UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/045,159	07/25/2018	Michael Francis	LNH-152US1

CONFIRMATION NO. 5408

23122
RATNERPRESTIA
2200 Renaissance Blvd
Suite 350
King of Prussia, PA 19406

FORMALITIES LETTER

OC00000101876054

Date Mailed: 08/28/2018

NOTICE TO FILE CORRECTED APPLICATION PAPERS

Filing Date Granted

An application number and filing date have been accorded to this application. The application is informal since it does not comply with the regulations for the reason(s) indicated below. Applicant is given TWO MONTHS from the date of this Notice within which to correct the informalities indicated below. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

The required item(s) identified below must be timely submitted to avoid abandonment:

- Replacement drawings in compliance with 37 CFR 1.84 and 37 CFR 1.121(d) are required. The drawings submitted are not acceptable because:
  - The application contains drawings and the specification contains a brief description of the drawings. However, the specification does not contain a brief description of the several views of the drawings as required by 37 CFR 1.74 and 37 CFR 1.77(b)(7) and/or a drawing(s) has not been labeled in accordance with 37 CFR 1.84(u)(1). If each figure is not labeled "Fig." with a consecutive Arabic numeral (1, 2, etc.) or an Arabic numeral and capital letter in the English alphabet (A, B, etc.), then the drawing(s) must be relabeled in accordance with 37 CFR 1.84(u)(1). In addition, if the brief description of the several views of the drawings does not refer to the figure(s) as properly labeled, then the specification must be amended to correspond to the figure(s) as properly labeled and a substitute specification in compliance with 37 CFR 1.52, 1.121(b)(3), and 1.125, is required.

Applicant is cautioned that correction of the above items may cause the specification and drawings page count to exceed 100 pages. If the specification and drawings exceed 100 pages, applicant will need to submit the required application size fee.

Replies must be received in the USPTO within the set time period or must include a proper Certificate of Mailing or Transmission under 37 CFR 1.8 with a mailing or transmission date within the set time period. For more information and a suggested format, see Form PTO/SB/92 and MPEP 512.

Replies should be mailed to:

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web, including a copy of this Notice and selecting the document description "Applicant response to Pre-Exam Formalities Notice". https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

For more information about EFS-Web please call the USPTO Electronic Business Center at 1-866-217-9197 or visit our website at http://www.uspto.gov/ebc.

If you are not using EFS-Web to submit your reply, you must include a copy of this notice.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/cnguyen/

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No:	16/045,159
Applicant:	LifeNet Health
Filed:	July 25, 2018
Title:	ALIGNED FIBER AND METHOD OF USE THEREOF
TC/A.U.:	TBD
Examiner:	TBD
Confirmation No.:	5408

INFORMATION DISCLOSURE STATEMENT TRANSMITTAL

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

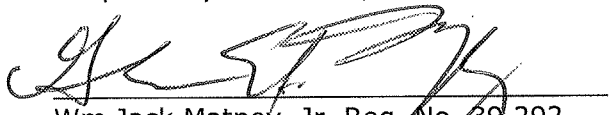
Pursuant to 37 C.F.R. §§ 1.97 and 1.98 and to the duty of disclosure set forth in 37 C.F.R. § 1.56, the Examiner in charge of the above-identified application is requested to consider and make of record the reference listed on the PTO/SB/08a submitted herewith. A copy of the reference listed on the PTO/SB/08a, other than U.S. patents, is enclosed.

Although the information submitted herewith may be "material" to the Examiner's consideration of the subject application, this submission is not intended to constitute an admission that such information is "prior art" as to the claimed invention.

In accordance with 37 C.F.R. § 1.97(g), the filing of this Information Disclosure Statement shall not be construed to mean that a search has been made.

The Information Disclosure Statement submitted herewith is being filed concurrently with the above-referenced application or is being filed within three months of the filing date of the above-referenced application, before mailing of the first Official Action on the merits or before the mailing of a first action on the merits after filing a Request for Continued Examination. No first Official Action has yet been received and it is presumed that none has yet been mailed. No fee or statement is required. 37 C.F.R. § 1.97(b).

Respectfully submitted,



Wm Jack Matney, Jr, Reg. No. 39,292
Glenn E. J. Murphy, Reg. No. 33,539
Attorneys for Applicants

WJM/GEM/nm

Enclosures: PTO/SB/08a
Notice of Allowance

Dated: September 14, 2018

2200 Renaissance Blvd
Suite 350
King of Prussia, PA 19406
(610) 407-0700

The Commissioner for Patents is hereby authorized to charge payment to Deposit Account No. **18-0350** of any fees associated with this communication.

4130144

PTO/SB/08a (03-15)
Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	16045159
Filing Date	2018-07-25
First Named Inventor	Michael Francis
Art Unit	TBD
Examiner Name	TBD
Attorney Docket Number	LNH-152US1

U.S.PATENTS

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					

If you wish to add additional U.S. Patent citation information please click the Add button.

U.S.PATENT APPLICATION PUBLICATIONS

Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					

If you wish to add additional U.S. Published Application citation information please click the Add button.

FOREIGN PATENT DOCUMENTS

Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1							☐

If you wish to add additional Foreign Patent Document citation information please click the Add button

NON-PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	16045159
	Filing Date	2018-07-25
	First Named Inventor	Michael Francis
	Art Unit	TBD
	Examiner Name	TBD
	Attorney Docket Number	LNH-152US1

	1	Notice of Allowance for Application No. 14/776,106, mailed August 15, 2018, 10 pages.	☐

If you wish to add additional non-patent literature document citation information please click the Add button

EXAMINER SIGNATURE

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	16045159
	Filing Date	2018-07-25
	First Named Inventor	Michael Francis
	Art Unit	TBD
	Examiner Name	TBD
	Attorney Docket Number	LNH-152US1

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

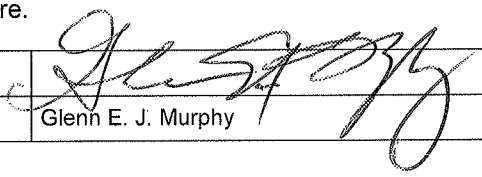
☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature		Date (YYYY-MM-DD)	2018-09-14
Name/Print	Glenn E. J. Murphy	Registration Number	33,539

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

PTO/AIA/96 (08-12)
Approved for use through 01/31/2013. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

STATEMENT UNDER 37 CFR 3.73(c)

Applicant/Patent Owner: Michael Francis et al.

Application No./Patent No.: 16/045,159 Filed/Issue Date: July 25, 2018

Titled: ALIGNED FIBER AND METHOD OF USE THEREOF

LIFENET HEALTH, a Corporation

(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that, for the patent application/patent identified above, it is (choose **one** of options 1, 2, 3 or 4 below):

1. ☑ The assignee of the entire right, title, and interest.

2. ☐ An assignee of less than the entire right, title, and interest (check applicable box):

   ☐ The extent (by percentage) of its ownership interest is _____%. Additional Statement(s) by the owners holding the balance of the interest must be submitted to account for 100% of the ownership interest.

   ☐ There are unspecified percentages of ownership. The other parties, including inventors, who together own the entire right, title and interest are:

   Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

3. ☐ The assignee of an undivided interest in the entirety (a complete assignment from one of the joint inventors was made). The other parties, including inventors, who together own the entire right, title, and interest are:

   Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

4. ☐ The recipient, via a court proceeding or the like (*e.g.*, bankruptcy, probate), of an undivided interest in the entirety (a complete transfer of ownership interest was made). The certified document(s) showing the transfer is attached.

The interest identified in option 1, 2 or 3 above (not option 4) is evidenced by either (choose **one** of options A or B below):

A. ☑ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel 046635, Frame 0001, or for which a copy thereof is attached.

B. ☐ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

   1. From: _____ To: _____

      The document was recorded in the United States Patent and Trademark Office at

      Reel _____, Frame _____, or for which a copy thereof is attached.

   2. From: _____ To: _____

      The document was recorded in the United States Patent and Trademark Office at

      Reel _____, Frame _____, or for which a copy thereof is attached.

This collection of information is required by 37 CFR 3.73(b). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PTO/AIA/96 (08-12)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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STATEMENT UNDER 37 CFR 3.73(c)

3. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

4. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

5. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

6. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet(s).

☐ As required by 37 CFR 3.73(c)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

Signature	September 14, 2018 Date
Glenn E. J. Murphy Printed or Typed Name	33,539 Title or Registration Number

[Page 2 of 2]

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No:	16/045,159
Applicants:	Michael Francis et al.
Filed:	July 25, 2018
Title:	ALIGNED FIBER AND METHOD OF USE THEREOF
T.C./A.U.:	To Be Assigned
Examiner:	To Be Assigned
Confirmation No.:	5408
Docket No.:	LNH-152US1

SECOND PRELIMINARY AMENDMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

Please amend the above-identified application as follows:

☒ **Amendments to the Specification** begin on page **2** of this paper.

☐ **Amendments to the Claims** are reflected in the listing of claims which begins on page of this paper.

☐ **Amendments to the Drawings** begin on page of this paper and include an attached replacement sheet(s).

☐ **Amendments to the Abstract** are on page of this paper. A clean version of the Abstract is on page of this paper.

☒ **Remarks/Arguments** begin on page **3** of this paper.

Amendments to the Specification:

Please replace the paragraph, beginning at page 1, line 17, with the following rewritten paragraph:

[0005] FIGURES 1A and 1D depicts representative field lines that are modulated between a split point negative voltage sources and a single positive voltage source ~~(A, D)~~, aiding in imparting alignment in the electrospinning scheme. FIGURES 1B and 1C model ~~T~~the change in field lines between a solid (B) and hollow (C) rod, respectively ~~is also modeled~~.

Please replace the paragraph, beginning at page 2, line 14, with the following rewritten paragraph:

[0012] FIGURE 8A depicts exemplary FFT analysis results ~~(a)~~ of the fibers described herein, and FIGURE 8B depicts an FFT analysis of ~~(b)~~ a control sample.

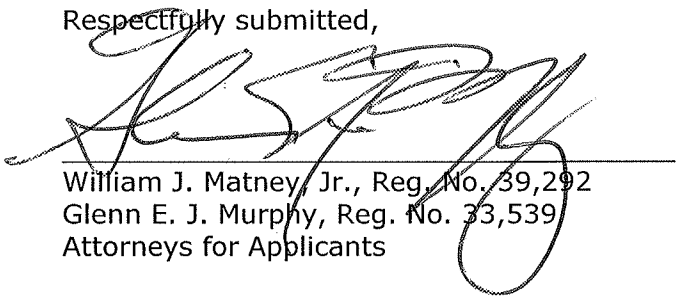
Please replace the paragraph, beginning at page 2, line 16, with the following rewritten paragraph:

[0013] FIGURES 9A, 9B and 9C depict exemplary scaffolds prepared into an elongated roll by folding an elongated sheet (FIGURE 9A), an elongated roll by rolling an elongated sheet (FIGURE 9B), and a composite of multiple layers of elongated sheets (FIGURE 9C).

Remarks/Arguments:

In response to the notice to file corrected application papers, Applicant amends the specification in the brief description of the drawings to refer to the figures as labeled. No new matter has been added.

Respectfully submitted,



William J. Matney, Jr., Reg. No. 39,292
Glenn E. J. Murphy, Reg. No. 33,539
Attorneys for Applicants

WJM/GEJM/nm

Dated: September 14, 2018

2200 Renaissance Blvd
Suite 350
King of Prussia, PA 19406
(610) 407-0700

The Director is hereby authorized to charge or credit Deposit Account No. **18-0350** for any additional fees, or any underpayment or credit for overpayment in connection herewith.

4130146

PTO/AIA/80 (07-12)
Approved for use through 11/30/2014. OMB 0651-0035
U.S. Patent and Trademark Office: U.S DEPARTMENT OF COMMERCE
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POWER OF ATTORNEY TO PROSECUTE APPLICATIONS BEFORE THE USPTO

I hereby revoke all previous powers of attorney given in the application identified in the attached statement under 37 CFR 3.73(c).

I hereby appoint:

■ Practitioners associated with Customer Number: 23122

OR

☐ Practitioner(s) named below (if more than ten patent practitioners are to be named, then a customer number must be used):

Name	Registration Number	Name	Registration Number

As attorney(s) or agent(s) to represent the undersigned before the United States Patent and Trademark Office (USPTO) in connection with any and all patent applications assigned only to the undersigned according to the USPTO assignment records or assignments documents attached to this form in accordance with 37 CFR 3.73(c).

Please change the correspondence address for the application identified in the attached statement under 37 CFR 3.73(c) to:

■ The address associated with Customer Number: 23122

OR

☐

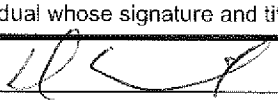
Firm or Individual Name			
Address			
City		State	Zip
Country			
Telephone		Email	

Assignee Name and Address: LIFENET HEALTH
1864 CONCERT DRIVE, VIRGINIA BEACH, VIRGINIA 23453

A copy of this form, together with a statement under 37 CFR 3.73(c) (Form PTO/AIA/96 or equivalent) is required to be Filed in each application in which this form is used. The statement under 37 CFR 3.73(c) may be completed by one of The practitioners appointed in this form, and must identify the application in which this Power of Attorney is to be filed.

SIGNATURE of Assignee of Record

The individual whose signature and title is supplied below is authorized to act on behalf of the assignee

Signature		Date	11-17-15
Name	D B Wilson	Telephone	7573482531
Title	EVP		

This collection of information is required by 37 CFR 1.31, 1.32 and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Acknowledgement Receipt

EFS ID:	33724130
Application Number:	16045159
International Application Number:	
Confirmation Number:	5408
Title of Invention:	ALIGNED FIBER AND METHOD OF USE THEREOF
First Named Inventor/Applicant Name:	Michael Francis
Customer Number:	23122
Filer:	Glenn E. Murphy/Nicole May
Filer Authorized By:	Glenn E. Murphy
Attorney Docket Number:	LNH-152US1
Receipt Date:	14-SEP-2018
Filing Date:	25-JUL-2018
Time Stamp:	14:57:52
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Preliminary Amendment	PreliminaryAmendment.pdf	61826 13edaf68ec72e7f812a37b17b47ff77d22be1e25	no	3

Warnings:

EMBZIM EX1002

Information:					
2	Transmittal Letter	IDS.pdf	44486 99a056b8a0a21aec18937068a458b5c5a43055fd	no	2
Warnings:					
Information:					
3	Information Disclosure Statement (IDS) Form (SB08)	PTOSB08a.pdf	192375 cd7c091f2326005a9d824c0228fd93f143663968	no	4
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
4	Non Patent Literature	NOA.pdf	1706813 b7b666ffd26251c4d369615a268349230ca1fddb	no	10
Warnings:					
Information:					
5	Assignee showing of ownership per 37 CFR 3.73	StatementUnder373c.pdf	155217 9bf240141db841fba3d61b09d6f9aa93dc83352d	no	3
Warnings:					
Information:					
6	Power of Attorney	POA.pdf	194547 910f1e1c22556b2a75d47c88de429d37494865d2	no	2
Warnings:					
The page size in the PDF is too large. The pages should be 8.5 x 11 or A4. If this PDF is submitted, the pages will be resized upon entry into the Image File Wrapper and may affect subsequent processing					
Information:					
			Total Files Size (in bytes):	2355264	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/045,159	07/25/2018	Michael Francis	LNH-152US1

CONFIRMATION NO. 5408

23122
RATNERPRESTIA
2200 Renaissance Blvd
Suite 350
King of Prussia, PA 19406

MISCELLANEOUS NOTICE

OC00000102386769

Date Mailed: 09/19/2018

A communication which cannot be delivered in electronic form has been mailed to the applicant.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/045,159	07/25/2018	Michael Francis	LNH-152US1

CONFIRMATION NO. 5408

23122
RATNERPRESTIA
2200 Renaissance Blvd
Suite 350
King of Prussia, PA 19406

FORMALITIES LETTER

OC00000102386713

Date Mailed: 09/19/2018

NOTICE OF INCOMPLETE REPLY (NONPROVISIONAL)

Filing Date Granted

The U.S. Patent and Trademark Office has received your reply on 09/14/2018 to the Notice to File Missing Parts (Notice) mailed 08/28/2018 and it has been entered into the nonprovisional application. The reply, however, does not include the following items required in the Notice. A complete reply must be timely filed to prevent ABANDONMENT of the above-identified application. Replies should be mailed to: Mail Stop Missing Parts, Commissioner for Patents, P.O. Box 1450, Alexandria VA 22313-1450.

Applicant is given **TWO MONTHS** from the date of the Notice to File Missing Parts (Notice) mailed 08/28/2018 within which to file all required items and pay any fees required below to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

Items Required to Avoid Abandonment:

The required items noted below SHOULD be filed along with any items required above. The filing date of this nonprovisional application will be the date of receipt of the items required above.

The application is informal since it does not comply with the regulations for the reason(s) indicated below.

The required item(s) identified below must be timely submitted to avoid abandonment:

- Replacement drawings in compliance with 37 CFR 1.84 and 37 CFR 1.121(d) are required. The drawings submitted are not acceptable because:
  - The application contains drawings and the specification contains a brief description of the drawings. However, the specification does not contain a brief description of the several views of the drawings as required by 37 CFR 1.74 and 37 CFR 1.77(b)(7) and/or a drawing(s) has not been labeled in accordance with 37 CFR 1.84(u)(1). If each figure is not labeled "Fig." with a consecutive Arabic numeral (1, 2, etc.) or an Arabic numeral and capital letter in the English alphabet (A, B, etc.), then the drawing(s) must be relabeled in accordance with 37 CFR 1.84(u)(1). In addition, if the brief description of the several views of the drawings does not refer to the figure(s) as properly labeled, then the specification must be amended to correspond to the figure(s) as properly labeled and a substitute specification in compliance with 37 CFR 1.52, 1.121(b)(3), and 1.125, is required.

Applicant is cautioned that correction of the above items may cause the specification and drawings page count to exceed 100 pages. If the specification and drawings exceed 100 pages, applicant will need to submit the required application size fee.

Replies must be received in the USPTO within the set time period or must include a proper Certificate of Mailing or Transmission under 37 CFR 1.8 with a mailing or transmission date within the set time period. For more information and a suggested format, see Form PTO/SB/92 and MPEP 512.

Replies should be mailed to:

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web, including a copy of this Notice and selecting the document description "Applicant response to Pre-Exam Formalities Notice". https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

For more information about EFS-Web please call the USPTO Electronic Business Center at 1-866-217-9197 or visit our website at http://www.uspto.gov/ebc.

If you are not using EFS-Web to submit your reply, you must include a copy of this notice.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/ebekele/

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/045,159	07/25/2018	Michael Francis	LNH-152US1

CONFIRMATION NO. 5408

23122
RATNERPRESTIA
2200 Renaissance Blvd
Suite 350
King of Prussia, PA 19406

POA ACCEPTANCE LETTER

OC00000102386611

Date Mailed: 09/19/2018

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 09/14/2018.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/ebekele/

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
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APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/045,159	07/25/2018	Michael Francis	LNH-152US1

CONFIRMATION NO. 5408

OC000000102386769

23122
RATNERPRESTIA
2200 Renaissance Blvd
Suite 350
King of Prussia, PA 19406

Date Mailed: 09/19/2018

NOTICE OF INCOMPLETE REPLY

Filing Date Granted

Applicant's reply to the Notice mailed on 08/28/2018 was received in the U.S. Patent and Trademark Office on 09/14/2018 and has been entered into the application. The reply, however, does not include the following item(s) required in the Notice.

The period for reply continues to run from the mailing date of the prior Notice. The item(s) listed below must be timely filed to avoid abandonment of the application. No new time period for reply is provided in this communication. If the period for reply set forth in the prior Notice has expired, this application will become abandoned unless applicant: (1) submits the following checked item(s), **and** (2) obtains an extension of time under 37 CFR 1. 136(a) (including the appropriate fee (37 CFR 1.17(a)). In no case may an applicant obtain an extension of time for more than FIVE (5) MONTHS beyond the date for reply set forth in the prior Notice.

A complete reply which includes the following checked item(s) must be timely filed to prevent ABANDONMENT of the above-identified application:

- ☑ A substitute specification.
- ☑ A statement that the substitute specification contains no new matter.
- ☐ A replacement abstract commencing on a separate sheet in compliance with 37 CFR 1.72(b) and 1.121 (deleting the drawings or flow diagrams).
- ☐ New and replacement drawing sheets.
- ☐ Replacement drawing sheets with the figures renumbered.
- ☐ A complete claim listing or a replacement claim listing with the claims renumbered.
- ☐ An amendment to the claims in compliance with 37 CFR 1.121 (deleting the drawings or flow diagrams).
- ☐ A replacement transmittal letter listing all of the files except the missing or unreadable file.
- ☐ A duplicate copy of the CD.
- ☐ A statement that the replacement CD contains no new matter.

Rev. 12/2008

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Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web.
https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

For more information about EFS-Web, please call the USPTO Electronic Business Center at 1-866-217-9197 or visit our website at http://www.uspto.gov/ebc.

Application Assistance Unit (571) 272-4200

Electronic Patent Application Fee Transmittal

Application Number:	16045159
Filing Date:	25-Jul-2018
Title of Invention:	ALIGNED FIBER AND METHOD OF USE THEREOF
First Named Inventor/Applicant Name:	Michael Francis
Filer:	Glenn E. Murphy/Nicole May
Attorney Docket Number:	LNH-152US1

Filed as Small Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension - 2 months with $0 paid	2252	1	300	300
Miscellaneous:				
			Total in USD ($)	**300**

Electronic Acknowledgement Receipt

EFS ID:	34714852
Application Number:	16045159
International Application Number:	
Confirmation Number:	5408
Title of Invention:	ALIGNED FIBER AND METHOD OF USE THEREOF
First Named Inventor/Applicant Name:	Michael Francis
Customer Number:	23122
Filer:	Glenn E. Murphy/Nicole May
Filer Authorized By:	Glenn E. Murphy
Attorney Docket Number:	LNH-152US1
Receipt Date:	28-DEC-2018
Filing Date:	25-JUL-2018
Time Stamp:	14:44:37
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$300
RAM confirmation Number	123118INTEFSW14451700
Deposit Account	180350
Authorized User	Nicole May

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

37 CFR 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Applicant Response to Pre-Exam Formalities Notice	PreliminaryAmendment.pdf	178182 f3cb9d089026a35da0ea4f175bd2d40041347627	no	6
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30525 82867229c8c5b1af3b4e894b232c71697b1135ca	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	208707		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No:	16/045,159
Applicants:	Michael Francis et al.
Filed:	July 25, 2018
Title:	ALIGNED FIBER AND METHOD OF USE THEREOF
T.C./A.U.:	To Be Assigned
Examiner:	To Be Assigned
Confirmation No.:	5408
Docket No.:	LNH-152US1

PRELIMINARY AMENDMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

In response to the Notice to File Corrected Application Papers dated August 28, 2018, and to the Notice of Incomplete Reply dated November 19, 2018, Applicant amends this application as follows:

☒ **Amendments to the Specification** begin on page **2** of this paper.

☐ **Amendments to the Claims** are reflected in the listing of claims which begins on page of this paper.

☐ **Amendments to the Drawings** begin on page of this paper and include an attached replacement sheet(s).

☐ **Amendments to the Abstract** are on page of this paper. A clean version of the Abstract is on page of this paper.

☒ **Remarks/Arguments** begin on page **4** of this paper.

Amendments to the Specification:

Please replace paragraph [0005] with the following rewritten paragraph:

[0005] FIGURES 1A ~~and 1D~~ depicts representative field lines that are modulated between ~~a~~ split point negative voltage sources and a single positive voltage source, aiding in imparting alignment in the electrospinning scheme. ~~FIGURES 1B and 1C model the change in field lines between a solid (B) and hollow (C) rod, respectively.~~

Please insert after paragraph [0005] the following new paragraphs [0005.1] to [0005.3]:

[0005.1] FIGURE 1B models field lines generated by a solid rod.

[0005.2] FIGURE 1C models field lines generated by a hollow rod.

[0005.3] FIGURE 1D depicts representative field lines that are modulated between split point negative voltage sources and a single positive voltage source, aiding in imparting alignment in the electrospinning scheme.

Please replace paragraph [0012] with the following rewritten paragraph:

[0012] FIGURE 8A depicts exemplary FFT analysis results of the fibers described herein~~, and FIGURE 8B depicts an FFT analysis of a control sample~~.

Please insert after paragraph [0012] the following new paragraph [0012.1]:

[0012.1] FIGURE 8B depicts an FFT analysis of a control sample.

Please replace paragraph [0013] with the following rewritten paragraph:

[0013] FIGURES 9A~~, 9B and 9C~~ depicts an exemplary scaffolds prepared into an elongated roll by folding an elongated sheet ~~(FIGURE 9A), an elongated roll by rolling an elongated sheet (FIGURE 9B), and a composite of multiple layers of elongated sheets (FIGURE 9C)~~.

Please insert after paragraph [0013] the following new paragraphs [0013.1] and [0013.2]:

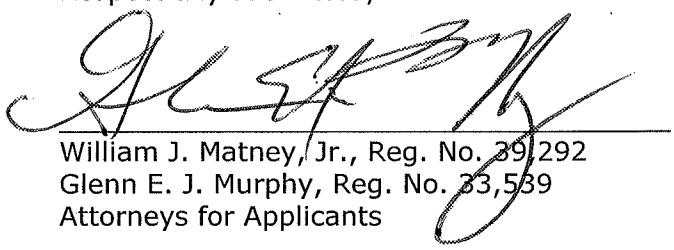
[0013.1] FIGURE 9B depicts an exemplary scaffold prepared into an elongated roll by rolling an elongated sheet.

[0013.2] FIGURE 9C depicts an exemplary scaffold prepared into an elongated roll from a composite of multiple layers of elongated sheets.

Remarks/Arguments:

In response to the Notice to File Corrected Application Papers mailed August 28, 2018, and to the Notice of Incomplete Reply mailed November 19, 2018, Applicant amends certain paragraphs in the Brief Description of the Drawings as set forth above. It is believed the specification so amended complies with 37 C.F.R. §§ 1.74 and 1.77(b)(7). In particular, the specification as amended contains a brief description of the several views of the drawings and refers to the figures as properly labeled. Acceptance of the application and forwarding to the appropriate Art Unit for examination are earnestly solicited.

Respectfully submitted,



William J. Matney, Jr., Reg. No. 39,292
Glenn E. J. Murphy, Reg. No. 33,539
Attorneys for Applicants

WJM/GEJM/nm

Dated: December 28, 2018

2200 Renaissance Blvd
Suite 350
King of Prussia, PA 19406
(610) 407-0700

The Director is hereby authorized to charge or credit Deposit Account No. **18-0350** for any additional fees, or any underpayment or credit for overpayment in connection herewith.

4199319

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/045,159	07/25/2018	Michael Francis	LNH-152US1

CONFIRMATION NO. 5408

FORMALITIES LETTER

23122
RATNERPRESTIA
2200 Renaissance Blvd
Suite 350
King of Prussia, PA 19406

OC000000102386713

Date Mailed: 09/19/2018

NOTICE OF INCOMPLETE REPLY (NONPROVISIONAL)

Filing Date Granted

The U.S. Patent and Trademark Office has received your reply on 09/14/2018 to the Notice to File Missing Parts (Notice) mailed 08/28/2018 and it has been entered into the nonprovisional application. The reply, however, does not include the following items required in the Notice. A complete reply must be timely filed to prevent ABANDONMENT of the above-identified application. Replies should be mailed to: Mail Stop Missing Parts, Commissioner for Patents, P.O. Box 1450, Alexandria VA 22313-1450.

Applicant is given **TWO MONTHS** from the date of the Notice to File Missing Parts (Notice) mailed 08/28/2018 within which to file all required items and pay any fees required below to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

Items Required to Avoid Abandonment:

The required items noted below SHOULD be filed along with any items required above. The filing date of this nonprovisional application will be the date of receipt of the items required above.

The application is informal since it does not comply with the regulations for the reason(s) indicated below.

The required item(s) identified below must be timely submitted to avoid abandonment:

- Replacement drawings in compliance with 37 CFR 1.84 and 37 CFR 1.121(d) are required. The drawings submitted are not acceptable because:
  - The application contains drawings and the specification contains a brief description of the drawings. However, the specification does not contain a brief description of the several views of the drawings as required by 37 CFR 1.74 and 37 CFR 1.77(b)(7) and/or a drawing(s) has not been labeled in accordance with 37 CFR 1.84(u)(1). If each figure is not labeled "Fig." with a consecutive Arabic numeral (1, 2, etc.) or an Arabic numeral and capital letter in the English alphabet (A, B, etc.), then the drawing(s) must be relabeled in accordance with 37 CFR 1.84(u)(1). In addition, if the brief description of the several views of the drawings does not refer to the figure(s) as properly labeled, then the specification must be amended to correspond to the figure(s) as properly labeled and a substitute specification in compliance with 37 CFR 1.52, 1.121(b)(3), and 1.125, is required.

Applicant is cautioned that correction of the above items may cause the specification and drawings page count to exceed 100 pages. If the specification and drawings exceed 100 pages, applicant will need to submit the required application size fee.

COPY

Replies must be received in the USPTO within the set time period or must include a proper Certificate of Mailing or Transmission under 37 CFR 1.8 with a mailing or transmission date within the set time period. For more information and a suggested format, see Form PTO/SB/92 and MPEP 512.

Replies should be mailed to:

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P.O. Box 1450
Alexandria VA 22313-1450

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For more information about EFS-Web please call the USPTO Electronic Business Center at 1-866-217-9197 or visit our website at http://www.uspto.gov/ebc.

If you are not using EFS-Web to submit your reply, you must include a copy of this notice.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/ebekele/

COPY

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 16/045,159

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	75		N/A	
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	330		N/A	
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	380		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	11 minus 20 =	*	x 50 =	0.00	OR		
INDEPENDENT CLAIMS (37 CFR 1.16(h))	4 minus 3 =	* 1	x 230 =	230			
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			0.00			
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				0.00			
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	1015		TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/045,159	07/25/2018	Michael Francis	LNH-152US1

CONFIRMATION NO. 5408

23122
RATNERPRESTIA
2200 Renaissance Blvd
Suite 350
King of Prussia, PA 19406

FORMALITIES LETTER

OC000000104807280

Date Mailed: 01/03/2019

NOTICE OF INCOMPLETE REPLY (NONPROVISIONAL)

Filing Date Granted

The U.S. Patent and Trademark Office has received your reply on 12/28/2018 to the Notice to File Missing Parts (Notice) mailed 08/28/2018 and it has been entered into the nonprovisional application. The reply, however, does not include the following items required in the Notice. A complete reply must be timely filed to prevent ABANDONMENT of the above-identified application. Replies should be mailed to: Mail Stop Missing Parts, Commissioner for Patents, P.O. Box 1450, Alexandria VA 22313-1450.

Applicant is given **TWO MONTHS** from the date of the Notice to File Missing Parts (Notice) mailed 08/28/2018 within which to file all required items and pay any fees required below to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

Items Required to Avoid Abandonment:

The required items noted below SHOULD be filed along with any items required above. The filing date of this nonprovisional application will be the date of receipt of the items required above.

The application is informal since it does not comply with the regulations for the reason(s) indicated below.

The required item(s) identified below must be timely submitted to avoid abandonment:

- Replacement drawings in compliance with 37 CFR 1.84 and 37 CFR 1.121(d) are required. The drawings submitted are not acceptable because:
  - The application contains drawings and the specification contains a brief description of the drawings. However, the specification does not contain a brief description of the several views of the drawings as required by 37 CFR 1.74 and 37 CFR 1.77(b)(7) and/or a drawing(s) has not been labeled in accordance with 37 CFR 1.84(u)(1). If each figure is not labeled "Fig." with a consecutive Arabic numeral (1, 2, etc.) or an Arabic numeral and capital letter in the English alphabet (A, B, etc.), then the drawing(s) must be relabeled in accordance with 37 CFR 1.84(u)(1). In addition, if the brief description of the several views of the drawings does not refer to the figure(s) as properly labeled, then the specification must be amended to correspond to the figure(s) as properly labeled and a substitute specification in compliance with 37 CFR 1.52, 1.121(b)(3), and 1.125, is required.

Applicant is cautioned that correction of the above items may cause the specification and drawings page count to exceed 100 pages. If the specification and drawings exceed 100 pages, applicant will need to submit the required application size fee.

Replies must be received in the USPTO within the set time period or must include a proper Certificate of Mailing or Transmission under 37 CFR 1.8 with a mailing or transmission date within the set time period. For more information and a suggested format, see Form PTO/SB/92 and MPEP 512.

Replies should be mailed to:

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web, including a copy of this Notice and selecting the document description "Applicant response to Pre-Exam Formalities Notice". https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

For more information about EFS-Web please call the USPTO Electronic Business Center at 1-866-217-9197 or visit our website at http://www.uspto.gov/ebc.

If you are not using EFS-Web to submit your reply, you must include a copy of this notice.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/hkquach/

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United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/045,159	07/25/2018	Michael Francis	LNH-152US1	5408

23122 7590 01/03/2019

RATNERPRESTIA
2200 Renaissance Blvd
Suite 350
King of Prussia, PA 19406

EXAMINER	
ART UNIT	PAPER NUMBER

NOTIFICATION DATE	DELIVERY MODE
01/03/2019	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

PCorrespondence@ratnerprestia.com

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address : COMMISIONER FOR PATENTS
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

NOTICE OF INCOMPLETE REPLY

Filing Date Granted

Applicant's reply to the Notice mailed on 19 September 2018 was received in the U.S. Patent and Trademark Office on 28 December 2018 and has been entered into the application. The reply, however, does not include the following item(s) required in the Notice.

The period for reply continues to run from the mailing date of the prior Notice. The item(s) listed below must be timely filed to avoid abandonment of the application. No new time period for reply is provided in this communication. If the period for reply set forth in the prior Notice has expired, this application will become abandoned unless applicant: (1) submits the following checked item(s), **and** (2) obtains an extension of time under 37 CFR 1. 136(a) (including the appropriate fee (37 CFR 1.17(a)). In no case may an applicant obtain an extension of time for more than FIVE (5) MONTHS beyond the date for reply set forth in the prior Notice.

A complete reply which includes the following checked item(s) must be timely filed to prevent ABANDONMENT of the above-identified application:

- ☑ A substitute specification.
- ☑ A statement that the substitute specification contains no new matter.
- ☐ A replacement abstract commencing on a separate sheet in compliance with 37 CFR 1.72(b) and 1.121 (deleting the drawings or flow diagrams).
- ☐ New and replacement drawing sheets.
- ☐ Replacement drawing sheets with the figures renumbered.
- ☐ A complete claim listing or a replacement claim listing with the claims renumbered.
- ☐ An amendment to the claims in compliance with 37 CFR 1.121 (deleting the drawings or flow diagrams).
- ☐ A replacement transmittal letter listing all of the files except the missing or unreadable file.
- ☐ A duplicate copy of the CD.
- ☐ A statement that the replacement CD contains no new matter.

Replies should be mailed to:

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web. https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

For more information about EFS-Web, please call the USPTO Electronic Business Center at 1-866-217-9197 or visit our website at https://www.uspto.gov/ebc

Any inquiries regarding this notice should be directed to the Application Assistance Unit at 571-272-4200

/erimando/

Application Assistance Unit
571-272-4200

SCORE Placeholder Sheet for IFW Content

Application Number: 16045159 Document Date: 02/27/2019

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded in PALM, and no paper documents or physical media exist. The TIFF images in the IFW record were created from the original documents that are stored in SCORE.

- Drawing

At the time of document entry (noted above):

- USPTO employees may access SCORE content via eDAN using the Supplemental Content tab, or via the SCORE web page.
- External customers may access SCORE content via PAIR using the Supplemental Content tab.

Form Revision Date: August 26, 2013

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No:	16/045,159
Applicants:	Michael Francis et al.
Filed:	July 25, 2018
Title:	ALIGNED FIBER AND METHOD OF USE THEREOF
Art Unit:	TBD
Examiner:	TBD
Confirmation No.:	5408
Docket No.:	LNH-152US1

RESPONSE TO NOTICE OF INCOMPLETE REPLY

Mail Stop: Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

In response to the Notice to File Corrected Application Papers dated August 28, 2018, and to the Notice of Incomplete Reply dated September 19, 2018, and January 3, 2019, respectively. Applicants hereby submit under 37 C.F.R. §§1.121(b)(3) and 1.125(c), a Substitute Specification (clean version), a Marked-Up Specification (showing edits) and replacement drawings. No new matter has been added. Applicants respectfully request that the Substitute Specification and replacement drawings (10 drawing sheets containing, FIGs. 1A, 1B, 1C, 1D, 2A, 2B, 2C, 2D, 2E, 3A, 3B, 3C, 3D, 4A, 4B, 4C, 5, 6A, 6B, 7A, 7B, 7C, 7D, 7E, 7F, 7G, 7H, 7I, 7J, 8A, 8B, 9A, 9B, and 9C) be entered and examined in the above-identified application.

The Director is hereby authorized to charge or credit Deposit Account No. **18-0350** for any additional fees, or any underpayment or credit for overpayment in connection herewith.

Respectfully submitted,

/ Valerie P. Hayes /
Valerie P. Hayes, Reg. No. 53,005
Attorneys for Applicants

WJM/VPH/er

Dated: February 27, 2019

RatnerPrestia
1090 Vermont Avenue, N.W.
Suite 1200
Washington D.C. 20005
Tel. 202-715-2898

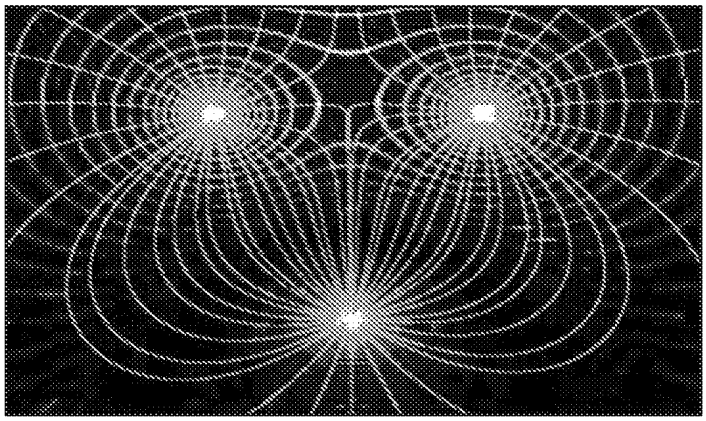


FIG. 1A

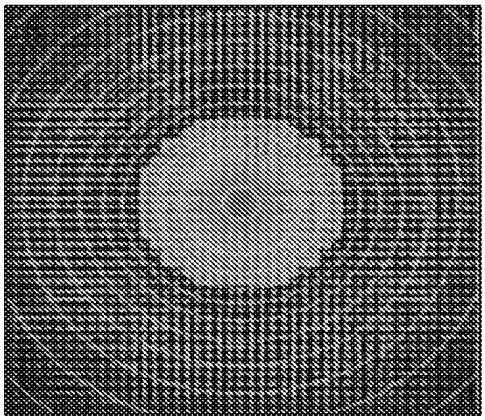


FIG. 1B

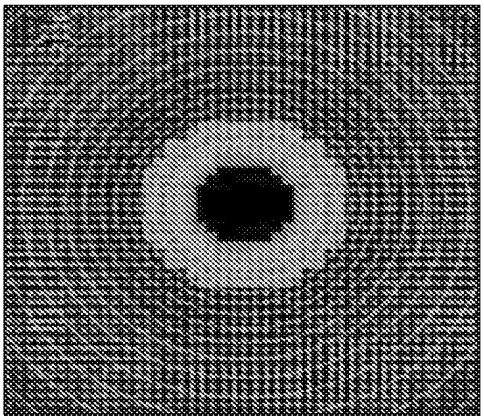


FIG. 1C

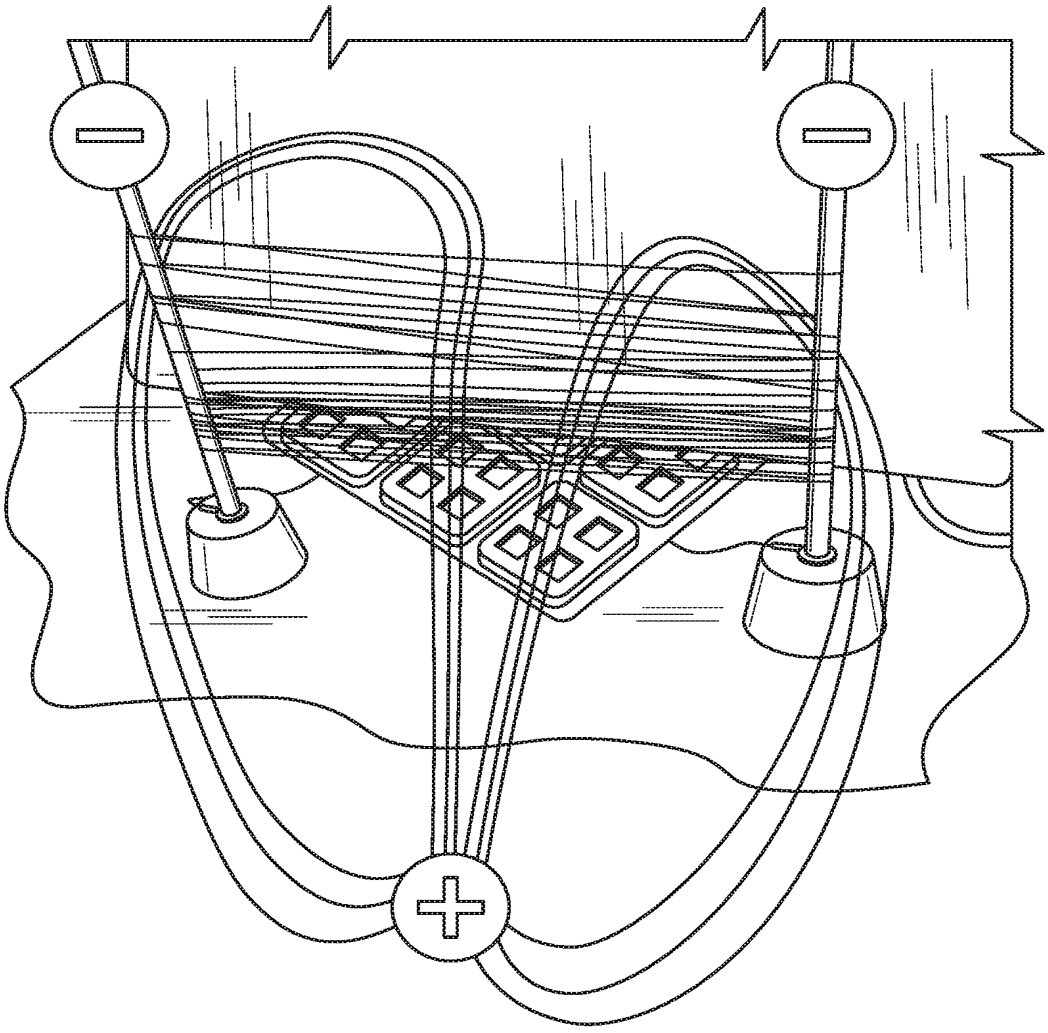


FIG. 1D

LYOPHILIZED BM

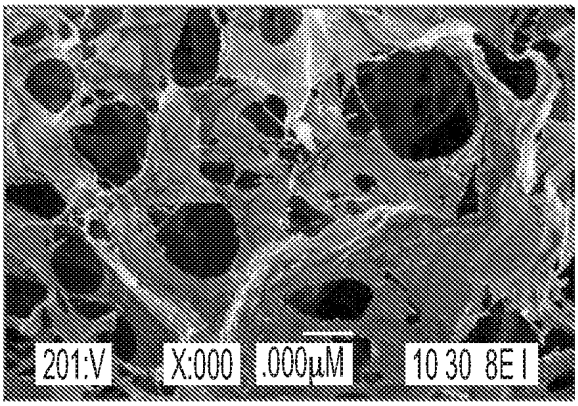


FIG. 2A

ELECTROSPUN BM

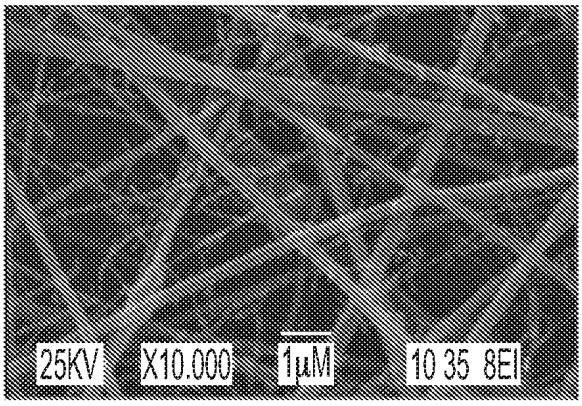
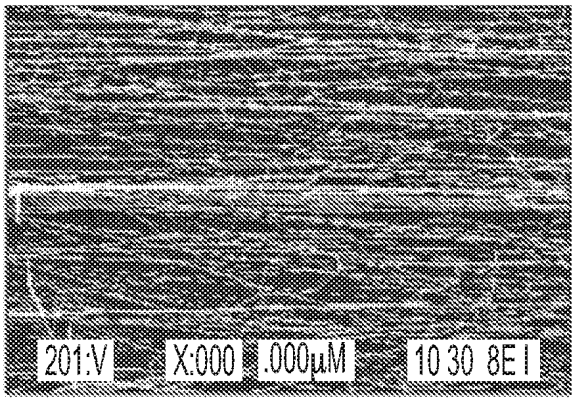
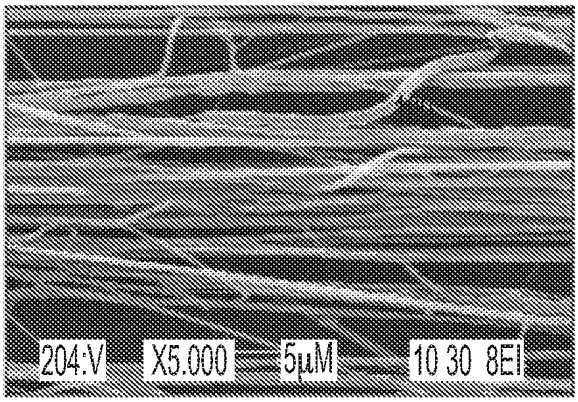


FIG. 2B



ALIGNED BM 1,000X

FIG. 2C



ALIGNED BM 5,000X

FIG. 2D

AVG DIAMETER (N=35, MEAN±SD)	FIBER SIZE RANGE	AVG FIBER ANGLE (N=35, MEAN±SD)	FIBER ANGLE RANGE
311±71 NM	152-458 NM	92.340±15.102 DEG	40-131 DEG

FIG. 2E

PHASE CONTRAST

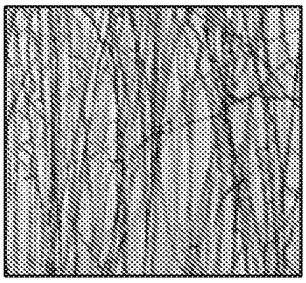


FIG. 3A

FOURIER SPECTRUM

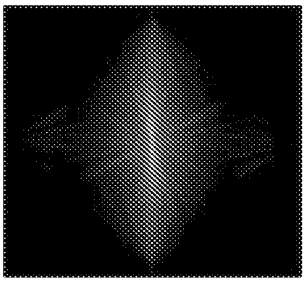


FIG. 3B

PHASE CONTRAST

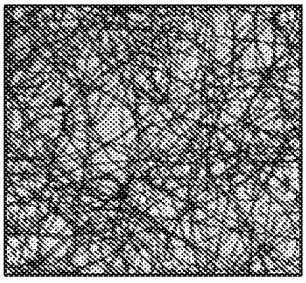


FIG. 3C

FOURIER SPECTRUM

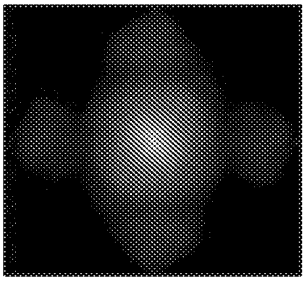


FIG. 3D

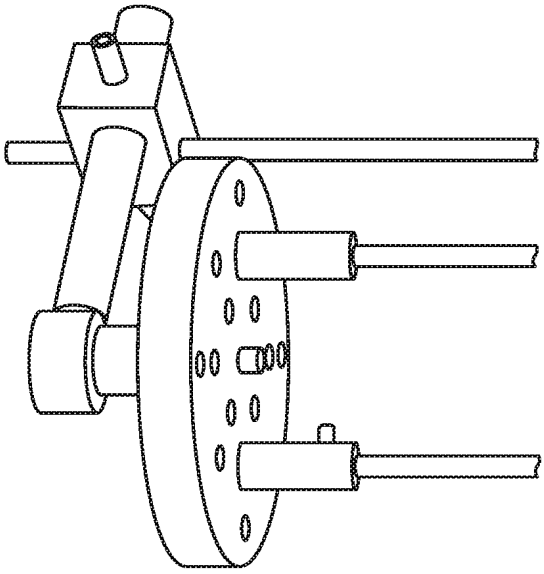


FIG. 4C

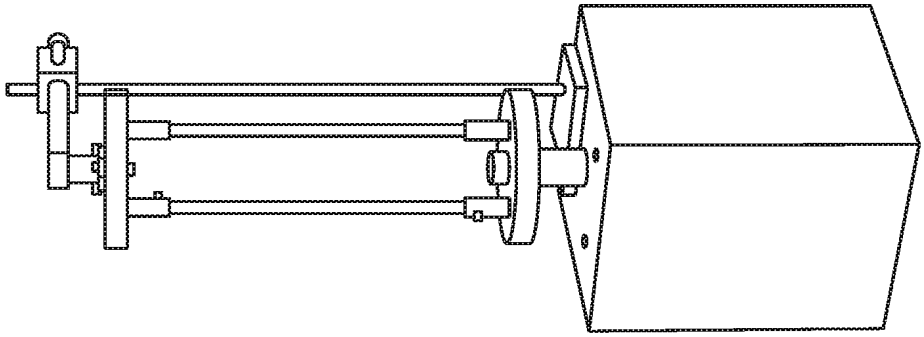


FIG. 4B

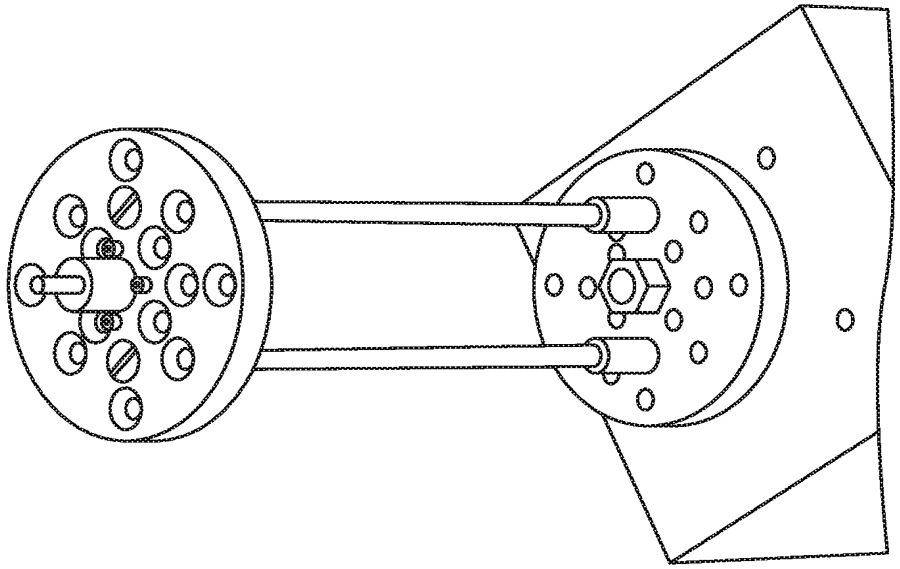


FIG. 4A

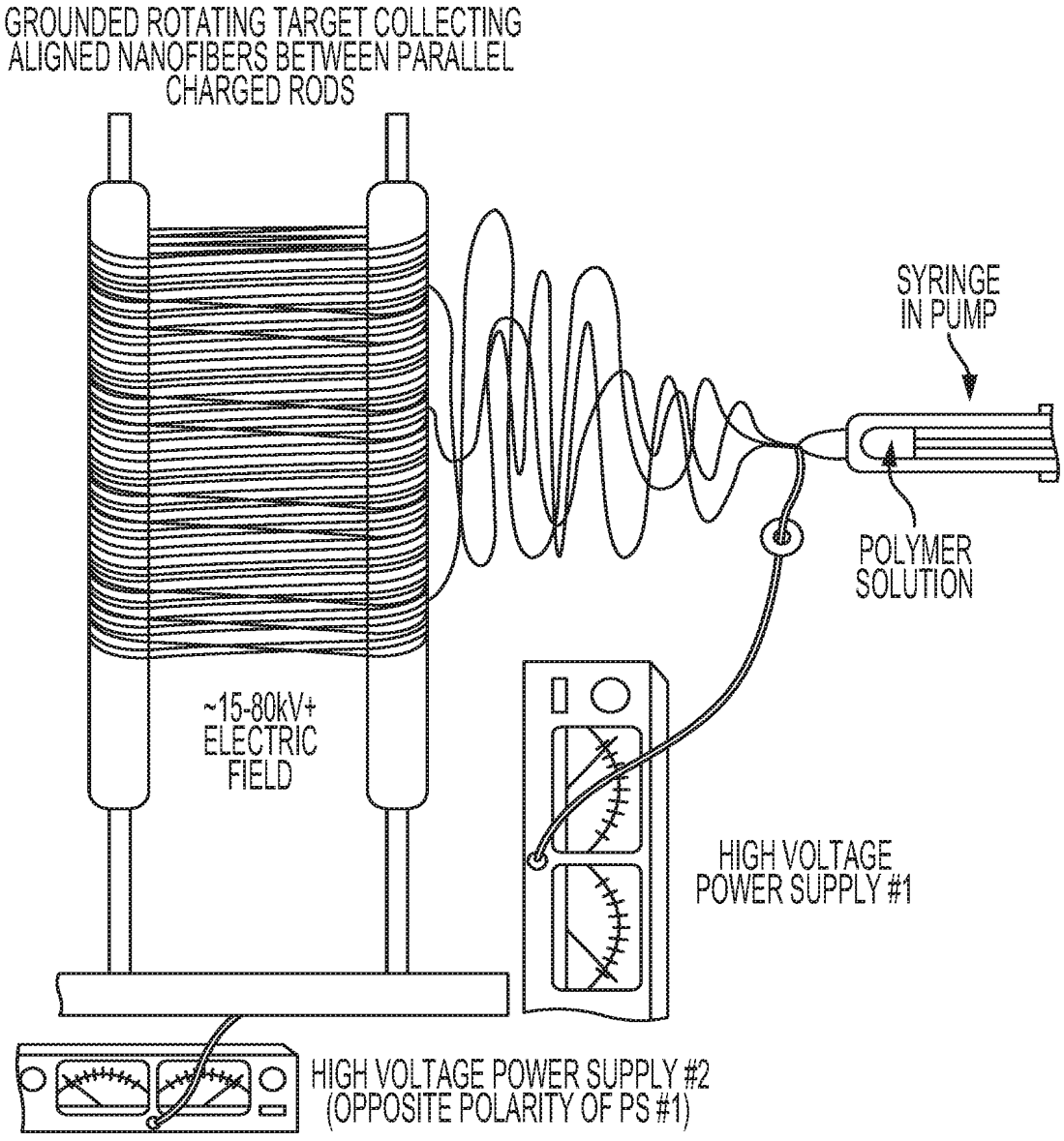

GROUNDED ROTATING TARGET COLLECTING ALIGNED NANOFIBERS BETWEEN PARALLEL CHARGED RODS
SYRINGE IN PUMP
POLYMER SOLUTION
~15-80kV+ ELECTRIC FIELD
HIGH VOLTAGE POWER SUPPLY #1
HIGH VOLTAGE POWER SUPPLY #2 (OPPOSITE POLARITY OF PS #1)

FIG. 5

DAY 2

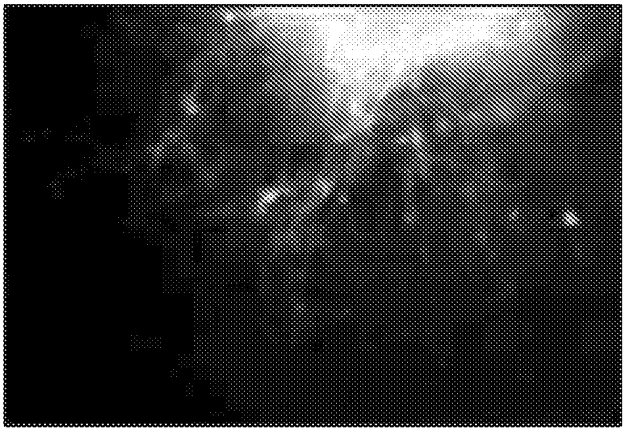


FIG. 6A

DAY 4

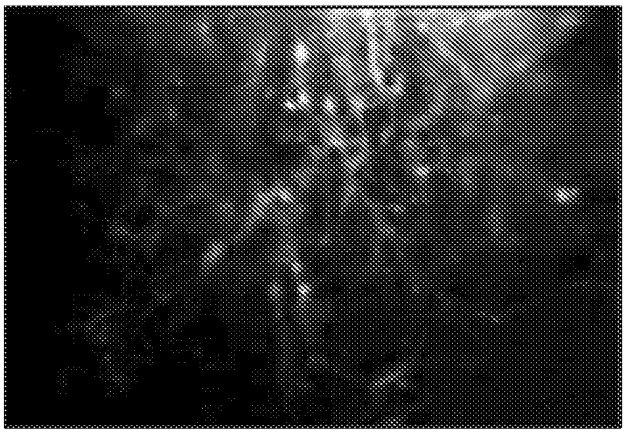


FIG. 6B

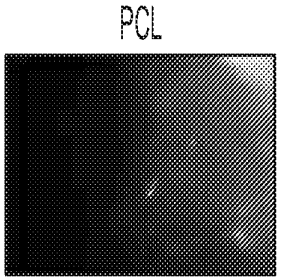


FIG. 7A

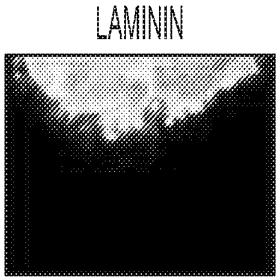


FIG. 7B

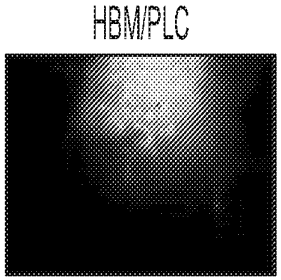


FIG. 7C

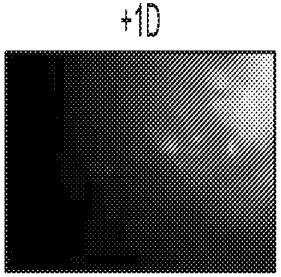


FIG. 7D

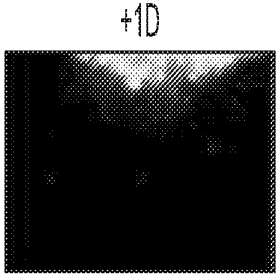


FIG. 7E

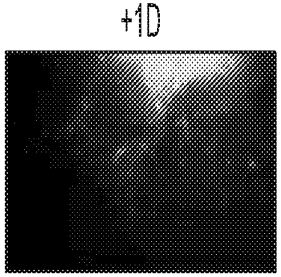


FIG. 7F

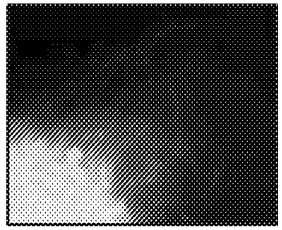


FIG. 7G



FIG. 7H

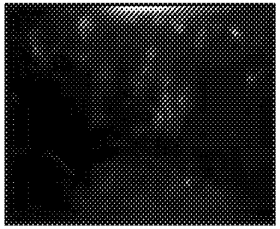


FIG. 7I

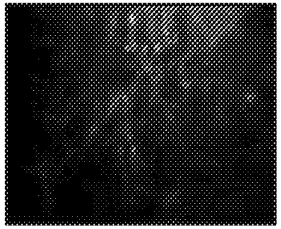


FIG. 7J

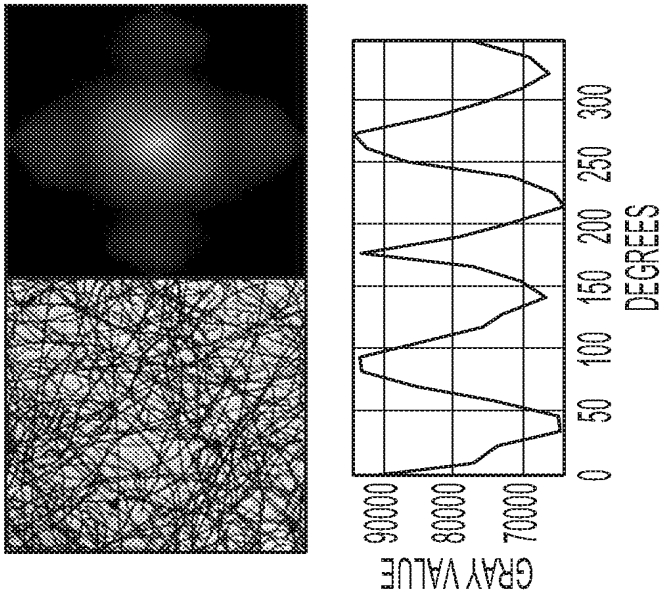

GRAY VALUE
90000
80000
70000
0
50
100
150
200
250
300
DEGREES

FIG. 8B

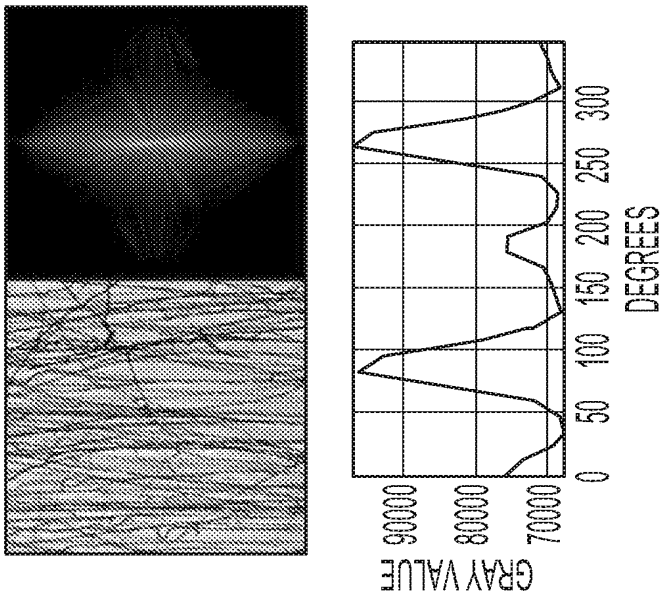

GRAY VALUE
90000
80000
70000
0
50
100
150
200
250
300
DEGREES

FIG. 8A

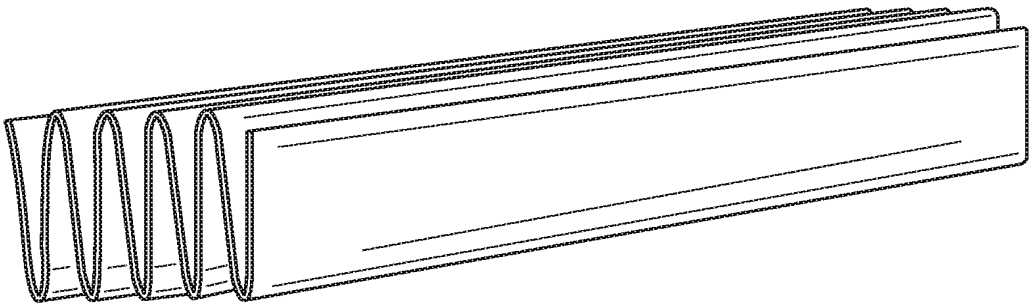


FIG. 9A

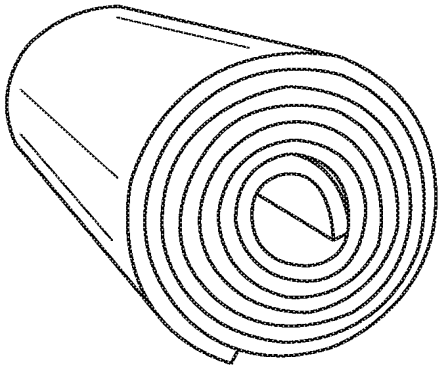


FIG. 9B

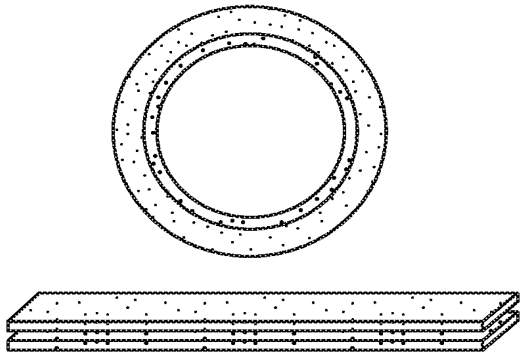


FIG. 9C

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ALIGNED FIBER AND METHOD OF USE THEREOF

Inventor

Michael Francis and Roy Ogle

Cross-Reference to Related Applications

[0001] This application is a Divisional Application of U.S. Application No. 14/776,106, filed September 14, 2015, which is a U.S. National Phase Application of PCT International Application PCT/US2014/025636, filed March 13, 2014, which claims priority benefit of U.S. Application No. 61/785,031, filed March 14, 2013, the disclosures of which are incorporated herein in their entirety.

Background of the Invention

[0002] The invention relates to an aligned fiber and method of use thereof.

Summary of the Invention

[0003] The invention relates to a scaffold comprising an aligned fiber. The invention further relates to a scaffold comprising one or more electrospun fibers. In some embodiments, wherein a fast Fourier transform (FFT) analysis result of the fibers have adjacent major peaks with about 180° apart from each other.

[0004] The invention further relates to methods culturing cells on the scaffold or aligned fiber described herein.

[0005] The invention also relates to methods of promoting differentiation of stem cells into osteoblasts, chondrocytes, ligament or tendon, the method comprising culturing the cells on the scaffold or aligned fiber described herein in conditions suitable for the cell differentiation

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Brief Description of the Drawings

[0006] FIGURE 1A depicts representative field lines that are modulated between split point negative voltage sources and a single positive voltage source , aiding in imparting alignment in the electrospinning scheme. .

[0007] FIGURE 1B models field lines generated by a solid rod.

[0008] FIGURE 1C models field lines generated by a hollow rod.

[0009] FIGURE 1D depicts representative filed lines that are modulated between split point negative voltage sources and a single positive voltage source, aiding in imparting alignment in the electrospinning scheme.

[0010] FIGURE 2A depicts raw basement membrane, FIGURE 2B depicts a randomly electrospun architecture, FIGURES 2C and 2D depict an aligned basement membrane generated by parallel charged rod aligned electrospinning as compared to FIGURES 2A and 2B, and FIGURE 2E provides calculations of the average cross-sectional fiber diameter of the electrospun fibers.

[0011] FIGURES 3A, 3B, 3C, and 3D depict a Fourier spectrum from charged rod aligned and randomly electrospun fibers. The aligned pattern/texture recognition is typical to that of a regular, repeating line pattern, with the random fibers consistent with random noise generation spectra, as commonly seen in pattern recognition from FFT analysis.

[0012] Figures 4A, 4B, and 4C depict a modeling of a rotating parallel rod mandrel having a mercury coupled bearing inserted into the top of the device to split the electrical charge between the rods.

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[0013] FIGURE 5 depicts the exemplary elements of the parallel electric charged rod electrospinning setup. Using two charged rods imparts alignment to the traveling fibers, optionally through the point charge splitting of the electric field.

[0014] FIGURES 6A and 6B depict an aligned heart base membrane fiber scaffold that allow dorsal root ganglia attachment and outgrowth.

[0015] FIGURES 7A, 7B, 7C, 7D, 7E, 7F, 7G, 7H, 7I, and 7J depict aligned placenta and heart base membrane fiber scaffolds that allow dorsal root ganglia attachment and outgrowth.

[0016] FIGURE 8A depicts exemplary FFT analysis results of the fibers described herein.

[0017] FIGURE 8B depicts an FFT analysis of a control sample.

[0018] FIGURES 9A depicts an exemplary scaffolds prepared into an elongated roll by folding an elongated sheet .

[0019] FIGURE 9B depicts an exemplary scaffold prepared into an elongated roll by rolling an elongated sheet.

[0020] FIGURE 9C depicts an exemplary scaffold prepared into an elongated roll from a composite of multiple layers of elongated sheets.

Detailed Description of the Invention

[0021] The invention relates to an electrospinning apparatus comprising (i) at least one spinneret comprising an electrified tip; and (ii) a collector comprising two rods and a platform connected to the two rods, wherein the two rods are configured split an electric field between them. In one aspect, the spinneret comprising an electrified tip may have two, three, four, five or more electrified tips. In another aspect, the collector comprising two rods may have three, four, five or

more rods. In additional aspect, the platform connected to the two rods may have three, four, give or more rods connected to the platform. As defined herein, "comprising two rods" refers to comprising at least two rods, including, for example, two, three, four, and five rods.

[0022] The invention also relates to an electrospinning apparatus comprising (i) two spinnerets, each of which comprises a tip electrified with an opposite charge to one another; and (ii) a collector comprising two rods and a platform connecting the two rods, wherein the two rods are grounded.

[0023] Electrospray/electrospinning techniques can be used to form particles and fibers as small as one nanometer in a principal direction. The phenomenon of electrospray involves the formation of a droplet of polymer melt at an end of a needle, the electric charging of that droplet, and an expulsion of parts of the droplet because of the repulsive electric force due to the electric charges. In electrospraying, a solvent present in the parts of the droplet evaporates and small particles are formed but not fibers. The electrospinning technique is similar to the electrospray technique. In electrospinning and during the expulsion, however, fibers are formed from the liquid as the parts are expelled.

[0024] In particular, for example, the electrospinning typically involves a polymer solution (or melt) maintained at its surface tension on the tip of a nozzle via a syringe in a pump. When sufficiently high voltage is introduced (*e.g.* 15-40kV) to the polymer or oligomer in solution to create a charge imbalance, the solution may be drawn towards a grounded collector through the static electric field. As the polymer erupts from the needle and the assembling polymer whip through space, the fiber may be subjected to a series of stretching and bending instabilities, resulting in plastic stretching and elongation to minimize these instabilities generated by repulsive electrostatic forces. As the polymer or oligomer travels through space and rapidly thins into a fine stream, the solvent may evaporate and the polymer or oligomer assembles into fibers, leaving dry nano- to micro-scale fibers of tailorable physical attributes on the collector in the typical electrospinning scheme. Additionally, when high electrical potential is applied to a low viscosity polymer or oligomer melt or solution, or when improperly high or low voltage is applied, electrospraying may occur, which is typified by the polymer jet breaking down into fine

droplets. It is therefore possible to produce particles or nano- to micro-sphere particles, fibers and bead-and-string type structures via this process from simply altering the solution or electrospinning parameters. Polymer solution properties, applied electrical potential, polymer molecular weight, polymer solution flow rate, distance between spinner and collector, ambient parameters (*e.g.* humidity, air velocity, temperature) and motion of the collecting target can be altered to form fibers of controlled fiber distribution, diameter and alignment via electrospinning.

[0025] The electrospinning apparatus as described herein in one aspect may include one or more spinneret. The term "spinneret" used herein refers to a single- or multi-pored device through which a polymer or oligomer solution is extruded to form fibers. In one aspect, the spinneret is selected from the group consisting of a multiple nozzle spinneret, a single syringe or capillary spinneret, and a compound spinneret. In another aspect, the spinnerets of the electrospinning apparatus may be differently charged, resulting in differently charged polymer or oligomer solutions extruded from them. For example, different voltages may be applied to two or more polymer or oligomer sources whereby a positively charged solution is combined in space with a negatively charged solution, which is sent to the grounded rotating rods of the mandrel.

[0026] The electrospinning apparatus according to some embodiments of the present invention includes at least one spinneret comprising an electrified tip. The spinneret may have an electrified tip having a charge, and the tip may have one or more pores. In some embodiments, the electrospinning apparatus may include two or more tips having different charges. In particular, for example, the electrospinning apparatus may include two tips having opposite charges to each other.

[0027] In some embodiments, the spinneret described herein may be replaced with a different ejecting device comprising a drum configured to rotate in contact with a reservoir of a polymer or oligomer solution herein. In one aspect, this drum may pull the polymer or oligomer solution as a thin film on the surface of the drum. In another aspect, the drum may be charged, resulting in charging of the solution. In additional embodiments, the spinneret described herein may be replaced with a different ejecting device comprising a flat surface in contact with a polymer or

oligomer solution, and the polymer or oligomer solution may form a polymer bubble(s) by applying air.

[0028] The electrospinning apparatus in one aspect may include a collector having at least two rods and a platform. In some embodiments, the collector may have, for example, three, four, five, six, seven, eight, nine, ten, twenty, thirty, or forty rods. Herein, the term "rod" used herein refers to a bar of material having a shape including, but not limited to prism, cylinder, pentagonal rod, hexagonal rod, square rod, and triangular rod. The rod may comprise an electrical conducting material (*e.g.* a metal). In some embodiments, the rod may be electrified with a charge opposite to a charge of the electrified tip extruding a solution to the rod. In another aspect, the rod may be made of a non-electrical conducting material (*e.g.* insulating plastic). In further aspect, the rod may be made of both electrical and non-electrical conducting materials. For example, the electrical conducting material may be coated on the non-electrical conducting material.

[0029] The rod described herein may be directly or indirectly connected and/or fixed to a platform. The platform refers to any support for the rod. In one aspect, the rod comprises a proximal part and distal end, and the proximal part and/or distal end may be connected and/or fixed to the platform. In another aspect, the rod may go through the platform to which the proximal part of the rod is connected and/or fixed.

[0030] In one aspect, the platform may be a part of a machine frame. In another aspect, the electrospinning apparatus described herein may further include a chamber enclosing the spinneret and the collector. The chamber may comprise the platform described herein. In another aspect, the platform may consist of or comprise a rotating shaft or a bearing that is directly or indirectly connected and/or fixed to the rod.

[0031] The platform according to some embodiments includes a bearing connected to the rod described herein. The bearing used herein refers to a guide for rotating the rod. In one aspect, the bearing may be a rotating shaft. In another aspect, the platform or the bearing used herein may use plain bearing, rolling-element bearings, jewel bearings, fluid bearings, magnetic

bearings, and/or flexure bearings. The bearing may include an electrical conductor configured to allow electrical conductance to the rods. For example, the bearing may have mercury.

[0032] In another aspect, the platform connected to the rod(s) may be configured to spin resulting in rotation of the rod(s) about the spinning axis of the platform. In some embodiments, the rods are configured to rotate at between about 0 and about 8000 RPM, between about 0 and about 7000 RPM, between about 0 and about 6000 RPM, between about 0 and about 5000 RPM, between about 0 and about 4000 RPM, between about 0 and about 3000 RPM, between about 0 and about 2000 RPM, between about 0 and about 1000 RPM, between about 0 and about 500 , between about 0 and about 300 RPM, between about 0 and about 100 RPM, between about 0 and about 50, between about 1 and about 2000 RPM, between about 1 and about 1000 RPM, between about 1 and about 500 , between about 1 and about 300 RPM, between about 1 and about 100 RPM, between about 1 and about 50, between about 1000 and about 8000 RPM, between about 2000 and about 8000 RPM, between about 3000 and about 8000 RPM, between about 4000 and about 8000 RPM, between about 5000 and about 8000 RPM, between about 1000 and about 6000 RPM, between about 2000 and about 6000 RPM, or between about 3000 and about 6000 RPM. In additional embodiments, the rods are configured to rotate at about 50 RPM, about 500 RPM, about 1500 RPM, about 2500 RPM, about 3500 RPM, about 4500 RPM, about 5500 RPM, about 6500 RPM, about 7500 RPM or lower. In additional embodiments, the rods are configured to rotate at about 500 RPM, about 1500 RPM, about 2500 RPM, about 3500 RPM, about 4500 RPM, about 5500 RPM, about 6500 RPM, about 7500 RPM or higher. In some embodiments, when the rods are rotating, the fibers may spin around the rods. In other embodiments, when the rods are rotating, the fibers may also move or jump from one rod to another rod. In yet additional embodiments, the rods are stationary. In yet further embodiments, when the rods are stationary, the fibers may move or jump from one rod to another through an electric field.

[0033] In some embodiments, an air driven (pneumatic) motor and an electric (DC) motor may be employed in the electrospinning. In further embodiments, the pneumatic motor may generate less stray field anomalies and allow capturing of more nanofiber product on the rod(s) compared to the DC motor.

[0034] In one aspect, the fibers align on the rod (*e.g.* by spinning around the rods or jumping to one rod to another) in a perpendicular angle to the rods. In another aspect, the fibers align in an angle about 91, 92, 93, 94, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155 ° or less. In another aspect, the fibers align in an angle about 90, 91, 92, 93, 94, 95, 98, 103, 108, 113, 118, 123, 128, 133, 138, 143, 148, 153 ° or more. In another aspect, the fibers align in an angle between about 90 and about 93 °, between about 90 and about 95 °, between about 90 and about 100 °, between about 90 and about 105 °, between about 90 and about 110 °, between about 90 and about 115 °, between about 93 and about 95 °, between about 93 and about 100 °, between about 93 and about 105 °, between about 93 and about 110 °, or between about 93 and about 115 °.

[0035] As used herein, the term "about" modifying, for example, the quantity of an ingredient in a composition, concentrations, volumes, process temperature, process time, yields, flow rates, pressures, and like values, and ranges thereof, refers to variation in the numerical quantity that can occur, for example, through typical measuring and handling procedures used for making compounds, compositions, concentrates or use formulations; through inadvertent error in these procedures; through differences in the manufacture, source, or purity of starting materials or ingredients used to carry out the methods; and like considerations. The term "about" also encompasses amounts that differ due to aging of, for example, a composition, formulation, or cell culture with a particular initial concentration or mixture, and amounts that differ due to mixing or processing a composition or formulation with a particular initial concentration or mixture. Whether modified by the term "about" the claims appended hereto include equivalents to these quantities. The term "about" further may refer to a range of values that are similar to the stated reference value. In certain embodiments, the term "about" refers to a range of values that fall within 10, 9, 8,7, 6, 5,4, 3, 2, 1 percent or less of the stated reference value.

[0036] The electrospinning apparatus according to some embodiments of the present invention includes a collector comprising two or more rods and a platform connected to the rods, wherein the rods are configured split an electric field between them. In one aspect, operating the

electrospinning apparatus described herein may result in forming a point charge splitting of an electric field, through which traveling polymer or oligomer solutions align. In another aspect, the rods may be grounded.

[0037] The split electric field in some embodiments is separated by an air insulator. In one aspect, with air as an insulator between electrically charged metal rods, electrospun fibers are collect between opposed metal rods in an aligned fashion. The electrospun fiber described herein may be a microfiber or a nanofiber.

[0038] The split electric field in some embodiments is insulated by non-conductive materials or environments placed between the rods (*e.g.* carbon fiber, PTFE [Teflon], mica, diamond, ceramic, rubbers, glass, vacuum, materials on the low ends of the triboelectric series in the high resistivity range, also including paper, cotton, wood, epoxy, plastics such as: ABS [acrylonitrile, butadiene, and styrene], polycarbonate, acetate, acrylic, delrin, fiberglass, FEP, high impact polystyrene [HIPS], kapton, kaptrex, kynan, macor, melamine, meldin 7001 unfilled polyimide, MICA, neoprene, nomex, noryl PPO, PolyEtherEther-Ketone [PEEK], polyethylene terephthalate [P.E.T], P.E.T.G, phenolics such as micarta phenolics, perfluoroalkoxy [PFA], Polycarbonate, polyester [mylar], polyolefins, polystyrene, polysufone, polyurethane,), Rexolite® 1422 &220, polyphenylene sulfide [Ryton], silicone/fiberglass, silicone rubber, techtron, Ultem® 1000, Vespel® SP-1 [polyimide], electrically insulating papers such as vulcanized fibre, tapes from the above materials, and foams of these materials, including neoprene foam, polystyrene foam, polyurethane foam, silicone foam, vinyl foam.

[0039] The term microfiber as used herein means a fiber comprising a diameter of about 1000 μm or less. The term nanofiber as used herein means a fiber comprising a diameter of about 1000 nm or less. Relative to the parent bulk material, nano-features can impart many extraordinary properties to macrostructures, including superior mechanical, electrical, optical and magnetic properties, adding surface functionality and yielding high surface area. Matrices of nanofibers of varying diameters may show a range of variable surface properties (*e.g.*

hydrophobicity and hydrophilicity), porosities, and usually superior mechanical properties (*e.g.* tensile strength, stiffness) relative to the material in other forms.

[0040] In some embodiments, the nanofiber described herein may have an average diameter of about 1000 nm or less, 900 nm or less, 800 nm or less, 700 nm or less, 600 nm or less, 500 nm or less, 400 nm or less, 300 nm or less, 200 nm or less, 100 nm or less, 50 nm or less, 20 nm or less, or 10 nm or less. In some embodiments, the nanofiber described herein may have an average diameter of about 1000 nm or more, 900 nm or more, 800 nm or more, 700 nm or more, 600 nm or more, 500 nm or more, 400 nm or more, 300 nm or more, 200 nm or more, 100 nm or more, 50 nm or more, 20 nm or more, 10 nm or more, or 1 nm or more.

[0041] In some embodiments, the microfiber described herein may have an average diameter of about 1000 μm or less, 900 μm or less, 800 μm or less, 700 μm or less, 600 μm or less, 500 μm or less, 400 μm or less, 300 μm or less, 200 μm or less, 100 μm or less, 50 μm or less, 20 μm or less, or 10 μm or less. In some embodiments, the microfiber described herein may have an average diameter of about 1000 μm or more, 900 μm or more, 800 μm or more, 700 μm or more, 600 μm or more, 500 μm or more, 400 μm or more, 300 μm or more, 200 μm or more, 100 μm or more, 50 μm or more, 20 μm or more, 10 μm or more, or 1 μm or more.

[0042] In some embodiments, the rods described herein may be set parallel to each other in the electrospinning apparatus. In other embodiments, the rods may not be set parallel to each other but at an angle, for example, at an angle smaller than 5°, 10°, 30°, 50°, 70°, or 90°. In other embodiments, the rods may not be set parallel to each other but at an angle, for example, at an angle bigger than 1°, 8°, 25°, 40°, 60°, or 75°. In additional embodiments, the rod described herein may be straight, curved or angled.

[0043] The rods described herein in some embodiments are separated from one another by an average distance of about 1 cm, about 2 cm, about 3 cm, about 5 cm, about 8 cm, about 10 cm, about 13 cm, about 15 cm, about 18 cm, about 20 cm, about 23 cm, about 25 cm, about 27 cm, about 30 cm or more. In one aspect, the rods are separated from one another by an average

distance of about 1 cm, about 2 cm, about 3 cm, about 5 cm, about 8 cm, about 10 cm, about 13 cm, about 15 cm, about 18 cm, about 20 cm, about 23 cm, about 25 cm, about 27 cm, about 30 cm, about 40 cm, about 50 cm, about 60 cm or less. In another aspect, the rods are separated from one another by a distance from about 1 cm to about 25 cm, from about 6 cm to about 25 cm, from about 12 cm to about 25 cm, from about 18 cm to about 25 cm, from about 23 cm to about 25 cm, from about 1 cm to about 35 cm, from about 6 cm to about 35 cm, from about 12 cm to about 35 cm, from about 18 cm to about 35 cm, from about 24 cm to about 35 cm, or from about 10 cm to about 20 cm.

[0044] The collector may be biocompatible. In fact, any of the other elements of the electrospinning apparatus described herein may be biocompatible.

[0045] The electrospinning apparatus described herein may also include a control mechanism configured to control the electric potential of the electrified tip. The electrospinning apparatus described herein may also include a reservoir for a solution. The electrospinning apparatus described herein may also include an electric source connected to the electrified tip.

[0046] The invention further relates to methods of electrospinning using the electrospinning apparatus described herein and methods of preparing an electrospun fiber by the electrospinning. The methods of electrospinning and methods of preparing an electrospun fiber by the electrospinning may comprise (i) extruding a solution from an electrified tip of a spinneret, and (ii) collecting the extruded solution on at least a part of a collector. The methods of electrospinning or preparing an electrospun fiber by the electrospinning may further comprise extruding two, three or more solutions from one, two, three or more electrified tips of a spinneret. The methods of electrospinning or preparing an electrospun fiber by the electrospinning may further comprise extruding two, three or more solutions from one, two, three or more electrified tips of one, two, three or more spinnerets. The methods of preparing an electrospun fiber by the electrospinning may further comprise coating the extruded solution with another electrospun fiber prepared by the methods described herein.

[0047] The solution that may be extruded using the electrospinning apparatus described herein may include, but is not limited to, solutions comprising collagen type I, adipose extracellular matrix, heart basement membrane extract or extracellular matrix, placenta basement membrane extract or extracellular matrix, and polycaprolactone. Additional materials that may be electrospun by the method described herein include, but are not limited to, poly(glycolic acid), poly(lactic acid), polydioxanone, poly (lactide-co-glycolide) copolymers, polyesters polysaccharides, polyhydroxyalka noates , starch, polylactic acid, cellulose, proteins, agar, silks, alginate, collagen/gelatin, carrageenan, elastin, pectin, resilin, konjac, adhesives, gums, polyamino acids, polysaccharides, soy, zein, wheat gluten, casein, chitin/chitosan, serum albumin, hyaluronic acid, lipids/s urfactants, xanthan, acetoglycerides, waxes, surfactants, dextran, emulsan, gelian, polyphenols, levan, lignin, curd, ian, tannin, polygalactosamine, humic acid, shellac, pullulan , poly-gamma-glutamic acid, elsinan, natural rubber, yeast glucans, and synthetic polymers from natural fats and oils, and the mixture thereof.

[0048] In one aspect, the collector of the electrospinning apparatus is a biocompatible support to be coated by the electrospun fiber on its surface and may be used as a part of the implant described herein. For example, the collector may comprise a bone matrix, and the electrospun fiber is collected on the surface of the bone matrix, resulting in coating of the bone matrix. The bone matrix may have two or more different electrospun fiber coatings on its surface.

[0049] The invention further relates to a method of coating a matrix on a surface comprising (i) extruding a solution from an electrified tip of a spinneret, and (ii) collecting the extruded solution on a portion of a collector, wherein the collector further comprises two rods and a platform connected to the two rods, the two rods are configured to split an electric field between them, and the portion of the collector comprises the matrix. The invention further relates to a method of coating a matrix on a surface comprising (i) extruding one or more solutions from electrified trips of two spinnerets, each of which comprises at least one tip electrified with an opposite charge to a tip of the other spinnerets, and (ii) collecting the extruded solution on a portion of a collector, wherein the collector further comprises two rods and a platform connected to the two

rods, the two rods are grounded, and the portion of the collector comprises the matrix. In some embodiments, the matrix is a bone matrix, and the solution comprises a bone matrix solution. In additional embodiments, the matrix may comprise a synthetic surface.

[0050] In some embodiments, the matrix is biocompatible. In further embodiments, the biocompatible matrix is an implantable biocompatible matrix that can function as a support system for the bone matrix described herein. A biocompatible matrix should be non-toxic, non-eliciting or stimulating severe inflammatory response or immunological rejections, and devoid of other undesired reactions at the implantation site. In one embodiment, the biocompatible matrix is bone matrix or cartilage or connective tissue.

[0051] In some embodiments, the biocompatible matrix is prepared into a form of an elongated roll, an elongated sheet, a composite of multiple layers of sheets, or a combination thereof before or after being coated with the electrospun fibers. For example, the biocompatible matrix may be prepared as shown in **figures 9A, 9B, and 9C**. In one aspect, the materials for the elongated roll and the elongated sheet may be different. In another aspect, the materials for each elongated sheet in the multiple layers of sheets may be different from each other. In another aspect, the electrospun fibers coated on the biocompatible matrix are aligned in one direction. For example, the electrospun fibers coated on the biocompatible matrix are aligned in the direction parallel or perpendicular to the length of the elongated sheet or the multiple layers. In another aspect, the biocompatible matrix is prepared by folding or rolling the sheet or sheets, along with the direction of nano fiber alignment to form an elongated roll implant. In one aspect, this biocompatible matrix in the form of an elongated sheet or a composite of multiple layers of sheets may be used to facilitate neuronal cell growth.

[0052] In some embodiments, the elongated roll implant may be prepared by combining a sheet or sheets to facilitate surgical implantation. The biomaterials to prepare the sheet and elongated roll may be the same, or different, as described previously.

[0053] In some embodiments, the elongated sheet described herein may have an average thickness of about 1000 μm or less, 900 μm or less, 800 μm or less, 700 μm or less, 600 μm or

less, 500 μm or less, 400 μm or less, 300 μm or less, 200 μm or less, 100 μm or less, 50 μm or less, 20 μm or less, or 10 μm or less. In one aspect, the elongated sheet described herein may have an average thickness of about 1000 μm or more, 900 μm or more, 800 μm or more, 700 μm or more, 600 μm or more, 500 μm or more, 400 μm or more, 300 μm or more, 200 μm or more, 100 μm or more, 50 μm or more, 20 μm or more, 10 μm or more, or 1 μm or more.

[0054] In some embodiments, the length of the elongated sheet or roll implant is about 0.1 cm or more, about 0.3 cm or more, about 0.5 cm or more, about 0.7 cm or more, about 1 cm or more, about 2 cm or more, about 3 cm or more, about 5 cm or more, about 8 cm or more, about 10 cm or more, about 13 cm or more, about 15 cm or more, about 18 cm or more, about 20 cm or more, about 23 cm or more, about 25 cm or more, about 27 cm or more, or about 30 cm or more. In one aspect, the length of the elongated sheet or roll implant is about 1 cm or less, about 2 cm or less, about 3 cm or less, about 5 cm or less, about 8 cm or less, about 10 cm or less, about 13 cm or less, about 15 cm or less, about 18 cm or less, about 20 cm or less, about 23 cm or less, about 25 cm or less, about 27 cm or less, about 30 cm or less, about 40 cm or less, about 50 cm or less, or about 60 cm or less.

[0055] In some embodiments, the biocompatible matrix includes but is not limited to, bone graft implants, synthetic bone graft materials in forms of particulates, sheet, or blocks, tendon and/or ligament in bone tunnels, prosthetic implant, for example, for hip, shoulder, knee, or ankle, and trabecular metal. In further embodiments, the bone graft implants may include allograft or xeno-graft. In yet further embodiments, the bone graft implants may include structural bone implants including, but not limited to, monolithic or composite spinal implants (e.g., VERTIGRATs from LifeNet Health) and bone struts or blocks. In yet further embodiments, the bone graft implants may include demineralized, or non-demineralized bone particulates, including, but not limited to, cortical, cancellous, or cortical cancellous bone. In other embodiments, the electrospun fibers may be formed on cell or tissue culture surface.

[0056] Suitable biocompatible matrices include, but are not limited to, porous biocompatible scaffolds into which bone cells or progenitor cells may migrate. Osteogenic or chondrogenic cells, i.e., cells involved in the process of deposition of new bone material or cartilagenous material, respectively, can often attach to such porous biocompatible matrices, which can then serve as scaffolding for bone and cartilage tissue growth. Cells involved in the process of deposition of new ligament or tendon material can also attach to such porous biocompatible matrices. For certain applications, the biocompatible matrix should have sufficient mechanical strength to maintain its three dimensional structure and help support the immobilization of the bone segments being united or grafted together. Porous biocompatible matrices which provide scaffolding for tissue growth can accelerate the deposition of new bone or the rate of bone growth and are said to be "osteoconductive." Osteoconductive biocompatible matrices are especially useful in the matrices described herein. Porous biocompatible matrices which provide scaffolding for tissue growth can accelerate the deposition of new cartilage or the rate of cartilage growth and are said to be "chondroconductive." Osteoconductive biocompatible matrices are especially useful in the matrices described herein. Chondroconductive biocompatible matrices are especially useful in the matrices described herein. Angiogenic (or vasculogenic) biocompatible matrices are especially useful in the pharmaceutical compositions described herein. The osteoinductive, angiogenic or chondroinductive activity of the surface coated with the electrospun fibers or may not be altered, including but not limited to, enhanced activity, relative to a surface without the electrospun fiber coating or a natural surface without any coating. Thus, the osteoconductive, angiogenic or chondroconductive activity of the biocompatible matrices treated with the electrospun fibers of the present invention may be enhanced compared to matrices not treated with the bone matrix described herein. Of course, the biocompatible matrices are considered to be osteoconductive, angiogenic or chondroconductive if cells within the biocompatible matrix begin to differentiate into more osteoblast-like or chondrocyte-like appearing or functional cells, respectively.

[0057] The biocompatible matrices according to some embodiments of the present invention can be derived from natural sources or they can be synthetic or a mixture of both. Biocompatible

matrices from natural sources may also comprise natural polymers, including, but not limited to, collagen, hyaluronic acid, alginate, albumin, fibrinogen-fibrin, chitosan, elasin, laminin, connective tissues, intervertebral disc, cortical or cancellous bone, demineralized or mineralized bone, fascia lata, dermis, muscle, ligament, tendon, cartilage including articular/hyaline cartilage, elastic cartilage, and fibrocartilage, a mixture thereof, and mixture of reconstituted tissue. Biocompatible matrices from synthetic sources refer to any material not produced by living organisms, which may include, not limited to, the synthetic material made up of organic components, inorganic components, or a mixture thereof. In some embodiments, a synthetic biocompatible matrix may comprise an organic synthetic polymer, such as poly(lactic-co-glycolic acid), polycaprolactone (PCL), polyglycolic acid (PGA), polylactic acid (PLA), polyhydroxybutyrate (PHB), Poly(ethylene glycol) (PEG), poly(ethylene oxide) (PEO)), and others. In some embodiments, a tissue, an organ, or biocompatible matrix comprising at least one of alginate, chitosan, collagen, gelatin, hyaluronic acid, a fibronectin, an elastin, a laminin, and a proteoglycan may be employed. In certain embodiments, a biocompatible matrix comprising inorganic components, such as hydroxyapatite, calcium sulfate, octacalcium phosphate, calcium phosphate, macroporous calcium metaphosphate ceramic, β-tricalcium phosphate, metal, metal alloy, and others, may be used. A biocompatible matrix used in certain embodiments of the present invention may be prepared by demineralizing, decellularizing or devitalizing a tissue or an organ and cells may be seeded onto the biocompatible matrix.

[0058] In some embodiments, the electrospun fibers described herein may be applied to a biocompatible matrix and may be incubated at conditions permitting the generation of a matrix that is partially or fully coated with the electrospun fibers. In some embodiments, incubation may be carried out at about 40 °C or lower, or between about 10 ºC and about 37 ºC, or about 20 ºC and about 37 ºC. Incubation may be carried out for between at least about 2 minutes and about 120 minutes, about 3 minutes and about 100 minutes, about 4 minutes and about 80 minutes, about 5 minutes and about 60 minutes, and about 5 minutes and about 30 minutes in certain embodiments. Incubation may be performed under static or dynamic conditions, such as with agitation, shaking, stirring, mixing, horizontal motion, rocking, and others.

[0059] In some embodiments of the present invention, a biocompatible matrix may be lyophilized before the electrospun fiber is applied to the biocompatible matrix. In certain embodiments, the electrospun fibers may be coated on the biocompatible matrix, and the coated matrix may be subsequently lyophilized. The lyophilized, coated matrix can then be rehydrated before it is used. Further, the cells can be seeded onto the matrix before implantation.

[0060] Examples of suitable osteoconductive or chondroconductive biocompatible matrices include but are not limited to, collagen (e.g., bovine dermal collagen), fibrin, calcium phosphate ceramics (e.g., hydroxyapatite and tricalcium phosphate), calcium sulfate, guanidine-extracted allogenic bone and combinations thereof. A number of suitable biocompatible matrices are commercially available, such as Collograft™ (Collagen Corporation), which is a mixture of hydroxyapatite, tricalcium phosphate and fibrillar collagen, and Interpore™ (Interpore International), which is a hydroxyapatite biomatrix formed by the conversion of marine coral calcium carbonate to crystalline hydroxyapatite.

[0061] A number of synthetic biodegradable polymers can serve as osteoconductive or chondroconductive biocompatible matrices with sustained release characteristics. Descriptions of these polymers can be found in Behravesh (1999) Clinical Orthopaedics 367, S118 and Lu (2000) Polymeric Delivery Vehicles for Bone Growth Factors in Controlled Drug Delivery: Designing Technologies for the Future, Park and Mrsny eds., American Chemical Society, which is incorporated herein in its entirety herein. Examples of these polymers include poly α-hydroxy esters such as polylactic acid/polyglycolic acid homopolymers and copolymers, polyphosphazenes (PPHOS), polyanhydrides and poly(propylene fumarates).

[0062] Polylactic acid/polyglycolic acid (PLGA) homo and copolymers are well known in the art as sustained release vehicles. The rate of release can be adjusted by the skilled artisan by variation of polylactic acid to polyglycolic acid ratio and the molecular weight of the polymer (see Anderson (1997) Adv. Drug Deliv. Rev. 28:5. The incorporation of PEG into the polymer as a blend to form microparticle matrices allows further alteration of the release profile of the

active ingredient (see Cleek (1997) J. Control Release 48, 259). Ceramics such as calcium phosphate and hydroxyapatite can also be incorporated into the formulation to improve mechanical qualities.

[0063] In one embodiment, the biocompatible matrices used in the methods of the present invention are other types of bone matrices. For example, the electrospun fibers prepared by the methods described herein can be coated on a bone matrix. As used herein, the other types of the bone matrix may be a biocompatible matrix derived from or including elements of natural bone. In some embodiments, the natural bone is mineralized, partially demineralized, demineralized, cancellous, cortical, or cortical cancellous bone. The bone matrices used herein may or may not include additional synthetic components not typically found in bone tissue. Other embodiments include methods utilizing a biocompatible matrix derived from cartilage, other soft tissues such as the dermis, connective tissue, fascia, small intestine submucosa, serous membrane, pericardium, tendon, ligament, muscle, adipose tissue, myelin, blood vessels, base membrane, amniotic membrane and others. A biocompatible matrix prepared from hyaline cartilage, fibrocartilage or elastic cartilage, may be employed in some embodiments. A biocompatible matrix may be prepared from hyaline cartilage found in the condyle, tibial plateau, femoral head, humeral head, costal cartilage, or fibrocartilage found in intervertebral discs, or elastic cartilage found in the epiglottis or ear. In certain embodiments, a biocompatible matrix derived from natural sources that has been optionally cleaned, disinfected, chemically modified, decellularized, particulated, homogenized, lyophilized, gamma ray irradiated, and/or plasticized may be used. Any of the biocompatible matrices used herein may or may not include additional synthetic components not typically found in such tissue.

[0064] In one specific embodiment, the bone or cartilage biocompatible matrices may be demineralized or decellularized, respectively. Examples of demineralized matrices and methods of making are described in U.S. Patent No. 6,189,537 and 6,305,379, which are incorporated herein in its entirety herein.

[0065] The biocompatible matrix, tissue, or organ used in certain embodiments of the present invention may be in the form of a powder, particulates, sheets, fibers, gels, putties, paste, blocks, cylinders, sponges, meshes, films, slices, curls, flakes, or wedges, among others. In certain embodiments of the present invention, the biocompatible matrix, tissue, or organ treated with the electrospun fibers by the methods described herein may be in the form of a powder, fibers, putty, or a sponge. In further embodiments, the sponge can include, for example, the implant having sponge-like structures disclosed in the co-pending, commonly-assigned patent application PCT/US09/04556 entitled "Composition for a Tissue Repair Implant and Methods of Making the Same" filed on August 7, 2009, which is incorporated herein in its entirety herein. The treated matrices can be used in any of the methods of the present invention.

[0066] The invention also related to a method of preparing an electrospun fiber by electrospinning comprising (i) extruding a solution from an electrified tip of a spinneret, and (ii) collecting the extruded solution on a portion of a collector comprising two rods and a platform connected to the two rods, wherein the two rods are configured to split an electric field between them. The invention further related to a method of preparing an electrospun fiber by electrospinning comprising (i) extruding one or more solutions from electrified trips of two spinnerets, each of which comprises at least one tip electrified with an opposite charge to a tip of the other spinnerets, and (ii) collecting the extruded solution on a part of a collector comprising two rods and a platform connecting the two rods, wherein the two rods are grounded. In some embodiments, the method of preparing an electrospun fiber further comprises applying an accessory polymer to the solution (*e.g.* as a copolymer, blend, or melt). An "accessory polymer" refers to a polymer that may be added to the electrospun fibers and have any effect on their physical, chemical, and/or biological properties (*e.g.* tensile strength, hydrophillicity, biocompatibility). For example, the accessory polymer may be selected from the group consisting of polycaprolactone, poly(glycolic acid), poly(lactic acid), polydioxanone, poly (lactide-co-glycolide) copolymers, polyesters polysaccharides, polyhydroxyalkanoates, starch, polylactic acid, cellulose, proteins, agar, silks, alginate, collagen/gelatin, carrageenan, elastin, pectin, resilin, konjac, adhesives, gums, polyamino acids, polysaccharides, soy, zein, wheat

gluten, casein, chitin/chitosan, serum albumin, hyaluronic acid, lipids/s urfactants, xanthan, acetoglycerides, waxes, surfactants, dextran, emulsan, gelian, polyphenols, levan, lignin, curd, ian, tannin, polygalactosamine, humic acid, shellac, pullulan , poly-gamma-glutamic acid, elsinan, natural rubber, yeast glucans, and synthetic polymers from natural fats and oils.

[0067] In one aspect, the method of preparing the electrospun fiber(s) according to some embodiments of the present invention may further comprise crosslinking the electrospun fiber(s). In some embodiments, the crosslinking may be performed by any conventional chemical crosslinking method (*e.g.* chemical reagent-promoted, chemically reactive linker-promoted and/or enzyme-promoted) and/or dehydrothermal crosslinking method (*e.g.* heat-promoted condensation), forming the covalently crosslinked electrospun fiber(s). In additional embodiments, the crosslinking comprises applying a cross-linking agent to the polymer or oligomer solutions to be electrospun. For example, the cross-linking agent may be selected from the group consisting of 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), EDC/hyaluronic acid, genipin, and glutaraldehyde.

[0068] In some embodiments, a method of preparing an electrospun fiber further comprises adding a bioactive factor to the solution. A "bioactive factor" refers to protein, carbohydrate, or mineral that has any effect on a cellular activity. Examples of bioactive factors include, but are not limited to, an osteogenic growth factor, collagen, glycosaminoglycans, osteonectin, bone sialo protein, an osteoinductive factor, a chondrogenic factor, a cytokine, a mitogenic factor, a chemotactic factor, a transforming growth factor (TGF), a fibroblast growth factor (FGF), an angiogenic factor, an insulin-like growth factor (IGF), a platelet-derived growth factor (PDGF), an epidermal growth factor (EGF), a vascular endothelial growth factor (VEGF), a nerve growth factor (NGF), a neurotrophin, a bone morphogenetic protein (BMP), osteogenin, osteopontin, osteocalcin, cementum attachment protein, erythropoietin, thrombopoietin, tumor necrosis factor (TNF), an interferon, a colony stimulating factor (CSF), stem cell derived factor-1 (SDF-1), or an interleukin, among others. The bioactive factor may be a BMP, PDGF, FGF, VEGF, TGF, insulin, among others. Examples of BMPs include but are not limited to BMP-2, BMP-3, BMP-4,

BMP-5, BMP-6, BMP-7, BMP-8, BMP-9, BMP-10, BMP-11, BMP-12, BMP-13, BMP-14, BMP-15, truncated BMPs described in PCT/US2012/053584, which is incorporated by reference in its entirety herein, and a mixture thereof.

[0069] The invention further relates to a scaffold comprising an aligned fiber. The invention further relates to a scaffold comprising one or more electrospun fibers. In some embodiments, a fast Fourier transform (FFT) analysis result of the fibers have adjacent major peaks with about 180° apart from each other. The "major peaks" herein refer to peaks higher than the average of the peaks in the result.

[0070] In one aspect, the scaffold may essentially consist of the aligned fiber and/or electrospun fiber. In another aspect, the scaffold may consist of the aligned fiber and/or electrospun fiber. The scaffold described herein may be biocompatible. A biocompatible matrix scaffold may be non-toxic, non-eliciting or stimulating severe inflammatory response or immunological rejections, and/or devoid of other undesired reactions at the implantation site.

[0071] Alignment of the fibers in the scaffold may be measured by a fast Fourier transform (FFT) analysis. For example, the FFT analysis may be performed by the methods described in *Measuring fiber alignment in electrospun scaffolds: a user's guide to the 2D fast Fourier transform approach*, Ayres CE, Jha BS, Meredith H, Bowman JR, Bowlin GL, Henderson SC, Simpson DG. J Biomater Sci Polym Ed. 2008; 19(5):603-21, which is incorporated by reference in its entirety herein. In some embodiments, FFT result of the fibers described herein may have adjacent major peaks with about 180° apart from each other as shown in **figure 8A, and figure 8B** depicts an FFT analysis of a control sample.

[0072] The aligned fiber, electrospun fiber, and/or scaffold of the present invention may comprise type I collagen. In some embodiments, total type I collagen may be present in the aligned fiber, electrospun fiber, and/or scaffold of the present invention in an amount from about 50% to about 95%, from about 60% to about 95%, from about 70% to about 95%, or from about 80% to about 95% by weight in it protein content. In additional embodiments, total type II

collagen may be present in the aligned fiber, electrospun fiber, and/or scaffold of the present invention in an amount about 10% or less, about 5% or less, about 3% or less, or about 1% or less by weight in it protein content. In further embodiments, collagen type II may be absent from aligned fiber, electrospun fiber, and/or scaffold of the present invention. The types of collagen that are present or absent in the composition can be easily assessed using routine methods in the art. Methods of identifying and quantifying type of collagen are well known in the art, as disclosed, for example in Schnaper, H.W. and Kleinman, H.K., Pediatr. Neprol., 7:96-104 (1993), which is incorporated by reference.

[0073] In one aspect, the aligned fiber, electrospun fiber, and/or scaffold described herein may have less than about 8 wt%, 7 wt%, 6 wt%, 5 wt%, 4 wt%, 3 wt%, 2 wt%, or 1 wt% residual calcium. In another aspect, the average residual calcium amount in the aligned fiber, electrospun fiber, and/or scaffold described herein may be less than about 8 wt%, 7 wt%, 6 wt%, 5 wt%, 4 wt%, 3 wt%, 2 wt%, or 1 wt% .

[0074] In one aspect, the aligned fiber and/or electrospun fiber described herein may have a length from about 0.1 cm to about 10 cm, from about 1 cm to about 10 cm, from about 0.1 cm to about 20 cm, from about 1 cm to about 20 cm, from about 10 cm to about 20 cm, from about 0.1 cm to about 30 cm, from about 1 cm to about 30 cm, from about 10 cm to about 30 cm, from about 20 cm to about 30 cm, from about 0.1 cm to about 40 cm, from about 1 cm to about 40 cm, from about 10 cm to about 40 cm, from about 20 cm to about 40 cm, from about 30 cm to about 40 cm, from about 0.1 cm to about 50 cm, from about 1 cm to about 50 cm, from about 10 cm to about 50 cm, from about 20 cm to about 50 cm, from about 30 cm to about 50 cm, from about 40 cm to about 50 cm, 0.1 cm to about 60 cm, from about 1 cm to about 60 cm, from about 10 cm to about 60 cm, from about 20 cm to about 60 cm, from about 30 cm to about 60 cm, or from about 40 cm to about 60 cm. In another aspect, the total the aligned fiber and/or electrospun fiber in the scaffold described herein may have an average length from about 0.1 cm to about 10 cm, from about 1 cm to about 10 cm, from about 0.1 cm to about 20 cm, from about 1 cm to about 20 cm, from about 10 cm to about 20 cm, from about 0.1 cm to about 30 cm, from about 1 cm to

about 30 cm, from about 10 cm to about 30 cm, from about 20 cm to about 30 cm, from about 0.1 cm to about 40 cm, from about 1 cm to about 40 cm, from about 10 cm to about 40 cm, from about 20 cm to about 40 cm, from about 30 cm to about 40 cm, from about 0.1 cm to about 50 cm, from about 1 cm to about 50 cm, from about 10 cm to about 50 cm, from about 20 cm to about 50 cm, from about 30 cm to about 50 cm, from about 40 cm to about 50 cm, 0.1 cm to about 60 cm, from about 1 cm to about 60 cm, from about 10 cm to about 60 cm, from about 20 cm to about 60 cm, from about 30 cm to about 60 cm, or from about 40 cm to about 60 cm.

[0075] In one aspect, the scaffold has an average porosity from about 60% to about 70%, from about 60% to about 80%, from about 60% to about 90%, from about 60% to about 95%, from about 70% to about 80%, from about 70% to about 90%, from about 70% to about 95%, from about 80% to about 90%, or from about 80% to about 95%.

[0076] In some embodiments, the aligned fiber, electrospun fiber, and/or scaffold comprises collagen type I, bone matrix, adipose extracellular matrix, heart basement membrane extract, heart basement membrane extracellular matrix, placenta basement membrane extract, placenta basement membrane extracellular matrix, brain-derived extracellular matrix, polycaprolactone, a biodegradable polymer, an accessory polymer described herein, or the mixture thereof.

[0077] In one aspect, the aligned fiber, electrospun fiber, and/or scaffold may comprise a bone matrix fiber comprising bone. In some embodiments, the bone matrix fiber may be prepared by the methods described in PCT/US2013/025226, which is incorporated herein in its entirety herein.

[0078] In another aspect, the aligned fiber, electrospun fiber, and/or scaffold may comprise heart basement membrane extract and/or heart basement membrane extracellular matrix. In some embodiments, the heart basement membrane extract and/or heart basement membrane extracellular matrix may be prepared by the methods described in PCT/US2011/49192, which is incorporated herein in its entirety herein. In an additional aspect, the placenta basement membrane extract and/or heart basement membrane extracellular matrix may be prepared by the

same method to prepare the heart basement membrane extract and/or heart basement membrane extracellular matrix.

[0079] In another aspect, the aligned fiber, electrospun fiber, and/or scaffold may comprise a biodegradable polymer. The biodegradable, biocompatible polymers may include, but is not limited to, ethylene vinyl acetate, polyanhydricles, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. The biodegradable, biocompatible polymers may further include a number of synthetic biodegradable polymers described herein.

[0080] In some embodiments, the aligned fiber and/or electrospun fiber in the scaffold is crosslinked. In additional embodiments, the aligned fiber, electrospun fiber, and/or scaffold comprises a bioactive factor described herein.

[0081] In additional embodiments, the aligned fiber, electrospun fiber, and/or scaffold may comprise an extracellular matrix component. For example, the extracellular matrix component may include, but is not limited to, collagen, glycosaminoglycans, osteocalcin, osteonectin, bone sialo protein, osteopontin, fibronectin, laminin, vitronectin, elastin or mixtures thereof.

[0082] In some embodiments, the scaffold of the present invention may further comprise a support. In additional embodiments, the fibers described herein may be attached to and/or cover the support.

[0083] In some embodiments, the scaffold of the present invention may be in a form of an elongated sheet or a composite of multiple layers of sheets similarly to the biocompatible matrix described herein. In additional embodiments, the scaffold may be prepared as shown in **figures 9A, 9B, and 9C**. In one aspect, the materials for the elongated roll and the elongated sheet may be different. In another aspect, the materials for each elongated sheet in the multiple layers of sheets may be different from each other. In another aspect, the electrospun fibers in the scaffold are aligned in one direction. For example, the electrospun fibers in the scaffold are aligned in the direction parallel or perpendicular to the length of the elongated sheet or the multiple layers. In

another aspect, the scaffold is prepared by folding or rolling the sheet or sheets, along with the direction of nano fiber alignment to form an elongated roll implant.

[0084] In some embodiments, the elongated roll implant may be prepared by combining a sheet or sheets to facilitate surgical implantation. The biomaterials to prepare the sheet and elongated roll may be the same, or different, as described previously.

[0085] In some embodiments, the elongated sheet scaffold described herein may have an average thickness of about 1000 μm or less, 900 μm or less, 800 μm or less, 700 μm or less, 600 μm or less, 500 μm or less, 400 μm or less, 300 μm or less, 200 μm or less, 100 μm or less, 50 μm or less, 20 μm or less, or 10 μm or less. In one aspect, the elongated sheet scaffold described herein may have an average thickness of about 1000 μm or more, 900 μm or more, 800 μm or more, 700 μm or more, 600 μm or more, 500 μm or more, 400 μm or more, 300 μm or more, 200 μm or more, 100 μm or more, 50 μm or more, 20 μm or more, 10 μm or more, or 1 μm or more.

[0086] In some embodiments, the length of the elongated sheet or roll implant scaffold is about 0.1 cm or more, about 0.3 cm or more, about 0.5 cm or more, about 0.7 cm or more, about 1 cm or more, about 2 cm or more, about 3 cm or more, about 5 cm or more, about 8 cm or more, about 10 cm or more, about 13 cm or more, about 15 cm or more, about 18 cm or more, about 20 cm or more, about 23 cm or more, about 25 cm or more, about 27 cm or more, or about 30 cm or more. In one aspect, the length of the elongated sheet or roll implant scaffold is about 1 cm or less, about 2 cm or less, about 3 cm or less, about 5 cm or less, about 8 cm or less, about 10 cm or less, about 13 cm or less, about 15 cm or less, about 18 cm or less, about 20 cm or less, about 23 cm or less, about 25 cm or less, about 27 cm or less, about 30 cm or less, about 40 cm or less, about 50 cm or less, or about 60 cm or less.

[0087] The scaffold according to some embodiments of the present invention may be used as an implant. An "implant" refers to any object that is designed to be placed partially or wholly within a patient's body for one or more therapeutic or prophylactic purposes such as for tissue augmentation, contouring, restoring physiological function, repairing or restoring tissues damaged by disease or trauma, and/or delivering therapeutic agents to normal, damaged or

diseased organs and tissues. A "bone matrix implant" refers to a medical device or implant that includes a volume replacement material for augmentation or reconstruction to replace a whole or part of a bone structure. A predetermined shape of the implant may be varied to fit the implant site.

[0088] The invention further relates to methods culturing cells on the scaffold or aligned fiber described herein. In some embodiments, the cells are selected from the group consisting of stem cells, adipose derived stem cells, dental pulp stem cells, fibroblasts, and dorsal root ganglia.

[0089] For example, the invention provides for growing and/or culturing cells on a scaffold produced by the method described herein *in vivo* or *in vitro*. "Growing and/or culturing cells on a scaffold" includes traditional cell culture methods as well as placing on a surface in any setting, such as in natural or synthetic biocompatible matrices or tissues. In some embodiments, the cells that are cultured on the scaffold are stem cells. In further embodiments, the scaffold can be used in *in vitro* methods for supporting cell growth and proliferation as well as for increasing osteogenesis, chondrogenesis, or ligament/tendon genesis in the stem cells cultured on the scaffold.

[0090] Any cell described herewith may be cultured on a scaffold produced by the method described herein for between about 15 minutes and about 4 weeks, about 2 hours and about 2 weeks, about 2 hours and about 1 week, about 2 hours and about 72 hours, about 24 hours and about 72 hours, or about 24 hours and about 96 hours, at between about 20 ℃ and about 40 ℃ or about 30°C and about 37°C, in an atmosphere containing between about 1% CO_2 and about 10% CO_2 or about 4% CO_2 and about 6% CO_2, in certain embodiments.

[0091] The invention also relates to methods of promoting nerve repair using the aligned fiber, electrospun fiber, and/or scaffold described herein. In some embodiments, the nerve repair includes, but is not limited to, central and peripheral nerve repair. The methods of promoting nerve repair may comprise implanting the aligned fiber, electrospun fiber, and/or scaffold into a nerve damaged site of a subject in need thereof. The method may further comprise culturing

dorsal root ganglia and/or stem cells on the aligned fiber, electrospun fiber, and/or scaffold prior to the implanting. The stem cells, prior to culture on the scaffold of the present invention, may be undifferentiated or partially differentiated cells. The nerve regenerating activity of the aligned fiber, electrospun fiber, and/or scaffold may or may not be altered, including but not limited to, enhanced activity, relative to other scaffolds without the aligned and/or electrospun fiber described herein. The scaffolds for promoting nerve repair according to some embodiments of the present invention are in a form of an elongated sheet or a composite of multiple layers of sheets as discussed herein.

[0092] The invention also relates to methods of promoting osteoinductivity, with the methods comprising culturing cells on an aligned fiber, electrospun fiber, and/or scaffold produced by the method described herein. The cells, prior to culture on the aligned fiber, electrospun fiber, and/or scaffold of the present invention, may be undifferentiated or partially differentiated cells. The osteoinductive activity of the aligned fiber, electrospun fiber, and/or scaffold may or may not be altered, including but not limited to, enhanced activity, relative to other scaffolds without the aligned and/or electrospun fiber described herein.

[0093] The invention also relates to methods of promoting chondroinductivity, with the methods comprising culturing cells on a aligned fiber, electrospun fiber, and/or scaffold produced by the method described herein. The cells, prior to culture on aligned fiber, electrospun fiber, and/or scaffold of the present invention, may be undifferentiated or partially differentiated cells. The chondroinductive activity of the aligned fiber, electrospun fiber, and/or scaffold may or may not be altered, including but not limited to, enhanced activity, relative to other scaffolds without the aligned and/or electrospun fiber described herein.

[0094] The invention also relates to methods of promoting ligament/tendon differentiation, with the methods comprising culturing cells on an aligned fiber, electrospun fiber, and/or scaffold produced by the method described herein. The cells, prior to culture on the aligned fiber, electrospun fiber, and/or scaffold of the present invention, may be undifferentiated or partially

differentiated cells. The ligament/tendon differentiation activity of the aligned fiber, electrospun fiber, and/or scaffold may or may not be altered, including but not limited to, enhanced activity, relative to other scaffolds without the aligned and/or electrospun fiber described herein.

[0095] In one aspect, *in vitro* alkaline phosphatase assays may be used to evaluate osteoinductivity in cells cultured on the scaffold prepared by the methods described herein. The ability of the aligned fiber, electrospun fiber, and/or scaffold prepared by the methods of the present invention to stimulate or induce the alkaline phosphatase expression in an otherwise non-bone forming cells, such as myoblast (C2C12 cells), would indicate that the aligned fiber, electrospun fiber, and/or scaffold of the present invention has osteoinductive activity. In these assays, cells cultured on other scaffolds without the aligned and/or electrospun fiber described herein are used as negative controls to show that the baseline alkaline phosphatase expression on non-bone forming cells. The baseline of the osteoblastic markers in the negative control need not be zero, meaning that the cells in the negative control group may have at least some level of phenotypic marker(s). Accordingly, an "osteoinductive" scaffold of the present invention would simply cause an increase in the osteoblastic markers in experimental cells over control grown on the other scaffolds without the aligned and/or electrospun fiber.

[0096] Moreover, osteoinductivity, chondroinductivity, and ligament/tendon differentiation may be determined in tissue culture by investigating the ability of the aligned fiber, electrospun fiber, and/or scaffold prepared by the methods of the present invention to differentiate or induce osteoblast phenotype, chondrocyte phenotype, ligament/tendon cell phenotype in cultured cells, such as primary cells, cell lines, or explants. For example, the cells may display increased production of a marker characteristic of osteoblasts and/or chondrocytes, such as alkaline phosphatase, etc. For example, the osteoinductive, chondroinductive, ligament/tendon differentiation potentials of the aligned fiber, electrospun fiber, and/or scaffold described herein may be more than 0.2, 0.4, 0.6, 0.8, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 times greater than the fiber and/or scaffolds without the aligned and/or electrospun fiber. In another example, the osteoinductive, chondroinductive, ligament/tendon differentiation potentials of the culture on the

scaffold and/or implant described herein may be more than 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 500 or even 1000 times greater than those of the aligned fiber, electrospun fiber, scaffold and/or implant without any aligned and/or electrospun fiber. Of course, this indicates that lower concentrations of modified growth factor, compared to unmodified growth factor are required to achieve the same effects.

[0097] Osteoinductivity, chondroinductivity, ligament/tendon differentiation, for assessing the bone, cartilage, ligament or tendon forming potential induced by the scaffold of the present invention in a location such as muscle, may also be evaluated using a suitable animal model.

[0098] The invention also relates to methods of promoting cell attachment, proliferation or maintaining the differentiated state or preventing de-differentiation of osteoblasts, chondrocytes, ligament cells, tendon cells and/or any cell type disclosed herein with the methods comprising culturing the cells on a scaffold produced by the method described herein. The proliferative activity of the aligned fiber, electrospun fiber, and/or scaffold may or may not be altered, including but not limited to, enhanced activity, relative to a fiber and/or scaffold without any aligned and/or electrospun fiber.

[0099] Mitogenicity may be assessed by investigating cell proliferation induced by the aligned fiber, electrospun fiber, and/or scaffold prepared by the methods of the present invention using various in vitro assays that measure metabolic activity, such as MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay, alamarBlue® assay, and others. The alamarBlue® assay uses a non-cytotoxic reduction-oxidation indicator to measure cell metabolic activity, making it a nondestructive assay for assessing the mitogenic activity of the aligned fiber, electrospun fiber, and/or scaffold described herein.

[0100] The invention also relates to methods of increasing or promoting osteogenesis, chondrogenesis, or ligament/tendon genesis in cells. The methods may comprise culturing the cells on an aligned fiber, electrospun fiber, and/or scaffold produced by the method described herein. The osteogenic, chondrogenic, ligament, or tendon inducing activity of the aligned fiber,

electrospun fiber, and/or scaffold may or may not be altered, including but not limited to, enhanced activity, relative to a fiber and/or scaffold without aligned and/or electrospun fiber described herein.

[0101] The invention also relates to methods of treating a tissue or organ defect or injury, for example, a musculoskeletal, dental or soft-tissue defect or injury, in an animal comprising administering (1) cells cultured on the scaffold produced by the methods described herein and/or (2) the aligned fiber, electrospun fiber, scaffold and/or implant described herein to the tissue or organ defect (e.g. osseous defects, defects in cartilage, ligament, tendon, spinal disk, and tendon insertion site to bone).

[0102] The invention further relates to methods of treating a tissue or an organ defect or injury, for example, including, but not limited to a musculoskeletal, dental or soft-tissue defect, or a nerve defect in an animal by applying a aligned fiber, electrospun fiber, and/or scaffold prepared by the methods described herein to the defect, and application to the defect may be accomplished by injecting the scaffold into the defect, inserting the aligned fiber, electrospun fiber, and/or scaffold between tissue or organ, or placing the aligned fiber, electrospun fiber, and/or scaffold on top of the defect. The present invention is also directed to treating a defect or injury in an organ by applying an aligned fiber, electrospun fiber, and/or scaffold to the defect. In one embodiment, the tissue defect may be treated by applying an elongated sheet described herein to surround the defect, or by applying an elongated roll implant described herein between the tissue defect or gap. The tissue defect or gap may be from the tissues including, but not limited to, bone, tendon, ligament, blood vessel, skin, intestine, nerve, and cartilage. Optionally, the elongated sheet may be applied around the tissue defect, and/or the elongated roll implant may be applied to facilitate surgery and healing. For example, the elongated implant roll may be applied between the tissue defect or gap, then the ends of the elongated implant roll may be sutured to the tissue. In some embodiments, the elongated sheet may be applied or sutured around the tissue defect or gap, covering the elongate implant roll and/or the suture sites of the elongated implant roll with tissues. In additional embodiments, the elongated roll may facilitate

the tissue growth through the defect gap. The elongated sheet can prevent adhesion of the elongated implant roll with surrounding tissue or prevent undesirable tissue ingrowth, for example, scar formation, to interfere the tissue regeneration or remodeling. The biomaterials used for preparing the enlongated sheet and the enlongated implant roll may be same or different, depending on the specific surgery and tissue regeneration needs. The tissue defect may occur in tendon, ligament, nerve and other tissue types enlisted previously.

[0103] In yet another embodiment, cells may be seeded onto an aligned fiber, electrospun fiber, and/or scaffold prepared by the methods provided herein. The cells seeded on the scaffold can be any cell, such as but not limited to, dorsal root ganglia, osteoblasts, chondrocytes, ligament cells, tendon cells, progenitor cells, and stem cells disclosed herein or otherwise known in the art. The seeded cells may be allowed to proliferate and possibly attach to the matrix. Methods of seeding cells onto matrices, such as collagen matrix coated with the aligned fiber, electrospun fiber, and/or scaffold prepared by the methods provided herein, are well known in the art.

[0104] While the invention has been described and illustrated herein by references to various specific materials, procedures and examples, it is understood that the invention is not restricted to the particular combinations of material and procedures selected for that purpose. Numerous variations of such details can be implied as will be appreciated by those skilled in the art. It is intended that the specification and examples be considered as exemplary, only, with the true scope and spirit of the invention being indicated by the following claims. All references, patents and patent applications referred to in this application are herein incorporated by reference in their entirety herein.

[0105] The following examples are illustrative and are not intended to limit the scope of the invention described herein.

[0106] Example 1:

[0107] 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) (CAS# 920-66-1) was from Acros Organics (Geel, Belgium, acros.com). Polycaprolactone (PCL) (B6003-1, B6003-2) was from Durect Lactone (Pelham, AL, absorbables.com). The electrospinning apparatus was designed using an Aladdin Programmable Syringe Pump from World Precision Instruments, Inc. (Sarasota, FL, wpiinc.com) and two adjustable high voltage power supplies from Gamma High Voltage Research (Ormond Beach, FL). The aligned metal rods used in the rotating motor, or statically, may be of steel, stainless steel, copper, or other heavy metal. A Mercotac® connector (#110T and 110TS) was used as an electrically conductive bearing. The following items were purchased from McMaster-Carr:

8965K42 Ultra Conductive Copper (Alloy 101) Rod, 1/4" Diameter

1256T14 Multipurpose Stainless Steel (Type 304) Mirror Finish #8, 1/4" Diameter

88855K51 High-Strength Stainless Steel (Type 17-4 Ph) 1/4" Diameter

86985K31 High-Strength Aluminum (Alloy 2024) 1/4" Diameter

8965K86 Ultra Conductive Copper (Alloy 101) Tube, 1/4" OD, .186" ID, .032" Wall Thk

8457K536 Stainless Steel Shape Type 304/304L, Round Tube, 1/4" OD

1968T11 High-Strength Aluminum Tube (Alloy 2024) 1/4" OD, .180" ID, .035" Wall Thickness

2706A4 Tube Cutter 1/4" to 1-5/8" Tube OD, 6" Open/Closed Length

4575N3 Miniature Flange-Mounted SS Ball Bearing Shielded, for 1/4" Shaft Diameter

6384K352 Steel Ball Bearing Flanged Double Sealed for 1/4" Shaft Dia, 11/16" OD

8600N3 Miniature Alum Base-Mnt SS Ball Brng--ABEC-3 for 1/4" Shaft Diameter

7200K3 NEMA 34 Face-Mount Brushless DC Motor 1/3 hp, 3450 rpm, Integrated Speed Control

6099K41 Stainless Steel One-Piece Set-Screw Coupling 1/2" Bore, 1-1/2" Length, 1" OD, with Keyway

8774K33	Static-Dissipative Clear Cast Acrylic Sheet 1/4" Thick, 12" X 24"
97042A516	18-8 Stainless Steel One-End Threaded Stud 1/2"-13 x 5/8" X 1-1/2"
99223A067	Acetal Hex Nut 1/2"-13 Thread
94564A023	Nylon Flat Point Socket Set Screw 6-32 X 1/4"
9986K21	Black Delrin Rod 4" Diameter, 1/2" Length
8576K15	Black Delrin Rod 1/2" Diameter, 5' Length
94922A050	Nylon 6/6 Acorn Nut Off-White, 1/2"-13 Thread Size, 3/4" W, 13/16" H
8572K61	White Delrin Rod 1" Diameter, 1' length
8582K21	White Delrin Rod 4" Diameter, 1/2" Length
8572K55	White Delrin Rod 1/2" Diameter
93140A839	Polycarbonate Machine Screw Flat Head Slotted, 1/4"-20 X 3/4"
95868A148	Nylon 6/6 SHCS 6-32 Thread, 1/2" Length, Off-White
7643A421	1" x 30', Gray, .012" Thk, High-Temp Self-Fusing Silicone Rubber Tape
7586K12	Adhesive-Backed Cable Holder Press in, 1/4" Maximum Bundle
60015K42	Tear-Resistant Rubber Vibration Damping Pad 6" X 6" X 3/8" Thick, 45 PSI Max Load
4056K42	Oil-Resistant Nitrile Vibration Damping Pad 6" X 6" X 5/16" Thick, 100 PSI Max Load
94605A541	Nylon 82 Deg Flat Head Slotted Machine Screw 1/4"-20 X 3/4"
94564A080	Nylon Flat Point Socket Set Screw 1/4"-20 X 1/2"
5537K26	Tinned Copper Expandable Mesh Sleeving 1/4" ID, 3/16" to 5/16" Bundle Dia
8491A614	Steel Press-Fit Drill Bushing/Liner 0.257" (F) ID, 13/32" OD, 1/2" Length
3504T21	Push-on Round FDA Cap Fits 3/8" OD, 1/2" Inside Height

92805K22 Push-on High-Temperature Silicone Rubber Cap Fits .6" Outside Diameter, 1-1/2" Inside Height

[0108] A high voltage power supply source was used to apply a +10-40kV DC voltage to parallel metal rods, with a -5-40kV DC field applied to the syringe/needle containing a polymer solution, with syringe placed in a syringe pump. The metal posts can be hollow or solid, and have been tested as rods of stainless steel, steel, copper, and aluminum, both solid and hollow using thin-walled tubing. The absolute DC field strength ranged from 15kV to 60kV. A programmable syringe pump was set to dispense the solution at 0.50-9.50 mL/hr, ideally at 6.5ml/hr for pure PCL, and 3ml/hr for heart basement membrane (HBM) and HBM/PCL mixtures. After approximately 5 minutes to 1 hour of electrospinning, the aligned fibers are collected for imaging (SEM and DIC).

[0109] Metal rods were used to align electrospun PCL. PCL dissolved in HFP (100mg/ml) at a +15kV reference, set 15cm apart, was electrospun between the rods, with PCL pumped at 5ml/hr over 5 minutes to the form the aligned nanofibers seen collecting between the rods. **Figure 1 (a, d)** shows the representative electric field lines and the know bending of these lines in a field with a split point charge (as simplified plane of point charges represented). This splitting of the electric charge field is believed to act on imparting alignment as they travel on the looping fibers in this warped electrostatic field. We have successfully aligned fibers with hollow and solid metal rods, and the modulation of the electric field lines by solid vs. hollow rods is also shown **(figure 1b and 1c)** (electric field diagrams created using the applet from cco.caltech.edu/~phys1/java/phys1/EField/EField.html).

[0110] For SEM images, aligned electrospun PCL and HBM/PCL blended samples were taken from the aligned rods and mounted on aluminum stubs using carbon stickers. The samples were then coated with gold at about 50nm thickness using a plasma-based sputter coater. Coated samples were imaged using a JOEL 6400 scanning electron microscope (SEM) with Orion image processing. Representative images of charged parallel rod aligned fibers, as compared to fibers generated in the traditional, randomly aligned fashion by collection onto a static piece of

grounded foil, can be seen in **Figures 2A, 2B, 2C, and 2D**. The average cross-sectional fiber diameter of the electrospun fibers generated from random and charged rod aligned basement membrane matrix was calculated by measuring 35 unique points in ImageJ64 (NIH shareware), with results shown in **Figure 2E**, along with the average angle of the fibers. A fast Fourier transform (FFT) was performed to indicate the degree of alignment as seen in **Figures 3A, 3B, 3C, and 3D** using the ImageJ64 FFT tool, with patterns of aligned fibers shown to match with those of know aligned Fourier spectra, as commonly used for pattern recognition.

[0111] Figures 4A, 4B, and 4C illustrate a design for rotating the charged parallel rods using a mercury coupled bearing to allow electrical conductance with the rotating charged rods mounted in plastic platforms attaching to a high speed motor (0-4000RPMs). The actual implementation of this rotating rod design is shown in **figure 5**. In another aspect, this rotating charged parallel point charged rod fiber alignment process may be modified so that electrospun fibers of opposite charge are propelled towards each other in the static field, whereby the fibers combine in a "whirlpool" fashion roughly equidistant apart in space. In this arrangement, the fiber charges are effectively neutralized and the fibers are then collected on the grounded rotating rods of the mandrel, which allows a thicker layer of fibers to be collected due to less fiber-to-fiber charge repulsion. Equally charged fibers are believed to have a repulsive effect on each other in the collected fibers. Charge neutralization of the fibers in this fashion theoretically negates this phenomenon to electrospin sheets that are up to about 1.5 times, 2.0 times, 3.0 times, or 3.4 times the thickness of conventionally electrospun fiber sheets. For example, two syringe pumps with basement membrane, each attached to a high voltage power supply may have one supply set to positive (+) voltage, and the other set to negative (-) voltage to generate the static electric field for electrospinning, with fibers collected on the rotating or static charged rods. The charges of equal voltage and opposite charge combine in space above the dual charged rod mandrel in a the vortex of the electric field, depositing as charge neutralized fibers on the rods, which allows for enhanced thickness of the collected aligned fibers over conventional methods.

[0112] By implementing a pneumatic motor in the conformations for electrospinning aligned (and random) nanofibers onto a mandrel as described above, on average 9.24% more material is collected on the mandrel(s) compared to using an electrical motor.

[0113] Example 2: Heart Base Membrane Fiber Scaffold

[0114] Heart basement membrane (HBM) and polycaprolactone (PCL) were dissolved separately in HFP at 100mg/ml, and mixed in a 10% ratio of HBM:PCL (0.100g HBM to 0.900g PCL), mixed at 4deg a total of 2hrs until fully into solution. The HBM:PCL was loaded into a glass 5ml syringe, loaded into a syringe pump set to expel at 1.5ml/ml, in a static +20kV electric field at the needle and -10kV to each of the parallel aligned collecting rods, then electrospun between the poles in a highly aligned fashion. The aligned fibers of HBM/PCL were collected and stored in a dessicator prior to use. To obtain dorsal root ganglion (DRGs), an 11 day old YFP-βIII Tubulin expressing transgenic mouse was sacrificed for collection of the spinal cord and removal of the DRGs, which were liberated from their capsule and briefly digested in trypsin, then placed upon the aligned HBM:PCL scaffold and cultured in basic neuron media containing B27 supplement. As shown in **figures 6A and 6B**, outgrowths from the DRGs were visualized under light and fluorescent microscopy at 1 and 2 days post explantation, with aligned neurons seen erupting from the bundle, with the neurite outgrowths seen tracing the aligned fibers.

[0115] Example 3: Dorsal Root Ganglia Attachment and Outgrowth on Placenta and Heart Basement Membrane Nanofibers.

[0116] Heart basement membrane HBM and polycaprolactone (PCL) were dissolved separately in HFP at 100mg/ml, and mixed in a 10% ratio of HBM:PCL (0.100g HBM to 0.900g PCL), mixed at 4deg a total of 2hrs until fully into solution. Each biopolymer was individually loaded into a glass 5ml syringe, loaded into a syringe pump set to expel at 2.5ml/hr, in a static +20kV electric field at the needle, then electrospun onto 10mm glass coverslips backed by a grounded piece of foil. The randomly aligned fibers were collected and stored in a dessicator prior to use. To obtain dorsal root ganglion (DRGs), a 10 day old YFP-βIII Tubulin expressing transgenic

mouse was sacrificed for collection of the spinal cord and removal of the DRGs, which were liberated from their capsule and briefly digested in trypsin, then placed upon the scaffolds and cultured in basic neuron media containing B27 supplement for 7 days. As shown in **figures 6A and 6B**, Outgrowths from the DRGs were again visualized under light and fluorescent microscopy at 1-7 days post explantation. Neurite outgrowth was seen tracing the electrospun fibers on HBM/PCL coated coverslips.

[0117] Example 4: Dorsal Root Ganglia Attachment and Outgrowth on Polycaprolactone Nanofibers (Comparative Example)

[0118] Polycaprolactone (PCL) was dissolved in HFP at 100mg/ml and mixed by vortexing at room temperature for 2hrs until fully into solution. The PCL biopolymer was loaded into a glass 5ml syringe, loaded into a syringe pump set to expel at 5ml/h, in a static +20kV electric field at the needle, then electrospun onto 10mm glass coverslips backed by a grounded piece of foil. The randomly aligned fibers of each group were collected and stored in a dessicator prior to use. To obtain dorsal root ganglion (DRGs), an 10 day old YFP-βIII Tubulin expressing transgenic mouse was sacrificed for collection of the spinal cord and removal of the DRGs, which were liberated from their capsule and briefly digested in trypsin, then placed upon the scaffolds and cultured in basic neuron media containing B27 supplement for 7 days (n=3). Outgrowths from the DRGs were visualized under light and fluorescent microscopy at 1-7 days post explantation. DRGs did not grow out on PCL and were found to be non-viable after 2-3 days in repeat trials (n=6).

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ALIGNED FIBER AND METHOD OF USE THEREOF

Inventor

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Cross-Reference to Related Applications

[0001] This application is a Divisional Application of U.S. Application No. 14/776,106, filed September 14, 2015, which is a U.S. National Phase Application of PCT International Application PCT/US2014/025636, filed March 13, 2014, which claims priority benefit of U.S. Application No. 61/785,031, filed March 14, 2013, the disclosures of which are incorporated herein in their entirety.

Background of the Invention

[0002] The invention relates to an aligned fiber and method of use thereof.

Summary of the Invention

[0003] The invention relates to a scaffold comprising an aligned fiber. The invention further relates to a scaffold comprising one or more electrospun fibers. In some embodiments, wherein a fast Fourier transform (FFT) analysis result of the fibers have adjacent major peaks with about 180° apart from each other.

[0004] The invention further relates to methods culturing cells on the scaffold or aligned fiber described herein.

[0005] The invention also relates to methods of promoting differentiation of stem cells into osteoblasts, chondrocytes, ligament or tendon, the method comprising culturing the cells on the scaffold or aligned fiber described herein in conditions suitable for the cell differentiation

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Brief Description of the Drawings

[0006] FIGURE 1A depicts representative field lines that are modulated between ~~a split~~split point negative voltage sources and a single positive voltage source ~~(A, D)~~, aiding in imparting alignment in the electrospinning scheme. ~~The change in field lines between a solid (B) and hollow (C) rod is also modeled~~.

[0007] FIGURE 1B models field lines generated by a solid rod.

[0008] FIGURE 1C models field lines generated by a hollow rod.

[0009] FIGURE 1D depicts representative filed lines that are modulated between split point negative voltage sources and a single positive voltage source, aiding in imparting alignment in the electrospinning scheme.

[0010] ~~FIGURE 2~~FIGURE 2A depicts raw basement membrane~~ (top left)~~, FIGURE 2B depicts a ~~next to it~~ randomly electrospun architecture~~ (to right),~~, FIGURES 2C and 2D depict ~~as compared to~~an aligned basement membrane generated by parallel charged rod aligned electrospinning ~~(bottom left and right)~~as compared to FIGURES 2A and 2B, and FIGURE 2E provides calculations of the average cross-sectional fiber diameter of the electrospun fibers.

[0011] ~~FIGURE 3 depicts~~FIGURES 3A, 3B, 3C, and 3D depict a Fourier spectrum from charged rod aligned and randomly electrospun fibers. The aligned pattern/texture recognition is typical to that of a regular, repeating line pattern, with the random fibers consistent with random noise generation spectra, as commonly seen in pattern recognition from FFT analysis.

[0012] ~~FIGURE 4 depicts~~Figures 4A, 4B, and 4C depict a modeling of a rotating parallel rod mandrel having a mercury coupled bearing inserted into the top of the device to split the electrical charge between the rods.

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[0013] FIGURE 5 depicts the exemplary elements of the parallel electric charged rod electrospinning setup. Using two charged rods imparts alignment to the traveling fibers, optionally through the point charge splitting of the electric field.

[0014] ~~FIGURE 6 depicts~~FIGURES 6A and 6B depict an aligned heart base membrane fiber scaffold that allow dorsal root ganglia attachment and outgrowth.

[0015] ~~FIGURE 7~~FIGURES 7A, 7B, 7C, 7D, 7E, 7F, 7G, 7H, 7I, and 7J depict ~~depicts~~ aligned placenta and heart base membrane fiber scaffolds that allow dorsal root ganglia attachment and outgrowth.

[0016] FIGURE 8A depicts exemplary FFT analysis results ~~(a)~~ of the fibers described herein ~~and (b) a control sample~~.

[0017] FIGURE 8B depicts an FFT analysis of a control sample.

[0018] FIGURES 9A~~, 9B and 9C depict~~ depicts an exemplary scaffolds prepared into an elongated roll by folding an elongated sheet ~~(9A), an elongated roll by rolling an elongated sheet (9B), and a composite of multiple layers of elongated sheets (9C)~~.

[0019] FIGURE 9B depicts an exemplary scaffold prepared into an elongated roll by rolling an elongated sheet.

[0020] FIGURE 9C depicts an exemplary scaffold prepared into an elongated roll from a composite of multiple layers of elongated sheets.

Detailed Description of the Invention

[0021] The invention relates to an electrospinning apparatus comprising (i) at least one spinneret comprising an electrified tip; and (ii) a collector comprising two rods and a platform connected

to the two rods, wherein the two rods are configured split an electric field between them. In one aspect, the spinneret comprising an electrified tip may have two, three, four, five or more electrified tips. In another aspect, the collector comprising two rods may have three, four, five or more rods. In additional aspect, the platform connected to the two rods may have three, four, give or more rods connected to the platform. As defined herein, "comprising two rods" refers to comprising at least two rods, including, for example, two, three, four, and five rods.

[0022] The invention also relates to an electrospinning apparatus comprising (i) two spinnerets, each of which comprises a tip electrified with an opposite charge to one another; and (ii) a collector comprising two rods and a platform connecting the two rods, wherein the two rods are grounded.

[0023] Electrospray/electrospinning techniques can be used to form particles and fibers as small as one nanometer in a principal direction. The phenomenon of electrospray involves the formation of a droplet of polymer melt at an end of a needle, the electric charging of that droplet, and an expulsion of parts of the droplet because of the repulsive electric force due to the electric charges. In electrospraying, a solvent present in the parts of the droplet evaporates and small particles are formed but not fibers. The electrospinning technique is similar to the electrospray technique. In electrospinning and during the expulsion, however, fibers are formed from the liquid as the parts are expelled.

[0024] In particular, for example, the electrospinning typically involves a polymer solution (or melt) maintained at its surface tension on the tip of a nozzle via a syringe in a pump. When sufficiently high voltage is introduced (*e.g.* 15-40kV) to the polymer or oligomer in solution to create a charge imbalance, the solution may be drawn towards a grounded collector through the static electric field. As the polymer erupts from the needle and the assembling polymer whip through space, the fiber may be subjected to a series of stretching and bending instabilities, resulting in plastic stretching and elongation to minimize these instabilities generated by repulsive electrostatic forces. As the polymer or oligomer travels through space and rapidly thins into a fine stream, the solvent may evaporate and the polymer or oligomer assembles into fibers, leaving dry nano- to micro-scale fibers of tailorable physical attributes on the collector in

the typical electrospinning scheme. Additionally, when high electrical potential is applied to a low viscosity polymer or oligomer melt or solution, or when improperly high or low voltage is applied, electrospraying may occur, which is typified by the polymer jet breaking down into fine droplets. It is therefore possible to produce particles or nano- to micro-sphere particles, fibers and bead-and-string type structures via this process from simply altering the solution or electrospinning parameters. Polymer solution properties, applied electrical potential, polymer molecular weight, polymer solution flow rate, distance between spinner and collector, ambient parameters (*e.g.* humidity, air velocity, temperature) and motion of the collecting target can be altered to form fibers of controlled fiber distribution, diameter and alignment via electrospinning.

[0025] The electrospinning apparatus as described herein in one aspect may include one or more spinneret. The term "spinneret" used herein refers to a single- or multi-pored device through which a polymer or oligomer solution is extruded to form fibers. In one aspect, the spinneret is selected from the group consisting of a multiple nozzle spinneret, a single syringe or capillary spinneret, and a compound spinneret. In another aspect, the spinnerets of the electrospinning apparatus may be differently charged, resulting in differently charged polymer or oligomer solutions extruded from them. For example, different voltages may be applied to two or more polymer or oligomer sources whereby a positively charged solution is combined in space with a negatively charged solution, which is sent to the grounded rotating rods of the mandrel.

[0026] The electrospinning apparatus according to some embodiments of the present invention includes at least one spinneret comprising an electrified tip. The spinneret may have an electrified tip having a charge, and the tip may have one or more pores. In some embodiments, the electrospinning apparatus may include two or more tips having different charges. In particular, for example, the electrospinning apparatus may include two tips having opposite charges to each other.

[0027] In some embodiments, the spinneret described herein may be replaced with a different ejecting device comprising a drum configured to rotate in contact with a reservoir of a polymer or oligomer solution herein. In one aspect, this drum may pull the polymer or oligomer solution as a thin film on the surface of the drum. In another aspect, the drum may be charged, resulting

in charging of the solution. In additional embodiments, the spinneret described herein may be replaced with a different ejecting device comprising a flat surface in contact with a polymer or oligomer solution, and the polymer or oligomer solution may form a polymer bubble(s) by applying air.

[0028] The electrospinning apparatus in one aspect may include a collector having at least two rods and a platform. In some embodiments, the collector may have, for example, three, four, five, six, seven, eight, nine, ten, twenty, thirty, or forty rods. Herein, the term "rod" used herein refers to a bar of material having a shape including, but not limited to prism, cylinder, pentagonal rod, hexagonal rod, square rod, and triangular rod. The rod may comprise an electrical conducting material (*e.g.* a metal). In some embodiments, the rod may be electrified with a charge opposite to a charge of the electrified tip extruding a solution to the rod. In another aspect, the rod may be made of a non-electrical conducting material (*e.g.* insulating plastic). In further aspect, the rod may be made of both electrical and non-electrical conducting materials. For example, the electrical conducting material may be coated on the non-electrical conducting material.

[0029] The rod described herein may be directly or indirectly connected and/or fixed to a platform. The platform refers to any support for the rod. In one aspect, the rod comprises a proximal part and distal end, and the proximal part and/or distal end may be connected and/or fixed to the platform. In another aspect, the rod may go through the platform to which the proximal part of the rod is connected and/or fixed.

[0030] In one aspect, the platform may be a part of a machine frame. In another aspect, the electrospinning apparatus described herein may further include a chamber enclosing the spinneret and the collector. The chamber may comprise the platform described herein. In another aspect, the platform may consist of or comprise a rotating shaft or a bearing that is directly or indirectly connected and/or fixed to the rod.

[0031] The platform according to some embodiments includes a bearing connected to the rod described herein. The bearing used herein refers to a guide for rotating the rod. In one aspect, the bearing may be a rotating shaft. In another aspect, the platform or the bearing used herein

may use plain bearing, rolling-element bearings, jewel bearings, fluid bearings, magnetic bearings, and/or flexure bearings. The bearing may include an electrical conductor configured to allow electrical conductance to the rods. For example, the bearing may have mercury.

[0032] In another aspect, the platform connected to the rod(s) may be configured to spin resulting in rotation of the rod(s) about the spinning axis of the platform. In some embodiments, the rods are configured to rotate at between about 0 and about 8000 RPM, between about 0 and about 7000 RPM, between about 0 and about 6000 RPM, between about 0 and about 5000 RPM, between about 0 and about 4000 RPM, between about 0 and about 3000 RPM, between about 0 and about 2000 RPM, between about 0 and about 1000 RPM, between about 0 and about 500 , between about 0 and about 300 RPM, between about 0 and about 100 RPM, between about 0 and about 50, between about 1 and about 2000 RPM, between about 1 and about 1000 RPM, between about 1 and about 500 , between about 1 and about 300 RPM, between about 1 and about 100 RPM, between about 1 and about 50, between about 1000 and about 8000 RPM, between about 2000 and about 8000 RPM, between about 3000 and about 8000 RPM, between about 4000 and about 8000 RPM, between about 5000 and about 8000 RPM, between about 1000 and about 6000 RPM, between about 2000 and about 6000 RPM, or between about 3000 and about 6000 RPM. In additional embodiments, the rods are configured to rotate at about 50 RPM, about 500 RPM, about 1500 RPM, about 2500 RPM, about 3500 RPM, about 4500 RPM, about 5500 RPM, about 6500 RPM, about 7500 RPM or lower. In additional embodiments, the rods are configured to rotate at about 500 RPM, about 1500 RPM, about 2500 RPM, about 3500 RPM, about 4500 RPM, about 5500 RPM, about 6500 RPM, about 7500 RPM or higher. In some embodiments, when the rods are rotating, the fibers may spin around the rods. In other embodiments, when the rods are rotating, the fibers may also move or jump from one rod to another rod. In yet additional embodiments, the rods are stationary. In yet further embodiments, when the rods are stationary, the fibers may move or jump from one rod to another through an electric field.

[0033] In some embodiments, an air driven (pneumatic) motor and an electric (DC) motor may be employed in the electrospinning. In further embodiments, the pneumatic motor may generate

less stray field anomalies and allow capturing of more nanofiber product on the rod(s) compared to the DC motor.

[0034] In one aspect, the fibers align on the rod (*e.g.* by spinning around the rods or jumping to one rod to another) in a perpendicular angle to the rods. In another aspect, the fibers align in an angle about 91, 92, 93, 94, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155 ° or less. In another aspect, the fibers align in an angle about 90, 91, 92, 93, 94, 95, 98, 103, 108, 113, 118, 123, 128, 133, 138, 143, 148, 153 ° or more. In another aspect, the fibers align in an angle between about 90 and about 93 °, between about 90 and about 95 °, between about 90 and about 100 °, between about 90 and about 105 °, between about 90 and about 110 °, between about 90 and about 115 °, between about 93 and about 95 °, between about 93 and about 100 °, between about 93 and about 105 °, between about 93 and about 110 °, or between about 93 and about 115 °.

[0035] As used herein, the term "about" modifying, for example, the quantity of an ingredient in a composition, concentrations, volumes, process temperature, process time, yields, flow rates, pressures, and like values, and ranges thereof, refers to variation in the numerical quantity that can occur, for example, through typical measuring and handling procedures used for making compounds, compositions, concentrates or use formulations; through inadvertent error in these procedures; through differences in the manufacture, source, or purity of starting materials or ingredients used to carry out the methods; and like considerations. The term "about" also encompasses amounts that differ due to aging of, for example, a composition, formulation, or cell culture with a particular initial concentration or mixture, and amounts that differ due to mixing or processing a composition or formulation with a particular initial concentration or mixture. Whether modified by the term "about" the claims appended hereto include equivalents to these quantities. The term "about" further may refer to a range of values that are similar to the stated reference value. In certain embodiments, the term "about" refers to a range of values that fall within 10, 9, 8,7, 6, 5,4, 3, 2, 1 percent or less of the stated reference value.

[0036] The electrospinning apparatus according to some embodiments of the present invention includes a collector comprising two or more rods and a platform connected to the rods, wherein the rods are configured split an electric field between them. In one aspect, operating the electrospinning apparatus described herein may result in forming a point charge splitting of an electric field, through which traveling polymer or oligomer solutions align. In another aspect, the rods may be grounded.

[0037] The split electric field in some embodiments is separated by an air insulator. In one aspect, with air as an insulator between electrically charged metal rods, electrospun fibers are collect between opposed metal rods in an aligned fashion. The electrospun fiber described herein may be a microfiber or a nanofiber.

[0038] The split electric field in some embodiments is insulated by non-conductive materials or environments placed between the rods (*e.g.* carbon fiber, PTFE [Teflon], mica, diamond, ceramic, rubbers, glass, vacuum, materials on the low ends of the triboelectric series in the high resistivity range, also including paper, cotton, wood, epoxy, plastics such as: ABS [acrylonitrile, butadiene, and styrene], polycarbonate, acetate, acrylic, delrin, fiberglass, FEP, high impact polystyrene [HIPS], kapton, kaptrex, kynan, macor, melamine, meldin 7001 unfilled polyimide, MICA, neoprene, nomex, noryl PPO, PolyEtherEther-Ketone [PEEK], polyethylene terephthalate [P.E.T], P.E.T.G, phenolics such as micarta phenolics, perfluoroalkoxy [PFA], Polycarbonate, polyester [mylar], polyolefins, polystyrene, polysufone, polyurethane,), Rexolite® 1422 &220, polyphenylene sulfide [Ryton], silicone/fiberglass, silicone rubber, techtron, Ultem® 1000, Vespel® SP-1 [polyimide], electrically insulating papers such as vulcanized fibre, tapes from the above materials, and foams of these materials, including neoprene foam, polystyrene foam, polyurethane foam, silicone foam, vinyl foam.

[0039] The term microfiber as used herein means a fiber comprising a diameter of about 1000 μm or less. The term nanofiber as used herein means a fiber comprising a diameter of about 1000 nm or less. Relative to the parent bulk material, nano-features can impart many extraordinary properties to macrostructures, including superior mechanical, electrical, optical and

magnetic properties, adding surface functionality and yielding high surface area. Matrices of nanofibers of varying diameters may show a range of variable surface properties (*e.g.* hydrophobicity and hydrophilicity), porosities, and usually superior mechanical properties (*e.g.* tensile strength, stiffness) relative to the material in other forms.

[0040] In some embodiments, the nanofiber described herein may have an average diameter of about 1000 nm or less, 900 nm or less, 800 nm or less, 700 nm or less, 600 nm or less, 500 nm or less, 400 nm or less, 300 nm or less, 200 nm or less, 100 nm or less, 50 nm or less, 20 nm or less, or 10 nm or less. In some embodiments, the nanofiber described herein may have an average diameter of about 1000 nm or more, 900 nm or more, 800 nm or more, 700 nm or more, 600 nm or more, 500 nm or more, 400 nm or more, 300 nm or more, 200 nm or more, 100 nm or more, 50 nm or more, 20 nm or more, 10 nm or more, or 1 nm or more.

[0041] In some embodiments, the microfiber described herein may have an average diameter of about 1000 μm or less, 900 μm or less, 800 μm or less, 700 μm or less, 600 μm or less, 500 μm or less, 400 μm or less, 300 μm or less, 200 μm or less, 100 μm or less, 50 μm or less, 20 μm or less, or 10 μm or less. In some embodiments, the microfiber described herein may have an average diameter of about 1000 μm or more, 900 μm or more, 800 μm or more, 700 μm or more, 600 μm or more, 500 μm or more, 400 μm or more, 300 μm or more, 200 μm or more, 100 μm or more, 50 μm or more, 20 μm or more, 10 μm or more, or 1 μm or more.

[0042] In some embodiments, the rods described herein may be set parallel to each other in the electrospinning apparatus. In other embodiments, the rods may not be set parallel to each other but at an angle, for example, at an angle smaller than 5°, 10°, 30°, 50°, 70°, or 90°. In other embodiments, the rods may not be set parallel to each other but at an angle, for example, at an angle bigger than 1°, 8°, 25°, 40°, 60°, or 75°. In additional embodiments, the rod described herein may be straight, curved or angled.

[0043] The rods described herein in some embodiments are separated from one another by an average distance of about 1 cm, about 2 cm, about 3 cm, about 5 cm, about 8 cm, about 10 cm,

about 13 cm, about 15 cm, about 18 cm, about 20 cm, about 23 cm, about 25 cm, about 27 cm, about 30 cm or more. In one aspect, the rods are separated from one another by an average distance of about 1 cm, about 2 cm, about 3 cm, about 5 cm, about 8 cm, about 10 cm, about 13 cm, about 15 cm, about 18 cm, about 20 cm, about 23 cm, about 25 cm, about 27 cm, about 30 cm, about 40 cm, about 50 cm, about 60 cm or less. In another aspect, the rods are separated from one another by a distance from about 1 cm to about 25 cm, from about 6 cm to about 25 cm, from about 12 cm to about 25 cm, from about 18 cm to about 25 cm, from about 23 cm to about 25 cm, from about 1 cm to about 35 cm, from about 6 cm to about 35 cm, from about 12 cm to about 35 cm, from about 18 cm to about 35 cm, from about 24 cm to about 35 cm, or from about 10 cm to about 20 cm.

[0044] The collector may be biocompatible. In fact, any of the other elements of the electrospinning apparatus described herein may be biocompatible.

[0045] The electrospinning apparatus described herein may also include a control mechanism configured to control the electric potential of the electrified tip. The electrospinning apparatus described herein may also include a reservoir for a solution. The electrospinning apparatus described herein may also include an electric source connected to the electrified tip.

[0046] The invention further relates to methods of electrospinning using the electrospinning apparatus described herein and methods of preparing an electrospun fiber by the electrospinning. The methods of electrospinning and methods of preparing an electrospun fiber by the electrospinning may comprise (i) extruding a solution from an electrified tip of a spinneret, and (ii) collecting the extruded solution on at least a part of a collector. The methods of electrospinning or preparing an electrospun fiber by the electrospinning may further comprise extruding two, three or more solutions from one, two, three or more electrified tips of a spinneret. The methods of electrospinning or preparing an electrospun fiber by the electrospinning may further comprise extruding two, three or more solutions from one, two, three or more electrified tips of one, two, three or more spinnerets. The methods of preparing an electrospun fiber by the electrospinning may further comprise coating the extruded solution with another electrospun fiber prepared by the methods described herein.

[0047] The solution that may be extruded using the electrospinning apparatus described herein may include, but is not limited to, solutions comprising collagen type I, adipose extracellular matrix, heart basement membrane extract or extracellular matrix, placenta basement membrane extract or extracellular matrix, and polycaprolactone. Additional materials that may be electrospun by the method described herein include, but are not limited to, poly(glycolic acid), poly(lactic acid), polydioxanone, poly (lactide-co-glycolide) copolymers, polyesters polysaccharides, polyhydroxyalka noates , starch, polylactic acid, cellulose, proteins, agar, silks, alginate, collagen/gelatin, carrageenan, elastin, pectin, resilin, konjac, adhesives, gums, polyamino acids, polysaccharides, soy, zein, wheat gluten, casein, chitin/chitosan, serum albumin, hyaluronic acid, lipids/s urfactants, xanthan, acetoglycerides, waxes, surfactants, dextran, emulsan, gelian, polyphenols, levan, lignin, curd, ian, tannin, polygalactosamine, humic acid, shellac, pullulan , poly-gamma-glutamic acid, elsinan, natural rubber, yeast glucans, and synthetic polymers from natural fats and oils, and the mixture thereof.

[0048] In one aspect, the collector of the electrospinning apparatus is a biocompatible support to be coated by the electrospun fiber on its surface and may be used as a part of the implant described herein. For example, the collector may comprise a bone matrix, and the electrospun fiber is collected on the surface of the bone matrix, resulting in coating of the bone matrix. The bone matrix may have two or more different electrospun fiber coatings on its surface.

[0049] The invention further relates to a method of coating a matrix on a surface comprising (i) extruding a solution from an electrified tip of a spinneret, and (ii) collecting the extruded solution on a portion of a collector, wherein the collector further comprises two rods and a platform connected to the two rods, the two rods are configured to split an electric field between them, and the portion of the collector comprises the matrix. The invention further relates to a method of coating a matrix on a surface comprising (i) extruding one or more solutions from electrified trips of two spinnerets, each of which comprises at least one tip electrified with an opposite charge to a tip of the other spinnerets, and (ii) collecting the extruded solution on a portion of a collector, wherein the collector further comprises two rods and a platform connected to the two

rods, the two rods are grounded, and the portion of the collector comprises the matrix. In some embodiments, the matrix is a bone matrix, and the solution comprises a bone matrix solution. In additional embodiments, the matrix may comprise a synthetic surface.

[0050] In some embodiments, the matrix is biocompatible. In further embodiments, the biocompatible matrix is an implantable biocompatible matrix that can function as a support system for the bone matrix described herein. A biocompatible matrix should be non-toxic, non-eliciting or stimulating severe inflammatory response or immunological rejections, and devoid of other undesired reactions at the implantation site. In one embodiment, the biocompatible matrix is bone matrix or cartilage or connective tissue.

[0051] In some embodiments, the biocompatible matrix is prepared into a form of an elongated roll, an elongated sheet, a composite of multiple layers of sheets, or a combination thereof before or after being coated with the electrospun fibers. For example, the biocompatible matrix may be prepared as shown in **figures 9A, 9B, and 9C**. In one aspect, the materials for the elongated roll and the elongated sheet may be different. In another aspect, the materials for each elongated sheet in the multiple layers of sheets may be different from each other. In another aspect, the electrospun fibers coated on the biocompatible matrix are aligned in one direction. For example, the electrospun fibers coated on the biocompatible matrix are aligned in the direction parallel or perpendicular to the length of the elongated sheet or the multiple layers. In another aspect, the biocompatible matrix is prepared by folding or rolling the sheet or sheets, along with the direction of nano fiber alignment to form an elongated roll implant. In one aspect, this biocompatible matrix in the form of an elongated sheet or a composite of multiple layers of sheets may be used to facilitate neuronal cell growth.

[0052] In some embodiments, the elongated roll implant may be prepared by combining a sheet or sheets to facilitate surgical implantation. The biomaterials to prepare the sheet and elongated roll may be the same, or different, as described previously.

[0053] In some embodiments, the elongated sheet described herein may have an average thickness of about 1000 μm or less, 900 μm or less, 800 μm or less, 700 μm or less, 600 μm or

less, 500 μm or less, 400 μm or less, 300 μm or less, 200 μm or less, 100 μm or less, 50 μm or less, 20 μm or less, or 10 μm or less. In one aspect, the elongated sheet described herein may have an average thickness of about 1000 μm or more, 900 μm or more, 800 μm or more, 700 μm or more, 600 μm or more, 500 μm or more, 400 μm or more, 300 μm or more, 200 μm or more, 100 μm or more, 50 μm or more, 20 μm or more, 10 μm or more, or 1 μm or more.

[0054] In some embodiments, the length of the elongated sheet or roll implant is about 0.1 cm or more, about 0.3 cm or more, about 0.5 cm or more, about 0.7 cm or more, about 1 cm or more, about 2 cm or more, about 3 cm or more, about 5 cm or more, about 8 cm or more, about 10 cm or more, about 13 cm or more, about 15 cm or more, about 18 cm or more, about 20 cm or more, about 23 cm or more, about 25 cm or more, about 27 cm or more, or about 30 cm or more. In one aspect, the length of the elongated sheet or roll implant is about 1 cm or less, about 2 cm or less, about 3 cm or less, about 5 cm or less, about 8 cm or less, about 10 cm or less, about 13 cm or less, about 15 cm or less, about 18 cm or less, about 20 cm or less, about 23 cm or less, about 25 cm or less, about 27 cm or less, about 30 cm or less, about 40 cm or less, about 50 cm or less, or about 60 cm or less.

[0055] In some embodiments, the biocompatible matrix includes but is not limited to, bone graft implants, synthetic bone graft materials in forms of particulates, sheet, or blocks, tendon and/or ligament in bone tunnels, prosthetic implant, for example, for hip, shoulder, knee, or ankle, and trabecular metal. In further embodiments, the bone graft implants may include allograft or xeno-graft. In yet further embodiments, the bone graft implants may include structural bone implants including, but not limited to, monolithic or composite spinal implants (e.g., VERTIGRATs from LifeNet Health) and bone struts or blocks. In yet further embodiments, the bone graft implants may include demineralized, or non-demineralized bone particulates, including, but not limited to, cortical, cancellous, or cortical cancellous bone. In other embodiments, the electrospun fibers may be formed on cell or tissue culture surface.

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[0056] Suitable biocompatible matrices include, but are not limited to, porous biocompatible scaffolds into which bone cells or progenitor cells may migrate. Osteogenic or chondrogenic cells, i.e., cells involved in the process of deposition of new bone material or cartilagenous material, respectively, can often attach to such porous biocompatible matrices, which can then serve as scaffolding for bone and cartilage tissue growth. Cells involved in the process of deposition of new ligament or tendon material can also attach to such porous biocompatible matrices. For certain applications, the biocompatible matrix should have sufficient mechanical strength to maintain its three dimensional structure and help support the immobilization of the bone segments being united or grafted together. Porous biocompatible matrices which provide scaffolding for tissue growth can accelerate the deposition of new bone or the rate of bone growth and are said to be "osteoconductive." Osteoconductive biocompatible matrices are especially useful in the matrices described herein. Porous biocompatible matrices which provide scaffolding for tissue growth can accelerate the deposition of new cartilage or the rate of cartilage growth and are said to be "chondroconductive." Osteoconductive biocompatible matrices are especially useful in the matrices described herein. Chondroconductive biocompatible matrices are especially useful in the matrices described herein. Angiogenic (or vasculogenic) biocompatible matrices are especially useful in the pharmaceutical compositions described herein. The osteoinductive, angiogenic or chondroinductive activity of the surface coated with the electrospun fibers or may not be altered, including but not limited to, enhanced activity, relative to a surface without the electrospun fiber coating or a natural surface without any coating. Thus, the osteoconductive, angiogenic or chondroconductive activity of the biocompatible matrices treated with the electrospun fibers of the present invention may be enhanced compared to matrices not treated with the bone matrix described herein. Of course, the biocompatible matrices are considered to be osteoconductive, angiogenic or chondroconductive if cells within the biocompatible matrix begin to differentiate into more osteoblast-like or chondrocyte-like appearing or functional cells, respectively.

[0057] The biocompatible matrices according to some embodiments of the present invention can be derived from natural sources or they can be synthetic or a mixture of both. Biocompatible

matrices from natural sources may also comprise natural polymers, including, but not limited to, collagen, hyaluronic acid, alginate, albumin, fibrinogen-fibrin, chitosan, elasin, laminin, connective tissues, intervertebral disc, cortical or cancellous bone, demineralized or mineralized bone, fascia lata, dermis, muscle, ligament, tendon, cartilage including articular/hyaline cartilage, elastic cartilage, and fibrocartilage, a mixture thereof, and mixture of reconstituted tissue. Biocompatible matrices from synthetic sources refer to any material not produced by living organisms, which may include, not limited to, the synthetic material made up of organic components, inorganic components, or a mixture thereof. In some embodiments, a synthetic biocompatible matrix may comprise an organic synthetic polymer, such as poly(lactic-co-glycolic acid), polycaprolactone (PCL), polyglycolic acid (PGA), polylactic acid (PLA), polyhydroxybutyrate (PHB), Poly(ethylene glycol) (PEG), poly(ethylene oxide) (PEO)), and others. In some embodiments, a tissue, an organ, or biocompatible matrix comprising at least one of alginate, chitosan, collagen, gelatin, hyaluronic acid, a fibronectin, an elastin, a laminin, and a proteoglycan may be employed. In certain embodiments, a biocompatible matrix comprising inorganic components, such as hydroxyapatite, calcium sulfate, octacalcium phosphate, calcium phosphate, macroporous calcium metaphosphate ceramic, β-tricalcium phosphate, metal, metal alloy, and others, may be used. A biocompatible matrix used in certain embodiments of the present invention may be prepared by demineralizing, decellularizing or devitalizing a tissue or an organ and cells may be seeded onto the biocompatible matrix.

[0058] In some embodiments, the electrospun fibers described herein may be applied to a biocompatible matrix and may be incubated at conditions permitting the generation of a matrix that is partially or fully coated with the electrospun fibers. In some embodiments, incubation may be carried out at about 40 °C or lower, or between about 10 ºC and about 37 ºC, or about 20 ºC and about 37 ºC. Incubation may be carried out for between at least about 2 minutes and about 120 minutes, about 3 minutes and about 100 minutes, about 4 minutes and about 80 minutes, about 5 minutes and about 60 minutes, and about 5 minutes and about 30 minutes in certain embodiments. Incubation may be performed under static or dynamic conditions, such as with agitation, shaking, stirring, mixing, horizontal motion, rocking, and others.

[0059] In some embodiments of the present invention, a biocompatible matrix may be lyophilized before the electrospun fiber is applied to the biocompatible matrix. In certain embodiments, the electrospun fibers may be coated on the biocompatible matrix, and the coated matrix may be subsequently lyophilized. The lyophilized, coated matrix can then be rehydrated before it is used. Further, the cells can be seeded onto the matrix before implantation.

[0060] Examples of suitable osteoconductive or chondroconductive biocompatible matrices include but are not limited to, collagen (e.g., bovine dermal collagen), fibrin, calcium phosphate ceramics (e.g., hydroxyapatite and tricalcium phosphate), calcium sulfate, guanidine-extracted allogenic bone and combinations thereof. A number of suitable biocompatible matrices are commercially available, such as Collograft™ (Collagen Corporation), which is a mixture of hydroxyapatite, tricalcium phosphate and fibrillar collagen, and Interpore™ (Interpore International), which is a hydroxyapatite biomatrix formed by the conversion of marine coral calcium carbonate to crystalline hydroxyapatite.

[0061] A number of synthetic biodegradable polymers can serve as osteoconductive or chondroconductive biocompatible matrices with sustained release characteristics. Descriptions of these polymers can be found in Behravesh (1999) Clinical Orthopaedics 367, S118 and Lu (2000) Polymeric Delivery Vehicles for Bone Growth Factors in Controlled Drug Delivery: Designing Technologies for the Future, Park and Mrsny eds., American Chemical Society, which is incorporated herein in its entirety herein. Examples of these polymers include poly α-hydroxy esters such as polylactic acid/polyglycolic acid homopolymers and copolymers, polyphosphazenes (PPHOS), polyanhydrides and poly(propylene fumarates).

[0062] Polylactic acid/polyglycolic acid (PLGA) homo and copolymers are well known in the art as sustained release vehicles. The rate of release can be adjusted by the skilled artisan by variation of polylactic acid to polyglycolic acid ratio and the molecular weight of the polymer (see Anderson (1997) Adv. Drug Deliv. Rev. 28:5. The incorporation of PEG into the polymer as a blend to form microparticle matrices allows further alteration of the release profile of the

active ingredient (see Cleek (1997) J. Control Release 48, 259). Ceramics such as calcium phosphate and hydroxyapatite can also be incorporated into the formulation to improve mechanical qualities.

[0063] In one embodiment, the biocompatible matrices used in the methods of the present invention are other types of bone matrices. For example, the electrospun fibers prepared by the methods described herein can be coated on a bone matrix. As used herein, the other types of the bone matrix may be a biocompatible matrix derived from or including elements of natural bone. In some embodiments, the natural bone is mineralized, partially demineralized, demineralized, cancellous, cortical, or cortical cancellous bone. The bone matrices used herein may or may not include additional synthetic components not typically found in bone tissue. Other embodiments include methods utilizing a biocompatible matrix derived from cartilage, other soft tissues such as the dermis, connective tissue, fascia, small intestine submucosa, serous membrane, pericardium, tendon, ligament, muscle, adipose tissue, myelin, blood vessels, base membrane, amniotic membrane and others. A biocompatible matrix prepared from hyaline cartilage, fibrocartilage or elastic cartilage, may be employed in some embodiments. A biocompatible matrix may be prepared from hyaline cartilage found in the condyle, tibial plateau, femoral head, humeral head, costal cartilage, or fibrocartilage found in intervertebral discs, or elastic cartilage found in the epiglottis or ear. In certain embodiments, a biocompatible matrix derived from natural sources that has been optionally cleaned, disinfected, chemically modified, decellularized, particulated, homogenized, lyophilized, gamma ray irradiated, and/or plasticized may be used. Any of the biocompatible matrices used herein may or may not include additional synthetic components not typically found in such tissue.

[0064] In one specific embodiment, the bone or cartilage biocompatible matrices may be demineralized or decellularized, respectively. Examples of demineralized matrices and methods of making are described in U.S. Patent No. 6,189,537 and 6,305,379, which are incorporated herein in its entirety herein.

[0065] The biocompatible matrix, tissue, or organ used in certain embodiments of the present invention may be in the form of a powder, particulates, sheets, fibers, gels, putties, paste, blocks, cylinders, sponges, meshes, films, slices, curls, flakes, or wedges, among others. In certain embodiments of the present invention, the biocompatible matrix, tissue, or organ treated with the electrospun fibers by the methods described herein may be in the form of a powder, fibers, putty, or a sponge. In further embodiments, the sponge can include, for example, the implant having sponge-like structures disclosed in the co-pending, commonly-assigned patent application PCT/US09/04556 entitled "Composition for a Tissue Repair Implant and Methods of Making the Same" filed on August 7, 2009, which is incorporated herein in its entirety herein. The treated matrices can be used in any of the methods of the present invention.

[0066] The invention also related to a method of preparing an electrospun fiber by electrospinning comprising (i) extruding a solution from an electrified tip of a spinneret, and (ii) collecting the extruded solution on a portion of a collector comprising two rods and a platform connected to the two rods, wherein the two rods are configured to split an electric field between them. The invention further related to a method of preparing an electrospun fiber by electrospinning comprising (i) extruding one or more solutions from electrified trips of two spinnerets, each of which comprises at least one tip electrified with an opposite charge to a tip of the other spinnerets, and (ii) collecting the extruded solution on a part of a collector comprising two rods and a platform connecting the two rods, wherein the two rods are grounded. In some embodiments, the method of preparing an electrospun fiber further comprises applying an accessory polymer to the solution (*e.g.* as a copolymer, blend, or melt). An "accessory polymer" refers to a polymer that may be added to the electrospun fibers and have any effect on their physical, chemical, and/or biological properties (*e.g.* tensile strength, hydrophillicity, biocompatibility). For example, the accessory polymer may be selected from the group consisting of polycaprolactone, poly(glycolic acid), poly(lactic acid), polydioxanone, poly (lactide-co-glycolide) copolymers, polyesters polysaccharides, polyhydroxyalkanoates, starch, polylactic acid, cellulose, proteins, agar, silks, alginate, collagen/gelatin, carrageenan, elastin, pectin, resilin, konjac, adhesives, gums, polyamino acids, polysaccharides, soy, zein, wheat

gluten, casein, chitin/chitosan, serum albumin, hyaluronic acid, lipids/s urfactants, xanthan, acetoglycerides, waxes, surfactants, dextran, emulsan, gelian, polyphenols, levan, lignin, curd, ian, tannin, polygalactosamine, humic acid, shellac, pullulan , poly-gamma-glutamic acid, elsinan, natural rubber, yeast glucans, and synthetic polymers from natural fats and oils.

[0067] In one aspect, the method of preparing the electrospun fiber(s) according to some embodiments of the present invention may further comprise crosslinking the electrospun fiber(s). In some embodiments, the crosslinking may be performed by any conventional chemical crosslinking method (*e.g.* chemical reagent-promoted, chemically reactive linker-promoted and/or enzyme-promoted) and/or dehydrothermal crosslinking method (*e.g.* heat-promoted condensation), forming the covalently crosslinked electrospun fiber(s). In additional embodiments, the crosslinking comprises applying a cross-linking agent to the polymer or oligomer solutions to be electrospun. For example, the cross-linking agent may be selected from the group consisting of 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), EDC/hyaluronic acid, genipin, and glutaraldehyde.

[0068] In some embodiments, a method of preparing an electrospun fiber further comprises adding a bioactive factor to the solution. A "bioactive factor" refers to protein, carbohydrate, or mineral that has any effect on a cellular activity. Examples of bioactive factors include, but are not limited to, an osteogenic growth factor, collagen, glycosaminoglycans, osteonectin, bone sialo protein, an osteoinductive factor, a chondrogenic factor, a cytokine, a mitogenic factor, a chemotactic factor, a transforming growth factor (TGF), a fibroblast growth factor (FGF), an angiogenic factor, an insulin-like growth factor (IGF), a platelet-derived growth factor (PDGF), an epidermal growth factor (EGF), a vascular endothelial growth factor (VEGF), a nerve growth factor (NGF), a neurotrophin, a bone morphogenetic protein (BMP), osteogenin, osteopontin, osteocalcin, cementum attachment protein, erythropoietin, thrombopoietin, tumor necrosis factor (TNF), an interferon, a colony stimulating factor (CSF), stem cell derived factor-1 (SDF-1), or an interleukin, among others. The bioactive factor may be a BMP, PDGF, FGF, VEGF, TGF, insulin, among others. Examples of BMPs include but are not limited to BMP-2, BMP-3, BMP-4,

BMP-5, BMP-6, BMP-7, BMP-8, BMP-9, BMP-10, BMP-11, BMP-12, BMP-13, BMP-14, BMP-15, truncated BMPs described in PCT/US2012/053584, which is incorporated by reference in its entirety herein, and a mixture thereof.

[0069] The invention further relates to a scaffold comprising an aligned fiber. The invention further relates to a scaffold comprising one or more electrospun fibers. In some embodiments, a fast Fourier transform (FFT) analysis result of the fibers have adjacent major peaks with about 180° apart from each other. The "major peaks" herein refer to peaks higher than the average of the peaks in the result.

[0070] In one aspect, the scaffold may essentially consist of the aligned fiber and/or electrospun fiber. In another aspect, the scaffold may consist of the aligned fiber and/or electrospun fiber. The scaffold described herein may be biocompatible. A biocompatible matrix scaffold may be non-toxic, non-eliciting or stimulating severe inflammatory response or immunological rejections, and/or devoid of other undesired reactions at the implantation site.

[0071] Alignment of the fibers in the scaffold may be measured by a fast Fourier transform (FFT) analysis. For example, the FFT analysis may be performed by the methods described in *Measuring fiber alignment in electrospun scaffolds: a user's guide to the 2D fast Fourier transform approach*, Ayres CE, Jha BS, Meredith H, Bowman JR, Bowlin GL, Henderson SC, Simpson DG. J Biomater Sci Polym Ed. 2008; 19(5):603-21, which is incorporated by reference in its entirety herein. In some embodiments, FFT result of the fibers described herein may have adjacent major peaks with about 180° apart from each other as shown in ~~**figure 8**~~**figure 8A, and figure 8B** depicts an FFT analysis of a control sample.

[0072] The aligned fiber, electrospun fiber, and/or scaffold of the present invention may comprise type I collagen. In some embodiments, total type I collagen may be present in the aligned fiber, electrospun fiber, and/or scaffold of the present invention in an amount from about 50% to about 95%, from about 60% to about 95%, from about 70% to about 95%, or from about 80% to about 95% by weight in it protein content. In additional embodiments, total type II

collagen may be present in the aligned fiber, electrospun fiber, and/or scaffold of the present invention in an amount about 10% or less, about 5% or less, about 3% or less, or about 1% or less by weight in it protein content. In further embodiments, collagen type II may be absent from aligned fiber, electrospun fiber, and/or scaffold of the present invention. The types of collagen that are present or absent in the composition can be easily assessed using routine methods in the art. Methods of identifying and quantifying type of collagen are well known in the art, as disclosed, for example in Schnaper, H.W. and Kleinman, H.K., Pediatr. Neprol., 7:96-104 (1993), which is incorporated by reference.

[0073] In one aspect, the aligned fiber, electrospun fiber, and/or scaffold described herein may have less than about 8 wt%, 7 wt%, 6 wt%, 5 wt%, 4 wt%, 3 wt%, 2 wt%, or 1 wt% residual calcium. In another aspect, the average residual calcium amount in the aligned fiber, electrospun fiber, and/or scaffold described herein may be less than about 8 wt%, 7 wt%, 6 wt%, 5 wt%, 4 wt%, 3 wt%, 2 wt%, or 1 wt% .

[0074] In one aspect, the aligned fiber and/or electrospun fiber described herein may have a length from about 0.1 cm to about 10 cm, from about 1 cm to about 10 cm, from about 0.1 cm to about 20 cm, from about 1 cm to about 20 cm, from about 10 cm to about 20 cm, from about 0.1 cm to about 30 cm, from about 1 cm to about 30 cm, from about 10 cm to about 30 cm, from about 20 cm to about 30 cm, from about 0.1 cm to about 40 cm, from about 1 cm to about 40 cm, from about 10 cm to about 40 cm, from about 20 cm to about 40 cm, from about 30 cm to about 40 cm, from about 0.1 cm to about 50 cm, from about 1 cm to about 50 cm, from about 10 cm to about 50 cm, from about 20 cm to about 50 cm, from about 30 cm to about 50 cm, from about 40 cm to about 50 cm, 0.1 cm to about 60 cm, from about 1 cm to about 60 cm, from about 10 cm to about 60 cm, from about 20 cm to about 60 cm, from about 30 cm to about 60 cm, or from about 40 cm to about 60 cm. In another aspect, the total the aligned fiber and/or electrospun fiber in the scaffold described herein may have an average length from about 0.1 cm to about 10 cm, from about 1 cm to about 10 cm, from about 0.1 cm to about 20 cm, from about 1 cm to about 20 cm, from about 10 cm to about 20 cm, from about 0.1 cm to about 30 cm, from about 1 cm to

about 30 cm, from about 10 cm to about 30 cm, from about 20 cm to about 30 cm, from about 0.1 cm to about 40 cm, from about 1 cm to about 40 cm, from about 10 cm to about 40 cm, from about 20 cm to about 40 cm, from about 30 cm to about 40 cm, from about 0.1 cm to about 50 cm, from about 1 cm to about 50 cm, from about 10 cm to about 50 cm, from about 20 cm to about 50 cm, from about 30 cm to about 50 cm, from about 40 cm to about 50 cm, 0.1 cm to about 60 cm, from about 1 cm to about 60 cm, from about 10 cm to about 60 cm, from about 20 cm to about 60 cm, from about 30 cm to about 60 cm, or from about 40 cm to about 60 cm.

[0075] In one aspect, the scaffold has an average porosity from about 60% to about 70%, from about 60% to about 80%, from about 60% to about 90%, from about 60% to about 95%, from about 70% to about 80%, from about 70% to about 90%, from about 70% to about 95%, from about 80% to about 90%, or from about 80% to about 95%.

[0076] In some embodiments, the aligned fiber, electrospun fiber, and/or scaffold comprises collagen type I, bone matrix, adipose extracellular matrix, heart basement membrane extract, heart basement membrane extracellular matrix, placenta basement membrane extract, placenta basement membrane extracellular matrix, brain-derived extracellular matrix, polycaprolactone, a biodegradable polymer, an accessory polymer described herein, or the mixture thereof.

[0077] In one aspect, the aligned fiber, electrospun fiber, and/or scaffold may comprise a bone matrix fiber comprising bone. In some embodiments, the bone matrix fiber may be prepared by the methods described in PCT/US2013/025226, which is incorporated herein in its entirety herein.

[0078] In another aspect, the aligned fiber, electrospun fiber, and/or scaffold may comprise heart basement membrane extract and/or heart basement membrane extracellular matrix. In some embodiments, the heart basement membrane extract and/or heart basement membrane extracellular matrix may be prepared by the methods described in PCT/US2011/49192, which is incorporated herein in its entirety herein. In an additional aspect, the placenta basement membrane extract and/or heart basement membrane extracellular matrix may be prepared by the

same method to prepare the heart basement membrane extract and/or heart basement membrane extracellular matrix.

[0079] In another aspect, the aligned fiber, electrospun fiber, and/or scaffold may comprise a biodegradable polymer. The biodegradable, biocompatible polymers may include, but is not limited to, ethylene vinyl acetate, polyanhydricles, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. The biodegradable, biocompatible polymers may further include a number of synthetic biodegradable polymers described herein.

[0080] In some embodiments, the aligned fiber and/or electrospun fiber in the scaffold is crosslinked. In additional embodiments, the aligned fiber, electrospun fiber, and/or scaffold comprises a bioactive factor described herein.

[0081] In additional embodiments, the aligned fiber, electrospun fiber, and/or scaffold may comprise an extracellular matrix component. For example, the extracellular matrix component may include, but is not limited to, collagen, glycosaminoglycans, osteocalcin, osteonectin, bone sialo protein, osteopontin, fibronectin, laminin, vitronectin, elastin or mixtures thereof.

[0082] In some embodiments, the scaffold of the present invention may further comprise a support. In additional embodiments, the fibers described herein may be attached to and/or cover the support.

[0083] In some embodiments, the scaffold of the present invention may be in a form of an elongated sheet or a composite of multiple layers of sheets similarly to the biocompatible matrix described herein. In additional embodiments, the scaffold may be prepared as shown in **figures 9A, 9B, and 9C**. In one aspect, the materials for the elongated roll and the elongated sheet may be different. In another aspect, the materials for each elongated sheet in the multiple layers of sheets may be different from each other. In another aspect, the electrospun fibers in the scaffold are aligned in one direction. For example, the electrospun fibers in the scaffold are aligned in the direction parallel or perpendicular to the length of the elongated sheet or the multiple layers. In

another aspect, the scaffold is prepared by folding or rolling the sheet or sheets, along with the direction of nano fiber alignment to form an elongated roll implant.

[0084] In some embodiments, the elongated roll implant may be prepared by combining a sheet or sheets to facilitate surgical implantation. The biomaterials to prepare the sheet and elongated roll may be the same, or different, as described previously.

[0085] In some embodiments, the elongated sheet scaffold described herein may have an average thickness of about 1000 μm or less, 900 μm or less, 800 μm or less, 700 μm or less, 600 μm or less, 500 μm or less, 400 μm or less, 300 μm or less, 200 μm or less, 100 μm or less, 50 μm or less, 20 μm or less, or 10 μm or less. In one aspect, the elongated sheet scaffold described herein may have an average thickness of about 1000 μm or more, 900 μm or more, 800 μm or more, 700 μm or more, 600 μm or more, 500 μm or more, 400 μm or more, 300 μm or more, 200 μm or more, 100 μm or more, 50 μm or more, 20 μm or more, 10 μm or more, or 1 μm or more.

[0086] In some embodiments, the length of the elongated sheet or roll implant scaffold is about 0.1 cm or more, about 0.3 cm or more, about 0.5 cm or more, about 0.7 cm or more, about 1 cm or more, about 2 cm or more, about 3 cm or more, about 5 cm or more, about 8 cm or more, about 10 cm or more, about 13 cm or more, about 15 cm or more, about 18 cm or more, about 20 cm or more, about 23 cm or more, about 25 cm or more, about 27 cm or more, or about 30 cm or more. In one aspect, the length of the elongated sheet or roll implant scaffold is about 1 cm or less, about 2 cm or less, about 3 cm or less, about 5 cm or less, about 8 cm or less, about 10 cm or less, about 13 cm or less, about 15 cm or less, about 18 cm or less, about 20 cm or less, about 23 cm or less, about 25 cm or less, about 27 cm or less, about 30 cm or less, about 40 cm or less, about 50 cm or less, or about 60 cm or less.

[0087] The scaffold according to some embodiments of the present invention may be used as an implant. An "implant" refers to any object that is designed to be placed partially or wholly within a patient's body for one or more therapeutic or prophylactic purposes such as for tissue augmentation, contouring, restoring physiological function, repairing or restoring tissues damaged by disease or trauma, and/or delivering therapeutic agents to normal, damaged or

diseased organs and tissues. A "bone matrix implant" refers to a medical device or implant that includes a volume replacement material for augmentation or reconstruction to replace a whole or part of a bone structure. A predetermined shape of the implant may be varied to fit the implant site.

[0088] The invention further relates to methods culturing cells on the scaffold or aligned fiber described herein. In some embodiments, the cells are selected from the group consisting of stem cells, adipose derived stem cells, dental pulp stem cells, fibroblasts, and dorsal root ganglia.

[0089] For example, the invention provides for growing and/or culturing cells on a scaffold produced by the method described herein *in vivo* or *in vitro*. "Growing and/or culturing cells on a scaffold" includes traditional cell culture methods as well as placing on a surface in any setting, such as in natural or synthetic biocompatible matrices or tissues. In some embodiments, the cells that are cultured on the scaffold are stem cells. In further embodiments, the scaffold can be used in *in vitro* methods for supporting cell growth and proliferation as well as for increasing osteogenesis, chondrogenesis, or ligament/tendon genesis in the stem cells cultured on the scaffold.

[0090] Any cell described herewith may be cultured on a scaffold produced by the method described herein for between about 15 minutes and about 4 weeks, about 2 hours and about 2 weeks, about 2 hours and about 1 week, about 2 hours and about 72 hours, about 24 hours and about 72 hours, or about 24 hours and about 96 hours, at between about 20 ºC and about 40 ºC or about 30°C and about 37°C, in an atmosphere containing between about 1% CO_2 and about 10% CO_2 or about 4% CO_2 and about 6% CO_2, in certain embodiments.

[0091] The invention also relates to methods of promoting nerve repair using the aligned fiber, electrospun fiber, and/or scaffold described herein. In some embodiments, the nerve repair includes, but is not limited to, central and peripheral nerve repair. The methods of promoting nerve repair may comprise implanting the aligned fiber, electrospun fiber, and/or scaffold into a nerve damaged site of a subject in need thereof. The method may further comprise culturing

dorsal root ganglia and/or stem cells on the aligned fiber, electrospun fiber, and/or scaffold prior to the implanting. The stem cells, prior to culture on the scaffold of the present invention, may be undifferentiated or partially differentiated cells. The nerve regenerating activity of the aligned fiber, electrospun fiber, and/or scaffold may or may not be altered, including but not limited to, enhanced activity, relative to other scaffolds without the aligned and/or electrospun fiber described herein. The scaffolds for promoting nerve repair according to some embodiments of the present invention are in a form of an elongated sheet or a composite of multiple layers of sheets as discussed herein.

[0092] The invention also relates to methods of promoting osteoinductivity, with the methods comprising culturing cells on an aligned fiber, electrospun fiber, and/or scaffold produced by the method described herein. The cells, prior to culture on the aligned fiber, electrospun fiber, and/or scaffold of the present invention, may be undifferentiated or partially differentiated cells. The osteoinductive activity of the aligned fiber, electrospun fiber, and/or scaffold may or may not be altered, including but not limited to, enhanced activity, relative to other scaffolds without the aligned and/or electrospun fiber described herein.

[0093] The invention also relates to methods of promoting chondroinductivity, with the methods comprising culturing cells on a aligned fiber, electrospun fiber, and/or scaffold produced by the method described herein. The cells, prior to culture on aligned fiber, electrospun fiber, and/or scaffold of the present invention, may be undifferentiated or partially differentiated cells. The chondroinductive activity of the aligned fiber, electrospun fiber, and/or scaffold may or may not be altered, including but not limited to, enhanced activity, relative to other scaffolds without the aligned and/or electrospun fiber described herein.

[0094] The invention also relates to methods of promoting ligament/tendon differentiation, with the methods comprising culturing cells on an aligned fiber, electrospun fiber, and/or scaffold produced by the method described herein. The cells, prior to culture on the aligned fiber, electrospun fiber, and/or scaffold of the present invention, may be undifferentiated or partially

differentiated cells. The ligament/tendon differentiation activity of the aligned fiber, electrospun fiber, and/or scaffold may or may not be altered, including but not limited to, enhanced activity, relative to other scaffolds without the aligned and/or electrospun fiber described herein.

[0095] In one aspect, *in vitro* alkaline phosphatase assays may be used to evaluate osteoinductivity in cells cultured on the scaffold prepared by the methods described herein. The ability of the aligned fiber, electrospun fiber, and/or scaffold prepared by the methods of the present invention to stimulate or induce the alkaline phosphatase expression in an otherwise non-bone forming cells, such as myoblast (C2C12 cells), would indicate that the aligned fiber, electrospun fiber, and/or scaffold of the present invention has osteoinductive activity. In these assays, cells cultured on other scaffolds without the aligned and/or electrospun fiber described herein are used as negative controls to show that the baseline alkaline phosphatase expression on non-bone forming cells. The baseline of the osteoblastic markers in the negative control need not be zero, meaning that the cells in the negative control group may have at least some level of phenotypic marker(s). Accordingly, an "osteoinductive" scaffold of the present invention would simply cause an increase in the osteoblastic markers in experimental cells over control grown on the other scaffolds without the aligned and/or electrospun fiber.

[0096] Moreover, osteoinductivity, chondroinductivity, and ligament/tendon differentiation may be determined in tissue culture by investigating the ability of the aligned fiber, electrospun fiber, and/or scaffold prepared by the methods of the present invention to differentiate or induce osteoblast phenotype, chondrocyte phenotype, ligament/tendon cell phenotype in cultured cells, such as primary cells, cell lines, or explants. For example, the cells may display increased production of a marker characteristic of osteoblasts and/or chondrocytes, such as alkaline phosphatase, etc. For example, the osteoinductive, chondroinductive, ligament/tendon differentiation potentials of the aligned fiber, electrospun fiber, and/or scaffold described herein may be more than 0.2, 0.4, 0.6, 0.8, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 times greater than the fiber and/or scaffolds without the aligned and/or electrospun fiber. In another example, the osteoinductive, chondroinductive, ligament/tendon differentiation potentials of the culture on the

scaffold and/or implant described herein may be more than 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 500 or even 1000 times greater than those of the aligned fiber, electrospun fiber, scaffold and/or implant without any aligned and/or electrospun fiber. Of course, this indicates that lower concentrations of modified growth factor, compared to unmodified growth factor are required to achieve the same effects.

[0097] Osteoinductivity, chondroinductivity, ligament/tendon differentiation, for assessing the bone, cartilage, ligament or tendon forming potential induced by the scaffold of the present invention in a location such as muscle, may also be evaluated using a suitable animal model.

[0098] The invention also relates to methods of promoting cell attachment, proliferation or maintaining the differentiated state or preventing de-differentiation of osteoblasts, chondrocytes, ligament cells, tendon cells and/or any cell type disclosed herein with the methods comprising culturing the cells on a scaffold produced by the method described herein. The proliferative activity of the aligned fiber, electrospun fiber, and/or scaffold may or may not be altered, including but not limited to, enhanced activity, relative to a fiber and/or scaffold without any aligned and/or electrospun fiber.

[0099] Mitogenicity may be assessed by investigating cell proliferation induced by the aligned fiber, electrospun fiber, and/or scaffold prepared by the methods of the present invention using various in vitro assays that measure metabolic activity, such as MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay, alamarBlue® assay, and others. The alamarBlue® assay uses a non-cytotoxic reduction-oxidation indicator to measure cell metabolic activity, making it a nondestructive assay for assessing the mitogenic activity of the aligned fiber, electrospun fiber, and/or scaffold described herein.

[0100] The invention also relates to methods of increasing or promoting osteogenesis, chondrogenesis, or ligament/tendon genesis in cells. The methods may comprise culturing the cells on an aligned fiber, electrospun fiber, and/or scaffold produced by the method described herein. The osteogenic, chondrogenic, ligament, or tendon inducing activity of the aligned fiber,

electrospun fiber, and/or scaffold may or may not be altered, including but not limited to, enhanced activity, relative to a fiber and/or scaffold without aligned and/or electrospun fiber described herein.

[0101] The invention also relates to methods of treating a tissue or organ defect or injury, for example, a musculoskeletal, dental or soft-tissue defect or injury, in an animal comprising administering (1) cells cultured on the scaffold produced by the methods described herein and/or (2) the aligned fiber, electrospun fiber, scaffold and/or implant described herein to the tissue or organ defect (e.g. osseous defects, defects in cartilage, ligament, tendon, spinal disk, and tendon insertion site to bone).

[0102] The invention further relates to methods of treating a tissue or an organ defect or injury, for example, including, but not limited to a musculoskeletal, dental or soft-tissue defect, or a nerve defect in an animal by applying a aligned fiber, electrospun fiber, and/or scaffold prepared by the methods described herein to the defect, and application to the defect may be accomplished by injecting the scaffold into the defect, inserting the aligned fiber, electrospun fiber, and/or scaffold between tissue or organ, or placing the aligned fiber, electrospun fiber, and/or scaffold on top of the defect. The present invention is also directed to treating a defect or injury in an organ by applying an aligned fiber, electrospun fiber, and/or scaffold to the defect. In one embodiment, the tissue defect may be treated by applying an elongated sheet described herein to surround the defect, or by applying an elongated roll implant described herein between the tissue defect or gap. The tissue defect or gap may be from the tissues including, but not limited to, bone, tendon, ligament, blood vessel, skin, intestine, nerve, and cartilage. Optionally, the elongated sheet may be applied around the tissue defect, and/or the elongated roll implant may be applied to facilitate surgery and healing. For example, the elongated implant roll may be applied between the tissue defect or gap, then the ends of the elongated implant roll may be sutured to the tissue. In some embodiments, the elongated sheet may be applied or sutured around the tissue defect or gap, covering the elongate implant roll and/or the suture sites of the elongated implant roll with tissues. In additional embodiments, the elongated roll may facilitate

the tissue growth through the defect gap. The elongated sheet can prevent adhesion of the elongated implant roll with surrounding tissue or prevent undesirable tissue ingrowth, for example, scar formation, to interfere the tissue regeneration or remodeling. The biomaterials used for preparing the enlongated sheet and the enlongated implant roll may be same or different, depending on the specific surgery and tissue regeneration needs. The tissue defect may occur in tendon, ligament, nerve and other tissue types enlisted previously.

[0103] In yet another embodiment, cells may be seeded onto an aligned fiber, electrospun fiber, and/or scaffold prepared by the methods provided herein. The cells seeded on the scaffold can be any cell, such as but not limited to, dorsal root ganglia, osteoblasts, chondrocytes, ligament cells, tendon cells, progenitor cells, and stem cells disclosed herein or otherwise known in the art. The seeded cells may be allowed to proliferate and possibly attach to the matrix. Methods of seeding cells onto matrices, such as collagen matrix coated with the aligned fiber, electrospun fiber, and/or scaffold prepared by the methods provided herein, are well known in the art.

[0104] While the invention has been described and illustrated herein by references to various specific materials, procedures and examples, it is understood that the invention is not restricted to the particular combinations of material and procedures selected for that purpose. Numerous variations of such details can be implied as will be appreciated by those skilled in the art. It is intended that the specification and examples be considered as exemplary, only, with the true scope and spirit of the invention being indicated by the following claims. All references, patents and patent applications referred to in this application are herein incorporated by reference in their entirety herein.

[0105] The following examples are illustrative and are not intended to limit the scope of the invention described herein.

[0106] Example 1:

[0107] 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) (CAS# 920-66-1) was from Acros Organics (Geel, Belgium, acros.com). Polycaprolactone (PCL) (B6003-1, B6003-2) was from Durect Lactone (Pelham, AL, absorbables.com). The electrospinning apparatus was designed using an Aladdin Programmable Syringe Pump from World Precision Instruments, Inc. (Sarasota, FL, wpiinc.com) and two adjustable high voltage power supplies from Gamma High Voltage Research (Ormond Beach, FL). The aligned metal rods used in the rotating motor, or statically, may be of steel, stainless steel, copper, or other heavy metal. A Mercotac® connector (#110T and 110TS) was used as an electrically conductive bearing. The following items were purchased from McMaster-Carr:

8965K42	Ultra Conductive Copper (Alloy 101) Rod, 1/4" Diameter
1256T14	Multipurpose Stainless Steel (Type 304) Mirror Finish #8, 1/4" Diameter
88855K51	High-Strength Stainless Steel (Type 17-4 Ph) 1/4" Diameter
86985K31	High-Strength Aluminum (Alloy 2024) 1/4" Diameter
8965K86	Ultra Conductive Copper (Alloy 101) Tube, 1/4" OD, .186" ID, .032" Wall Thk
8457K536	Stainless Steel Shape Type 304/304L, Round Tube, 1/4" OD
1968T11	High-Strength Aluminum Tube (Alloy 2024) 1/4" OD, .180" ID, .035" Wall Thickness
2706A4	Tube Cutter 1/4" to 1-5/8" Tube OD, 6" Open/Closed Length
4575N3	Miniature Flange-Mounted SS Ball Bearing Shielded, for 1/4" Shaft Diameter
6384K352	Steel Ball Bearing Flanged Double Sealed for 1/4" Shaft Dia, 11/16" OD
8600N3	Miniature Alum Base-Mnt SS Ball Brng--ABEC-3 for 1/4" Shaft Diameter
7200K3	NEMA 34 Face-Mount Brushless DC Motor 1/3 hp, 3450 rpm, Integrated Speed Control
6099K41	Stainless Steel One-Piece Set-Screw Coupling 1/2" Bore, 1-1/2" Length, 1" OD, with Keyway

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8774K33	Static-Dissipative Clear Cast Acrylic Sheet 1/4" Thick, 12" X 24"
97042A516	18-8 Stainless Steel One-End Threaded Stud 1/2"-13 x 5/8" X 1-1/2"
99223A067	Acetal Hex Nut 1/2"-13 Thread
94564A023	Nylon Flat Point Socket Set Screw 6-32 X 1/4"
9986K21	Black Delrin Rod 4" Diameter, 1/2" Length
8576K15	Black Delrin Rod 1/2" Diameter, 5' Length
94922A050	Nylon 6/6 Acorn Nut Off-White, 1/2"-13 Thread Size, 3/4" W, 13/16" H
8572K61	White Delrin Rod 1" Diameter, 1' length
8582K21	White Delrin Rod 4" Diameter, 1/2" Length
8572K55	White Delrin Rod 1/2" Diameter
93140A839	Polycarbonate Machine Screw Flat Head Slotted, 1/4"-20 X 3/4"
95868A148	Nylon 6/6 SHCS 6-32 Thread, 1/2" Length, Off-White
7643A421	1" x 30', Gray, .012" Thk, High-Temp Self-Fusing Silicone Rubber Tape
7586K12	Adhesive-Backed Cable Holder Press in, 1/4" Maximum Bundle
60015K42	Tear-Resistant Rubber Vibration Damping Pad 6" X 6" X 3/8" Thick, 45 PSI Max Load
4056K42	Oil-Resistant Nitrile Vibration Damping Pad 6" X 6" X 5/16" Thick, 100 PSI Max Load
94605A541	Nylon 82 Deg Flat Head Slotted Machine Screw 1/4"-20 X 3/4"
94564A080	Nylon Flat Point Socket Set Screw 1/4"-20 X 1/2"
5537K26	Tinned Copper Expandable Mesh Sleeving 1/4" ID, 3/16" to 5/16" Bundle Dia
8491A614	Steel Press-Fit Drill Bushing/Liner 0.257" (F) ID, 13/32" OD, 1/2" Length
3504T21	Push-on Round FDA Cap Fits 3/8" OD, 1/2" Inside Height

92805K22 Push-on High-Temperature Silicone Rubber Cap Fits .6" Outside Diameter, 1-1/2" Inside Height

[0108] A high voltage power supply source was used to apply a +10-40kV DC voltage to parallel metal rods, with a -5-40kV DC field applied to the syringe/needle containing a polymer solution, with syringe placed in a syringe pump. The metal posts can be hollow or solid, and have been tested as rods of stainless steel, steel, copper, and aluminum, both solid and hollow using thin-walled tubing. The absolute DC field strength ranged from 15kV to 60kV. A programmable syringe pump was set to dispense the solution at 0.50-9.50 mL/hr, ideally at 6.5ml/hr for pure PCL, and 3ml/hr for heart basement membrane (HBM) and HBM/PCL mixtures. After approximately 5 minutes to 1 hour of electrospinning, the aligned fibers are collected for imaging (SEM and DIC).

[0109] Metal rods were used to align electrospun PCL. PCL dissolved in HFP (100mg/ml) at a +15kV reference, set 15cm apart, was electrospun between the rods, with PCL pumped at 5ml/hr over 5 minutes to the form the aligned nanofibers seen collecting between the rods. **Figure 1 (a, d)** shows the representative electric field lines and the know bending of these lines in a field with a split point charge (as simplified plane of point charges represented). This splitting of the electric charge field is believed to act on imparting alignment as they travel on the looping fibers in this warped electrostatic field. We have successfully aligned fibers with hollow and solid metal rods, and the modulation of the electric field lines by solid vs. hollow rods is also shown **(figure 1b and 1c)** (electric field diagrams created using the applet from cco.caltech.edu/~phys1/java/phys1/EField/EField.html).

[0110] For SEM images, aligned electrospun PCL and HBM/PCL blended samples were taken from the aligned rods and mounted on aluminum stubs using carbon stickers. The samples were then coated with gold at about 50nm thickness using a plasma-based sputter coater. Coated samples were imaged using a JOEL 6400 scanning electron microscope (SEM) with Orion image processing. Representative images of charged parallel rod aligned fibers, as compared to fibers generated in the traditional, randomly aligned fashion by collection onto a static piece of

grounded foil, can be seen in ~~figure 2~~**Figures 2A, 2B, 2C, and 2D**. The average cross-sectional fiber diameter of the electrospun fibers generated from random and charged rod aligned basement membrane matrix was calculated by measuring 35 unique points in ImageJ64 (NIH shareware), with results shown in ~~figure 2~~**Figure 2E**, along with the average angle of the fibers. A fast Fourier transform (FFT) was performed to indicate the degree of alignment as seen in ~~figure 3~~**Figures 3A, 3B, 3C, and 3D** using the ImageJ64 FFT tool, with patterns of aligned fibers shown to match with those of know aligned Fourier spectra, as commonly used for pattern recognition.

[0111] ~~**Figures 4** illustrates~~**Figures 4A, 4B, and 4C illustrate** a design for rotating the charged parallel rods using a mercury coupled bearing to allow electrical conductance with the rotating charged rods mounted in plastic platforms attaching to a high speed motor (0-4000RPMs). The actual implementation of this rotating rod design is shown in **figure 5**. In another aspect, this rotating charged parallel point charged rod fiber alignment process may be modified so that electrospun fibers of opposite charge are propelled towards each other in the static field, whereby the fibers combine in a "whirlpool" fashion roughly equidistant apart in space. In this arrangement, the fiber charges are effectively neutralized and the fibers are then collected on the grounded rotating rods of the mandrel, which allows a thicker layer of fibers to be collected due to less fiber-to-fiber charge repulsion. Equally charged fibers are believed to have a repulsive effect on each other in the collected fibers. Charge neutralization of the fibers in this fashion theoretically negates this phenomenon to electrospin sheets that are up to about 1.5 times, 2.0 times, 3.0 times, or 3.4 times the thickness of conventionally electrospun fiber sheets. For example, two syringe pumps with basement membrane, each attached to a high voltage power supply may have one supply set to positive (+) voltage, and the other set to negative (-) voltage to generate the static electric field for electrospinning, with fibers collected on the rotating or static charged rods. The charges of equal voltage and opposite charge combine in space above the dual charged rod mandrel in a the vortex of the electric field, depositing as charge neutralized fibers on the rods, which allows for enhanced thickness of the collected aligned fibers over conventional methods.

[0112] By implementing a pneumatic motor in the conformations for electrospinning aligned (and random) nanofibers onto a mandrel as described above, on average 9.24% more material is collected on the mandrel(s) compared to using an electrical motor.

[0113] Example 2: Heart Base Membrane Fiber Scaffold

[0114] Heart basement membrane (HBM) and polycaprolactone (PCL) were dissolved separately in HFP at 100mg/ml, and mixed in a 10% ratio of HBM:PCL (0.100g HBM to 0.900g PCL), mixed at 4deg a total of 2hrs until fully into solution. The HBM:PCL was loaded into a glass 5ml syringe, loaded into a syringe pump set to expel at 1.5ml/ml, in a static +20kV electric field at the needle and -10kV to each of the parallel aligned collecting rods, then electrospun between the poles in a highly aligned fashion. The aligned fibers of HBM/PCL were collected and stored in a dessicator prior to use. To obtain dorsal root ganglion (DRGs), an 11 day old YFP-βIII Tubulin expressing transgenic mouse was sacrificed for collection of the spinal cord and removal of the DRGs, which were liberated from their capsule and briefly digested in trypsin, then placed upon the aligned HBM:PCL scaffold and cultured in basic neuron media containing B27 supplement. As shown in ~~figure 6~~**figures 6A and 6B**, outgrowths from the DRGs were visualized under light and fluorescent microscopy at 1 and 2 days post explantation, with aligned neurons seen erupting from the bundle, with the neurite outgrowths seen tracing the aligned fibers.

[0115] Example 3: Dorsal Root Ganglia Attachment and Outgrowth on Placenta and Heart Basement Membrane Nanofibers.

[0116] Heart basement membrane HBM and polycaprolactone (PCL) were dissolved separately in HFP at 100mg/ml, and mixed in a 10% ratio of HBM:PCL (0.100g HBM to 0.900g PCL), mixed at 4deg a total of 2hrs until fully into solution. Each biopolymer was individually loaded into a glass 5ml syringe, loaded into a syringe pump set to expel at 2.5ml/hr, in a static +20kV electric field at the needle, then electrospun onto 10mm glass coverslips backed by a grounded piece of foil. The randomly aligned fibers were collected and stored in a dessicator prior to use.

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To obtain dorsal root ganglion (DRGs), a 10 day old YFP-βIII Tubulin expressing transgenic mouse was sacrificed for collection of the spinal cord and removal of the DRGs, which were liberated from their capsule and briefly digested in trypsin, then placed upon the scaffolds and cultured in basic neuron media containing B27 supplement for 7 days. As shown in ~~**figure 6**~~**figures 6A and 6B**, Outgrowths from the DRGs were again visualized under light and fluorescent microscopy at 1-7 days post explantation. Neurite outgrowth was seen tracing the electrospun fibers on HBM/PCL coated coverslips.

[0117] Example 4: Dorsal Root Ganglia Attachment and Outgrowth on Polycaprolactone Nanofibers (Comparative Example)

[0118] Polycaprolactone (PCL) was dissolved in HFP at 100mg/ml and mixed by vortexing at room temperature for 2hrs until fully into solution. The PCL biopolymer was loaded into a glass 5ml syringe, loaded into a syringe pump set to expel at 5ml/h, in a static +20kV electric field at the needle, then electrospun onto 10mm glass coverslips backed by a grounded piece of foil. The randomly aligned fibers of each group were collected and stored in a dessicator prior to use. To obtain dorsal root ganglion (DRGs), an 10 day old YFP-βIII Tubulin expressing transgenic mouse was sacrificed for collection of the spinal cord and removal of the DRGs, which were liberated from their capsule and briefly digested in trypsin, then placed upon the scaffolds and cultured in basic neuron media containing B27 supplement for 7 days (n=3). Outgrowths from the DRGs were visualized under light and fluorescent microscopy at 1-7 days post explantation. DRGs did not grow out on PCL and were found to be non-viable after 2-3 days in repeat trials (n=6).

Electronic Patent Application Fee Transmittal

Application Number:	16045159
Filing Date:	25-Jul-2018
Title of Invention:	ALIGNED FIBER AND METHOD OF USE THEREOF
First Named Inventor/Applicant Name:	Michael Francis
Filer:	Valerie P. Hayes/Elizabeth Raufi
Attorney Docket Number:	LNH-152US1

Filed as Small Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension - 4 months with $300 paid	2254	1	800	800
Miscellaneous:				
			Total in USD ($)	**800**

Electronic Acknowledgement Receipt

EFS ID:	35262397
Application Number:	16045159
International Application Number:	
Confirmation Number:	5408
Title of Invention:	ALIGNED FIBER AND METHOD OF USE THEREOF
First Named Inventor/Applicant Name:	Michael Francis
Customer Number:	23122
Filer:	Valerie P. Hayes/Elizabeth Raufi
Filer Authorized By:	Valerie P. Hayes
Attorney Docket Number:	LNH-152US1
Receipt Date:	27-FEB-2019
Filing Date:	25-JUL-2018
Time Stamp:	12:22:38
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$800
RAM confirmation Number	022719INTEFSW12232100
Deposit Account	180350
Authorized User	Elizabeth Raufi

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

37 CFR 1.16 (National application filing, search, and examination fees)

37 CFR 1.17 (Patent application and reexamination processing fees)

37 CFR 1.19 (Document supply fees)

37 CFR 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Applicant Response to Pre-Exam Formalities Notice	Response_Incomplete_Reply.pdf	83300 3ebf1e6c2e0a0abd8c8cdacb03cdb71c6ec428df	no	2
Warnings:					
Information:					
2	Drawings-only black and white line drawings	Replacement_Drawings.pdf	753294 9c1e4f1106b0b106b32593d0880aad541d3649a4	no	10
Warnings:					
Information:					
3	Specification	Clean_Copy_Specifcation.pdf	234864 e72003259f7b2b31a95a99153ff260bf542e91ef	no	37
Warnings:					
Information:					
4	Specification	Markedup_Copy_Specification.pdf	237095 fff23cdb377243ff6594200f37e80354a6630b06	no	37
Warnings:					
Information:					
5	Fee Worksheet (SB06)	fee-info.pdf	31036 5d801223fc6a13c68dbbd5b37b7518b50e76bc4a	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	1339589		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
16/045,159	07/25/2018		1015	LNH-152US1	11	4

CONFIRMATION NO. 5408

UPDATED FILING RECEIPT

23122
RATNERPRESTIA
2200 Renaissance Blvd
Suite 350
King of Prussia, PA 19406

OC000000106254158

Date Mailed: 03/01/2019

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Michael Francis, Virginia Beach, VA;
Roy Ogle, Virginia Beach, VA;

Applicant(s)

LifeNet Health, Virginia Beach, VA;

Power of Attorney: The patent practitioners associated with Customer Number 23122

Domestic Priority data as claimed by applicant

This application is a DIV of 14/776,106 09/14/2015 PAT 10137223
which is a 371 of PCT/US2014/025636 03/13/2014
which claims benefit of 61/785,031 03/14/2013

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 08/27/2018

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 16/045,159**

Projected Publication Date: 06/06/2019

Non-Publication Request: No

Early Publication Request: No
**** SMALL ENTITY ****
Title

ALIGNED FIBER AND METHOD OF USE THEREOF

Preliminary Class

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

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Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

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NOT GRANTED

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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 16/045,159

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	75		N/A	
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	330		N/A	
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	380		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	11 minus 20 =	*	x 50 =	0.00	OR		
INDEPENDENT CLAIMS (37 CFR 1.16(h))	4 minus 3 =	* 1	x 230 =	230			
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			0.00			
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				0.00			
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	1015		TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/045,159	07/25/2018	Michael Francis	LNH-152US1

CONFIRMATION NO. 5408

23122
RATNERPRESTIA
2200 Renaissance Blvd
Suite 350
King of Prussia, PA 19406

PUBLICATION NOTICE

OC000000108723250

Title:ALIGNED FIBER AND METHOD OF USE THEREOF

Publication No.US-2019-0167842-A1
Publication Date:06/06/2019

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

The publication may be accessed through the USPTO's publically available Searchable Databases via the Internet at www.uspto.gov. The direct link to access the publication is currently http://www.uspto.gov/patft/.

The publication process established by the Office does not provide for mailing a copy of the publication to applicant. A copy of the publication may be obtained from the Office upon payment of the appropriate fee set forth in 37 CFR 1.19(a)(1). Orders for copies of patent application publications are handled by the USPTO's Public Records Division. The Public Records Division can be reached by telephone at (571) 272-3150 or (800) 972-6382, by facsimile at (571) 273-3250, by mail addressed to the United States Patent and Trademark Office, Public Records Division, Alexandria, VA 22313-1450 or via the Internet.

In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently https://portal.uspto.gov/pair/PublicPair. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

Further assistance in electronically accessing the publication, or about PAIR, is available by calling the Patent Electronic Business Center at 1-866-217-9197.

Office of Data Managment, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/045,159	07/25/2018	Michael Francis	LNH-152US1	5408

23122 7590 12/24/2020

RATNERPRESTIA
2200 Renaissance Blvd
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King of Prussia, PA 19406

EXAMINER
MARVICH, MARIA

ART UNIT	PAPER NUMBER
1633	

NOTIFICATION DATE	DELIVERY MODE
12/24/2020	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

PCorrespondence@ratnerprestia.com

Office Action Summary	Application No. 16/045,159	Applicant(s) Francis et al.	
	Examiner MARIA MARVICH	Art Unit 1633	AIA (FITF) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL.** 2b) ☑ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 25-35 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 25-35 is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☑ The specification is objected to by the Examiner.

11)☑ The drawing(s) filed on 2/27/2019 is/are: a)☑ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☑ Notice of References Cited (PTO-892)

2) ☑ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

DETAILED ACTION

The present application is being examined under the pre-AIA first to invent provisions.

Claims 25-35 are pending.

This application is a Divisional Application of U.S. Application No. 14/776,106, filed September 14, 2015, now U.S. Patent 10,137,223, which is a U.S. National Phase Application of PCT International Application PCT/US2014/025636, filed March 13, 2014, which claims priority benefit of U.S. Application No. 61/785,031, filed March 14, 2013 the disclosures of which are incorporated herein in their entirety.

Information Disclosure Statement

Information disclosure statements filed 2/24/15 and 1/8/16 have been identified and the documents considered. The corresponding signed and initialed PTO Form 1449 has been mailed with this action. The documents listed as Search Reports and office actions *have been considered* but have been crossed off the 1449 so that it will not appear on the face of any patent issuing from the instant application.

Claim Objections

Claims 25, 26 , 27 and 31 are objected to because of the following informalities: grammatically, in claim 25, the phrase "a fast Fourier transform (FFT) analysis result of the fiber have adjacent major peaks with about 180° apart from each other" is awkward. The analysis result of the fiber is not accurate. As well, the analysis result can't have adjacent major peaks. It appears the claim should recite –the result of a fast Fourier transform (FFT) analysis of the fibers

demonstrate adjacent that the fibers have major adjacent peaks that are about 180° apart from each other--.

An abbreviation is only necessary once as it establishes how the term is to be referenced. Hence, it is not necessary to continually abbreviate a term throughout the claims. This is true of FFT.

Appropriate correction is required.

Claim Rejections - 35 USC § 112

> The following is a quotation of 35 U.S.C. 112(b):
> (b) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.
>
> The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:
> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 25-35 are rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor (or for applications subject to pre-AIA 35 U.S.C. 112, the applicant), regards as the invention.

The dependent claims are included in the rejection because they fail to address or clarify the basis of the rejection as discussed in detail for the independent claims.

Claim 25 is rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. The omitted steps are: where the tissue defect and the applying step occurs. There is no indication of a target for the application nor source of the tissue defect. It appears that the tissue and application is in a subject with the tissue defect. However,

the claims are lacking this step. As well, it is not clear to what "to surround the tissue defect" refers. In general, as a conclusion, this statement is incomplete. The claim should indicate with clarity the end step of the method i.e. wherein the application of the scaffold at the tissue defect surround the tissue defect with the scaffold--. These issues are true of claim 26.

Claims 27 and 31 also lack a conclusory step such that it is clear when the goals of the claim have been met. This makes the claims incomplete and unclear.

Claim 31 lacks proper antecedent basis for the term "the cells" in line 2. There are two types of cells in the claim and the claims are not clear as to which "the cells" refers.

Claim 32 is rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. The omitted steps are: what the conditions are that are suitable for cell differentiation.

Claim 33 is vague and indefinite in that the metes and bounds of the term "derived from" are unclear. It is unclear the nature and number of steps required to obtained a "derivative" of the tissues. The term implies a number of different steps that may or may not result in a change in the functional characteristics of the cell from the source that it is "derived from".

Claim Rejections - 35 USC § 112, first paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 25-35 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of treating a subject with a defect or injury in a system selected from the bone, tendon, ligament, blood vessel, skin, intestine, nerve, and cartilage, the

method comprising inserting directly to the site of the defect or the injury the scaffold which comprises heart basement extract, heart basement membrane extracellular matrix, placenta basement membrane extract or placenta basement membrane extracellular matrix in combination with autologous cells selected from autologous stem cells, autologous fibroblasts and autologous dorsal root ganglia wherein the fibers of the scaffold have adjacent major peaks about 180° apart from one another as measured by a fast Fourier transformer, wherein the implantation of the scaffold treats the tissue defect or the injury and for methods of promoting differentiation of stem cells into osteoblasts, chondrocytes, ligament or tendon or when the method comprises culturing the stem cells or differentiation of the stem cells on the scaffold, the method requires use of scaffold comprising heart basement extract, heart basement membrane extracellular matrix, placenta basement membrane extract or placenta basement membrane extracellular matrix and for differentiation the conditions are differentiation media wherein the culturing results in differentiation of the stem cells on the scaffold, does not reasonably provide enablement for any other embodiment. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make or use the invention commensurate in scope with these claims.

The test of enablement is whether one skilled in the art could make and use the claimed invention from the disclosures in the patent coupled with information known in the art without undue experimentation (*United States v. Telectronics, Inc.*, 8 USPQ2d 1217 (Fed. Cir. 1988)). Whether undue experimentation is required is not based on a single factor but is rather a conclusion reached by weighing many factors (See *Ex parte Forman*, 230 USPQ 546 (Bd. Pat. App. & Inter, 1986) and In *re Wands*, 8USPQ2d 1400 (Fed. Cir. 1988); these factors include the

following:

The claims have been evaluated in light of the art at the time of filing and found not to be commensurate in scope with the specification. The MPEP teaches, "However, claims reading on significant numbers of inoperative embodiments would render claims non- enabled when the specification does not clearly identify the operative embodiments and undue experimentation is involved in determining those that are operative. Atlas Powder Co. v. E.I. duPont de Nemours & Co., 750 F.2d 1569, 1577, 224 USPQ 409, 414 (Fed. Cir. 1984); In re Cook, 439 F.2d 730, 735, 169 USPQ 298,302 (CCPA 1971). (see MPEP 2164.08(b).

1) Nature of invention. Instant claim 25 and 26 are drawn to methods of treating a subject with a tissue defect or a gap in a tissue by use of scaffolds. The nature of the invention is tissue engineering.

2) Scope of the invention. The scope of the invention is fairly broad in that the claims do not indicate the material comprising the scaffold nor do the claims indicate to where the treatment and application are performed.

3) Number of working examples and guidance. Applicants teach construction of a scaffold comprised of heart basement membrane and polycaprolactone wherein dorsal root ganglion were seeded thereon leading to development of neurite outgrowths (example 2 and 3).

4) State of the art. The art of tissue engineering has been under examination since 1988 and as set forth by Hutmacher et al is a multidisciplinary art (see page 245).

The state of the art just before the time of fling was such that the predictability was quite low (Werner et al).

> Reviewing the literature, it can be concluded that it is by no means clear what defines an ideal scaffold–cell or scaffold–neotissue construct, even for a specific tissue type. Since some tissues perform multiple functional roles, it is unlikely that a single scaffold would serve as a universal foundation for the regeneration of even a single tissue. In this context it is salutary to recall one

> of the many definitions of biocompatibility of materials, which determines the suitability of a particular material, within the context of the functional role played by the materials following implantation. The considerations for scaffold design are hence complex and include material composition, architecture, structural mechanics, surface properties, degradation properties and products, together with the composition of any added biological components and, of course, the changes in all of these factors with time.
>
> For each envisioned application, successful TECs will have certain minimum requirements for biochemical and physical properties. Scaffolds may be required to provide sufficient initial mechanical strength and stiffness to substitute for the mechanical function of the diseased or damaged tissue which the TEC aims at repairing or regenerating.

Even into 2019, this predictability was still of concern. Sharma taches, 2019, page 1.

> These tissue-engineered constructs can be helpful in restoring and repairing malfunctioned tissues and organs at the high social and economic point of view (WHO Scientific Group, 2003; Kang et al., 2015). It is challenging to incorporate these bioengineered materials due to limitations of onsite target and side effects such as cell toxicity, interfering with immune systems, and transporting mechanisms. Also, designing and developing a system based on biological features such as mechanical stress at the targeted site, strength, complex viscoelastic, nonlinear, and anisotropic mechanical features have constantly been an area of consideration, as illustrated in **Figure 1**.

Not all disorders and tissues operate the same. .

> The accuracy and efficiency of bioengineered constructs are very challenging, as they require a deep understanding with regard to mechanisms of regeneration. New treatment approaches are required, as designing new nanostructures and constructs can provide more precise, target-specific scaffolds with varying functionalities. For example, only drugs and/or heart transplantation solutions are available for cardiac diseases. In summary, bio-mimicked constructs possess advanced architectures and surface topography like native tissues and organs to recover intrinsic properties of damaged tissues and organs.

At the time of filing use of PCL was quite limited as a scaffold for *in* vivo delivery. Use was typically limited to fabrication of 3D structures in culture (see e.g. Dash and Konkimalla). There is some discovery of use as a bone scaffold (see page 251 if Hutmacher et al and Table 3 Dash and Konkimalla).

5) Unpredictability of the art. The physiological art is recognized as unpredictable. (MPEP 2164.03.) In cases involving predictable factors, such as mechanical or electrical elements, a single embodiment provides broad enablement in the sense that, once imagined, other embodiments can be made without difficulty and their performance characteristics

predicted by resort to known scientific laws. In cases involving unpredictable factors, such as most chemical reactions and physiological activity, the scope of enablement obviously varies inversely with the degree of unpredictability of the factors involved.

In this case, applicants' claims suffer from gaps in the methods which lead to a complicated and lacking set of steps. These appear to be mostly omission such as to whom or what the scaffold is being administered and exactly what the steps for the method require. The remedies are set forth in the scope of enablement above.

The second issue with the claims involves the large breadth. First is the type of defect or gap to be treated. The claims do not limit the target tissue or defect. And yet, the state of engineering must be developed uniquely for each tissue type. This is set forth above and also would be obvious to any practitioner based upon the milieu and complexity of each structure. Secondly, the claims use any scaffold so long as the fibers are aligned as set forth in the claims. However, tissues engineering structures especially to treat tissue defects/gaps require many other components such as collagen, cells and structure for more complicated tissues. As set forth by the art, the type of biomaterial is critical. The ability to form structures and interact with cells are defined by the biomaterial. The specification does not describe any structures other than polycaprolactone that can mediate the aligned fiber formation as set forth and these experiments demonstrate that the method requires heart basement extract, heart basement membrane extracellular matrix, placenta basement membrane extract or placenta basement membrane extracellular matrix in combination with autologous cells selected from autologous stem cells, autologous fibroblasts and autologous dorsal root ganglia.

Consequently, the prior art (and post-filing art) when combined with the lack of any disclosed direct experimental test of Applicant's hypothesis, shows that one of skill in the art at

the time the invention was made would have had no basis to reasonably predict or conclude the claimed sequences could be identified given the lack of details necessary to identify those meeting the necessary functions. Though not controlling, the lack of working examples, is, nevertheless, a factor to be considered in a case involving both physiological activity and an undeveloped art. When a patent applicant chooses to forego exemplification and bases utility on broad terminology and general allegations, he runs the risk that unless one with ordinary skill in the art would accept the allegations as obviously valid and correct, the PTO may, properly, ask for evidence to substantiate them. Ex parte Sudilovsky, 21 USPQ2d 1702, 1705 (BPAI 1991); In re Novak, 134 USPA 335 (CCPA 1962); In re Fouche, 169 USPQ 429 (CCPA 1971).

6) Amount of experimentation required. The art of tissue engineering at the time of filing was an improving art but still wrought with a number of obstacles as set forth above. When considering complicated structures, the instant specification does not advance the art. Given the generic nature of the claims, Given the lack of guidance in the specification, the large and diverse group of administration recited and the highly unpredictable nature of the art, it is concluded that a person of skill in the art would have had to conduct undue experimentation in order to practice the claimed invention.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to MARIA MARVICH whose telephone number is (571)272-0774. The examiner can normally be reached on 6 am- 3 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christopher Babic can be reached on 571-272-8507. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/MARIA MARVICH/
Primary Examiner, Art Unit 1633

Notice of References Cited	Application/Control No. 16/045,159	Applicant(s)/Patent Under Reexamination Francis et al.	
	Examiner MARIA MARVICH	Art Unit 1633	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
	A					
	B					
	C					
	D					
	E					
	F					
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	I					
	J					
	K					
	L					
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FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Sharma et al, State-of-Art Functional Biomaterials for Tissue Engineering, Front. Mater., 2019, pages 1-10
	V	Hutmacher et al, State of the art and future directions of scaffold-based bone engineering from a biomaterials perspective, J Tissue Eng Regen Med 2007; 1: 245–260.
	W	Dash and Konkimalla, Poly-ϵ-caprolactone based formulations for drug delivery and tissue engineering: A review, Journal of Controlled Release, Volume 158, Issue 1, 28 February 2012, Pages 15-33
	X	Werner et al, State of the art and future directions of scaffold-based bone engineering from a biomaterials perspective, J Tissue Eng Regen Med 2007; 1: 245–260.

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/045,159	Francis et al.
	Examiner	Art Unit
	MARIA MARVICH	1633

CPC - Searched*		
Symbol	Date	Examiner

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
SEARCH- USPAT, PGPUB, EPO, JPO, Derwent, USOCR, IBM-IDB	12/17/2020	MM
inventor search	12/17/2020	MM
npl search	12/17/2020	MM

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

Web Search History

date, time	web site	search string
12/18/2020 10:25:11 PM	Google	fiber peaks fourier 180
12/18/2020 10:25:22 PM	Google	fiber peaks fourier 180 degrees apart
12/18/2020 10:25:49 PM	Google	180° apart fibers fourier
12/18/2020 10:44:00 PM	Google	adipose derived stem cells
12/18/2020 10:57:43 PM	Google	human scaffolds made of polycaprolactone
12/18/2020 11:05:08 PM	Google	state of art scaffolds to correct tissue defects
12/18/2020 11:05:24 PM	Google	state of art scaffolds to correct tissue defects what types of tissues
12/18/2020 11:10:38 PM	Google	what are teh conditions necessary to differentate stem cells into osteoblasts
12/18/2020 11:22:04 PM	Google	polycaprolactone for tissue engineering
12/18/2020 11:22:29 PM	Google	polycaprolactone for tissue engineering what types of tissues
12/18/2020 11:24:20 PM	Google	applications of pcl
12/18/2020 11:24:27 PM	Google	applications of pcl in tissue engineering
12/18/2020 11:25:46 PM	Google	Polycaprolactone-based biomaterials for tissue engineering and drug delivery: Current scenario and challenges
12/18/2020 11:25:59 PM	Google	pdf Polycaprolactone-based biomaterials for tissue engineering and drug delivery: Current scenario and challenges
12/18/2020 11:27:36 PM	Google	can you use polycaprolactone to engineer heart
12/18/2020 11:27:56 PM	Google	can you use polycaprolactone to engineer stomach
12/19/2020 8:14:11 AM	Google	Towards the development of polycaprolactone based amphiphilic block copolymers: molecular design, self-assembly and biomedical applications
12/19/2020 12:38:52 PM	Google	polycaprolactone based biomedical applications
12/19/2020 12:39:49 PM	Google	pcl and pcl-based materials in biomedical applications
12/19/2020 12:41:37 PM	Google	polycaprolactone based tissue engineering
12/19/2020 1:05:30 PM	Google	Evaluation of a gelatin modified poly(ε-caprolactone) film as a scaffold for lung disease

date, time	web site	search string
12/19/2020 1:08:29 PM	Google	PCL-Based Composite Scaffold Matrices for Tissue Engineering Applications
12/19/2020 1:10:10 PM	Google	polycaprolactone works for what types of tissue engineering
12/19/2020 1:12:54 PM	Google	polycaprolactone reivew use in tissues
12/19/2020 1:13:41 PM	Google	polycaprolactone review use in tissues
12/19/2020 1:29:13 PM	Google	, The enhancement of VEGF-mediated angiogenesisby polycaprolactone scaffolds with surface cross-linked hepari
12/19/2020 1:29:39 PM	Google	, A hybrid biomimetic nanomatrix composedof electrospun polycaprolactone and bioactive peptide amphiphiles for cardio-vascular implants, Acta Biomate

PE2E SEARCH - Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
L1	2	16/045159	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 09:39 PM
L2	1372	Francis adj2 Michael OR Ogle adj2 Roy	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 09:39 PM
L3	36	L2 AND (fiber).clm.	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 09:39 PM
L4	6	L2 AND (fiber).clm. AND sheet SAME peak.clm.	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 09:39 PM
L5	9	L2 AND (fiber).clm. SAME sheet	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 09:41 PM
L6	5	L2 AND (fiber).clm. SAME sheet AND fourier	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 09:41 PM
L7	5	L2 AND (fiber).clm. SAME sheet AND fourier SAME peak	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 09:42 PM
L9	5	L2 AND (fiber).clm. SAME sheet AND fourier SAME peak AND tissue	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 09:50 PM
L10	5	L2 AND (fiber).clm. SAME sheet AND fourier SAME peak AND tissue SAME fiber	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 09:50 PM
L11	5	L2 AND (fiber).clm. SAME sheet AND fourier SAME peak AND tissue SAME scaffold	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 09:50 PM

L12	5	L2 AND (fiber).clm. SAME sheet AND fourier SAME peak AND tissue SAME scaffold AND 180$	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 09:51 PM
L13	4	14/776106	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 09:53 PM
L15	4	14/776106 AND tissue ADJ defect	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 09:59 PM
L16	0	14/776106 AND scaffold SAME cultr$	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 10:00 PM
L17	4	14/776106 AND scaffold SAME cell	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 10:00 PM
L18	4	14/776106 AND cultur$	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 10:00 PM
L19	4	14/776106 AND differentiation	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 10:01 PM
L20	64	"6189537".pn. OR "6305379".pn. OR "20020090725" OR "20040037813" OR "20070225631" OR "20080213389"	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 10:18 PM
L21	12	L20 AND (adminster$ OR adminstration OR application OR apply$ OR adhere) adj10 scaffold	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 10:19 PM
L22	12	L20 AND (adminster$ OR administration OR application OR apply$ OR adhere) adj10 scaffold	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 10:19 PM
L23	12	L20 AND (adminster$ OR administration OR application OR apply$ OR adhere) adj10 scaffold	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 10:19 PM

		AND scaffold SAME fiber					
L24	0	L20 AND (adminster$ OR administration OR application OR apply$ OR adhere) adj10 scaffold AND scaffold SAME fiber SAME sheet	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 10:19 PM
L25	12	L20 AND (adminster$ OR administration OR application OR apply$ OR adhere) adj10 scaffold AND scaffold SAME fiber	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 10:19 PM
L26	12	L20 AND (administer$ OR administration OR application OR apply$ OR adhere) adj10 scaffold AND scaffold SAME fiber	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 10:24 PM
L27	0	L20 AND (administer$ OR administration OR application OR apply$ OR adhere) adj10 scaffold AND scaffold SAME fiber AND fiber SAME 180$	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 10:24 PM
L28	22	L20 AND (administer$ OR administration OR application OR apply$ OR adhere) adj10 scaffold AND scaffold SAME fiber andfourier	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 10:24 PM
L29	0	L20 AND (administer$ OR administration OR application OR apply$ OR adhere) adj10 scaffold AND scaffold SAME fiber AND fourier	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 10:24 PM
L30	3	L20 AND fiber SAME fourier	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 10:24 PM
L31	2	L20 AND aligned ADJ fiber	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 10:26 PM
L32	15	L2 AND (fiber).clm. AND fiber SAME (align OR aligned OR fourier)	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 10:27 PM

L33	9	L2 AND (fiber).clm. AND fiber SAME (align OR aligned OR fourier) AND tissue.clm.	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 10:28 PM
L34	7	L2 AND (fiber).clm. AND fiber SAME (fourier) AND tissue.clm.	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 10:29 PM
L35	2	16/045159 AND tissue SAME (defect AND gap)	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 11:00 PM
L36	6	differentation SAME media SAME stem.clm.	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 11:11 PM
L37	0	differentation SAME stem.clm. SAME scaffold	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 11:11 PM
L38	0	differentate SAME stem.clm. SAME scaffold	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 11:12 PM
L39	0	differentate SAME stem SAME scaffold	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 11:12 PM
L40	2	differentation SAME stem SAME scaffold	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 11:12 PM
L41	49	differentation SAME media SAME stem	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/18 11:12 PM
L42	3612	scaffold SAME stem	USPAT	OR	ON	ON	2020/12/18 11:39 PM
L43	195	scaffold SAME stem.clm.	USPAT	OR	ON	ON	2020/12/18 11:40 PM
L44	89	scaffold SAME polycaprolactone.clm.	USPAT	OR	ON	ON	2020/12/18 11:43 PM
L45	4	14/776106 AND defect	US-PGPUB; USPAT; USOCR; FIT (CN, FR,	OR	ON	ON	2020/12/18 11:46 PM

			WO); FPRS; EPO; JPO; DERWENT; IBM_TDB				
L46	4	14/776106 AND skin	US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	ON	2020/12/19 07:38 AM

PE2E SEARCH - Search History (Interference)

There are no Interference searches to show.

Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (03-15)
Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT
(Not for submission under 37 CFR 1.99)

Application Number	16045159
Filing Date	2018-07-25
First Named Inventor	Michael Francis
Art Unit	TBD
Examiner Name	TBD
Attorney Docket Number	LNH-152US1

U.S.PATENTS

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
/M.M/	1	6189537	B1	2001-02-20	Wolfinbarger, L.	
/M.M/	2	6305379	B1	2001-10-23	Wolfinbarger, L.	

If you wish to add additional U.S. Patent citation information please click the Add button.

U.S.PATENT APPLICATION PUBLICATIONS

Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
/M.M/	1	20020090725	A1	2002-07-11	Simpson et al.	
/M.M/	2	20040037813	A1	2004-02-26	Simpson et al.	
/M.M/	3	20070225631	A1	2007-09-27	Bowlin et al.	
/M.M/	4	20080213389	A1	2008-09-04	Lelkes et al.	

/MARIA MARVICH/ 12/18/2020

EFS Web 2.1.17

EXAMINER: REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	16045159
	Filing Date	2018-07-25
	First Named Inventor	Michael Francis
	Art Unit	TBD
	Examiner Name	TBD
	Attorney Docket Number	LNH-152US1

/M.M/	5	20090018643	A1	2009-01-15	Hashi et al.	
/M.M/	6	20100233115	A1	2010-09-16	Patel et al.	
/M.M/	7	20110236974	A1	2011-09-29	Ogle et al.	
/M.M/	8	20120221025	A1	2012-08-30	Simpson et al.	
/M.M/	9	20120273993	A1	2012-11-01	Shoseyov et al.	

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FOREIGN PATENT DOCUMENTS

Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
/M.M/	1	2012027592	WO	A2	2012-03-01	LifeNet Health		☐
/M.M/	2	2013033680	WO	A1	2013-03-07	LifeNet Health		☐
/M.M/	3	2013119873	WO	A1	2013-08-15	LifeNet Health		☐
/M.M/	4	2010016942	WO	A1	2010-02-11	Lifenet Health		☐

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	16045159
	Filing Date	2018-07-25
	First Named Inventor	Michael Francis
	Art Unit	TBD
	Examiner Name	TBD
	Attorney Docket Number	LNH-152US1

NON-PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T5
/M.M/	1	Anderson et al., "Biodegradation and Biocompatibility of PLA and PLGA Microspheres", Advanced Drug Delivery Reviews, 1997, Vol. 28, pp. 5-24	☐
/M.M/	2	Ayres et al., "Measuring Fiber Alignment in Electrospun Scaffolds: A User's Guide to the 2D Fast Fourier Transform Approach," Journal of Biomaterials Science, Polymer Edition, (2008), Vol. 19, No. 5, pp. 603-621	☐
/M.M/	3	Behravesh et al., "Synthetic Biodegradable Polymers for Orthopaedic Applications," Clinical Orthopaedics and Related Research, No. 367S, pp. S118-S125	☐
/M.M/	4	Cleek et al., "Microparticles of Poly(DL-lactic-co-glycolic acid)/poly(ethylene glycol) Blends for Controlled Drug Delivery," Journal of Controlled Release, Vol. 48 1997 pp. 259-268	☐
/M.M/	5	European Communication for European Application No. 14 773 677.1, dated November 6, 2017 - 8 pages	☐
/M.M/	6	Extended Search Report for European Application No. 14 773 677.1, dated October 7, 2016 - 6 pages	☐
/M.M/	7	Huang et al., "Electrospun Collagen-Chitosan-TPU Nanofibrous Scaffolds For Tissue Engineered tubular Grafts", Colloids and Surfaces B: Biointerfaces, Vol. 82 2011, pp. 307-315.	☐
/M.M/	8	International Search Report for International Application No. PCT/US2014/025636, dated July 10, 2014 - 8 pages	☐
/M.M/	9	Lu et al., "Polymeric Delivery Vehicles for Bone Growth Factors," 2000, Park & Mrsny; Controlled Drug Delivery, ACS Symposium Series; American Chemical Society, pp. 124-138	☐

/MARIA MARVICH/ 12/18/2020

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	16045159
	Filing Date	2018-07-25
	First Named Inventor	Michael Francis
	Art Unit	TBD
	Examiner Name	TBD
	Attorney Docket Number	LNH-152US1

/M.M/	10	Schnaper et al., "Regulation of Cell Function by Extracellular Matrix," Pediatr. Nephrol, 1993, Vol. 7 - pp. 96-104	☐
/M.M/	11	~~Supplementary European Seach Report for European Application No. 14 773 677.1, dated October 25, 2016 - 3 pages~~	☐
/M.M/	12	Zeugoils et al., "Electro-spinning of Pure Collagen Nano-fibres - Just an Expensive Way to Make Gelatin", Biomaterials, Vol. 29, 2008, pp. 2293-2305	☐
/M.M/	13	~~Entire patent prosecution history of U.S. Patent Application No.14/776,106, filed September 14, 2015, entitled,~~ "ALIGNED FIBER AND METHOD OF USE THEREOF."	☐

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EXAMINER SIGNATURE

Examiner Signature	/MARIA MARVICH/	Date Considered	12/18/2020

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	16045159
	Filing Date	2018-07-25
	First Named Inventor	Michael Francis
	Art Unit	TBD
	Examiner Name	TBD
	Attorney Docket Number	LNH-152US1

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

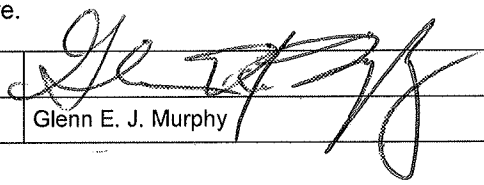
☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature		Date (YYYY-MM-DD)	2018-07-26
Name/Print	Glenn E. J. Murphy	Registration Number	33,539

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

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6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	16045159
Filing Date	2018-07-25
First Named Inventor	Michael Francis
Art Unit	TBD
Examiner Name	TBD
Attorney Docket Number	LNH-152US1

U.S.PATENTS

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					

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Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
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FOREIGN PATENT DOCUMENTS

Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
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NON-PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
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	Attorney Docket Number	LNH-152US1

/M.M/	1	Notice of Allowance for Application No. 14/776,106, mailed August 15, 2018, 10 pages.	☐

If you wish to add additional non-patent literature document citation information please click the Add button

EXAMINER SIGNATURE

Examiner Signature	/MARIA MARVICH/	Date Considered	12/18/2020

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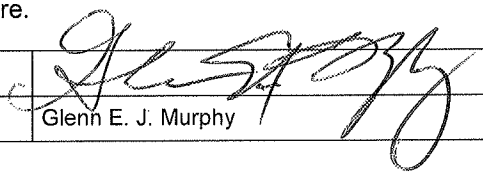
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 16/045,159	Filing Date 07/25/2018	☐ To be Mailed

ENTITY: ☐ LARGE ☑ SMALL ☐ MICRO

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A		N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A		N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*		x $50 =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*		x $230 =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).				
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))					
* If the difference in column 1 is less than zero, enter "0" in column 2.				TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT	04/26/2021	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 20	Minus	** 20	= 0		x $50 =	0
	Independent (37 CFR 1.16(h))	* 4	Minus	*** 4	= 0		x $240 =	0
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	0

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=		x $0 =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=		x $0 =	
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.	SLIE
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".	/DEIDRE C KING-WOODWARD/
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Electronic Patent Application Fee Transmittal

Application Number:	16045159
Filing Date:	25-Jul-2018
Title of Invention:	ALIGNED FIBER AND METHOD OF USE THEREOF
First Named Inventor/Applicant Name:	Michael Francis
Filer:	Valerie P. Hayes/Kathy Spina
Attorney Docket Number:	LNH-152US1

Filed as Small Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension - 1 month with $0 paid	2251	1	110	110
Miscellaneous:				
			Total in USD ($)	**110**

Electronic Acknowledgement Receipt

EFS ID:	42552775
Application Number:	16045159
International Application Number:	
Confirmation Number:	5408
Title of Invention:	ALIGNED FIBER AND METHOD OF USE THEREOF
First Named Inventor/Applicant Name:	Michael Francis
Customer Number:	23122
Filer:	Valerie P. Hayes/Kathy Spina
Filer Authorized By:	Valerie P. Hayes
Attorney Docket Number:	LNH-152US1
Receipt Date:	26-APR-2021
Filing Date:	25-JUL-2018
Time Stamp:	16:03:05
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$110
RAM confirmation Number	E20214PG03333541
Deposit Account	180350
Authorized User	Kathy Spina

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37 CFR 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Amendment/Req. Reconsideration-After Non-Final Reject	Amendment_LNH-152US1.pdf	128409 e49d7c5085d7826f602ce393799185f1e51f8b84	no	6
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30604 95e670a9be79fc15bd13cb14c279abfb4529521a	no	2
Warnings:					
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If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No:	16/045,159
Applicant:	LifeNet Health
Filed:	July 25, 2018
Title:	ALIGNED FIBER AND METHOD OF USE THEREOF
T.C./A.U.:	1633
Examiner:	MARVICH, MARIA
Confirmation No.:	5408
Docket No.:	LNH-152US1

AMENDMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

In response to the non-final Office Action dated December 24, 2020, please amend the above-identified application as follows:

☐ **Amendments to the Specification** begin on page of this paper.

☒ **Amendments to the Claims** are reflected in the listing of claims, which begins on page **2** of this paper.

☐ **Amendments to the Drawings** begin on page of this paper and include an attached replacement sheet(s).

☐ **Amendments to the Abstract** are on page of this paper. A clean version of the Abstract is on page of this paper.

☒ **Remarks/Arguments** begin on page **5** of this paper.

Amendments to the Claims:

This list of claims replaces all prior versions and lists of claims in the application:

1.-24. (Canceled)

25. (Currently Amended) A method of treating a tissue defect in a subject comprising

~~applying~~ implanting at the tissue defect a scaffold comprising one or more crosslinked ~~electrospun~~ fibers comprising collagen, wherein a result of a fast Fourier transform (FFT) analysis ~~result~~ of the fibers demonstrates that the fibers have major adjacent ~~major~~ peaks ~~with~~ that are about 180° apart from each other, and ~~wherein the scaffold is in a form of one or more elongated sheets~~,

~~to surround~~ surrounding the tissue defect with the scaffold to repair a tendon, ligament or nerve defect.

26. (Currently Amended) A method of treating a tissue defect ~~or gap~~ in a subject comprising

~~applying~~ implanting a scaffold comprising one or more crosslinked ~~electrospun~~ fibers comprising collagen, wherein the fibers are anisotropic based on a ~~fastlourier transform (FFT)~~ FFT analysis ~~result of the fibers have adjacent major peaks with about 180° apart from each other~~, and ~~wherein the scaffold is in the form of one or more elongated rolls,~~

wherein the scaffold is inserted into ~~between~~ the tissue defect ~~or gap~~to repair a tendon, ligament or nerve.

27. (Currently Amended) A method of cell culture comprising

~~culturing~~ placing cells on a scaffold comprising one or more aligned ~~electrospun~~ fibers, wherein a result of a ~~fast Fourier transform (FFT)~~ FFT analysis ~~result~~ of the fibers demonstrates that the fibers have major adjacent ~~major~~ peaks ~~with~~ that are about 180° apart from each other, and

growing the cells in conditions suitable for the cell culture.

28. (Currently Amended) The method of claim 27, wherein the cells are ~~selected from the group consisting of~~ stem cells, adipose derived stem cells, dental pulp stem cells, fibroblasts, and/or dorsal root ganglia.

29. (Original) The method of claim 27, wherein the cells are dorsal root ganglia.

30. (Original) The method of claim 27, wherein the cells are dorsal root ganglia, and the fibers comprise heart basement membrane extract, heart basement membrane extracellular matrix, placenta basement membrane extract, or placenta basement membrane extracellular matrix.

31. (Currently Amended) A method of promoting differentiation of stem cells into osteoblasts, chondrocytes, ligament or tendon, the method comprising

~~culturing~~placing the stem cells on a scaffold comprising one or more ~~electrospun~~ aligned fibers, wherein a result of a ~~fast Fourier transform (FFT)~~ FFT analysis ~~result~~ of the fibers demonstrates that the fibers have major adjacent ~~major~~ peaks ~~with~~ that are about 180° apart from each other, and

growing the stem cells *in vitro* in conditions suitable for the cell differentiation.

32. (Original) The method of claim 31, wherein the stem cells are progenitor cells or adult stem cells.

33. (Currently Amended) The method of claim 32, wherein the progenitor cells or the adult stem cells are ~~derived or~~ isolated from placenta, bone marrow, adipose tissue, blood vessel, amniotic fluid, synovial fluid, synovial membrane, pericardium, periosteum, dura, peripheral blood, umbilical blood, menstrual blood, baby teeth, nucleus pulposus, brain, skin, hair follicle, intestinal crypt, neural tissue, or muscle.

34. (Cancelled.)

35. (Currently Amended) The method of ~~claim 34~~claim 33, wherein the stem cells are ~~selected from the group consisting of~~ adipose derived stem cells and/or dental pulp stem cells.

36. (New) The method of claim 25, wherein the scaffold is in the form of one or more elongated sheets.

37. (New) The method of claim 25, wherein the scaffold is a composite of multiple layers of one or more sheets.

38. (New) The method of claim 25, wherein the tissue defect is a nerve defect.

39. (New) The method of claim 25, wherein the tissue defect is a ligament defect.

40. (New) The method of claim 25, wherein the tissue defect is a tendon defect.

41. (New) The method of claim 26, wherein the scaffold is in the form of one or more elongated sheets.

42. (New) The method of claim 26, wherein the scaffold is a composite of multiple layers of one or more sheets.

43. (New) The method of claim 26, wherein the repair is to a nerve.

44. (New) The method of claim 26, wherein the repair is to a ligament.

45. (New) The method of claim 26, wherein the repair is to a tendon.

Remarks/Arguments:

Status:

Claims 25-33 and 35-45 are pending in the application. Claims 25-28, 31, 33, and 35 have been amended, and claim 34 has been canceled. New claims 36-45 have been added and are supported by at least paragraphs [50-51], [56], [76], [83-84], [87-98], and [101-103] of U.S. Patent Publication 2019/0167842. No prohibited new matter has been introduced by these new claims or amendments.

Claims 25-35 were rejected.

Claim Objections:

Claims 25, 26, 27 and 31 were objected to for allegedly being awkward and for expressing both "fast Fourier transform" and its abbreviation, "FFT," in each independent claim. It is noted that the allegedly awkward, objected to language is recited in granted parent patent, U.S. Patent No. 10,137,223. Although Applicant disagrees with the Examiner's assertions and without acquiescing to the merits of these objections, each of these claims have been amended as requested by the Examiner. It is believed that these objections have been overcome.

Rejection Under 35 U.S.C. § 112, second paragraph

Claims 25-35 were rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor (or for applications subject to pre-AIA 35 U.S.C. 112, the applicant), regards as the invention for the reasons provided on pages 3-4 of the Office Action. Without acquiescing to the merits of this rejection, Applicant has amended claims 25-28, 31, 33, and 35 to provide additional elements and further clarity. Applicant requests reconsideration and withdrawal of the §112 rejection for indefiniteness.

Rejection Under 35 U.S.C. § 112, first paragraph

Claims 25-35 were rejected under 35 U.S.C. 112, first paragraph, as the specification allegedly does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with the claims

for the reasons provided on pages 4-9 of the Office Action. Without acquiescing to the merits of this rejection, Applicant has amended claims 25-28, 31, 33, and 35 to provide additional elements and further clarity. Applicant requests reconsideration and withdrawal of the §112 rejection for lack of enablement.

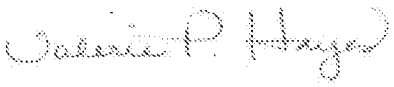
Conclusion

For the foregoing reasons, Applicant respectfully requests the Office to withdraw the rejections and allow the pending application.

As Applicant's remarks above with respect to the Examiner's rejections are sufficient to overcome the rejections, Applicant's silence as to any assertions by the Examiner in the Office Action or certain requirements that may be applicable to such rejections is not a concession by Applicant that such assertions are accurate or such requirements have been met, and Applicant reserves the right to analyze and dispute such in the future.

To the extent necessary, if any, a petition for an extension of time under 37 C.F.R. § 1.136 is hereby made. The Director is hereby authorized to charge or credit Deposit Account No. 18-0350 for any additional fees, or any underpayment or credit for overpayment in connection herewith.

Respectfully submitted,



William J. Matney, Jr., Reg. No. 39,292
Valerie P. Hayes, Reg. No. 53,005
Attorneys for Applicants

WJM/VPH/ks
Dated: April 26, 2021

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The Director is hereby authorized to charge or credit Deposit Account No. **18-0350** for any additional fees, or any underpayment or credit for overpayment in connection herewith.

UNITED STATES DEPARTMENT OF COMMERCE
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/045,159	07/25/2018	Michael Francis	LNH-152US1	5408

23122 7590 07/19/2021

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EXAMINER
MARVICH, MARIA

ART UNIT	PAPER NUMBER
1633	

NOTIFICATION DATE	DELIVERY MODE
07/19/2021	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

PCorrespondence@ratnerprestia.com

Office Action Summary	Application No. 16/045,159	Applicant(s) Francis et al.	
	Examiner MARIA MARVICH	Art Unit 1633	AIA (FITF) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☑ Responsive to communication(s) filed on 4/26/2021.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☑ This action is **FINAL.** 2b) ☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 25-33 and 35-45 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 25-33 and 35-45 is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☑ The drawing(s) filed on 2/27/2019 is/are: a)☑ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☑ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

DETAILED ACTION

The present application is being examined under the pre-AIA first to invent provisions.

This office action is in response to an amendment filed 4/26/2021.

Claims 25-33 and 35-45 are pending.

This application is a Divisional Application of U.S. Application No. 14/776,106, filed September 14, 2015, now U.S. Patent 10,137,223, which is a U.S. National Phase Application of PCT International Application PCT/US2014/025636, filed March 13, 2014, which claims priority benefit of U.S. Application No. 61/785,031, filed March 14, 2013 the disclosures of which are incorporated herein in their entirety.

Response to Amendments

Information disclosure statements filed 2/24/15 and 1/8/16 have been identified and the documents considered. The corresponding signed and initialed PTO Form 1449 has been mailed with this action. The documents listed as Search Reports and office actions *have been considered* but have been crossed off the 1449 so that it will not appear on the face of any patent issuing from the instant application.

Claim Objections

Claims 25, 31 is objected to because of the following informalities: in claim 25, each of "tendon" and "ligament" requires the article "a". In claim 31, the preposition "in" is missing prior to "*in vitro*" in line 7.

For consistency, claims38-40 and 43-45 should refer to "the" tissue, ligament or nerve

defect.

Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of 35 U.S.C. 112(b):
(b) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:
The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 25-33 and 35-45 are rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor (or for applications subject to pre-AIA 35 U.S.C. 112, the applicant), regards as the invention.

The dependent claims are included in the rejection because they fail to address or clarify the basis of the rejection as discussed in detail for the independent claims.

Claim 25 lacks proper antecedent basis for the term “the fibers” in line 2. There is insufficient antecedent basis for this limitation in the claim.

Claim Rejections - 35 USC § 112, first paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 25-33 and 35-45 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of treating a subject with a tissue defect *that is a*

tendon, a ligament or a nerve defect, the method comprising surrounding the tissue defect with a scaffold by implantation thereof wherein the scaffold comprises *at least two fibers* that are cross-linked wherein the *at least two fibers* comprise collagen, wherein FFT analysis of the scaffold demonstrates that the *at least two fibers* have major adjacent peaks that are about 180^0 apart from each other ***or*** are anisotropic *wherein autologous cells for treatment of the tendon, ligament or nerve defect are seeded on the scaffold and the implantation of the seeded scaffold* treats the tissue defect, the ligament defect or the nerve defect;

or a method of cell culture (or differentiation), the method comprising placing *the cells* on the scaffold wherein the scaffold comprises at least two fibers that are cross-linked wherein the fibers comprise collagen and growing the cells in condition suitable for the cell culture (or the differentiation), wherein FFT analysis of the scaffold demonstrates that the at least two fibers have major adjacent peaks that are about 180^0 apart from each other comprising collagen, does not reasonably provide enablement for any other embodiment. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make or use the invention commensurate in scope with these claims. **This rejection is based in part on the previous rejection. However, the amendment to recite that the scaffold comprises one or more cross-linked fibers has led to new rejections. The italicized portions are at issue with the claims.**

The test of enablement is whether one skilled in the art could make and use the claimed invention from the disclosures in the patent coupled with information known in the art without undue experimentation (*United States v. Telectronics, Inc.*, 8 USPQ2d 1217 (Fed. Cir. 1988)). Whether undue experimentation is required is not based on a single factor but is rather a conclusion reached by weighing many factors (See *Ex parte Forman*, 230 USPQ 546 (Bd. Pat.

App. & Inter, 1986) and In *re Wands*, 8USPQ2d 1400 (Fed. Cir. 1988); these factors include the following:

The claims have been evaluated in light of the art at the time of filing and found not to be commensurate in scope with the specification. The MPEP teaches, "However, claims reading on significant numbers of inoperative embodiments would render claims non- enabled when the specification does not clearly identify the operative embodiments and undue experimentation is involved in determining those that are operative. Atlas Powder Co. v. E.I. duPont de Nemours & Co., 750 F.2d 1569, 1577, 224 USPQ 409, 414 (Fed. Cir. 1984); In re Cook, 439 F.2d 730, 735, 169 USPQ 298,302 (CCPA 1971). (see MPEP 2164.08(b).

1) Nature of invention. Instant claim 25 and 26 are drawn to methods of treating a subject with a tissue defect or a gap in a tissue by use of scaffolds. The nature of the invention is tissue engineering. Claims 27 and 31 are drawn to *in vitro* cell culture or differentiation.

2) Scope of the invention. The scope of the invention is fairly broad in that the claims do not indicate the material comprising the scaffold nor do the claims indicate to where the treatment and application are performed.

3) Number of working examples and guidance. Applicants teach construction of a scaffold comprised of heart basement membrane and polycaprolactone wherein dorsal root ganglion were seeded thereon leading to development of neurite outgrowths (example 2 and 3).

4) State of the art. The art of tissue engineering has been under examination since 1988 and as set forth by Hutmacher et al is a multidisciplinary art (see page 245).

The state of the art just before the time of fling was such that the predictability was quite low (Werner et al).

> Reviewing the literature, it can be concluded that it is by no means clear what defines an ideal scaffold–cell or scaffold–neotissue construct, even for a specific tissue type. Since some tissues

> perform multiple functional roles, it is unlikely that a single scaffold would serve as a universal foundation for the regeneration of even a single tissue. In this context it is salutary to recall one of the many definitions of biocompatibility of materials, which determines the suitability of a particular material, within the context of the functional role played by the materials following implantation. The considerations for scaffold design are hence complex and include material composition, architecture, structural mechanics, surface properties, degradation properties and products, together with the composition of any added biological components and, of course, the changes in all of these factors with time.
>
> For each envisioned application, successful TECs will have certain minimum requirements for biochemical and physical properties. Scaffolds may be required to provide sufficient initial mechanical strength and stiffness to substitute for the mechanical function of the diseased or damaged tissue which the TEC aims at repairing or regenerating.

Even into 2019, this predictability was still of concern. Sharma taches, 2019, page 1.

> These tissue-engineered constructs can be helpful in restoring and repairing malfunctioned tissues and organs at the high social and economic point of view (WHO Scientific Group, 2003; Kang et al., 2015). It is challenging to incorporate these bioengineered materials due to limitations of onsite target and side effects such as cell toxicity, interfering with immune systems, and transporting mechanisms. Also, designing and developing a system based on biological features such as mechanical stress at the targeted site, strength, complex viscoelastic, nonlinear, and anisotropic mechanical features have constantly been an area of consideration, as illustrated in **Figure 1**.

Not all disorders and tissues operate the same. .

> The accuracy and efficiency of bioengineered constructs are very challenging, as they require a deep understanding with regard to mechanisms of regeneration. New treatment approaches are required, as designing new nanostructures and constructs can provide more precise, target-specific scaffolds with varying functionalities. For example, only drugs and/or heart transplantation solutions are available for cardiac diseases. In summary, bio-mimicked constructs possess advanced architectures and surface topography like native tissues and organs to recover intrinsic properties of damaged tissues and organs.

At the time of filing use of PCL was quite limited as a scaffold for *in* vivo delivery. Use was typically limited to fabrication of 3D structures in culture (see e.g. Dash and Konkimalla). There is some discovery of use as a bone scaffold (see page 251 if Hutmacher et al and Table 3 Dash and Konkimalla).

5) Unpredictability of the art. The physiological art is recognized as unpredictable. (MPEP 2164.03.) In cases involving predictable factors, such as mechanical or electrical elements, a single embodiment provides broad enablement in the sense that, once imagined,

other embodiments can be made without difficulty and their performance characteristics predicted by resort to known scientific laws. In cases involving unpredictable factors, such as most chemical reactions and physiological activity, the scope of enablement obviously varies inversely with the degree of unpredictability of the factors involved.

Amount of experimentation required. The art of tissue engineering at the time of filing was an improving art but still wrought with a number of obstacles as set forth above. This is complicated by the claims which have gaps. First, the claims build a scaffold with "one or more crosslinked fibers". It is not clear how to crosslink one fiber and how to have major peaks form one fiber. As well, the claims require implantation of the scaffold and then surrounding of the tissue defect. It is not clear how both steps are accomplished. If the scaffold is implanted than it is not clear how it can then be used to surround the defect. Third, the specification and the art teach that for repair of defects such as tendon, ligament and nerve, cells are required to build back the defect. However, the claims simply require use of a scaffold comprising fibers. There is no indication that the fibers are provided for any other reason than a support for growth of the cells.

Consequently, the prior art (and post-filing art) when combined with the lack of any disclosed direct experimental test of Applicant's hypothesis, shows that one of skill in the art at the time the invention was made would have had no basis to reasonably predict or conclude the claimed sequences could be identified given the lack of details necessary to identify those meeting the necessary functions. Though not controlling, the lack of working examples, is, nevertheless, a factor to be considered in a case involving both physiological activity and an undeveloped art. When a patent applicant chooses to forego exemplification and bases utility on broad terminology and general allegations, he runs the risk that unless one with ordinary skill in

the art would accept the allegations as obviously valid and correct, the PTO may, properly, ask for evidence to substantiate them. Ex parte Sudilovsky, 21 USPQ2d 1702, 1705 (BPAI 1991); In re Novak, 134 USPA 335 (CCPA 1962); In re Fouche, 169 USPQ 429 (CCPA 1971).

Given the generic nature of the claims, Given the lack of guidance in the specification, the large and diverse group of administration recited and the highly unpredictable nature of the art, it is concluded that a person of skill in the art would have had to conduct undue experimentation in order to practice the claimed invention.

Claim Rejections - 35 USC § 102

In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of the statutory basis for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

The following is a quotation of the appropriate paragraphs of pre-AIA 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claim 26 is rejected under pre-AIA 35 U.S.C. 102(b) as being anticipated Patel et al (WO 2012078472) and Simpson et al (US 20120221025; see entire document). **This is a new rejection necessitated by applicant's amendment.**

Simpson et al teach construction of fibrous scaffolds (see e.g. ¶0035) which is anisotropic (see e.g. ¶0011). The fibers comprise collagen (see e.g. 0049). These are used to repair nerve damage (see e.g. abstract). The fiber anisotropy is measured using Transform analysis (see e.g. ¶0100).

Yeo et al teach construction of fibrous scaffolds with collagen (see e.g. ¶0189) which is anisotropic (see e.g. ¶0323). These are used to repair tendon, ligament or nerve damage (see e.g. ¶0168). As evidenced above, the fiber anisotropy is measured using Fourier Transform analysis.

Double Patenting

A rejection based on double patenting of the "same invention" type finds its support in the language of 35 U.S.C. 101 which states that "whoever invents or discovers any new and useful process ... may obtain a patent therefor ..." (Emphasis added). Thus, the term "same invention," in this context, means an invention drawn to identical subject matter. See *Miller v. Eagle Mfg. Co.*, 151 U.S. 186 (1894); *In re Ockert*, 245 F.2d 467, 114 USPQ 330 (CCPA 1957); and *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970).

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970);and, *In re Thorington,* 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b).

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claim 26 is provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-25 of copending Application No.

16/989,307 in view of Patel et al (WO 2012078472) and Simpson et al (US 20120221025; see entire document). **This is a new rejection necessitated by applicants' amendment.**

Although the conflicting claims are not identical, they are not patentably distinct from each other because both sets of claims are drawn to scaffold wherein the fibers are analuyzd by FFT for anistropic. Such scaffolds comprising thereof are known to be used for culturing and for treatment of defects as demonstrated by Patel and Simpson. Furthermore, the prohibition against obviousness double patenting is moot as the parent restriction has been withdrawn and no other safe harbor has been established.

Additionally, if a patent resulting from the instant claims was issued and transferred to an assignee different from the assignee holding the copending Application No. 16/989,307, then two different assignees would hold a patent to the claimed invention of copending Application No. 16/989,307, and thus improperly there would be possible harassment by multiple assignees.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Conclusion

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period

will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to MARIA MARVICH whose telephone number is (571)272-0774. The examiner can normally be reached on 8 am - 5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christopher Babic can be reached on 571-272-8507. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/MARIA MARVICH/
Primary Examiner, Art Unit 1633
90x

Notice of References Cited	Application/Control No. 16/045,159	Applicant(s)/Patent Under Reexamination Francis et al.	
	Examiner MARIA MARVICH	Art Unit 1633	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
*	A	US-20120221025-A1	08-2012	Simpson; David	A61F2/02	606/152
	B					
	C					
	D					
	E					
	F					
	G					
	H					
	I					
	J					
	K					
	L					
	M					

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
	N	WO 2012078472	06-2012	WIPO	Patel et al	
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
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	X	

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Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/045,159	Francis et al.
	Examiner	Art Unit
	MARIA MARVICH	1633

CPC - Searched*		
Symbol	Date	Examiner

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

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Search Notes		
Search Notes	Date	Examiner
SEARCH- USPAT, PGPUB, EPO, JPO, Derwent, USOCR, IBM-IDB	12/17/2020	MM
inventor search	12/17/2020	MM
npl search	12/17/2020	MM
search updated, search notes attached	07/13/2021	MM

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 June 2012 (14.06.2012)

WIPO | PCT

(10) International Publication Number
WO 2012/078472 A2

(51) International Patent Classification:
A61L 27/44 (2006.01) *DOW 5/00* (2006.01)
B32B 27/02 (2006.01) *A61F 2/02* (2006.01)
A61L 27/38 (2006.01)

(21) International Application Number:
PCT/US2011/063142

(22) International Filing Date:
2 December 2011 (02.12.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/419,869 5 December 2010 (05.12.2010) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

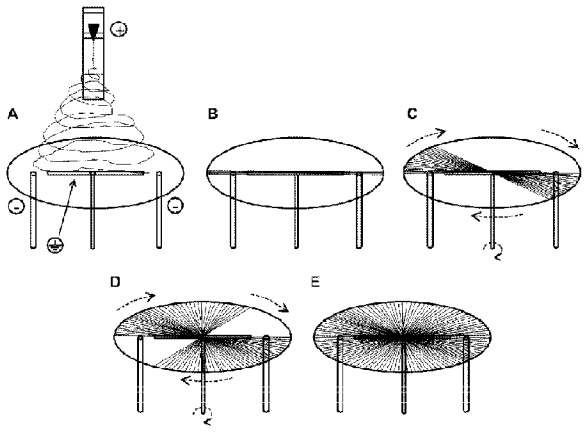
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

(54) Title: FIBROUS POLYMER SCAFFOLDS HAVING DIAMETRICALLY PATTERNED POLYMER FIBERS

FIGURE 5



(57) Abstract: Fibrous polymer scaffolds having at least one layer of diametrically patterned fibers, as well as methods of manufacture, are provided. The scaffolds can be formed of multiple layers and the extent and direction of alignment of each layer can be controlled. Fabrication systems and methods for producing such scaffolds including apparatuses and processes are also provided.

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FIBROUS POLYMER SCAFFOLDS HAVING DIAMETRICALLY PATTERNED POLYMER FIBERS

CROSS-REFERENCE TO RELATED APPLICATIONS

[001] This application claims the benefit of U.S. Provisional Patent Application No. 61/419,869 filed December 5, 2010, which is incorporated by reference herein in its entirety.

FIELD

[002] The disclosure relates to fibrous scaffolds with at least one layer of diametrically patterned polymer fibers finding application as medical devices for the repair of wounds, surgical incisions and/or biopsies and for promoting and/or improving the regeneration of anatomical biological components *(i.e.,* biological tissues including, without limitation, abdominal wall, amniotic sac, bladder, blood vessels, brain, cardiac tissue, fascia, gingival tissue, intestine, ligaments, meningeal tissue, mucous membranes, muscle, nerves, skin, spinal cord, stomach, tendons, etc.).

BACKGROUND

[003] Electrospinning is a technique known in the art for producing articles composed of polymer fibers. See U.S. Patent No. 4,323,525. More recently, U.S. Patent No. 7,276,271 disclosed a fibrous polymer tubular structure. Substrates with nanoscale aligned geometries are disclosed in U.S. Patent No. 6,676,675. The use of electrospun fibrous polymer grafts in medical applications is disclosed in U.S. Patent No. 7,172,765.

[004] Modified electrospinning methods for forming directed fibers are also disclosed in U.S. Patent No. 7,135,134. PCT Publication Nos. WO 2007/146261 and WO 2007/090102 disclose various aspects of polymer fibrous scaffold sheets.

SUMMARY

[005] There is a need in the art for fibrous polymer scaffolds having at least one layer of diametrically patterned polymer fibers for promoting rapid tissue growth across tissue defects, and for providing flexibility and tensile strength in multiple directions. Such scaffolds provide stability in a wide range of applications, particularly biomedical applications. Further, there is a need for apparatuses designed to manufacture such scaffolds. The present disclosure addresses these and other needs.

[006] Fibrous polymer scaffolds having at least a first layer of diametrically patterned polymer fibers are disclosed. Fibrous polymer scaffolds with at least one layer of diametrically patterned fibers may be used to repair, heal and/or regenerate wounded tissues

and organs. These uses can include, without limitation, uses as tissue substitutes and/or tissue regeneration matrices for wounds and/or defects in biological tissues including, without limitation, abdominal wall, amniotic sac, bladder, blood vessels, brain, cardiac tissue, fascia, gingival tissue, intestine, ligaments, meningeal tissue, mucous membranes, muscle, nerves, skin, spinal cord, stomach, tendons, etc., uses in combination with cells, uses in combination with biomolecules, and uses in combination with pharmaceutically acceptable excipients. In various embodiments, the diametrically patterned fiber layer of the fibrous polymer scaffold is placed in contact with a tissue defect or wound. In various embodiments the convergence region of the diametrically patterned fiber layer is positioned over the center of the tissue defect or wound. The diametrically patterned fibers direct cell and tissue growth from the entire periphery of the defect toward the center.

[007] In various aspects, the fibrous polymer scaffolds have a second layer that can include aligned, unaligned or randomly oriented fibers. In various aspects, the scaffolds can include a plurality of layers including, without limitation, three, four, five, six, seven, eight, nine, ten or more layers.

[008] The polymer scaffolds of the disclosure can be formed into a variety of shapes. In various aspects, the polymer scaffolds can be in the shape of a membrane. The membrane may take any desired geometrical shape including, without limitation, a square, rectangle, circle, oval and other shapes as in **Fig.** 1. The shape of the scaffolds may be designed to correspond to a specific tissue or specific tissue defect.

[009] Additional layers can be formed from different polymers than the first layer, or the same polymer as the first layer. In some embodiments, the polymer fibers of the first layer and the polymer fibers of the secondary layers comprise a single, unbroken polymer fiber. In some embodiments, the polymer fibers of the first layer and the polymer fibers of the secondary layers comprise multiple, unbroken polymer fibers. In some embodiments, at least one polymer fiber in the first layer and at least one polymer fiber in the secondary layers is the same, continuous polymer fiber. In some embodiments, the fibers of the first layer and the fibers of the secondary layers comprise two polymers present in two continuous fibers. In some embodiments, the fibers of the first layer and the fibers of the secondary layers comprise three polymers present in three continuous fibers. In some embodiments, the fibers of the first layer and the fibers of the secondary layers comprise four polymers present in four continuous fibers. In some embodiments, the fibers of the first layer and the fibers of the secondary layers comprise five polymers present in five continuous fibers.

[0010] In some embodiments, the first layer is formed from diametrically patterned polymer fibers. The secondary layers can be formed from aligned polymer fibers, or from polymer fibers that are substantially unaligned or randomly oriented. In those embodiments where the secondary layers are formed from aligned polymer fibers, the orientation of the aligned fibers of the secondary layers can be offset from the alignment of the fibers of the first layer.

[0011] In various aspects, the scaffold ranges from about 100 microns to about 500 microns thick. In some embodiments, the thickness of the first layer is less than the thickness of the secondary layers. In some embodiments, the thickness of the first layer is greater than about 1 micron and less than about 70 microns thick. In some embodiments, the thickness of the first layer is greater than about 70 microns and less than about 150 microns thick. In some embodiments, the thickness of the first layer is greater than about 150 microns and less than about 300 microns thick. In some embodiments, the thickness of the secondary layers of fibers is greater than about 150 microns and less than about 330 microns thick. In some embodiments, the total thickness of the scaffold is about 100 to about 200 microns, about 150 to about 250 microns, about 300 to about 400 microns, or about 500 microns. In some embodiments, the total thickness of the scaffold is greater than about 500 microns.

[0012] In various aspects, the polymer fibers of the first layer and the polymer fibers of the secondary layers are comprised of biodegradable polymers.

[0013] In various aspects, the polymer fibers of the fibrous polymer scaffolds can further optionally comprise an additive selected from the group consisting of poly(propylene glycol), poly(ethylene oxide), triethyl citrate, glycerol, poly(ethylene glycol), and combinations thereof.

[0014] In various aspects, the polymer fibers of the scaffolds can further optionally comprise a salt. In some embodiments, the salt can be sodium acetate.

[0015] In various aspects, kits for the repair of wounds, surgical incisions or biopsies are provided. In some embodiments, the kits comprise at least one fibrous polymer scaffold and instructions for using the scaffold to repair wounds, surgical incisions and/or biopsies.

[0016] In various aspects, methods of implanting fibrous polymer scaffolds are provided. In some embodiments, the methods are directed toward treating an injury or defect in a subject and comprise applying a scaffold to an injury or defect site on or in said subject, in an amount, and under conditions, sufficient to treat the injury.

[0017] The polymer fibers comprising any one or more of the layers can comprise a material selected from an aliphatic polyester, a poly(alkylene) oxide, poly(L-lactic acid), 70/30 L-

lactide/e-caprolactone co-polymer, poly(caprolactone), poly(DL-lactide-co-caprolactone), poly(D-lactide-co-caprolactone), poly(L-lactide-co-caprolactone), poly(lactide-co-glycolic acid), poly(vinylpyrrolidine), poly(dimethylsiloxane), poly(lysine), collagen, laminin, fibronectin, elastin, alginate, fibrin, hyaluronic acid, proteoglycans, polypeptides, poly(ethylene-co-vinyl) alcohol, poly(vinyl) alcohol, poly(ethylene) glycol, poly(propylene) glycol, poly-L-lactide-co-glycolide-co-e-caprolactone, poly(tetrafluoroethylene), poly(dioxanone), polyglactin 910 and combinations thereof.

[0018] The aliphatic polyester can be selected from D-lactide, L- lactide, poly(lactic acid), poly(lactide) glycolic acid, poly(glycolic acid), poly(glycolide), glycolide, poly(lactide-co-glycolide), poly(lactic acid-co-glycolic acid), epsilon-caprolactone, poly(epsilon-caprolactone) and a combination thereof. The poly(alkylene) oxide can be selected from poly(ethylene) oxide and poly(propylene) oxide.

[0019] The polymer fibers of the first layer and the polymer fibers of the second layer can comprise poly(L-lactide-co-caprolactone). The polymer fibers of the first layer and the polymer fibers of the second layer can comprise poly(L-lactic acid). The polymer fibers of the first layer and the polymer fibers of the second layer can comprise poly(L-lactide-co-caprolactone) and poly(L-lactic acid). One of a plurality of continuous fibers can comprise poly(L-lactide-co-caprolactone) and another of a plurality of continuous fibers can comprise poly(L-lactic acid).

[0020] The fibrous polymer scaffolds can also comprise an additive. The additive can be selected from poly(propylene glycol), poly(ethylene oxide), triethyl citrate, glycerol, poly(ethylene glycol), and combinations thereof. The additive can be poly(propylene glycol). The additive can be present in an amount ranging from about 0.1% to about 10% (wt%) of the total weight of the polymer. The additive can be present in an amount selected from about 1.0% to about 2.0%, about 2.0% to about 3.0%, about 3.0% to about 4.0%, about 4.0% to about 5.0%, about 5.0% to about 6.0%, about 6.0% to about 7.0%, about 7.0% to about 8.0%, about 8.0% to about 9.0%, and about 9.0% to about 10.0% of the total weight of the polymer.

[0021] In various aspects, kits for the repair of wounds, surgical incisions or biopsies are disclosed, the kits comprising (i) a fibrous polymer scaffold comprising at least a first layer of diametrically aligned polymer fibers and a second layer of polymer fibers, wherein the fibers of the second layer are substantially unaligned, and wherein at least one fiber in the first layer of fibers and at least one fiber in the second layer of fibers is the same continuous fiber; and (ii) instructions for using the scaffold to repair wounds, surgical incisions or biopsies by

promoting the regeneration of anatomical biological components. The anatomical biological components can be selected from abdominal wall, amniotic sac, bladder, blood vessels, brain, cardiac tissue, fascia, gingival tissue, intestine, ligaments, meningeal tissue, mucous membranes, muscle, nerves, skin, spinal cord, stomach, and tendons. The first layer of fibers of the scaffold and the second layer of fibers of the scaffold can comprise a single continuous fiber. The first layer of fibers of the scaffold and the second layer of fibers of the scaffold can comprise two continuous fibers. The fibers of the second layer of the scaffold can have a random orientation. The fibers of the second layer can be aligned, in which case they will be offset from the diametrical alignment of the fibers of the first layer. The first layer of the scaffold can be the innermost layer and the second layer can be the outermost layer. The scaffold can be a membrane.

[0022] In various aspects, methods of implanting fibrous polymer scaffolds are provided, the methods comprising: contacting a defect in a tissue of a subject with a single layer of a fibrous polymer scaffold, the scaffold comprising at least a first layer of diametrically patterned polymer fibers. The defect can be selected from wounds, surgical incisions and biopsies. The contacting can comprise placing the scaffold over the defect. The contacting can comprise placing the scaffold completely within the defect. The contacting can comprise placing the scaffold underneath the defect. The scaffold can be secured in place at the defect. The securing can comprise suturing the scaffold and/or gluing the scaffold.

[0023] In various aspects, fibrous polymer scaffolds are disclosed comprising at least a first layer of diametrically aligned polymer fibers and a second layer of polymer fibers having a different alignment than the fibers in the first layer. At least one fiber in the first layer of fibers and at least one fiber in the second layer of fibers can be the same continuous fiber. The first and second layers of fibers can comprise a single continuous fiber. The scaffold can be a membrane. The first layer of diametrically aligned fibers can be greater than 1 micron and less than 70 microns thick. The first layer of diametrically aligned fibers can be greater than 70 microns and less than 150 microns thick. The first layer of diametrically aligned fibers can be greater than 150 microns and less than 300 microns thick. The second layer of diametrically aligned fibers can be greater than 150 microns and less than 330 microns thick. The total thickness of all layers can be greater than 20 microns and less than 150 microns. The total thickness of all layers can be greater than 150 microns and less than 250 microns. The total thickness of all layers can be greater than 250 microns and less than 300 microns. The total thickness of all layers can be greater than 300 microns and less than 400 microns.

The second layer can comprise fibers uniaxially aligned in parallel with an axis of the fibrous scaffold.

[0024] The fibers can comprise a material selected from the group consisting of aliphatic polyesters, polyhydroxyalkanoates, polyurethanes, polyalkylene oxides, polydimethylsiloxane, polyvinylalcohol, polyvinylpyrrolidone, polylysine, collagen, gelatin, laminin, fibronectin, elastin, alginate, fibrin, hyaluronic acid, proteoglycans, polypeptides, polysaccharides and combinations thereof. The aliphatic polyester can be selected from the group consisting of D-lactide, L- lactide, poly(lactic acid), poly(lactide) glycolic acid, poly(glycolic acid), poly(glycolide), glycolide, poly(lactide-co-glycolide) or PLGA, poly(lactic acid-co-glycolic acid), epsilon-caprolactone, poly(epsilon-caprolactone), and combinations thereof. The aliphatic polyester can be poly(L-lactide). The aliphatic polyester can be poly(L-lactide-co-caprolactone.

[0025] The scaffolds can further comprise an additive. The additive can be selected from triethyl citrate, glycerol, poly(ethylene glycol), poly(propylene glycol), glycerol, and combinations thereof. The additive can be from 0.01% to 25% of the weight of the polymer.

[0026] The fibrous polymer scaffolds can comprise a salt selected from NaCl, KH2P04, K2HP04, KI03, KC1, MgS04, MgC12, NaHC03, CaC12, salt of acetic acid, salt of ascorbic acid, salt of citric acid, salt of lactic acid, salt of glycolic acid, and mixtures thereof.

[0027] In other aspects, apparatuses for making fibrous polymer scaffolds are provided. In some embodiments, an apparatus for making fibrous polymer scaffolds is provided that includes a rotational collector assembly having an electrically insulating disk in front of and conformally contacting a thin grounded metal strip extending across the diameter of the disk and two electrically charged point electrodes positioned equidistant from the center of the disk. A charged electrically conducting spinneret configured to release a polymer solution onto the rotating disk is positioned normal disk surface.

[0028] In various aspects, the rate of revolution of the rotational assemblies of the apparatuses can be varied. In some embodiments, the rotational assemblies are rotated at a rate of greater than 1 revolution per minute (RPM), greater than 5 RPM, greater than 20 RPM, greater than 100 RPM, greater than 800 RPM, greater than 1,000 RPM, greater than 2,000 RPM, greater than 3,000 RPM, greater than 4,000 RPM, or greater than 5, 000 RPM during the formation of the layers. In some embodiments, the rotational assemblies are rotated at a rate of less than 5, 000 RPM, less than 4,000 RPM, less than 3,000 RPM, less than 2,000 RPM, less than 1,000 RPM, less than 800 RPM, less than 100 RPM, less than 20 RPM, less than 5 RPM, or less than 1 RPM during the formation of the layers. In some

embodiments, the rotational assemblies are rotated during the formation of at least one layer of fibers at a rate selected from 1 RPM, 5 RPM, 10 RPM, 15 RPM, 20 RPM, 25 RPM, 30 RPM and 35 RPM. In some embodiments, the rotational assemblies are rotated at a rate of 20 RPM during the formation of at least one layer of fibers.

[0029] The rotational assembly can be positioned a distance of 5 cm to 30 cm from a spinneret from which a polymer solution is released. The spinneret can move parallel to the diametrical axis of the rotational assembly during formation of at least one layer. The spinneret can traverse the length parallel to the diametrical axis of the electrospinning assembly at a rate of lcm/min.

[0030] In some embodiments, the apparatuses are configured for the polymer-containing spinneret to move laterally to the diametrical surfaces of the rotational assemblies during formation of one or more layers.

BRIEF DESCRIPTION OF THE DRAWINGS

[0031] **Fig. 1** depicts examples of membranes according to the present disclosure of various shapes and having diametrically patterned fibers. This figure also provides examples of a convergence region.

[0032] **Fig. 2** depicts an electrospinning assembly with a rotational disk collector according to the present disclosure.

[0033] **Fig. 3A** is a simple disk rotational assembly for collecting a first layer of unaligned polymer fibers produced by an electrospinning process according to the present disclosure. **A** resulting unaligned nanofiber membrane is also depicted.

[0034] **Fig. 3B** depicts a front view and a side view of another rotational disk assembly for collecting a first layer of diametrically patterned nanofibers produced by an electrospinning process according to the present disclosure. The process incorporates electrically charged conductive arms near the spinneret to restrict lateral whipping of the nanofiber and also uses electrically charged or grounded conductive components in contact with the back of the collecting disk to guide diametrical nanofiber deposition.

[0035] **Fig. 4** depicts another rotational disk assembly for collecting a first layer of diametrically patterned nanofibers according to the present disclosure. The figure shows that, in certain embodiments, a grounded conductive element may either be a thin conductive strip located behind the rotating disk or a conductive circular disk element affixed to the front of the large rotating disk.

[0036] **Fig. 5A-E** depicts an example of a step-by-step process in which diametrically patterned nanofibers are deposited evenly on a collector assembly via rotation of the collector according to the present disclosure.

[0037] **Fig. 6A-C** depicts an example of a secondary layer of unaligned fibers being deposited onto a first layer of diametrically patterned fibers according to the present disclosure.

[0038] **Fig. 6D** Depicts an example of a nanofiber membrane having a first layer of diametrically patterned fibers and a second layer of unaligned fibers according to the present disclosure.

[0039] **Fig. 7A** depicts scanning electron microscopy images of nanofibers from a membrane having a layer of diametrically patterned fibers made according to Example 6 of the present disclosure.

[0040] **Fig. 7B** depicts scanning electron microscopy images of nanofibers from a membrane having a layer of unaligned (i.e. random) fibers made according to Example 6 of the present disclosure.

[0041] **Figs. 8A, 8B and 8C** depict fluorescent microscopy images of cellular migration and wound coverage of mesenchymal stem cells on nanofiber membranes in an *in vitro* wound healing experiment performed pursuant to Example 11. Cells were seeded onto membranes having different types of nanofiber alignment (8A: random, 8B: uniaxial, 8C: diametrical) and were allowed to migrate into an induced gap defect. Fluorescent staining of the cellular cytoskeleton shows enhanced wound coverage on membranes with diametrically patterned fibers.

[0042] **Fig. 9A** depicts test samples used for mechanical testing of nanofiber membranes with varying degrees of alignment according to Example 12 of the present disclosure.

[0043] **Fig. 9B** depicts the mechanical properties of the nanofiber membrane test samples shown in **Fig. 9A** as determined by tensile testing according to Example 12 of the present disclosure.

DETAILED DESCRIPTION

Definitions

[0044] "Aligned" refers to the orientation of fibers in a fibrous polymer scaffold wherein at least 80% of the fibers are oriented in a defined direction and their orientation forms either a single axis or multiple axes of alignment. The orientation of any given fiber can deviate from a given axis of alignment and the deviation can be expressed as the angle formed between the

alignment axis and orientation of the fiber. A deviation angle of 0° exhibits perfect alignment with the given axis and 90° (or -90°) exhibits orthogonal alignment of the fiber with respect to the given axis of alignment. When multiple axes of alignment exist in a given layer, the alignment of a particular fiber is determined in relation to its closest axis. In exemplary embodiments, the standard deviation of the aligned fibers from their closest axes of alignment can be an angle selected from between 0° and 1°, between 0° and 3°, between 0° and 5°, between 0° and 10°, between 0° and 20°, or between 0° and 25°. Where the fibers of a layer of a fibrous polymer scaffold are comprised of a single fiber that loops over itself, the term "aligned" refers to the portion of the fiber spanning either between two points on the periphery of the membrane or between a point on the periphery and a point in a defined region of the membrane, such as a fiber convergence region.

[0045] The term "convergence region," as used herein in the context of a fibrous polymer scaffold, refers to an area through which at least 80% of diametrical fibers in the scaffold intersect. A convergence region does not necessarily refer to the center or middle of the scaffold. An example of a convergence region is depicted in **Fig. 1.**

[0046] The term "diametrical axis," as used herein in the context of a fibrous polymer scaffold, refers to any straight axis that spans between two discrete points located on the periphery of the scaffold while also passing through a defined convergence region located within the boundary of the periphery.

[0047] The term "diametrical fiber," as used herein in the context of a fibrous polymer scaffold, refers to a fiber within the scaffold that spans two points on the periphery of the scaffold and is aligned along a diametrical axis of the scaffold.

[0048] The term "diametrically patterned" or "diametrically aligned," as used herein in the context of a fibrous polymer scaffold, refers to a plurality of diametrical fibers that are oriented along individual diametrical axes sharing a defined convergence region within the scaffold. In a diametrically patterned scaffold, there are multiple diametrical axes that pass through a defined convergence region.

[0049] The term "membrane," as used herein, refers to a fibrous polymer scaffold that is essentially flat, or planar in shape. As used herein, a membrane has at least a first layer of fibers and may or may not have additional layers of fibers. In varying embodiments, a membrane can have more than two layers of fibers. Each layer of a membrane may have a different orientation of fiber alignment.

[0050] The term "periphery," as used herein in the context of a fibrous polymer scaffold, refers to the boundary line encompassing the plane of the scaffold. The periphery of a scaffold is not necessarily circular.

[0051] The term "uniaxially aligned," as used herein in the context of a fibrous polymer scaffold, refers to aligned fibers that are oriented parallel to a single axis of alignment in a scaffold.

[0052] Reference is now made in detail to certain embodiments of scaffolds, methods of use, and methods of making. The disclosed embodiments are not intended to be limiting of the claims. To the contrary, the claims are intended to cover all alternatives, modifications, and equivalents.

Diametrically Patterned Polymer Fibers

[0053] Fibrous polymer scaffolds of this disclosure comprise at least one layer of diametrically patterned polymer fibers. The current disclosure comprises diametrical fibers that each span two edges of the scaffold and also pass through a convergence region with no bends or discontinuities of the fibers at the convergence region. Diametrical patterning of a plurality of fibers within the fibrous polymer scaffold results in diametrical fibers sharing a defined convergence region within the scaffold. The diametrical fibers are thereby arranged in a pattern in which the fibers appear to radiate from the convergence point toward the periphery of the scaffold.

[0054] Diametrical patterning within a scaffold is distinguished from radial patterning or radial alignment wherein the radially aligned fibers span straight across only one edge and the convergence region within the scaffold and may have discontinuities at the convergence region. A radially aligned fiber only spans a location between the center, or convergence region, of a scaffold and the periphery of a scaffold. Radially aligned fibers thus do not cross the entire scaffold. In this regard, because of the electrospinning process, scaffolds comprising radially aligned fibers may have discontinuities at the convergence region. In contrast, a diametrically aligned fiber crosses the entire scaffold, from one discrete point on the periphery of the scaffold to another, and also passes across a convergence region of the scaffold. Fibrous polymer scaffolds provided according to the current disclosure comprise diametrically aligned fibers and thus may be expected to demonstrate higher tensile strength than fibrous polymer scaffolds comprising radially aligned fibers. This is because diametrical fibers are diametrically aligned straight through a convergence region in a scaffold, whereas radially aligned fibers loop back over themselves within the convergence region, thereby producing a fiber structure with reduced organization of the tensile elements

in that region. Diametrically patterned fibrous polymer scaffolds may also be expected to promote enhanced wound healing and cell migration characteristics when compared to radially aligned fibrous polymer scaffolds, particularly near the convergence region, because the diametrical fibers are diametrically aligned straight through the convergence region whereas radially aligned fibers may have discontinuities, thereby reducing overall alignment in that region.

[0055] Fibrous polymer scaffolds can be made through electrospinning, as described herein. The individual fibers within one or more layers of the scaffolds can be diametrically aligned during electrospinning, as described herein. In several aspects, the individual polymer fibers comprising one or more layers of the scaffolds are diametrically aligned during electrospinning.

Diametrically Patterned Fibrous Polymer Scaffolds

[0056] The present disclosure relates to a fibrous polymer scaffold with one or more layers of fibers wherein at least one layer comprises diametrically patterned fibers. In some embodiments, the fibrous polymer scaffold may be in the shape of a membrane or sheet. In some embodiments, the membrane or sheet may be a circle, oval, rectangle, square or other shape. The present disclosure is generally directed to fibrous scaffolds having at least one layer of diametrically patterned polymer fibers, apparatuses and methods of their manufacture, methods of use, and kits comprising the scaffolds.

[0057] Scaffolds generally refer to, but are not limited to, membrane structures formed from polymer microfibers or polymer fibers as described herein. In various embodiments, greater than 50%, greater than 60%, greater than 70%, greater than 80%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98%, or greater than 99% of a scaffold is w/w comprised of the polymer fibers as described herein.

[0058] Scaffolds can include, without limitation, membrane structures formed from polymer microfibers or nanofibers as described herein. Diametrically patterned polymer scaffolds disclosed herein can be diametrically patterned fibrous polymer scaffolds, such as nanofiber polymer scaffolds. In various aspects, the fibrous polymer scaffolds can also be micropattemed polymer scaffolds. The scaffolds of the present disclosure contain at least one layer and can contain a plurality of layers. In some embodiments, the composition has between about 1 and about 10 fibrous layers. In various aspects, scaffolds can include more than two layers. In some embodiments, the first and second layers are adjacent, and one or more additional layers can be disposed on the outside of the second layer.

[0059] In various aspects, a single layer of fibers in a scaffold can be constructed from one, two, three, four, five, or more continuous, unbroken polymer fibers. Similarly, in various aspects a plurality of layers of fibers in a single scaffold can also be constructed from one, two, three, four, five or more continuous, unbroken polymer fibers. Each polymer fiber may be deposited via the use of a spinneret, as described herein. For example, when it is desired for a single layer of a scaffold to have diametrically patterned polymer fibers, the fiber can loop back upon itself at the edges and pass through the convergence region of the scaffold repeatedly. Where the diametrically aligned fibers are comprised of one, two, three or more continuous polymer fibers, each continuous fiber can loop back upon itself to form a diametrically aligned layer of fibers.

[0060] In some embodiments, the scaffolds can comprise multiple layers of fibers wherein the layers are differentiated by chemical composition or average fiber alignment within each individual layer. Scaffolds of multiple layers of polymer fibers may have multiple differential alignments for each layer, providing tensile strength, suture retention strength, elasticity, flexibility, and rigidity while minimizing delamination of the layers of fibers.

[0061] Each layer can contain fibers in a unique pattern or alignment, wherein each layer of the multilayer polymer scaffolds can optionally be unaligned, diametrically aligned, or aligned with a non-diametrical orientation. For example, at least one layer may contain diametrically patterned fibers and at least one further layer may contain randomly oriented fibers.

[0062] The layers of fibers in a single scaffold can be distinguished by differential alignment of the fibers composing each of the layers. Each layer of the multilayer polymer scaffolds can have polymer fibers that are unaligned, diametrically aligned, or aligned with a non-diametrical orientation. For example, the first layer of the scaffold membranes may comprise diametrically patterned fibers around a convergence region of the scaffold membrane. A second layer of fibers may comprise fibers that are uniaxially aligned or that are randomly oriented, thereby providing a difference in the alignment of the fibers between the two layers. The different alignment of polymer fibers between the different layers of the scaffolds provides additional mechanical integrity and tensile strength in multiple directions. In some embodiments, the multilayer scaffolds display greater structural integrity due to the presence of at least one layer comprising randomly oriented fibers. Randomly oriented fibers can provide mechanical integrity to the scaffold membranes by increasing its flexibility, tensility and/or suturability.

[0063] Each layer of fibers in a single scaffold may comprise diametrically aligned fibers or randomly oriented fibers. For example, in embodiments with at least two adjacent layers that are formed from continuous polymer fibers, each layer can comprise diametrically aligned fibers, each layer can comprise non-diametrically aligned fibers, each layer can comprise randomly oriented fibers, or the individual layers may comprise differing alignment. The layers of fibers can thus be distinguished by differential alignment of fibers composing the layers. For example, a first layer can comprise diametrically aligned fibers and a second layer of fibers can comprise unaligned fibers. In some embodiments, a first layer of a multilayer polymer scaffold comprises diametrically aligned fibers and a second layer of the scaffold comprises randomly oriented fibers, wherein the fibers of the first layer and the fibers of the second layer are formed from at least one continuous polymer fiber.

[0064] In various embodiments, a single layer of a scaffold can have an alignment which can be defined relative to at least one axis of the layer. In some embodiments, the scaffold has an asymmetrical shape such as an oval or rectangle. In some embodiments, a single layer of a scaffold having an asymmetrical shape can be defined relative to a long axis of the scaffold. In some embodiments, a single layer of a scaffold having an asymmetrical shape can be defined relative to a short axis of the scaffold. In some embodiments, a scaffold having an asymmetrical shape can have at least one layer of polymer fibers that are aligned essentially parallel to its long axis. In some embodiments, a scaffold having an asymmetrical shape can have at least one layer of polymer fibers that are aligned essentially parallel to its short axis. In some embodiments, fibers can be randomly aligned, or orthogonally aligned, with respect to an axis of the scaffold. In some embodiments, the alignment of fibers in a single layer of a scaffold having an asymmetrical shape may be at an angle relative to either the short axis of the scaffold, or the long axis of the scaffold. In some embodiments, the angle can be from 0° to 90°. In some embodiments, the angle can be from 10° to 80°. In some embodiments, the angle can be from 20° to 70°. In some embodiments, the angle can be from 30° to 60°. In some embodiments, the angle can be from 40° to 50°. In some embodiments, the angle can be 45°.

[0065] In various aspects, scaffolds can include more than two layers. In various aspects, scaffolds can comprise a plurality of layers of fibers. In some embodiments, the first layer of polymer fibers contacts the second layer of polymer fibers, or the first and second layers are adjacent, and one or more additional layers can be disposed on the outside of one of the two layers as a third layer, a fourth layer, a fifth layer, and so on. In other embodiments, one of the plurality of layers in a scaffold can be one or more intervening, non-electrospun, non-

polymer and/or non-fibrous layers. In various embodiments, an intervening layer can be an adhesive layer. In some embodiments, the adhesives can be any biocompatible adhesive known in the art. In some embodiments, an intervening layer can provide additional mechanical strength and/or integrity, flexibility, tensility, or other desired properties. In some embodiments, an intervening layer can comprise a molecule or therapeutic compound.

[0066] In certain embodiments, the fibrous polymer scaffold comprises a first layer of diametrically patterned fibers and a second layer of uniaxially aligned fibers with average fiber alignment parallel to one axis of the scaffold. In certain embodiments, the fibrous polymer scaffold comprises a first layer of diametrically patterned fibers and a second layer of unaligned fibers.

[0067] Fibrous layers of polymer fibers may also be oriented in the same direction (or in a similar random orientation) continuously or discontinuously. For example, in embodiments with at least two adjacent layers that are formed from discontinuous fibers, each layer can comprise diametrically patterned fibers within a membrane. In embodiments where at least two adjacent layers are formed from continuous fibers, the layers can differ in their alignment relative to a particular axis or set of axes as disclosed herein.

[0068] The fibrous scaffolds have specific properties, including the ability to retain a specific shape or geometry, flexibility, and alignment of fiber components in the absence of any non-fiber component. The scaffolds can thus be formed into specific or desired shapes or geometries including, without limitation, square, round, oval, and rectangular shaped membranes. The scaffolds may optionally be cut or trimmed into any desired shape or size without any loss of function.

[0069] The mechanical integrity of the diametrically patterned polymer scaffolds allow them to be readily suturable, with minimal leak points created from the use of sutures with the scaffolds. Additionally, the structure of the scaffolds make them sufficiently rigid such that they hold their shape, remain in place at a defect site in a subject, and are not prone to falling apart in the hands of the user, thereby facilitating their use.

[0070] The layers of fibers comprising a scaffold can be distinguished by differential alignment of fibers between the layers. For example, in the context of a membrane, the first layer of the scaffold may comprise diametrically patterned fibers around the convergence region of the membrane. The second layer of fibers can be selected from diametrically aligned, uniaxially aligned, and unaligned. Different alignment of polymer fibers between different layers can provide additional tensile strength in multiple directions.

[0071] In various aspects, a layer of a scaffold can be constructed from a single unbroken polymer fiber, or from a plurality of unbroken polymer fibers. For example, where fibers are patterned diametrically within a scaffold membrane, the fiber can loop back and forth upon itself during manufacture of the layer. For example, layers of fibers diametrically aligned along diametrical axes within a single layer of a scaffold membrane traverse these axes of the layer and are connected at the periphery of the scaffold.

[0072] As described herein, in certain embodiments, different layers of fibers can be constructed from the same fiber (continuous) or different fibers (discontinuous). In some embodiments, each layer of the scaffolds can be comprised of at least one continuous polymer fiber and at least one polymer fiber can also be continuous between the layers of the scaffolds as well, as described herein. The use of continuous fibers between the layers of the scaffolds makes the layers resistant to separation as the polymer chemistry between the layers is similar. The layers thus resist delamination or separation, contributing to the mechanical integrity and strength of the scaffolds.

[0073] In various aspects, the fibrous polymer scaffolds having at least one layer of diametrically patterned fibers are produced by electrospinning polymers onto the surface of a rotational disk assembly. In some aspects, the rotational disk assembly comprises an electrically insulating disk with its back surface in conformal contact with a grounded metal strip and two or more charged point electrodes. In some aspects, the rotational disk assembly comprises an electrically insulating disk with its front face in conformal contact with a smaller electrically conducting disk and its back face in conformal contact with two or more charged point electrodes, as disclosed herein.

Fibrous Polymer Scaffold Membranes

[0074] In some embodiments, the polymer scaffold has the shape of a sheet or membrane. The individual fibers within the membrane can be diametrically aligned during electrospinning using a rotating disc as a collector, as disclosed herein. The individual fibers within a different layer of the membrane can be randomly oriented during electrospinning using a rotating disc as a collector.

[0075] In some embodiments, the polymer scaffold has the shape of a membrane and has diametrically patterned polymer fibers. The fibers within the membrane create a diametrical pattern by spanning across two edges and a convergence region of the membrane. Examples of diametrically patterned polymer fibers are depicted in figure 1.

[0076] In various aspects, the disclosure relates to multilayer fibrous scaffold membranes. In some embodiments, the scaffolds may comprise at least one biodegradable polymer, one or more salts and, optionally, one or more additives. In some embodiments, the membrane is a shape that is substantially planar or sheet-like, having at least one flat surface. In some embodiments, a scaffold membrane in the shape of a membrane can be of varying thickness, with a homogeneous or a heterogeneous structure and/or composition. In some embodiments, the membrane can be substantially planar or sheet-like with a curvature such that at least one surface is concave or convex.

[0077] In various aspects, the scaffold membranes can be used in tissue engineering to improve, regenerate or replace biological tissues. Diametrically patterned fibers within a scaffold membrane promote growth of tissues from the periphery toward the convergence region on the surface of the scaffold, thereby allowing the scaffold membranes to repair and/or replace a defect in a tissue of a subject. Analogously, non-diametrically aligned fibers promote the growth of tissues along the scaffold membranes in the direction of fiber alignment. In some embodiments, repairing and/or replacing a defect comprises contacting the scaffold membranes with the defect such that the scaffold membranes patch and/or cover all or any portion of a defect in a patient, thereby taking the place of at least some of the biological tissue that would otherwise be present in the defect. Scaffold membranes of the present disclosure can be used to repair and/or replace a defect when placed on top of a defect, below a defect, or within the physical boundaries of a defect. In some embodiments, scaffold membranes can repair and/or replace a defect when used as an onlay. In some embodiments, scaffold membranes can repair and/or replace a defect when sutured in place. In some embodiments, the convergence region of the diametrical fibers may be positioned in the center of the wound or defect such that new tissue growth is directed specifically toward the center of the wound or defect from all points on the periphery. In various embodiments, scaffold membranes of the present disclosure can completely or partially repair and/or replace a defect, at the option of the user.

[0078] Therefore, in several aspects, the fibrous scaffold membranes can be used medically to patch and/or regrow tissue in a subject, particularly in defect areas including, without limitation, surgical wounds, non-surgical wounds, biopsy defects, and areas of tissue damage or trauma.

Continuous Fibers In Individual Layers and Between Layers

[0079] As described herein, in certain embodiments, different layers of fibers can be constructed from the same fiber (continuous) or different fibers (discontinuous). In various

embodiments, all of the layers of multilayer scaffolds are comprised of a single polymer fiber that is continuous between the layers. In various embodiments, all of the layers of multilayer scaffolds are comprised of two continuous polymer fibers. It will be understood that the total number of continuous polymer fibers that comprise the layers of the scaffolds can be increased or decreased as may be desired.

[0080] In some embodiments, the multilayer fibrous polymer scaffolds are constructed of a plurality of layers that collectively comprise a single, continuous polymer fiber. In other embodiments, the multilayer fibrous polymer scaffolds are constructed of a plurality of layers that collectively comprise at least two, at least three, at least four, or at least five continuous polymer fibers.

[0081] In some embodiments, the polymer fibers of a first layer of the scaffolds can be continuous with the polymer fibers of a second layer of the scaffolds. For example, if a two-layer scaffold created from a single polymer is desired, the polymer stream used to create the first layer can be used to create the second layer without stopping the flow of polymer from the spinneret, and thus both layers will be constructed with a single, continuous polymer fiber. In some embodiments, a two-layer scaffold is created from a single polymer, wherein the polymer stream used to create the first layer is used to create the second layer without stopping the flow of polymer from the spinneret, thus both layers are constructed from a single, continuous polymer fiber. In some embodiments, a two-layer scaffold is created from two polymers, wherein the polymer streams used to create the first layer are used to create the second layer without stopping the flow of the polymers from the spinnerets, thus both layers are constructed from two continuous polymer fibers. In some embodiments, a two-layer scaffold is created from three polymers, wherein the polymer streams used to create the first layer are used to create the second layer without stopping the flow of the polymers from the spinnerets, thus both layers are constructed from three continuous polymer fibers. In some embodiments, a two-layer scaffold is created from four polymers, wherein the polymer streams used to create the first layer are used to create the second layer without stopping the flow of the polymers from the spinnerets, thus both layers are constructed from four continuous polymer fibers. In some embodiments, a two-layer scaffold is created from five polymers, wherein the polymer streams used to create the first layer are used to create the second layer without stopping the flow of the polymers from the spinnerets, thus both layers are constructed from five continuous polymer fibers.

[0082] In various embodiments, the continuous layers of fibers may be formed of the same polymer solution. In various embodiments, the continuous layers of fibers may be formed of

two or more different polymer solutions. A series of different polymer solutions can be sequentially fed through the same tube into the same electrospinner jet to create a continuous flow even while changing the composition of the solution used to spin fibers.

[0083] In some embodiments, the layers of fibers may be discontinuous. The discontinuity between fibrous layers may be achieved, for example, by: (i) pausing or stopping the process of fiber formation (*i. e.*, solution flow and/or mandrel rotation) for an amount of time sufficient to break the trajectory of fiber-forming solution and/or cause solvent in the deposited layer to evaporate; and/or (ii) physically cutting or disturbing (*i.e.*, putting objects in the pathway) the jet trajectory of fiber-forming solution with or without stopping the process (*i.e.* , solution flow and/or mandrel rotation).

[0084] Non-continuously formed fibrous layers (in addition to continuously formed fibrous layers) may be associated with each other in various degrees depending upon the application desired. The fibrous layers may be randomly intertwined, non-covalently associated, chemically adhered, physically fused, etc. Thus, even though the fibrous layers can be formed in a non-continuous process of more than one electrospinning session, the layers can be made essentially integral.

Polymers, Additives, Salts

[0085] A variety of polymers from synthetic and/or natural sources can be used to compose the fibrous polymer scaffolds. A fiber can be made from a polymer comprising one monomer or subunit or from a polymer comprising a plurality of monomers or subunits. For example, lactic or polylactic acid or glycolic or polyglycolic acid can be utilized to form poly(lactide) (PLA) or poly(L-lactide) (PLLA) nanofibers or poly(glycolide) (PGA) nanofibers. Fibers can also be made from polymers comprising more than one monomer or subunit thus forming a co-polymer, terpolymer, etc. For example, lactic or polylactic acid and be combined with glycolic acid or polyglycolic acid to form the copolymer poly(lactide-co-glycolide) (PLGA). Other copolymers of use in the present disclosure include poly(ethylene-co-vinyl) alcohol.

[0086] The polymer(s) may be natural polymers, biological polymers, synthetic polymers, or a combination thereof. In various embodiments, the polymer fibers used to create the scaffolds of the present disclosure are selected from aliphatic polyesters, polyhydroxyalkanoates, polyurethanes, poly(ester urethane), poly(ether urethane), poly(trimethylene carbonate), poly(glycerol sebacate), poly(pyrrole), poly(acetylene), polyalkylene oxides, polydimethylsiloxane, polyvinylalcohol, polyvinylpyrrolidone, polylysine, collagen, gelatin, laminin, fibronectin, elastin, alginate, fibrin, hyaluronic acid,

proteoglycans, polypeptides, polysaccharides, silk fibroin and combinations and copolymers thereof.

[0087] In some embodiments, a fiber comprises a single polymer or subunit which is a member selected from aliphatic polyesters, polyhydroxyalkanoates, polyurethanes, poly(ester urethane), poly(ether urethane), poly(trimethylene carbonate), poly(glycerol sebacate), poly(pyrrole), poly(acetylene), polyalkylene oxides, polydimethylsiloxane, polyvinylalcohol, polyvinylpyrrolidone, polylysine, collagen, gelatin, laminin, fibronectin, elastin, alginate, fibrin, hyaluronic acid, proteoglycans, polypeptides, polysaccharides and combinations and copolymers thereof.

[0088] In some embodiments, a fiber comprises two different polymers or subunits which are members selected from aliphatic polyesters, polyhydroxyalkanoates, polyurethanes, poly(ester urethane), poly(ether urethane), poly(trimethylene carbonate), poly(glycerol sebacate), poly(pyrrole), poly(acetylene), polyalkylene oxides, polydimethylsiloxane, polyvinylalcohol, polyvinylpyrrolidone, polylysine, collagen, gelatin, laminin, fibronectin, elastin, alginate, fibrin, hyaluronic acid, proteoglycans, polypeptides, polysaccharides, silk fibroin and combinations and copolymers thereof.

[0089] In some embodiments, a fiber comprises three different polymers or subunits which are members selected from aliphatic polyesters, polyhydroxyalkanoates, polyurethanes, poly(ester urethane), poly(ether urethane), poly(trimethylene carbonate), poly(glycerol sebacate), poly(pyrrole), poly(acetylene), polyalkylene oxides, polydimethylsiloxane, polyvinylalcohol, polyvinylpyrrolidone, polylysine, collagen, gelatin, laminin, fibronectin, elastin, alginate, fibrin, hyaluronic acid, proteoglycans, polypeptides, polysaccharides, silk fibroin and combinations and copolymers thereof.

[0090] In some embodiments, a fiber comprises four different polymers or subunits which are members selected from aliphatic polyesters, polyhydroxyalkanoates, polyurethanes, poly(ester urethane), poly(ether urethane), poly(trimethylene carbonate), poly(glycerol sebacate), poly(pyrrole), poly(acetylene), polyalkylene oxides, polydimethylsiloxane, polyvinylalcohol, polyvinylpyrrolidone, polylysine, collagen, gelatin, laminin, fibronectin, elastin, alginate, fibrin, hyaluronic acid, proteoglycans, polypeptides, polysaccharides, silk fibroin, and combinations and copolymers thereof.

[0091] In another embodiment, a fiber comprises five different polymers or subunits which are members selected from aliphatic polyesters, polyhydroxyalkanoates, polyurethanes, poly(ester urethane), poly(ether urethane), poly(trimethylene carbonate), poly(glycerol sebacate), poly(pyrrole), poly(acetylene), polyalkylene oxides, polydimethylsiloxane,

polyvinylalcohol, polyvinylpyrrolidone, polylysine, collagen, gelatin, laminin, fibronectin, elastin, alginate, fibrin, hyaluronic acid, proteoglycans, polypeptides, polysaccharides, silk fibroin, and combinations and copolymers thereof.

[0092] The aliphatic polyester can be linear or branched. In some embodiments, the aliphatic polyester is linear and is selected from D-lactic acid, L-lactic acid, lactide, poly(lactic acid), poly(lactide) glycolic acid, poly(glycolic acid), poly(glycolide), glycolide, poly(lactide-co-glycolide), poly(lactic acid-co-glycolic acid), polycaprolactone and combinations thereof. In some embodiments, the aliphatic polyester is branched and is selected from D-lactic acid, L-lactic acid, lactide, poly(lactic acid), poly(lactide) glycolic acid, poly(glycolic acid), poly(glycolide), glycolide, poly(lactide-co-glycolide), poly(lactic acid-co-glycolic acid), polycaprolactone and combinations thereof. In some embodiments, the aliphatic polyester is conjugated to a linker or a biomolecule.

[0093] In some embodiments, the polyalkylene oxide is selected from polyethylene oxide, polyethylene glycol, polypropylene oxide, polypropylene glycol and combinations thereof.

[0094] The fibrous polymer scaffolds can comprise a fiber of at least one composition. In some embodiments, the fibrous polymer scaffolds comprise a number of different types of fibers selected from at least one fiber, at least two fibers, at least three fibers, at least four fibers, at least five fibers, at least six fibers, at least seven fibers, at least eight fibers, at least nine fibers and at least ten fibers.

[0095] In some embodiments, the fiber or fibers of the fibrous polymer scaffolds are biodegradable. In some embodiments, the fibers of the fibrous polymer scaffolds comprise biodegradable polymers. In some embodiments, the biodegradable polymers comprise a monomer which is a member selected from lactic acid and glycolic acid. In another embodiment, the biodegradable polymers are poly(lactic acid), poly(glycolic acid) or a copolymer thereof. In some embodiments, the biodegradable polymers are those which are approved by the FDA for clinical use, such as poly(lactic acid) and poly(glycolic acid).

[0096] In various aspects, scaffolds comprising biodegradable polymers can be used to guide the morphogenesis of tissue and/or to gradually degrade after the assembly of the tissue. The degradation rate of the polymers can be tailored by one of skill in the art to match the tissue generation rate. For example, if a polymer that biodegrades quickly is desired, a combination of approximately 50:50 polylactic acid to glycolic acid or polyglycolic acid can be selected to form the copolymer poly(lactide-co-glycolide). Additional ways to increase polymer biodegradability can involve selecting a hydrophilic copolymer (for example, polyethylene glycol), decreasing the molecular weight of the polymer, as higher molecular weight often

means a slower degradation rate, and decreasing the fiber density in the scaffolds, as lower fiber density can lead to more water absorption and faster degradation.

[0097] The tissue can include, without limitation, abdominal wall, amniotic sac, bladder, blood vessels, brain, cardiac tissue, fascia, gingival tissue, intestine, ligaments, meningeal tissue, mucous membranes, muscle, nerves, skin, spinal cord, stomach, tendons, etc. In some embodiments, scaffolds comprising biodegradable polymers can be used to guide the morphogenesis of dura mater and gradually degrade after the assembly, repair or replacement of the dura mater.

[0098] In various embodiments, greater than 50%, greater than 60%, greater than 70%, greater than 80%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98%, or greater than 99% of a single layer of a scaffold is w/w comprised of at least one of the polymers described herein.

[0099] To improve flexibility and hydrophilic properties of the polymer fibers, an additive may be blended with the polymer. In various embodiments, the additive is present in the scaffold in an amount of from 0.1% to 10% of the total weight of the polymer. In various embodiments, the additive is present in the scaffold in an amount of from 1.0% to 2.0%, from 2.0% to 3.0%, from 3.0% to 4.0%, from 4.0% to 5.0%, from 5.0% to 6.0%, from 6.0% to 7.0%, from 7.0% to 8.0%, from 8.0% to 9.0%, or from 9.0% to 10.0% of the total weight of the polymer. In some embodiments, the additive is 3.1% of the total weight of the polymer. In some embodiments, the additive is 6.2% of the total weight of the polymer.

[00100] The additive can be a plasticizer including, but not limited to, triethyl citrate, poly(ethylene glycol), poly(propylene glycol), glycerol, and similar materials. In various embodiments, the plasticizer is present in the scaffold in an amount of from 0.1% - 25% of the total weight of the polymer.

[00101] Poly(propylene glycol) can be used as a plasticizer for certain rigid polymers, including, without limitation, poly(L-lactide-co-caprolactone), it can also be used to increase water uptake into the scaffold and to allow for the formation of a thick layer of unaligned polymer fibers on top of another layer of polymer fibers, such as a diametrically aligned fiber layer. In some embodiments, the additive is poly(propylene glycol). In some embodiments, the additive is poly(propylene glycol) with an average molecular weight between 100-10,000. In some embodiments, the additive is poly(propylene glycol) with an average molecular weight between 100-1,000. In some embodiments, poly(propylene glycol) is blended with the polymer in an amount of from 0.01% - 25% of the total weight of the polymer. In some

embodiments, poly(propylene glycol) is blended with the polymer in an amount of from 0.01% - 10% of the total weight of the polymer.

[00102] To improve electrospinning and to decrease the average diameter of the polymer fibers, a dopant may be added to the polymer solution. In various embodiments, the dopant is a salt. Salt produces an excess charge to facilitate the electrospinning process. Salts can include, for example, NaCl, KH_2PO_4, K_2HPO_4, KIO_3, KCl, $MgSO_4$, $MgCl_2$, NaHCC>3, $CaCl_2$, $NaCH_3COOH$, as well as salts of organic compounds including, for example, acetic acid, ascorbic acid, citric acid, lactic acid, glycolic acid, and mixtures thereof. In some embodiments, the salt is sodium acetate. In some embodiments, the salt is anhydrous sodium acetate.

[00103] In various embodiments, the fibers comprise a copolymer of L-lactide and epsilon-caprolactone, with molar ratios of L-lactide between 40% - 90%, and molar ratios of epsilon-caprolactone between 10% - 60%. For example, the fibers can comprise a copolymer of L-lactide and epsilon-caprolactone with molar ratios of L-lactide between 50% - 80% and molar ratios of epsilon-caprolactone between 20% - 50%. In an additional example, the fibers can comprise a copolymer of L-lactide and epsilon-caprolactone with molar ratios of L-lactide between 50% - 70% and molar ratios of epsilon-caprolactone between 30% - 50%.

[00104] In various embodiments, the fibers are formed from polymer solutions, wherein one or more polymers are dissolved in a solvent. In some embodiments, the polymer solution is at least 20% (weight/volume) of poly(L-lactide) dissolved in a solvent. In this embodiment, polymer fibers comprising 100 wt% poly(L-lactide) are formed upon electrospinning.

[00105] In some embodiments, the polymer solution is poly(L-lactide), glycerol and sodium acetate dissolved in hexafluoroisopropanol at weight/volume concentrations of 20 wt%, 2 wt% and 0.2 wt%, respectively. In this embodiment, polymer fibers comprising 90 wt% poly(L-lactide are formed), 9 wt% glycerol and 1 wt% sodium acetate are formed upon electrospinning.

[00106] In some embodiments, the polymer solution is poly(L-lactide), triethyl citrate, and sodium acetate dissolved in hexafluoroisopropanol at weight/volume concentrations of 25 wt%, 6 wt% and 0.2 wt%, respectively. In this embodiment, polymer fibers comprising 80.1 wt% poly(L-lactide), 19.2 wt% triethyl citrate and 0.7 wt% sodium acetate are formed upon electrospinning.

[00107] In some embodiments, the polymer solution is poly(L-lactide-co-caprolactone) (lactide:caprolactone molar ratio: 70:30), poly(propylene glycol) (MW: 425) and sodium acetate dissolved in hexafluoroisopropanol at weight/volume concentrations of 15 wt%, 1 wt% and 0.2 wt%, respectively. In this embodiment, polymer fibers comprising 93 wt% poly(L-lactide-co-caprolactone), 6 wt% poly(propylene glycol) and 1 wt% sodium acetate are formed upon electrospinning.

[00108] In some embodiments, the polymer solution is poly(DL-lactide-co-caprolactone)(lactide:caprolactone molar ratio: 85:15) and sodium acetate dissolved in hexafluoroisopropanol at weight/volume concentrations of 20 wt% and 0.2 wt%, respectively. In this embodiment, polymer fibers comprising 99 wt% poly(DL-lactide-co-caprolactone) and 1 wt% sodium acetate are formed upon electrospinning.

[00109] In some embodiments, the polymer solution is poly(L-lactide-co-caprolactone) (lactide:caprolactone molar ratio: 70:30) and sodium acetate dissolved in hexafluoroisopropanol at weight/volume concentrations of 15 wt% and 0.2 wt%, respectively. In this embodiment, polymer fibers comprising 99 wt% poly(L-lactide-co-caprolactone) and 1 wt% sodium acetate are formed upon electrospinning.

[00110] In some embodiments, the polymer solution is poly(L-lactide), poly(lactide-co-glycolide) and sodium acetate dissolved in hexafluoroisopropanol at weight/volume concentrations of 15 wt%, 15 wt% and 0.2 wt%, respectively. In this embodiment, polymer fibers comprising 49.7 wt% poly(l-lactide), 49.7 wt% poly(lactide-co-glycolide) and 0.6 wt% sodium acetate are formed upon electrospinning.

[00111] In some embodiments, the polymer solution is poly(L-lactide) and sodium acetate dissolved in hexafluoroisopropanol at weight/volume concentrations of 28 wt% and 0.2 wt%, respectively. In this embodiment, polymer fibers comprising 99.3 wt% poly(L-lactide) and 0.7 wt% sodium acetate are formed upon electrospinning.

[00112] In some embodiments, the polymer solution is poly(dioxanone) and sodium acetate dissolved in hexafluoroisopropanol at weight/volume concentrations of 10 wt% and 0.2 wt%, respectively. In this embodiment, polymer fibers comprising 98 wt% poly(dioxanone) and 2 wt% sodium acetate are formed upon electrospinning.

[00113] In some embodiments, the polymer solution is poly(dioxanone) dissolved in hexafluoroisopropanol at weight/volume concentration of 10 wt%. In this embodiment, polymer fibers comprising 100 wt% poly(dioxanone) are formed upon electrospinning.

[00114] In some embodiments, the polymer solution is collagen dissolved in hexafluoroisopropanol at weight/volume concentrations of 8 wt%. In this embodiment, polymer fibers comprising 100 wt% collagen are formed upon electrospinning.

[00115] In some embodiments, the polymer solution is poly(ethylene oxide) dissolved in 90% ethanol, 10% water at weight/volume concentration of 8 wt%. In this embodiment, polymer fibers composed of 100 wt% poly(ethylene oxide) are formed upon electrospinning.

[00116] In various embodiments, the polymer fibers of one or more layers of the scaffolds comprise one or more materials selected from an aliphatic polyester, a poly(alkylene) oxide, poly(L-lactic acid), 70/30 poly (L-lactide-co-caprolactone), poly(caprolactone), poly(DL-lactide-co-caprolactone), poly(lactide-co-glycolic acid), poly(vinylpyrrolidine), poly(dimethylsiloxane), poly(lysine), collagen, laminin, fibronectin, elastin, alginate, fibrin, hyaluronic acid, proteoglycans, polypeptides, silk fibroin, poly(urethanes), poly(ester urethanes), poly(ether urethanes), poly(trimethylene carbonate), poly(glycerol sebacate), poly(pyrrole), poly(acetylene), poly(ethylene-co-vinyl) alcohol, poly(vinyl) alcohol, poly(ethylene) glycol, poly(propylene) glycol, poly-L-lactide-co-glycolide-co-e-caprolactone, poly(tetrafluoroethylene), poly(dioxanone), polyglactin 910, and/or combinations and copolymers thereof.

[00117] In various embodiments, the polymer fibers of one or more layers of the scaffolds comprise one or more materials selected from aliphatic polyesters, polyhydroxyalkanoates, polyurethanes, polyalkylene oxides, polydimethylsiloxane, polyvinylalcohol, polyvinylpyrrolidone, polylysine, collagen, gelatin, laminin, fibronectin, elastin, alginate, fibrin, hyaluronic acid, proteoglycans, polypeptides, polysaccharides and combinations thereof.

[00118] The aliphatic polyesters can be, for example, lactic acid (D- or L-), lactide, poly(lactic acid), poly(lactide) glycolic acid, poly(glycolic acid), poly(glycolide), glycolide, poly(lactide-co-glycolide), poly(lactic acid-co-glycolic acid), epsilon-caprolactone, poly(epsilon-caprolactone), and/or combinations and copolymers thereof.

[00119] The polyalkylene oxides can be, for example poly(ethylene) oxide, poly(propylene) oxide, and/or combinations thereof.

[00120] In some embodiments, the fibers of one or more layers of the scaffolds comprise one or more materials selected from poly(L-lactide-co-caprolactone), poly(L-lactic acid), and/or combinations thereof.

[00121] In some embodiments, the first layer of fibers and the second layer of fibers in a multilayer scaffold comprise a single continuous polymer fiber comprising poly(L-lactide-co-caprolactone) and poly(propylene glycol). In some embodiments the continuous polymer fiber also comprises sodium acetate. In some embodiments, the continuous fiber comprises 92.6% poly(L-lactide-co-caprolactone), 6.2% poly(propylene glycol) and 1.2% sodium acetate.

[00122] Poly (L-lactide-co-caprolactone) is a flexible elastomeric polymer. In various embodiments, poly(propylene glycol) is added to poly (L-lactide-co-caprolactone) as it serves to plasticize poly (L-lactide-co-caprolactone), to increase water uptake into the scaffold membrane, and it allows for the formation of a thicker unaligned fiber layer as a second layer, on top of a diametrically aligned fiber layer. Poly (L-lactic acid) is a rigid polymer. In various embodiments, poly(L-lactic acid) is used to provide structural integrity to a scaffold. In some embodiments, a combination of poly (L-lactide-co-caprolactone) polymer fibers and poly(L-lactic acid) polymer fibers is used to generate scaffolds as the use of poly (L-lactic acid) makes the scaffolds more rigid and less elastic than poly (L-lactide-co-caprolactone) with poly(propylene glycol) alone.

[00123] In some embodiments, the first layer of fibers and the second layer of fibers comprise two continuous polymer fibers, wherein one of the polymer fibers comprises poly(L-lactide-co-caprolactone), and the other polymer fiber comprises poly(L-lactic acid). In some embodiments, one of the two continuous fibers comprises 100% poly(L-lactide-co-caprolactone). In some embodiments, one of the two continuous fibers comprises 100% poly(L-lactic acid).

[00124] In some embodiments, the first layer of fibers and the second layer of fibers comprise a single continuous polymer fiber, wherein continuous fiber comprises poly(L-lactide-co-caprolactone), poly(propylene glycol) and sodium acetate. In some embodiments, the fiber comprises 92.6% poly(L-lactide-co-caprolactone), 6.2% poly(propylene glycol) and 1.2% sodium acetate.

[00125] In some embodiments, the first layer of fibers and the second layer of fibers comprise two continuous polymer fibers, wherein the first of the two continuous fibers comprises poly(L-lactide-co-caprolactone) and poly(propylene glycol), and the second of the two continuous fibers comprises poly(L-lactic acid). In some embodiments both fibers also comprise sodium acetate. In some embodiments, one of the two continuous fibers comprises 92.6% poly(L-lactide-co-caprolactone), 6.2% poly(propylene glycol) and 1.2% sodium

acetate. In some embodiments, one the two continuous fibers comprises 98.7% poly(L-lactic acid) and 1.3% sodium acetate.

[00126] In some embodiments, the first layer of fibers and the second layer of fibers comprise two continuous polymer fibers, wherein the first of the two continuous fibers comprises poly(L-lactide-co-caprolactone) and poly(propylene glycol), and the second of the two continuous fibers comprises poly(glycolic acid). In some embodiments both fibers also comprise sodium acetate. In some embodiments, one of the two continuous fibers comprises 92.6% poly(L-lactide-co-caprolactone), 6.2% poly(propylene glycol) and 1.2% sodium acetate. In some embodiments, one of the two continuous fibers comprises greater than 98% poly(glycolic acid) and less than 2% sodium acetate.

[00127] Poly (L-lactide-co-caprolactone) is a flexible elastomeric polymer. In various embodiments, poly(propylene glycol) is added to poly (L-lactide-co-caprolactone) to plasticize poly (L-lactide-co-caprolactone), to increase water uptake into the scaffold membrane, and to generate a thicker unaligned fiber layer as a second layer of the scaffold, on top of a diametrically aligned fiber layer.

[00128] Poly (glycolic acid) is a rigid polymer. In various embodiments, poly (glycolic acid) is used to provide structural integrity to a scaffold and because it biodegrades quickly.

[00129] In some embodiments, a combination of polymer streams of poly (L-lactide-co-caprolactone) with poly(propylene glycol) and poly (glycolic acid) is used to generate scaffolds because the addition of poly (glycolic acid) may operate to make the scaffold more rigid and less elastic than poly (L-lactide-co-caprolactone) with poly(propylene glycol) alone. Additionally, poly(glycolic acid) has a faster rate of biodegradation than poly (L-lactide-co-caprolactone) or poly(L-lactic acid).

[00130] In some embodiments, the first layer of fibers and the second layer of fibers comprise two continuous polymer fibers, wherein the first of the two continuous fibers comprises poly(L-lactide-co-caprolactone) and poly(propylene glycol), and the second of the two continuous fibers comprises collagen. In some embodiments both fibers also comprise sodium acetate. In some embodiments, one of the two continuous fibers comprises 92.6% poly(L-lactide-co-caprolactone), 6.2% poly(propylene glycol) and 1.2% sodium acetate. In some embodiments, one of the two continuous fibers comprises greater than 98% collagen and less than 2% sodium acetate.

[00131] Collagen is a rigid polymer that is present in a large number of biological tissues. In some embodiments, collagen is used as one of a plurality of polymer fibers comprising the scaffolds to provide structural integrity and because it biodegrades quickly. Collagen also provides cell adhesion regions within the scaffolds.

[00132] In some embodiments, a combination of polymer streams of poly(L-lactide-co-caprolactone) with poly(propylene glycol) and collagen is used to generate scaffolds because the addition of collagen may make the scaffolds more rigid and less elastic than poly (L-lactide-co-caprolactone) with poly(propylene glycol) alone. Additionally, because collagen has a faster rate of biodegradation than poly (L-lactide-co-caprolactone), the rate of biodegradation can be tailored to suit several specific uses.

[00133] In various embodiments, two polymer streams can be used to generate the fibrous polymer scaffolds. In some embodiments, the two polymer streams comprise a first stream of poly (L-lactide-co-caprolactone) with poly(propylene glycol) and a second stream of poly(L-lactic acid), wherein the relative population of the two polymer fibers may be varied when creating a scaffold. In general, the higher the population of poly(L-lactic acid) fibers, the more rigid the scaffold will be. The higher the population of poly (L-lactide-co-caprolactone) with poly(propylene glycol) fibers, the more elastic the scaffold. In some embodiments, the relative population of the two polymer fibers is selected from: 50% poly (L-lactide-co-caprolactone) with poly(propylene glycol) and 50% poly(L-lactic acid); 90% poly (L-lactide-co-caprolactone) with poly(propylene glycol) and 10% poly(L-lactic acid); 80% poly (L-lactide-co-caprolactone) with poly(propylene glycol) and 20% poly(L-lactic acid); 70% poly (L-lactide-co-caprolactone) with poly(propylene glycol) and 30% poly(L-lactic acid); 60% poly (L-lactide-co-caprolactone) with poly(propylene glycol) and 40% poly(L-lactic acid); 40% poly (L-lactide-co-caprolactone) with poly(propylene glycol) and 60% poly(L-lactic acid); 30% poly (L-lactide-co-caprolactone) with poly(propylene glycol) and 70% poly(L-lactic acid); 20% poly (L-lactide-co-caprolactone) with poly(propylene glycol) and 80% poly(L-lactic acid); and 10% poly (L-lactide-co-caprolactone) with poly(propylene glycol) and 90% poly(L-lactic acid).

[00134] In some embodiments, the two polymer streams comprise a first stream of poly (L-lactide-co-caprolactone) with poly(propylene glycol) and a second stream of poly(glycolic acid), wherein the relative population of the two polymer fibers may be varied when creating a scaffold. In general, the higher the population of poly(glycolic acid) fibers, the more rigid the scaffold will be. Additionally, the biodegradation rate increases with an increase in the poly(glycolic acid) population. The higher the population of poly (L-lactide-

co-caprolactone) with poly(propylene glycol) fibers, the more elastic the scaffold. In some embodiments, the relative population of the two polymer fibers is selected from: 50% poly (L-lactide-co-caprolactone) with poly(propylene glycol) and 50% poly(glycolic acid); 90% poly (L-lactide-co-caprolactone) with poly(propylene glycol) and 10% poly(glycolic acid); 80% poly (L-lactide-co-caprolactone) with poly(propylene glycol) and 20% poly(glycolic acid); 70% poly (L-lactide-co-caprolactone) with poly(propylene glycol) and 30% poly(glycolic acid); 60% poly (L-lactide-co-caprolactone) with poly(propylene glycol) and 40% poly(glycolic acid); 40% poly (L-lactide-co-caprolactone) with poly(propylene glycol) and 60% poly(glycolic acid), 30% poly (L-lactide-co-caprolactone) with poly(propylene glycol) and 70% poly(glycolic acid); 20% poly (L-lactide-co-caprolactone) with poly(propylene glycol) and 80% poly(glycolic acid); and 10% poly (L-lactide-co-caprolactone) with poly(propylene glycol) and 90% poly(glycolic acid).

[00135] In some embodiments, the two polymer streams comprise a first stream of poly (L-lactide-co-caprolactone) with poly(propylene glycol) and a second stream of collagen, wherein the relative population of the two polymer fibers may be varied when creating a scaffold. In general, the higher the population of collagen fibers, the more rigid the scaffold will be. Additionally, the biodegradation rate increases with an increase in the collagen population. The higher the population of poly (L-lactide-co-caprolactone) with poly(propylene glycol) fibers, the more elastic the scaffold. In some embodiments, the relative population of the two polymer fibers is selected from: 50% poly (L-lactide-co-caprolactone) with poly(propylene glycol) and 50% collagen; 90% poly (L-lactide-co-caprolactone) with poly(propylene glycol) and 10% collagen; 80% poly (L-lactide-co-caprolactone) with poly(propylene glycol) and 20% collagen; 70% poly (L-lactide-co-caprolactone) with poly(propylene glycol) and 30% collagen; 60% poly (L-lactide-co-caprolactone) with poly(propylene glycol) and 40% collagen; 40% poly (L-lactide-co-caprolactone) with poly(propylene glycol) and 60% collagen; 30% poly (L-lactide-co-caprolactone) with poly(propylene glycol) and 70% collagen; 20% poly (L-lactide-co-caprolactone) with poly(propylene glycol) and 80% collagen; and 10% poly (L-lactide-co-caprolactone) with poly(propylene glycol) and 90% collagen.

Plasma Treatment

[00136] It is known in the art that various chemical and physical properties of a substrate can be altered through surface exposure to plasma, an ionized gas consisting of positive ions and free electrons in proportions resulting in essentially no net electrical charge. Diametrically patterned fibrous polymer scaffolds may be treated with plasma to influence

scaffold properties such as hydrophilicity/wettability, cell binding, attachment of biomolecules, adhesion to other polymers or substrates. Treating a scaffold with plasma can include, for example, placing the scaffold into contact with plasma for a period of time. The type and duration of the effects of plasma treatment depend on the type of gas used in plasma formation and the duration of scaffold exposure to the plasma.

[00137] In various embodiments, the type of gas used to create the plasma is selected without limitation from oxygen, hydrogen, nitrogen, helium, argon, freon, neon, air, nitrous oxide, carbon dioxide and combinations thereof.

[00138] In various embodiments, the plasma treatment lasts for a duration selected from greater than 1 s, greater than 2 s, greater than 3 s, greater than 4 s, greater than 5 s, greater than 10 s, greater than 15 s, greater than 30 s, greater than 45 s, greater than 60s, greater than 2 min, greater than 5 min, greater than 10 min, greater than 20 min, greater than 30 min, or greater than 60 min. In some embodiments, the scaffold is exposed to plasma for a duration selected from less than 60 min, less than 30 min, less than 20 min, less than 10 min, less than 5 min, less than 2 min, less than 60 s, less than 45 s, less than 30 s, less than 15 s, less than 10 s, less than 5 s, less than 4 s, less than 3 s, less than 2 s, or less than 1 s. In some embodiments, the scaffold is exposed to plasma for a duration of 60 s.

[00139] In various embodiments, fibrous polymer scaffolds made from naturally hydrophobic polymers may be treated with plasma in order to increase hydrophilicity/wettability. In one embodiment, a diametrically patterned fibrous polymer scaffold comprising poly(L-lactide-co-caprolactone) and poly(propylene glycol) is exposed to oxygen plasma in order to increase hydrophilicity/wettability.

[00140] In various embodiments, all surfaces of the fibrous polymer scaffold may be exposed to the plasma treatment. In various embodiments, select surfaces may be exposed to the plasma treatment while others are blocked or masked from treatment. In one embodiment, the first layer of fibrous polymer membrane is directly exposed to plasma treatment while the last layer is blocked from plasma treatment through close contact with a masking surface, thereby resulting in different final properties for first layer and the last layer in a single scaffold.

[00141] In various embodiments, the plasma treatment may be applied after the complete fibrous scaffold has been formed. In various embodiments, the plasma treatment may be applied in between the formation of two discrete layers. In various embodiments, more than one plasma treatment may be applied at various points during the creation of the scaffold.

Dimensions

[00142] The fibrous polymer scaffolds of the disclosure can have a variety of dimensions which can be varied to suit the needs of a particular application. In various embodiments, the scaffolds are nanofiber polymer scaffolds. Nanofiber polymer scaffolds have submicron-scale features, with each layer comprising polymer fibers having an average fiber diameter of, for example, between lOnm and I,OOOηιη and/or between 50nm and I,OOOηιη. In various embodiments, nanofiber polymer scaffolds can resemble the physical structure at the area of treatment, such as native collagen fibrils or other extracellular matrices.

[00143] In various embodiments, the scaffolds are microfiber polymer scaffolds. Microfiber polymer scaffolds have micron-scale features, with each layer comprising polymer fibers having an average fiber diameter of, for example, between 1,OOOηιη and 50,000nm and/or between I,OOOηιη and 20,000nm. In various embodiments, microfiber polymer scaffolds can resemble the physical structure at the area of treatment, such as native collagen fibrils or other extracellular matrices.

[00144] The layers of fibers within a single scaffold may have the same thickness and/or composition with respect to one another. For some embodiments, the thickness and/or composition may be varied within a single layer.

[00145] The fiber diameters can vary both between and within layers. In some embodiments, the fiber diameters can vary from lnm - IO,OOOηιη. In some embodiments, the fiber diameters can vary from lOOnm - 2000nm. Different fiber diameters may be desirable for different applications. In formulating a desired fiber diameter, one should consider whether any components *(i.e.,* drugs, growth factors, differentiation factors, etc.) will be seeded between fibers and/or the size of any cells expected to be attracted to and deposited along the fibers *in vivo.*

[00146] In some embodiments, the scaffold membranes have a diameter selected from greater than 1 cm, greater than 2 cm, greater than 3 cm, greater than 4 cm, greater than 5 cm, greater than 6 cm, greater than 7 cm, greater than 8 cm, greater than 9 cm, greater than 10 cm, greater than 11 cm, greater than 12 cm, greater than 13 cm, greater than 14 cm, greater than 15 cm, greater than 16 cm, greater than 17 cm, greater than 18 cm, greater than 19 cm, greater than 20 cm, greater than 21 cm, greater than 22 cm, greater than 23 cm, greater than 24 cm, greater than 25 cm, greater than 26 cm, greater than 27 cm, greater than 28 cm, greater than 29 cm, or greater than 30 cm. In some embodiments, the scaffold membranes have a diameter selected from less than 30 cm, less than 29 cm, less than 28 cm, less than 27

cm, less than 26 cm, less than 25 cm, less than 24 cm, less than 23 cm, less than 22 cm, less than 2 1 cm, less than 20 cm, less than 19 cm, less than 18 cm, less than 17 cm, less than 16 cm, less than 15 cm, less than 14 cm, less than 13 cm, less than 12 cm, less than 11 cm, less than 10 cm, less than 9 cm, less than 8 cm, less than 7 cm, less than 6 cm, less than 5 cm, less than 4 cm, less than 3 cm, less than 2 cm, or less than 1 cm.

[00147] The thickness of the layers of the scaffolds can vary from layer to layer. In various embodiments, the first layer of the scaffolds comprises diametrically aligned fibers and is thinner than a second layer of the scaffolds, which can comprise aligned or unaligned fibers.

[00148] The thickness or diameter of the polymer fibers can be varied from layer to layer within a single scaffold by controlling several factors. For example, fiber diameter may be decreased by: (i) adding more salt to the polymer solution; (ii) using a more polar solvent in the polymer solution; (iii) increasing the distance between the spinneret(s) and the mandrel; (iv) increasing the voltage; (v) increasing the concentration of the polymer solution; (vi) decreasing the flow rate of the polymer solution; and (vii) increasing the spin rate of the mandrel. *See, e.g.,* U.S. Patent Application Publication No. 2007/0269481 at paragraphs [0027], [0030], [0127], [0139], [0162], [0165], [0176], and [0179].

[00149] In some embodiments, the diameter of the fibers comprising a fibrous polymer scaffold ranges from lnm - 10,000 nm. In some embodiments, the average diameter of the fibers comprising a fibrous polymer scaffold ranges from 500nm - I,OOOηιη. In various embodiments, the average diameter of the fibers is within the range of lOnm - I,OOOηιη.

[00150] In some embodiments, the average diameter of the fiber or fibers comprising a single layer of a multilayer fibrous polymer scaffold is within a range selected from: lOOnm to 10 microns; I,OOOηιη to 20,000nm; lOnm to I,OOOηιη; 2,000nm to IO,OOOηιη; 0.5nm to lOOnm; 0.5nm to 50nm; lnm to 35nm; 2nm to 25nm; 90nm to about I,OOOηιη; and 500nm to I,OOOηιη.

[00151] In various embodiments, greater than 50%, greater than 60%, greater than 70%, greater than 80%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98%, or greater than 99% of the fibers in a single layer of a scaffold have the same average diameter.

[00152] The dimensions of each of the fibrous layers comprising a scaffold can be controlled. In some embodiments, the first layer of a fibrous polymer scaffold comprises

diametrically aligned polymer fibers and has a thickness ranging from 1 micron to 70 microns. In various embodiments, each layer of a multilayer polymer scaffold has a thickness selected from: greater than 1 micron; greater than 20 microns; greater than 30 microns; greater than 40 microns; greater than 50 microns; greater than 60 microns; greater than 70 microns; greater than 80 microns; greater than 90 microns; greater than 100 microns; greater than 120 microns; greater than 140 microns; greater than 160 microns; greater than 180 microns; greater than 200 microns; greater than 220 microns; greater than 240 microns; greater than 260 microns; greater than 280 microns; and greater than 300 microns.

[00153] In various embodiments, the first layer of a multilayer polymer scaffold comprises diametrically aligned fibers and has an average thickness selected from: less than 600 microns; less than 580 microns; less than 560 microns; less than 540 microns; less than 520 microns; less than 500 microns; less than 480 microns; less than 460 microns; less than 440 microns; less than 420 microns; less than 400 microns; less than 380 microns; less than 360 microns; less than 340 microns; less than 330 microns; less than 320 microns; less than 300 microns; less than 280 microns; less than 260 microns; less than 240 microns; less than 220 microns; less than 200 microns; less than 180 microns; less than 160 microns; less than 140 microns; less than 120 microns; less than 100 microns; less than 90 microns; less than 80 microns; less than 70 microns; less than 60 microns; less than 50 microns; less than 40 microns; less than 30 microns; and less than 20 microns.

[00154] In various embodiments, the total thickness of the entire fibrous polymer scaffold is selected from: greater than 20 microns; greater than 40 microns; greater than 60 microns; greater than 80 microns; greater than 100 microns; greater than 120 microns; greater than 140 microns; greater than 160 microns; greater than 180 microns; greater than 200 microns; greater than 220 microns; greater than 240 microns; greater than 260 microns; greater than 280 microns; greater than 300 microns; greater than 320 microns; greater than 340 microns; greater than 360 microns; greater than 380 microns; greater than 400 microns; greater than 420 microns; greater than 440 microns; greater than 460 microns; greater than 480 microns; greater than 500 microns; greater than 520 microns; greater than 540 microns; greater than 560 microns; greater than 580 microns; and greater than 600 microns.

Therapeutic Uses

[00155] The fibrous polymer scaffolds can be used in any manner known in the art. These uses can include, without limitation, uses as tissue substitutes and/or tissue regeneration matrices for wounds and/or defects in biological tissues including, without limitation, abdominal wall, amniotic sac, bladder, blood vessels, brain, cardiac tissue, fascia,

gingival tissue, intestine, ligaments, meningeal tissue, mucous membranes, muscle, nerves, skin, spinal cord, stomach, tendons, etc., uses in combination with cells, uses in combination with biomolecules, and uses in combination with pharmaceutically acceptable excipients, as described in PCT Publication No. WO 2007/090102. Similarly, uses in compositions, and as grafts for nerves, skin, vascular tissue, and muscle may be used as described in PCT Publication No. WO 2007/090102.

[00156] A diametrically aligned layer of polymer fibers can affect cell alignment, cell migration and cellular function. Diametrically aligned polymer fibers can induce and direct cell migration along the direction of the fiber orientation, thus enhancing tissue regeneration in such a direction.

[00157] The diametrically aligned polymer fibers function by first promoting directed outgrowth of tissue in contact with the diametrically aligned fibers and second by continuously guiding the regenerating tissue in the direction of the axis or axes of alignment of the diametrically aligned fibers. Therefore, when placed in contact with a wound or defect, a layer of diametrically patterned polymer fibers promotes healing by promoting directed outgrowth of tissue in contact with the diametrically aligned fibers at the periphery of the wound or defect and by continuously guiding the regenerating tissue across the wound or defect gap along the direction of alignment of the diametrically aligned fibers toward the convergence region of the scaffold, which can be positioned at the center of the tissue defect or at any other desired location.

[00158] Therefore, diametrically patterned polymer fibers can be used to help regenerate a variety of tissues including, without limitation, abdominal wall, amniotic sac, bladder, blood vessels, brain, cardiac tissue, fascia, gingival tissue, intestine, ligaments, meningeal tissue, mucous membranes, muscle, nerves, skin, spinal cord, stomach, tendons, etc. For example, the diametrically patterned layer of a multilayer fibrous polymer scaffold can enhance and specifically direct the growth of biological tissue from the entire periphery toward the center of a tissue defect. In some embodiments, the scaffolds can be used in this manner to promote rapid cell and/or tissue coverage within a wound or defect area.

[00159] The scaffolds can be sized to completely cover, or to partially cover, a wound or defect gap in a tissue. In some embodiments, the scaffolds are cut or trimmed into a desired shape and sized to completely cover, or to partially cover, a wound or defect. In various embodiments, the scaffolds are used to patched large tissue defects. In some embodiments, defect to be patched can have a length selected from greater than 1 cm, greater than 2 cm, greater than 3 cm, greater than 4 cm, greater than 5 cm, greater than 6 cm, greater

than 7 cm, greater than 8 cm, greater than 9 cm, greater than 10 cm, greater than 15 cm, greater than 20 cm, greater than 25 cm, greater than 30 cm. In some embodiments, defect gaps to be bridged can have a length selected from less than 30 cm, less than 25 cm, less than 20 cm, less than 15 cm, less than 10 cm, less than 9 cm, less than 8 cm, less than 7 cm, less than 6 cm, less than 5 cm, less than 4 cm, less than 3 cm, less than 2 cm, or less than 1 cm.

[00160] In some embodiments, defects to be patched can have a width selected from greater than 1 cm, greater than 2 cm, greater than 3 cm, greater than 4 cm, greater than 5 cm, greater than 6 cm, greater than 7 cm, greater than 8 cm, greater than 9 cm, greater than 10 cm, greater than 15 cm, greater than 20 cm, greater than 25 cm, or greater than 30 cm. In some embodiments, defect gaps to be bridged can have a width selected from less than 30 cm, less than 25 cm, less than 20 cm, less than 15 cm, less than 10 cm, less than 9 cm, less than 8 cm, less than 7 cm, less than 6 cm, less than 5 cm, less than 4 cm, less than 3 cm, less than 2 cm, or less than 1 cm.

[00161] In some embodiments, defects to be patched can have a diameter selected from greater than 1 cm, greater than 2 cm, greater than 3 cm, greater than 4 cm, greater than 5 cm, greater than 6 cm, greater than 7 cm, greater than 8 cm, greater than 9 cm, greater than 10 cm, greater than 15 cm, greater than 20 cm, greater than 25 cm, or greater than 30 cm. In some embodiments, defect gaps to be bridged can have a width selected from less than 30 cm, less than 25 cm, less than 20 cm, less than 15 cm, less than 10 cm, less than 9 cm, less than 8 cm, less than 7 cm, less than 6 cm, less than 5 cm, less than 4 cm, less than 3 cm, less than 2 cm, or less than 1 cm.

[00162] The wound or defect area can be asymmetrical, having a long axis and a short axis. In various embodiments, the layer of diametrically patterned fibers within a scaffold can be contacted with an asymmetrical wound or defect such that the convergence region of the scaffold is positioned at the center of the wound or defect area to promote rapid cell ingrowth and coverage of the defect or wound from the entire periphery toward the center.

[00163] In several aspects, diametrically patterned fibrous polymer scaffolds can be contacted with a subject to replace, regenerate or improve a biological function and/or a biological tissue. In some embodiments, the scaffolds replace, regenerate or improve the structure and/or function of a biological tissue, wherein the biological tissue is selected from abdominal wall, amniotic sac, bladder, blood vessels, brain, cardiac tissue, fascia, gingival tissue, intestine, ligaments, meningeal tissue, mucous membranes, muscle, nerves, skin, spinal cord, stomach, tendons, and other biological tissues in a subject.

[00164] In several aspects, the present disclosure provides methods of treating an injury or defect in a subject, the methods comprising contacting the subject with a therapeutically effective amount of the scaffolds of the disclosure, sufficient to treat the injury or defect. A therapeutically effective amount is an amount of contact between a scaffold and a subject sufficient to provide the desired local or systemic effect or to affect the desired therapeutic result. In some embodiments, the subject is contacted with one or more scaffolds at the site of the injury or defect. In some embodiments, the defect or injury is selected from wounds, defects arising due to disease, defects arising due to infection, surgical incisions and/or biopsies; the foregoing can include, without limitation, a severed meninx, a damaged meninx, severed fascia, damaged fascia, a severed nerve, a damaged nerve, a severed muscle, a damaged muscle, a severed blood vessel, a damaged blood vessel, a skin wound and bruised skin.

[00165] In several aspects, the present disclosure provides methods of growing tissue in a subject, the methods comprising contacting the subject with a therapeutically effective amount of a scaffold disclosed herein, sufficient to facilitate growth of the tissue. In some embodiments, the tissue is a biologically-simple connective tissue having a sheet-like architecture. In some embodiments, the tissue is a member selected from meninx tissue, fascia, skin tissue, cardiac tissue, gastrointestinal tissue, stomach tissue, tissue of the abdominal wall, muscle tissue, vascular tissue, nerve tissue, and similar tissues. In some embodiments, the tissue is a biological tissue selected from abdominal wall, amniotic sac, bladder, blood vessels, brain, cardiac tissue, fascia, gingival tissue, intestine, ligaments, meningeal tissue, mucous membranes, muscle, nerves, skin, spinal cord, stomach, tendons, and other biological tissues.

[00166] In several aspects, the present disclosure provides methods of treating an injury or defect in a subject, the methods comprising contacting the defect or injury in a subject with a fibrous polymer scaffold such that the scaffold at least partially replaces the defect or injury. The scaffolds of the present disclosure can be used *in vitro* or *in vivo* to test for their efficacy.

[00167] Scaffolds are suitable for use with a variety of subjects. In some embodiments, the subject is an animal. In some embodiments, the animal is selected from a human, a dog, a cat, a horse, a rat and a mouse.

[00168] In several aspects, diametrically aligned layers of polymer fibers comprise biodegradable polymers. These layers can be used to guide the morphogenesis of tissue types having anisotropic structure, *e.g.*, biological tissues such as nerve, skin, blood vessel, skeletal

muscle, cardiac muscle, tendon and ligament. These diametrically aligned layers can also be used in the development of three-dimensional tissue constructs. Using the scaffolds described herein, three-dimensional constructs of nerve tissue, spinal cord tissue, skin tissue, vascular tissue, muscle tissue, and many other tissues can be created.

[00169] Diametrically patterned fibrous polymer scaffolds of the present disclosure have numerous characteristics that are useful in wound healing. For example, the scaffolds can be both nano-porous and breathable, thus preventing microbes and infectious particles from crossing through to the wound or defect, while allowing air flow and moisture penetration, which are important to natural wound healing. Additionally, in some embodiments the polymer fibers comprising the scaffolds are biodegradable, which allows for temporary wound coverage followed by eventual ingrowth of new tissue.

[00170] The materials comprising the scaffolds can be varied to approximate the characteristics of natural tissue including, for example, mechanical strength, rate of degradation, and rate of tissue regeneration.

[00171] The scaffolds of the present disclosure are useful for clinical and personal wound care and soft tissue regeneration. In some embodiments, a diametrically patterned fibrous polymer scaffold can be used as a wound dressing or as a graft to treat external skin wounds. In a clinical setting, the scaffolds can be used to treat wounds resulting from trauma, burns, ulcers, abrasions, lacerations, surgery, or other damage or trauma. A user can use the scaffolds to cover and protect the wound area, to temporarily replace lost or damaged skin tissue, and to guide new tissue generation and wound healing into the damaged area. The scaffolds can be used as an onlay, or may be secured to the wound area using sutures, adhesives, or overlaying bandages. The scaffolds may be cut to match the size of the wound, or may overlap the wound edges.

[00172] The scaffolds may be tailored for personal/home care by combining the scaffold with an adhesive to create a diametrically patterned fibrous polymer scaffold bandage. The adhesive can serve to hold the scaffold in place on a wound or defect area and can be removed when the fibers degrade or fuse with the tissue. The scaffolds may be secured with any suitable adhesive including, for example, liquid or gel adhesives.

[00173] Large sized scaffolds can be used as gauze to absorb fluid and protect large wounds or defects. When used in this manner, the scaffolds can be wrapped around a wounded area, secured with tape, or with secondary bandages.

[00174] The scaffolds can be used to treat internal soft tissue wounds or defects in biological tissues including, without limitation, the amniotic sac, ulcers in the gastrointestinal tract or mucous membranes, gingival damage or recession, internal surgical incisions or biopsies, etc. The scaffolds can be used as an onlay or may be sutured or adhered into place to fill or cover the damaged tissue area. The nano-scale architecture of nanofibrous scaffolds closely mimics the architecture of the extracellular matrix of many common soft tissues. For example, nano-scale polymer fibers are structurally similar to collagen fibrils found in skin and other tissues. This architecture may prevent scar formation in a wound or defect by providing an organized, diametrically aligned layer of a scaffold for cells to migrate into a wound.

[00175] Diametrically patterned polymer fibers in the scaffolds can be used to keep cells aligned and organized during regrowth into the defect or wound area, rather than allowing them to arrange randomly. Random arrangement of cells typically results in the formation of scar tissue and thus the scaffolds may be used to minimize the formation of scar tissue.

[00176] In several embodiments, a molecule is covalently attached, either directly or through a linker, to the fibrous polymer scaffolds, and the molecule is capable of either covalently or non-covalently attaching to a member selected from an extracellular matrix component, a growth factor, a differentiation factor and combinations thereof. In some embodiments, the molecule is covalently attached to the scaffold through a linker, and the linker is selected from di-amino poly(ethylene glycol), poly(ethylene glycol) and combinations thereof. The molecule can be structural or alternatively can be related to a tissue. In some embodiments, the extracellular matrix component is selected from laminin, collagen, fibronectin, elastin, vitronectin, fibrinogen, polylysine and combinations thereof. In some embodiments, the growth factor is selected from acidic fibroblast growth factor, basic fibroblast growth factor, nerve growth factor, brain-derived neurotrophic factor, insulin-like growth factor, platelet derived growth factor, transforming growth factor beta, vascular endothelial growth factor, epidermal growth factor, keratinocyte growth factor and combinations thereof. In some embodiments, the differentiation factor is selected from stromal cell derived factor, sonic hedgehog, bone morphogenic proteins, notch ligands, Wnt and combinations thereof. Molecules can also be incorporated with the scaffolds either during electrospinning or post-fabrication. The molecules can be incorporated via blending, direct covalent attachment, attachment through various linkers, or by adsorption. In some embodiments, the molecules are organic molecules typically made by living organisms. In

some embodiments, the molecules are selected from nucleotides, amino acids, sugars, fatty acids, steroids, nucleic acids, polypeptides, peptides, peptide fragments, carbohydrates, lipids, and combinations thereof *(e.g. ,* glycoproteins, ribonucleoproteins, lipoproteins, or the like). In some embodiments, the molecules are selected from receptor molecules, extracellular matrix components or biochemical factors. Biochemical factors can include, for example, growth factors or differentiation factors. In some embodiments, the molecule is selected from glycosaminoglycans and proteoglycans.

[00177] The following are examples of certain specific uses of the scaffolds of the disclosure and are not intended to be limiting. Rather, the present disclosure is intended to include uses of the disclosed polymer scaffolds with a plurality of biological tissues including, without limitation, abdominal wall, amniotic sac, bladder, blood vessels, brain, cardiac tissue, fascia, gingival tissue, intestine, ligaments, meningeal tissue, mucous membranes, muscle, nerves, skin, spinal cord, stomach, tendons, etc.

Uses Involving Meninges

[00178] In some embodiments, the scaffolds are used to repair, regenerate and/or replace a severed or damaged meninx, or one or more severed or damaged meninges. One use is for the regeneration of damaged dura mater. The dura mater is a membrane that surrounds the brain and spinal cord. It is the outermost of the three meninges, which help protect the brain and spinal cord and provide a retention barrier for the cerebrospinal fluid. In a normal subject, the dura mater is attached directly to the skull, or to the bones of the vertebral canal in the spinal cord. Damage to the dura mater can be caused by trauma, surgical incisions, subdural hematoma, epidural hematoma, meningitis, meningiomas, tumor involvement, etc. The tissue of the dura mater is capable of repair and/or regeneration but it is unable to do so efficiently over large gaps or defects.

[00179] The scaffolds described herein can serve as replacements for all known dural graft matrices, such as xenogeneic collagen-based dural graft substitutes, which are currently a popular form of treatment for dura mater defects.

[00180] In some embodiments, the scaffolds may be used to repair the dura mater at one or more locations where it is damaged or otherwise contains a defect and is no longer in continuity. The scaffolds may be used in all situations involving discontinuous and/or damaged dura mater. In some embodiments, the scaffolds are used where the defect gap is large enough to either greatly hinder or prevent direct regeneration of the dura mater. In some embodiments, the scaffolds are contacted with a defect gap in the dura mater and at

least partially bridge the defect gap to enhance and direct the regeneration of dura mater into the defect gap.

[00181] In some embodiments, the scaffolds are placed and/or contacted with a defect gap such that a single layer of a scaffold, comprising diametrically patterned polymer fibers, contacts the defect site and the remaining layers of the scaffold do not contact the defect. In some embodiments, the diametrically patterned fibers in contact with the defect promote rapid cell and tissue growth from the entire periphery toward the center of the defect and/or toward the convergence region of the scaffold. Rapid cell and tissue coverage of the defect helps lower the risk for complications such as cerebrospinal fluid leakage, neural tissue adhesions and infections.

[00182] In various embodiments, the scaffolds are used as an onlay either on top of, or underneath, the dura mater to bridge the wound or defect. In some embodiments, the scaffolds are inserted directly into the wound or defect. In some embodiments, the scaffolds are put in contact with a wound or defect so that a layer of diametrically aligned fibers contacts the wound or defect. In some embodiments, the scaffolds are sutured in place.

[00183] In various embodiments, the polymer content of the scaffolds is adjusted to match the architecture and physical properties of the dura mater. Dura mater is composed mainly of collagen and elastic fibers embedded in an amorphous extracellular substance. Various studies have observed the tensile strength of human cranial and spinal dura mater as being between 6-20 MPa *(see, e.g.,* J. Neurosurg. 86:1012-1017 (1997); J. Biomech. Eng. 4:541-544 (1998); and Anesth. Analg. 88:1317-21 (1999)). The dura mater is also characterized as being tough, inelastic and flexible. In some embodiments, the scaffolds are created to have the tensile strength, elasticity and flexibility similar to that of human dura mater by comprising at least two continuous polymer fibers, wherein the first of the two continuous fibers comprises poly(L-lactide-co-caprolactone) plus poly(propylene glycol) the second of the two continuous fibers comprises poly(L-lactic acid), and both fibers further comprise sodium acetate. In some embodiments, the scaffolds are created to have the tensile strength and flexibility similar to that of human dura mater by comprising at least two continuous polymer fibers, wherein both of the continuous fibers comprise poly(L-lactide-co-caprolactone) plus poly(propylene glycol) and sodium acetate.

Uses Involving Fascia

[00184] In some embodiments, the scaffolds are used to repair, regenerate and/or replace severed or damaged fascia. One use is for the regeneration of damaged abdominal fascia. Fascia comprises both superficial fascia and deep fascia. In a normal subject,

superficial fascia surrounds organs, glands and neurovascular bundles, fills otherwise unoccupied space in the subject's body and can serve to store fat and water, can serve as a passageway for lymph, nerve and blood vessels, and can act as a protective padding. In a normal subject, deep fascia interpenetrates and surrounds the muscles, bones, nerves and blood vessels of the body where it serves to protect and insulate these structures. Damage to the fascia can be caused by trauma, surgical incisions, tumor involvement, etc. The tissue of fascia is capable of repair and/or regeneration but it is unable to do so efficiently over large gaps or defects.

[00185] The scaffolds described herein can serve as a replacement for all known means of fascia repair including surgical sutures, which are currently a popular form of treatment for fascia defects but are far from perfect.

[00186] In some embodiments, the scaffolds may be used to repair fascia at one or more locations where it is damaged or otherwise contains a defect and is no longer in continuity. The scaffolds may be used in all situations involving discontinuous and/or damaged fascia. In some embodiments, the scaffolds are used where the defect gap is large enough to either greatly hinder or prevent direct regeneration of fascia. In some embodiments, the scaffolds are contacted with a defect gap in fascia and at least partially bridge the defect gap to enhance and direct the regeneration of fascia into the defect gap.

[00187] In some embodiments, the scaffolds are placed and/or contacted with a defect gap such that a single layer of a scaffold, comprising diametrically patterned polymer fibers, contacts the defect site and the remaining layers of the scaffold do not contact the defect. In some embodiments, the diametrically patterned fibers in contact with the fascia defect promote rapid cell and fascia tissue growth from the entire periphery toward the center of the defect and/or toward the convergence region of the scaffold. Rapid cell and fascia tissue coverage of the defect helps lower the risk for complications such as infections, hernias and tissue adhesions.

[00188] In various embodiments, the scaffolds are used as an onlay either on top of, or underneath, the fascia in order to bridge the wound or defect. In some embodiments, the scaffolds are inserted directly into the wound or defect. In some embodiments, the scaffolds are put in contact with a wound or defect so that a layer of diametrically aligned fibers contacts the wound or defect. In some embodiments, the scaffolds are sutured in place.

[00189] In various embodiments, the polymer content of the scaffolds is adjusted to match the architecture and physical properties of fascia. Fascia is composed mainly of collagen and the amount of elastin fibers included in the fascia determines the level of

extensibility and resilience the fascia will have. Other collagen-rich soft tissues, such as tendons, ligaments, and skin, display mechanical properties that strongly depend on the collagen fiber quantity and orientation. Accordingly, the mechanical properties of fascia can correlate to the underlying dense collagen structure. The observed elastic spring constant for collagen in humans is generally about 3.7 GPa - about 4.0 GPa. In some embodiments, the polymer content of the scaffolds is adjusted to closely match an elastic spring constant of about 3.7 GPa - about 4.0 GPa. In some embodiments, the polymer content of the scaffolds is adjusted to closely match the physical properties of collagen. In some embodiments, the physical properties of collagen may be matched in a scaffold that comprises at least two continuous polymer fibers, wherein the first of the two continuous fibers comprises poly(L-lactide-co-caprolactone) plus poly(propylene glycol) the second of the two continuous fibers comprises poly(L-lactic acid), and both fibers further comprise sodium acetate. In some embodiments, the physical properties of collagen may be matched in a scaffold that comprises at least one continuous polymer fiber, wherein the continuous fiber comprises poly(L-lactide-co-caprolactone) plus poly(propylene glycol) and sodium acetate.

Uses Involving Skin

[00190] In some embodiments, the scaffolds are used to repair, regenerate and/or replace damaged skin. One use is for the regeneration of damaged skin. The skin is the outer covering of the body of an animal. The skin is the largest organ of the integumentary system and is made up of epidermal and dermal tissue layers. Damage to the skin can be caused by trauma, burns, surgical incisions, tumor involvement, etc. The skin is capable of repair and/or regeneration but it is unable to do so efficiently over large gaps or defects without creating scar tissue.

[00191] The scaffolds described herein can serve as a replacement for all known means of skin repair including, without limitation, surgical sutures or stitches, adhesive bandages, gauze bandages, and synthetic skin replacements, each of which are currently popular forms of treatment for skin defects but are far from perfect.

[00192] In some embodiments, the scaffolds may be used to repair skin at one or more locations where it is damaged or otherwise contains a defect and is no longer in continuity. The scaffolds may be used in all situations involving discontinuous and/or damaged skin. In some embodiments, the scaffolds are used where the defect gap is large enough to either greatly hinder or prevent direct regeneration of skin without the generation of scar tissue. In some embodiments, the scaffolds are contacted with a defect gap in skin and at least partially bridge the defect gap to enhance and direct the regeneration of the skin into the defect gap.

[00193] In some embodiments, the scaffolds are placed and/or contacted with a defect gap such that a single layer of a scaffold, comprising diametrically patterned polymer fibers, contacts the defect site and the remaining layers of the scaffold do not contact the defect. In some embodiments, the diametrically patterned fibers in contact with the defect promote rapid cell and tissue growth from the entire periphery toward the center of the defect and/or toward the convergence region of the scaffold. Rapid cell and tissue coverage of the defect helps lower the risk for complications such as infections.

[00194] In various embodiments, the scaffolds are used as an onlay either on top of, or underneath, the skin in order to bridge the wound or defect. In some embodiments, the scaffolds are inserted directly into the wound or defect. In some embodiments, the scaffolds are put in contact with a wound or defect so that a layer of diametrically aligned fibers contacts the wound or defect. In some embodiments, the scaffolds are sutured in place. In some embodiments, the scaffolds are held in place via the use of secondary bandages. In some embodiments, the scaffolds are held in place by adhesives.

[00195] In various embodiments, the polymer content of the scaffolds is adjusted to match the architecture and physical properties of the skin. The underlying structure of skin is composed primarily of collagen. Other collagen-rich soft tissues, such as tendons and ligaments, display mechanical properties that strongly depend on the collagen fiber quantity and orientation. Accordingly, the mechanical properties of skin can correlate to the underlying collagen structure. The observed elastic spring constant for collagen in humans is generally about 3.7 GPa - about 4.0 GPa. In some embodiments, the polymer content of the scaffolds is adjusted to closely match an elastic spring constant of about 3.7 GPa - about 4.0 GPa. In some embodiments, the polymer content of the scaffolds is adjusted to closely match the physical properties of skin. In some embodiments, the physical properties of skin may be matched in a scaffold that comprises at least two continuous polymer fibers, wherein the first of the two continuous fibers comprises poly(L-lactide-co-caprolactone) plus poly(propylene glycol) the second of the two continuous fibers comprises poly(L-lactic acid), and both fibers further comprise sodium acetate. In some embodiments, the physical properties of skin may be matched in a scaffold that comprises at least one continuous polymer fiber, wherein the continuous fiber comprises poly(L-lactide-co-caprolactone) plus poly(propylene glycol) and sodium acetate.

Uses Involving Cardiac Tissue

[00196] In some embodiments, the scaffolds are used to repair, regenerate and/or replace damaged cardiac tissue. One use is for the regeneration of damaged cardiac tissue.

Cardiac tissue is primarily a highly oxidative, striated muscle tissue composed of cardiac myocytes. Damage to cardiac tissue can be caused by trauma, surgical incisions, tumor involvement, myocardial infarction, coronary heart disease, cardiomyopathy, ischemic heart disease, etc. Cardiac tissue is capable of repair and/or regeneration but it is unable to do so efficiently over large gaps or defects.

[00197] The scaffolds described herein can serve as a replacement for known means of cardiac tissue repair including surgical sutures, which are currently a popular form of treatment for defects of cardiac tissue but are far from perfect.

[00198] In some embodiments, the scaffolds may be used to repair cardiac tissue at one or more locations where it is damaged or otherwise contains a defect and is no longer in continuity. The scaffolds may be used in all situations involving discontinuous and/or damaged cardiac tissue. In some embodiments, the scaffolds are used where the defect gap is large enough to either greatly hinder or prevent direct regeneration of cardiac tissue. In some embodiments, the scaffolds are contacted with a defect gap in cardiac tissue and at least partially bridge the defect gap and enhance and direct the regeneration of cardiac tissue into the defect gap.

[00199] In some embodiments, the scaffolds are placed and/or contacted with a defect gap such that a single layer of a scaffold, comprising diametrically patterned polymer fibers, contacts the defect site and the remaining layers of the scaffold do not contact the defect. In some embodiments, the diametrically patterned fibers in contact with the defect promote rapid cell and tissue growth from the entire periphery toward the center of the defect and/or toward the convergence region of the scaffold.

[00200] In various embodiments, the scaffolds are used as an onlay either on top of, or underneath, the cardiac tissue in order to bridge the wound or defect. In some embodiments, the scaffolds are inserted directly into the wound or defect. In some embodiments, the scaffolds are put in contact with a wound or defect so that a layer of diametrically aligned fibers contacts the wound or defect. In some embodiments, the scaffolds are sutured in place.

[00201] In various embodiments, the polymer content of the scaffolds is adjusted to match the architecture and physical properties of cardiac tissue. Cardiac tissue is composed mainly of the proteins actin and myosin. Accordingly, the mechanical properties of cardiac tissue can correlate to the average properties of both actin and myosin. The observed elastic modulus of the lateral wall of the myocardium has been shown to be about 3.40 kPa - 3.78 kPa. In some embodiments, the polymer content of the scaffolds can be adjusted to closely match an elastic modulus of about 3.40 kPa - 3.78 kPa. In some embodiments, the polymer

content of the scaffolds can be adjusted to closely match the physical properties of cardiac tissue. In some embodiments, the physical properties of cardiac tissue may be matched in a scaffold that comprises at least two continuous polymer fibers, wherein the first of the two continuous fibers comprises poly(L-lactide-co-caprolactone) plus poly(propylene glycol) the second of the two continuous fibers comprises poly(L-lactic acid), and both fibers further comprise sodium acetate. In some embodiments, the physical properties of cardiac tissue may be matched in a scaffold that comprises at least one continuous polymer fiber, wherein the continuous fiber comprises poly(L-lactide-co-caprolactone) plus poly(propylene glycol) and sodium acetate.

Implantation of the Scaffolds

[00202] In various aspects, methods of implanting fibrous polymer scaffolds are provided. The scaffolds may be implanted into a subject according to any method known in the art. These methods include, without limitation, implantation by topical application *(e.g.* non-invasive implantation such as by placing a scaffold on the surface of the skin), onlay implantation, sutured implantation, and other methods. In various embodiments, the scaffolds can be implanted using the methods described in Zerris, *et al.,* "Repair of the Dura Mater With Processed Collagen Devices", *Journal of Biomedical Materials Research Part B: Applied Biomaterials,* published online 26 April 2007 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jbm.b.30831. In various embodiments, the scaffolds can be implanted using the methods described in Mello et al., "Duraplasty with biosynthetic cellulose: An experimental study", *J. Neurosurg.,* 86: 143-150 (1997).

[00203] In some embodiments, methods of implanting scaffolds comprise contacting a wound, injury or defect in a tissue of a subject with a scaffold. In some embodiments, methods of implanting scaffolds comprise contacting a wound, injury or defect in a tissue of a subject with a single layer of a fibrous polymer scaffold. In some embodiments, the polymer fibers comprising the layer in contact with the wound, injury or defect are diametrically aligned. In some embodiments, the scaffold in contact with the wound, injury or defect at least partially replaces the defect. In some embodiments, the scaffold in contact with the wound, injury or defect completely replaces the defect.

[00204] In various embodiments, methods of implanting scaffolds comprise contacting a wound, injury or defect with one or more scaffolds in an amount, and under conditions, sufficient to treat the injury. In some embodiments, the amount of scaffolds is the amount necessary to at least partially replace the wound, injury or defect. In some embodiments, the amount of scaffolds is the amount necessary to completely replace the wound, injury or

defect. In some embodiments, the conditions comprise contacting at least one layer of diametrically aligned fibers with the wound, injury or defect in order to allow the diametrically aligned fibers to provide specific guidance cues that direct regenerating tissue efficiently along the diametrically aligned layer and into/across the wound, injury or defect.

[00205] In various embodiments, the layer that contacts the wound, injury or defect comprises diametrically patterned polymer fibers. In some embodiments, the convergence region of the diametrically patterned layer is positioned over the center of the tissue wound or defect. In some embodiments, the layer that contacts the wound, injury or defect is the first layer of a scaffold membrane.

[00206] The wound, injury or defect can be wounds of any type, injuries of any type, surgical incisions, biopsies, etc.

[00207] In some embodiments, contacting a wound, injury or defect with a scaffold comprises placing the scaffolds over the defect. In some embodiments, contacting a wound, injury or defect with a scaffold comprises placing the scaffolds completely within the boundaries of the wound, injury or defect. In some embodiments, contacting a wound, injury or defect with a scaffold comprises placing the scaffolds underneath the defect wound, injury or defect.

[00208] In various embodiments, the method also comprises securing the scaffolds in place. In some embodiments, securing the scaffolds in place comprises suturing the scaffolds in place. In some embodiments, the scaffolds are placed on top of the wound, injury or defect and sutured into place. In some embodiments, the scaffolds are placed underneath the wound, injury or defect and sutured into place. In some embodiments, the scaffolds are placed within the boundaries of the wound, injury or defect and sutured into place.

[00209] In some embodiments, methods of implanting the scaffolds to repair or replace a defect in a meninx are provided. In some embodiments, the scaffolds are implanted into a subject to repair a defect in the dura mater. In some embodiments, the defect is created by an injury or disease. In some embodiments, the defect in the dura mater is surgically created. In some embodiments, the method comprises exposing the dura mater by performing any number of standard surgical methods including, without limitation, frontotemporoparietal craniotomy. In some embodiments, a portion of the exposed dura mater is excised and removed to surgically create a defect in the dura mater.

[00210] In some embodiments, the methods comprise contacting the defect in the dura mater with one or more scaffolds. In some embodiments, the contacting comprises

contacting the defect with a layer of diametrically patterned polymer fibers. In some embodiments, the contacting comprises using the scaffold to completely cover the dural defect. In some embodiments, the contacting comprises using the scaffold to partially cover the dural defect. In some embodiments, the convergence region of the diametrically patterned layer of fibers is positioned over the center of the dural wound or defect.

[00211] In some embodiments, the methods comprise contacting the dural defect with a scaffold, wherein the scaffold is placed on top of the defect, superficial to the dura mater. In some embodiments, the methods comprise contacting the dural defect with a scaffold, wherein the scaffold is placed underneath the defect, deep to the dura mater. In some embodiments, the methods comprise contacting the dural defect with a scaffold, wherein the scaffold is placed within the boundaries of the defect, on the same plane as the dura mater.

[00212] In some embodiments, the methods comprise completely covering the dural defect with a scaffold and suturing the scaffold in place. In some embodiments, the methods comprise partially covering the dural defect with a scaffold and suturing the scaffold in place. In some embodiments, the methods comprise placing the scaffold within the boundaries of the dural defect and suturing the scaffold in place. In some embodiments, the methods comprise placing the scaffold underneath the dural defect and suturing the scaffold in place.

Kits Including the Scaffolds

[00213] In various embodiments, the fibrous polymer scaffolds described herein can be included as part of a kit. These kits can comprise, *inter alia,* instructions, such as in the form of an instruction manual, that teach methods of the disclosure and/or that describe the use of the scaffolds as components of the kit. In some embodiments, the kits comprise at least one scaffold and instructions for using the scaffold. In some embodiments, the kits comprise at least one scaffold and instructions for implanting the scaffold in the brain of a subject. In some embodiments, the kit comprises at least one scaffold and instructions for implanting the scaffold in the spinal cord of a subject. In some embodiments, the kits comprise at least one scaffold and instructions for using the scaffold to repair or replace damaged dura mater. In some embodiments, the kits comprise at least one scaffold membrane and instructions for using the scaffold membrane.

[00214] In some embodiments, the kits indicate that the diametrically aligned fibers of a layer of the scaffolds can function by promoting directed outgrowth of tissue located at the periphery of a damaged or defective area and in contact with the diametrically aligned layer of the scaffolds, and by continuously guiding regenerating tissue into/across the damaged or

defective area in the direction of the diametrically aligned fibers of the first layer and toward the center of the defect until the damage or defect is healed.

[00215] In some embodiments, the kits comprise at least one scaffold and instructions for using the scaffold to repair wounds, injuries or defects by promoting the regeneration of anatomical biological components. The anatomical biological components can include, without limitation, abdominal wall, amniotic sac, bladder, blood vessels, brain, cardiac tissue, fascia, gingival tissue, intestine, ligaments, meningeal tissue, mucous membranes, muscle, nerves, skin, spinal cord, stomach and tendons.

[00216] The kits can include instructions for using the scaffolds in situations where one or more biological components or tissues are damaged or otherwise contain a defect and are no longer in continuity. In some embodiments, the kits indicate that the scaffolds can be used where the damage or defect is large enough to either greatly hinder or prevent direct regeneration of a biological component or tissue. In some embodiments, the kit indicates that the scaffolds can at least partially bridge the damage or defect and enhance and direct the regeneration of the biological component or tissue into/across the damage or defect.

[00217] In some embodiments, the kits indicate that a damaged or defective area of a biological component or tissue can be contacted with the scaffolds such that a layer of the scaffolds containing diametrically patterned polymer fibers contacts the damaged or defective site and the remaining layers of the scaffolds do not contact the damaged or defective area. In some embodiments, the kits can indicate that the convergence region of the diametrically patterned fiber layer can be positioned over the center of the wound defect to direct tissue growth from the periphery toward the center of the defect.

[00218] In some embodiments, the kits comprise at least one scaffold membrane and indications that the scaffold membrane is sided, in that the scaffold has one side comprising diametrically patterned fibers and another side comprising randomly oriented fibers. In some embodiments, the instructions include directions for placing a diametrically aligned layer of fibers present in the scaffold membrane in contact with a tissue. In some embodiments, the instructions include directions for placing a diametrically aligned layer of fibers present in a scaffold membrane in contact with a tissue and positioning the convergence region of the scaffold membrane over the center of the tissue defect. In some embodiments, the tissue is meningeal tissue.

[00219] In various embodiments, the kits indicate that the scaffolds can be shaped and sized by the user to match the specific requirements of the subject's wound, injury or defect. In some embodiments, the kit includes scaffold membranes that are largely circular in shape

and indicates that the scaffolds may be sized to completely cover, or to partially cover, the subject's wound, injury or defect. In various embodiments, the kit indicates that the scaffolds can be used to patch large wounds, injuries or defects.

[00220] In some embodiments, the kit indicates that the wounds, injuries or defects to be patched can have a length selected from greater than 1 cm, greater than 2 cm, greater than 3 cm, greater than 4 cm, greater than 5 cm, greater than 6 cm, greater than 7 cm, greater than 8 cm, greater than 9 cm, greater than 10 cm, greater than 15 cm, greater than 20 cm, greater than 25 cm, greater than 30 cm. In some embodiments, the kit indicates that the wounds, injuries or defects to be patched can have a length selected from less than 30 cm, less than 25 cm, less than 20 cm, less than 15 cm, less than 10 cm, less than 9 cm, less than 8 cm, less than 7 cm, less than 6 cm, less than 5 cm, less than 4 cm, less than 3 cm, less than 2 cm, or less than 1 cm.

[00221] In some embodiments, the kit indicates that the wounds, injuries or defects to be patched can have a width selected from greater than 1 cm, greater than 2 cm, greater than 3 cm, greater than 4 cm, greater than 5 cm, greater than 6 cm, greater than 7 cm, greater than 8 cm, greater than 9 cm, greater than 10 cm, greater than 15 cm, greater than 20 cm, greater than 25 cm, greater than 30 cm. In some embodiments, the kit indicates that the wounds, injuries or defects to be bridged can have a width selected from less than 30 cm, less than 25 cm, less than 20 cm, less than 15 cm, less than 10 cm, less than 9 cm, less than 8 cm, less than 7 cm, less than 6 cm, less than 5 cm, less than 4 cm, less than 3 cm, less than 2 cm, or less than 1 cm.

[00222] In some embodiments, the kit indicates that the wounds, injuries or defects to be patched can have a diameter selected from greater than 1 cm, greater than 2 cm, greater than 3 cm, greater than 4 cm, greater than 5 cm, greater than 6 cm, greater than 7 cm, greater than 8 cm, greater than 9 cm, greater than 10 cm, greater than 15 cm, greater than 20 cm, greater than 25 cm, greater than 30 cm. In some embodiments, the kit indicates that the wounds, injuries or defects to be bridged can have a diameter selected from less than 30 cm, less than 25 cm, less than 20 cm, less than 15 cm, less than 10 cm, less than 9 cm, less than 8 cm, less than 7 cm, less than 6 cm, less than 5 cm, less than 4 cm, less than 3 cm, less than 2 cm, or less than 1 cm.

[00223] In various embodiments, the kits indicate that the scaffolds described herein can be used as an onlay either on top of, or underneath, a wound, injury or defect. In some embodiments, the kits indicate that the scaffolds can be placed within the boundaries of a wound, injury or defect. In some embodiments, the kits indicate that the scaffolds can be

placed within the boundaries of discontinuous tissue to bridge a wound, injury or defect gap. In some embodiments, the kits indicate that the scaffolds can be sutured in place.

[00224] In some embodiments, the scaffolds in the kits are marked so as to allow a user to differentiate between a side comprising diametrically patterned polymer fibers and a side comprising unaligned polymer fibers.

[00225] In some embodiments, the scaffolds described herein are included as a component of a kit to be used to replace or repair a severed or damaged meninx, or one or more severed or damaged meninges. In some embodiments, the kit is for use in the regeneration of damaged dura mater in the brain of a subject. The kits can comprise, *inter alia,* an instruction manual that teaches the use of the scaffolds of the disclosure in the regeneration of the dura mater of the brain.

[00226] In some embodiments, the kit is for use in the regeneration of damaged dura mater in the spine or spinal cord of a subject. The kits can comprise, *inter alia,* an instruction manual that teaches the use of the scaffolds of the disclosure in the regeneration of the dura mater of the spine or spinal cord.

[00227] In some embodiments, the scaffolds described herein are included as a component of a kit to be used to replace or repair severed or damaged fascia. In some embodiments, the kit is for use in the regeneration of damaged fascia in a subject. These kits can comprise, *inter alia,* an instruction manual that teaches the use of the scaffolds of the disclosure in the regeneration of the fascia.

[00228] In some embodiments, the scaffolds described herein are included as a component of a kit to be used to replace or repair severed or damaged skin. In some embodiments, the kit is for use in the regeneration of damaged skin in a subject. These kits can comprise, *inter alia,* an instruction manual that teaches the use of the scaffolds of the disclosure in the regeneration of the skin.

[00229] In some embodiments, the scaffolds described herein are included as a component of a kit to be used to replace or repair severed or damaged cardiac tissue. In some embodiments, the kit is for use in the regeneration of damaged cardiac tissue in a subject. These kits can comprise, *inter alia,* an instruction manual that teaches the use of the scaffolds of the disclosure in the regeneration of the cardiac tissue.

Creation of Fibrous Polymer Scaffolds - Rotational Assemblies

[00230] The present disclosure provides technology that adapts the electrospinning process to directly fabricate fibrous polymer scaffolds wherein at least one layer comprises

diametrically patterned fibers. In various embodiments, the fibers can be formed by using the electrospinning apparatus and motor assemblies as described in International Patent Publication No. WO 2007/090102. In various embodiments, the fibers can be formed using the electrospinning apparatus and assemblies described in U.S. Non-Provisional Patent Application No. 12/575,432.

[00231] An electrospinning apparatus is shown in **Fig. 2,** which depicts an electrospinning apparatus capable of producing the fibrous polymer scaffolds described herein. A polymer solution, which contains polymer dissolved in a solvent, is contained within a syringe assembly 205. The syringe assembly 205 is part of a syringe pump 210 assembly in which a computer controls the rate at which the polymer solution exits the syringe 205 by controlling pressure or flow rate. Optionally, different flow rates can be provided and controlled to selected spinnerets 215. The flow rate can be changed to suit the desired physical characteristics of the polymer scaffolds, *i.e.,* membrane thickness, layer thickness, fiber diameter, etc. The syringe pump 210 assembly feeds the polymer solution to a syringe needle, also referred to as a spinneret 215, that sits on a platform. The spinneret 215 has a tip geometry which allows for jet formation and transportation of the polymer stream 200, without interference. An electrical charge of magnitude ranging from about 10 kV to about 30 kV is applied to the spinneret 215 by a high voltage power supply 220 through a wire 225.

[00232] The spinneret 215 is positioned such that a jet of polymer solution 200 is directed towards a rotating collecting disk 230. The disk 230 is electrically charged with a polarity opposite that of the spinneret 215 at a magnitude ranging from about 1 kV to about 10 kV such that an electric field is created between the charged spinneret 215 and the collecting disk 230. Alternatively, the disk 230 may be electrically grounded rather than oppositely charged. The electric field causes a jet of the polymer solution 200 to be ejected from the spinneret 215 and spray toward the collecting disk 230, forming micro- or nanometer diameter polymer fibers that deposit on the surface of the disk 230.

[00233] The collecting disk 230 is attached to a drill chuck in the frame of the electrospinner via a connecting mandrel on the back of the disk, and the drill chuck is connected either to a high voltage power supply 220 or to electrical ground via a wire 235. The drill chuck is also attached to a non-conducting bearing, and the entire collector assembly (non-conducting bearing, drill chuck, and collecting disk 230) is connected to a motor 240. The motor 240 is linked to a speed control which controls the rate at which the motor spins the collector assembly. Optionally, different spin rates can be provided. The

spin rate may be changed to suit the desired physical characteristics of the polymer scaffolds, i.e., membrane thickness, layer thickness, fiber diameter, etc.

[00234] During the electrospinning process, the spinneret 215 can be optionally traversed parallel to the surface of the collecting disk 230. The traversing motion of the spinneret 215 can be specifically controlled to produce specific amounts of fiber deposition at various points or regions on the collecting disk 230. The traversing motion can also be used to increase the overall area of fiber deposition on the collecting disk.

[00235] The alignment of the fibers depositing on the collector is directly influenced by the arrangement, geometries, and electrical charges of the various elements within the electrospinning area, including the needle platform, the collector itself and other nearby components. Using a disk collector of uniform geometry and electrical charge (as in **Fig. 3A),** and an electrospinning area that is free from additional electrical charges, the fibers in the electric field are free to whip in all directions, thereby depositing on the disk with random alignment. To obtain nanofibers or nanofiber layers having more complex types of alignment, such as diametrical alignment, more complex electrospinning assemblies may be employed.

Multi-Component Rotational Assemblies and Fibrous Scaffolds

[00236] In various embodiments, a multi-component rotational disk assembly device is disclosed that can provide diametrical alignment of fibers.

[00237] To produce diametrically patterned fibers, additional components can be added to the rotating disk electrospinning assembly. An example of a multi-component disk assembly is depicted in **Fig. 3B.** The collector 300 comprises a thin, electrically-insulating circular disk positioned in front of three electrically conductive elements: one rectangular strip 305 in the center and two flanking mandrels 310 positioned a set distance away from each end of the strip.

[00238] The conductive components in the electrospinning setup can be electrically charged as depicted in **Fig. 3B:** the spinneret 315 can be positively charged, the mandrels 310 can be negatively charged, and the strip 305 can be grounded. The multi-component collector assembly can be used to create both a first layer of diametrically aligned fibers and one or more subsequent layers of differentially *(i.e.,* unaligned) fibers without changing any electrospinning conditions. After electrospinning a first layer of diametrically aligned fibers, a time delay can be used in which a critical density of the diametrically aligned fibers covers the region over the conductive strip 305, after which unaligned fibers can be deposited.

[00239] In various embodiments, the two outer electrically conducting mandrels 310 and the center conducting strip 305 can be constructed of any conductive material, such as stainless steel. The two outer mandrels 310 can be grounded or charged. The center strip 305 can be charged, grounded, or a floating potential. An electrically insulating material is used for the disk 300 in front of the conducting components. In various embodiments the electrically insulating material may be nylon, polyurethane, polyvinylidene fluoride (PVDF), polyaryletheretherketone (PEEK), polytetrafluoroethylene (PTFE) or Teflon™ (various fluoropolymers), or poly(vinyl chloride) (PVC). If polymer material is used for the insulating regions, it can have varying thickness to customize the insulating properties.

[00240] The dimensions of the various components in the collector assembly may vary to accommodate the desired nanofiber deposition. Component conditions that may be altered include, without limitation: the diameter of the conducting mandrels 310, the length of the conducting strip 305, the spacing between the strip 305 and the mandrels 310, and the diameter of the insulating disk 300.

[00241] In various aspects, during operation, the multi-component disk assembly is rotated around its central axis and the outer mandrels 310 are electrically charged oppositely to the polymer solution 320 being released from the spinneret 315. For example, the polymer solution 320/spinneret 315 may be positively charged, and the outer mandrels 310 negatively charged. Alternatively, the polymer solution 320/spinneret 315 may be negatively charged and the outer mandrels 310 positively charged. Alternatively, the outer mandrels 310 may be grounded.

[00242] With reference to **Fig. 3B,** diametrically aligned fibers deposit across the length of the conductive strip 305 spanning between the conducting mandrels 310. The fibers form an arc spanning between the points of the two conducting mandrels 310. The arcing fibers have the opposite charge to mandrels 310. The arcing fibers lose their charge shortly after deposition and settle onto the disk 300 across the center strip 305 in a diametrically aligned manner. There is thus a constant deposition of arcing fibers and a constant settling of diametrical fibers onto the collecting disk 300 during electrospinning. Eventually, as the depositing fibers form a layer of increasing electrical insulation on the collector 300, the fibers no longer arc between the conducting mandrels 310. The diametrically aligned fibers thus form on the surface of the insulating disk 300 between the conducting mandrels 310 and over the conducting strip 305.

[00243] Alternatively, the strip 305 behind the insulating disk 300 can be replaced with a conductive disk 400 centered on the front of the insulating disk 300 as depicted in **Fig. 4**

and the polymer solution 320 produces fibers having comparable diametrical alignment. Like the strip 305, the disk 400 can be constructed of any conductive material, such as stainless steel. The dimensions (i.e., diameter) of the disk 400 may be adjusted to suit the desired size of the deposition area and resulting scaffold. The depositing diametrical fibers span between the conducting mandrels 310 and deposit across the diameter of the conductive disk 400.

[00244] In some embodiments, the disk collector is rotated about its central axis during the electrospinning process to produce even deposition of the diametrically patterned fibers, as in **Fig. 5.**

[00245] It will be understood that additional regions or components within this rotating disk system can be used so long as insulating regions are used to separate adjacent conductive regions. For example, in certain embodiments, some of the conductive regions in a multi-component disk embodiment are neither grounded nor charged.

[00246] With reference to **Fig. 6,** upon the diametrically aligned fibers reaching a critical density through the electrospinning process, the subsequent deposition of fibers through the same rotation rate and potential applied to the mandrels, strip, and spinneret results in the formation of a second layer of fibers having a different orientation from the first diametrically aligned fibers.

[00247] In certain embodiments, the second layer of fibers can be randomly aligned, with no prevalent alignment axis *(i.e.,* no net alignment or direction). Alternatively if, after the diametrically aligned layer has deposited, the configuration of the collecting disk is altered sufficiently, the depositing fibers can be uniaxially aligned. This uniaxial alignment can be achieved by removing the insulating disk from the current assembly and placing it onto a multi-component conveyor belt or drum assembly as described in U.S. Patent Publication No. 2010/0233115. By varying the geometry and charges of the components in the electrospinning area as described, different layers of nanofiber alignment can be achieved within a single scaffold, including random alignment, uniaxial alignment, and diametrically patterned alignment.

[00248] In some embodiments, the electrospinning components can be rearranged during the electrospinning process without interruption of the polymer fiber such that the resulting scaffold comprises a single, unbroken polymer fiber. Because of the connection between layers via the unbroken polymer fiber, the different layers cannot be delaminated *(i.e.* separated completely), or shift along their axis without breaking the single fiber. Accordingly, a multilayer layer scaffold can be constructed that does not readily allow delamination of the layers.

[00249] Alternatively, if desired, multilayer scaffolds can be constructed wherein the flow of the polymer solution is stopped at the end of one layer and started at the beginning of the next layer, resulting in a broken polymer fiber, or multiple individual fibers. In such instances, the mechanical continuity between the layers depends at least in part on the electrospinning gap time between the layers.

[00250] In one embodiment the continuous or non-continuous layers of fibers may be formed of the same polymer solution. In another embodiment, the continuous or non-continuous layers of fibers may be formed of two or more different polymer solutions. A series of different polymer solutions can be sequentially fed through the same tube into the same electrospinner jet to create a continuous flow even while changing the composition of the solution used to spin fibers.

[00251] During the electrospinning process, the spinneret that dispenses the solution may either remain stationary or may move laterally, parallel to the collecting surface of the disk. The spinneret can move in any direction depending upon the desired fiber alignment pattern and scaffold thickness. The spinneret may also encircle the scaffold and/or spray upward from the bottom of the scaffold. The spinneret and mandrel keep the polymer solution moving to ensure substantially even layers of fibers deposited throughout the shape of the scaffold. However, depending upon the specific application, in some embodiments it may be desirable for parts of the scaffold to be thicker than others and the spinneret movement or collector rotation can be adjusted accordingly to accommodate a scaffold of differential thickness.

[00252] An example of a first layer of diametrically patterned polymer fibers in a fibrous polymer scaffold, which can be produced using any of the electrospinning apparatuses and techniques described herein, is depicted in **Fig. 7A.** An example of a second layer of unaligned or randomly oriented polymer fibers in a fibrous polymer scaffold, which can be produced using any of the electrospinning apparatuses and techniques described herein, is depicted in **Fig. 7B.**

Rotational Assemblies With Conductive Arms

[00253] In various embodiments, a rotational assembly device can includes one or more conductive arms such as, for example, those disclosed in U.S. Patent Publication No. 2010/0233115.

[00254] The conductive arms are electrically conductive components that can be attached to any of the electrospinning apparatuses disclosed herein. When electrically

charged, the conductive arms can be used to repel an electrospinning polymer fiber. Therefore, the direction of polymer fiber deposition can be controlled via the use of one or more conductive arms. In some embodiments, conductive arms can be used to guide the deposition of an electrospinning fiber into a diametrical alignment with respect to a collecting surface. The conductive arms may be used to create fiber alignment on a simple single-component collector or may be used to further enhance alignment on a multi-component collector.

[00255] Under typical electrospinning conditions, a positively charged polymer fiber is ejected from a spinneret away from a positively charged spinneret-holder plate, and toward a negatively charged collector *(e.g.* a disk or other collector). This is typically done at a distance away from the collector such that there is an air gap between the tip of the spinneret and the collector, through which the polymer fiber travels *(see, e.g.,* **Fig. 3A).** In the air gap between the spinneret and the collector, the nascent polymer fiber is free to move as it proceeds through the air gap to the collector. It is typical for the polymer fiber to move erratically, in several directions, as it is guided generally by the electric field from the spinneret to the collector.

[00256] By introducing additional, positively charged elements such as conductive arms into the vicinity of the electrospinning fiber, the direction of deposition of the polymer fiber can be controlled. The positively charged polymer fiber is repelled from the positive charge of the conducting arms and will thus tend to move in a direction away from the positively charged conducting arms toward a negatively charged element. Alternatively, if the polymer fiber is negatively charged, the conductive arms can be likewise negatively charged to repel and control the fiber.

[00257] An example of an apparatus with a pair of positively charged conducting arms is shown in **Fig. 3B.** In the depicted embodiment, the conductive arms 350 are attached to a spinneret 315 platform, also known as a spinneret holder plate. In the depicted embodiment, the dimensions of the arms 350 are equal. In various embodiments, the conductive arms 350 may be attached to the spinneret-holder plate itself, or may be free-standing elements, existing apart from the spinneret-holder plate.

[00258] As shown in **Fig. 3B,** the conductive arms 350 extend downward from the spinneret 315, toward the desired collector 300, which in the depicted embodiment is a multi-component disk. The conductive arms 350 thus extend into the air gap between the spinneret 315 and the collector 300. During electrospinning, a positive charge is applied to the spinneret-holder plate. When the conductive arms 350 are in direct contact with the

spinneret-holder plate, the positive charge is also imparted to the conductive arms 350. A positively charged polymer fiber 320 is ejected from a spinneret tip 315 as described previously. The polymer fiber 320 proceeds through the air gap between the conductive arms 350, toward the disk 300 assembly, which has negatively charged components. Because the conductive arms 350 are positively charged, the positively charged polymer fiber 320 will be repelled by the charge of the conductive arms 350 as it travels from the spinneret tip 315 to the collector 300.

[00259] The means of attaching the conductive arms to the spinneret holder plate can be varied. In some embodiments, the conductive arms can be attached to the plate by a clip. In other embodiments, the conductive arms can be attached to the plate by any other mechanical means including, for example, by welding them to the plate, by screwing them into place at the plate, or by other means of attachment. In some embodiments, the conductive arms are not attached to the plate itself, but are rather present in the air gap by some other means including, for example, by a stand that holds each of the conductive arms in place at the air gap.

[00260] Any number of conductive arms can be employed. In some embodiments, a single conductive arm is attached to a spinneret-holder plate in an electrospinning apparatus. In some embodiments, two conductive arms are attached to a spinneret-holder plate in an electrospinning apparatus. In some embodiments, three conductive arms are attached to a spinneret-holder plate in an electrospinning apparatus. In some embodiments, four conductive arms are attached to a spinneret-holder plate in an electrospinning apparatus. In some embodiments, five or more conductive arms are attached to a spinneret-holder plate in an electrospinning apparatus. In some embodiments, a single conductive arm is introduced into the air gap in the vicinity of a spinneret-holder plate in an electrospinning apparatus, but is not connected to the plate. In some embodiments, two conductive arms are introduced into the air gap in the vicinity of a spinneret-holder plate in an electrospinning apparatus, but are not connected to the plate. In some embodiments, three conductive arms are introduced into the air gap in the vicinity of a spinneret-holder plate in an electrospinning apparatus, but are not connected to the plate. In some embodiments, four conductive arms are introduced into the air gap in the vicinity of a spinneret-holder plate in an electrospinning apparatus, but are not connected to the plate. In some embodiments, five or more conductive arms are introduced into the air gap in the vicinity of a spinneret-holder plate in an electrospinning apparatus, but are not connected to the plate.

[00261] The position and/or orientation of the conductive arms can be altered to prevent the polymer fiber from moving in one or more specific directions and to encourage the polymer fiber to move in one or more other directions as described in U.S. Patent Publication No. 2010/0233115. In some embodiments, the conductive arms are oriented such that they direct the polymer fiber to move and/or deposit along a diametrical axis or axes of a collector. For example, as shown in **Fig. 3B,** by positioning the inner surfaces of the conductive arms 350 such that they flank both the spinneret 315 and the conducting strip 305 of a multi-component disk collector in an orientation that is both parallel to the long axis of the strip 305 and orthogonal to the surface of the collector 300, the whipping of the fiber 320 is restricted between the arms 350 and is encouraged along the long axis of the strip 305, thereby producing diametrical alignment of the fibers on the collector 300.

[00262] The shape and size of the conductive arms can be altered. In some embodiments, the conductive arms are rectangular in shape. In some embodiments, the conductive arms are oval in shape. In some embodiments, the conductive arms are square in shape. In some embodiments, more than one conductive arm is used and each arm is independently a shape selected from rectangular, square and oval.

[00263] In some embodiments, the conductive arms have a length of greater than 1 cm, greater than 2 cm, greater than 3 cm, greater than 4 cm, greater than 5 cm, greater than 6 cm, greater than 7 cm, greater than 8 cm, greater than 9 cm, greater than 10 cm, greater than 11 cm, greater than 12 cm, greater than 13 cm, greater than 14 cm, greater than 15 cm, greater than 16 cm, greater than 17 cm, greater than 18 cm, greater than 19 cm, or greater than 20 cm. In some embodiments, the conductive arms have a length of less than 20 cm, less than 19 cm, less than 18 cm, less than 17 cm, less than 16 cm, less than 15 cm, less than 14 cm, less than 13 cm, less than 12 cm, less than 11 cm, less than 10 cm, less than 9 cm, less than 8 cm, less than 7 cm, less than 6 cm, less than 5 cm, less than 4 cm, less than 3 cm, less than 2 cm, or less than 1 cm.

[00264] In some embodiments, the conductive arms have a width of greater than 0.1 cm, greater than 0.2 cm, greater than 0.3 cm, greater than 0.4 cm, greater than 0.5 cm, greater than 0.6 cm, greater than 0.7 cm, greater than 0.8 cm, greater than 0.9 cm, greater than 1 cm, greater than 2 cm, greater than 3 cm, greater than 4 cm, greater than 5 cm, greater than 6 cm, greater than 7 cm, greater than 8 cm, greater than 9 cm, or greater than 10 cm. In some embodiments, the conductive arms have a width of less than 10 cm, less than 9 cm, less than 8 cm, less than 7 cm, less than 6 cm, less than 5 cm, less than 4 cm, less than 3 cm, less than

2 cm, less than 1 cm, less than 0.9 cm, less than 0.8 cm, less than 0.7 cm, less than 0.6 cm, less than 0.5 cm, less than 0.4 cm, less than 0.3 cm, less than 0.2 cm, or less than 0.1 cm.

[00265] In some embodiments, the conductive arms have a thickness of greater than 0.1 cm, greater than 0.2 cm, greater than 0.3 cm, greater than 0.4 cm, greater than 0.5 cm, greater than 0.6 cm, greater than 0.7 cm, greater than 0.8 cm, greater than 0.9 cm, greater than 100 cm, greater than 1.1 cm, greater than 1.2 cm, greater than 1.3 cm, greater than 1.4 cm, greater than 1.5 cm, greater than 1.6 cm, greater than 1.7 cm, greater than 1.8 cm, greater than 1.9 cm, or greater than 2.0 cm. In some embodiments, the conductive arms have a thickness of less than 2.0 cm, less than 1.9 cm, less than 1.8 cm, less than 1.7 cm, less than 1.6 cm, less than 1.5 cm, less than 1.4 cm, less than 1.3 cm, less than 1.2 cm, less than 1.1 cm, less than 1.0 cm, less than 0.9 cm, less than 0.8 cm, less than 0.7 cm, less than 0.6 cm, less than 0.5 cm, less than 0.4 cm, less than 0.3 cm, less than 0.2 cm, or less than 0.1 cm.

[00266] In various embodiments, the electrically conducting arms can be constructed of any conductive material. Conductive materials can include metals such as, for example, aluminum, copper, silver, gold, iron, nickel, titanium and alloys including, without limitation, steels, bronze, brass and conductive polymers such as polyaniline, polypyrrole, polyacetylene, polythiopene. In some embodiments, the conducting arms are constructed of the same material as the collector used in the electrospinning apparatus.

[00267] By deliberately placing the conductive arms within the electrospinning area, the overall electric field within the electrospinning area in any electrospinning apparatus, inclusive of those disclosed herein, can be altered. The conducting arms can thus be used to guide the movement of a polymer fiber during electrospinning. In various embodiments, the conductive arms can be used to improve the overall alignment of the polymer fibers deposited in a scaffold. In some embodiments, two rectangular shaped conductive arms can be employed in an electrospinning apparatus such that a flat face of each conductive arm is oriented opposite the other, as depicted in **Fig. 3B.** As shown in the embodiment depicted in **Fig. 3B,** the presence of the conductive arms allows for greater control of the direction of polymer fiber movement, and this the direction of polymer fiber deposition along a collector.

[00268] Differences in spinneret height can be used to help control the degree of polymer fiber alignment when conductive arms are employed. As noted herein, the distance between the spinneret tip and the top surface of the collector can have an effect on the resulting deposition of the polymer fibers along a collector. In various aspects, conductive arms can be employed with an electrospinning apparatus when the spinneret tip is positioned at any height above the collector. In some embodiments, the conductive arms can be

employed when the spinneret tip is positioned at lower heights. In some embodiments, conductive arms can be used to direct polymer fiber alignment and deposition when the spinneret tip is positioned greater than 5 cm, greater than 6 cm, greater than 7 cm, greater than 8 cm, greater than 9 cm, greater than 10 cm, greater than 11cm, greater than 12 cm, greater than 13cm, greater than 14cm, greater than 15cm, greater than 16cm, greater than 17cm, greater than 18cm, greater than 19cm, greater than 20cm, greater than 21cm, greater than 22cm, greater than 23cm, greater than 24cm, greater than 25cm, greater than 26cm, greater than 27cm, greater than 28cm, greater than 29cm, or greater than 30cm above the collector. In some embodiments, conductive arms can be used to direct polymer fiber alignment and deposition when the spinneret tip is positioned less than 30cm, less than 29cm, less then 28cm, less than 27cm, less than 26cm, less than 25cm, less than 24cm, less than 23cm, less than 22cm, less than 21cm, less than 20cm, less than 19cm, less than 18cm, less than 17cm, less than 16cm, less than 15cm, less than 14cm, less than 13cm, less than 12cm, less than 11cm, less than 10cm, less than 9cm, less than 8cm, less than 7cm, less than 6cm, or less than 5cm above the collector. In some embodiments, conductive arms can be used to direct polymer fiber alignment and deposition when the spinneret tip is positioned approximately 15cm above the collector. In some embodiments, conductive arms can be used to direct polymer fiber alignment and deposition when the spinneret tip is positioned approximately 12cm above the collector. In some embodiments, conductive arms can be used to direct polymer fiber alignment and deposition when the spinneret tip is positioned approximately 11cm above the collector. In some embodiments, conductive arms can be used to direct polymer fiber alignment and deposition when the spinneret tip is positioned approximately 10cm above the collector. During a typical electrospinning process, at lower spinneret heights alignment of polymer fibers is more difficult because the fibers do not have sufficient time to arch in advance of deposition and thus the fibers tend to deposit in a more random orientation. While not wishing to be limited to any particular theory or embodiment, it is believed that, at lower spinneret heights, the conductive arms make up for a lack of arching fibers in terms of overall alignment by controlling the direction of fiber alignment for a greater distance across the air gap between the spinneret tip and the collector. In some embodiments, the conductive arms can be employed to contain the movement of the polymer fiber for a larger fraction of its travel across the air gap from the spinneret tip to the collector.

[00269] The conductive arms alter the overall electric field in the electrospinning area to manipulate fiber movement and deposition. However, differences in the electric field in the electrospinning area also cause differences in the minimum required voltage to achieve an

electrospinning fiber. The electrospinning process and required voltages can be specifically controlled by varying the size and geometry of the conductive arms, and thus, manipulating the induced electric field. In some embodiments, two conducting arms having a width of from 0.25cm - 2.0 cm are used during electrospinning. In some embodiments, two conductive arms of 1.5 cm width are used during electrospinning. In some embodiments, two conductive arms of 0.5cm width are used during electrospinning. In some embodiments, the voltage required for electrospinning can be reduced by using conductive arms with a narrow width.

[00270] Fiber alignment can be altered within the various layers of a single scaffold by altering the orientation of the conductive arms for each layer accordingly. For example, during a single electrospinning run, the orientation of the conductive arms can be changed. During a single electrospinning run, conductive arms can be added or removed at various points. In some embodiments, the orientation of the conductive causes the fibers to deposit along the diametrical axes of the collector during electrospinning, as depicted, for example, in **Fig. 3B.** In some embodiments, the orientation of the conductive arms is changed during electrospinning. In some embodiments, conductive arms are removed at the start of a particular layer. In some embodiments, conductive arms are added at the start of a particular layer.

[00271] Polymer fiber alignment is dependent on the orientation of the conductive arms during electrospinning. In some embodiments, the inner surfaces of the arms that flank the spinneret are oriented parallel to the diametrical axes of the collector and orthogonal to the surface of the collector during electrospinning and the polymer fibers in the resulting layer of the scaffold are deposited parallel to the diametrical axes of the collector. In various embodiments, the inner surfaces of the conductive arms are oriented parallel or perpendicular to a particular single axis of the collector and orthogonal to the surface of the collector during electrospinning and the polymer fibers in the resulting layer of the scaffold are uniaxially aligned. In various embodiments, the conductive arms are removed from the electrospinning area and the polymer fibers in the resulting area are randomly aligned.

[00272] In various aspects, the conductive arms can be used during electrospinning to improve polymer fiber alignment in a scaffold. In some embodiments, conductive arms can be used in an electrospinning apparatus having a simple single-component disk collector (such as the one as in **Fig. 3A**) to improve fiber alignment by orienting the inner surfaces of the conductive arms parallel to the diametrical axes of the collector and orthogonal to the surface of the collector. In some embodiments, conductive arms can be used in conjunction

with an electrospinning apparatus having a more complex multi-component disk collector (such as the one as in **Fig. 3B**) to improve fiber alignment further by orienting the conductive arms parallel to the axis of alignment formed by the conductive collector components. In some embodiments, the conductive arms can be used to continue producing fibers with diametrical or uniaxial alignment even after the initial fibers have already created an insulating layer on the surface of the collector.

EXAMPLES

[00273] The following examples describe in detail the preparation and properties of certain fibrous polymer scaffolds, and apparatuses and methods used for their preparation. It will be apparent to those skilled in the art that many modifications, both to materials and methods, may be practiced without departing from the scope of the disclosure.

[00274] Although the electrospinning apparatuses and differentially aligned fibrous scaffold fabrication methods may be performed with other electrospinning systems, designs and techniques, a description of various functional systems is provided below.

Example 1

Production of a Layer of Randomly Oriented Polymer Fibers

[00275] To produce a fibrous scaffold, an electrospinning assembly was used as depicted in **Fig. 2.** First, a polymer solution used to form the fibers was loaded into a syringe and bubbles were eliminated. The polymer solution was prepared within a chemical hood and the solution source and loaded syringe were stored upright to reduce bubbles. A piece of polytetrafluoroethylene (PTFE) *(i.e.*, Teflon™) tube was cut to a sufficient length to connect the syringe pump and the spinneret platform, while minimizing the distance between them.

[00276] Male and female luer-lock connectors were attached to the tube. A 25g stainless steel needle was attached to the female luer-lock connector on the tube. The syringe was attached to the male connector on the tube. All luer-lock connections were tightly sealed.

[00277] The syringe assembly was attached to the syringe pump. The syringe pump modulated the pressure and flow of the polymer solution to the spinneret while preventing blockage. The spinneret was attached to a slot in the needle platform. The syringe plunger was pushed manually until the polymer solution reached the needle reservoir. The syringe pump panel was pushed and locked to contact the syringe plunger. The flow rate, syringe size and total solution volume were adjusted via the pump digital display.

[00278] The spinneret platform was adjusted to the appropriate position to optimize the distance between the tip of the spinneret and the surface of the collector substrate. The spinneret assembly was stationary and centered during the fiber deposition process as polymer solution was allowed to pass through the spinneret tip.

[00279] Alignment of polymer fibers depositing on a collector during electrospinning is directly influenced by the arrangement, geometries, and electrical charges of the various elements within the electrospinning area, including the needle platform, the collector itself and other nearby components. In this example, an electrically grounded collector with a uniform circular geometry was used as depicted in **Fig. 3A.** In this example, the collector was a stainless steel disk (13.5cm diameter) with a thin circular disk of polyvinyldifluoride (0.03" thick, 13.5cm diameter) secured on top. The spinneret assembly was stationary and centered during the fiber deposition process as polymer solution was allowed to pass through the positively charged spinneret tip during the electrospinning process. The collector was connected to a motorized rotational assembly via a rod and the collector was rotated at a constant speed to ensure even deposition of the fibers. Randomly oriented fibers were deposited on the polyvinyldifluoride surface of the collector.

Example 2

Production of a Layer of Diametrically Patterned Polymer Fibers

[00280] To produce diametrically patterned polymer fibers, an electrospinning assembly as depicted in **Fig. 3B** was utilized as shown. The collector was a thin circular disk of polyvinyldifluoride (0.03" thick, 13.5cm diameter) positioned in front of three electrically conductive stainless steel elements: one rectangular strip (5.0cm length, 2.0mm width) in the center and two flanking mandrels (3.0mm diameter) positioned 1.0cm away from each end of the strip. Two rectangular stainless steel conductive arms (7cm length, 2 mm width) were also placed on either side the spinneret (each 2.75cm away) parallel to the orientation of the strip behind the polyvinyldifluoride disk.

[00281] The conductive components in the electrospinning setup were electrically charged as depicted in **Fig. 3B:** the spinneret and arms were positively charged, the mandrels were negatively charged, and the strip was grounded. The spinneret assembly was stationary and centered during the fiber deposition process as polymer solution was allowed to pass through the positively charged spinneret tip. The polyvinyldifluoride disk was connected to a motorized rotational assembly via a rod passing through the center of the conductive strip. The polyvinyldifluoride disk was rotated at a constant speed to ensure even deposition of the

fibers while the strip and mandrels behind the disk remained stationary. Diametrically patterned fibers were deposited on the surface of the collector.

[00282] In another trial, the strip behind the polyvinyldifluoride disk was replaced with a stainless steel disk (5.0cm diameter) placed in front of the collector as depicted in **Fig. 4** and fibers were produced having comparable diametrical alignment. **Fig. 5** presents a step-by-step depiction of the rotational motion of the collector utilized in this example that resulted in a uniform layer of diametrically patterned fibers.

Example 3

Production of a Multiple Layer Scaffold

[00283] A fibrous scaffold having multiple layers was created using the electrospinning assemblies described herein. The electrospinning assembly was first arranged as in Example 2 for generating diametrically patterned fibers in a first layer of the scaffold. Rotation of the collector assembly was started. The negative polarity power supply was turned on, set to -6 V, and connected to the mandrels behind the disk to impart a negative charge to them.

[00284] The syringe pump was activated to allow the flow of the polymer solution. When a droplet of the polymer solution had formed at the tip of the spinneret, a positive polarity power supply was connected to the spinneret assembly including the conductive arms, and turned on in order to impart a positive charge to the polymer solution and arms. The positive voltage was incrementally increased until a jet of polymer solution formed and a Taylor cone stabilized.

[00285] The positive and negative voltages were adjusted as necessary to ensure even fiber deposition on the surface of the collector. The Taylor cone was stabilized at the tip of the spinneret so that the cone where the fiber jet extended out toward collector substrate did not change in size. The voltage was decreased if the cone was observed to decrease in size. The voltage was increased if the cone was observed to increase in size.

[00286] A first layer of diametrically patterned fibers was allowed to form on the surface of the collector. The spinneret assembly was stationary and centered during formation of the diametrically patterned layer of fibers as the polymer solution was allowed to pass through the spinneret tip and diametrically patterned fibers formed as shown in **Fig. 5.**

[00287] After electrospinning the diametrically patterned layer to the desired thickness, the polymer flow rate was stopped, the power supplies were turned off, the collector rotation was stopped, and the polyvinyldifluoride disk with deposited fibers was transferred onto the

front of a conductive stainless steel disk as described in Example 1. The conductive mandrels were removed from the collector area and the conductive arms were removed from the spinneret assembly.

[00288] Rotation was then started for the new collector assembly and the polymer flow rate was turned back on. Negative voltage was applied to the collector and positive voltage was applied to the spinneret assembly, as before. Randomly oriented fibers were deposited as a second layer on top of the diametrically patterned fibers during this second electrospinning step as shown in **Fig. 6.**

[00289] During production of the randomly oriented layer of fibers, the spinneret assembly was offset with respect to the center of the collector surface to create a more even thickness for the overall multilayer scaffold. Electrospinning proceeded until the desired thickness of the randomly oriented fiber layer was achieved.

[00290] After electrospinning was complete, the positive and negative power supplies, collector rotation motor, and syringe pump were stopped simultaneously. The resulting fibrous scaffold had a first layer of diametrically patterned fibers and a second layer of randomly oriented fibers as shown in **Fig. 6.**

Example 4

Production of a Fibrous Scaffold Having Randomly Oriented Fibers Made Using Poly (L-lactide-co-caprolactone) with Poly(propylene glycol)

[00291] A fibrous scaffold having a single layer of randomly oriented fibers was created using the electrospinning processes described herein. A polymer solution of poly (L-lactide-co-caprolactone) (PLCL), having a molar weight ratio of 70:30 lactide:caprolactone (Purac Biomaterials, Amsterdam Netherlands), sodium acetate (Sigma-Aldrich St. Louis, MO) and poly(propylene glycol) (PPG) (Acros Organics, NJ) dissolved in hexafluoroisopropanol (Matrix Scientific, Columbia, SC) was used to fabricate scaffolds, as described herein. The concentration of PLCL, sodium acetate and PPG was such that the final polymer fiber composition was 92.6% poly (L-lactide-co-caprolactone), 6.2% poly(propylene glycol), and 1.2% sodium acetate.

[00292] The electrospinning assembly was arranged as described in Example 1. The polymer solution was loaded onto a syringe pump and delivered to the spinneret at a rate of 3 ml/hr. The spinneret was centered and positioned a distance of 13.5 cm from the stainless steel and polyvinyldifluoride collector disk assembly. The collector disk assembly was electrically grounded and rotated at 20 RPM. The electrospinning process was started by

charging the polymer solution with a positive high voltage power supply set at +16 kV. The electrospinning process proceeded for 20 minutes.

[00293] After electrospinning was complete, the positive high voltage power supply, collector rotation motor, and syringe pump were stopped simultaneously. The fibrous scaffold was air dried for 48 hr and then removed from the polyvinyldifluoride collector disk. The resulting fibrous scaffold had a layer of randomly oriented fibers and a thickness of 180 μιη.

Example 5

Production of a Fibrous Scaffold Having Diametrically Patterned Fibers Made Using Poly (L-lactide-co-caprolactone) with Poly(propylene glycol)

[00294] A fibrous scaffold having a single layer of diametrically patterned fibers was created using the electrospinning processes described herein. A polymer solution of poly (L-lactide-co-caprolactone) (PLCL), having a molar weight ratio of 70:30 lactide:caprolactone (Purac Biomaterials, Amsterdam Netherlands), sodium acetate (Sigma-Aldrich St. Louis, MO) and poly(propylene glycol) (PPG) (Acros Organics, NJ) dissolved in hexafluoroisopropanol (Matrix Scientific, Columbia, SC) was used to fabricate scaffolds, as described herein. The concentration of PLCL, sodium acetate and PPG was such that the final polymer fiber composition was 92.6% poly (L-lactide-co-caprolactone), 6.2% poly(propylene glycol), and 1.2% sodium acetate.

[00295] The electrospinning assembly was setup as described in Example 2. The polymer solution was loaded onto a syringe pump and delivered to the spinneret at a rate of 1 ml/hr. The spinneret assembly including the conducting arms was centered and positioned a distance of 13 cm from the collector disk assembly. Two conducting arms were used as shown in **Fig. 3B.** Each arm had a length of 7 cm and a width of 0.9 cm. The spacing between the arms was 7 cm, with the needle positioned directly in between. The stainless steel mandrels were positioned such that the mandrel ends were in contact with the back surface of the polyvinyldifluoride disk and spaced 7 cm horizontally apart from each other. A metal strip having length of 5 cm was centered within the 7 cm space between the mandrel ends and made to contact the back surface of the polyvinyldifluoride disk as illustrated in **Fig. 3B.** The metal mandrels were charged with a negative high voltage power supply set to -6kV. The metal strip was electrically grounded. The polyvinyldifluoride disk was rotated at 20 RPM around its center axis. The electrospinning process was started by charging the polymer solution with a positive high voltage power supply set to +17.5 kV. The electrospinning process proceeded for 20 minutes.

[00296] After electrospinning was complete, the positive and negative high voltage power supplies, collector rotation motor, and syringe pump were stopped simultaneously. The fibrous scaffold was removed from the polyvinyldifluoride collector disk. The resulting fibrous scaffold had a layer of diametrically patterned fibers and a thickness of 30 μιη.

Example 6

Production of a Multilayer Fibrous Scaffold Having a First Layer of Diametrically Patterned Fibers and a Second Layer of Unaligned Fibers Made Using Poly (L-lactide-co-caprolactone) with Poly(propylene glycol)

[00297] A fibrous scaffold having a first layer of diametrically patterned fibers and a second layer of unaligned fibers was created using the electrospinning processes described herein. A polymer solution of poly (L-lactide-co-caprolactone) (PLCL), having a molar weight ratio of 70:30 lactide:caprolactone (Purac Biomaterials, Amsterdam Netherlands), sodium acetate (Sigma-Aldrich St. Louis, MO) and poly(propylene glycol) (PPG) (Acros Organics, NJ) dissolved in hexafluoroisopropanol (Matrix Scientific, Columbia, SC) was used to fabricate scaffolds, as described herein. The concentration of PLCL, sodium acetate and PPG was such that the final polymer fiber composition was 92.6% poly (L-lactide-co-caprolactone), 6.2% poly(propylene glycol), and 1.2% sodium acetate.

[00298] The first layer of diametrically patterned fibers was created using the electrospinning process as described in Example 5. After the electrospinning process was completed, the fibrous scaffold having a first layer of diametrically patterned fibers remained on the polyvinyldifluoride disk. The polyvinyldifluoride disk was removed from the collector assembly and attached to a stainless steel disk as described in Example 1. The conducting arms were removed from the spinneret assembly and the metal strip and metal mandrels were removed from the collector disk assembly.

[00299] The stainless steel disk with the attached polyvinyldifluoride disk was re-attached to the collector assembly. The spinneret assembly was offset horizontally 2 cm from the center point of the polyvinyldifluoride disk assembly, to allow for uniform fiber deposition across the polyvinyldifluoride disk. The polymer solution delivery was re-started at a flow rate of 3 ml/hr. The collector assembly was electrically grounded and rotated at 20 RPM. The electrospinning process was started by charging the polymer solution with a positive high voltage power supply set to +16 kV. Electrospinning proceeded for 45 minutes.

[00300] After electrospinning was complete, the positive high voltage power supply, collector rotation motor, and syringe pump were stopped simultaneously. The fibrous scaffold was removed from the polyvinyldifluoride disk assembly. The resulting bi-layered

fibrous scaffold had a first layer of diametrically patterned fibers and a second layer of unaligned fibers. The scaffold thickness was 200 μιη at the edges and 240 μιη at the center. Scanning Electron Microscopy (SEM) images in **Figs. 7A and 7B** confirm the expected alignment of the fibers at various regions on the nanofiber membrane. On the surface of the membrane designed to have randomly oriented fibers, SEM confirms that the fibers are randomly oriented as shown in **Fig. 7B.** On the side of the membrane designed to have diametrically patterned fibers, SEM shows that fibers at various regions around the periphery are diametrically aligned towards the convergence region of the membrane (all periphery areas were ~2cm from the center of the membrane) as illustrated in **Fig. 7A.** SEM from the convergence region shows fibers overlapping at various angles, as expected, in **Fig. 7A.**

Example 7

Production of a Multilayer Fibrous Scaffold Having a First Layer of Diametrically Patterned Fibers Made Using Poly (L-lactide-co-caprolactone) with Poly(propylene glycol) and a Second Layer of Unaligned Fibers Made Using Poly (L-lactic acid)

[00301] A fibrous scaffold having a first layer of diametrically patterned PLCL fibers and a second layer of unaligned poly(L-lactide) (PLLA) fibers is created using the electrospinning processes described herein. A polymer solution of poly (L-lactide-co-caprolactone) (PLCL), having a molar weight ratio of 70:30 lactide:caprolactone (Purac Biomaterials, Amsterdam Netherlands), sodium acetate (Sigma-Aldrich St. Louis, MO) and poly(propylene glycol) (PPG) (Acros Organics, NJ) dissolved in hexafluoroisopropanol (Matrix Scientific, Columbia, SC) is used to fabricate the first PLCL fiber layer, as described herein. The concentration of PLCL, sodium acetate and PPG is such that the final polymer fiber composition is 92.6% poly (L-lactide-co-caprolactone), 6.2% poly(propylene glycol), and 1.2% sodium acetate. A polymer solution of PLLA (Sigma Aldrich, St. Louis, MO) and sodium acetate (Sigma-Aldrich St. Louis, MO) dissolved in hexafluoroisopropanol (Matrix Scientific, Columbia, SC) is used to fabricate the second PLLA fiber layer, as described herein. The concentration of PLLA and sodium acetate is such that the final polymer fiber composition is 98.7% PLLA, and 1.3% sodium acetate.

[00302] The first layer of diametrically patterned PLCL fibers is created using the electrospinning process as described in Example 5. After the electrospinning process is completed, the fibrous scaffold having a first layer of diametrically patterned fibers remains on the polyvinyldifluoride disk. The polyvinyldifluoride disk is removed from the collector assembly and attached to a stainless steel disk as described in Example 1. The conducting

arms are removed from the spinneret assembly and the metal strip and metal mandrels are removed from the collector disk assembly.

[00303] The stainless steel disk with the attached polyvinyldifluoride disk is re-attached to the collector assembly. The PLLA polymer solution is loaded onto the syringe pump and delivered to the spinneret. The spinneret assembly is offset horizontally 2 cm from the center point of the polyvinyldifluoride disk assembly, to allow for uniform fiber deposition across the polyvinyldifluoride disk. The PLLA polymer solution delivery is started at a flow rate of 1 ml/hr. The collector assembly is electrically grounded and rotated at 20 RPM. The electrospinning process is started by charging the polymer solution with a positive high voltage power supply set to +16 kV. The electrospinning process proceeds for 20 minutes.

[00304] After electrospinning is complete, the positive high voltage power supply, collector rotation motor, and syringe pump are stopped simultaneously. The fibrous scaffold is removed from the polyvinyldifluoride disk assembly. The resulting bi-layered fibrous scaffold has a first layer of diametrically patterned 92.6% PLCL, 6.2% PPG, and 1.2% sodium acetate fibers and a second layer of unaligned 98.7% PLLA and 1.3% sodium acetate fibers.

Example 8

Production of a Multilayer Fibrous Scaffold Having a First Layer of Diametrically Patterned Fibers and a Second Layer of Unaligned Fibers Made Using Poly (L-lactide-co-caprolactone) with Poly(propylene glycol) in an Uninterrupted Production Process

[00305] A fibrous scaffold having a first layer of diametrically patterned fibers and a second layer of unaligned fibers is created using the electrospinning processes described herein. A polymer solution of poly (L-lactide-co-caprolactone) (PLCL), having a molar weight ratio of 70:30 lactide:caprolactone (Purac Biomaterials, Amsterdam Netherlands), sodium acetate (Sigma-Aldrich St. Louis, MO) and poly(propylene glycol) (PPG) (Acros Organics, NJ) dissolved in hexafluoroisopropanol (Matrix Scientific, Columbia, SC) is used to fabricate scaffolds, as described herein. The concentration of PLCL, sodium acetate and PPG is such that the final polymer fiber composition is 92.6% poly (L-lactide-co-caprolactone), 6.2% poly(propylene glycol), and 1.2% sodium acetate.

[00306] The first layer of diametrically patterned fibers is created using the electrospinning process as described in Example 5. After 20 minutes of electrospinning the diametrically patterned fiber layer, the electrospinning process is not stopped. The conducting arms of the spinneret assembly are receded from the spinneret assembly to remove their

influence on the trajectory of the electrospinning jet. The conducting metal strip and negatively charged mandrel ends at the back of the collector disk assembly are receded until they no longer are in contact with the back surface of the disk. The negative high voltage power supply connected to the mandrels is turned off. The spinneret assembly is offset horizontally 2 cm from the center point of the polyvinyldifluoride disk. The flow rate of the polymer solution is increased to 3 ml/hr and the positive voltage is adjusted to maintain a stable Taylor cone and electrospinning jet. The electrospinning process proceeds for an additional 20 minutes.

[00307] After electrospinning is complete, the positive high voltage power supply, collector rotation motor, and syringe pump are stopped simultaneously. The fibrous scaffold is removed from the polyvinyldifluoride disk assembly. The resulting bi-layered fibrous scaffold has a first layer of diametrically patterned fibers and a second layer of unaligned fibers, wherein at least one fiber spans both the first and second layers of the fibrous polymer scaffold.

Example 9

Production of a Multilayer Fibrous Scaffold Having a First Layer of Diametrically Patterned Fibers and a Second Layer of Uniaxially Aligned Fibers Made Using Poly (L-lactide-co-caprolactone) with Poly(propylene glycol)

[00308] A fibrous scaffold having a first layer of diametrically patterned fibers and a second layer of uniaxially aligned fibers is created using the electrospinning processes described herein. A polymer solution of poly (L-lactide-co-caprolactone) (PLCL), having a molar weight ratio of 70:30 lactide:caprolactone (Purac Biomaterials, Amsterdam Netherlands), sodium acetate (Sigma-Aldrich St. Louis, MO) and poly(propylene glycol) (PPG) (Acros Organics, NJ) dissolved in hexafluoroisopropanol (Matrix Scientific, Columbia, SC) is used to fabricate scaffolds, as described herein. The concentration of PLCL, sodium acetate and PPG is such that the final polymer fiber composition is 92.6% poly (L-lactide-co-caprolactone), 6.2% poly(propylene glycol), and 1.2% sodium acetate.

[00309] The first layer of diametrically patterned fibers is created using the electrospinning process as described in Example 5. After the electrospinning process is completed, the fibrous scaffold having a single layer of diametrically patterned fibers remains on the polyvinyldifluoride disk.

[00310] The polyvinyldifluoride disk with the fibrous scaffold is removed from the collector assembly and attached to the surface of a conveyor belt assembly as described in U.S. Patent Publication No. 2010/0233115. The conveyor belt is 11 cm wide and made of

polyvinyldifluoride. The center 7 cm wide region of the belt is covered with a 0.002" thick stainless steel shim. The conveyor belt is attached to a 1/8" diameter rotating mandrel at the top end and a belt tensioner at the bottom end. The rotating mandrel is an electrically insulating ceramic mandrel coated with electrically conducting stainless steel such that the central 10 cm section of the mandrel is bare ceramic and the two flanking regions are stainless steel coated. The conveyor belt is positioned around the mandrel such that the stainless steel coating of the mandrel overlaps 0.5 cm under the ends of the polyvinyldifluoride belt. The top end of the conveyor belt assembly is positioned 12 cm below the electrospinner spinneret. The spinneret assembly's conductive arms are positioned such that they are perpendicular to the long axis of the rotating mandrel.

[00311] The stainless steel coated regions of the rotating mandrel are charged with a negative high voltage power supply set to -6 kV. The belt is rotated at 20 RPM. The flow rate of the PLCL polymer solution is set to 1 ml/hr. The electrospinning process is started by charging the polymer solution with a positive high voltage power supply set to +15 kV. The electrospinning process proceeds for 20 minutes. During the electrospinning process the fibers align uniaxially across the width of the 7cm stainless steel section of the conveyor belt. The process deposits a fiber of uniaxially aligned fibers on top of the diametrically patterned fiber layer on the polyvinyldifluoride disk.

[00312] After electrospinning is complete, the positive and negative high voltage power supplies, collector rotation motor, and syringe pump are stopped simultaneously. The fibrous scaffold is removed from the polyvinyldifluoride disk and conveyor belt assembly. The resulting bi-layered fibrous scaffold has a first layer of diametrically patterned fibers and a second layer of uniaxially aligned fibers.

Example 10

Production of a Multilayer Fibrous Scaffold Having a First Layer of Diametrically Patterned Fibers Made Using Poly (L-lactide-co-caprolactone) with Poly(propylene glycol) and a Second Layer of Uniaxially Aligned Fibers Made Using Poly (L-lactic acid)

[00313] A fibrous scaffold having a first layer of diametrically patterned PLCL fibers and a second layer of uniaxially aligned PLLA fibers is created using the electrospinning processes described herein. A polymer solution of poly (L-lactide-co-caprolactone) (PLCL), having a molar weight ratio of 70:30 lactide:caprolactone (Purac Biomaterials, Amsterdam Netherlands), sodium acetate (Sigma-Aldrich St. Louis, MO) and poly(propylene glycol) (PPG) (Acros Organics, NJ) dissolved in hexafluoroisopropanol (Matrix Scientific, Columbia, SC) is used to fabricate the first fibrous scaffold layer, as described herein. The

concentration of PLCL, sodium acetate and PPG is such that the final polymer fiber composition is 92.6% poly (L-lactide-co-caprolactone), 6.2% poly(propylene glycol), and 1.2% sodium acetate. A polymer solution of PLLA (Sigma Aldrich, St. Louis, MO) and sodium acetate (Sigma-Aldrich St. Louis, MO) dissolved in hexafluoroisopropanol (Matrix Scientific, Columbia, SC) is used to fabricate the second PLLA fiber layer, as described herein. The concentration of PLLA and sodium acetate is such that the final polymer fiber composition is 98.7% PLLA, and 1.3% sodium acetate.

[00314] The first layer of diametrically patterned fibers is created using the electrospinning process as described in Example 5. After the electrospinning process is completed, the fibrous scaffold comprising diametrically patterned fibers remains on the polyvinyldifluoride disk.

[00315] The polyvinyldifluoride disk with the fibrous scaffold is removed from the collector assembly and attached to the surface of a conveyor belt assembly as per Example 9. The conveyor belt is 11 cm wide and comprises polyvinyldifluoride. The center 7 cm wide region of the belt is covered with a 0.002" thick stainless steel shim. The conveyor belt is attached to a 1/8" diameter rotating mandrel at the top end and a belt tensioner at the bottom end. The rotating mandrel is an electrically insulating ceramic mandrel coated with electrically conducting stainless steel such that the central 10 cm section of the mandrel is bare ceramic and the two flanking regions are stainless steel coated. The conveyor belt is positioned around the mandrel such that the stainless steel coating of the mandrel overlaps 0.5 cm under the ends of the polyvinyldifluoride belt. The top end of the conveyor belt assembly is positioned 12 cm below the electrospinner spinneret. The spinneret assembly's conductive arms are positioned such that they are perpendicular to the long axis of the rotating mandrel.

[00316] The stainless steel coated regions of the rotating mandrel are charged with a negative high voltage power supply set to -6 kV. The belt is rotated at 20 RPM. The PLLA polymer solution is loaded onto the syringe pump and delivered to the spinneret at a flow rate of 1 ml/hr. The electrospinning process is started by charging the polymer solution with a positive high voltage power supply set to +15 kV. The electrospinning process proceeds for 20 minutes. During the electrospinning process the PLLA fibers align uniaxially across the width of the 7cm stainless steel section of the conveyor belt. The process deposits a fiber of uniaxially aligned PLLA fibers on top of the diametrically patterned PLCL fiber layer on the polyvinyldifluoride disk.

[00317] After electrospinning is complete, the positive and negative high voltage power supplies, collector rotation motor, and syringe pump are stopped simultaneously. The fibrous scaffold is removed from the polyvinyldifluoride disk and conveyor belt assembly. The resulting bi-layered fibrous scaffold has a first layer of diametrically patterned PLCL fibers and a second layer of uniaxially aligned PLLA fibers.

Example 11

***In vitro* Wound Healing Assay Demonstrating the Effectiveness of Diametrically Patterned Nanofibers for Influencing Cell Migration and Closing Gap Defects**

[00318] To test the efficacy of diametrically patterned nanofibers for enhancing cell migration and wound healing, an *in vitro* model was used. Nanofiber membranes having a first layer of diametrically patterned fibers and a second layer of unaligned fibers were produced as described in example 6. Each membrane was cut into a 2X2cm square with the convergence region of the diametrical fibers positioned at the center of the square. Membranes having either uniaxially or randomly oriented fiber layers were used as controls. A circular disk (1cm D) was placed onto the center of each membrane in order to prevent cell attachment underneath and human mesenchymal stem cells (MSCs) were then seeded at confluency on the membranes around the periphery of each disk. Following attachment of the cells to the nanofibers, each disk was removed leaving an initial circular gap defect, a simulated wound, where no cells were present.

[00319] Immediately prior to seeding, MSCs were stained using Dil cell tracker (Invitrogen, Carlsbad, CA) to determine the initial "wound" edges and to monitor the progression of "wound" coverage. Samples were maintained for 7 days, during which time the cells migrated from the periphery of each gap defect "wound" towards the center. Samples were then fluorescently stained for total actin using Alexa Fluor 488 phalloidin (Invitrogen, Carlsbad, CA) to view the cytoskeletal structure of the cells within the wound area.

[00320] **Fig. 8** depicts the cellular wound coverage on the various nanofiber membranes. On randomly oriented nanofibers, the MSC population showed a correspondingly random orientation and the wound coverage after 7 days was distinctly incomplete with very few cells reaching the center of the wound. The uniaxially aligned fibers induced corresponding uniaxial alignment of the cells and the wound coverage was much greater. However, the elongated shape of the wound on day 7 indicates that cell migration was much faster along the axis of fiber alignment than perpendicular to that axis. On the diametrically patterned fibers, the cells oriented toward the center of the wound at all

points around the periphery, and migration was subsequently enhanced toward the center from all directions. Wound coverage on the diametrical fibers was nearly complete after 7 days with only a few small areas remaining uncovered.

Example 12

Tensile Testing of a Multilayer Fibrous Scaffold Having a First Layer of Diametrically Patterned Fibers and a Second Layer of Unaligned Fibers Made Using Poly (L-lactide-co-caprolactone) with Poly(propylene glycol)

[00321] To investigate the mechanical properties of nanofibrous membranes with various fiber orientation, nanofiber membranes were produced as described herein. One set of samples was produced having a first layer of diametrically patterned fibers and a second layer of randomly oriented fibers. Another set of samples was produced having a first layer of uniaxially aligned fibers and a second layer of randomly aligned fibers.

[00322] Samples were subjected to uniaxial load using a Chatillon TCD225 Series Digital Force Tester equipped with a 100 N load cell and a pair of Universal Wedge Grips (GF-9 series, rated to 2.5 kN) (Ametek, Inc., Paoli, PA). Each sample was cut to 6X1 cm and fully wetted in Sorensen's buffer (pH 7.4) prior to loading in order to better simulate physiological conditions. Due to their inherent structural anisotropy, the nanofibrous samples were cut and tested under various orientations as depicted by the dashed lines in **Fig. 9A.** Samples with a first layer of uniaxially aligned fibers were tested with the tensile load oriented either parallel or perpendicular to the primary axis of fiber alignment. Samples with a first layer of diametrically patterned fibers were tested with tensile load either oriented directly across the convergence region of the membrane or offset 2 cm from the convergence region as depicted in **Fig. 9A.** Ultimate tensile stress and strain were calculated from the load-at-failure, elongation and cross sectional area measurements.

[00323] Mechanical test results in **Fig. 9B** indicate no significant difference in ultimate sample elongation for the various test conditions. However, tensile strength data show that samples with uniaxially aligned fibers were much stronger along the axis of alignment than perpendicular to that axis, which corresponds to the anisotropic nature of the fiber architecture. Conversely, the tensile strength of the samples with diametrically patterned fibers was roughly intermediate between the strengths either parallel or perpendicular to the uniaxial fibers. Additionally, the tensile strength for the diametrically patterned samples was approximately the same when the sample was tested across the convergence region of the diametrical fibers or offset from the convergence region. These results demonstrate that the mechanical properties of the nanofibrous membranes depend on the fiber orientation and

architecture, and that these properties can be specifically tailored by controlling the electrospinning process.

[00324] While illustrative embodiments of the disclosure are disclosed herein, it will be appreciated that numerous modifications and other embodiments may be devised by those skilled in the art. For example, the features for the various embodiments can be used in other embodiments. Therefore, it will be understood that the appended claims are intended to cover all such modifications and embodiments that come within the spirit and scope of the present disclosure.

CLAIMS

What is claimed is:

1. A fibrous polymer scaffold, comprising:
a first layer of diametrically patterned polymer fibers.

2. The fibrous polymer scaffold of claim 1, comprising:
a second layer of polymer fibers.

3. The fibrous polymer scaffold of claim 2, wherein the polymer fibers of the second layer are randomly oriented.

4. The fibrous polymer scaffold of claim 2, wherein the polymer fibers of the second layer are uniaxially aligned.

5. The fibrous polymer scaffold of claim 2, wherein at least one polymer fiber in the first layer and at least one polymer fiber in the second layer is the same polymer fiber that is continuous between the layers.

6. The fibrous polymer scaffold of claim 1, wherein the polymer fibers of the first layer comprise a material selected from: an aliphatic polyester, a poly(alkylene) oxide, poly(L-lactic acid), 70/30 L-lactide/e-caprolactone co-polymer, poly(caprolactone), poly(DL-lactide-co-caprolactone), poly(D-lactide-co-caprolactone), poly(L-lactide-co-caprolactone), poly(lactide-co-glycolic acid), poly(vinylpyrrolidine), poly(dimethylsiloxane), poly(lysine), collagen, laminin, fibronectin, elastin, alginate, fibrin, hyaluronic acid, proteoglycans, polypeptides, polysaccharides, silk fibroin, poly(ethylene-co- vinyl) alcohol, poly(vinyl) alcohol, poly(ethylene) glycol, poly(propylene) glycol, poly-L-lactide-co-glycolide-co-e-caprolactone, poly(tetrafluoroethylene), poly(dioxanone), polyglactin 910, poly(urethanes), poly(ester urethane), poly(ether urethane), poly(trimethylene carbonate), poly(glycerol sebacate), poly(pyrrole), poly(acetylene), poly(hydroxyalkanoates), and copolymers and combinations thereof.

7. The fibrous polymer scaffold of claim 2, wherein the polymer fibers of the second layer comprise a material selected from: an aliphatic polyester, a poly(alkylene) oxide, poly(L-lactic acid), 70/30 L-lactide/e-caprolactone co-polymer, poly(caprolactone), poly(DL-lactide-co-caprolactone), poly(D-lactide-co-caprolactone), poly(L-lactide-co-caprolactone), poly(lactide-co-glycolic acid), poly(vinylpyrrolidine), poly(dimethylsiloxane), poly(lysine), collagen, laminin, fibronectin, elastin, alginate, fibrin, hyaluronic acid, proteoglycans, polypeptides,

polysaccharides, silk fibroin, poly(ethylene-co- vinyl) alcohol, poly(vinyl) alcohol, poly(ethylene) glycol, poly(propylene) glycol, poly-L-lactide-co-glycolide-co- ε-caprolactone, poly(tetrafluoroethylene), poly(dioxanone), polyglactin 910, poly(urethanes), poly(ester urethane), poly(ether urethane), poly(trimethylene carbonate), poly(glycerol sebacate), poly (pyrrole), poly(acetylene), poly(hydroxyalkanoates), and copolymers and combinations thereof.

8. The fibrous polymer scaffold of any one of claims 1-7, wherein the scaffold is a membrane.

9. The fibrous polymer scaffold of any one of claims 1-8, comprising an additive selected from poly(propylene glycol), poly(ethylene oxide), triethyl citrate, glycerol, poly (ethylene glycol), and combinations thereof.

10. The fibrous polymer scaffold of claim 9, wherein the additive is poly(propylene glycol).

11. The fibrous polymer scaffold of any one of claims 1-10, comprising a salt selected from NaCl, KH_2P0_4, K_2HP0_4, $KI0_3$, KC1, $MgS0_4$, $MgCl_2$, $NaHC0_3$, $CaCl_2$, sodium acetate, salt of acetic acid, salt of ascorbic acid, salt of citric acid, salt of lactic acid, salt of glycolic acid, and combinations thereof.

12. The fibrous polymer scaffold of claim 11, wherein the salt is sodium acetate.

13. The fibrous polymer scaffold of any one of claims 1-12, wherein the polymer fibers comprise poly(L-lactide-co-caprolactone), poly(propylene glycol) and sodium acetate.

14. The fibrous polymer scaffold of claim 1, comprising a cell.

15. The fibrous polymer scaffold of claim 1, comprising a biomolecule.

16. An electrospinning apparatus for producing fibrous polymer scaffolds comprising a first layer of diametrically patterned polymer fibers, comprising:

(i) a spinneret assembly;

(ii) a rotational disk assembly; and

(iii) an electrical field;

(iv) wherein an electrically charged polymer solution is electrospun from the spinneret toward the surface of the rotational disk assembly.

17. The apparatus of claim 16 wherein the spinneret assembly comprises a pair of conductive arms.

18. The apparatus of claim 17, comprising an electrically insulating disk, an electrically grounded metal strip or disk, and two or more electrically charged point electrodes in conformal contact with the back surface of the rotational disk assembly;

wherein the disk assembly orients deposition of the electrospun fibers along a diametrical axis of the surface of the disk assembly.

19. A method of treating an injury in a subject, comprising: applying a fibrous polymer scaffold comprising a first layer of diametrically patterned polymer fibers such that:
    (i) the first layer contacts a defect in a tissue of the subject; and
    (ii) a convergence region of the first layer is placed in contact with the center of the defect.

20. A method of enhancing tissue growth within an injury in a subject, comprising: applying a fibrous polymer scaffold comprising a first layer of diametrically patterned polymer fibers such that:
    (i) the first layer contacts a defect in a tissue of the subject; and
    (ii) a convergence region of the first layer is placed in contact with the center of the tissue defect.

21. The method of claim 20, wherein the injury comprises a defect in a tissue selected from abdominal wall, amniotic sac, bladder, blood vessels, brain, cardiac tissue, fascia, gingival tissue, intestine, ligaments, meningeal tissue, mucous membranes, muscle, nerves, skin, spinal cord, stomach and tendons.

22. A kit for the repair of wounds, surgical incisions or biopsies comprising:
    (i) a fibrous polymer scaffold comprising a first layer of diametrically patterned polymer fibers; and
    (ii) instructions for using the scaffold to repair wounds, surgical incisions or biopsies by promoting the regeneration of at least one anatomical biological component selected from abdominal wall, amniotic sac, bladder, blood vessels, brain, cardiac tissue, fascia, gingival tissue, intestine, ligaments, meningeal tissue, mucous membranes, muscle, nerves, skin, spinal cord, stomach and tendons.

FIGURE 1

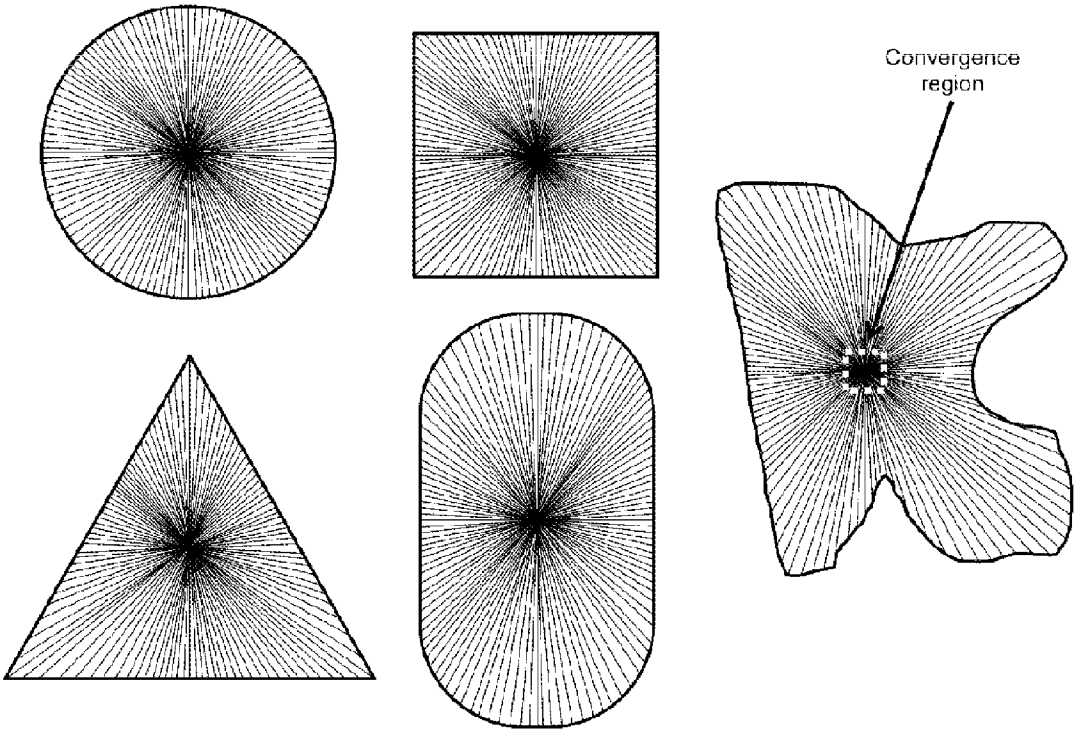


FIGURE 2

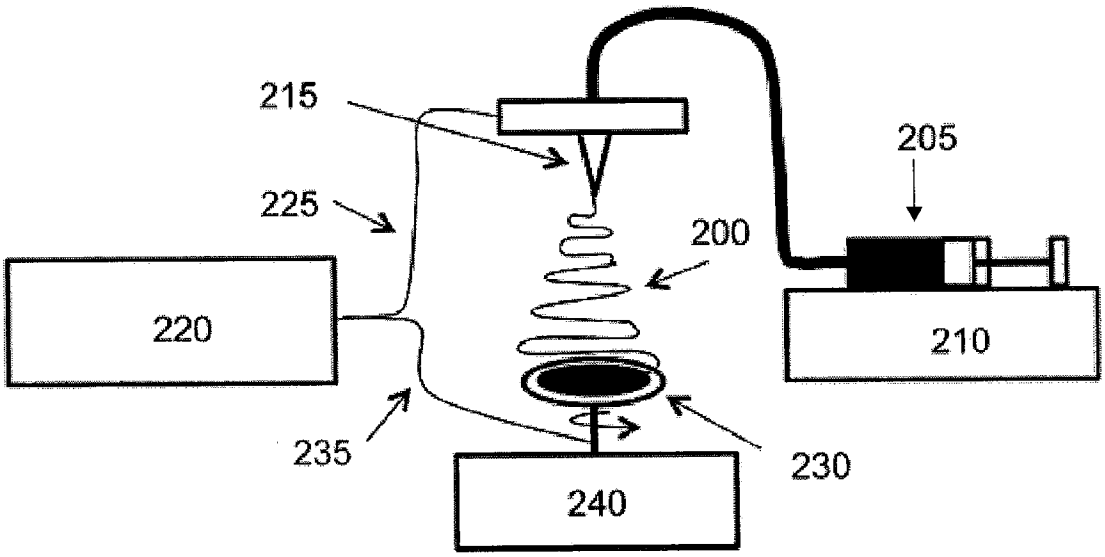


FIGURE 3

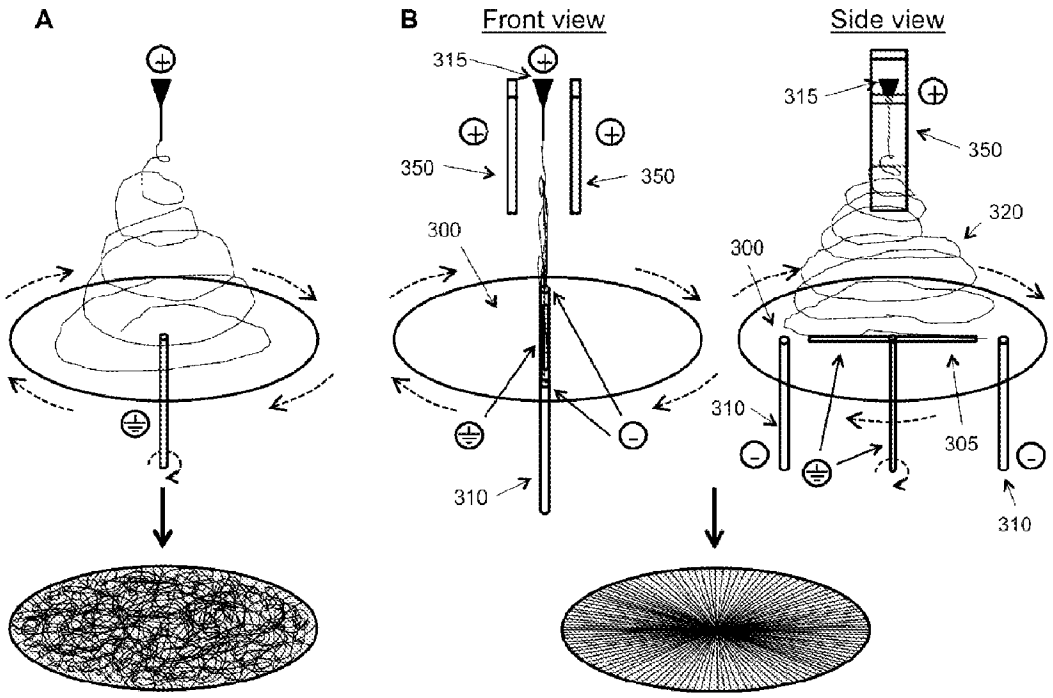

A
B
Front view
Side view
315
350
350
300
310
315
350
320
300
310
305
310

FIGURE 4

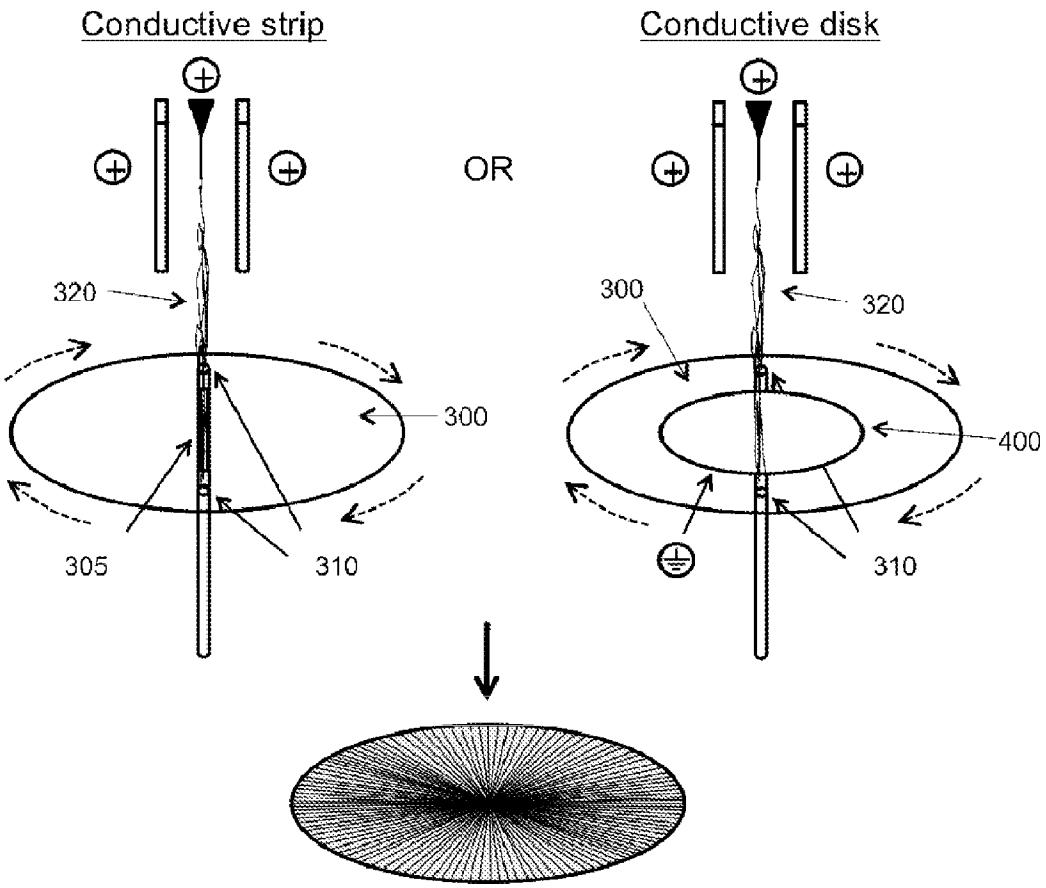

Conductive strip
OR
Conductive disk
320
300
305
310
300
320
400
310

FIGURE 5

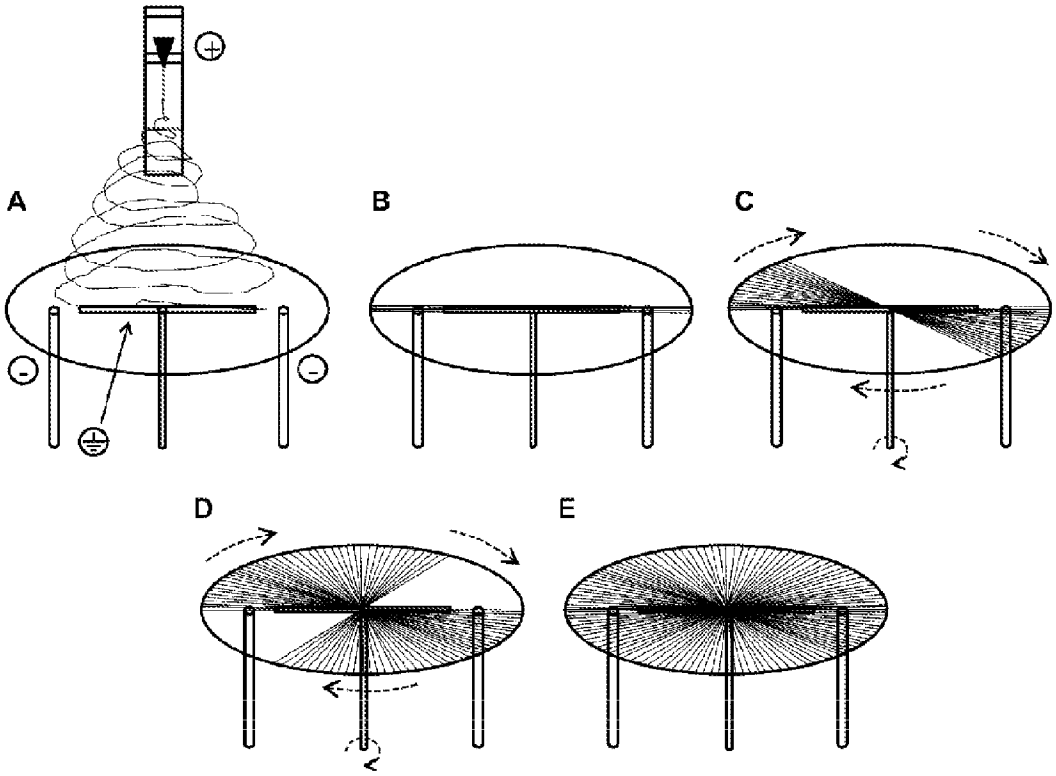


FIGURE 6

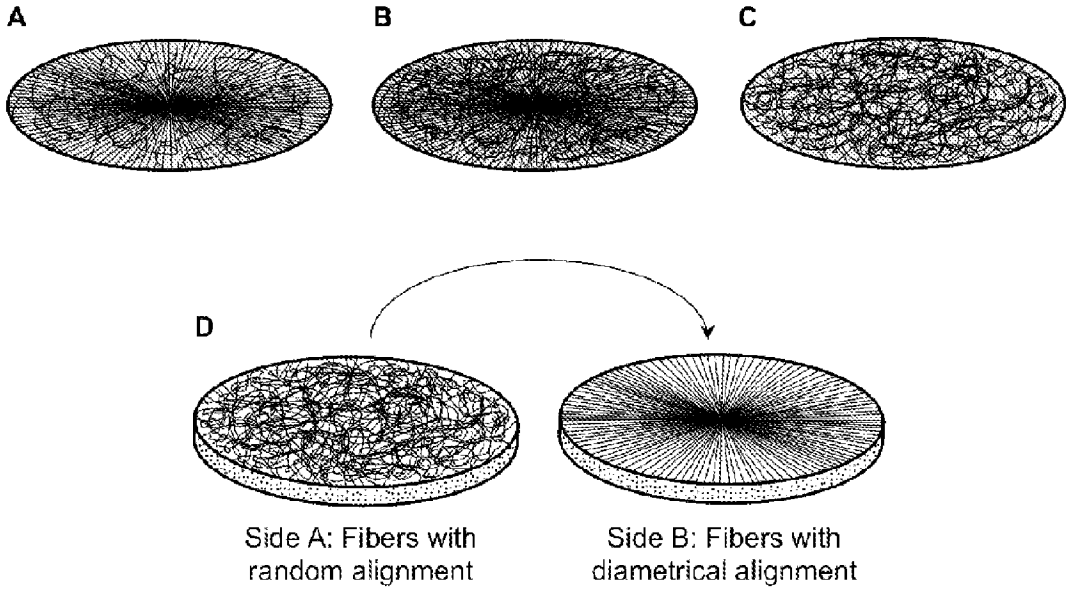

A
B
C
D
Side A: Fibers with random alignment
Side B: Fibers with diametrical alignment

FIGURE 7

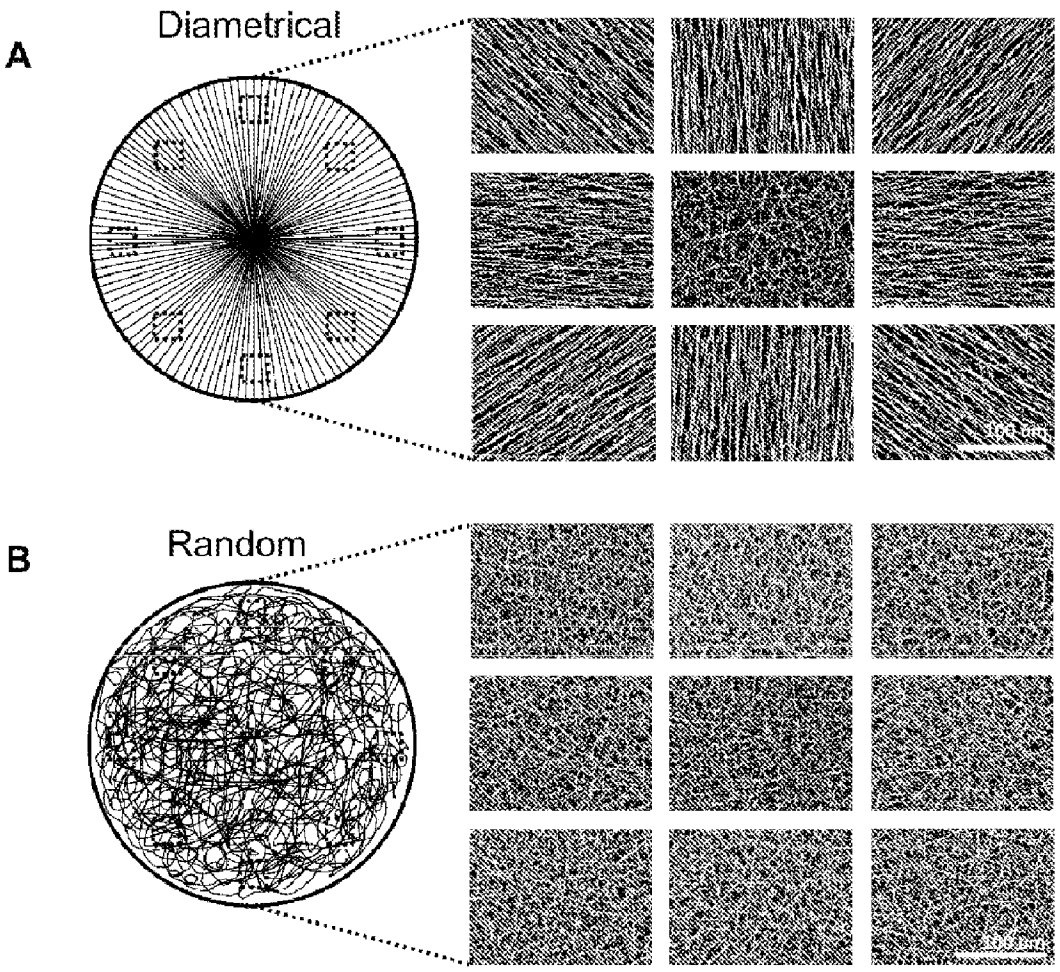


FIGURE 8

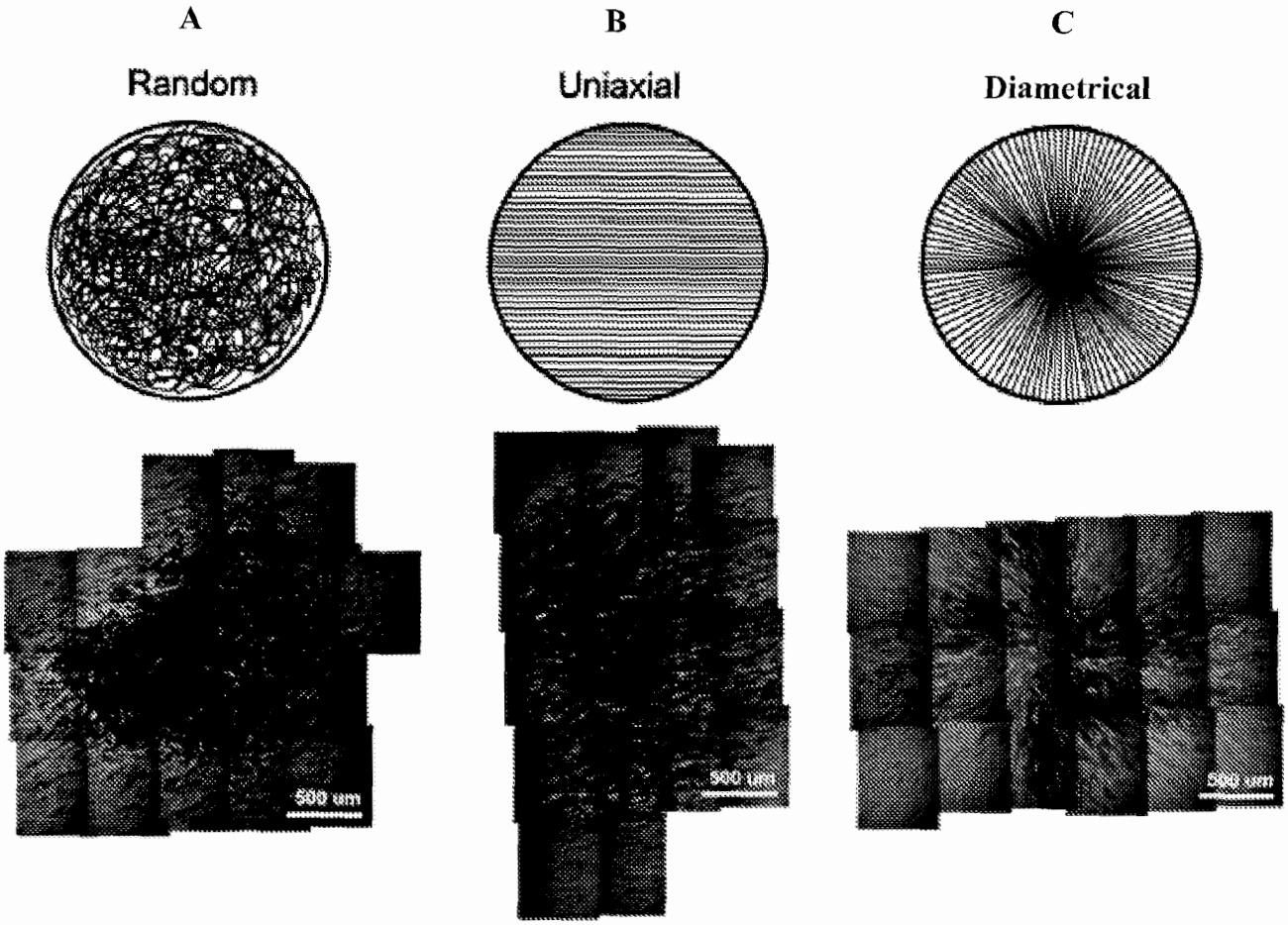
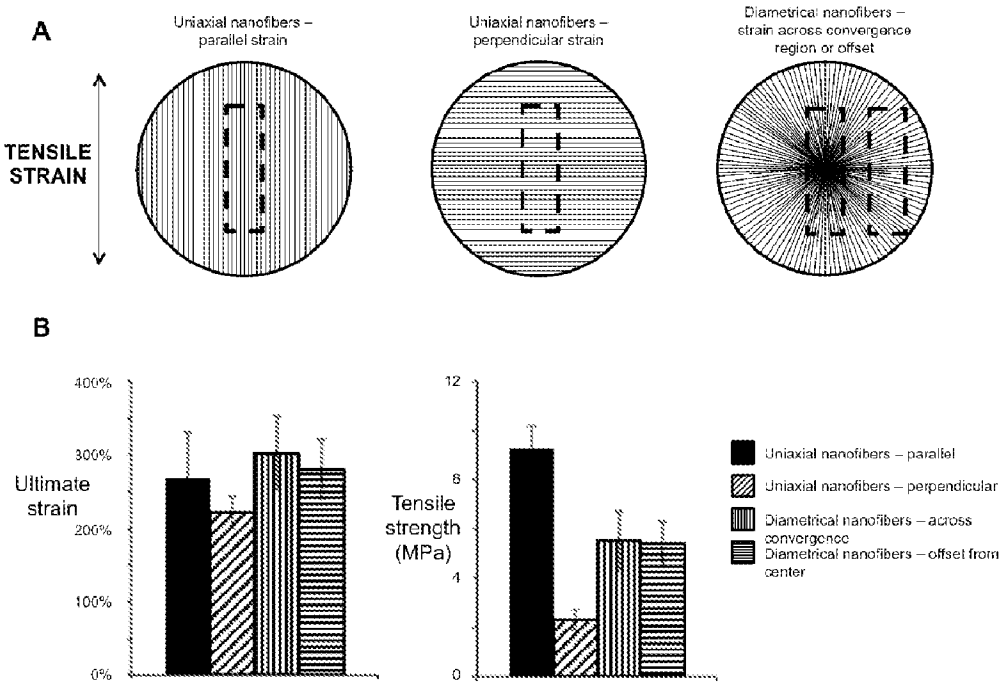


FIGURE 9


A
Uniaxial nanofibers – parallel strain
Uniaxial nanofibers – perpendicular strain
Diametrical nanofibers – strain across convergence region or offset
TENSILE STRAIN
B
400%
300%
200%
100%
0%
Ultimate strain
12
8
4
0
Tensile strength (MPa)
Uniaxial nanofibers – parallel
Uniaxial nanofibers – perpendicular
Diametrical nanofibers – across convergence
Diametrical nanofibers – offset from center

PE2E SEARCH - Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
L47	2	16/045159 AND fiber	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/07 04:04 PM
L48	2	16/045159 AND fiber SAME crosslink$	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/07 04:05 PM
L49	2	16/045159 AND fiber SAME 180$	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/07 04:06 PM
L50	2	16/045159 AND major ADJ peak	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/07 04:54 PM
L51	15561	(scaffold OR tissue adj2 support) near20 fiber	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/07 05:14 PM
L52	3214	(scaffold OR tissue adj2 support) near20 fiber AND (fourier OR fiber near10 align$ OR peak near10 180$)	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/07 05:14 PM
L53	1248	(scaffold OR tissue adj2 support) near20 fiber SAME (fourier OR fiber near10 align$ OR peak near10 180$)	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/07 05:52 PM
L54	491	L53 AND @ad<"20130314"	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/07 05:57 PM
L55	434	(scaffold OR tissue adj2 support) near20 fiber SAME (fourier OR fiber near10 cross-link$ OR peak near10 180$)	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/07 08:32 PM
L56	189	L55 AND @ad<"20130314"	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/07 08:32 PM

L57	337	(scaffold OR tissue adj2 support) near20 fiber SAME (fourier OR fiber near4 cross-link$ OR peak near10 180$)	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/07 08:40 PM
L58	150	L57 AND @ad<"20130314"	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/07 08:41 PM
L59	2	16/045159 AND fiber SAME collagen	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/12 01:54 PM
L60	2	16/045159 AND polycaprolactone	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/12 05:23 PM
L61	2	16/045159 AND basement	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/13 10:32 AM
L62	2	16/045159 AND scaffold SAME cell SAME defect	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/13 10:37 AM
L63	2	16/045159 AND cell SAME defect	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/13 10:38 AM
L64	2	16/045159 AND cell	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/13 10:39 AM
L65	2	16/045159 AND DRG	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/13 10:42 AM
L66	2	16/045159 AND tendon SAME cell	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/13 10:46 AM
L67	0	(scaffold OR tissue adj2 support) near20 fiber SAME (fourier OR peak) SAME (anisotropic near10	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/13 11:12 AM

		180$)					
L68	0	fiber SAME (fourier OR peak) SAME (anisotropic near10 180$)	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/13 11:18 AM
L69	33281	fiber SAME (fourier SAME peak OR anisotropic)	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/13 11:19 AM
L70	2295	fiber SAME (fourier SAME peak)	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/13 11:19 AM
L71	44	fiber SAME (fourier SAME peak) SAME scaffold	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/13 11:19 AM
L72	392	fiber SAME (anisotropic) SAME scaffold	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/13 11:19 AM
L73	428	L72 OR L71	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/13 11:20 AM
L74	160	L73 AND @ad<"20130314"	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/13 11:20 AM
L75	143	L72 AND @ad<"20130314"	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/13 11:21 AM
L76	108	fiber near20 anisotropic AND scaffold SAME (tendon OR ligament OR nerve) SAME fiber	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/13 11:22 AM
L77	34	L76 AND @ad<"20130314"	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/13 11:22 AM
L78	5	L76 AND @ad<"20130314" AND (fourier OR fft)	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO;	OR	ON	ON	2021/07/13 11:23 AM

			JPO; DERWENT; IBM_TDB)				
L79	0	"8,940,092".pn. AND fiber SAME collagen	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/13 11:49 AM
L80	0	"8,940,092".pn. AND collagen	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/13 11:49 AM
L81	0	"8,940,092".pn. AND basement	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/13 11:50 AM
L82	30	L76 AND @ad<"20130314" AND collagen SAME fiber	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/13 11:50 AM
L83	5	L76 AND @ad<"20130314" AND collagen SAME fiber AND fourier	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/13 01:46 PM
L84	0	13/394415 AND tendon	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/13 02:06 PM
L85	0	13/394415 AND ligament	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/13 02:06 PM
L86	2	13/394415 AND nerve	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/13 02:06 PM
L87	8	fiber near20 major ADJ peak AND scaffold SAME (tendon OR ligament OR nerve) SAME fiber AND fourier	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/07/13 02:08 PM

PE2E SEARCH - Search History (Interference)

There are no Interference searches to show.

Web Search History

date, time	web site	search string
7/12/2021 5:45:57 PM	Google	scaffold for tendon repair
7/12/2021 5:46:03 PM	Google	scaffold for tendon repair material
7/12/2021 5:46:24 PM	Google	scaffold for tendon repair material polycaprolactone
7/13/2021 10:16:59 AM	Google	traeting autoimmune disease with anti-cd33
7/13/2021 10:50:32 AM	Google	tendon cells
7/13/2021 10:51:08 AM	Google	cells to differentiate into tendons ligaments
7/13/2021 11:24:12 AM	Google	fft anisotropic
7/13/2021 11:24:21 AM	Google	fft anisotropic of fiber
7/13/2021 11:24:35 AM	Google	anistrophy
7/13/2021 11:24:55 AM	Google	measuring anisotrophy fft
7/13/2021 11:41:36 AM	Google	Measuring fiber alignment in electrospun scaffolds: a user's guide to the 2D fast Fourier transform approach
7/13/2021 11:53:16 AM	Google	Preparation and anisotropic mechanical behavior of highly-oriented electrospun poly(hutylene terephthalate) fibers
7/13/2021 11:53:50 AM	Google	fft peaks 180 degree

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/045,159	07/25/2018	Michael Francis	LNH-152US1	5408

23122 7590 01/11/2022

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King of Prussia, PA 19406

EXAMINER
MARVICH, MARIA

ART UNIT	PAPER NUMBER
1633	

NOTIFICATION DATE	DELIVERY MODE
01/11/2022	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

PCorrespondence@ratnerprestia.com

Applicant-Initiated Interview Summary	**Application No.** 16/045,159	**Applicant(s)** Francis et al.		
	Examiner MARIA MARVICH	**Art Unit** 1633	**AIA (First Inventor to File) Status** No	**Page** 1 of 1

All Participants (applicant, applicants representative, PTO personnel)	**Title**	**Type**
MARIA MARVICH	Primary Examiner	Telephonic
Valerie Hayes	Attorney of Record	
Jack Matney	Attorney of Record	

Date of Interview: 06 January 2022

Issues Discussed:

35 U.S.C. 112

Amendments to overcome issues raised in the 112, first paragraph.

35 U.S.C. 102

Claim 26 is rejected as anticipated by Patel and Simpson. The reference to Yeo in the body of the document is incorrect. The reference teaches the fibers are anisotropic and therefore they are cross-linked.

Non-statutory Double Patenting

An TD will be filed to overcome this rejection.

/MARIA MARVICH/ Primary Examiner, Art Unit 1633	

Applicant is reminded that a complete written statement as to the substance of the interview must be made of record in the application file. It is the applicants responsibility to provide the written statement, unless the interview was initiated by the Examiner and the Examiner has indicated that a written summary will be provided. See MPEP 713.04
Please further see:
MPEP 713.04
Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews, paragraph (b)
37 CFR § 1.2 Business to be transacted in writing

Applicant recordation instructions: The formal written reply to the last Office action must include the substance of the interview. (See MPEP section 713.04). If a reply to the last Office action has already been filed, applicant is given a non-extendable period of the longer of one month or thirty days from this interview date, or the mailing date of this interview summary form, whichever is later, to file a statement of the substance of the interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 16/045,159	Filing Date 07/25/2018	☐ To be Mailed

ENTITY: ☐ LARGE ☑ SMALL ☐ MICRO

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A		N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A		N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*		x $50 =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*		x $230 =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).				
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))					
* If the difference in column 1 is less than zero, enter "0" in column 2.				TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT	01/18/2022	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 14	Minus	** 20	= 0		x $50 =	0
	Independent (37 CFR 1.16(h))	* 4	Minus	*** 4	= 0		x $240 =	0
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	0

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=		x $0 =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=		x $0 =	
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.	SLIE
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".	/DEIDRE C KING-WOODWARD/
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".	
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.	

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Doc Code: A.NE.AFCP
Document Description: After Final Consideration Pilot Program Request

PTO/SB/434 (05-13)

CERTIFICATION AND REQUEST FOR CONSIDERATION UNDER THE AFTER FINAL CONSIDERATION PILOT PROGRAM 2.0

Practitioner Docket No.:	Application No.:	Filing Date:
LNH-152US1	16/045,159	07/25/2018
First Named Inventor:	**Title:**	
Michael Francis	ALIGNED FIBER AND METHOD OF USE THEREOF	

APPLICANT HEREBY CERTIFIES THE FOLLOWING AND REQUESTS CONSIDERATION UNDER THE AFTER FINAL CONSIDERATION PILOT PROGRAM 2.0 (AFCP 2.0) OF THE ACCOMPANYING RESPONSE UNDER 37 CFR 1.116.

1. The above-identified application is (i) an original utility, plant, or design nonprovisional application filed under 35 U.S.C. 111(a) [a continuing application (*e.g.*, a continuation or divisional application) is filed under 35 U.S.C. 111(a) and is eligible under (i)], or (ii) an international application that has entered the national stage in compliance with 35 U.S.C. 371(c).
2. The above-identified application contains an outstanding final rejection.
3. Submitted herewith is a response under 37 CFR 1.116 to the outstanding final rejection. The response includes an amendment to at least one independent claim, and the amendment does not broaden the scope of the independent claim in any aspect.
4. This certification and request for consideration under AFCP 2.0 is the only AFCP 2.0 certification and request filed in response to the outstanding final rejection.
5. Applicant is willing and available to participate in any interview requested by the examiner concerning the present response.
6. This certification and request is being filed electronically using the Office's electronic filing system (EFS-Web).
7. Any fees that would be necessary consistent with current practice concerning responses after final rejection under 37 CFR 1.116, *e.g.*, extension of time fees, are being concurrently filed herewith. [There is no additional fee required to request consideration under AFCP 2.0.]
8. By filing this certification and request, applicant acknowledges the following:
    - Reissue applications and reexamination proceedings are not eligible to participate in AFCP 2.0.
    - The examiner will verify that the AFCP 2.0 submission is compliant, *i.e.*, that the requirements of the program have been met (see items 1 to 7 above). For compliant submissions:
        - The examiner will review the response under 37 CFR 1.116 to determine if additional search and/or consideration (i) is necessitated by the amendment and (ii) could be completed within the time allotted under AFCP 2.0. If additional search and/or consideration is required but cannot be completed within the allotted time, the examiner will process the submission consistent with current practice concerning responses after final rejection under 37 CFR 1.116, *e.g.*, by mailing an advisory action.
        - If the examiner determines that the amendment does not necessitate additional search and/or consideration, or if the examiner determines that additional search and/or consideration is required and could be completed within the allotted time, then the examiner will consider whether the amendment places the application in condition for allowance (after completing the additional search and/or consideration, if required). If the examiner determines that the amendment does not place the application in condition for allowance, then the examiner will contact the applicant and request an interview.
            - The interview will be conducted by the examiner, and if the examiner does not have negotiation authority, a primary examiner and/or supervisory patent examiner will also participate.
            - If the applicant declines the interview, or if the interview cannot be scheduled within ten (10) calendar days from the date that the examiner first contacts the applicant, then the examiner will proceed consistent with current practice concerning responses after final rejection under 37 CFR 1.116.

Signature	Date
/Valerie P. Hayes/	01/18/2022
Name (Print/Typed) Valerie P. Hayes	**Practitioner Registration No.** 53005

***Note**: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.*

[✓] * Total of 1 forms are submitted.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

PTO/SB/31 (02-14)
Approved for use through 11/30/2020. OMB 0651-0031
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

NOTICE OF APPEAL FROM THE EXAMINER TO THE PATENT TRIAL AND APPEAL BOARD

Docket Number (Optional): LNH-152US1

I hereby certify that this correspondence is being facsimile transmitted to the USPTO, EFS-Web transmitted to the USPTO, or or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to "Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450" [37 CFR 1.8(a)]

on 01/18/2022

Signature /Kathy Spina/

Typed or printed name Kathy Spina

In re Application of: Michael Francis

Application Number	Filed
16/045,159	07/25/2018

For ALIGNED FIBER AND METHOD OF USE THEREOF

Art Unit	Examiner
1633	MARVICH, MARIA

Applicant hereby **appeals** to the Patent Trial and Appeal Board from the last decision of the examiner.

The fee for this Notice of Appeal is (37 CFR 41.20(b)(1)) $ 840

☑ Applicant claims small entity status. See 37 CFR 1.27. Therefore, the fee shown above is reduced by half, and the resulting fee is: $ 420

☐ A check in the amount of the fee is enclosed.

☑ Payment by credit card. Form PTO-2038 is attached.

☑ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. 18-0350 .

☐ A petition for an extension of time under 37 CFR 1.136(a) (PTO/SB/22) is enclosed.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

I am the

☐ applicant/inventor.

☐ assignee of record of the entire interest. See 37 CFR 3.71. Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96)

☑ attorney or agent of record. Registration number 53005.

☐ attorney or agent acting under 37 CFR 1.34. Registration number if acting under 37 CFR 1.34. _____

/Valerie P. Hayes/
Signature

Valerie P. Hayes
Typed or printed name

610-407-0700
Telephone number

01/18/2022
Date

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

☑ *Total of 1 forms are submitted.

This collection of information is required by 37 CFR 41.31. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11, 1.14 and 41.6. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
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3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
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6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
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9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal

Application Number:	16045159
Filing Date:	25-Jul-2018
Title of Invention:	ALIGNED FIBER AND METHOD OF USE THEREOF
First Named Inventor/Applicant Name:	Michael Francis
Filer:	Valerie P. Hayes/Kathy Spina
Attorney Docket Number:	LNH-152US1

Filed as Small Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
NOTICE OF APPEAL	2401	1	420	420
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Extension - 3 months with $0 paid	2253	1	740	740
Miscellaneous:				
			Total in USD ($)	**1160**

Electronic Acknowledgement Receipt

EFS ID:	44765980
Application Number:	16045159
International Application Number:	
Confirmation Number:	5408
Title of Invention:	ALIGNED FIBER AND METHOD OF USE THEREOF
First Named Inventor/Applicant Name:	Michael Francis
Customer Number:	23122
Filer:	Valerie P. Hayes/Kathy Spina
Filer Authorized By:	Valerie P. Hayes
Attorney Docket Number:	LNH-152US1
Receipt Date:	18-JAN-2022
Filing Date:	25-JUL-2018
Time Stamp:	15:04:30
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$1160
RAM confirmation Number	E20221HF05011266
Deposit Account	180350
Authorized User	Kathy Spina

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

37 CFR 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Amendment after Notice of Allowance (Rule 312)	AF_Response_LNH-152US1.pdf	114868 615a3cd6dfdca3df287414296bbcc86b713f021b	no	8
Warnings:					
Information:					
2	After Final Consideration Program Request	AF_2_0_LNH-152US1.pdf	1011531 2c13db1a9b5dfd7a2ec34508d490e06e72dbd0b5	no	2
Warnings:					
Information:					
3	Notice of Appeal Filed	LNH-152US1_Notice_Appeal.pdf	898257 685ee4872152e000acc575fc8a5a2bfcd602b74c	no	2
Warnings:					
Information:					
4	Fee Worksheet (SB06)	fee-info.pdf	40611 d1f108a052af43a1159b7e6bf0737c837f2e7567	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	2065267		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No:	16/045,159
Applicant:	LifeNet Health
Filed:	July 25, 2018
Title:	ALIGNED FIBER AND METHOD OF USE THEREOF
T.C./A.U.:	1633
Examiner:	MARVICH, MARIA
Confirmation No.:	5408
Docket No.:	LNH-152US1

AMENDMENT UNDER 37 C.F.R. § 1.116

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

In response to the Final Office Action dated July 19, 2021, please amend the above-identified application as follows:

☐ **Amendments to the Specification** begin on page of this paper.

☒ **Amendments to the Claims** are reflected in the listing of claims, which begins on page **2** of this paper.

☐ **Amendments to the Drawings** begin on page of this paper and include an attached replacement sheet(s).

☐ **Amendments to the Abstract** are on page of this paper. A clean version of the Abstract is on page of this paper.

☒ **Remarks/Arguments** begin on page **5** of this paper.

Amendments to the Claims:

This list of claims replaces all prior versions and lists of claims in the application:

1.-24. (Canceled)

25. (Currently Amended) A method of treating a tissue defect in a subject comprising

implanting at the tissue defect a scaffold comprising ~~one or more~~ crosslinked fibers comprising collagen, wherein a result of a fast Fourier transform (FFT) analysis of the fibers demonstrates that the fibers have major adjacent peaks that are about 180° apart from each other, and ,

~~surrounding~~ applying the scaffold to the tissue defect ~~with the scaffold~~ to repair a tendon, a ligament or a nerve defect.

26. (Cancelled)

27. (Currently Amended) A method of cell culture comprising

placing cells on a scaffold comprising ~~one or more~~ aligned fibers, wherein a result of a FFT analysis of the fibers demonstrates that the fibers have major adjacent peaks that are about 180° apart from each other, and

growing the cells in conditions suitable for the cell culture.

28. (Previously Presented) The method of claim 27, wherein the cells are stem cells, adipose derived stem cells, dental pulp stem cells, fibroblasts, and/or dorsal root ganglia.

29. (Original) The method of claim 27, wherein the cells are dorsal root ganglia.

30. (Original) The method of claim 27, wherein the cells are dorsal root ganglia, and the fibers comprise heart basement membrane extract, heart basement membrane extracellular matrix, placenta basement membrane extract, or placenta basement membrane extracellular matrix.

31. (Currently Amended) A method of promoting differentiation of stem cells into osteoblasts, chondrocytes, ligament or tendon, the method comprising

placing the stem cells on a scaffold comprising ~~one or more~~ aligned fibers, wherein a result of a FFT analysis of the fibers demonstrates that the fibers have major adjacent peaks that are about 180° apart from each other, and

growing the stem cells in *in vitro* ~~in conditions~~conditions suitable for the cell differentiation.

32. (Original) The method of claim 31, wherein the stem cells are progenitor cells or adult stem cells.

33. (Previously Presented) The method of claim 32, wherein the progenitor cells or the adult stem cells are isolated from placenta, bone marrow, adipose tissue, blood vessel, amniotic fluid, synovial fluid, synovial membrane, pericardium, periosteum, dura, peripheral blood, umbilical blood, menstrual blood, baby teeth, nucleus pulposus, brain, skin, hair follicle, intestinal crypt, neural tissue, or muscle.

34. (Cancelled.)

35. (Previously Presented) The method of claim 33, wherein the stem cells are adipose derived stem cells and/or dental pulp stem cells.

36. (Previously Presented) The method of claim 25, wherein the scaffold is in the form of one or more elongated sheets.

37. (Previously Presented) The method of claim 25, wherein the scaffold is a composite of multiple layers of one or more sheets.

38. (Currently Amended) The method of claim 25, wherein the tissue defect is ~~a nerve~~the nerve defect.

39. (Currently Amended) The method of claim 25, wherein the tissue defect is ~~a ligament~~the ligament defect.

40. (Currently Amended) The method of claim 25, wherein the tissue defect is ~~a tendon~~the tendon defect.

41. (Cancelled)

42. (Cancelled)

43. (Cancelled).

44. (Cancelled)

45. (Cancelled)

Remarks/Arguments:

Status

By this Response, claims 25, 27, 31, and 38-40 are amended to clarify features of Applicant's invention and are supported by the specification in least at paragraph [0102] of Applicant's Published Specification US 2019/0167842. Claims 26 and 41-45 are cancelled without prejudice or disclaimer. No prohibited new matter is added. Claims 25, 27-33, and 35-40 remain pending for consideration in the application.

Reconsideration and withdrawal of the objections and rejections in view of the above amendments and the following remarks are respectfully requested.

Applicant-Initiated Interview Summary

As a preliminary matter, Applicant's representatives Wm. Jack Matney and Valerie Hayes appreciate the opportunity to conduct a telephonic interview with Examiner Maria Marvich on January 6, 2022. During the telephonic interview, features of Applicant's invention were discussed in view of the §112, first paragraph rejection. Based upon Applicant's explanation, a better understanding of Applicant's invention was reached, and the Examiner requested that similar amendments and remarks be included in Applicant's Response. The Examiner further clarified that the identification of "Yeo" as a reference applied in the §102 rejection is a typographical error.

The amendments and remarks presented in this Response are consistent with the discussion with the Examiner, and provide the substance of the interview. The formal reply to the final Office Action follows. Also, as suggested by the Examiner during the telephonic interview, a Request under the After-Final Continuation Practice ("AFCP") 2.0 Program is filed herewith.

Claim Objections

Claims 25, 31, 38-40, and 43-45 are objected to because of informalities. Claims 25, 31, and 38-40 are amended, and claims 43-45 are cancelled without prejudice. Accordingly, Applicant believes the objections are overcome. Withdrawal of the objections is requested.

Rejection Under 35 U.S.C. §112, second paragraph

Claims 25-33 and 35-45 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite. Without acquiescing to the merits of this rejection, claims 25, 27, 31, and 38-40

have been amended. Claims 26 and 41-45 have been cancelled without prejudice or disclaimer. Thus, the rejection, as applied to these cancelled claims, is rendered moot. Reconsideration and withdrawal of the §112, second paragraph rejection are requested.

Rejection Under 35 U.S.C. §112, first paragraph

In the Office Action, claims 25-33 and 35-45 are rejected under 35 U.S.C. §112, first paragraph because the specification, while being enabling "for a method of treating a subject with a tissue defect…" or " a method of cell culture (or differentiation)…" allegedly does not provide enablement for any other embodiment or enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make or use the invention commensurate in scope with claims for the reasons provided on pages 4-8 of the Office Action. Without acquiescing to the merits of this rejection, and based upon the telephonic interview with the Examiner on January 6, 2022, Applicant respectfully traverses the rejection.

During the telephonic interview, Examiner Marvich indicated issues with respect to claiming one versus multiple fibers, and completeness as to whether independent claims 25 and 26 were sufficient because they did not include a recitation of growing or culturing cells.

Regarding the first item of one versus multiple fibers, Applicant has amended claims 25, 27, and 31 to delete "one or more" as an adjective describing the fibers so that the claims recite either "crosslinked fibers" or "aligned fibers."

Regarding growing or culturing cells, Applicant submits the invention disclosure provides that including cultured cells is an alternative feature. See, e.g., Applicant's Published Specification US 2019/0167842, paragraphs [0087]-[0093] and [0101]-[0103]. For example, the specification provides, at para [0091] (emphasis added):

> The invention also relates to methods of promoting nerve repair using the aligned fiber, electrospun fiber, and/or scaffold described herein. In some embodiments, the nerve repair includes, but is not limited to, central and peripheral nerve repair. <u>The methods of promoting nerve repair may comprise implanting the aligned fiber, electrospun fiber, and/or scaffold into a nerve damaged site of a subject in need thereof. The method may further comprise culturing dorsal root ganglia and/or stem cells on the aligned fiber, electrospun fiber, and/or scaffold prior to the implanting.</u>

Similarly, in other portions of Applicant's specification, the invention *optionally* offers cell culturing, (1) including both cultured cells and scaffold, or (2) fiber, scaffold and/or implant without cultured cells, for example, at para [0101] (emphases added):

> Applicant's invention also relates to methods of treating a tissue or organ defect or injury, for example, a musculoskeletal, dental or soft-tissue defect or injury, in an animal comprising administering (1) cells cultured on the scaffold produced by the methods described herein and/or (2) the aligned fiber, electrospun fiber, scaffold and/or implant described herein to the tissue or organ defect (e.g. osseous defects, defects in cartilage, ligament, tendon, spinal disk, and tendon insertion site to bone) (*id.*, [0101]).

Further, based upon the above and as discussed with the Examiner during the telephonic interview, Applicant believes that the claims of the application are both complete and enabled as required by 35 U.S.C. § 112, first paragraph.

Reconsideration and withdrawal of the §112, first paragraph rejection are requested.

Rejection Under 35 U.S.C. § 102(b)

Claim 26 is rejected under §102(b) as being anticipated by Patel et al. (WO 2012078471, hereafter "Patel") and Simpson et al. (US 20120221025, hereafter "Simpson"). Without reaching the merits of the rejection, Applicant notes that claim 26 is canceled without prejudice or disclaimer. Thus, the rejection is rendered moot. Withdrawal of the §102 rejection is requested.

Double Patenting Rejection

Claim 26 is provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-25 of co-pending Application No. 16/989,307 (Applicant's reference: LNH-152US2) in view of Patel and Simpson.

Claim 26 is cancelled without prejudice or disclaimer. Thus, the provisional double patenting rejection is rendered moot. Withdrawal of the double patenting rejection is requested.

Conclusion

For the foregoing reasons, Applicant respectfully request the Office to withdraw the objections and rejections, and allow the pending application

As Applicant's remarks above with respect to the Examiner's objections and rejection are sufficient to overcome the objections and rejections, Applicant's silence as to any assertions by the Examiner in the Office Action or certain requirements that may be applicable to such rejections is not a concession by Applicant that such assertions are accurate or such requirements have been met, and Applicant reserves the right to analyze and dispute such in the future.

To the extent necessary, if any, a petition for an extension of time under 37 C.F.R. § 1.136 is hereby made. The Director is hereby authorized to charge or credit Deposit Account No. 18-0350 for any additional fees, or any underpayment or credit for overpayment in connection herewith.

Respectfully submitted,



William J. Matney, Jr., Reg. No. 39,292
Valerie P. Hayes, Reg. No. 53,005
Attorneys for Applicants

WJM/VPH/ks
Dated: January 18 2022

1090 Vermont Avenue NW,
Suite 1200
Washington, DC 20005
(202) 715-2898

The Director is hereby authorized to charge or credit Deposit Account No. **18-0350** for any additional fees, or any underpayment or credit for overpayment in connection herewith.

PTO/SB/26 (08-11)
Approved for use through 11/30/2020. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TERMINAL DISCLAIMER TO OBVIATE A DOUBLE PATENTING REJECTION OVER A "PRIOR" PATENT	Docket Number (Optional) LNH-152US1

In re Application of: Michael Francis

Application No.: 16/045,159

Filed: 07/25/2018

For: ALIGNED FIBER AND METHOD OF USE THEREOF

The owner*, LifeNet Health, of 100 percent interest in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of **prior patent** No. 10,137,223 as the term of said **prior patent** is presently shortened by any terminal disclaimer. The owner hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and the **prior patent** are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the owner does not disclaim the terminal part of the term of any patent granted on the instant application that would extend to the expiration date of the full statutory term of the **prior patent**, "as the term of said **prior patent** is presently shortened by any terminal disclaimer," in the event that said **prior patent** later:

expires for failure to pay a maintenance fee;
is held unenforceable;
is found invalid by a court of competent jurisdiction;
is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321;
has all claims canceled by a reexamination certificate;
is reissued; or
is in any manner terminated prior to the expiration of its full statutory term as presently shortened by any terminal disclaimer.

Check either box 1 or 2 below, if appropriate.

1. ☐ For submissions on behalf of a business/organization (e.g., corporation, partnership, university, government agency, etc.), the undersigned is empowered to act on behalf of the business/organization.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

2. ☑ The undersigned is an attorney or agent of record. Reg. No. 53005

/Valerie P. Hayes/ — Signature
02/04/2022 — Date

Valerie P. Hayes — Typed or printed name

610-407-0700 — Telephone Number

☑ Terminal disclaimer fee under 37 CFR 1.20(d) included.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

*Statement under 37 CFR 3.73(b) is required if terminal disclaimer is signed by the assignee (owner). Form PTO/SB/96 may be used for making this certification. See MPEP § 324.

This collection of information is required by 37 CFR 1.321. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
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5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal

Application Number:	16045159
Filing Date:	25-Jul-2018
Title of Invention:	ALIGNED FIBER AND METHOD OF USE THEREOF
First Named Inventor/Applicant Name:	Michael Francis
Filer:	Valerie P. Hayes/Kathy Spina
Attorney Docket Number:	LNH-152US1

Filed as Small Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
STATUTORY OR TERMINAL DISCLAIMER	2814	1	170	170
		Total in USD ($)		**170**

Electronic Acknowledgement Receipt

EFS ID:	44917173
Application Number:	16045159
International Application Number:	
Confirmation Number:	5408
Title of Invention:	ALIGNED FIBER AND METHOD OF USE THEREOF
First Named Inventor/Applicant Name:	Michael Francis
Customer Number:	23122
Filer:	Valerie P. Hayes/Kathy Spina
Filer Authorized By:	Valerie P. Hayes
Attorney Docket Number:	LNH-152US1
Receipt Date:	04-FEB-2022
Filing Date:	25-JUL-2018
Time Stamp:	15:13:43
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$170
RAM confirmation Number	E202224F14290074
Deposit Account	180350
Authorized User	Kathy Spina

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

37 CFR 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Terminal Disclaimer Filed	Terminal_Disclaimer-LNH-152US1.pdf	915004 1d206aff53b79fd0f5a2ee3066d44f1476c24893	no	2
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	37901 2d03458b925bfef9c8c924f6c3a0deffb1111366	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	952905		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Application Number	Application/Control No.	Applicant(s)/Patent under Reexamination	
* 16/045,159 *	16/045,159	Francis et al.	
	Examiner	**Art Unit**	
	MARVICH, MARIA	1633	

Document Code - DISQ	**Internal Document - DO NOT MAIL**

TERMINAL DISCLAIMER	☑ APPROVED	☐ DISAPPROVED
Date Filed: **04 February 2022**	**This patent is subject to a Terminal Disclaimer**	

Approved/Disapproved by:

/PAMELA A YOUNG/

Technology Center: OPLC

Telephone: (571)272-3622

_____

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/045,159	07/25/2018	Michael Francis	LNH-152US1	5408

23122 7590 02/08/2022

RATNERPRESTIA
2200 Renaissance Blvd
Suite 350
King of Prussia, PA 19406

EXAMINER
MARVICH, MARIA

ART UNIT	PAPER NUMBER
1633	

NOTIFICATION DATE	DELIVERY MODE
02/08/2022	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

PCorrespondence@ratnerprestia.com

Examiner-Initiated Interview Summary	**Application No.** 16/045,159	**Applicant(s)** Francis et al.		
	Examiner MARIA MARVICH	**Art Unit** 1633	**AIA (First Inventor to File) Status** No	**Page** 1 of 1

All Participants (applicant, applicants representative, PTO personnel)	**Title**	**Type**
MARIA MARVICH	Primary Examiner	Telephonic
Valerie Hayes	Attorney of Record	

Date of Interview: 03 February 2022

Issues Discussed:

Non-statutory Double Patenting

Applicants will submit a TD disclaiming the terminal portion of any patent granted on this application which would extend beyond the expiration date of U.S. Patent 10,137,223. Claims 11-15 were found to overlap the instant claims 27-33 and 35. This will place the case in condition for allowance.

/MARIA MARVICH/ Primary Examiner, Art Unit 1633	

Applicant is reminded that a complete written statement as to the substance of the interview must be made of record in the application file. It is the applicants responsibility to provide the written statement, unless the interview was initiated by the Examiner and the Examiner has indicated that a written summary will be provided. See MPEP 713.04
Please further see:
MPEP 713.04
Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews, paragraph (b)
37 CFR § 1.2 Business to be transacted in writing

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
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Alexandria, Virginia 22313-1450
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

23122 7590 02/28/2022

RATNERPRESTIA
2200 Renaissance Blvd
Suite 350
King of Prussia, PA 19406

EXAMINER
MARVICH, MARIA

ART UNIT	PAPER NUMBER
1633	

DATE MAILED: 02/28/2022

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/045,159	07/25/2018	Michael Francis	LNH-152US1	5408

TITLE OF INVENTION: ALIGNED FIBER AND METHOD OF USE THEREOF

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	$600	$0.00	$0.00	$600	05/31/2022

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Maintenance fees are due in utility patents issuing on applications filed on or after Dec. 12, 1980. It is patentee's responsibility to ensure timely payment of maintenance fees when due. More information is available at www.uspto.gov/PatentMaintenanceFees.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via EFS-Web.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

By fax, send to: (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

23122 7590 02/28/2022
RATNERPRESTIA
2200 Renaissance Blvd
Suite 350
King of Prussia, PA 19406

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via EFS-Web or by facsimile to (571) 273-2885, on the date below.

(Typed or printed name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/045,159	07/25/2018	Michael Francis	LNH-152US1	5408

TITLE OF INVENTION: ALIGNED FIBER AND METHOD OF USE THEREOF

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	$600	$0.00	$0.00	$600	05/31/2022

EXAMINER	ART UNIT	CLASS-SUBCLASS
MARVICH, MARIA	1633	435-395000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/AIA/122 or PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/AIA/47 or PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 _____
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. Fees submitted: ❑ Issue Fee ❑ Publication Fee (if required) ❑ Advance Order - # of Copies _____

4b. Method of Payment: *(Please first reapply any previously paid fee shown above)*

❑ Electronic Payment via EFS-Web ❑ Enclosed check ❑ Non-electronic payment by credit card (Attach form PTO-2038)

❑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. _____

5. **Change in Entity Status** (from status indicated above)

❑ Applicant certifying micro entity status. See 37 CFR 1.29

❑ Applicant asserting small entity status. See 37 CFR 1.27

❑ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/045,159	07/25/2018	Michael Francis	LNH-152US1	5408

23122 7590 02/28/2022

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Suite 350
King of Prussia, PA 19406

EXAMINER	
MARVICH, MARIA	
ART UNIT	PAPER NUMBER
1633	

DATE MAILED: 02/28/2022

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

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The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 16/045,159	Applicant(s) Francis et al.	
	Examiner MARIA MARVICH	Art Unit 1633	AIA (FITF) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☑ This communication is responsive to an AFCP 1/18/2022.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

3. ☑ The allowed claim(s) is/are 25,27-33 and 35-40 . As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information , please see **http://www.uspto.gov/patents/init_events/pph/index.jsp** or send an inquiry to **PPHfeedback@uspto.gov.**

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some* c) ☐ None of the:

   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____ .

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____ .

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____.
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____.
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date. _____.
5. ☑ Examiner's Amendment/Comment
6. ☐ Examiner's Statement of Reasons for Allowance
7. ☑ Other FORM 2323.

/MARIA MARVICH/
Primary Examiner, Art Unit 1633

DETAILED ACTION

The present application is being examined under the pre-AIA first to invent provisions.

This office action is in response to an after final amendment filed 1/18/2022.

Claims 25, 27-33 and 35-40 are pending.

This application is a Divisional Application of U.S. Application No. 14/776,106, filed September 14, 2015, now U.S. Patent 10,137,223, which is a U.S. National Phase Application of PCT International Application PCT/US2014/025636, filed March 13, 2014, which claims priority benefit of U.S. Application No. 61/785,031, filed March 14.

Terminal Disclaimer

The terminal disclaimers filed on 2/4/2022 disclaiming the terminal portion of any patent granted on this application which would extend beyond the expiration date of U.S. 10,137,223 has been reviewed and is accepted. The terminal disclaimer has been recorded.

Conclusion

Claims renumbered in the following order: 25, 36, 37, 38, 39, 40, 27, 28, 29, 30, 31, 32, 33, 35.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to MARIA MARVICH whose telephone number is (571)272-0774. The examiner can normally be reached on 8 am - 5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christopher Babic can be reached on 571-272-8507. The fax phone number for the

organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/MARIA MARVICH/
Primary Examiner, Art Unit 1633

AFCP 2.0 Decision

Application No.	Applicant(s)	
16/045,159	Francis et al.	
Examiner	**Art Unit**	**AIA (FITF) Status**
MARIA MARVICH	1633	No

This is in response to the After Final Consideration Pilot request filed 18 January 2022.

1. **Improper Request** – The AFCP 2.0 request is improper for the following reason(s) and the after final amendment submitted with the request will be treated under pre-pilot procedure.

   ☐ An AFCP 2.0 request form PTO/SB/434 (or equivalent document) was not submitted.

   ☐ A non-broadening amendment to at least one independent claim was not submitted.

   ☐ The request is not the first proper AFCP 2.0 request submitted in response to the most recent final rejection.

   ☐ Other: _____

2. **Proper Request**

   A. After final amendment submitted with the request will not be treated under AFCP 2.0.
   The after final amendment cannot be reviewed and a search conducted within the guidelines of the pilot program.

   ☐ The after final amendment will be treated under pre-pilot procedure.

   B. Updated search and/or completed additional consideration.
   The examiner performed an updated search and/or completed additional consideration of the after final amendment within the time authorized for the pilot program. The result(s) of the updated search and/or completed additional consideration are:

   ☑ 1. All of the rejections in the most recent final Office action are overcome and a Notice of Allowance is issued herewith.

   ☐ 2. The after final amendment would not overcome all of the rejections in the most recent final Office action . See attached interview summary for further details.

   ☐ 3. The after final amendment was reviewed, and it raises a new issue(s). See attached interview summary for further details.

   ☐ 4. The after final amendment raises new issues, but would overcome all of the rejections in the most recent final Office action. A decision on determining allowability could not be made within the guidelines of the pilot. See attached interview summary for further details, including any newly discovered prior art.

   ☐ 5. Other: _____

   Examiner Note: Please attach an interview summary when necessary as described above.

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/045,159	Francis et al.
	Examiner	**Art Unit**
	MARIA MARVICH	1633

CPC						
Symbol					**Type**	**Version**
A61L	/	27	/	18	F	2013-01-01
A61L	/	27	/	24	I	2013-01-01
A61L	/	27	/	26	I	2013-01-01
A61L	/	27	/	3604	I	2013-01-01
A61L	/	27	/	3633	I	2013-01-01
A61L	/	27	/	38	I	2013-01-01
A61L	/	27	/	383	I	2013-01-01
A61L	/	27	/	56	I	2013-01-01
D01D	/	5	/	0076	I	2013-01-01
D01D	/	5	/	0092	I	2013-01-01
C12N	/	5	/	0618	I	2013-01-01
A61F	/	2240	/	001	A	2013-01-01
A61L	/	2400	/	12	A	2013-01-01
A61F	/	2	/	08	A	2013-01-01
C12N	/	2533	/	30	A	2013-01-01
C12N	/	2533	/	54	A	2013-01-01
C12N	/	2533	/	90	A	2013-01-01

CPC Combination Sets								
Symbol					**Type**	**Set**	**Ranking**	**Version**
A61L	/	27	/	26	I	1	1	2013-01-01
C08L	/	67	/	04	I	1	2	2013-01-01
A61L	/	27	/	18	I	2	1	2013-01-01
C08L	/	67	/	04	I	2	2	2013-01-01

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	14	
/MARIA MARVICH/ Primary Examiner, Art Unit 1633	07 February 2022	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	25	None

U.S. Patent and Trademark Office — Part of Paper No.: 20220207

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/045,159	Francis et al.
	Examiner	Art Unit
	MARIA MARVICH	1633

INTERNATIONAL CLASSIFICATION				
CLAIMED				
A61F	/	2	/	08
A61L	/	27	/	24
A61L	/	27	/	26
A61K	/	38	/	17
A61L	/	27	/	38

NON-CLAIMED				
	/		/	

US ORIGINAL CLASSIFICATION	
CLASS	SUBCLASS
435	395

CROSS REFERENCES(S)						
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)					
424	442					
435	325	398	377			

NONE		Total Claims Allowed:	
(Assistant Examiner)	(Date)	14	
/MARIA MARVICH/ Primary Examiner, Art Unit 1633	07 February 2022	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	25	None

U.S. Patent and Trademark Office

Part of Paper No.: 20220207

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/045,159	Francis et al.
	Examiner	**Art Unit**
	MARIA MARVICH	1633

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☑ T.D. ☐ R.1.47

CLAIMS

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
1	25	10	30												
2	36	11	31												
3	37	12	32												
4	38	13	33												
5	39	14	35												
6	40														
7	27														
8	28														
9	29														

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 14	
/MARIA MARVICH/ Primary Examiner, Art Unit 1633 (Primary Examiner)	07 February 2022 (Date)	O.G. Print Claim(s) 25	O.G. Print Figure None

U.S. Patent and Trademark Office Part of Paper No.: 20220207

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/045,159	Francis et al.
	Examiner	Art Unit
	MARIA MARVICH	1633

CPC - Searched*		
Symbol	Date	Examiner

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
SEARCH- USPAT, PGPUB, EPO, JPO, Derwent, USOCR, IBM-IDB	12/17/2020	MM
inventor search	12/17/2020	MM
npl search	12/17/2020	MM
search updated, search notes attached	07/13/2021	MM
search updated, search notes attached	02/02/2022	MM

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner
see search notes		02/02/2022	MM

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No:	16/045,159
Applicant:	LifeNet Health
Filed:	July 25, 2018
Title:	ALIGNED FIBER AND METHOD OF USE THEREOF
T.C./A.U.:	1633
Examiner:	MARVICH, MARIA
Confirmation No.:	5408
Docket No.:	LNH-152US1

OK to enter
MM
2/7/2022

AMENDMENT UNDER 37 C.F.R. § 1.116

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

In response to the Final Office Action dated July 19, 2021, please amend the above-identified application as follows:

☐ **Amendments to the Specification** begin on page of this paper.

☒ **Amendments to the Claims** are reflected in the listing of claims, which begins on page **2** of this paper.

☐ **Amendments to the Drawings** begin on page of this paper and include an attached replacement sheet(s).

☐ **Amendments to the Abstract** are on page of this paper. A clean version of the Abstract is on page of this paper.

☒ **Remarks/Arguments** begin on page **5** of this paper.

Bibliographic Data

Application No: 16/045,159

Foreign Priority claimed: ○ Yes ◉ No

35 USC 119 (a-d) conditions met: ☐ Yes ☐ No ☐ Met After Allowance

Verified and Acknowledged: /MARIA MARVICH/ Examiner's Signature | Initials

Title: ALIGNED FIBER AND METHOD OF USE THEREOF

FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
07/25/2018	435	1633	LNH-152US1
RULE			

APPLICANTS

LifeNet Health, Virginia Beach, VA,

INVENTORS

Michael Francis, Virginia Beach, VA, UNITED STATES

Roy Ogle, Virginia Beach, VA, UNITED STATES

CONTINUING DATA

This application is a DIV of 14776106 09/14/2015 PAT 10137223

14776106 is a 371 of PCT/US2014/025636 03/13/2014

PCT/US2014/025636 has PRO of 61785031 03/14/2013

FOREIGN APPLICATIONS

IF REQUIRED, FOREIGN LICENSE GRANTED**

08/27/2018

**** SMALL ENTITY ****

STATE OR COUNTRY

UNITED STATES

ADDRESS

RATNERPRESTIA

2200 Renaissance Blvd

Suite 350

King of Prussia, PA 19406

UNITED STATES

FILING FEE RECEIVED

$1,015

Web Search History

date, time	web site	search string
2/2/2022 2:32:27 PM	Google	alligned fibers
2/2/2022 2:32:55 PM	Google	alligned fibers fourier transform
2/2/2022 2:34:43 PM	Google	heart basement extract
2/2/2022 2:36:00 PM	Google	heart basement membrane extract
2/2/2022 2:36:17 PM	Google	heart basement membrane extract fibers
2/2/2022 2:38:13 PM	Google Images	heart basement membrane extract fibers
2/2/2022 2:59:24 PM	Google	alligned fibers fourier scaffold

PE2E SEARCH - Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
L88	3	"20120221025"	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/01/06 01:23 PM
L89	3	"20120221025" AND cross$	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/01/06 01:23 PM
L90	2	"20120221025" AND cross-link$	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/01/06 01:23 PM
L91	2	16/045159	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/01/06 01:24 PM
L92	1	16/045159 AND anisotropic	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/01/06 01:24 PM
L93	2	16/045159 AND (fourier OR FFT)	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/02/02 01:39 PM
L94	2	16/045159 AND heart ADJ basement	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/02/02 02:37 PM
L95	2132	((("FRANCIS") near3 ("Michael")) OR (("OGLE") near3 ("Roy"))).INV.	(US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT)	OR	ON	ON	2022/02/02 04:45 PM
L96	26	L95 AND scaffold.clm.	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/02/02 04:54 PM
L97	18	L95 AND scaffold.clm. AND fourier	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/02/02 05:04 PM
L98	12	L95 AND scaffold.clm.	(US-PGPUB; USPAT;	OR	ON	ON	2022/02/02

		AND fourier SAME fiber	USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)				05:04 PM
L99	3	L95 AND scaffold.clm. AND fourier SAME fiber near20 ("180" OR alligned)	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/02/02 05:05 PM
L100	3	L95 AND scaffold.clm. AND fft SAME fiber near20 ("180" OR alligned)	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/02/02 05:06 PM
L101	5	L95 AND scaffold.clm. AND fft SAME fiber near20 ("180" OR aligned)	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/02/02 05:08 PM
L102	5	L95 AND scaffold.clm. AND fft SAME fiber near20 ("180" OR aligned) AND aligned	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/02/02 05:08 PM
L103	5	L95 AND scaffold.clm. AND fft SAME fiber near20 ("180" OR aligned)	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/02/02 05:09 PM
L104	1455	scaffold SAME fiber near20 ("180" OR aligned)	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/02/02 05:22 PM
L105	739	scaffold adj20 fiber near20 ("180" OR aligned)	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/02/02 05:22 PM
L106	122	scaffold adj20 fiber near20 ("180" OR aligned) AND fourier	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/02/02 05:23 PM
L107	39	L106 AND @ad<"20130314" AND collagen SAME fiber	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/02/02 05:24 PM
L108	42	L106 AND @ad<"20130314"	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/02/02 05:24 PM

L109	42	L108 AND (scaffold near20 tissue)	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/02/02 06:34 PM
L110	90	scaffold adj20 fiber near20 ("180" OR aligned) AND fourier AND fiber near20 collagen	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/02/02 06:36 PM
L111	33	L110 AND @ad<"20130314"	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/02/02 06:36 PM
L112	55	scaffold adj20 fiber near20 ("180" OR aligned) AND fourier AND fiber near20 collagen SAME scaffold	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/02/02 06:52 PM
L113	18	L112 AND @ad<"20130314"	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/02/02 06:53 PM
L114	4	scaffold adj20 fiber near20 ("180") AND fourier AND fiber near20 collagen SAME scaffold	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/02/02 06:57 PM
L115	0	L114 AND @ad<"20130314"	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/02/02 06:58 PM
L116	5	scaffold adj20 fiber near20 ("180") AND fourier	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/02/02 06:59 PM
L117	0	scaffold adj20 fiber AND ("180") near10 fourier	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/02/02 06:59 PM
L118	14	fiber SAME ("180") near10 fourier	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/02/02 07:02 PM
L119	0	L115 AND @ad<"20130314"	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/02/02 07:02 PM

L120	14	fiber SAME ("180$") near10 fourier	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/02/02 07:02 PM
L121	3	fiber SAME (180°) near10 fourier	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/02/02 07:03 PM
L122	194	(180°) near10 fourier	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/02/02 07:04 PM
L123	1	(180°) near10 fourier AND scaffold SAME fiber	(US-PGPUB; USPAT; USOCR; FIT (CN, FR, WO); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/02/02 07:04 PM

PE2E SEARCH - Search History (Interference)

There are no Interference searches to show.

Electronic Acknowledgement Receipt

EFS ID:	45132810
Application Number:	16045159
International Application Number:	
Confirmation Number:	5408
Title of Invention:	ALIGNED FIBER AND METHOD OF USE THEREOF
First Named Inventor/Applicant Name:	Michael Francis
Customer Number:	23122
Filer:	Valerie P. Hayes/Kathy Spina
Filer Authorized By:	Valerie P. Hayes
Attorney Docket Number:	LNH-152US1
Receipt Date:	03-MAR-2022
Filing Date:	25-JUL-2018
Time Stamp:	12:03:03
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Amendment after Notice of Allowance (Rule 312)	Amendment_312_LNH-152US1.pdf	102944 5cbbac602fb850f73d70d3e3746f6f9700079ea2	no	4

Warnings:

EMBZIM EX1002

Information:	
Total Files Size (in bytes):	102944

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No:	16/045,159
Applicant:	LifeNet Health
Filed:	July 25, 2018
Title:	ALIGNED FIBER AND METHOD OF USE THEREOF
T.C./A.U.:	1633
Examiner:	MARVICH, MARIA
Confirmation No.:	5408
Docket No.:	LNH-152US1

AMENDMENT UNDER 37 C.F.R. § 1.312
Expedited Procedure

Mail Stop Issue Fee
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

Responsive to the Notice of Allowance dated February 28, 2022, please amend the above-identified application as follows:

☐ **Amendments to the Specification** begin on page of this paper.

☒ **Amendments to the Claims** are reflected in the listing of claims, which begins on page **2** of this paper.

☐ **Amendments to the Drawings** begin on page of this paper and include an attached replacement sheet(s).

☐ **Amendments to the Abstract** are on page of this paper. A clean version of the Abstract is on page of this paper.

☒ **Remarks/Arguments** begin on page **4** of this paper.

Amendments to the Claims:

This list of claims replaces all prior versions and lists of claims in the application:

1.-24. (Canceled)

25. (Currently Amended) A method of treating a tissue defect in a subject comprising implanting at the tissue defect a scaffold comprising crosslinked fibers comprising collagen, wherein a result of a fast Fourier transform (FFT) analysis of the fibers demonstrates that the fibers have major adjacent peaks that are about 180° apart from each other, ~~and ,~~and

applying the scaffold to the tissue defect to repair a tendon, a ligament or a nerve defect.

26. (Cancelled)

27. (Previously Presented) A method of cell culture comprising

placing cells on a scaffold comprising aligned fibers, wherein a result of a FFT analysis of the fibers demonstrates that the fibers have major adjacent peaks that are about 180° apart from each other, and

growing the cells in conditions suitable for the cell culture.

28. (Previously Presented) The method of claim 27, wherein the cells are stem cells, adipose derived stem cells, dental pulp stem cells, fibroblasts, and/or dorsal root ganglia.

29. (Original) The method of claim 27, wherein the cells are dorsal root ganglia.

30. (Original) The method of claim 27, wherein the cells are dorsal root ganglia, and the fibers comprise heart basement membrane extract, heart basement membrane extracellular matrix, placenta basement membrane extract, or placenta basement membrane extracellular matrix.

31. (Previously Presented) A method of promoting differentiation of stem cells into osteoblasts, chondrocytes, ligament or tendon, the method comprising

placing the stem cells on a scaffold comprising aligned fibers, wherein a result of a FFT analysis of the fibers demonstrates that the fibers have major adjacent peaks that are about 180° apart from each other, and

growing the stem cells in *in vitro* conditions suitable for the cell differentiation.

32. (Original) The method of claim 31, wherein the stem cells are progenitor cells or adult stem cells.

33. (Previously Presented) The method of claim 32, wherein the progenitor cells or the adult stem cells are isolated from placenta, bone marrow, adipose tissue, blood vessel, amniotic fluid, synovial fluid, synovial membrane, pericardium, periosteum, dura, peripheral blood, umbilical blood, menstrual blood, baby teeth, nucleus pulposus, brain, skin, hair follicle, intestinal crypt, neural tissue, or muscle.

34. (Cancelled.)

35. (Previously Presented) The method of claim 33, wherein the stem cells are adipose derived stem cells and/or dental pulp stem cells.

36. (Previously Presented) The method of claim 25, wherein the scaffold is in the form of one or more elongated sheets.

37. (Previously Presented) The method of claim 25, wherein the scaffold is a composite of multiple layers of one or more sheets.

38. (Previously Presented) The method of claim 25, wherein the tissue defect is the nerve defect.

39. (Previously Presented) The method of claim 25, wherein the tissue defect is the ligament defect.

40. (Previously Presented) The method of claim 25, wherein the tissue defect is the tendon defect.

41 - 45. (Cancelled)

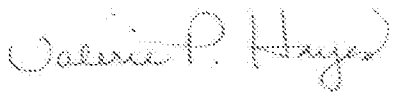
Remarks/Arguments:

Claim 1 is amended to correct a minor typographical error. Upon entry of the foregoing amendment, claims 25, 27-33, and 35-40 remain pending in the above-captioned application. No new matter is added.

Applicant submits the amendments go to matters of form only and should not raise any substantive issues requiring further consideration or search regarding this allowed application. As such, entry of the amendments should be proper at this stage of prosecution under 37 C.F.R. §1.312.

The Director is hereby authorized to charge or credit Deposit Account No. **18-0350** for any additional fees, or any underpayment or credit for overpayment in connection herewith.

Respectfully submitted,



William J. Matney, Jr., Reg. No. 39,292
Valerie P. Hayes, Reg. No. 53,005
Attorneys for Applicants

WJM/VPH/ks

Dated: March 3, 2022

1090 Vermont Avenue NW,
Suite 1200
Washington, DC 20005
(202) 715-2898

The Director is hereby authorized to charge or credit Deposit Account No. **18-0350** for any additional fees, or any underpayment or credit for overpayment in connection herewith.

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/045,159	07/25/2018	Michael Francis	LNH-152US1	5408

23122 7590 03/25/2022

RATNERPRESTIA
2200 Renaissance Blvd
Suite 350
King of Prussia, PA 19406

EXAMINER
MARVICH, MARIA

ART UNIT	PAPER NUMBER
1633	

NOTIFICATION DATE	DELIVERY MODE
03/25/2022	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

PCorrespondence@ratnerprestia.com

Response to Rule 312 Communication	Application No.	Applicant(s)	
	16/045,159	Francis et al.	
	Examiner	Art Unit	AIA (FITF) Status
	MARIA MARVICH	1633	No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

1. ☑ The amendment filed on 03 March 2022 under 37 CFR 1.312 has been considered, and has been:

a) ☑ entered.

b) ☐ entered as directed to matters of form not affecting the scope of the invention.

c) ☐ disapproved because the amendment was filed after the payment of the issue fee.

Any amendment filed after the date the issue fee is paid must be accompanied by a petition under 37 CFR 1.313(c)(1) and the required fee to withdraw the application from issue.

d) ☐ disapproved. See explanation below.

e) ☐ entered in part. See explanation below.

f) ☐ not entered because the supplemental or corrected Application Data sheet (ADS)

☐ was not accompanied by a petition to accept an unintentionally delayed claim under 37 CFR 1.55 or 27 CFR 1.78;

☐ did not identify the information being changed in accordance with 37 CFR 1.76(c)(2);

☐ was not properly signed in accordance with 37 CFR 1.76(e) (or 37 CFR 1.33(b) for applications filed prior to September 16, 2012).

/MARIA MARVICH/
Primary Examiner, Art Unit 1633

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No:	16/045,159
Applicant:	LifeNet Health
Filed:	July 25, 2018
Title:	ALIGNED FIBER AND METHOD OF USE THEREOF
T.C./A.U.:	1633
Examiner:	MARVICH, MARIA
Confirmation No.:	5408
Docket No.:	LNH-152US1

AMENDMENT UNDER 37 C.F.R. § 1.312
Expedited Procedure

OK to enter
MM
3/16/2022

Mail Stop Issue Fee
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

Responsive to the Notice of Allowance dated February 28, 2022, please amend the above-identified application as follows:

☐ **Amendments to the Specification** begin on page of this paper.

☒ **Amendments to the Claims** are reflected in the listing of claims, which begins on page **2** of this paper.

☐ **Amendments to the Drawings** begin on page of this paper and include an attached replacement sheet(s).

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☒ **Remarks/Arguments** begin on page **4** of this paper.

Doc Code: MFEE.C.AD

Document Description: Maintenance Fee Address Change

PTO/SB/47 (10-18)
Approved for use through 09/30/2021. OMB 0651-0016
U.S. Patent and Trademark Office; U. S. DEPARTMENT OF COMMERCE
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"FEE ADDRESS" INDICATION FORM

Address to:
Mail Stop M Correspondence
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

- OR -

Fax to:
571-273-6500

INSTRUCTIONS: The issue fee must have been paid for application(s) listed on this form. In addition, only an address represented by a Customer Number can be established as the fee address for maintenance fee purposes (hereafter, fee address). A fee address should be established when correspondence related to maintenance fees should be mailed to a different address than the correspondence address for the application. **When to check the first box below**: If you have a Customer Number to represent the fee address. **When to check the second box below**: If you have no Customer Number representing the desired fee address, in which case a completed Request for Customer Number (PTO/SB/125) must be attached to this form. For more information on Customer Numbers, see the Manual of Patent Examining Procedure (MPEP) § 403.

For the following listed application(s), please recognize as the "Fee Address" under the provisions of 37 CFR 1.363 the address associated with:

☑ Customer Number: 00197

OR

☐ The attached Request for Customer Number (PTO/SB/125) form.

PATENT NUMBER (if known)	APPLICATION NUMBER
	16/045,159

Completed by (check one):

☐ Applicant/Inventor

☑ Attorney or Agent of record 53005 (Reg. No.)

☐ Assignee of record of the entire interest. See 37 CFR 3.71. Statement under 37 CFR 3.73(b) (Form PTO/SB/96) is enclosed or was filed on _____ (Date)

/Valerie P. Hayes/
Signature

Valerie P. Hayes
Typed or printed name

610-407-0700
Requester's telephone number

03/28/2022
Date

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. If the assignee is a juristic entity, this form must be signed by a patent practitioner (attorney or agent) of record. Submit multiple forms if more than one signature is required, see below*.

☑ * Total of 1 forms are submitted.

This collection of information is required by 37 CFR 1.363. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 5 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Mail Stop M Correspondence, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal

Application Number:	16045159
Filing Date:	25-Jul-2018
Title of Invention:	ALIGNED FIBER AND METHOD OF USE THEREOF
First Named Inventor/Applicant Name:	Michael Francis
Filer:	Valerie P. Hayes/Kathy Spina
Attorney Docket Number:	LNH-152US1

Filed as Small Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
UTILITY APPL ISSUE FEE	2501	1	600	600

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**600**

Electronic Acknowledgement Receipt

EFS ID:	45331722
Application Number:	16045159
International Application Number:	
Confirmation Number:	5408
Title of Invention:	ALIGNED FIBER AND METHOD OF USE THEREOF
First Named Inventor/Applicant Name:	Michael Francis
Customer Number:	23122
Filer:	Valerie P. Hayes/Kathy Spina
Filer Authorized By:	Valerie P. Hayes
Attorney Docket Number:	LNH-152US1
Receipt Date:	28-MAR-2022
Filing Date:	25-JUL-2018
Time Stamp:	16:10:28
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$600
RAM confirmation Number	E20223RG11473769
Deposit Account	180350
Authorized User	Kathy Spina

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

37 CFR 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Issue Fee Payment (PTO-85B)	Issue_Fee_Transmittal_LNH-152US1.pdf	267264 b2314cf3d20ebf4b529e90c188aa2d6b1e15d490	no	1
Warnings:					
Information:					
2	Maintenance Fee Address Change	Fee_Address_Indication_LNH-152US1.pdf	925276 a66e238b271f7474f9234f0444b669a245966378	no	2
Warnings:					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	37895 5ccddc38d91930315149c4ddecda276177b3f4aa	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	1230435		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via EFS-Web.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

By fax, send to: (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

23122 7590 02/28/2022
RATNERPRESTIA
2200 Renaissance Blvd
Suite 350
King of Prussia, PA 19406

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via EFS-Web or by facsimile to (571) 273-2885, on the date below.

Kathy Spina	(Typed or printed name)
/Kathy Spina/	(Signature)
March 28, 2022	(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/045,159	07/25/2018	Michael Francis	LNH-152US1	5408

TITLE OF INVENTION: ALIGNED FIBER AND METHOD OF USE THEREOF

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	$600	$0.00	$0.00	$600	05/31/2022

EXAMINER	ART UNIT	CLASS-SUBCLASS
MARVICH, MARIA	1633	435-395000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

☐ Change of correspondence address (or Change of Correspondence Address form PTO/AIA/122 or PTO/SB/122) attached.

☒ "Fee Address" indication (or "Fee Address" Indication form PTO/AIA/47 or PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 RatnerPrestia
2
3

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE: LIFENET HEALTH

(B) RESIDENCE: (CITY and STATE OR COUNTRY): VIRGINIA

Please check the appropriate assignee category or categories (will not be printed on the patent) : ☐ Individual ☒ Corporation or other private group entity ☐ Government

4a. Fees submitted: ☒ Issue Fee ☐ Publication Fee (if required) ☐ Advance Order - # of Copies _____

4b. Method of Payment: *(Please first reapply any previously paid fee shown above)*

☒ Electronic Payment via EFS-Web ☐ Enclosed check ☐ Non-electronic payment by credit card (Attach form PTO-2038)

☒ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. 18-0350

5. **Change in Entity Status** (from status indicated above)

☐ Applicant certifying micro entity status. See 37 CFR 1.29

☐ Applicant asserting small entity status. See 37 CFR 1.27

☐ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature /Valerie P. Hayes/ Date March 28, 2022

Typed or printed name Valerie P. Hayes Registration No. 53005

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
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Alexandria, Virginia 22313-1450
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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/045,159	05/03/2022	11318227	LNH-152US1	5408

23122 7590 04/13/2022

RATNERPRESTIA
2200 Renaissance Blvd
Suite 350
King of Prussia, PA 19406

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 591 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Patents Stakeholder Experience (OPSE), Stakeholder Support Division (SSD) at (571)-272-4200.

INVENTOR(s) (Please see PAIR WEB site http://pair.uspto.gov for additional inventors):

Michael Francis, Virginia Beach, VA;
Roy Ogle, Virginia Beach, VA;

APPLICANT(s) (Please see PAIR WEB site http://pair.uspto.gov for additional applicants):

LifeNet Health, Virginia Beach, VA;

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The USA offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to encourage and facilitate business investment. To learn more about why the USA is the best country in the world to develop technology, manufacture products, and grow your business, visit SelectUSA.gov.