

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A SUBMISSION UNDER 35 U.S.C. 371		Attorney Docket No. 067949-5058
		U.S. Application No. (if known, see 37 CFR 1.5)
International Application No. PCT/US2014/025636	International Filing Date 13 March 2014	Priority Date Claimed 14 March 2013
Title of Invention Aligned Fiber and Method of Use Thereof		
First Named Inventor Michael FRANCIS		
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information. 1. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). NOTE: The express request under 35 U.S.C. 371(f) will not be effective unless the requirements under 35 U.S.C. 371(c)(1), (2), and (4) for payment of the basic national fee, copy of the International Application and English translation thereof (if required), and the oath or declaration of the inventor(s) have been received. 2. ☐ A copy of the International Application (35 U.S.C. 371(c)(2)) is attached hereto (not required if the International Application was previously communicated by the International Bureau or was filed in the United States Receiving Office (RO/US)). 3. An English language translation of the International Application (35 U.S.C. 371(c)(2)) a. ☐ is attached hereto. b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4). 4. An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)) a. ☐ is attached. b. ☐ was previously filed in the international phase under PCT Rule 4.17(iv). Items 5 to 8 below concern amendments made in the international phase. <u>PCT Article 19 and 34 amendments</u> 5. ☐ Amendments to the claims under PCT Article 19 are attached (not required if communicated by the International Bureau) (35 U.S.C. 371(c)(3)). 6. ☐ English translation of the PCT Article 19 amendment is attached (35 U.S.C. 371(c)(3)). 7. ☐ English translation of annexes (Article 19 and/or 34 amendments only) of the International Preliminary Examination Report is attached (35 U.S.C. 371(c)(5)). <u>Cancellation of amendments made in the international phase</u> 8a. ☐ Do not enter the amendment made in the international phase under PCT Article 19. 8b. ☐ Do not enter the amendment made in the international phase under PCT Article 34. NOTE: A proper amendment made in English under Article 19 or 34 will be entered in the U.S. national phase application absent a clear instruction from applicant not to enter the amendment(s). The following items 9 to 17 concern a document(s) or information included. 9. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98. 10. ☐ A preliminary amendment. 11. ☒ An Application Data Sheet under 37 CFR 1.76. 12. ☐ A substitute specification. NOTE: A substitute specification cannot include claims. See 37 CFR 1.125(b). 13. ☐ A power of attorney and/or change of address letter. 14. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.3 and 37 CFR 1.821-1.825. 15. ☐ Assignment papers (<i>cover sheet and document(s)</i>). Name of Assignee: _____ 16. ☐ 37 CFR 3.73(c) Statement (<i>when there is an Assignee</i>). _____		

This collection of information is required by 37 CFR 1.414 and 1.491-1.492. 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 15 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: Mail Stop PCT, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 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.

U.S. APPLN. No. (if known – see 37 CFR 1.5)	INTERNATIONAL APPLICATION No. PCT/US2014/025636	ATTORNEY DOCKET No. 067949-5058
17. ☒ Other items or information: WO 2014/160019A1 (cover page only); PCT/IB/304; PCT/IB/308 (first and second notices); PCT/ISA/210 (ISR); and PCT/ISA/237 (WOP)		
The following fees have been submitted.		CALCULATIONS PTO USE ONLY
18. ☒ Basic national fee (37 CFR 1.492(a)) \$280		\$ 280.00
19. ☒ Examination fee (37 CFR 1.492(c)) If the written opinion prepared by ISA/US or the international preliminary examination report prepared by IPEA/US indicates all claims satisfy provisions of PCT Article 33(1)-(4) \$0 All other situations \$720		\$ 720.00
20. ☒ Search fee (37 CFR 1.492(b)) If the written opinion prepared by ISA/US or the international preliminary examination report prepared by IPEA/US indicates all claims satisfy provisions of PCT Article 33(1)-(4) \$0 Search fee (37 CFR 1.445(a)(2)) has been paid on the international application to the USPTO as an International Searching Authority \$120 International Search Report prepared by an ISA other than the US and provided to the Office or previously communicated to the US by the IB \$480 All other situations \$600		\$ 120.00
TOTAL OF 18, 19, and 20 =		\$ 1120.00
☐ Additional fee for specification and drawings filed in paper over 100 sheets (excluding sequence listing in compliance with 37 CFR 1.821(c) or (e) in an electronic medium or computer program listing in an electronic medium) (37 CFR 1.492(j)). Fee for each additional 50 sheets of paper or fraction thereof \$400		
Total Sheets	Extra Sheets	RATE
- 100 =	/ 50 =	x \$400
Surcharge of \$140.00 for furnishing any of the search fee, examination fee, or the oath or declaration after the date of commencement of the national stage (37 CFR 1.492(h)).		\$
CLAIMS	NUMBER FILED	RATE
Total claims	- 20 =	x \$80
Independent claims	- 3 =	x \$420
MULTIPLE DEPENDENT CLAIM(S) (if applicable)		+ \$780
Processing fee of \$140.00 for furnishing the English translation later than 30 months from the earliest claimed priority date (37 CFR 1.492(i)).		\$
TOTAL OF ABOVE CALCULATIONS =		\$ 1120.00
☒ Applicant asserts small entity status. See 37 CFR 1.27. Fees above are reduced by 1/2.		
☐ Applicant certifies micro entity status. See 37 CFR 1.29. Fees above are reduced by 3/4. Applicant must attach form PTO/SB/15A or B or equivalent.		
TOTAL NATIONAL FEE =		\$ 560.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property.		\$
TOTAL FEES ENCLOSED =		\$ 560.00
		Amount to be refunded:
		\$
		Amount to be charged:
		\$

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.

- a. ☐ A check in the amount of \$ _____ to cover the above fees is enclosed.
- b. ☒ Please charge my Deposit Account No. 50-0310 in the amount of \$ 560.00 to cover the above fees.
- c. ☒ The Director is hereby authorized to charge additional fees which may be required, or credit any overpayment, to Deposit Account No. 50-0310 as follows:
- i. ☐ any required fee.
- ii. ☒ any required fee except for excess claims fees required under 37 CFR 1.492(d) and (e) and multiple dependent claim fee required under 37 CFR 1.492(f).
- d. ☐ Fees are to be charged to a credit card. **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. The PTO-2038 should only be mailed or faxed to the USPTO. However, when paying the basic national fee, the PTO-2038 may NOT be faxed to the USPTO.

ADVISORY: If filing by EFS-Web, do **NOT** attach the PTO-2038 form as a PDF along with your EFS-Web submission. Please be advised that this is **not** recommended and by doing so your **credit card information may be displayed via PAIR**. To protect your information, it is recommended to pay fees online by using the electronic payment method.

NOTE: Where an appropriate time limit under 37 CFR 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the International Application to pending status.

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

- ☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013, and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.

NOTE 1: By providing this statement under 37 CFR 1.55 or 1.78, **this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.**

NOTE 2: A U.S. national stage application may not claim priority to the international application of which it is the national phase. The filing date of a U.S. national stage application is the international filing date. See 35 U.S.C. 363.

Correspondence Address

- ☒ The address associated with Customer Number: 09629 **OR** ☐ Correspondence address below

Name					
Address					
City		State		Zip Code	
Country				Telephone	
Email					

Signature	/Janice H. Lee/	Date	2015-09-14
Name (Print/Type)	Janice H. Lee	Registration No. (Attorney/Agent)	64526

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.

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.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	067949-5058
		Application Number	
Title of Invention	Aligned Fiber and Method of Use Thereof		
The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76. This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.			

Secrecy Order 37 CFR 5.2

☐	Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)
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Inventor Information:

Inventor 1					Remove	
Legal Name						
Prefix	Given Name	Middle Name	Family Name	Suffix		
	Michael		FRANCIS			
Residence Information (Select One) ☒ US Residency ☐ Non US Residency ☐ Active US Military Service						
City	Virginia Beach	State/Province	VA	Country of Residence i	US	
Mailing Address of Inventor:						
Address 1		1864 Concert Drive				
Address 2						
City	Virginia Beach	State/Province	VA			
Postal Code	23453	Country i	US			
Inventor 2					Remove	
Legal Name						
Prefix	Given Name	Middle Name	Family Name	Suffix		
	Roy		OGLE			
Residence Information (Select One) ☒ US Residency ☐ Non US Residency ☐ Active US Military Service						
City	Virginia Beach	State/Province	VA	Country of Residence i	US	
Mailing Address of Inventor:						
Address 1		1864 Concert Drive				
Address 2						
City	Virginia Beach	State/Province	VA			
Postal Code	23453	Country i	US			
All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the Add button.						
Add						

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below. For further information see 37 CFR 1.33(a).
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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	067949-5058
		Application Number	
Title of Invention	Aligned Fiber and Method of Use Thereof		

☐ An Address is being provided for the correspondence information of this application.

Customer Number	09629		
Email Address	wapatents@morganlewis.com	Add Email	Remove Email

Application Information:

Title of the Invention	Aligned Fiber and Method of Use Thereof		
Attorney Docket Number	067949-5058	Small Entity Status Claimed	☒
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	10	Suggested Figure for Publication (if any)	

Filing By Reference :

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32). Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	☒ Customer Number	☐ US Patent Practitioner	☐ Limited Recognition (37 CFR 11.9)
Customer Number	09629		

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	067949-5058
		Application Number	
Title of Invention	Aligned Fiber and Method of Use Thereof		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.

When referring to the current application, please leave the application number blank.

Prior Application Status	Pending	Remove	
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	a 371 of international	PCT/US2014/025636	2014-03-13
Prior Application Status	Expired	Remove	
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
PCT/US2014/025636	Claims benefit of provisional	61785031	2013-03-14
Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the Add button.			Add

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX) the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

			Remove
Application Number	Country ⁱ	Filing Date (YYYY-MM-DD)	Access Code ^j (if applicable)
Additional Foreign Priority Data may be generated within this form by selecting the Add button.			Add

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	067949-5058
		Application Number	
Title of Invention	Aligned Fiber and Method of Use Thereof		

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013. ☐ NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.
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Authorization to Permit Access:

☒ Authorization to Permit Access to the Instant Application by the Participating Offices
If checked, the undersigned hereby grants the USPTO authority to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the World Intellectual Property Office (WIPO), and any other intellectual property offices in which a foreign application claiming priority to the instant patent application is filed access to the instant patent application. See 37 CFR 1.14(c) and (h). This box should not be checked if the applicant does not wish the EPO, JPO, KIPO, WIPO, or other intellectual property office in which a foreign application claiming priority to the instant patent application is filed to have access to the instant patent application. In accordance with 37 CFR 1.14(h)(3), access will be provided to a copy of the instant patent application with respect to: 1) the instant patent application-as-filed; 2) any foreign application to which the instant patent application claims priority under 35 U.S.C. 119(a)-(d) if a copy of the foreign application that satisfies the certified copy requirement of 37 CFR 1.55 has been filed in the instant patent application; and 3) any U.S. application-as-filed from which benefit is sought in the instant patent application. In accordance with 37 CFR 1.14(c), access may be provided to information concerning the date of filing this Authorization.

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.
--

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	067949-5058
		Application Number	
Title of Invention	Aligned Fiber and Method of Use Thereof		

Applicant 1				Remove	
If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.					
Clear					
☒ Assignee		☐ Legal Representative under 35 U.S.C. 117		☐ Joint Inventor	
☐ Person to whom the inventor is obligated to assign.			☐ Person who shows sufficient proprietary interest		
If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:					
Name of the Deceased or Legally Incapacitated Inventor :					
If the Applicant is an Organization check here. ☒					
Organization Name		LifeNet Health			
Mailing Address Information For Applicant:					
Address 1		1864 Concert Drive			
Address 2					
City		Virginia Beach	State/Province		VA
Country ⁱ	US	Postal Code		23453	
Phone Number			Fax Number		
Email Address					
Additional Applicant Data may be generated within this form by selecting the Add button. Add					

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.	
Assignee 1	
Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.	
Remove	
If the Assignee or Non-Applicant Assignee is an Organization check here. ☒	

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	067949-5058
		Application Number	
Title of Invention	Aligned Fiber and Method of Use Thereof		

Organization Name	LifeNet Health
-------------------	----------------

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1	1864 Concert Drive		
Address 2			
City	Virginia Beach	State/Province	VA
Country i	US	Postal Code	23453
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button.

Signature:

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Signature	/Janice H. Lee/		Date (YYYY-MM-DD)	2015-09-14	
First Name	Janice H.	Last Name	Lee	Registration Number	64526

Additional Signature may be generated within this form by selecting the Add button.

This collection of information is required by 37 CFR 1.76. 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 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet 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

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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 the Freedom of Information Act requires disclosure of these records.
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.

(51) International Patent Classification:
A61F 2/08 (2006.01)(21) International Application Number:
PCT/US2014/025636(22) International Filing Date:
13 March 2014 (13.03.2014)

(25) Filing Language: English

(26) Publication Language: English

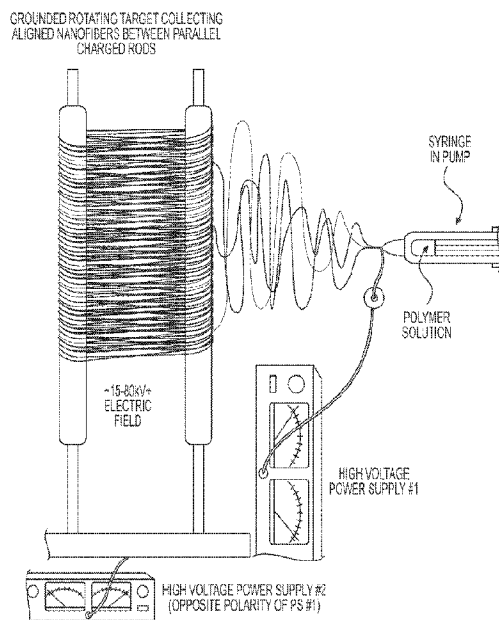
(30) Priority Data:
61/785,031 14 March 2013 (14.03.2013) US(71) Applicant: **LIFENET HEALTH** [US/US]; 1864 Concert Drive, Virginia Beach, VA 23453 (US).(72) Inventors: **FRANCIS, Michael**; 1864 Concert Drive, Virginia Beach, VA 23453 (US). **OGLE, Roy**; 1864 Concert Drive, Virginia Beach, VA 23453 (US).(74) Agents: **BUCK, Todd B.** et al.; Morgan, Lewis & Bockius LLP, 1111 Pennsylvania Avenue, NW, Washington, DC 20004 (US).

(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, BN, 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, IR, IS, JP, KE, KG, 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, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, 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, 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, KM, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) Title: **ALIGNED FIBER AND METHOD OF USE THEREOF****FIG. 5**

(57) Abstract: A scaffold comprising an aligned fiber. The invention further relates to a scaffold comprising one or more electrospun fibers wherein a fast Fourier transform (FFT) analysis result of the fibers have adjacent major peaks with about 180° apart from each other. Methods for promoting differentiation of stem cells into osteoblasts, chondrocytes, ligament or tendon, the method comprising culturing the cells on the scaffold or aligned fiber in conditions suitable for the cell differentiation.

ADVANCE E-MAIL

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING SUBMISSION,
OBTENTION OR TRANSMITTAL
OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

BUCK, Todd B.
Morgan, Lewis & Bockius LLP
1111 Pennsylvania Avenue, NW
Washington, DC 20004
ETATS-UNIS D'AMERIQUE

Date of mailing (<i>day/month/year</i>) 24 April 2014 (24.04.2014)	
Applicant's or agent's file reference 67949-5058WO	IMPORTANT NOTIFICATION
International application No. PCT/US2014/025636	International filing date (<i>day/month/year</i>) 13 March 2014 (13.03.2014)
International publication date (<i>day/month/year</i>) Not yet published	Priority date (<i>day/month/year</i>) 14 March 2013 (14.03.2013)
Applicant LIFENET HEALTH	

The applicant is hereby notified of the date of receipt (or of obtaining by the International Bureau) of the priority document(s) relating to all earlier application(s) whose priority is claimed. Unless otherwise indicated by the letters "NR", in the right-hand column or by an asterisk appearing next to the date of receipt, **the priority document concerned was submitted or transmitted to or obtained by the International Bureau in compliance with Rule 17.1(a), (b) or (b-bis)**. This Form replaces any previously issued notification concerning submission, transmittal or obtaining of priority documents.

<u>Priority date</u>	<u>Priority application No.</u>	<u>Country or regional Office or PCT receiving Office</u>	<u>Date of receipt of priority document</u>
14 March 2013 (14.03.2013)	61/785,031	US	21 April 2014 (21.04.2014)

The letters "NR" denote a priority document which, on the date of mailing of this Form, had not yet been received or obtained by the International Bureau in compliance with Rule 17.1(a), (b) or (b-bis). Where the applicant has failed to either submit, request to prepare and transmit, or to request the International Bureau to obtain the priority document within the applicable time limit under that Rule, the attention of the applicant is directed to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

An asterisk "*" next to a date of receipt, denotes a priority document submitted or transmitted to or obtained by the International Bureau but not in compliance with Rule 17.1(a), (b) or (b-bis) (the priority document was received after the time limit prescribed in Rule 17.1(a); the request to prepare and transmit the priority document was submitted to the receiving Office after the applicable time limit under Rule 17.1(b) or the request to the International Bureau to obtain the priority document was made after the applicable time limit under Rule 17.1(b-bis)). Even though the priority document was not furnished in compliance with Rule 17.1(a), (b) or (b-bis), the International Bureau will nevertheless transmit a copy of the document to the designated Offices, for their consideration. In case such a copy is not accepted by the designated Office as the priority document, Rule 17.1(c) provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Agnès Wittmann-Regis
Facsimile No. +41 22 338 89 70	e-mail pt06.pct@wipo.int Telephone No. +41 22 338 74 06



ADVANCE E-MAIL

From the INTERNATIONAL BUREAU

PCT

FIRST NOTICE INFORMING THE APPLICANT OF
THE COMMUNICATION OF THE INTERNATIONAL
APPLICATION (TO DESIGNATED OFFICES WHICH
DO NOT APPLY THE 30 MONTH TIME LIMIT
UNDER ARTICLE 22(1))

(PCT Rule 47.1(c))

To:

BUCK, Todd B.
Morgan, Lewis & Bockius LLP
1111 Pennsylvania Avenue, NW
Washington, DC 20004
ETATS-UNIS D'AMERIQUE

Date of mailing (day/month/year) 16 October 2014 (16.10.2014)		
Applicant's or agent's file reference 67949-5058WO		IMPORTANT NOTICE
International application No. PCT/US2014/025636	International filing date (day/month/year) 13 March 2014 (13.03.2014)	Priority date (day/month/year) 14 March 2013 (14.03.2013)
Applicant LIFENET HEALTH		

1. **ATTENTION:** For any designated Office(s), for which the time limit under Article 22(1), as in force from 1 April 2002 (30 months from the priority date), **does apply**, please see Form PCT/IB/308(Second and Supplementary Notice) (to be issued promptly after the expiration of 28 months from the priority date).

2. Notice is hereby given that the following designated Office(s), for which the time limit under Article 22(1), as in force from 1 April 2002, **does not apply**, has/have requested that the communication of the international application, as provided for in Article 20, be effected under Rule 93bis.1. The International Bureau has effected that communication on the date indicated below:
02 October 2014 (02.10.2014)

None

In accordance with Rule 47.1(c-bis)(i), those Offices will accept the present notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

3. The following designated Offices, for which the time limit under Article 22(1), as in force from 1 April 2002, **does not apply**, have not requested, as at the time of mailing of the present notice, that the communication of the international application be effected under Rule 93bis.1 :

LU, TZ, UG

In accordance with Rule 47.1(c-bis)(ii), those Offices accept the present notice as conclusive evidence that the Contracting State for which that Office acts as a designated Office does not require the furnishing, under Article 22, by the applicant of a copy of the international application.

4. TIME LIMITS for entry into the national phase

For the designated Office(s) listed above, and unless a demand for international preliminary examination has been filed before the expiration of **19 months** from the priority date (see Article 39(1)), the applicable time limit for entering the national phase will, **subject to what is said in the following paragraph**, be **20 MONTHS** from the priority date.

In practice, **time limits other than the 20-month time limit** will continue to apply, for various periods of time, in respect of certain of the designated Offices listed above. For **regular updates on the applicable time limits** (20 or 21 months, or other time limit), Office by Office, refer to the *PCT Gazette*, the *PCT Newsletter* and the *PCT Applicant's Guide*, Volume II, National Chapters, all available from WIPO's Internet site, at <http://www.wipo.int/pct/en/index.html>.

It is the applicant's **sole responsibility** to monitor all these time limits.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Agnès Wittmann-Regis
Facsimile No. +41 22 338 82 70	e-mail: pt06.pct@wipo.int



ADVANCE E-MAIL

From the INTERNATIONAL BUREAU

PCT

SECOND AND SUPPLEMENTARY NOTICE
INFORMING THE APPLICANT OF THE
COMMUNICATION OF THE INTERNATIONAL
APPLICATION (TO DESIGNATED OFFICES
WHICH APPLY THE 30 MONTH TIME
LIMIT UNDER ARTICLE 22(1))

(PCT Rule 47.1(c))

To:

BUCK, Todd B.
Morgan, Lewis & Bockius LLP
1111 Pennsylvania Avenue, NW
Washington, DC 20004
ETATS-UNIS D'AMERIQUE

Date of mailing (day/month/year) 16 July 2015 (16.07.2015)		
Applicant's or agent's file reference 67949-5058WO		IMPORTANT NOTICE
International application No. PCT/US2014/025636	International filing date (day/month/year) 13 March 2014 (13.03.2014)	Priority date (day/month/year) 14 March 2013 (14.03.2013)
Applicant LIFENET HEALTH		

- ATTENTION:** For any designated Office(s), for which the time limit under Article 22(1), as in force from 1 April 2002 (30 months from the priority date), **does not apply**, please see Form PCT/IB/308(First Notice) issued previously.
- Notice is hereby given that the following designated Office(s), for which the time limit under Article 22(1), as in force from 1 April 2002, **does apply**, has/have requested that the communication of the international application, as provided for in Article 20, be effected under Rule 93bis.1. The International Bureau has effected that communication on the date indicated below:
02 October 2014 (02.10.2014)

AZ, BY, CN, EP, HU, KG, KP, KR, MD, MK, MZ, NA, NG, PG, RU, SY, TM

In accordance with Rule 47.1(c-bis)(i), those Offices will accept the present notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

- The following designated Offices, for which the time limit under Article 22(1), as in force from 1 April 2002, **does apply**, have not requested, as at the time of mailing of the present notice, that the communication of the international application be effected under Rule 93bis.1 :

AE, AG, AL, AM, AO, AP, AT, AU, BA, BB, BG, BH, BN, BR, BW, BZ, CA, CH, CL, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EA, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, ID, IL, IN, IR, IS, JP, KE, KN, KZ, LA, LC, LK, LR, LS, LT, LY, MA, ME, MG, MN, MW, MX, MY, NI, NO, NZ, OA, OM, PA, PE, PH, PL, PT, QA, RO, RS, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, TH, TJ, TN, TR, TT, UA, US, UZ, VC, VN, ZA, ZM, ZW

In accordance with Rule 47.1(c-bis)(ii), those Offices accept the present notice as conclusive evidence that the Contracting State for which that Office acts as a designated Office does not require the furnishing, under Article 22, by the applicant of a copy of the international application.

4. TIME LIMITS for entry into the national phase

For the designated or elected Office(s) listed above, the applicable time limit for entering the national phase will, **subject to what is said in the following paragraph**, be **30 MONTHS** from the priority date.

In practice, **time limits other than the 30-month time limit** will continue to apply, for various periods of time, in respect of certain of the designated or elected Office(s) listed above. For **regular updates on the applicable time limits** (30 or 31 months, or other time limit), Office by Office, refer to the *PCT Gazette*, the *PCT Newsletter* and the *PCT Applicant's Guide*, Volume II, National Chapters, all available from WIPO's Internet site, at <http://www.wipo.int/pct/en/index.html>.

It is the applicant's **sole responsibility** to monitor all these time limits.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Agnès Wittmann-Regis e-mail: pt06.pct@wipo.int
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number		
	Filing Date		2015-09-14
	First Named Inventor	Michael Francis	
	Art Unit		
	Examiner Name		
	Attorney Docket Number	067949-5058	

U.S.PATENTS						Remove
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
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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
	1	20100233115	A1	2010-09-16	PATEL et al.	
	2	20110236974	A1	2011-09-29	OGLE et al.	
	3	20120221025	A1	2012-08-30	SIMPSON et al.	

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

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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							☐

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number		
	Filing Date		2015-09-14
	First Named Inventor	Michael Francis	
	Art Unit		
	Examiner Name		
	Attorney Docket Number	067949-5058	

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

NON-PATENT LITERATURE DOCUMENTS			Remove
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
	1	HUANG et al. (2011) Electrospun Collagen-Chitosan-TPU Nanofibrous Scaffolds for Tissue Engineered Tubular Grafts, Colloids Surf. B. Biointerfaces, 82:307-315	☐
	2	Copy of International Search Report and Written Opinion dated July 10, 2014; International Application No. PCT/US2014/025636	☐

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

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	
Filing Date	2015-09-14
First Named Inventor	Michael Francis
Art Unit	
Examiner Name	
Attorney Docket Number	067949-5058

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	/Janice H. Lee/	Date (YYYY-MM-DD)	2015-09-14
Name/Print	Janice H. Lee	Registration Number	64526

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 records.
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.

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

To:

TODD B. BUCK
MORGAN, LEWIS & BOCKIUS LLP
1111 PENNSYLVANIA AVENUE, NW
WASHINGTON, DC 20004

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT AND
THE WRITTEN OPINION OF THE INTERNATIONAL
SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

Applicant's or agent's file reference 67949-5058WO	Date of mailing (day/month/year)
International application No. PCT/US 14/25636	International filing date (day/month/year) 13 March 2014 (13.03.2014)
Applicant LIFENET HEALTH	

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

Filing of amendments and statement under Article 19:

The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes
1211 Geneva 20, Switzerland, Facsimile No.: +41 22 338 82 70

For more detailed instructions, see *PCT Applicant's Guide*, International Phase, paragraphs 9.004 – 9.011.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.
3. ☐ **With regard to any protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:
- ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with any request to forward the texts of both the protest and the decision thereon to the designated Offices.
- ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. Following the expiration of 30 months from the priority date, these comments will also be made available to the public.

Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90bis.1 and 90bis.3).

Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide*, National Chapters.

Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer Lee W. Young PCT Helpdesk: 571-272-4300 Telephone No. PCT OSP: 571-272-7774
---	---

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

To:

TODD B. BUCK
MORGAN, LEWIS & BOCKIUS LLP
1111 PENNSYLVANIA AVENUE, NW
WASHINGTON, DC 20004

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT AND
THE WRITTEN OPINION OF THE INTERNATIONAL
SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

Date of mailing
(day/month/year)

10 JUL 2014

Applicant's or agent's file reference
67949-5058WO

FOR FURTHER ACTION See paragraphs 1 and 4 below

International application No.
PCT/US 14/25636

International filing date
(day/month/year) 13 March 2014 (13.03.2014)

Applicant LIFENET HEALTH

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

Filing of amendments and statement under Article 19:

The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes
1211 Geneva 20, Switzerland, Facsimile No.: +41 22 338 82 70

For more detailed instructions, see *PCT Applicant's Guide*, International Phase, paragraphs 9.004 – 9.011.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.
3. ☐ **With regard to any protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:
- ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with any request to forward the texts of both the protest and the decision thereon to the designated Offices.
- ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. Following the expiration of 30 months from the priority date, these comments will also be made available to the public.

Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90*bis*.1 and 90*bis*.3).

Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide*, National Chapters.

Name and mailing address of the ISA/
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-3201

Authorized officer

Lee W. Young

PCT Helpdesk: 571-272-4300

Telephone No. PCT OSP: 571-272-7774

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 67949-5058WO	FOR FURTHER ACTION see Form PCT/ISA/220 as well as, where applicable, item 5 below.	
International application No. PCT/US 14/25636	International filing date (<i>day/month/year</i>) 13 March 2014 (13.03.2014)	(Earliest) Priority Date (<i>day/month/year</i>) 14 March 2013 (14.03.2013)
Applicant LIFENET HEALTH		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6bis(a)).

c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☒ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☐ the text is approved as submitted by the applicant.

☒ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. 5

☐ as suggested by the applicant.

☒ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☐ none of the figures is to be published with the abstract.

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 5-35
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Box No. IV Text of the abstract (Continuation of item 5 of the first sheet)

A scaffold comprising an aligned fiber. The invention further relates to a scaffold comprising one or more electrospun fibers wherein a fast Fourier transform (FFT) analysis result of the fibers have adjacent major peaks with about 180° apart from each other. Methods for promoting differentiation of stem cells into osteoblasts, chondrocytes, ligament or tendon, the method comprising culturing the cells on the scaffold or aligned fiber in conditions suitable for the cell differentiation.

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61F 2/08 (2014.01)

USPC - 530/356; 623/13.11

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - A61F 2/08 (2014.01)

USPC - 530/356; 623/13.11

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

IPC(8) - A61F 2/08 (2014.01)

USPC - 530/356; 623/13.11; 623/11.11; 623/917 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Patbase, Google, Freepatentsonline

Search terms used: scaffold electrospun biocompatible fast fourier FFT peaks separate degree aligned

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Huang et al., "Electrospun collage-chitosan-TPU nanofibrous scaffolds for tissue engineered tubular grafts" Colloids and Surfaces B: Biointerfaces 82 (2011) 307-315, 01 February 2011 (01.02.2011), pg 308, 309; Fig 4E	1-4
A	US 2010/0233115 A1 (Patel et al.) 16 September 2010 (16.09.2010), entire document, especially Fig 16	1-4
A	US 2011/0236974 A1 (Ogle et al.) 29 September 2011 (29.09.2011), entire document	1-4
A	US 2012/0221025 A1 (Simpson et al.) 30 August 2012 (30.08.2012), entire document	1-4

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

13 June 2014 (13.06.2014)

Date of mailing of the international search report

10 JUL 2014

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-3201

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: TODD B. BUCK
MORGAN, LEWIS & BOCKIUS LLP
1111 PENNSYLVANIA AVENUE, NW
WASHINGTON, DC 20004

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

Date of mailing
(day/month/year)

10 JUL 2014

Applicant's or agent's file reference
67949-5058WO

FOR FURTHER ACTION

See paragraph 2 below

International application No.

PCT/US 14/25636

International filing date (day/month/year)

13 March 2014 (13.03.2014)

Priority date (day/month/year)

14 March 2013 (14.03.2013)

International Patent Classification (IPC) or both national classification and IPC

IPC(8) - A61F 2/08 (2014.01)

USPC - 530/356; 623/13.11

Applicant LIFENET HEALTH

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-3201

Date of completion of this opinion

13 June 2014 (13.06.2014)

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 14/25636

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:
 - ☒ the international application in the language in which it was filed.
 - ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).
2. ☐ This opinion has been established taking into account **therectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43bis.1(a))
3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:
 - a. (means)
 - ☐ on paper
 - ☐ in electronic form
 - b. (time)
 - ☐ in the international application as filed
 - ☐ together with the international application in electronic form
 - ☐ subsequently to this Authority for the purposes of search
4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
5. Additional comments:

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 14/25636

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 5-35

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search (*specify*):

☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 5-35 are so unclear that no meaningful opinion could be formed (*specify*):

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed (*specify*):

☒ no international search report has been established for said claims Nos. 5-35

☐ a meaningful opinion could not be formed without the sequence listing: the applicant did not, within the prescribed time limit

☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.

☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.

☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13ter.1(a) or (b).

☐ See Supplemental Box for further details.

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.

PCT/US 14/25636

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	None	YES
	Claims	1-4	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-4	NO
Industrial applicability (IA)	Claims	1-4	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-4 lack novelty under PCT Article 33(2) as being anticipated by the article entitled "Electrospun collagen.chitosan.TPU nanofibrous scaffolds for tissue engineered tubular grafts" by Huang et al. (hereinafter Huang).

Regarding claim 1, Huang teaches a scaffold comprising one or more electrospun fibers (abstract; pg 308, col 1; pg 309, col 2, aligned nanofibrous scaffolds based on collagen.chitosan.thermoplastic polyurethane (TPU) blends were electrospun to mimic the componential and structural aspects of the native extracellular matrix, while an optimal proportion was found to keep the balance between biocompatibility and mechanical strength), wherein a fast Fourier transform (FFT) analysis result of the fibers have adjacent major peaks with about 180 degrees apart from each other (Fig 4E; pg 309, col 2, two peaks at 175 degrees and 355 degrees (180 degrees apart)).

Regarding claim 2, Huang teaches the scaffold according to claim 1, wherein the scaffold essentially consists of the electrospun fibers (abstract; pg 308, col 1; pg 309, col 2).

Regarding claim 3, Huang teaches the scaffold according to claim 1, wherein the scaffold consists of the electrospun fibers (abstract; pg 308, col 1; pg 309, col 2, electrospinning is the only method disclosed for forming the fibers).

Regarding claim 4, Huang teaches the scaffold according to any of claims 1-3, wherein the scaffold is biocompatible (abstract; pg 308, col 1, Collagen.chitosan electrospun complex and their biocompatibility have been reported previously).

Claims 1-4 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

Electronic Patent Application Fee Transmittal

Application Number:				
Filing Date:				
Title of Invention:	Aligned Fiber and Method of Use Thereof			
First Named Inventor/Applicant Name:	Michael Francis			
Filer:	Janice Hyeseung Lee			
Attorney Docket Number:	067949-5058			
Filed as Small Entity				
Filing Fees for U.S. National Stage under 35 USC 371				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Basic National Stage Fee	2631	1	140	140
Natl Stage Search Fee - U.S. was the ISA	2641	1	60	60
Natl Stage Exam Fee - all other cases	2633	1	360	360
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				560

Electronic Acknowledgement Receipt

EFS ID:	23482244
Application Number:	14776106
International Application Number:	PCT/US2014/025636
Confirmation Number:	1091
Title of Invention:	Aligned Fiber and Method of Use Thereof
First Named Inventor/Applicant Name:	Michael Francis
Customer Number:	9629
Filer:	Janice Hyeseung Lee
Filer Authorized By:	
Attorney Docket Number:	067949-5058
Receipt Date:	14-SEP-2015
Filing Date:	
Time Stamp:	15:19:30
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	\$ 560
RAM confirmation Number	1761
Deposit Account	500310
Authorized User	LEE, HYESEUNG

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

File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal of New Application	067949-5058-pto1390-09-14-2015.pdf	220540	no	4
			0db456bf62a7de3600c2f142acc093e7a619259		
Warnings:					
Information:					
2	Application Data Sheet	067949-5058-ADS-09-14-2015.pdf	1819204	no	7
			a21c4d57469fa616f369f3d0be4c54a756c172ea		
Warnings:					
Information:					
3	Documents submitted with 371 Applications	067949-5058-WO2014160019-CoverPageOnly-09-14-2015.pdf	151416	no	1
			5e4408ef7916e7f914f769d97eae455d8eb5ec69		
Warnings:					
Information:					
4	Documents submitted with 371 Applications	067949-5058-IB304-09-14-2015.pdf	145114	no	1
			69b155841ab1ea9ad25e3698f96422c462695bf4		
Warnings:					
Information:					
5	Documents submitted with 371 Applications	067949-5058-IB308-FirstNotice-09-14-2015.pdf	144137	no	1
			aeae7d23de8cb778e79a6f118f434316d1606f7a		
Warnings:					
Information:					
6	Documents submitted with 371 Applications	067949-5058-IB308-SecondNotice-09-14-2015.pdf	153080	no	1
			6496a6d2c6b1a0121f9bf132aa8320c695e4493a		
Warnings:					
Information:					
7	Information Disclosure Statement (IDS) Form (SB08)	067949-5058-IDS-09-14-2015.pdf	1035389	no	4
			399e4ac36d23dac0ba54e1b5a3713788c725f849		
Warnings:					
Information:					

8	Non Patent Literature	067949-5058- Huang-09-14-2015.pdf	1950274 5b6bf6099bf631e5c8e1aa7b97608fda3b03f21f	no	9
Warnings:					
Information:					
9	Other Reference-Patent/App/Search documents	067949-5058-ISR- WOP-09-14-2015.pdf	1177084 052828491d052d84b12452d042f59cd5243c41e8	no	10
Warnings:					
Information:					
10	Fee Worksheet (SB06)	fee-info.pdf	34953 0c8e244fe94b3308eee64d144be7d6ee9d6ee760	no	2
Warnings:					
Information:					
Total Files Size (in bytes):			6831191		
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. <u>New Applications Under 35 U.S.C. 111</u> 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. <u>National Stage of an International Application under 35 U.S.C. 371</u> 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. <u>New International Application Filed with the USPTO as a Receiving Office</u> 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.					

616387

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

April 19, 2014

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: 61/785,031

FILING DATE: *March 14, 2013*

RELATED PCT APPLICATION NUMBER: *PCT/US14/25636*

THE COUNTRY CODE AND NUMBER OF YOUR PRIORITY APPLICATION, TO BE USED FOR FILING ABROAD UNDER THE PARIS CONVENTION, IS *US61/785,031*



Certified by

David J. Kyros

Under Secretary of Commerce
for Intellectual Property
and Director of the United States
Patent and Trademark Office

Electronic Acknowledgement Receipt

EFS ID:	15231516
Application Number:	61785031
International Application Number:	
Confirmation Number:	9544
Title of Invention:	ALIGNED FIBER AND METHOD OF USE THEREOF
First Named Inventor/Applicant Name:	Michael Francis
Customer Number:	9629
Filer:	Hyeseung Janice Lee
Filer Authorized By:	
Attorney Docket Number:	067949-5058-PR
Receipt Date:	14-MAR-2013
Filing Date:	
Time Stamp:	19:26:26
Application Type:	Provisional

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	\$ 125
RAM confirmation Number	27506
Deposit Account	500310
Authorized User	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
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1	Transmittal of New Application	ProvisionalTransmittal.pdf	145152 f9d758d3646be0c6446f6bcb5f28745ea68f15e6	no	2
Warnings:					
Information:					
2		067949-5058_Specification.pdf	1541533 a18bf55f942e08bf9fab11dde3079cd1b85ea97	yes	44
	Multipart Description/PDF files in .zip description				
	Document Description		Start	End	
	Specification		1	30	
	Claims		31	34	
	Drawings-other than black and white line drawings		35	44	
Warnings:					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	29826 d3e0c5ecfb784ad5fd806fdadc450f6b4d879e9	no	2
Warnings:					
Information:					
Total Files Size (in bytes):			1716511		
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. <u>New Applications Under 35 U.S.C. 111</u> 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. <u>National Stage of an International Application under 35 U.S.C. 371</u> 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. <u>New International Application Filed with the USPTO as a Receiving Office</u> 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.					

Figure 1.

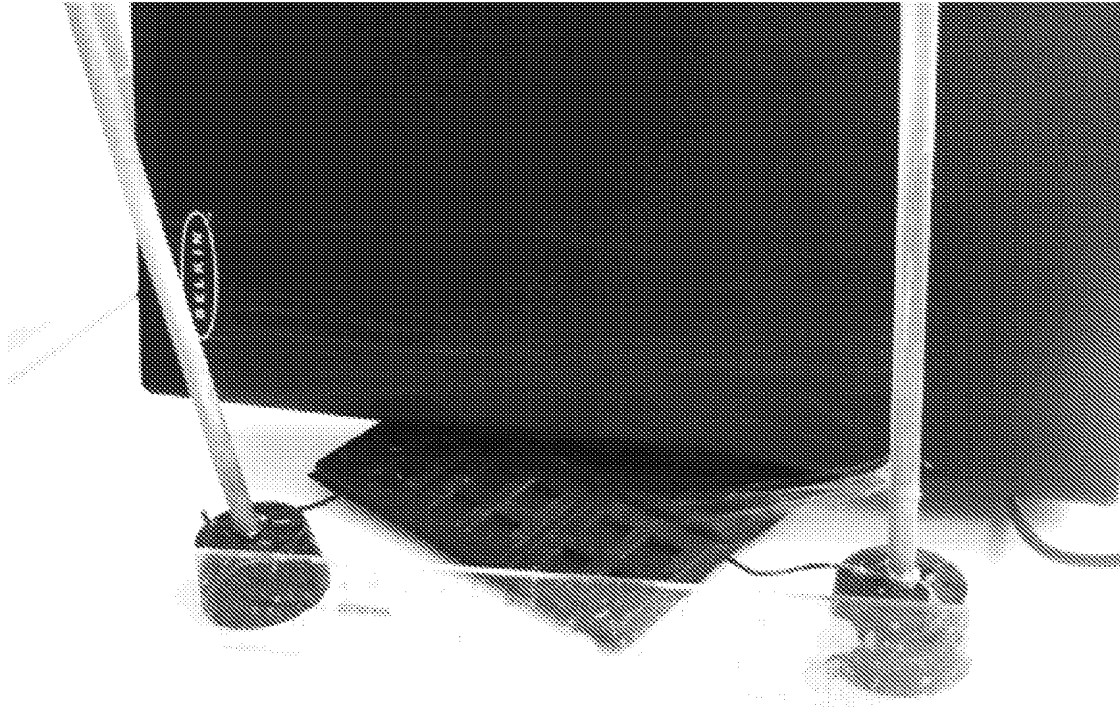


Figure 2.

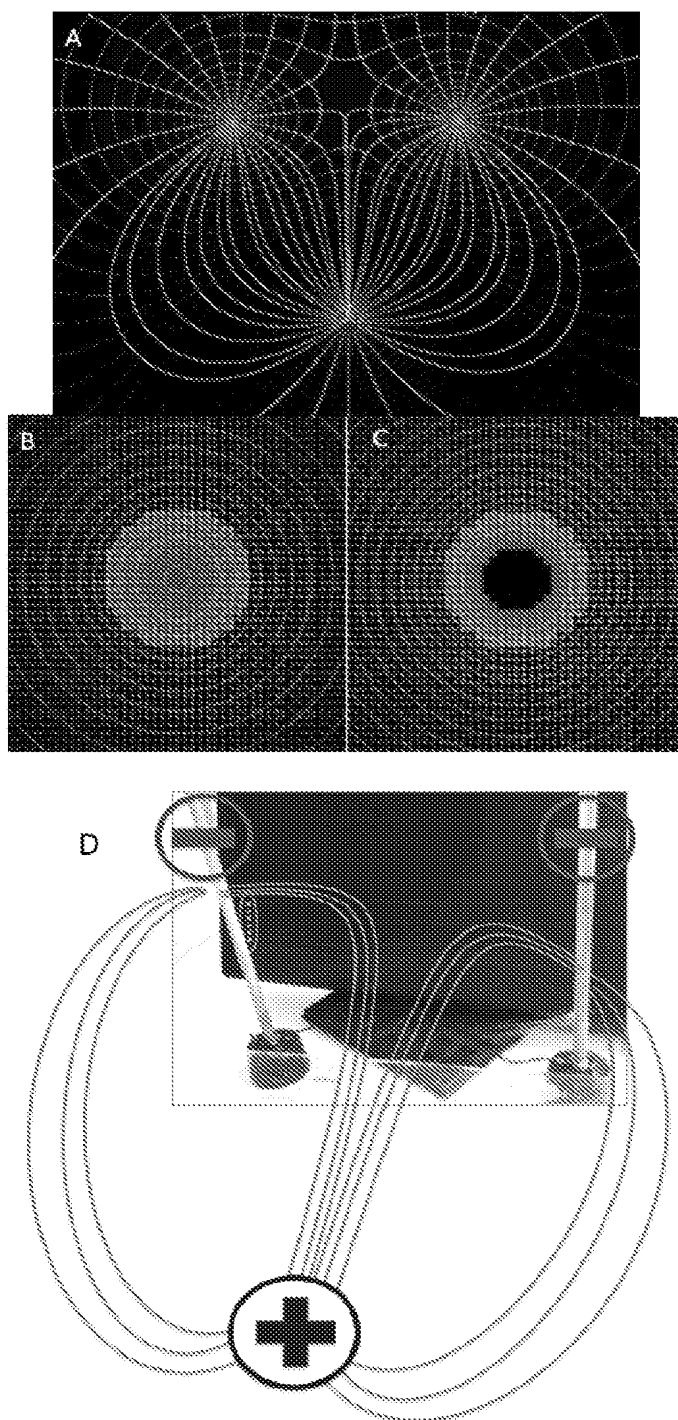
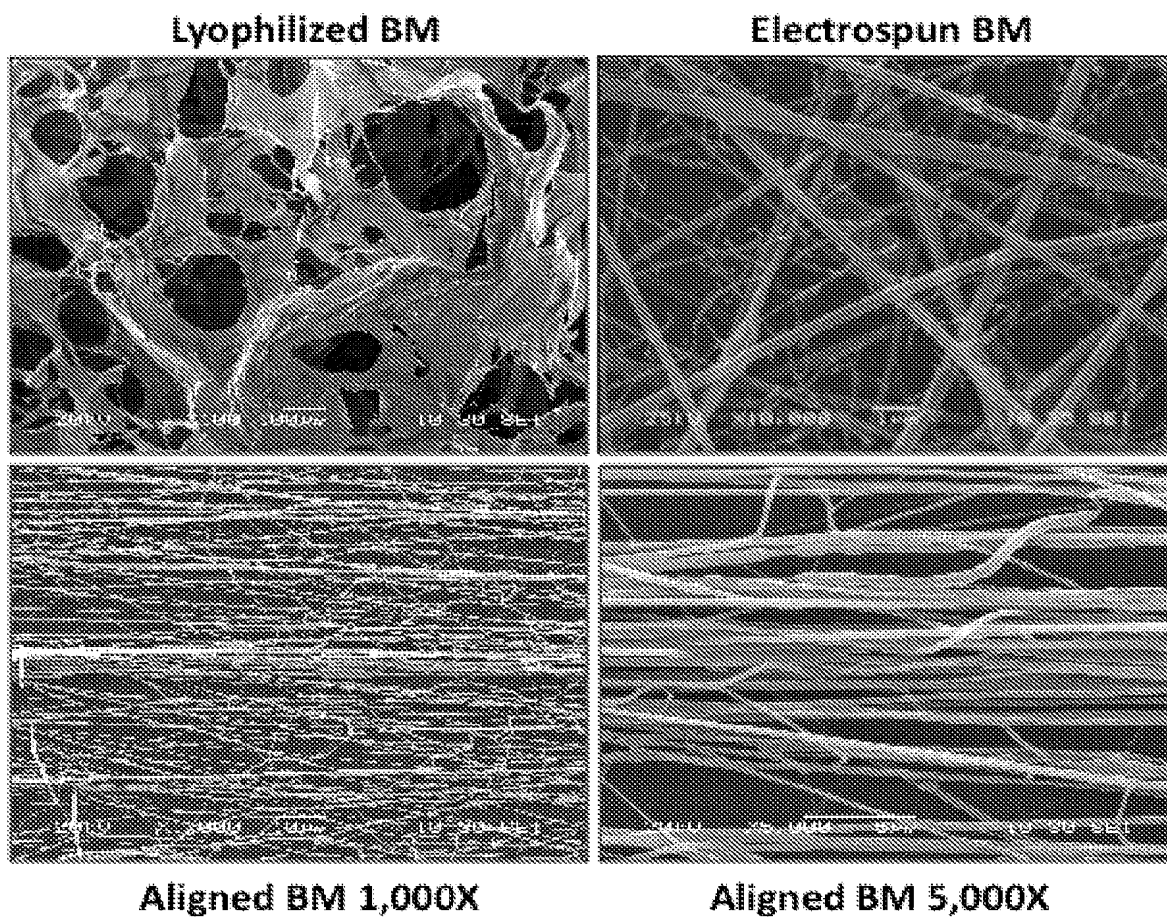


Figure 3.



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

Figure 4.

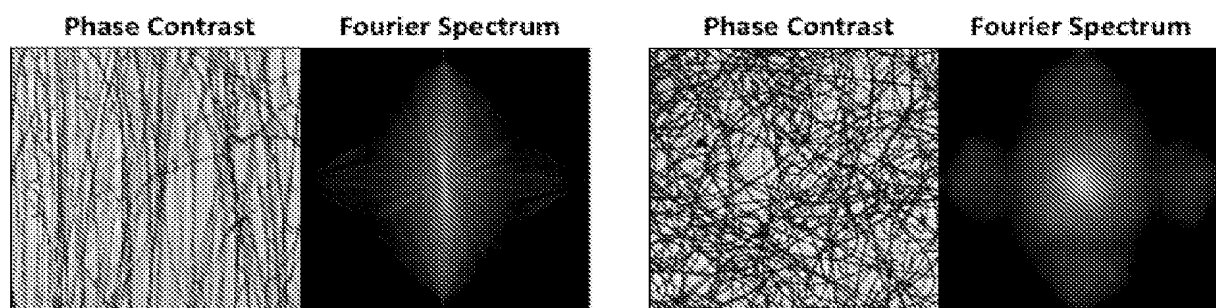


Figure 5.

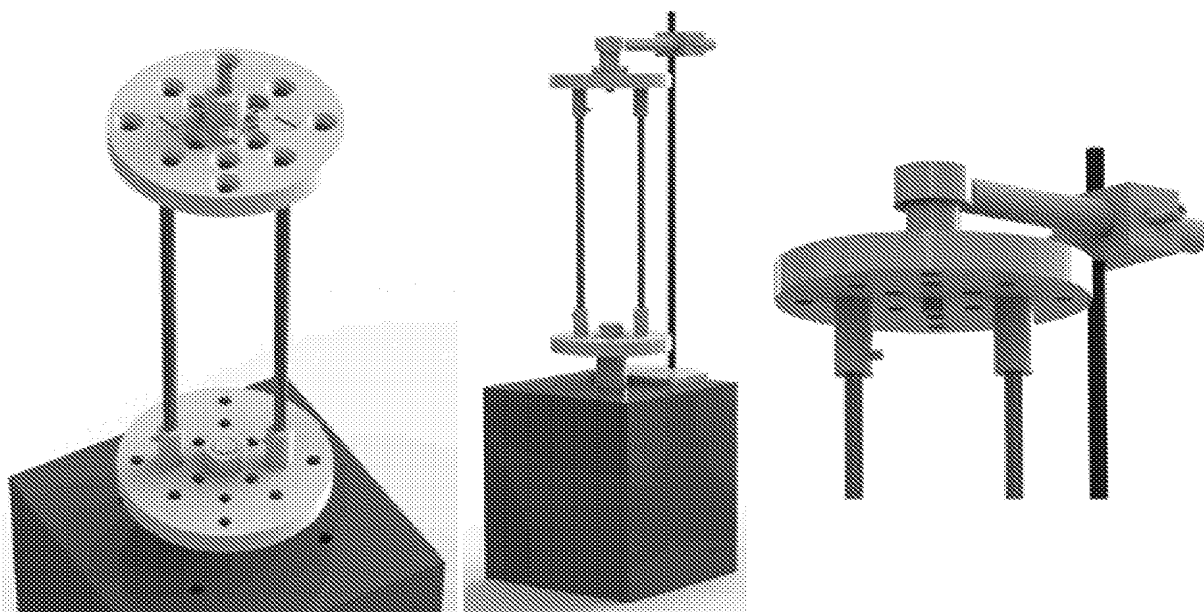


Figure 6.

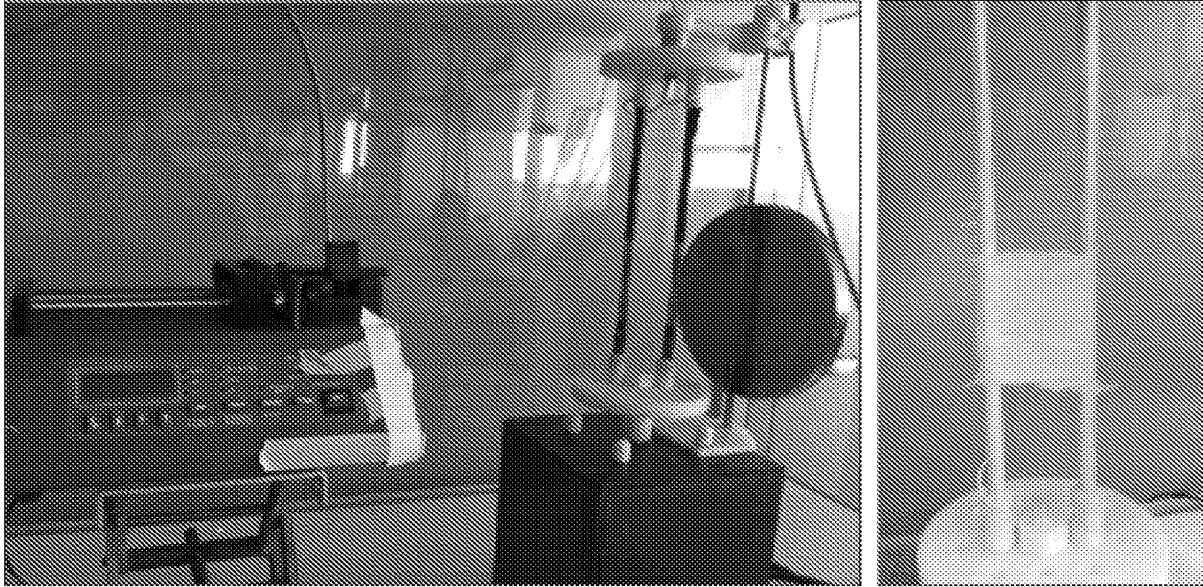


Figure 7.

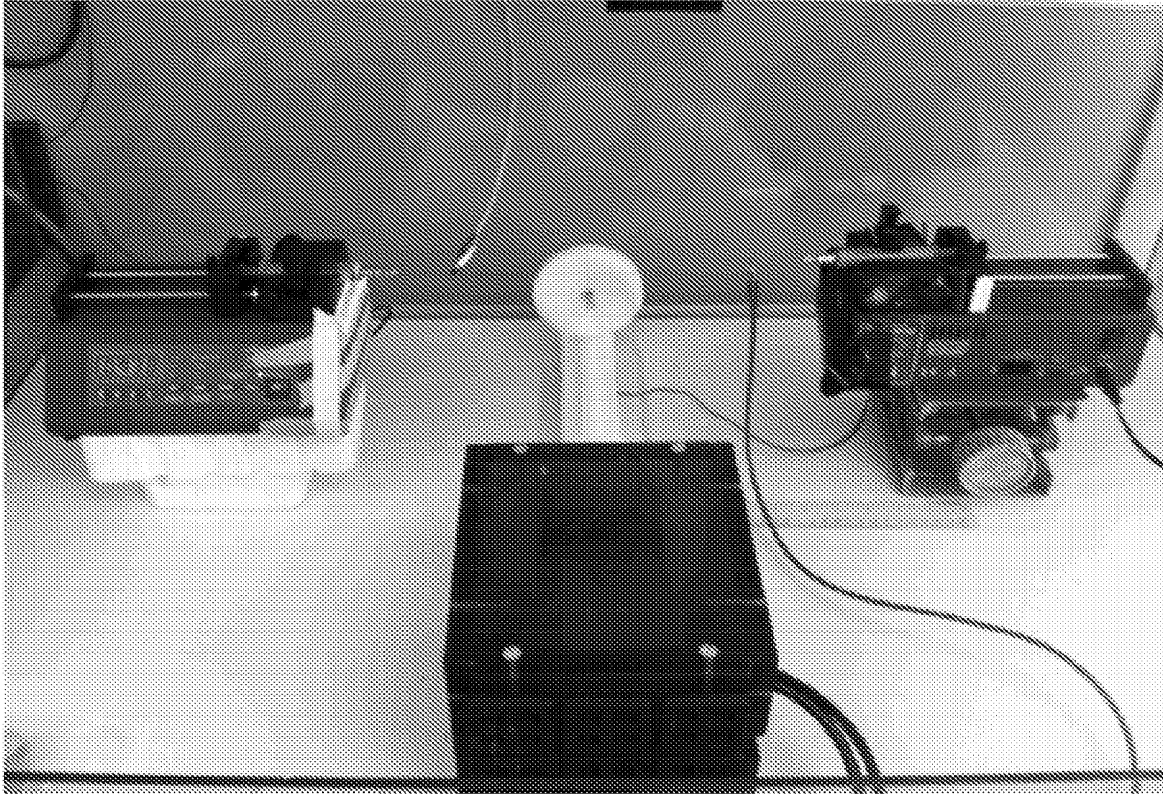
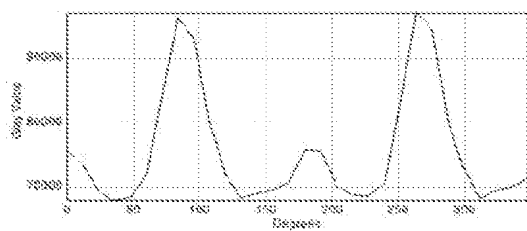


Figure 8.

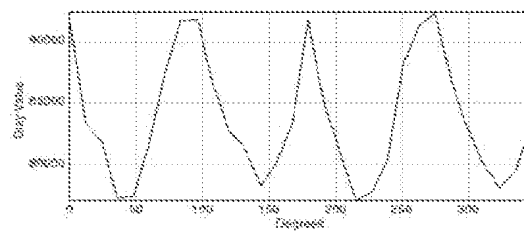
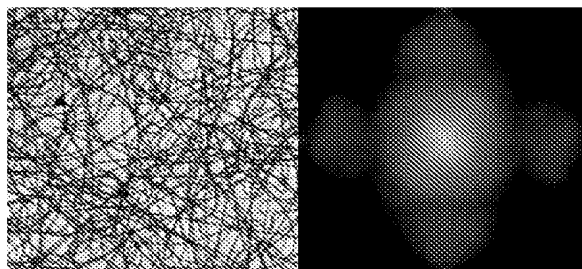
Figure 9.

Figure 10.

(a)



(b)



CLAIMS

1. A scaffold comprising one or more electrospun fibers, wherein a fast Fourier transform (FFT) analysis result of the fibers have adjacent major peaks with about 180° apart from each other.
2. The scaffold according to claim 1, wherein the scaffold essentially consists of the electrospun fibers.
3. The scaffold according to claim 1, wherein the scaffold consists of the electrospun fibers.
4. The scaffold according to any of claims 1-3, wherein the scaffold is biocompatible.
5. The scaffold according to any of claims 1-4, wherein the fiber comprise type I collagen in its native triple helical structure.
6. The scaffold according to any of claims 1-5, wherein the fiber has less than about 8 wt% residual calcium.
7. The scaffold according to any of claims 1-6, wherein the fiber has an average diameter from about 5 nm to about 1000 nm.
8. The scaffold according to any of claims 1-7, wherein the fiber has an average diameter of less than about 100 nm.
9. The scaffold according to any of claims 1-8, wherein the fiber has an average length from about 1 cm to about 50 cm.
10. The scaffold according to any of claims 1-9, wherein the fiber has an average length from about 10 cm to about 50 cm.
11. The scaffold according to any of claims 1-10, wherein the scaffold has an average porosity from about 60% to about 90%.
12. The scaffold according to any of claims 1-11, wherein the 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, an accessory polymer, or the mixture thereof.

13. The scaffold according to any of claims 1-11, wherein the 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, or polycaprolactone.
14. The scaffold according to any of claims 1-11, wherein the scaffold comprises heart basement membrane extract or heart basement membrane extracellular matrix.
15. The scaffold according to any of claims 1-11, wherein the scaffold comprises placenta basement membrane extract or placenta basement membrane extracellular matrix.
16. The scaffold according to any of claims 1-15, wherein the scaffold comprises an accessory polymer.
17. The scaffold according to claim 16, wherein the accessory polymer is 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/surfactants, xanthan, acetoglycerides, waxes, surfactants, dextran, emulsan, gelatin, polyphenols, levan, lignin, curd, tannin, polygalactosamine, humic acid, shellac, pullulan, poly-gamma-glutamic acid, elastin, natural rubber, yeast glucans, synthetic polymers from natural fats and oils.
18. The scaffold according to any of claims 1-17, wherein the electrospun fibers are crosslinked.
19. The scaffold according to any of claims 1-18, wherein the scaffold comprises a bioactive factor.
20. The scaffold according to claim 19, wherein the bioactive factor is selected from the group consisting of an osteogenic growth factor, collagen, glycosaminoglycans, osteonectin, bone sialoprotein, 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), and an interleukin.

21. The scaffold according to any of claims 1-20, wherein the scaffold comprises an extracellular matrix component.
22. The scaffold according to claim 21, wherein the extracellular matrix component is selected from the group consisting of collagen, glycosaminoglycans, osteocalcin, osteonectin, bone sialo protein, osteopontin, fibronectin, laminin, and mixtures thereof.
23. A method of cell culture comprising culturing cells on the scaffold according to any of claims 1-22 in condition suitable for the cell culture.
24. The method of claim 23, wherein the cells are selected from the group consisting of stem cells, adipose derived stem cells, dental pulp stem cells, fibroblasts, and dorsal root ganglia.
25. The method of claim 23, wherein the cells are dorsal root ganglia.
26. The method of claim 23, wherein the cells are dorsal root ganglia, and the fiber comprise heart basement membrane extract, heart basement membrane extracellular matrix, placenta basement membrane extract, or placenta basement membrane extracellular matrix.
27. A method of promoting differentiation of stem cells into osteoblasts, chondrocytes, ligament or tendon, the method comprising culturing the cells on the scaffold according to any of claims 1-22 in conditions suitable for the cell differentiation.
28. The method of claim 27, wherein the stem cells are progenitor cells or adult stem cells.
29. The method of claim 28, 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.
30. The method of claim 27, wherein the stem cells are pluripotent stem cells.

31. The method of claim 30, wherein the stem cells are selected from the group consisting of adipose derived stem cells and dental pulp stem cells.

ALIGNED FIBER AND METHOD OF USE THEREOF

Inventor:

Michael Francis and Roy Ogle

Background of the Invention

[0001] The invention relates to an aligned fiber and method of use thereof.

Summary of the Invention

[0002] 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.

[0003] The invention further relates to methods culturing cells on the scaffold or aligned fiber described herein.

[0004] 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

Brief Description of the Drawings

[0005] FIGURE 1 depicts metal rods that are used to align electrospun PCL. PCL dissolved in HFP (100mg/ml) at a +15kV reference, set 15cm apart, is electrospun between the rods, with PCL pumped at 5ml/hr over 5 minutes to the form the aligned nanofibers seen collecting between the rods.

[0006] FIGURE 2 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. The change in field lines between a solid (B) and hollow (C) rod is also modeled.

[0007] FIGURE 3 depicts raw basement membrane (top left) next to it randomly electrospun architecture (to right), as compared to aligned basement membrane generated by parallel charged rod aligned electrospinning (bottom left and right).

[0008] FIGURE 4 depicts 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.

[0009] FIGURE 5 depicts 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.

[0010] FIGURE 6 depicts the elements of the rotating parallel electric charged rod electrospinning setup in the left image. Using two charged rods imparts alignment to the traveling fibers, likely through the point charge splitting of the electric field. A sheet of aligned fibers formed after 1 hour of electrospinning is shown on the right.

[0011] FIGURE 7 depicts two syringes pumps with basement membrane, each attached to a high voltage power supply, with one supply set to positive (+) voltage, the other set to negative (-) voltage to generate the static electric field for electrospinning, with fibers collected on the rotating or static charged rods, which are earthed in this schema. 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.

[0012] FIGURE 8 depicts aligned heart base membrane fiber scaffold that allow dorsal root ganglia attachment and outgrowth.

[0013] FIGURE 9 depicts aligned placenta and heart base membrane fiber scaffolds that allow dorsal root ganglia attachment and outgrowth.

[0014] FIGURE 10 depicts exemplary FFT analysis results (a) of the fibers described herein and (b) a control sample.

Detailed Description of the Invention

[0015] 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, five 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.

[0016] 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.

[0017] 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.

[0018] 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.

[0019] 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.

[0020] 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.

[0021] 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.

[0022] 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.

[0023] 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.

[0024] 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.

[0025] 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.

[0026] 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 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.

[0027] 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.

[0028] 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 93 °, between about 93 and about 93 °, 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 °.

[0029] 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.

[0030] 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.

[0031] 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.

[0032] 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, kyanan, 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, polysulfone, 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.

[0033] 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.

[0034] 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.

[0035] 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.

[0036] 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.

[0037] 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.

[0038] The collector may be biocompatible. In fact, any of the other elements of the electrospinning apparatus described herein may be biocompatible.

[0039] 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.

[0040] 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.

[0041] 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, 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/surfactants, xanthan, acetoglycerides, waxes, surfactants, dextran, emulsan, gelatin, polyphenols, levan, lignin, curd, inulin, tannin, polygalactosamine, humic acid, shellac, pullulan, poly-gamma-glutamic acid, elastin, natural rubber, yeast glucans, and synthetic polymers from natural fats and oils, and the mixture thereof.

[0042] 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.

[0043] 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.

[0044] 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.

[0045] 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 xenograft. 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.

[0046] 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.

[0047] 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.

[0048] 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 °C and about 37 °C, or about 20 °C and about 37 °C. 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.

[0049] 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.

[0050] 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 CollograftTM (Collagen Corporation), which is a mixture of hydroxyapatite, tricalcium phosphate and fibrillar collagen, and InterporeTM (Interpore International), which is a hydroxyapatite biomatrix formed by the conversion of marine coral calcium carbonate to crystalline hydroxyapatite.

[0051] 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 Behravesch (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).

[0052] 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.

[0053] 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.

[0054] 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.

[0055] 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.

[0056] 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, hydrophilicity, 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/surfactants, xanthan, acetoglycerides, waxes, surfactants, dextran, emulsan, gelatin, polyphenols, levan, lignin, curd, inulin, tannin, polygalactosamine, humic acid, shellac, pullulan, poly-gamma-glutamic acid, elsinan, natural rubber, yeast glucans, and synthetic polymers from natural fats and oils.

[0057] 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.

[0058] 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.

[0059] 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.

[0060] 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.

[0061] 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 10.

[0062] 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. Nephrol.*, 7:96-104 (1993), which is incorporated by reference.

[0063] 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% .

[0064] 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.

[0065] 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%.

[0066] 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.

[0067] 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.

[0068] 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.

[0069] 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, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. The biodegradable, biocompatible polymers may further include a number of synthetic biodegradable polymers described herein.

[0070] 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.

[0071] 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.

[0072] 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.

[0073] 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.

[0074] 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.

[0075] 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.

[0076] 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 °C and about 40 °C or about 30°C and about 37°C, in an atmosphere containing between about 1% CO₂ and about 10% CO₂ or about 4% CO₂ and about 6% CO₂, in certain embodiments.

[0077] 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.

[0078] The invention also relates to methods of promoting osteoinductivity, 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 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.

[0079] 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.

[0080] The invention also relates to methods of promoting ligament/tendon differentiation, 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 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.

[0081] 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.

[0082] 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.

[0083] 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.

[0084] 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.

[0085] 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.

[0086] 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 a 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.

[0087] 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).

[0088] 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.

[0089] 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.

[0090] 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.

[0091] The following examples are illustrative and are not intended to limit the scope of the invention described herein.

[0092] Example 1:

[0093] 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, can be of steel, stainless steel, copper, or other heavy metal, as used in the illustrated drawing in **figure 5**. 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

[0094] 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).

[0095] A photo of electrospun PCL is seen collecting across steel rods set 20cm apart in **figure 1**, with **figure 2 (a, d)** showing the representative electric field lines and the known 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 2b, 2c**) (electric field diagrams created using the applet from cco.caltech.edu/~phys1/java/phys1/EField/EField.html).

[0096] 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 3**. 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 3**, 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 4** using the ImageJ64 FFT tool, with patterns of aligned fibers shown to match with those of known aligned Fourier spectra, as commonly used for pattern recognition.

[0097] **Figures 5** illustrates 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 6** along with a picture of a sheet of aligned fibers formed in this fashion for reference. **Figure 7** shows a slight modification of this rotating charged parallel point charged rod fiber alignment process whereby 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, which we appreciated as being able to electrospin sheets that are up to around 3.4 times the thickness of conventionally electrospun fiber sheets.

[0098] 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.

[0099] Example 2: Heart Base Membrane Fiber Scaffold

[00100] 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- β III 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 8**, 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.

[00101] Example 3: Dorsal Root Ganglia Attachment and Outgrowth on Placenta and Heart Basement Membrane Nanofibers.

[00102] 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- β III 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 9**, 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.

[00103] Example 4: Dorsal Root Ganglia Attachment and Outgrowth on Polycaprolactone Nanofibers (Comparative Example)

[00104] 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- β III 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).

Provisional Application for Patent Cover Sheet

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c)

Inventor(s)

Inventor 1

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All Inventors Must Be Listed – Additional inventor information blocks may be generated within this form by selecting the **Add** button.**Add****Title of Invention**

ALIGNED FIBER AND METHOD OF USE THEREOF

Attorney Docket Number (if applicable)

067949-5058-PR

Correspondence Address

Direct all correspondence to (select one):

☒ The address corresponding to Customer Number☐ Firm or Individual Name

Customer Number

09629

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.☐ Yes, the name of the U.S. Government agency and the Government contract number are:

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Entity Status

Applicant claims small entity status under 37 CFR 1.27

- ☒ Yes, applicant qualifies for small entity status under 37 CFR 1.27
☐ No

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Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

Signature

Please see 37 CFR 1.4(d) for the form of the signature.

Signature	/H. Janice Lee/			Date (YYYY-MM-DD)	2013-03-14
First Name	H. Janice	Last Name	Lee	Registration Number (if appropriate)	64526

This collection of information is required by 37 CFR 1.51. 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 8 hours 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. This form can only be used when in conjunction with **EFS-Web**. If this form is mailed to the **USPTO**, it may cause delays in handling the provisional application.

DOCUMENT MADE AVAILABLE UNDER THE PATENT COOPERATION TREATY (PCT)

International application number:	PCT/US2014/025636
International filing date:	13 March 2014 (13.03.2014)
Document type:	Certified copy of priority document
Document details:	Country/Office: US
	Number: 61/785,031
	Filing date: 14 March 2013 (14.03.2013)
Date of receipt at the International Bureau:	21 April 2014 (21.04.2014)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a),(b) or (b-bis)

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

PCT/US14/25636

International Application No.

13 MAR 2014 (13.03.14)

International Filing Date

RO/US

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference
(if desired) (12 characters maximum) 67949-5058WO

Box No. I	TITLE OF INVENTION	
ALIGNED FIBER AND METHOD OF USE THEREOF		
Box No. II	APPLICANT	
☐ This person is also inventor		
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)		Telephone No.
LIFENET HEALTH 1864 Concert Drive Virginia Beach, VA 23453 United States of America		Facsimile No.
		Applicant's registration No. with the Office
E-mail authorization: Marking one of the check-boxes below authorizes the receiving Office, the International Searching Authority, the International Bureau and the International Preliminary Examining Authority to use the e-mail address indicated in this Box to send, notifications issued in respect of this international application to that e-mail address if those offices are willing to do so. ☐ as advance copies followed by paper notifications; or ☐ exclusively in electronic form (no paper notifications will be sent).		
E-mail address:		
State (that is, country) of nationality: US		State (that is, country) of residence: US
This person is applicant for the purposes of: ☒ all designated States ☐ the States indicated in the Supplemental Box		
Box No. III	FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)	
☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.		
Box No. IV	AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE	
The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:		☒ agent ☐ common representative
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)		Telephone No.
BUCK, Todd B. Morgan, Lewis & Bockius LLP 1111 Pennsylvania Avenue, NW Washington, DC 20004 United States of America		202.739.3000
		Facsimile No.
		202.739.3001
		Agent's registration No. with the Office
		48,574
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E-mail address: patents@morganlewis.com		
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Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

FRANCIS, Michael**1864 Concert Drive****Virginia Beach, VA 23453****United States of America**

This person is:

☐ applicant only☐ applicant and inventor☒ inventor only (If this check-box is marked, do not fill in below.)

Applicant's registration No. with the Office

State (that is, country) of nationality:

State (that is, country) of residence:

This person is applicant for the purposes of:

☐ all designated States☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

OGLE, Roy**1864 Concert Drive****Virginia Beach, VA 23453****United States of America**

This person is:

☐ applicant only☐ applicant and inventor☒ inventor only (If this check-box is marked, do not fill in below.)

Applicant's registration No. with the Office

State (that is, country) of nationality:

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This person is:

☐ applicant only☐ applicant and inventor☐ inventor only (If this check-box is marked, do not fill in below.)

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This person is applicant for the purposes of:

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Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

This person is:

☐ applicant only☐ applicant and inventor☐ inventor only (If this check-box is marked, do not fill in below.)

Applicant's registration No. with the Office

State (that is, country) of nationality:

State (that is, country) of residence:

This person is applicant for the purposes of:

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☒ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Supplemental Box

If the Supplemental Box is not used, this sheet should not be included in the request.

1. *If, in any of the Boxes, except Boxes Nos. VIII(i) to (v) for which a special continuation box is provided, the space is insufficient to furnish all the information: in such case, write "Continuation of Box No." (indicate the number of the Box) and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:*
 - (i) *if more than one person is to be indicated as applicant and/or inventor and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;*
 - (ii) *if, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;*
 - (iii) *if, in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;*
 - (iv) *if, in addition to the agent(s) indicated in Box No. IV, there are further agents: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;*
 - (v) *if, in Box No. VI, there are more than three earlier applications whose priority is claimed: in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI.*
2. *If the applicant intends to make an indication of the wish that the international application be treated, in certain designated States, as an application for a patent of addition, certificate of addition, inventor's certificate of addition or utility certificate of addition: in such case, write the name or two-letter code of each designated State concerned and the indication "patent of addition," "certificate of addition," "inventor's certificate of addition" or "utility certificate of addition," the number of the parent application or parent patent or other parent grant and the date of grant of the parent patent or other parent grant or the date of filing of the parent application (Rules 4.11(a)(i) and 49bis.1(a) or (b)).*
3. *If the applicant intends to make an indication of the wish that the international application be treated, in the United States of America, as a continuation or continuation-in-part of an earlier application: in such case, write "United States of America" or "US" and the indication "continuation" or "continuation-in-part" and the number and the filing date of the parent application (Rules 4.11(a)(ii) and 49bis.1(d)).*

Box No. IV. Agent or common Representative:
(continue)

Boswell, Mary Jane
 Buck, Todd, B.
 Busby, Robert W.
 Cangro, Bradford A.
 Cherukuri, Kavitha B.
 Connor, David E.
 DU, Harry
 Gaybrick, Robert J.
 Goodell, Robert J.
 Lee, H. Janice
 Lowen, Gregory T.
 Matney, William Jackson, Jr.
 Ohara, Toyomi
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 Peterson, Jeremy
 Shu, Haining
 Snodgrass, Jacob, A.
 Smyth, Robert
 Sullivan, Mark J.
 You, Xiaobin
 Zele, John D.

and all of MORGAN, LEWIS & BOCKIUS LLP, 1111
 Pennsylvania Avenue, NW, Washington, DC 20004
 United States of America

The address, telephone number and facsimile
 number of all of the above are as indicated
 in Box IV.

Box No. V DESIGNATIONS

The filing of this request constitutes under Rule 4.9(a) the designation of all Contracting States bound by the PCT on the international filing date, for the grant of every kind of protection available and, where applicable, for the grant of both regional and national patents.

However,

- ☐ DE Germany is not designated for any kind of national protection
- ☐ JP Japan is not designated for any kind of national protection
- ☐ KR Republic of Korea is not designated for any kind of national protection

(The check-boxes above may only be used to exclude (irrevocably) the designations concerned if, at the time of filing or subsequently under Rule 26bis.1, the international application contains in Box No. VI a priority claim to an earlier national application filed in the particular State concerned, in order to avoid the ceasing of the effect, under the national law, of this earlier national application.)

Box No. VI PRIORITY CLAIM AND DOCUMENT

The priority of the following earlier application(s) is hereby claimed:

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is:		
		national application: country or Member of WTO	regional application: regional Office	international application: receiving Office
item (1) 14 March 2013 (14.03.2013)	61/785,031	US		
item (2)				
item (3)				

☐ Further priority claims are indicated in the Supplemental Box.

Furnishing the priority document(s):

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) (only if the earlier application(s) was filed with the receiving Office which, for the purposes of this international application, is the receiving Office) identified above as:

☐ all items ☒ item (1) ☐ item (2) ☐ item (3) ☐ other, see Supplemental Box

☐ The International Bureau is requested to obtain from a digital library a certified copy of the earlier application(s) identified above, using, where applicable, the access code(s) indicated below (if the earlier application(s) is available to it from a digital library):

☐ item (1) access code _____ ☐ item (2) access code _____ ☐ item (3) access code _____ ☐ other, see Supplemental Box

Restore the right of priority: the receiving Office is requested to restore the right of priority for the earlier application(s) identified above or in the Supplemental Box as item(s) _____. (See also the Notes to Box No. VI; further information must be provided to support a request to restore the right of priority.)

Incorporation by reference: where an element of the international application referred to in Article 11(1)(iii)(d) or (e) or a part of the description, claims or drawings referred to in Rule 20.5(a) is not otherwise contained in this international application but is completely contained in an earlier application whose priority is claimed on the date on which one or more elements referred to in Article 11(1)(iii) were first received by the receiving Office, that element or part is, subject to confirmation under Rule 20.6, incorporated by reference in this international application for the purposes of Rule 20.6.

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) (if more than one International Searching Authority is competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):

ISA/ US

Box No. IX CHECK LIST for EFS-Web filings - this sheet is only to be used when filing an international application with RO/US via EFS-Web			
This international application contains the following:	Number of sheets	This international application is accompanied by the following item(s) (<i>mark the applicable check-boxes below and indicate in right column the number of each item</i>):	Number of items
(a) request form PCT/RO/101 (including any declarations and supplemental sheets) :	5	1. ☒ fee calculation sheet :	1
(b) description (excluding any sequence listing part of the description, see (f), below) :	33	2. ☐ original separate power of attorney :	
(c) claims :	4	3. ☐ original general power of attorney :	
(d) abstract :	1	4. ☐ copy of general power of attorney; reference number: :	
(e) drawings (if any) :	9	5. ☐ priority document(s) identified in Box No. VI as item(s) :	
(f) sequence listing part of the description in the form of an image file (e.g. PDF) :		6. ☐ Translation of international application into (language): :	
Total number of sheets (including the sequence listing part of the description if filed as an image file) :	52	7. ☐ separate indications concerning deposited microorganism or other biological material :	
(g) sequence listing part of the description		8. ☐ (<i>only where item (f) is marked in the left column</i>) copy of the sequence listing in electronic form (Annex C/ST.25 text file) not forming part of the international application but furnished only for the purposes of international search under Rule 13ter :	
☐ filed in the form of an Annex C/ST.25 text file		9. ☐ (<i>only where item (f) is marked in the left column</i>) a statement confirming that "the information recorded in electronic form submitted under Rule 13ter is identical to the sequence listing as contained in the international application" as filed via EFS-Web: :	
☐ WILL BE filed separately on physical data carrier(s), on the same day and in the form of an Annex C/ST.25 text file		10. ☐ copy of results of earlier search(es) (Rule 12bis.1(a)) .. :	
Indicate type and number of physical data carrier(s) :		11. ☒ other (<i>specify</i>): Transmittal Letter :	1
Figure of the drawings which should accompany the abstract:		Language of filing of the international application:	ENGLISH
Box No. X SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE <i>Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).</i>			
Todd B. Buck			
/Todd B. Buck, Reg. no. 48,574/			
13-03-2014			

For receiving Office use only			
1. Date of actual receipt of the purported international application:	13 MAR 2014 (13.03.14)	2. Drawings:	☐ received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:		☐ not received:	
4. Date of timely receipt of the required corrections under PCT Article 11(2):		6. ☐ Transmittal of search copy delayed until search fee is paid	
5. International Searching Authority (if two or more are competent): ISA / US			

For International Bureau use only
Date of receipt of the record copy by the International Bureau:

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44bis)

Applicant's or agent's file reference 67949-5058WO	FOR FURTHER ACTION	See item 4 below
International application No. PCT/US2014/025636	International filing date (<i>day/month/year</i>) 13 March 2014 (13.03.2014)	Priority date (<i>day/month/year</i>) 14 March 2013 (14.03.2013)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant LIFENET HEALTH		

1.	This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 bis.1(a).																								
2.	This REPORT consists of a total of 5 sheets, including this cover sheet.																								
In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.																									
3.	This report contains indications relating to the following items: <table style="width: 100%;"> <tr> <td style="width: 10%; text-align: center;">☒</td> <td style="width: 30%;">Box No. I</td> <td style="width: 80%;">Basis of the report</td> </tr> <tr> <td style="text-align: center;">☐</td> <td>Box No. II</td> <td>Priority</td> </tr> <tr> <td style="text-align: center;">☒</td> <td>Box No. III</td> <td>Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</td> </tr> <tr> <td style="text-align: center;">☐</td> <td>Box No. IV</td> <td>Lack of unity of invention</td> </tr> <tr> <td style="text-align: center;">☒</td> <td>Box No. V</td> <td>Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</td> </tr> <tr> <td style="text-align: center;">☐</td> <td>Box No. VI</td> <td>Certain documents cited</td> </tr> <tr> <td style="text-align: center;">☐</td> <td>Box No. VII</td> <td>Certain defects in the international application</td> </tr> <tr> <td style="text-align: center;">☐</td> <td>Box No. VIII</td> <td>Certain observations on the international application</td> </tr> </table>	☒	Box No. I	Basis of the report	☐	Box No. II	Priority	☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability	☐	Box No. IV	Lack of unity of invention	☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement	☐	Box No. VI	Certain documents cited	☐	Box No. VII	Certain defects in the international application	☐	Box No. VIII	Certain observations on the international application
☒	Box No. I	Basis of the report																							
☐	Box No. II	Priority																							
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability																							
☐	Box No. IV	Lack of unity of invention																							
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement																							
☐	Box No. VI	Certain documents cited																							
☐	Box No. VII	Certain defects in the international application																							
☐	Box No. VIII	Certain observations on the international application																							
4.	The International Bureau will communicate this report to designated Offices in accordance with Rules 44bis.3(c) and 93bis.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44bis .2).																								

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Date of issuance of this report 15 September 2015 (15.09.2015) Authorized officer Agnès Wittmann-Regis e-mail: pt06.pct@wipo.int
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Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: TODD B. BUCK
MORGAN, LEWIS & BOCKIUS LLP
1111 PENNSYLVANIA AVENUE, NW
WASHINGTON, DC 20004

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

Date of mailing
(day/month/year)

10 JUL 2014

Applicant's or agent's file reference
67949-5058WO

FOR FURTHER ACTION

See paragraph 2 below

International application No.

PCT/US 14/25636

International filing date (day/month/year)

13 March 2014 (13.03.2014)

Priority date (day/month/year)

14 March 2013 (14.03.2013)

International Patent Classification (IPC) or both national classification and IPC

IPC(8) - A61F 2/08 (2014.01)

USPC - 530/356; 623/13.11

Applicant LIFENET HEALTH

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-3201

Date of completion of this opinion

13 June 2014 (13.06.2014)

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US 14/25636

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:
 - ☒ the international application in the language in which it was filed.
 - ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).
2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43bis.1(a))
3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:
 - a. (means)
 - ☐ on paper
 - ☐ in electronic form
 - b. (time)
 - ☐ in the international application as filed
 - ☐ together with the international application in electronic form
 - ☐ subsequently to this Authority for the purposes of search
4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
5. Additional comments:

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US 14/25636

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 5-35

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search (*specify*):

☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 5-35 are so unclear that no meaningful opinion could be formed (*specify*):

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed (*specify*):

☒ no international search report has been established for said claims Nos. 5-35

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit

☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.

☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.

☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13~~ter~~.1(a) or (b).

☐ See Supplemental Box for further details.

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.

PCT/US 14/25636

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	None	YES
	Claims	1-4	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-4	NO
Industrial applicability (IA)	Claims	1-4	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-4 lack novelty under PCT Article 33(2) as being anticipated by the article entitled "Electrospun collagen.chitosan.TPU nanofibrous scaffolds for tissue engineered tubular grafts" by Huang et al. (hereinafter Huang).

Regarding claim 1, Huang teaches a scaffold comprising one or more electrospun fibers (abstract; pg 308, col 1; pg 309, col 2, aligned nanofibrous scaffolds based on collagen.chitosan.thermoplastic polyurethane (TPU) blends were electrospun to mimic the componential and structural aspects of the native extracellular matrix, while an optimal proportion was found to keep the balance between biocompatibility and mechanical strength), wherein a fast Fourier transform (FFT) analysis result of the fibers have adjacent major peaks with about 180 degrees apart from each other (Fig 4E; pg 309, col 2, two peaks at 175 degrees and 355 degrees (180 degrees apart)).

Regarding claim 2, Huang teaches the scaffold according to claim 1, wherein the scaffold essentially consists of the electrospun fibers (abstract; pg 308, col 1; pg 309, col 2).

Regarding claim 3, Huang teaches the scaffold according to claim 1, wherein the scaffold consists of the electrospun fibers (abstract; pg 308, col 1; pg 309, col 2, electrospinning is the only method disclosed for forming the fibers).

Regarding claim 4, Huang teaches the scaffold according to any of claims 1-3, wherein the scaffold is biocompatible (abstract; pg 308, col 1, Collagen.chitosan electrospun complex and their biocompatibility have been reported previously).

Claims 1-4 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.



(51) International Patent Classification:
A61F 2/08 (2006.01)

(21) International Application Number:
PCT/US2014/025636

(22) International Filing Date:
13 March 2014 (13.03.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/785,031 14 March 2013 (14.03.2013) US

(71) Applicant: **LIFENET HEALTH** [US/US]; 1864 Concert Drive, Virginia Beach, VA 23453 (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, BN, 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, IR, IS, JP, KE, KG, 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, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, 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, 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, KM, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) Title: ALIGNED FIBER AND METHOD OF USE THEREOF

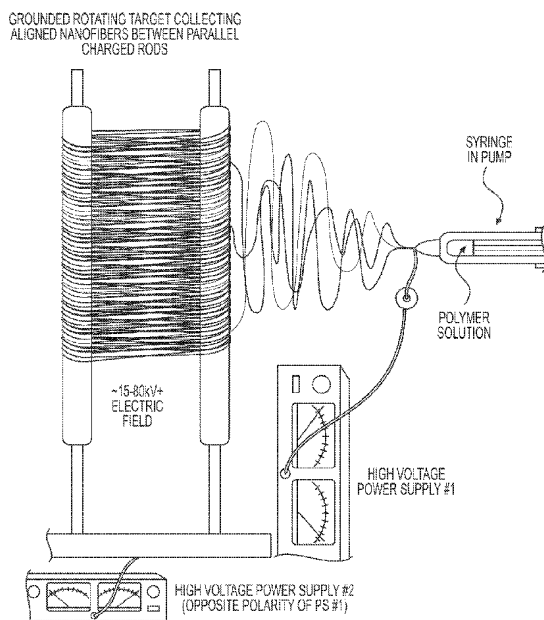


FIG. 5

(57) Abstract: A scaffold comprising an aligned fiber. The invention further relates to a scaffold comprising one or more electrospun fibers wherein a fast Fourier transform (FFT) analysis result of the fibers have adjacent major peaks with about 180° apart from each other. Methods for promoting differentiation of stem cells into osteoblasts, chondrocytes, ligament or tendon, the method comprising culturing the cells on the scaffold or aligned fiber in conditions suitable for the cell differentiation.

ALIGNED FIBER AND METHOD OF USE THEREOF**Inventor**

Michael Francis and Roy Ogle

Background of the Invention

[0001] The invention relates to an aligned fiber and method of use thereof.

Summary of the Invention

[0002] 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.

[0003] The invention further relates to methods culturing cells on the scaffold or aligned fiber described herein.

[0004] 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

Brief Description of the Drawings

[0005] FIGURE 1 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. The change in field lines between a solid (B) and hollow (C) rod is also modeled.

[0006] FIGURE 2 depicts raw basement membrane (top left) next to it randomly electrospun architecture (to right), as compared to aligned basement membrane generated by parallel charged rod aligned electrospinning (bottom left and right).

[0007] FIGURE 3 depicts 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.

[0008] FIGURE 4 depicts 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.

[0009] 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.

[0010] FIGURE 6 depicts aligned heart base membrane fiber scaffold that allow dorsal root ganglia attachment and outgrowth.

[0011] FIGURE 7 depicts aligned placenta and heart base membrane fiber scaffolds that allow dorsal root ganglia attachment and outgrowth.

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

[0013] FIGURES 9A, 9B and 9C depict 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).

Detailed Description of the Invention

[0014] 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, five 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.

[0015] 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.

[0016] 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.

[0017] 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.

[0018] 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.

[0019] 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.

[0020] 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.

[0021] 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.

[0022] 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.

[0023] 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.

[0024] 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.

[0025] 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.

[0026] 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.

[0027] 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 °.

[0028] 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.

[0029] 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.

[0030] 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 collected between opposed metal rods in an aligned fashion. The electrospun fiber described herein may be a microfiber or a nanofiber.

[0031] 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, kyan, 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, polysulfone, 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.

[0032] 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.

[0033] 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.

[0034] 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.

[0035] 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.

[0036] 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.

[0037] The collector may be biocompatible. In fact, any of the other elements of the electrospinning apparatus described herein may be biocompatible.

[0038] 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.

[0039] 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.

[0040] 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, 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/surfactants, xanthan, acetoglycerides, waxes, surfactants, dextran, emulsan, gelatin, polyphenols, levan, lignin, curd, inulin, tannin, polygalactosamine, humic acid, shellac, pullulan, poly-gamma-glutamic acid, elastin, natural rubber, yeast glucans, and synthetic polymers from natural fats and oils, and the mixture thereof.

[0041] 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.

[0042] 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.

[0043] 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.

[0044] 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.

[0045] 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.

[0046] 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.

[0047] 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.

[0048] 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 xenograft. 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.

[0049] 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 cartilaginous 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.

[0050] 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.

[0051] 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 °C and about 37 °C, or about 20 °C and about 37 °C. 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.

[0052] 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.

[0053] 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 CollograftTM (Collagen Corporation), which is a mixture of hydroxyapatite, tricalcium phosphate and fibrillar collagen, and InterporeTM (Interpore International), which is a hydroxyapatite biomatrix formed by the conversion of marine coral calcium carbonate to crystalline hydroxyapatite.

[0054] 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 Behravesch (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 Mersny 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).

[0055] 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.

[0056] 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.

[0057] 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.

[0058] 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.

[0059] 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 tips 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, hydrophilicity, 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/surfactants, xanthan, acetoglycerides, waxes, surfactants, dextran, emulsan, gelatin, polyphenols, levan, lignin, curd, tannin, polygalactosamine, humic acid, shellac, pullulan, poly- γ -glutamic acid, elastin, natural rubber, yeast glucans, and synthetic polymers from natural fats and oils.

[0060] 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.

[0061] 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.

[0062] 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.

[0063] 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.

[0064] 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**.

[0065] 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 its 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 its 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. Nephrol.*, 7:96-104 (1993), which is incorporated by reference.

[0066] 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% .

[0067] 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.

[0068] 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%.

[0069] 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.

[0070] 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.

[0071] 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.

[0072] 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, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. The biodegradable, biocompatible polymers may further include a number of synthetic biodegradable polymers described herein.

[0073] 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.

[0074] 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.

[0075] 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.

[0076] 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.

[0077] 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.

[0078] 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.

[0079] 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.

[0080] 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.

[0081] 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.

[0082] 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.

[0083] 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 °C and about 40 °C or about 30°C and about 37°C, in an atmosphere containing between about 1% CO₂ and about 10% CO₂ or about 4% CO₂ and about 6% CO₂, in certain embodiments.

[0084] 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.

[0085] 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.

[0086] 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.

[0087] 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.

[0088] 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.

[0089] 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.

[0090] 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.

[0091] 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.

[0092] 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.

[0093] 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.

[0094] 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).

[0095] 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.

[0096] 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.

[0097] 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.

[0098] The following examples are illustrative and are not intended to limit the scope of the invention described herein.

[0099] Example 1:

[00100] 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

[00101] 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).

[00102] 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).

[00103] 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**. 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**, 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** 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.

[00104] **Figures 4** illustrates 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.

[00105] 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.

[00106] Example 2: Heart Base Membrane Fiber Scaffold

[00107] 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- β III 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**, 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.

[00108] Example 3: Dorsal Root Ganglia Attachment and Outgrowth on Placenta and Heart Basement Membrane Nanofibers.

[00109] 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- β III 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**, 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.

[00110] Example 4: Dorsal Root Ganglia Attachment and Outgrowth on Polycaprolactone Nanofibers (Comparative Example)

[00111] 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- β III 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).

CLAIMS

1. A scaffold comprising one or more electrospun fibers, wherein a fast Fourier transform (FFT) analysis result of the fibers have adjacent major peaks with about 180° apart from each other.
2. The scaffold according to claim 1, wherein the scaffold essentially consists of the electrospun fibers.
3. The scaffold according to claim 1, wherein the scaffold consists of the electrospun fibers.
4. The scaffold according to any of claims 1-3, wherein the scaffold is biocompatible.
5. The scaffold according to any of claims 1-4, wherein the fiber comprise type I collagen in its native triple helical structure.
6. The scaffold according to any of claims 1-5, wherein the fiber has less than about 8 wt% residual calcium.
7. The scaffold according to any of claims 1-6, wherein the fiber has an average diameter from about 5 nm to about 1000 nm.
8. The scaffold according to any of claims 1-7, wherein the fiber has an average diameter of less than about 100 nm.
9. The scaffold according to any of claims 1-8, wherein the fiber has an average length from about 1 cm to about 50 cm.
10. The scaffold according to any of claims 1-9, wherein the fiber has an average length from about 10 cm to about 50 cm.
11. The scaffold according to any of claims 1-10, wherein the scaffold has an average porosity from about 60% to about 90%.
12. The scaffold according to any of claims 1-11, wherein the 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, an accessory polymer, or the mixture thereof.

13. The scaffold according to any of claims 1-11, wherein the 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, or polycaprolactone.
14. The scaffold according to any of claims 1-11, wherein the scaffold comprises heart basement membrane extract or heart basement membrane extracellular matrix.
15. The scaffold according to any of claims 1-11, wherein the scaffold comprises placenta basement membrane extract or placenta basement membrane extracellular matrix.
16. The scaffold according to any of claims 1-15, wherein the scaffold comprises an accessory polymer.
17. The scaffold according to claim 16, wherein the accessory polymer is 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/surfactants, xanthan, acetoglycerides, waxes, surfactants, dextran, emulsan, gelatin, polyphenols, levan, lignin, curd, tannin, polygalactosamine, humic acid, shellac, pullulan, poly-gamma-glutamic acid, elsinan, natural rubber, yeast glucans, synthetic polymers from natural fats and oils.
18. The scaffold according to any of claims 1-17, wherein the electrospun fibers are crosslinked.
19. The scaffold according to any of claims 1-18, wherein the scaffold comprises a bioactive factor.
20. The scaffold according to claim 19, wherein the bioactive factor is selected from the group consisting of an osteogenic growth factor, collagen, glycosaminoglycans, osteonectin, bone sialoprotein, 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), and an interleukin.

21. The scaffold according to any of claims 1-20, wherein the scaffold comprises an extracellular matrix component.

22. The scaffold according to claim 21, wherein the extracellular matrix component is selected from the group consisting of collagen, glycosaminoglycans, osteocalcin, osteonectin, bone sialo protein, osteopontin, fibronectin, laminin, and mixtures thereof.

23. The scaffold according to any of claims 1-21, wherein the scaffold is in a form of one or more elongated sheet.

24. The scaffold according to any of claims 1-21, wherein the scaffold is in a form of one or more elongated roll.

25. A method of treating a tissue defect comprising applying the scaffold according to claim 23 to surround the tissue defect.

26. A method of treating a tissue defect or gap comprising applying the scaffold according to claim 24 between the tissue defect or gap.

27. A method of cell culture comprising culturing cells on the scaffold according to any of claims 1-24 in condition suitable for the cell culture.

28. 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 dorsal root ganglia.

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

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

31. A method of promoting differentiation of stem cells into osteoblasts, chondrocytes, ligament or tendon, the method comprising culturing the cells on the scaffold according to any of claims 1-24 in conditions suitable for the cell differentiation.

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

33. 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. The method of claim 31, wherein the stem cells are pluripotent stem cells.

35. The method of claim 34, wherein the stem cells are selected from the group consisting of adipose derived stem cells and dental pulp stem cells.

1/10

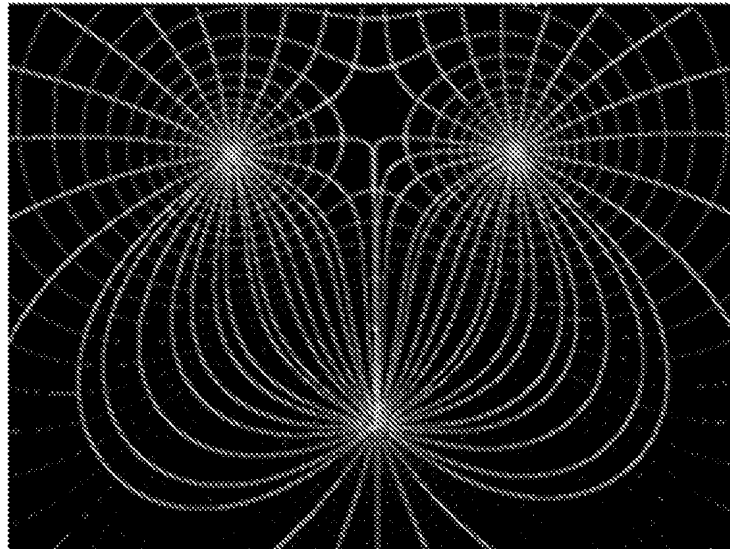


FIG. 1A

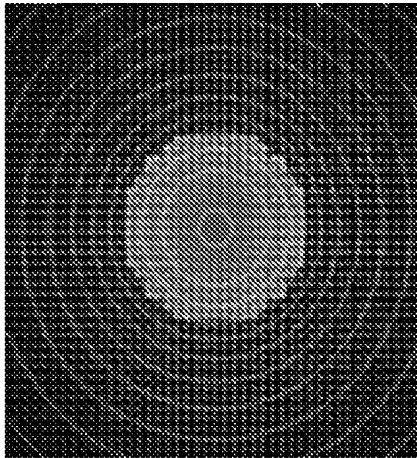


FIG. 1B

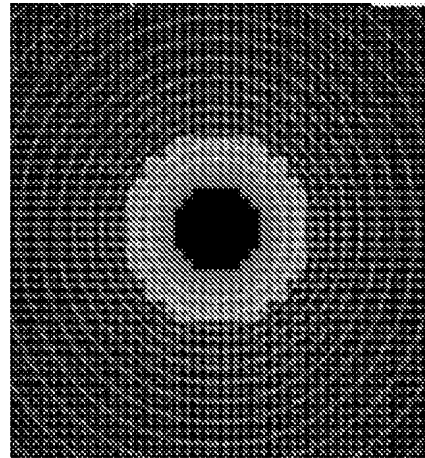
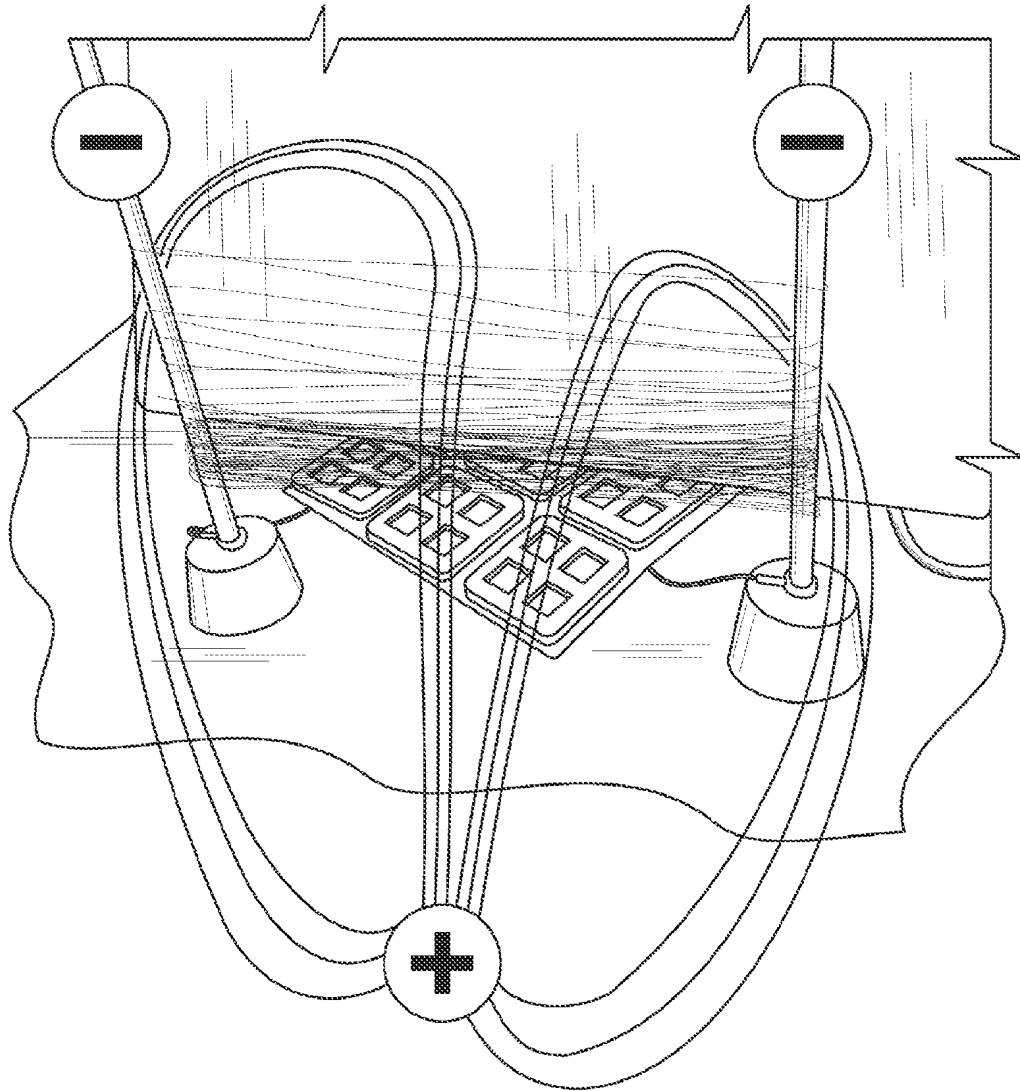
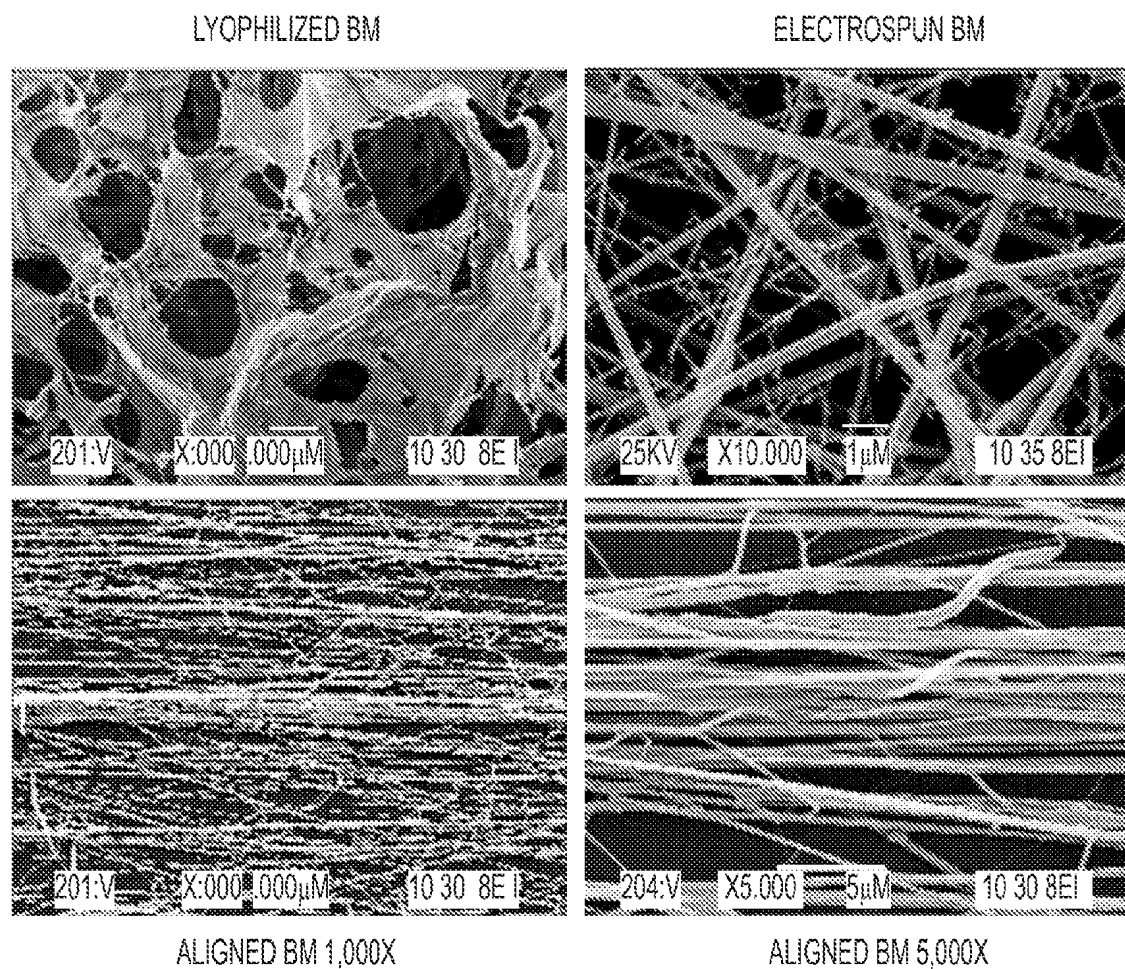


FIG. 1C

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**FIG. 1D**

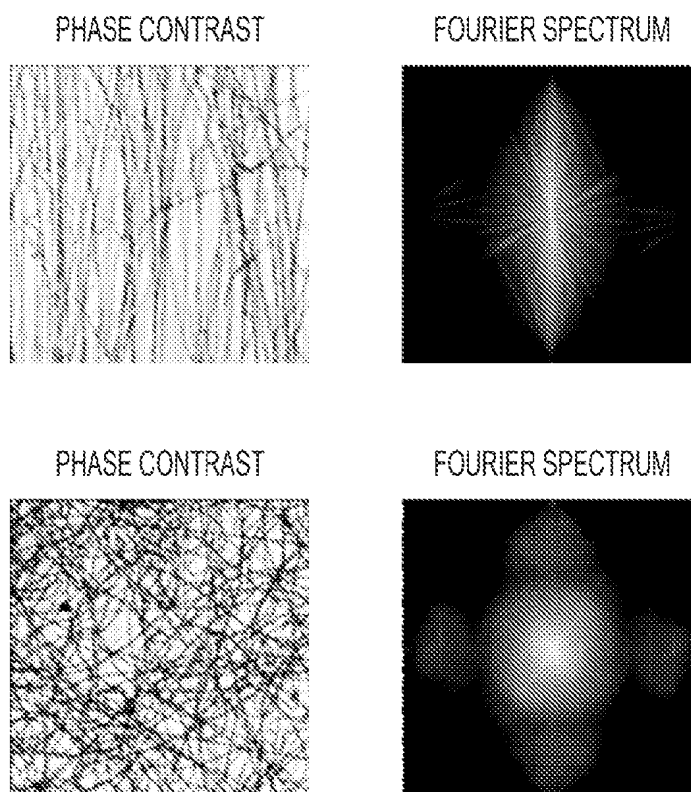
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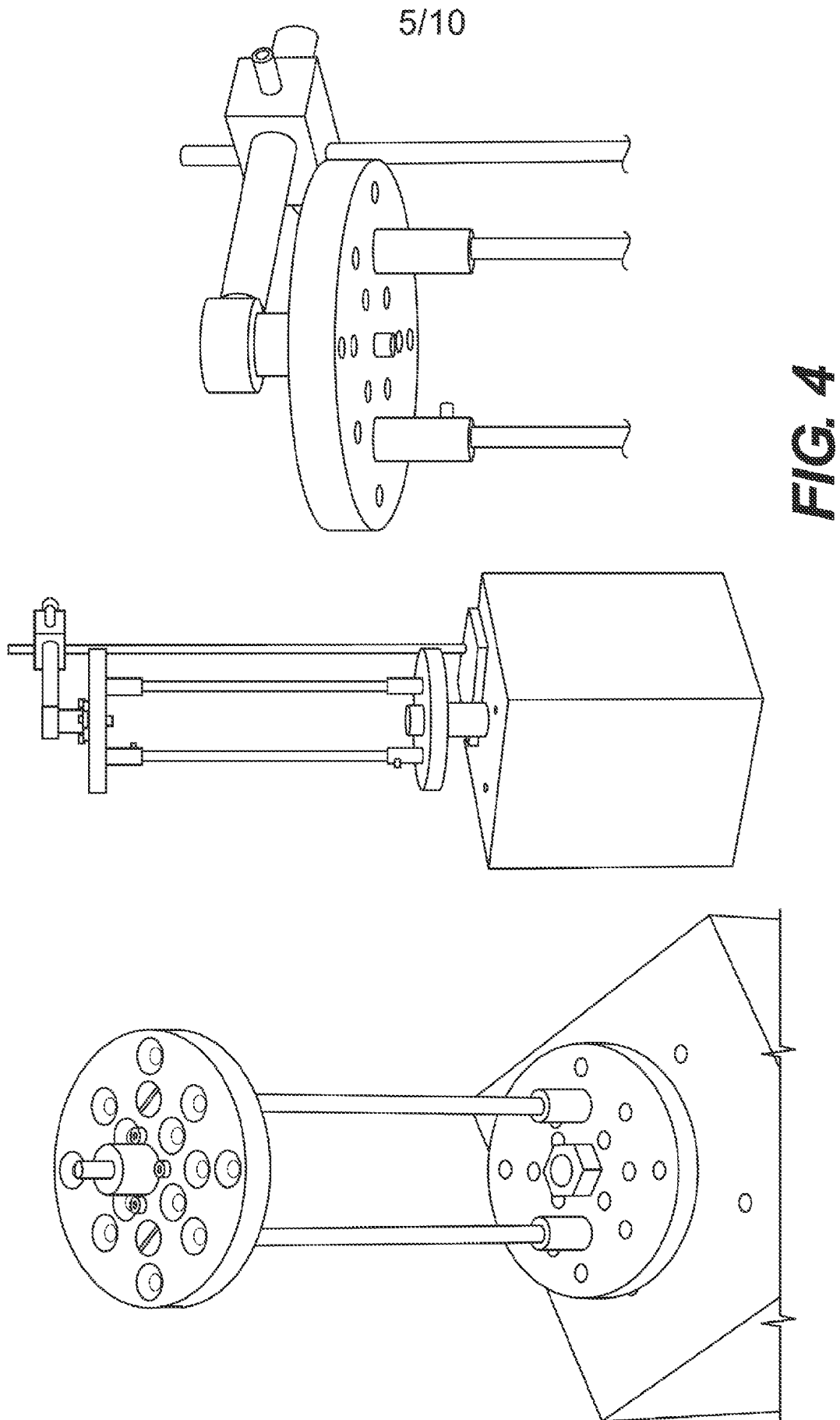


AVG DIAMETER (N=35, MEAN $\pm$ SD)	FIBER SIZE RANGE	AVG FIBER ANGLE (N=35, MEAN $\pm$ SD)	FIBER ANGLE RANGE
311 $\pm$ 71 NM	152-458 NM	92.340 $\pm$ 15.102 DEG	40-131 DEG

FIG. 2

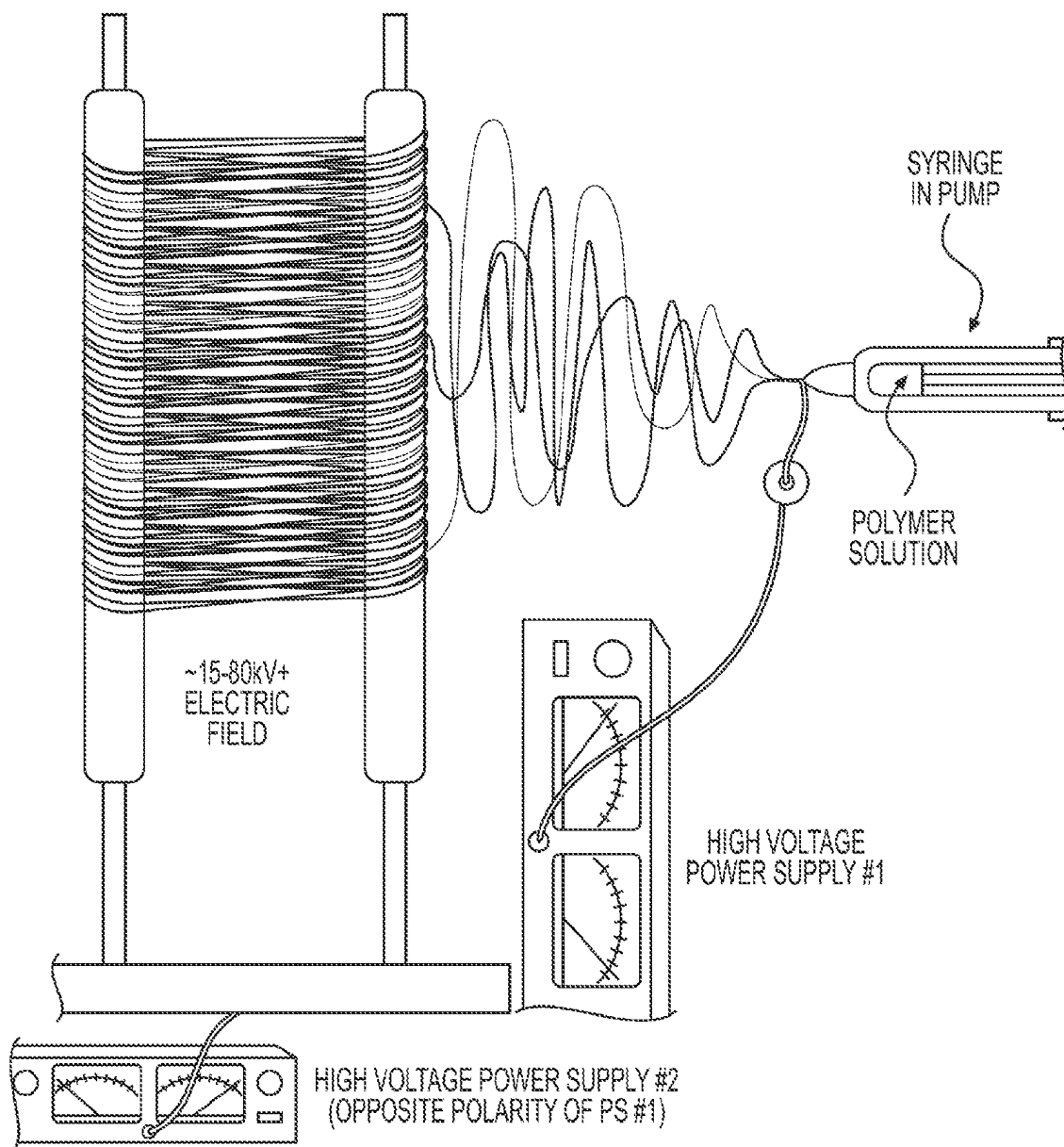
4/10

**FIG. 3**



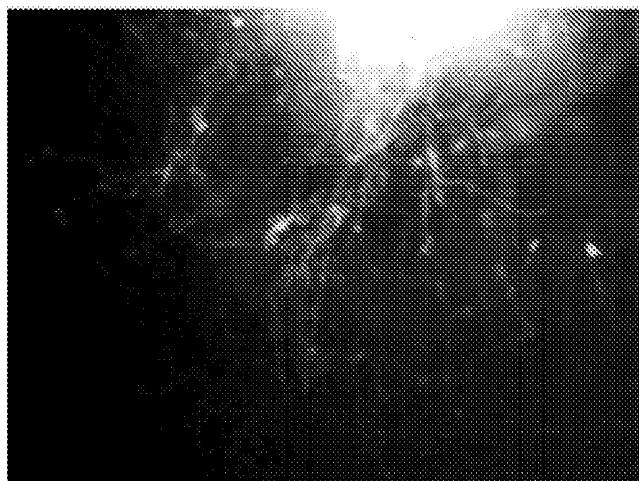
6/10

GROUNDING ROTATING TARGET COLLECTING
ALIGNED NANOFIBERS BETWEEN PARALLEL
CHARGED RODS

**FIG. 5**

7/10

DAY 2



DAY 4

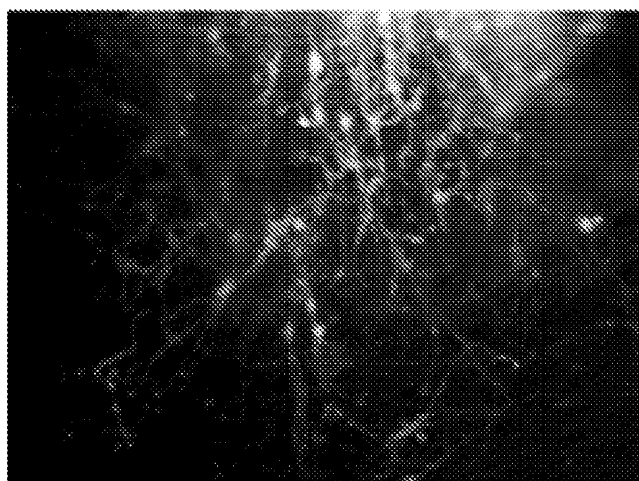


FIG. 6

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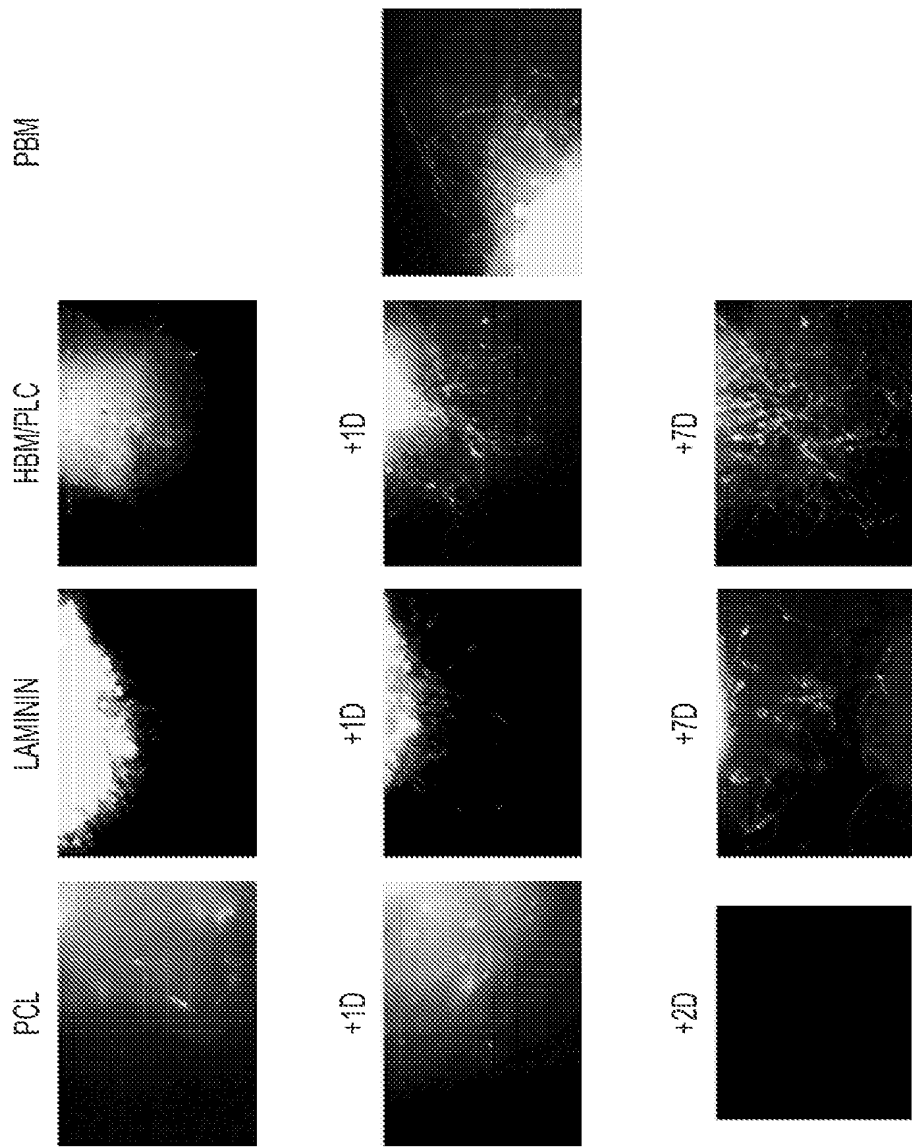


FIG. 7

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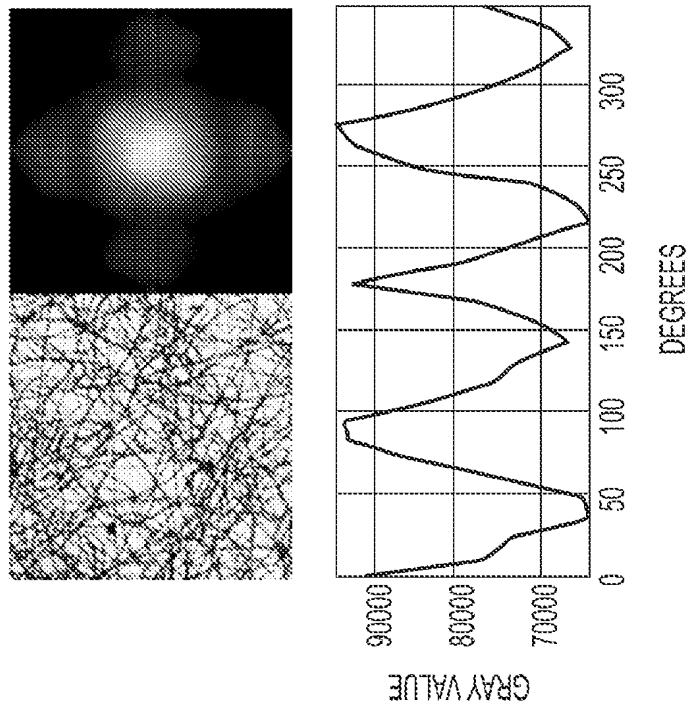


FIG. 8A

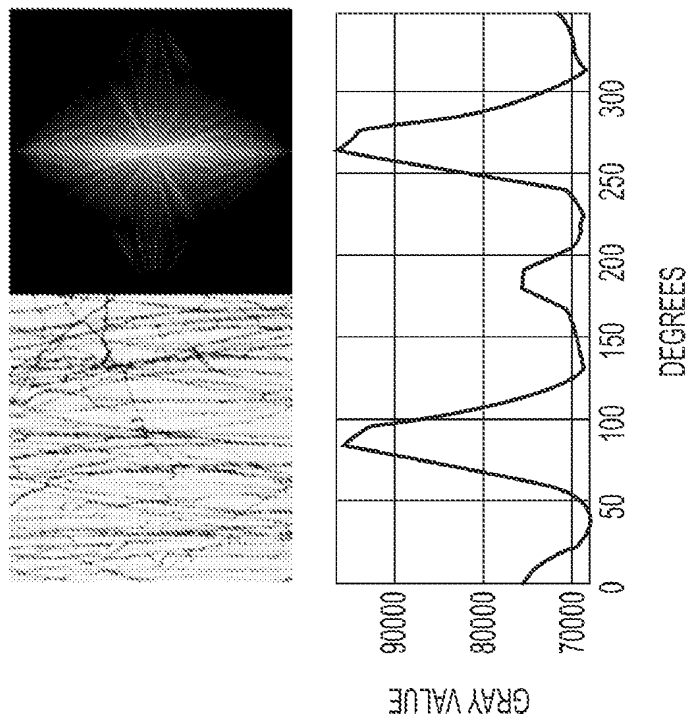


FIG. 8A

10/10

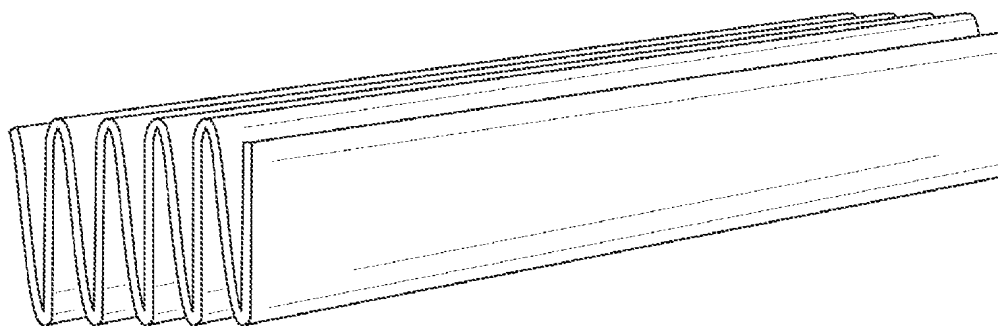


FIG. 9A

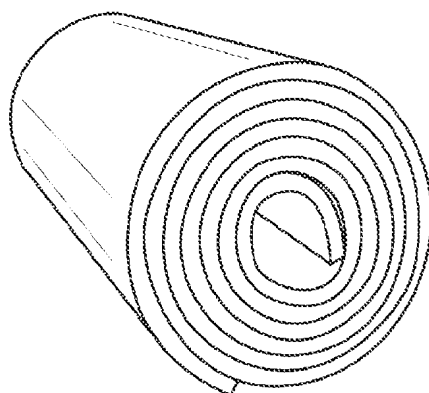


FIG. 9B

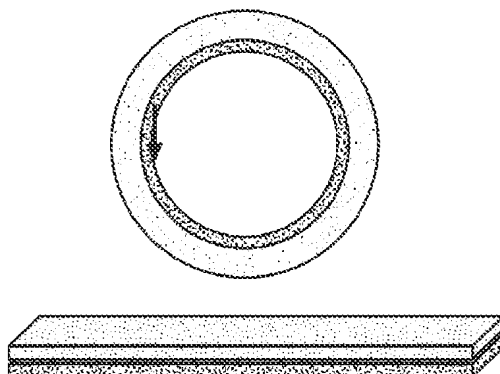


FIG. 9C

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U.S. APPLICATION NUMBER NO.	FIRST NAMED INVENTOR	ATTY. DOCKET NO.
14/776,106	Michael FRANCIS	067949-5058

9629

MORGAN LEWIS & BOCKIUS LLP (WA)
1111 PENNSYLVANIA AVENUE NW
WASHINGTON, DC 20004

INTERNATIONAL APPLICATION NO.

PCT/US14/25636

I.A. FILING DATE	PRIORITY DATE
03/13/2014	03/14/2013

CONFIRMATION NO. 1091
371 FORMALITIES LETTER



Date Mailed: 10/23/2015

Notification of Insufficiency Under 37 CFR 1.492 and/or 1.495 (DO/EO/US)

The following items have been submitted by the applicant or the International Bureau to the United States Patent and Trademark Office as a Designated / Elected Office (37 CFR 1.495).

- Indication of Small Entity Status
- Priority Document
- Copy of the International Application filed on 09/14/2015
- Copy of the International Search Report filed on 09/14/2015
- Information Disclosure Statements filed on 09/14/2015
- U.S. Basic National Fees filed on 09/14/2015
- Assignee Statement for PGPUB filed on 09/14/2015
- Priority Documents filed on 09/14/2015
- Authorization to Permit Access filed on 09/14/2015
- Application Data Sheet (37 CFR 1.76) filed on 09/14/2015

The following items **MUST** be furnished within the period set forth below:

- Additional claim fees of **\$600** as a small entity, including any required multiple dependent claim fee, are required. Applicant must submit the additional claim fees or cancel the additional claims for which fees are due.

SUMMARY OF FEES DUE:

Total additional fees required for this application is **\$600** for a Small Entity:

Applicant is notified that the above-identified application contains the deficiencies noted below. No period for reply is set forth in this notice for correction of these deficiencies. However, if a deficiency relates to the inventor's oath or declaration, the applicant must file an oath or declaration in compliance with 37 CFR 1.63, or a substitute statement in compliance with 37 CFR 1.64, executed by or with respect to each actual inventor no later than the expiration of the time period set in the "Notice of Allowability" to avoid abandonment. See 37 CFR 1.495(c).

- Properly executed inventor's oath or declaration for the following inventor(s) has not been submitted: **Michael FRANCIS** and **Roy OGLE**

Total additional claim fee(s) for this application is **\$600**

- **\$600** for **15** total claims over 20.

ALL OF THE ITEMS SET FORTH ABOVE MUST BE SUBMITTED WITHIN TWO (2) MONTHS FROM THE DATE OF THIS NOTICE. FAILURE TO PROPERLY RESPOND WILL RESULT IN ABANDONMENT.

page 1 of 2

The time period set above may be extended by filing a petition and fee for extension of time under the provisions of 37 CFR 1.136(a).

Applicant is reminded that any communications to the United States Patent and Trademark Office must be mailed to the address given in the heading and include the U.S. application no. shown above (37 CFR 1.5)

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web.

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VALERIE A JACKSON

Telephone: (703) 756-1443



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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
14/776,106	09/14/2015		630	067949-5058	35	1

CONFIRMATION NO. 1091

FILING RECEIPT

9629

MORGAN LEWIS & BOCKIUS LLP (WA)
1111 PENNSYLVANIA AVENUE NW
WASHINGTON, DC 20004



0000000078213340

Date Mailed: 10/23/2015

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

Assignment For Published Patent Application

LIFENET HEALTH, Virginia Beach, VA

Power of Attorney: None

Domestic Priority data as claimed by applicant

This application is a 371 of PCT/US14/25636 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 - A proper **Authorization to Permit Access to Application by Participating Offices** (PTO/SB/39 or its equivalent) has been received by the USPTO.

If Required, Foreign Filing License Granted: 10/21/2015

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 14/776,106**

Projected Publication Date: 01/28/2016

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

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MULTIPLE DEPENDENT CLAIM FEE CALCULATION SHEET Substitute for Form PTO-1360 (For use with Form PTO/SB/06)							Application Number 14776106		Filing Date				
							Applicant(s) Michael FRANCIS						
							* May be used for additional claims or amendments						
CLAIMS	AS FILED		AFTER FIRST AMENDMENT		AFTER SECOND AMENDMENT			*		*		*	
	Indep	Depend	Indep	Depend	Indep	Depend		Indep	Depend	Indep	Depend	Indep	Depend
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3		1											
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875						Application or Docket Number 14/776,106			
APPLICATION AS FILED - PART I									
(Column 1)		(Column 2)		SMALL ENTITY		OR OTHER THAN SMALL ENTITY			
FOR	NUMBER FILED	NUMBER EXTRA	RATE(\$)	FEE(\$)		RATE(\$)	FEE(\$)		
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	140		N/A			
SEARCH FEE (37 CFR 1.16(k), (l), or (m))	N/A	N/A	N/A	60		N/A			
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	360		N/A			
TOTAL CLAIMS (37 CFR 1.16(j))	35	minus 20 = *	15	x 40 =	600				
INDEPENDENT CLAIMS (37 CFR 1.16(h))	1	minus 3 = *		x 210 =	0.00				
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					
			TOTAL	1160		TOTAL			
* If the difference in column 1 is less than zero, enter "0" in column 2.									
APPLICATION AS AMENDED - PART II									
(Column 1)		(Column 2)		(Column 3)		SMALL ENTITY		OR OTHER THAN SMALL ENTITY	
AMENDMENT A	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE(\$)	ADDITIONAL FEE(\$)		RATE(\$)	ADDITIONAL FEE(\$)	
	Total (37 CFR 1.16(i))	*	Minus **	=	x =		x =		
	Independent (37 CFR 1.16(h))	*	Minus ***	=	x =		x =		
	Application Size Fee (37 CFR 1.16(s))								
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))								
			TOTAL ADD'L FEE			TOTAL ADD'L FEE			
(Column 1)		(Column 2)		(Column 3)		SMALL ENTITY		OR OTHER THAN SMALL ENTITY	
AMENDMENT B	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE(\$)	ADDITIONAL FEE(\$)		RATE(\$)	ADDITIONAL FEE(\$)	
	Total (37 CFR 1.16(i))	*	Minus **	=	x =		x =		
	Independent (37 CFR 1.16(h))	*	Minus ***	=	x =		x =		
	Application Size Fee (37 CFR 1.16(s))								
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))								
			TOTAL ADD'L FEE			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
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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
14/776,106	09/14/2015	Michael FRANCIS	067949-5058

CONFIRMATION NO. 1091

PUBLICATION NOTICE



OC000000080157421

9629
MORGAN LEWIS & BOCKIUS LLP (WA)
1111 PENNSYLVANIA AVENUE NW
WASHINGTON, DC 20004

Title: Aligned Fiber and Method of Use Thereof

Publication No. US-2016-0022865-A1

Publication Date: 01/28/2016

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 Office of Public Records. The Office of Public Records can be reached by telephone at (703) 308-9726 or (800) 972-6382, by facsimile at (703) 305-8759, by mail addressed to the United States Patent and Trademark Office, Office of Public Records, 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 <http://pair.uspto.gov/>. 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 Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No: 14/776,106
Applicants: Michael Francis et al.
Filed: September 14, 2015
Title: ALIGNED FIBER AND METHOD OF USE THEREOF
T.C./A.U.: To Be Assigned
Examiner: To Be Assigned
Confirmation No.: 1091
Docket No.: LNH-152US

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 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. (Original) A scaffold comprising one or more electrospun fibers, wherein a fast Fourier transform (FFT) analysis result of the fibers have adjacent major peaks with about 180° apart from each other.

2.-4. (Cancelled)

5. (Currently Amended) The scaffold according to ~~any of claims 1-4~~claim 1, wherein the fibers comprise type I collagen in its native triple helical structure.

6. (Currently Amended) The scaffold according to ~~any of claims 1-5~~claim 1, wherein the fiber has less than about 8 wt% residual calcium.

7.-10. (Cancelled)

11. (Currently Amended) The scaffold according to ~~any of claims 1-10~~claim 1, wherein the scaffold has an average porosity from about 60% to about 90%.

12. (Currently Amended) The scaffold according to ~~any of claims 1-11~~claim 1, wherein the 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, an accessory polymer, or the mixture thereof.

13.-16. (Cancelled)

17. (Currently Amended) The scaffold according to ~~claim 16~~claim 12, wherein the accessory polymer is 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/surfactants, xanthan, acetoglycerides, waxes, surfactants, dextran, emulsan, gelatin, polyphenols, levan, lignin, curd, inulin, tannin, polygalactosamine,

humic acid, shellac, pullulan, poly-gamma-glutamic acid, elsinan, natural rubber, yeast glucans, synthetic polymers from natural fats and oils.

18. (Cancelled)

19. (Currently Amended) The scaffold according to ~~any of claims 1-18~~claim 1, wherein the scaffold comprises a bioactive factor.

20. (Original) The scaffold according to claim 19, wherein the bioactive factor is selected from the group consisting of 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), and an interleukin.

21. (Currently Amended) The scaffold according to ~~any of claims 1-20~~claim 1, wherein the scaffold comprises an extracellular matrix component.

22. (Original) The scaffold according to claim 21, wherein the extracellular matrix component is selected from the group consisting of collagen, glycosaminoglycans, osteocalcin, osteonectin, bone sialo protein, osteopontin, fibronectin, laminin, and mixtures thereof.

23.-26. (Cancelled)

27. (Currently Amended) A method of cell culture comprising culturing cells on the scaffold according to ~~any of claims 1-24~~claim 1 in condition suitable for the cell culture.

28. (Original) 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 dorsal root ganglia.

29.-30. (Cancelled)

31. (Currently Amended) A method of promoting differentiation of stem cells into osteoblasts, chondrocytes, ligament or tendon, the method comprising culturing the cells on the scaffold according to ~~any of claims 1-24~~claim 1 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. (Original) 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.-35. (Cancelled)

Remarks/Arguments:

Claims 1-35 were pending. Claims 2-4, 7-10, 13-16, 18, 23-26, 29, 30, 34, and 35 are canceled. Claims 5, 6, 11, 12, 17, 19, 21, 27 and 31 are amended to omit multiple dependencies and correct dependencies required by the claim cancellations. No new matter has been added to the application. Examination and allowance of the claims as amended 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: April 25, 2016

P.O. Box 980
Valley Forge, PA 19482
(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.

3115179

Electronic Patent Application Fee Transmittal

Application Number:	14776106			
Filing Date:	14-Sep-2015			
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:	067949-5058			
Filed as Small Entity				
Filing Fees for U.S. National Stage under 35 USC 371				
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 \$0 paid	2254	1	1100	1100
Miscellaneous:				
Total in USD (\$)				1100

Electronic Acknowledgement Receipt

EFS ID:	25586956
Application Number:	14776106
International Application Number:	
Confirmation Number:	1091
Title of Invention:	Aligned Fiber and Method of Use Thereof
First Named Inventor/Applicant Name:	Michael FRANCIS
Customer Number:	9629
Filer:	Glenn E. Murphy/Nicole May
Filer Authorized By:	Glenn E. Murphy
Attorney Docket Number:	067949-5058
Receipt Date:	25-APR-2016
Filing Date:	14-SEP-2015
Time Stamp:	15:46:14
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	Credit Card
Payment was successfully received in RAM	\$ 1100
RAM confirmation Number	2260
Deposit Account	180350
Authorized User	MURPHY, GLENN E.J.

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

Charge any Additional Fees required under 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	Preliminary Amendment	PreliminaryAmendment.pdf	162951	no	5
			c3e4a3d216eaa825c2077b61a19ab3a5393f3ce0		

Warnings:

Information:

2	Fee Worksheet (SB06)	fee-info.pdf	30506	no	2
			95c135fa85c5d3052f07b28ce28dc769a8966902		

Warnings:

Information:

Total Files Size (in bytes):	193457
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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.

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.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 14/776,106	Filing Date 09/14/2015	☐ To be Mailed
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ENTITY: ☐ LARGE ☒ SMALL ☐ MICRO**APPLICATION AS FILED – PART I**

(Column 1)

(Column 2)

FOR	NUMBER FILED	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 \$ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X \$ =	
☐ 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	04/25/2016	CLAIMS REMAINING AFTER AMENDMENT	Minus	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE (\$)	ADDITIONAL FEE (\$)
	Total (37 CFR 1.16(i))	* 15	Minus	** 20	= 0	X \$40 =	0
	Independent (37 CFR 1.16(h))	* 1	Minus	***3	= 0	X \$210 =	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	Minus	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE (\$)	ADDITIONAL FEE (\$)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X \$ =
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X \$ =
	☐ 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.

** 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 number found in the appropriate box in column 1.

LIE
/CORALIA BETANCOURT/

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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U.S. APPLICATION NUMBER NO.	FIRST NAMED INVENTOR	ATTY. DOCKET NO.
14/776,106	Michael FRANCIS	067949-5058

9629

MORGAN LEWIS & BOCKIUS LLP (WA)
1111 PENNSYLVANIA AVENUE NW
WASHINGTON, DC 20004

INTERNATIONAL APPLICATION NO.

PCT/US14/25636

I.A. FILING DATE	PRIORITY DATE
03/13/2014	03/14/2013

CONFIRMATION NO. 1091
371 ACCEPTANCE LETTER



Date Mailed: 05/13/2016

NOTICE OF ACCEPTANCE OF APPLICATION UNDER 35 U.S.C 371 AND 37 CFR 1.495

The applicant is hereby advised that the United States Patent and Trademark Office, in its capacity as a Designated / Elected Office (37 CFR 1.495), has ACCEPTED the above identified international application for national patentability examination in the United States Patent and Trademark Office.

The United States Application Number assigned to the application is shown above. A Filing Receipt will be issued for the present application in due course. **THE DATE APPEARING ON THE FILING RECEIPT AS THE "FILING DATE or 371(c) DATE" IS THE DATE ON WHICH THE LAST OF THE 35 U.S.C. 371 (c)(1) and (c)(2) REQUIREMENTS HAS BEEN RECEIVED IN THE OFFICE. THIS DATE IS SHOWN BELOW.** The filing date of the above identified application is the international filing date of the international application (Article 11(3) and 35 U.S.C. 363)

09/14/2015

DATE OF RECEIPT OF 35 U.S.C.
371(c)(1) and (c)(2) REQUIREMENTS

The following items have been received:

- Indication of Small Entity Status
- Copy of the International Application filed on 09/14/2015
- Copy of the International Search Report filed on 09/14/2015
- Information Disclosure Statements filed on 09/14/2015
- U.S. Basic National Fees filed on 09/14/2015
- Assignee Statement for PGPUB filed on 09/14/2015
- Priority Documents filed on 09/14/2015
- Authorization to Permit Access filed on 09/14/2015
- Application Data Sheet (37 CFR 1.76) filed on 09/14/2015

Applicant is notified that the above-identified application contains the deficiencies noted below. No period for reply is set forth in this notice for correction of these deficiencies. However, if a deficiency relates to the inventor's oath or declaration, the applicant must file an oath or declaration in compliance with 37 CFR 1.63, or a substitute statement in compliance with 37 CFR 1.64, executed by or with respect to each actual inventor no later than the expiration of the time period set in the "Notice of Allowability" to avoid abandonment. See 37 CFR 1.495(c).

- Properly executed inventor's oath or declaration for the following inventor(s) has not been submitted: **Michael FRANCIS** and **Roy OGLE**

Applicant is reminded that any communications to the United States Patent and Trademark Office must be mailed to the address given in the heading and include the U.S. application no. shown above (37 CFR 1.5)

VALERIE A JACKSON

Telephone: (703) 756-1443

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875						Application or Docket Number 14/776,106			
APPLICATION AS FILED - PART I									
(Column 1)		(Column 2)		SMALL ENTITY		OR OTHER THAN SMALL ENTITY			
FOR	NUMBER FILED	NUMBER EXTRA	RATE(\$)	FEE(\$)		RATE(\$)	FEE(\$)		
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	140		N/A			
SEARCH FEE (37 CFR 1.16(k), (l), or (m))	N/A	N/A	N/A	60		N/A			
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	360		N/A			
TOTAL CLAIMS (37 CFR 1.16(j))	15	minus 20 = *	x 40 =	0.00	OR				
INDEPENDENT CLAIMS (37 CFR 1.16(h))	1	minus 3 = *	x 210 =	0.00					
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					
			TOTAL	560		TOTAL			
* If the difference in column 1 is less than zero, enter "0" in column 2.									
APPLICATION AS AMENDED - PART II									
(Column 1)		(Column 2)		(Column 3)		SMALL ENTITY		OR OTHER THAN SMALL ENTITY	
AMENDMENT A	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE(\$)	ADDITIONAL FEE(\$)		RATE(\$)	ADDITIONAL FEE(\$)	
	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))					OR			
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))					OR			
			TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE			
(Column 1)		(Column 2)		(Column 3)		SMALL ENTITY		OR OTHER THAN SMALL ENTITY	
AMENDMENT B	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE(\$)	ADDITIONAL FEE(\$)		RATE(\$)	ADDITIONAL FEE(\$)	
	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))					OR			
	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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Alexandria, Virginia 22313-1450
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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
14/776,106	09/14/2015	1655	630	067949-5058	15	1

CONFIRMATION NO. 1091
UPDATED FILING RECEIPT

9629
MORGAN LEWIS & BOCKIUS LLP (WA)
1111 PENNSYLVANIA AVENUE NW
WASHINGTON, DC 20004



Date Mailed: 05/13/2016

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

Assignment For Published Patent Application

LIFENET HEALTH, Virginia Beach, VA

Power of Attorney: None

Domestic Priority data as claimed by applicant

This application is a 371 of PCT/US14/25636 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: No

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: 10/21/2015

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 14/776,106**

Projected Publication Date: Not Applicable

Non-Publication Request: No

Early Publication Request: No

**** SMALL ENTITY ****

Title

Aligned Fiber and Method of Use Thereof

Preliminary Class

424

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).

LICENSE FOR FOREIGN FILING UNDER
Title 35, United States Code, Section 184
Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign Assets Control, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

SelectUSA

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 U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop technology, manufacture products, deliver services, and grow your business, visit <http://www.SelectUSA.gov> or call +1-202-482-6800.

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.

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	757 348-2931
Title	ESR		

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.

STATEMENT UNDER 37 CFR 3.73(c)

Applicant/Patent Owner: Michael Francis et al.

Application No./Patent No.: 14/776,106 Filed/Issue Date: September 14, 2015

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 039966, Frame 0432, 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.

[Page 1 of 2]

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.

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

Glenn E. J. Murphy

Printed or Typed Name

October 11, 2016

Date

33,539

Title or Registration Number

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:	14776106			
Filing Date:	14-Sep-2015			
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:	067949-5058			
Filed as Small Entity				
Filing Fees for U.S. National Stage under 35 USC 371				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
OATH/DECL > 30 MOS FROM 371 COMMENCEMENT	2617	1	70	70
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				70

Electronic Acknowledgement Receipt

EFS ID:	27173783
Application Number:	14776106
International Application Number:	
Confirmation Number:	1091
Title of Invention:	Aligned Fiber and Method of Use Thereof
First Named Inventor/Applicant Name:	Michael FRANCIS
Customer Number:	9629
Filer:	Glenn E. Murphy/Nicole May
Filer Authorized By:	Glenn E. Murphy
Attorney Docket Number:	067949-5058
Receipt Date:	11-OCT-2016
Filing Date:	14-SEP-2015
Time Stamp:	11:54:22
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	\$ 70
RAM confirmation Number	101116INTEFSW11551100
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	Oath or Declaration filed	DEC-Francis.pdf	177189	no	2
			e14b1d897721a827b80199f4cab7ba0a29dae3f7		
Warnings:					
Information:					
2	Oath or Declaration filed	DEC-Ogle.pdf	111507	no	2
			9d31b824574775325216a0ca7cedb97464b9cf0f		
Warnings:					
Information:					
3	Power of Attorney	POA.pdf	194664	no	2
			42932756fb8cb3b46ec05b77df2d3ce60129f843		
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:					
4	Assignee showing of ownership per 37 CFR 3.73	StatementUnder373c.pdf	151745	no	3
			e0a818fd505b471a53440fdffc869084934227b		
Warnings:					
Information:					
5	Fee Worksheet (SB06)	fee-info.pdf	30511	no	2
			6df8350b97c9e2d5f56c5f7fbd8ae91755f116ab		
Warnings:					
Information:					
Total Files Size (in bytes):			665616		

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.

COMBINED DECLARATION/ASSIGNMENT - Patent Application		Docket Number: <u>LNH-152US</u>
Whereas, the Assignor, comprising the following named inventors:		
ASSIGNOR(s)/ INVENTOR(s)	1. Michael Francis	2. Roy Ogle
	3.	4.
	5.	6.
made an invention entitled: <u>ALIGNED FIBER AND METHOD OF USE THEREOF</u>		
for which an application for Letters Patent of the United States (the Application): (Select one of the following):		
☐ is submitted concurrently herewith ☒ was filed in the U.S. Patent and Trademark Office on <u>09/14/2015</u> , as U.S. Patent Application Serial No.: <u>14/776,106</u>		
ASSIGNEE (Full Name and Address)	LifeNet Health 1864 Concert Drive Virginia Beach, VA 23453	
is desirous of acquiring the entire interest in and to said invention and the Letters Patent to be obtained therefor. Now, therefore, in consideration of the payment by Assignee to Assignor of a sum corresponding to One Dollar (\$1.00), and for other good and valuable consideration, the receipt of which is hereby acknowledged, Assignor, intending to be legally bound, hereby sells, assigns and transfers to Assignee, its successors and assigns, the full and exclusive right, title and interest in and to said invention, all applications for Letters Patent for said invention, including the Application, all divisions, continuations, and continuations-in-part thereof and non-provisional applications claiming priority thereto, all rights to claim priority based thereon, and all Letters Patent, including reissues, to be obtained therefor, including any and all foreign patent rights in this invention corresponding thereto and authorizes Assignee to file such applications for Letters Patent in the United States and any and all other countries. Assignor hereby warrants that no assignment, sale, agreement or encumbrance has been or will be made or entered into which would conflict with this Assignment. Assignor agrees it shall be legally bound, upon request of the Assignee or its successors or assigns or a legal representative thereof, to supply all information and evidence of which the Assignor has knowledge or possession, relating to the making and practice of said invention, to testify in any legal proceeding relating thereto, to execute all instruments proper to patent the invention in the United States of America and foreign countries in the name of the Assignee, and to execute all instruments proper to carry out the intent of this instrument. If the invention requires a biological deposit, Assignor also grants to Assignee such control over any deposit made by Assignor as may be necessary to the validity of the patent rights assigned herein. Assignor authorizes Assignee's attorney to insert the serial number and filing date of the aforesaid application for United States Letters Patent and/or the Attorney Docket or file designation for this application if not identified above. If the Assignor includes more than one individual, these obligations shall apply to these individuals both individually and collectively. In witness whereof, this Assignment is executed on the day indicated below.		

COMBINED DECLARATION/ASSIGNMENT -
Patent Application

Docket Number: LNH-152US

Declaration:

Note to Inventor: 37 C.F.R. § 1.63(c) states: "A person may not execute an oath or declaration for an application unless that person has reviewed and understands the contents of the application, including the claims, and is aware of the duty to disclose to the Office all information known to the person to be material to patentability as defined in § 1.56."

As a below named inventor, I declare that the Application was made or authorized to be made by me.

I believe I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

	Typed or Printed Name	Signature	Date
SIGNATURE	1. Michael Francis		<u>9.26.16</u>
		* Witness	
	2. Roy Ogle		
		* Witness	
	3.		
		* Witness	
	4.		
		* Witness	
	5.		
		* Witness	
	6.		
		* Witness	

Application for United States Letters Patent:

Serial No.: 14/776,106

Filed: 09/14/2015

Attorney Docket Number: LNH-152US

Total Number of Assignment Pages 2

* Witness signature recommended, mandatory for some countries

**COMBINED DECLARATION/ASSIGNMENT -
Patent Application**

Docket Number: LNH-152US

Whereas, the Assignor, comprising the following named inventors:

ASSIGNOR(s)/ INVENTOR(s)	1. Michael Francis	2. Roy Ogle
	3.	4.
	5.	6.

made an invention entitled: ALIGNED FIBER AND METHOD OF USE THEREOF

for which an application for Letters Patent of the United States (the Application):
(Select one of the following):

- ☐ is submitted concurrently herewith
☒ was filed in the U.S. Patent and Trademark Office on 09/14/2015,
as U.S. Patent Application Serial No. 14/776,106

ASSIGNEE
(Full Name and Address)

LifeNet Health
1864 Concert Drive
Virginia Beach, VA 23453

is desirous of acquiring the entire interest in and to said invention and the Letters Patent to be obtained therefor.

Now, therefore, in consideration of the payment by Assignee to Assignor of a sum corresponding to One Dollar (\$1.00), and for other good and valuable consideration, the receipt of which is hereby acknowledged, Assignor, intending to be legally bound, hereby sells, assigns and transfers to Assignee, its successors and assigns, the full and exclusive right, title and interest in and to said invention, all applications for Letters Patent for said invention, including the Application, all divisions, continuations, and continuations-in-part thereof and non-provisional applications claiming priority thereto, all rights to claim priority based thereon, and all Letters Patent, including reissues, to be obtained therefor, including any and all foreign patent rights in this invention corresponding thereto and authorizes Assignee to file such applications for Letters Patent in the United States and any and all other countries.

Assignor hereby warrants that no assignment, sale, agreement or encumbrance has been or will be made or entered into which would conflict with this Assignment.

Assignor agrees it shall be legally bound, upon request of the Assignee or its successors or assigns or a legal representative thereof, to supply all information and evidence of which the Assignor has knowledge or possession, relating to the making and practice of said invention, to testify in any legal proceeding relating thereto, to execute all instruments proper to patent the invention in the United States of America and foreign countries in the name of the Assignee, and to execute all instruments proper to carry out the intent of this instrument.

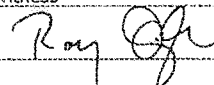
If the invention requires a biological deposit, Assignor also grants to Assignee such control over any deposit made by Assignor as may be necessary to the validity of the patent rights assigned herein.

Assignor authorizes Assignee's attorney to insert the serial number and filing date of the aforesaid application for United States Letters Patent and/or the Attorney Docket or file designation for this application if not identified above.

If the Assignor includes more than one individual, these obligations shall apply to these individuals both individually and collectively.

In witness whereof, this Assignment is executed on the day indicated below.

COMBINED DECLARATION/ASSIGNMENT - Patent Application	Docket Number: LNH-152US
Declaration: Note to Inventor: 37 C.F.R. § 1.63(c) states: "A person may not execute an oath or declaration for an application unless that person has reviewed and understands the contents of the application, including the claims, and is aware of the duty to disclose to the Office all information known to the person to be material to patentability as defined in § 1.56." As a below named inventor, I declare that the Application was made or authorized to be made by me. I believe I am the original inventor or an original joint inventor of a claimed invention in the application. I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.	

	Typed or Printed Name	Signature	Date
SIGNATURE	1. Michael Francis	_____	_____
		* Witness	_____
	2. Roy Ogle		10-5-16
		* Witness	_____
	3.	_____	_____
		* Witness	_____
	4.	_____	_____
		* Witness	_____
	5.	_____	_____
		* Witness	_____
	6	_____	_____
		* Witness	_____

Application for United States Letters Patent:	
Serial No.:	<u>14/776,106</u>
Filed:	<u>09/14/2015</u>
Attorney Docket Number:	<u>LNH-152US</u>
Total Number of Assignment Pages <u>2</u>	* Witness signature recommended, mandatory for some countries



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
14/776,106	09/14/2015	Michael FRANCIS	067949-5058

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

CONFIRMATION NO. 1091
POA ACCEPTANCE LETTER



Date Mailed: 10/18/2016

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 10/11/2016.

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.

/nhassani/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number		14776106	
	Filing Date		2015-09-14	
	First Named Inventor	Michael Francis		
	Art Unit	1653		
	Examiner Name	Douglas F. White		
	Attorney Docket Number	LNH-152US		

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	20080213389		2008-09-04	Lelkes	

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	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	14776106
Filing Date	2015-09-14
First Named Inventor	Michael Francis
Art Unit	1653
Examiner Name	Douglas F. White
Attorney Docket Number	LNH-152US

1	Supplementary European Search Report for European Application no. 14773677.1, dated October 25, 2016, 3 pages.	☐
2	Extended Search Report for European Application No. 14773677.1, dated October 7, 2016, 6 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	14776106
Filing Date	2015-09-14
First Named Inventor	Michael Francis
Art Unit	1653
Examiner Name	Douglas F. White
Attorney Docket Number	LNH-152US

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).

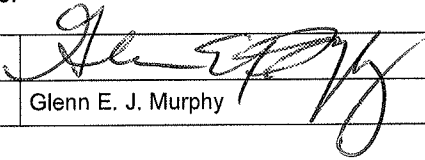
☐ See attached certification statement.

☐ 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)	2017-02-02
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 records.
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.

Electronic Acknowledgement Receipt

EFS ID:	28241373
Application Number:	14776106
International Application Number:	
Confirmation Number:	1091
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:	067949-5058
Receipt Date:	02-FEB-2017
Filing Date:	14-SEP-2015
Time Stamp:	13:23:38
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	no
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	IDS.pdf	45424	no	2
			6fd6c91ac58279ecfbc15a058983b9392d34986c		

Warnings:

Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	PTOSB08a.pdf	202275	no	4
			a46203388c74cc8853215b115fff9658a1b482ee		
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
3	Non Patent Literature	SupplementarySearchReport.pdf	379785	no	3
			eef5ac00014c0155b10b36f31ee91abcca47ab5e		
Warnings:					
Information:					
4	Non Patent Literature	ExtendedSearchReport.pdf	633854	no	6
			c819d213224c7279a7d072c4fd0d98564ed5a28f		
Warnings:					
Information:					
Total Files Size (in bytes):			1261338		
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. <u>New Applications Under 35 U.S.C. 111</u> 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. <u>National Stage of an International Application under 35 U.S.C. 371</u> 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. <u>New International Application Filed with the USPTO as a Receiving Office</u> 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: 14/776,106
Applicant: LifeNet Health
Filed: September 14, 2015
Title: ALIGNED FIBER AND METHOD OF USE THEREOF
TC/A.U.: 1653
Examiner: Douglas F. White
Confirmation No.: 1091

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 references listed on the PTO/SB/08a submitted herewith. A copy of the references 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,



Glenn E. J. Murphy, Reg. No. 33,539
Attorney for Applicants

GEM/nm

Enclosures: PTO/SB/08a
Supplementary European Search Report
Extended European Search Report

Dated: February 2, 2017

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.

3482062



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.
14/776,106	09/14/2015	Michael FRANCIS	067949-5058	1091

23122 7590 09/01/2017
RATNERPRESTIA
2200 Renaissance Blvd
Suite 350
King of Prussia, PA 19406

EXAMINER

WHITE, DOUGLAS F

ART UNIT	PAPER NUMBER
----------	--------------

1653

NOTIFICATION DATE	DELIVERY MODE
-------------------	---------------

09/01/2017

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. 14/776,106	Applicant(s) FRANCIS ET AL.	
	Examiner DOUGLAS F. WHITE	Art Unit 1653	AIA (First Inventor to File) 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 2 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) 1,5,6,11,12,17,19-22,27,28 and 31-33 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) _____ is/are rejected.
- 8) ☐ Claim(s) _____ is/are objected to.
- 9) ☒ Claim(s) 1, 5-6, 11-12, 17, 19-22, 27-28 and 31-33 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 _____ 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) | 3) ☐ Interview Summary (PTO-413)
Paper No(s)/Mail Date. _____ |
| 2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b)
Paper No(s)/Mail Date _____ | 4) ☐ Other: _____ |

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

DETAILED ACTION

Election/Restrictions

Restriction is required under 35 U.S.C. 121 and 372.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

In accordance with 37 CFR 1.499, applicant is required, in reply to this action, to elect a single invention to which the claims must be restricted.

Group I, claim(s) 1, 5-6, 11-12, 17, and 19-22, drawn to a product— a scaffold comprising one or more electrospun fibers.

Group II, claim(s) 27-28 and 31-33, drawn to a method – a method of culturing cells.

The groups of inventions listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

According to PCT Rule 13.2, unity of invention exists only when the shared or corresponding technical feature is a contribution over the prior art. The special technical feature shared by the groups of inventions is a scaffold comprising one or more electrospun fibers. However, such a scaffold comprising one or more electrospun fibers is taught (anticipated) by the prior art (see, e.g., Huang et al. cited on the corresponding PCT search report). Thus, the technical feature that would otherwise bind the claims together is not a special technical feature and, therefore, the claims lack unity – as further discussed below.

REQUIREMENT FOR UNITY OF INVENTION

As provided in 37 CFR 1.475(a), a national stage application shall relate to one invention only or to a group of inventions so linked as to form a single general inventive concept (“requirement of unity of invention”). Where a group of inventions is claimed in a national stage application, the requirement of unity of invention shall be fulfilled only when there is a technical relationship among those inventions involving one or more of the same or corresponding special technical features. The expression “special technical features” shall mean those technical features that define a contribution which each of the claimed inventions, considered as a whole, makes over the prior art.

The determination whether a group of inventions is so linked as to form a single general inventive concept shall be made without regard to whether the inventions are claimed in separate claims or as alternatives within a single claim. See 37 CFR 1.475(e).

WHEN CLAIMS ARE DIRECTED TO MULTIPLE CATEGORIES OF INVENTIONS

As provided in 37 CFR 1.475(b), a national stage application containing claims to different categories of invention will be considered to have unity of invention if the claims are drawn only to one of the following combinations of categories:

- (1) A product and a process specially adapted for the manufacture of said product; or
- (2) A product and process of use of said product; or
- (3) A product, a process specially adapted for the manufacture of the said product, and a use of the said product; or

(4) A process and an apparatus or means specifically designed for carrying out the said process; or

(5) A product, a process specially adapted for the manufacture of the said product, and an apparatus or means specifically designed for carrying out the said process.

Otherwise, unity of invention might not be present. See 37 CFR 1.475(c).

The examiner has required restriction between product or apparatus claims and process claims. Where applicant elects claims directed to the product/apparatus, and all product/apparatus claims are subsequently found allowable, withdrawn process claims that include all the limitations of the allowable product/apparatus claims should be considered for rejoinder. All claims directed to a nonelected process invention must include all the limitations of an allowable product/apparatus claim for that process invention to be rejoined.

In the event of rejoinder, the requirement for restriction between the product/apparatus claims and the rejoined process claims will be withdrawn, and the rejoined process claims will be fully examined for patentability in accordance with 37 CFR 1.104. Thus, to be allowable, the rejoined claims must meet all criteria for patentability including the requirements of 35 U.S.C. 101, 102, 103 and 112. Until all claims to the elected product/apparatus are found allowable, an otherwise proper restriction requirement between product/apparatus claims and process claims may be maintained. Withdrawn process claims that are not commensurate in scope with an allowable product/apparatus claim will not be rejoined. See MPEP § 821.04. Additionally, in order for rejoinder to occur, applicant is advised that the process claims should be amended during prosecution to require the limitations of the product/apparatus claims. **Failure to do so may result in no rejoinder.** Further, note that the prohibition against double patenting rejections of 35 U.S.C. 121 does not apply where the restriction requirement is withdrawn by the examiner before the patent issues. See MPEP § 804.01.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to DOUGLAS F. WHITE whose telephone number is (571)270-5561. The examiner can normally be reached on Monday-Friday; 7-3:30 EST.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at <http://www.uspto.gov/interviewpractice>.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Sharmila Landau can be reached on (571) 272-0614. 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.

/DOUGLAS F. WHITE/
Examiner, Art Unit 1653

/Christopher R Tate/
Primary Examiner, Art Unit 1655

Appln. No.: 14/776,106
Response Dated September 11, 2017
In Reply to Office Action of September 1, 2017

LNH-152US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No: 14/776,106
Applicant: LifeNet Health
Filed: September 14, 2015
Title: ALIGNED FIBER AND METHOD OF USE THEREOF
TC/A.U.: 1653
Examiner: Douglas F. White
Confirmation No.: 1091
Docket No.: LNH-152US

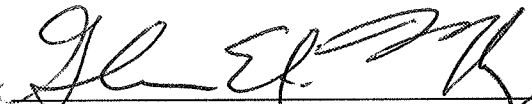
RESPONSE TO RESTRICTION REQUIREMENT

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

Dear Sirs:

In response to the restriction requirement in the office communication of September 1, 2017, Applicant elects Group I (claims 1, 5, 6, 11, 12, 17, and 19-22), without traverse.

Respectfully submitted,



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

WJM/GEJM/nm

Dated: September 11, 2017

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.

3751814

Electronic Acknowledgement Receipt

EFS ID:	30318947
Application Number:	14776106
International Application Number:	
Confirmation Number:	1091
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:	067949-5058
Receipt Date:	11-SEP-2017
Filing Date:	14-SEP-2015
Time Stamp:	11:28:46
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	no
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Response to Election / Restriction Filed	ResponseToRestrictionRequirement.pdf	25516 be8674b5e1785c4e2a36d9fc8eef64d7ded54825	no	1

Warnings:

Information:	
Total Files Size (in bytes):	25516
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. <u>New Applications Under 35 U.S.C. 111</u> 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. <u>National Stage of an International Application under 35 U.S.C. 371</u> 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. <u>New International Application Filed with the USPTO as a Receiving Office</u> 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.	

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number		14776106
	Filing Date		2015-09-14
	First Named Inventor	Michael Francis	
	Art Unit	1653	
	Examiner Name	Douglas F. White	
	Attorney Docket Number	LNH-152US	

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 ²ⁱ	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, page(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		14776106
	Filing Date		2015-09-14
	First Named Inventor	Michael Francis	
	Art Unit	1653	
	Examiner Name	Douglas F. White	
	Attorney Docket Number	LNH-152US	

1	ZEUGOLIS, D.I., et al., "Electro-spinning of pure collagen nano-fibres - Just an expensive way to make gelatin?", March 3, 2008, pp. 2293-2305, Vol. 29(15), Biomaterials, Elsevier Science Publishers	☐
2	European Communication for European Application No. 14 773 677.1, dated November 6, 2017, 8 pages	☐

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

EXAMINER SIGNATURE

Examiner Signature		Date Considered	
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*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		14776106
	Filing Date		2015-09-14
	First Named Inventor	Michael Francis	
	Art Unit	1653	
	Examiner Name	Douglas F. White	
	Attorney Docket Number	LNH-152US	

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).

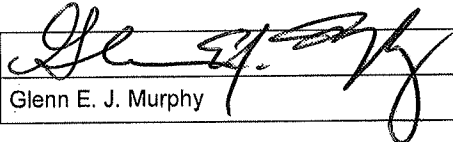
OR

☐ 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)	2017-12-06
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 records.
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.

Electronic Acknowledgement Receipt

EFS ID:	31142468
Application Number:	14776106
International Application Number:	
Confirmation Number:	1091
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:	067949-5058
Receipt Date:	06-DEC-2017
Filing Date:	14-SEP-2015
Time Stamp:	14:15:07
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	no
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	IDS.pdf	43214	no	2
			56c06e2e99fbd51fec06a51a502f6cb4d781f0ac		

Warnings:

Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	PTOSB08a.pdf	213768	no	4
			5ccb056c6db27e9326ea49d6cb0d314bd795437		
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
3	Non Patent Literature	Zeugolis.pdf	1666352	no	14
			bc84e8dbee1fa35c5485b9f5e4b1fd4869ebc295		
Warnings:					
Information:					
4	Non Patent Literature	EPComm.pdf	329482	no	8
			cbc84b76f73303abaf974f142305479de02d4e		
Warnings:					
Information:					
			Total Files Size (in bytes):	2252816	
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. <u>New Applications Under 35 U.S.C. 111</u> 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. <u>National Stage of an International Application under 35 U.S.C. 371</u> 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. <u>New International Application Filed with the USPTO as a Receiving Office</u> 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: 14/776,106
Applicant: LifeNet Health
Filed: September 14, 2015
Title: ALIGNED FIBER AND METHOD OF USE THEREOF
TC/A.U.: 1653
Examiner: Douglas F. White
Confirmation No.: 1091

INFORMATION DISCLOSURE STATEMENT TRANSMITTAL

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

Commissioner:

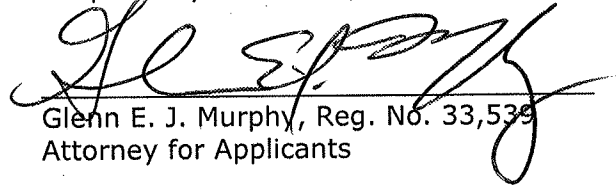
Pursuant to 37 C.F.R. §§ 1.56, 1.97 and 1.98, Applicants ask the Examiner in charge of this application to consider and make of record the references listed on the enclosed PTO/SB/08a. Copies of references listed on the PTO/SB/08a (other than U.S. patents) are enclosed.

Although the Examiner may find the information submitted here to be material to the examination of this application, this filing is not an admission that the information is prior art as to the claimed invention.

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

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

Respectfully submitted,



Glenn E. J. Murphy, Reg. No. 33,539
Attorney for Applicants

GEM/nm

Enclosures: PTO/SB/08a
(1) Reference
European Communication

Dated: December 6, 2017

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.

3839028



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/776,106	09/14/2015	Michael FRANCIS	067949-5058	1091

23122 7590 12/15/2017
RATNERPRESTIA
2200 Renaissance Blvd
Suite 350
King of Prussia, PA 19406

EXAMINER

WHITE, DOUGLAS F

ART UNIT	PAPER NUMBER
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1653

NOTIFICATION DATE	DELIVERY MODE
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12/15/2017

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. 14/776,106	Applicant(s) FRANCIS ET AL.	
	Examiner DOUGLAS F. WHITE	Art Unit 1653	AIA (First Inventor to File) 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 9/11/17 and 12/06/17.
☐ 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) 1,5,6,11,12,17,19-22,27,28 and 31-33 is/are pending in the application.
5a) Of the above claim(s) 27,28 and 31-33 is/are withdrawn from consideration.
- 6) ☐ Claim(s) ____ is/are allowed.
- 7) ☒ Claim(s) 1,5,6,11,12,17 and 19-22 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 ____ 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) | 3) ☐ Interview Summary (PTO-413)
Paper No(s)/Mail Date. ____. |
| 2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b)
Paper No(s)/Mail Date ____. | 4) ☐ Other: ____. |

DETAILED ACTION

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

Claims 1,5,6,11,12,17 and 19-22 have been examined on the merits.

Election/Restrictions

Applicant's election without traverse of the Group I (Claims 1,5,6,11,12,17 and 19-22) invention in the reply filed on 09/11/17 is acknowledged.

Claims 27,28 and 31-33 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on 09/11/17.

Claim Rejections - 35 USC § 102

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(s) 1, 11-12, and 17 are rejected under pre-AIA 35 U.S.C. 102(b) as being anticipated by Huang et al. (Colloids Surf. B. Biointerfaces, 2011, 82:307-315; IDS reference, hereinafter “Huang”).

Huang discloses a scaffold comprising one or more electrospun fibers (abstract; pg. 308, col 1; pg. 309, col 2, aligned nanofibrous scaffolds based on collagen, chitosan, thermoplastic polyurethane (TPU) blends were electrospun to mimic the componential and structural aspects of

Art Unit: 1653

the native extracellular matrix, while an optimal proportion was found to keep the balance between biocompatibility and mechanical strength), wherein a fast Fourier transform (FFT) analysis result of the fibers have adjacent major peaks with about 180 degrees apart from each other (Fig 4E; pg. 309, col 2, two peaks at 175 degrees and 355 degrees (180 degrees apart)). Huang discloses that the scaffold has an average porosity from about 60% to about 90% (Table 1).

Therefore, the reference is deemed to anticipate the instant claims above.

Claim Rejections - 35 USC § 103

The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Claims 1,5,6,11,12,17 and 19-22 are rejected under 35 U.S.C. 103(a) as obvious over Simpson et al. (US PG PUB 20040037813; hereinafter “Simpson”).

Simpson beneficially teaches a scaffold comprising one or more electrospun fibers (abstract; paragraph [0092]). Simpson teaches that the secondary structure of collagen is not modified in the process of making the scaffold, as shown by Fourier transform infrared spectroscopy (paragraph [0361]).

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Simpson also teaches that the collagen used in electroprocessing may be derived from a natural source (including collagens produced by or contained within the tissue of living organisms, autologous collagen, collagen from a conspecific organism, or collagen from another species, and some collagens include collagen types I) (paragraph [0091]). Simpson teaches that the electroprocessed collagen compositions include additional electroprocessed materials (including amino acids, peptides, denatured peptides such as gelatin from denatured collagen, polypeptides, proteins, carbohydrates, lipids, nucleic acids, glycoproteins, minerals, lipoproteins, glycolipids, glycosaminoglycans, and proteoglycans. materials for electroprocessing with collagen are naturally occurring extracellular matrix materials and blends of naturally occurring extracellular matrix materials, including, fibrin, elastin, laminin, fibronectin, chitin, chitosan, alginates hyaluronic acid, chondroitin 4-sulfate, chondroitin 6-sulfate, dermatan sulfate, heparan sulfate, heparin, and keratan sulfate, and proteoglycans) (paragraphs [0059], [0096]-[0097]).

Simpson also teaches that the electroprocessed collagen compositions can further comprise molecules such as glycoproteins, lipoproteins, glycolipids, glycosaminoglycans, proteoglycans, and growth factors [including transforming growth factor- α ("TGF- α "), transforming growth factor- β ("TGF- β "), platelet-derived growth factors including the AA, AB and BB isoforms ("PDGF"), fibroblast growth factors ("FGF"), including FGF acidic isoforms 1 and 2, FGF basic form 2, and FGF 4, 8, 9 and 10, nerve growth factors ("NGF") including NGF 2.5 s, NGF 7.0 s and beta NGF and neurotrophins, brain derived neurotrophic factor, cartilage derived factor, bone growth factors (BGF), basic fibroblast growth factor, insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF), granulocyte colony stimulating factor (G-CSF), insulin like growth factor (IGF) I and II, hepatocyte growth factor, glial neurotrophic

Art Unit: 1653

growth factor (GDNF), stem cell factor (SCF), keratinocyte growth factor (KGF), transforming growth factors (TGF), including TGFs alpha, beta, betal, beta2, and beta3, skeletal growth factor, bone matrix derived growth factors, and bone derived growth factors and mixtures thereof] (paragraphs [0009] and [0116]). It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to make a scaffold comprising one or more electrospun fibers based upon the beneficial teachings provided by Simpson. If not expressly taught, the adjustment of particular conventional working conditions is deemed merely a matter of judicious selection, design choice and routine optimization which is well within the purview of the skilled artisan.

From the teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention. Therefore, the invention as a whole was *prima facie* obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary.

Conclusion

No claim is allowed.

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to DOUGLAS F. WHITE whose telephone number is (571)270-5561. The examiner can normally be reached on Monday-Friday; 7-3:30 EST.

Art Unit: 1653

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If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Sharmila Landau can be reached on (571) 272-0614. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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/DOUGLAS F. WHITE/
Examiner, Art Unit 1653

/Christopher R Tate/
Primary Examiner, Art Unit 1655

Notice of References Cited	Application/Control No. 14/776,106		Applicant(s)/Patent Under Reexamination FRANCIS ET AL.	
	Examiner DOUGLAS F. WHITE		Art Unit 1653	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-2002/0090725 A1	07-2002	Simpson, David G.	A61L15/32	435/402
*	B	US-2004/0037813 A1	02-2004	Simpson, David G.	A61F2/08	424/93.7
*	C	US-2007/0225631 A1	09-2007	Bowlin; Gary L.	A61K38/363	602/52
*	D	US-2009/0018643 A1	01-2009	Hashi; Craig	A61F2/82	623/1.15
*	E	US-2012/0273993 A1	11-2012	Shoseyov; Oded	A61L27/24	264/202
	F	US-				
	G	US-				
	H	US-				
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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		14776106	
	Filing Date		2015-09-14	
	First Named Inventor	Michael Francis		
	Art Unit	1653		
	Examiner Name	Douglas F. White		
	Attorney Docket Number	LNH-152US		

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	14776106
	Filing Date	2015-09-14
	First Named Inventor	Michael Francis
	Art Unit	1653
	Examiner Name	Douglas F. White
	Attorney Docket Number	LNH-152US

1	ZEUGOLIS, D.I., et al., "Electro-spinning of pure collagen nano-fibres - Just an expensive way to make gelatin?", March 3, 2008, pp. 2293-2305, Vol. 29(15), Biomaterials, Elsevier Science Publishers	☐
2	European Communication for European Application No. 14 773 677.1, dated November 6, 2017, 8 pages	☐

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	First Named Inventor	Michael Francis
	Art Unit	1653
	Examiner Name	Douglas F. White
	Attorney Docket Number	LNH-152US

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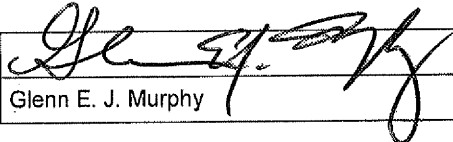
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Signature		Date (YYYY-MM-DD)	2017-12-06
Name/Print	Glenn E. J. Murphy	Registration Number	33,539

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CONFIRMATION NO. 1091

SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.	
14/776,106	09/14/2015	435	1653	067949-5058	
RULE					
APPLICANTS LIFENET HEALTH, Virginia Beach,, VA INVENTORS Michael FRANCIS, Virginia Beach, VA; Roy OGLE, Virginia Beach, VA; ** CONTINUING DATA ***** This application is a 371 of PCT/US14/25636 03/13/2014 which claims benefit of 61/785,031 03/14/2013 ** FOREIGN APPLICATIONS ***** ** IF REQUIRED, FOREIGN FILING LICENSE GRANTED *** SMALL ENTITY ** 10/21/2015					
Foreign Priority claimed ☒ Yes ☐ No 35 USC 119(a-d) conditions met ☒ Yes ☐ No Verified and Acknowledged <u>/DOUGLAS F WHITE/</u> Examiner's Signature	☐ Met after Allowance Initials	STATE OR COUNTRY VA	SHEETS DRAWINGS 10	TOTAL CLAIMS 15	INDEPENDENT CLAIMS 1
ADDRESS RATNERPRESTIA 2200 Renaissance Blvd Suite 350 King of Prussia, PA 19406 UNITED STATES					
TITLE Aligned Fiber and Method of Use Thereof					
FILING FEE RECEIVED 700	FEES: Authority has been given in Paper No. _____ to charge/credit DEPOSIT ACCOUNT No. _____ for following:		☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other _____ ☐ Credit		

Search Notes 	Application/Control No. 14776106	Applicant(s)/Patent Under Reexamination FRANCIS ET AL.
	Examiner DOUGLAS F WHITE	Art Unit 1653

CPC- SEARCHED		
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CPC COMBINATION SETS - SEARCHED		
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SEARCH NOTES		
Search Notes	Date	Examiner
EAST- Inventor and text search	12/7/2017	DFW
PALM- Inventor search	12/7/2017	DFW

INTERFERENCE SEARCH			
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	First Named Inventor	Michael Francis		
	Art Unit			
	Examiner Name			
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	1	20100233115	A1	2010-09-16	PATEL et al.	
	2	20110236974	A1	2011-09-29	OGLE et al.	
	3	20120221025	A1	2012-08-30	SIMPSON et al.	

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First Named Inventor	Michael Francis	
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	1	HUANG et al. (2011) Electrospun Collagen-Chitosan-TPU Nanofibrous Scaffolds for Tissue Engineered Tubular Grafts, Colloids Surf. B. Biointerfaces, 82:307-315	☐
	2	Copy of International Search Report and Written Opinion dated July 10, 2014; International Application No. PCT/US2014/025636	☐

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EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	186	(electrospun adj fibers) same collagen	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/12/10 20:05
L2	84	L1 and calcium	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/12/10 20:05
L3	58	L2 and (ECM or (extracellular adj matrix))	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/12/10 20:05
L4	42	L3 and native	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/12/10 20:05
L5	13	L4 and helical	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/12/10 20:05
L6	6	L5 and Fourier	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/12/10 20:05
L7	6	L6 and pore\$5	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/12/10 20:05
L8	6	L6 and poro\$5	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/12/10 20:06
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S9	36	S8 and ECM	US-PGPUB; USPAT; USOCR; EPO; JPO;	OR	OFF	2017/11/21 16:50

			DERWENT			
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S13	84	S12 and calcium	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/12/07 15:41
S14	58	S13 and (ECM or (extracellular adj matrix))	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/12/07 15:41
S15	42	S14 and native	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/12/07 15:41
S16	186	(electrospun adj fibers) same collagen	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/12/10 07:03
S17	84	S16 and calcium	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/12/10 07:03
S18	58	S17 and (ECM or (extracellular adj matrix))	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/12/10 07:03
S19	42	S18 and native	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/12/10 07:03
S20	13	S19 and helical	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/12/10 07:03
S21	6	S20 and Fourier	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/12/10 07:05

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	First Named Inventor	Michael Francis		
	Art Unit	1653		
	Examiner Name	Douglas F. White		
	Attorney Docket Number	LNH-152US		

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	1	20080213389		2008-09-04	Lelkes	

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Application Number	14776106
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Art Unit	1653
Examiner Name	Douglas F. White
Attorney Docket Number	LNH-152US

1	Supplementary European Search Report for European Application no. 14773677.1, dated October 25, 2016, 3 pages.	☐
2	Extended Search Report for European Application No. 14773677.1, dated October 7, 2016, 6 pages.	☐

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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).

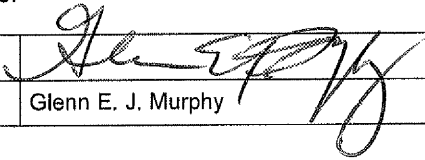
OR

- ☐ 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)	2017-02-02
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 records.
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.



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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/776,106	09/14/2015	Michael FRANCIS	LNH-152US	1091

23122 7590 01/23/2018
RATNERPRESTIA
2200 Renaissance Blvd
Suite 350
King of Prussia, PA 19406

EXAMINER

WHITE, DOUGLAS F

ART UNIT	PAPER NUMBER
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1653

NOTIFICATION DATE	DELIVERY MODE
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01/23/2018

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

<i>Applicant-Initiated Interview Summary</i>	Application No. 14/776,106	Applicant(s) FRANCIS ET AL.	
	Examiner DOUGLAS F. WHITE	Art Unit 1653	

All participants (applicant, applicant's representative, PTO personnel):

(1) DOUGLAS F. WHITE.

(3) William Mattney.

(2) Glenn Murphy.

(4) Jing Song Chen.

Date of Interview: 17 January 2017.

Type: ☐ Telephonic ☒ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☒ No.

If Yes, brief description: _____.

Issues Discussed ☐101 ☐112 ☒102 ☒103 ☐Others

(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: 1.

Identification of prior art discussed: Huang et al. and Simpson et al.

Substance of Interview

(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

Discussed various amendments to the claims so as to better define and possibly distinguish the invention over Huang et al. - including amending claim 1 to recite --A scaffold comprising one or more electrospun fibers comprising collagen, wherein a fast Fourier transform (FFT) analysis result of the fibers have adjacent major peaks with about 180° apart from each other-- . Applicant also argued that Simpson et al. do not teach that a fast Fourier transform (FFT) analysis result of the fibers have adjacent major peaks with about 180° apart from each other. Applicants also argued that the device used in the instant application and those found in the prior art of record were different and in the instant case, such a difference allowed for greater alignment of fibers. Applicants' representative plans to file a response shortly detailing such amendments and arguments.

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.

☐ Attachment

/Christopher R Tate/
Primary Examiner, Art Unit 1655

Summary of Record of Interview Requirements

Manual of Patent Examining Procedure (MPEP), Section 713.04, Substance of Interview Must be Made of Record

A complete written statement as to the substance of any face-to-face, video conference, or telephone interview with regard to an application must be made of record in the application whether or not an agreement with the examiner was reached at the interview.

Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews

Paragraph (b)

In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for reply to Office action as specified in §§ 1.111, 1.135. (35 U.S.C. 132)

37 CFR §1.2 Business to be transacted in writing.

All business with the Patent or Trademark Office should be transacted in writing. The personal attendance of applicants or their attorneys or agents at the Patent and Trademark Office is unnecessary. The action of the Patent and Trademark Office will be based exclusively on the written record in the Office. No attention will be paid to any alleged oral promise, stipulation, or understanding in relation to which there is disagreement or doubt.

The action of the Patent and Trademark Office cannot be based exclusively on the written record in the Office if that record is itself incomplete through the failure to record the substance of interviews.

It is the responsibility of the applicant or the attorney or agent to make the substance of an interview of record in the application file, unless the examiner indicates he or she will do so. It is the examiner's responsibility to see that such a record is made and to correct material inaccuracies which bear directly on the question of patentability.

Examiners must complete an Interview Summary Form for each interview held where a matter of substance has been discussed during the interview by checking the appropriate boxes and filling in the blanks. Discussions regarding only procedural matters, directed solely to restriction requirements for which interview recordation is otherwise provided for in Section 812.01 of the Manual of Patent Examining Procedure, or pointing out typographical errors or unreadable script in Office actions or the like, are excluded from the interview recordation procedures below. Where the substance of an interview is completely recorded in an Examiners Amendment, no separate Interview Summary Record is required.

The Interview Summary Form shall be given an appropriate Paper No., placed in the right hand portion of the file, and listed on the "Contents" section of the file wrapper. In a personal interview, a duplicate of the Form is given to the applicant (or attorney or agent) at the conclusion of the interview. In the case of a telephone or video-conference interview, the copy is mailed to the applicant's correspondence address either with or prior to the next official communication. If additional correspondence from the examiner is not likely before an allowance or if other circumstances dictate, the Form should be mailed promptly after the interview rather than with the next official communication.

The Form provides for recordation of the following information:

- Application Number (Series Code and Serial Number)
- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (telephonic, video-conference, or personal)
- Name of participant(s) (applicant, attorney or agent, examiner, other PTO personnel, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). Note: Agreement as to allowability is tentative and does not restrict further action by the examiner to the contrary.
- The signature of the examiner who conducted the interview (if Form is not an attachment to a signed Office action)

It is desirable that the examiner orally remind the applicant of his or her obligation to record the substance of the interview of each case. It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview.

A complete and proper recordation of the substance of any interview should include at least the following applicable items:

- 1) A brief description of the nature of any exhibit shown or any demonstration conducted,
- 2) an identification of the claims discussed,
- 3) an identification of the specific prior art discussed,
- 4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the Examiner,
- 5) a brief identification of the general thrust of the principal arguments presented to the examiner,
(The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he or she feels were or might be persuasive to the examiner.)
- 6) a general indication of any other pertinent matters discussed, and
- 7) if appropriate, the general results or outcome of the interview unless already described in the Interview Summary Form completed by the examiner.

Examiners are expected to carefully review the applicant's record of the substance of an interview. If the record is not complete and accurate, the examiner will give the applicant an extendable one month time period to correct the record.

Examiner to Check for Accuracy

If the claims are allowable for other reasons of record, the examiner should send a letter setting forth the examiner's version of the statement attributed to him or her. If the record is complete and accurate, the examiner should place the indication, "Interview Record OK" on the paper recording the substance of the interview along with the date and the examiner's initials.

Appln. No.: 14/776,106
Amendment Dated February 21, 2018
Reply to Office Action of December 15, 2017

LNH-152US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No: 14/776,106
Applicant: LifeNet Health
Filed: September 14, 2015
Title: ALIGNED FIBER AND METHOD OF USE THEREOF
TC/A.U.: 1653
Examiner: Douglas F. White
Confirmation No.: 1091
Docket No.: LNH-152US

AMENDMENT

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

Commissioner:

Applicant responds to the office action dated December 15, 2017 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. (Currently Amended) A scaffold comprising one or more electrospun fibers comprising collagen, wherein a fast Fourier transform (FFT) analysis result of the fibers have adjacent major peaks with about 180° apart from each other.
- 2.-4. (Cancelled)
5. (Previously Presented) The scaffold according to claim 1, wherein the fibers comprise type I collagen in its native triple helical structure.
6. (Previously Presented) The scaffold according to claim 1, wherein the fiber has less than about 8 wt% residual calcium.
- 7.-10. (Cancelled)
11. (Previously Presented) The scaffold according to claim 1, wherein the scaffold has an average porosity from about 60% to about 90%.
12. (Previously Presented) The scaffold according to claim 1, wherein the 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, an accessory polymer, or the mixture thereof.
- 13.-16. (Cancelled)
17. (Previously Presented) The scaffold according to claim 12, wherein the accessory polymer is 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/surfactants, xanthan, acetoglycerides, waxes, surfactants, dextran, emulsan, gelatin, polyphenols, levan, lignin, curd, inulin, tannin, polygalactosamine,

humic acid, shellac, pullulan, poly-gamma-glutamic acid, elsinan, natural rubber, yeast glucans, synthetic polymers from natural fats and oils.

18. (Cancelled)

19. (Previously Presented) The scaffold according to claim 1, wherein the scaffold comprises a bioactive factor.

20. (Original) The scaffold according to claim 19, wherein the bioactive factor is selected from the group consisting of 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), and an interleukin.

21. (Previously Presented) The scaffold according to claim 1, wherein the scaffold comprises an extracellular matrix component.

22. (Original) The scaffold according to claim 21, wherein the extracellular matrix component is selected from the group consisting of collagen, glycosaminoglycans, osteocalcin, osteonectin, bone sialo protein, osteopontin, fibronectin, laminin, and mixtures thereof.

23.-26. (Cancelled)

27. (Withdrawn) A method of cell culture comprising culturing cells on the scaffold according to claim 1 in condition suitable for the cell culture.

28. (Withdrawn) 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 dorsal root ganglia.

29.-30. (Cancelled)

31. (Withdrawn) A method of promoting differentiation of stem cells into osteoblasts, chondrocytes, ligament or tendon, the method comprising culturing the cells on the scaffold according to claim 1 in conditions suitable for the cell differentiation.

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

33. (Withdrawn) 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.-35. (Cancelled)

Remarks/Arguments:

Status and Disposition of the Claims

Claims 1, 5, 6, 11, 12, 17, 19-22, 27, 28, and 31-33 are pending in the application. Claims 27, 28, and 31-33 are withdrawn under restriction. Claims 1, 5, 6, 11, 12, 17, and 19-22 stand rejected.

Claim 1 is amended to recite a scaffold comprising one or more electrospun fibers comprising collagen in addition to its other limitations. Support for this amendment is found throughout the specification, for example at page 10, paragraph [0040]. No new matter has been added to the application.

Examiner Interview Summary

Applicant thanks Examiner White for the courtesy of an interview held by video conference at the USPTO on January 17, 2018. In attendance for Applicant were Applicant's counsel of record Glenn E. J. Murphy and William J. Matney, Jr., as well as Dr. Jingsong Chen, Vice President of Intellectual Property and General Manager, Life Sciences Division of Applicant Lifenet Health.

The substance of the interview included a discussion of the pending rejections for anticipation and obviousness over the Huang and Simpson references. Applicant's representatives pointed out the differences between the claimed scaffold and those disclosed in the references as well as the differences between the apparatus used to prepare the scaffold according to the invention and the apparatus used to prepare the scaffolds of the prior art. No agreement was reached as to the allowability of any claim. Applicants proposed to enter an amendment limiting the claimed scaffold as recited in amended claim 1.

Claims Rejections – 35 U.S.C. § 102

Claims 1, 11, 12, and 17 were rejected for anticipation over Huang et al., *Coll. Surf. B: Biointerfaces* 82:307-15 (2011). For the reasons that follow, Applicants respectfully submit that this rejection should not be maintained against the claims as amended herein.

Huang discloses a scaffold comprising electrospun fibers based on thermoplastic polyurethane (TPU) blended with various natural materials including collagen and chitosan. The office has alleged that Huang at Fig. 4E discloses a scaffold wherein a Fast Fourier Transform (FFT) analysis shows fibers having adjacent major peaks about 180° apart from each other. This disclosure, however, is not anticipatory of claim 1 as amended herein, at least for the reason that the FFT analysis in Huang's Fig. 4E relate to pure thermoplastic polyurethane fibers that comprise no collagen. In contrast, Huang's electrospun fibers comprising TPU, collagen, and chitosan did not exhibit the degree of alignment as recited in present claim 1; an FFT analysis of these fibers showed major peaks closer than 180° apart from each other. See Huang, Fig. 4F. Therefore, Huang does not disclose a scaffold comprising one or more electrospun fibers comprising collagen, wherein a fast Fourier transform (FFT) analysis result of the fibers have adjacent major peaks with about 180° apart from each other. For this reason claims 1, 11, 12, and 17 should not be rejected again as anticipated by Huang.

Claim Rejections – 35 U.S.C. § 103

Claims 1, 5, 6, 11, 12, 17 and 19-22 were rejected for obviousness over Simpson et al., U.S. Pat. Pub. No. 2004/0037813 A1. For the reasons that follow, this rejection also should not be maintained over the claims as amended herein.

Simpson is directed to the formation and use of electro-processed collagen in the form of electrospun fibers. Simpson does not disclose a scaffold as recited in claim 1, nor has the Office produced any evidence that such alignment would be inherent in scaffolds of Simpson. Rather, the evidence as a whole tends to show that Simpson's teachings would not lead to the claimed scaffold material.

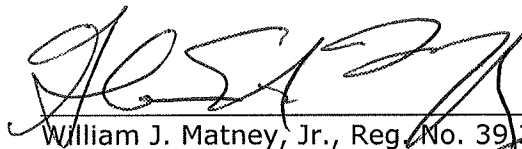
As is explained in further detail in the present application, the claimed scaffolds comprising electrospun collagen fibers may be obtained using a particular apparatus described in instant specification, wherein the electrospun fibers are deposited onto a collector comprising rotating, rod-shaped electrodes. See, e.g., spec. at [0029], [00103-04]. In contrast, both Huang and Simpson disclose an electrodeposition apparatus in which the fiber material is depositing on a rotating cylindrical collector. Huang further discloses that using an apparatus with a cylindrical collector to prepare electrospun fibers comprising collagen did not produce a scaffold having the claimed fiber alignment. See Huang, Fig. 4F. A person of skill, knowing that the device of Huang did not produce the claimed fiber alignment, would not have expected

to obtain the claimed fiber alignment using the similar device disclosed in Simpson. Rather, one of skill would reasonably have expected that similar devices processing similar materials would produce similar results, i.e., scaffolds of electrospun fibers lacking the claimed fiber alignment. It follows that the scaffold of claim 1 would not have been obvious in view of Simpson, and that claims 1, 5, 6, 11, 12, 17 and 19-22 should not be rejected again for obviousness over Simpson et al., U.S. Pat. Pub. No. 2004/0037813 A1.

Conclusion

In view of the amendments and arguments presented herein, Applicant respectfully asks for reconsideration and allowance of all claims under examination.

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: February 21, 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.

3919236

Electronic Acknowledgement Receipt

EFS ID:	31843201
Application Number:	14776106
International Application Number:	
Confirmation Number:	1091
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-152US
Receipt Date:	21-FEB-2018
Filing Date:	14-SEP-2015
Time Stamp:	11:40:16
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	no
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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.pdf	243121	no	7
			f2efd8984993d02f2694a92b04f0e3c1b5476992		

Warnings:

Information:	
Total Files Size (in bytes):	243121
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. <u>New Applications Under 35 U.S.C. 111</u> 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. <u>National Stage of an International Application under 35 U.S.C. 371</u> 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. <u>New International Application Filed with the USPTO as a Receiving Office</u> 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.	

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.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 14/776,106	Filing Date 09/14/2015	☐ To be Mailed
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ENTITY: ☐ LARGE ☒ SMALL ☐ MICRO**APPLICATION AS FILED – PART I**

(Column 1)

(Column 2)

FOR	NUMBER FILED	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), (l), 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 \$ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X \$ =	
☐ 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	02/21/2018	CLAIMS REMAINING AFTER AMENDMENT	MINUS	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE (\$)	ADDITIONAL FEE (\$)
	Total (37 CFR 1.16(i))	* 15	Minus	** 20	= 0	X \$50 =	0
	Independent (37 CFR 1.16(h))	* 1	Minus	***3	= 0	X \$230 =	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	MINUS	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE (\$)	ADDITIONAL FEE (\$)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X \$ =
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X \$ =
	☐ 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.

** 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 number found in the appropriate box in column 1.

LIE
KIM DOWNING

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.**

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



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

NOTICE OF ALLOWANCE AND FEE(S) DUE

23122 7590 06/12/2018
RATNERPRESTIA
2200 Renaissance Blvd
Suite 350
King of Prussia, PENNSYLVANIA 19406
UNITED STATES OF AMERICA

EXAMINER

WHITE, DOUGLAS F

ART UNIT

PAPER NUMBER

1653

DATE MAILED: 06/12/2018

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/776,106	09/14/2015	Michael FRANCIS	LNH-152US	1091

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	\$500	\$0.00	\$0.00	\$500	09/12/2018

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 and an extra copy of the form should be submitted. 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: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: **Mail** **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (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)

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.

23122 7590 06/12/2018
RATNERPRESTIA
 2200 Renaissance Blvd
 Suite 350
 King of Prussia, PENNSYLVANIA 19406
 UNITED STATES OF AMERICA

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 facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/776,106	09/14/2015	Michael FRANCIS	LNH-152US	1091

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	\$500	\$0.00	\$0.00	\$500	09/12/2018

EXAMINER	ART UNIT	CLASS-SUBCLASS
WHITE, DOUGLAS F	1653	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/SB/122) attached.

☐ "Fee Address" indication (or "Fee Address" Indication form 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 has been filed for recordation as set forth in 37 CFR 3.11. 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. The following fee(s) are submitted:

☐ Issue Fee

☐ Publication Fee (No small entity discount permitted)

☐ Advance Order - # of Copies _____

4b. Payment of Fee(s): (**Please first reapply any previously paid issue fee shown above**)

☐ A check is enclosed.

☐ Payment by credit card. Form PTO-2038 is attached.

☐ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

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 PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/776,106	09/14/2015	Michael FRANCIS	LNH-152US	1091
23122	7590	06/12/2018	EXAMINER	
RATNERPRESTIA 2200 Renaissance Blvd Suite 350 King of Prussia, PENNSYLVANIA 19406 UNITED STATES OF AMERICA			WHITE, DOUGLAS F	
			ART UNIT	PAPER NUMBER
			1653	
DATE MAILED: 06/12/2018				

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 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, 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

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 enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 14/776,106	Applicant(s) FRANCIS et al.	
	Examiner DOUGLAS F WHITE	Art Unit 1653	AIA 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 the communication filed 21 February 2018.
☐ 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 See Continuation Sheet. 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 areply 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. <u>20180606</u> .	5. ☒ Examiner's Amendment/Comment 6. ☐ Examiner's Statement of Reasons for Allowance 7. ☐ Other _____.
--	--

/DOUGLAS F WHITE/ Examiner, Art Unit 1653	/CHRISTOPHER R TATE/ Primary Examiner, Art Unit 1655
--	---

Continuation of 3. The allowed claim(s) is/are: 1,5-6,11-12,17,19-22,27-28 and 31-33

Notice of Pre-AIA or AIA Status

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

The response with amendments filed 21 February 2018 has been received and entered.

Examiner's Comment

The claims have been examined on the merits and found allowable – as amended within the Examiner's Amendment set forth below. Please also refer to the Rejoinder statement below.

Rejoinder

Claims 1, 5-6, 11-12, 17, and 19-22 are directed to an allowable product. Pursuant to the procedures set forth in MPEP § 821.04(B), claims 27-28 and 31-33, directed to the process of making or using an allowable product, previously withdrawn from consideration as a result of a restriction requirement, are hereby rejoined and fully examined for patentability under 37 CFR 1.104.

Because all claims previously withdrawn from consideration under 37 CFR 1.142 have been rejoined, **the restriction requirement as set forth in the Office action mailed on [4] is hereby withdrawn.** In view of the withdrawal of the restriction requirement as to the rejoined inventions, applicant(s) are advised that if any claim presented in a continuation or divisional application is anticipated by, or includes all the limitations of, a claim that is allowable in the present application, such claim may be subject to provisional statutory and/or nonstatutory double patenting rejections over the claims of the instant application. Once the restriction requirement is withdrawn, the provisions of 35 U.S.C. 121 are no longer applicable. See *In re Ziegler*, 443 F.2d 1211, 1215, 170 USPQ 129, 131-32 (CCPA 1971). See also MPEP § 804.01.

Examiner's Amendment

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it **MUST** be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with Glenn Murphy on 06 June 2018.

IN THE CLAIMS:

In claim 27, at line 2, the phrase "in condition" has been omitted and replaced with the phrase --under conditions--.

In claim 31, at line 2, the phrase "in conditions" has been omitted and replaced with the phrase --under conditions--.

Conclusion

Claims 1, 5-6, 11-12, 17, 19-22, 27-28 and 31-33 are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to DOUGLAS F WHITE whose telephone number is (571)270-5561. The examiner can normally be reached on Monday-Thursday 8am-6:30pm.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at <http://www.uspto.gov/interviewpractice>.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Sharmila G. Landau can be reached on 571-272-0614. 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.

/DOUGLAS F. WHITE/
Examiner, Art Unit 1653

/CHRISTOPHER R TATE/
Primary Examiner, Art Unit 1655

Issue Classification 	Application/Control No. 14/776,106	Applicant(s)/Patent Under Reexamination FRANCIS et al.
	Examiner DOUGLAS F WHITE	Art Unit 1653

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	/	2	/	08	A	2013-01-01
A61F	/	2240	/	001	A	2013-01-01
A61L	/	2400	/	12	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

/DOUGLAS F WHITE/ Examiner, Art Unit 1653 (Assistant Examiner)	06 June 2018 (Date)	Total Claims Allowed: 15	
/CHRISTOPHER R TATE/ Primary Examiner, Art Unit 1655 (Primary Examiner)	08 June 2018 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure 5

Issue Classification 	Application/Control No. 14/776,106	Applicant(s)/Patent Under Reexamination FRANCIS et al.	
	Examiner DOUGLAS F WHITE	Art Unit 1653	

INTERNATIONAL CLASSIFICATION					
CLAIMED					
A61K	/	38	/	17	
C07K	/	1	/	00	
C07K	/	14	/	00	
C07K	/	16	/	00	
C07K	/	17	/	00	
C09H	/	1	/	00	
NON-CLAIMED					
	/		/		

US ORIGINAL CLASSIFICATION	
CLASS	SUBCLASS
435	395

CROSS REFERENCES(S)						
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)					

/DOUGLAS F WHITE/ Examiner, Art Unit 1653 (Assistant Examiner)	06 June 2018 (Date)	Total Claims Allowed: 15	
/CHRISTOPHER R TATE/ Primary Examiner, Art Unit 1655 (Primary Examiner)	08 June 2018 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure 5

Issue Classification 	Application/Control No. 14/776,106	Applicant(s)/Patent Under Reexamination FRANCIS et al.
	Examiner DOUGLAS F WHITE	Art Unit 1653

☒ 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

/DOUGLAS F WHITE/ Examiner, Art Unit 1653 (Assistant Examiner)	06 June 2018 (Date)	Total Claims Allowed: 15	
/CHRISTOPHER R TATE/ Primary Examiner, Art Unit 1655 (Primary Examiner)	08 June 2018 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure 5

<i>Search Notes</i> 	Application/Control No. 14/776,106	Applicant(s)/Patent Under Reexamination FRANCIS et al.
	Examiner DOUGLAS F WHITE	Art Unit 1653

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
EAST- Inventor and text search	12/7/2017	DFW
PALM- Inventor search	12/7/2017	DFW
Updated	06/06/2018	DFW

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner
EAST search	attached	06/06/2018	DFW

	/DOUGLAS F WHITE/ Examiner.Art Unit 1653
--	---

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S2	19	((Roy) near2 (Ogle)).INV.	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/11/21 16:27
S3	3	"20080213389"	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/11/21 16:29
S4	6	(US-20100233115-\$ US-20110236974-\$ US-20120221025-\$).DID.	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/11/21 16:46
S5	185	(electrospun adj fibers) same collagen	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/11/21 16:48
S6	16634	"I6" and calcium	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/11/21 16:49
S7	18924	"I5" and calcium	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/11/21 16:49
S8	84	S5 and calcium	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/11/21 16:49
S9	36	S8 and ECM	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/11/21 16:50
S10	58	S8 and (ECM or (extracellular adj matrix))	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/11/21 16:52
S11	1	((Micheal) near2 (Francis)).INV.	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/12/07 15:32
S12	186	(electrospun adj fibers) same collagen	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/12/07 15:41
S13	84	S12 and calcium	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/12/07 15:41
S14	58	S13 and (ECM or (extracellular adj matrix))	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/12/07 15:41
S15	42	S14 and native	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/12/07 15:41
S16	186	(electrospun adj fibers) same collagen	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/12/10 07:03
S17	84	S16 and calcium	US-PGPUB; USPAT; USOCR; EPO; JPO;	OR	OFF	2017/12/10 07:03

			DERWENT			
S18	58	S17 and (ECM or (extracellular adj matrix))	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/12/10 07:03
S19	42	S18 and native	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/12/10 07:03
S20	13	S19 and helical	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/12/10 07:03
S21	6	S20 and Fourier	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	OFF	2017/12/10 07:05

EAST Search History (Interference)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	2	(electrospun adj fibers) same collagen.clm.	USPAT	OR	OFF	2018/06/07 12:08
L2	74	(electrospun adj fibers) and collagen.clm.	USPAT	OR	OFF	2018/06/07 12:09
L3	1	l2 and Fourier.clm.	USPAT	OR	OFF	2018/06/07 12:09

6/ 7/ 2018 12:24:53 PM
C:\ Users\ dwhite3\ Documents\ EAST\ Workspaces\ 14776106.wsp

REQUEST FOR CONTINUED EXAMINATION(RCE)TRANSMITTAL **(Submitted Only via EFS-Web)**

Application Number	14/776,106	Filing Date	2015-09-14	Docket Number (if applicable)	LNH-152US	Art Unit	1653
First Named Inventor	Michael Francis et al.			Examiner Name	Douglas F. White		

This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.
Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8, 1995, or to any design application. The Instruction Sheet for this form is located at WWW.USPTO.GOV

SUBMISSION REQUIRED UNDER 37 CFR 1.114

Note: If the RCE is proper, any previously filed unentered amendments and amendments enclosed with the RCE will be entered in the order in which they were filed unless applicant instructs otherwise. If applicant does not wish to have any previously filed unentered amendment(s) entered, applicant must request non-entry of such amendment(s).

☐ Previously submitted. If a final Office action is outstanding, any amendments filed after the final Office action may be considered as a submission even if this box is not checked.

☐ Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____

☐ Other _____

☒ Enclosed

☒ Amendment/Reply

☒ Information Disclosure Statement (IDS)

☐ Affidavit(s)/ Declaration(s)

☒ Other Replacement Drawing

MISCELLANEOUS

☐ Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a period of months _____
(Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)

☐ Other _____

FEES

The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed.

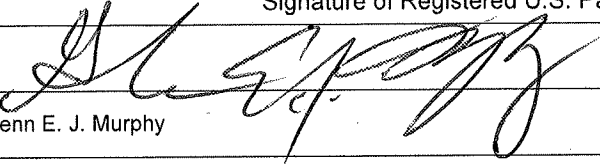
☒ The Director is hereby authorized to charge any underpayment of fees, or credit any overpayments, to
Deposit Account No 180350

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

☒ Patent Practitioner Signature

☐ Applicant Signature

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.

Signature of Registered U.S. Patent Practitioner			
Signature		Date (YYYY-MM-DD)	2018-07-27
Name	Glenn E. J. Murphy	Registration Number	33539

This collection of information is required by 37 CFR 1.114. 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.

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 the Freedom of Information Act requires disclosure of these records.
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.

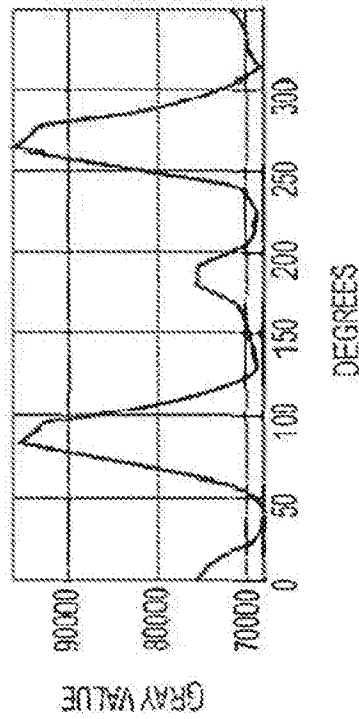
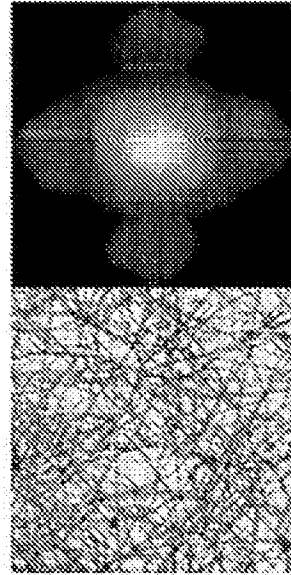
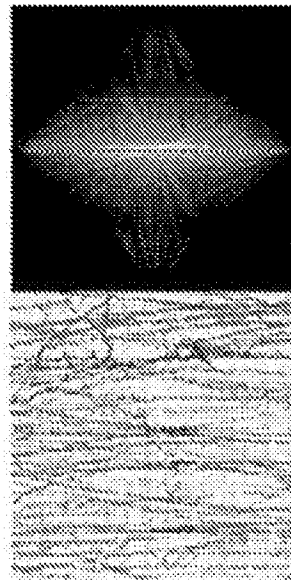


FIG. 8A

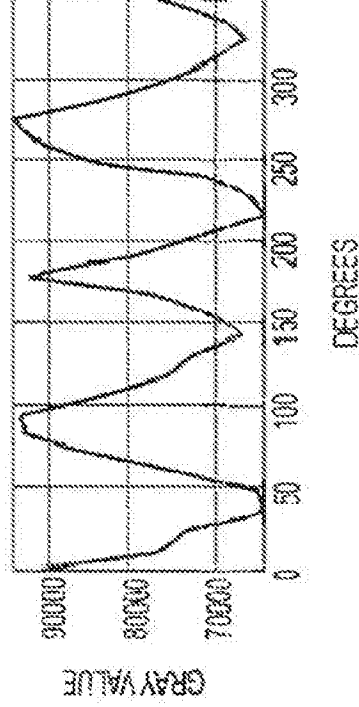


FIG. 8B

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No: 14/776,106
Applicant: LifeNet Health
Filed: September 14, 2015
Title: ALIGNED FIBER AND METHOD OF USE THEREOF
TC/A.U.: 1653
Examiner: Douglas F. White
Confirmation No.: 1091

INFORMATION DISCLOSURE STATEMENT TRANSMITTAL

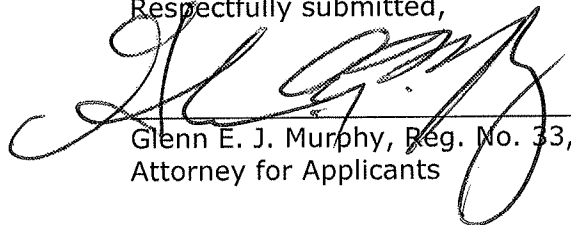
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

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, Applicants submit the information listed on the attached form PTO/SB/08a and ask that it be considered and made of record in this application. Although the Examiner may determine the information submitted here to be material to examining this application, this submission is not an admission that any such information is prior art to the claimed invention. Further, in accordance with 37 C.F.R. § 1.97(g), the Office may not construe the filing of this Information Disclosure Statement to mean that a search has been made.

This Information Disclosure Statement is being filed concurrently with the above-referenced application or with a Request for Continued Examination under 37 CFR 1.114.

Respectfully submitted,



Glenn E. J. Murphy, Reg. No. 33,539
Attorney for Applicants

GEM/nm

Enclosures: PTO/SB/08a
(10) References

Dated: July 27, 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.

4080636

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number		14776106
	Filing Date		2015-09-14
	First Named Inventor	Michael Francis et al.	
	Art Unit	1653	
	Examiner Name	Douglas F. White	
	Attorney Docket Number	LNH-152US	

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

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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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Examiner Initial*	Cite No	Foreign Document Number ³	Country Code ²	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	2012027592	WO	A2	2012-03-01	LifeNet Health		☐
	2	2013033680	WO	A1	2013-03-07	LifeNet Health		☐
	3	2013119873	WO	A1	2013-08-15	LifeNet Health		☐

**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	14776106
Filing Date	2015-09-14
First Named Inventor	Michael Francis et al.
Art Unit	1653
Examiner Name	Douglas F. White
Attorney Docket Number	LNH-152US

4	2010016942	WO	A1	2010-02-11	Lifenet Health	☐
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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
	1	Anderson et al., "Biodegradation and Biocompatibility of PLA and PLGA Microspheres", Advanced Drug Delivery Reviews, 1997, Vol. 28, pp. 5-24	☐
	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	☐
	3	Behravesh et al., "Synthetic Biodegradable Polymers for Orthopaedic Applications," Clinical Orthopaedics and Related Research, No. 367S, pp. S118-S125	☐
	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	☐
	5	Lu et al., "Polymeric Delivery Vehicles for Bone Growth Factors," 2000, Park & Mersny; Controlled Drug Delivery, ACS Symposium Series; American Chemical Society, pp. 124-138	☐
	6	Schnaper et al., "Regulation of Cell Function by Extracellular Matrix," Pediatr. Nephrol, 1993, Vol. 7 - pp. 96-104	☐

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.

**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**

(Not for submission under 37 CFR 1.99)

Application Number	14776106
Filing Date	2015-09-14
First Named Inventor	Michael Francis et al.
Art Unit	1653
Examiner Name	Douglas F. White
Attorney Docket Number	LNH-152US

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	14776106
Filing Date	2015-09-14
First Named Inventor	Michael Francis et al.
Art Unit	1653
Examiner Name	Douglas F. White
Attorney Docket Number	LNH-152US

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).

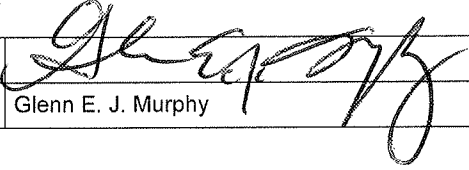
OR

☐ 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)	2018-07-27
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

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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 the Freedom of Information Act requires disclosure of these records.
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.

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 March 2012 (01.03.2012)



(10) International Publication Number
WO 2012/027592 A2

(51) International Patent Classification:
C12N 5/0735 (2010.01)

(21) International Application Number:
PCT/US2011/049192

(22) International Filing Date:
25 August 2011 (25.08.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/376,966 25 August 2010 (25.08.2010) US

(71) Applicant (for all designated States except US):
LIFENET HEALTH [US/US]; 1864 Concert Drive, Virginia Beach, VA 23453 (US).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **OGLE, Roy** [US/US]; 9590 9th Bay St, Norfolk, VA 23518 (US).

(74) Agents: **BUCK, Todd B.** et al.; Morgan, Lewis & Bockius LLP, 1111 Pennsylvania Avenue, NW, Washington, DC 20004 (US).

(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, 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, 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).

Published:

— without international search report and to be republished upon receipt of that report (Rule 48.2(g))

(54) Title: BASEMENT MEMBRANE COMPOSITIONS AND APPLICATIONS THEREOF

(57) Abstract: The invention relates to cell support compositions comprising a basement membrane extract isolated from cardiac or smooth muscle. The invention also relates to methods of using the cell support compositions for supporting cellular functions.



WO 2012/027592 A2

Basement Membrane Compositions and Applications Thereof

Inventor:

Roy Ogle

Cross-Reference to Related Applications

[0001] This application claims priority to U.S. Provisional Application Serial No. 61/376,966, filed 25 August 2010, which is incorporated by reference in its entirety.

Background of the Invention

Field of the Invention

[0002] The invention relates to cell support compositions comprising a basement membrane extract isolated from cardiac or smooth muscle. The invention also relates to methods of using the cell support compositions for supporting cellular functions.

Background

[0003] Basement membranes are thin, continuous sheets that separate an epithelium from adjacent stroma and surround nerves, muscle fibers, smooth muscle cells and adipose cells. As shown in many species, basement membranes appear early during embryogenesis and cover the inner cell mass, suggesting a critical role for basement membrane in early development. All basement membranes contain members of 3 extracellular matrix families: laminins, collagen IV and proteoglycans, along with the glycoprotein, nidogen/entactin. The basement membrane is a nanoscale network of filaments that interacts to form a lattice. This network, in part, results from the assembly of independent networks of laminins and collagen IVs, joined together by the nidogens and other associated proteins.

[0004] Basement membrane preparations have been used for cell biology research to provide a physiological environment promoting cell attachment, growth, and differentiation. Thus for culturing embryonic and other pluripotent stem cells *in vitro*, the basement membrane promotes cell growth and can be used to promote the differentiation of certain cell types.

[0005] Among the most broadly employed basement membrane preparations is the basement membrane-rich matrix from Engelbreth Holm-Swarm (EHS) murine sarcoma, known by its trade name MATRIGEL™ (BD Biosciences). MATRIGEL™ has been a useful product to facilitate cell growth,

development and differentiation for a broad assortment of cell types including stem cells, epithelial cells, endothelial cells, adipocytes, neurons, all forms of muscle cells and their precursors. MATRIGEL™ has been used commonly as an alternative to mouse embryonic fibroblast feeder layer to propagate pluripotent stem cells.

[0006] However, MATRIGEL™ has limited utility in many applications that arises from its origin as a mouse tumor or limits in the biological activity of the components that comprise it. Many products and processes for making these products require that the products or processes used to make the products be free of animal products. Clearly mouse-derived products are not suitable, for example, for the culture of human cells where the cells are grown to generate biologics products for human use such as antibodies or other proteins. The mouse-derived products are unsuitable for fabrication of tissue engineering scaffolds or other forms of implantation in humans due to concerns of passage of murine pathogens or elicitation of unwanted immune response. Beyond the concern of species difference is that of the composition itself. For example, MATRIGEL™ contains only laminin type I and alpha 1 and alpha 2 chains of collagen type IV. There are 6 chains of type IV collagen and 15 chains of laminin found in human cells, each assumed to impart specific biological functions to the basement membranes in which they occur.

[0007] There are undoubtedly components of the basement membrane of each tissue that are unique to that given tissue, although this area of research still requires much greater elucidation. For example, the heart is known not to contain a specific population of stem cells that give rise to cardiomyocytes. Rather, the cardiac tissue contains specific signaling molecules like the chemokines, SDF-1, which recruits circulating stem cells to the heart upon damage. Likewise, the basement membrane composition of tissues like smooth muscle or cardiac muscle constitute a unique signature for each tissue.

Summary of the Invention

[0008] The invention relates to cell supports compositions comprising a basement membrane extract isolated from cardiac or smooth muscle tissue. The compositions comprise molecules from extracellular matrices including, but not limited laminin, collagen, perlecan, and nidogen/entactin. In some embodiments, the composition may further comprise additional heparan sulfate proteoglycans.

[0009] The invention also relates to methods of using the novel compositions for supporting cellular functions, including but not limited to promotion of cell attachment, proliferation, differentiation, or maintenance of the phenotype of differentiated cells, or maintenance of the totipotency, pluripotency, multipotency of stem cells or progenitor cells through multiple culture passages *in vitro*

[0010] The invention also provides methods of applying or injecting the cell support compositions of the present invention in a subject in need of treatment such treatment.

[0011] The invention also relates to articles of manufacture comprising the cell support compositions of the present invention. For example, the invention provides for cell culture surfaces comprising the cell support compositions of the present invention.

Brief Description of the Drawings

[0012] Figure 1 depicts the protein content of the cell support compositions of the present invention. Equivalent amounts of protein from 4 preparations of human heart basement membrane extract (HBME) from individual donors and MATRIGEL™ (MG) were resolved by electrophoresis on 5% SDS-polyacrylamide gels. The pattern of polypeptides in HBME was very reproducible and distinct from that of MATRIGEL™. The majority of the protein in MATRIGEL™ is present as laminin 1—the alpha 1 chain migrates at 400 kD and the beta 1 and gamma 1 chains co-migrate at about 200 kD. A number of the polypeptide bands in the region of 180-400 kD in the HBME samples reacted positively with monospecific antibodies against known laminin chains. Similarly, specific antibodies to type IV collagen (alpha 1 and alpha 2 chains), type I collagen, nidogen/entactin and perlecan were able to bind to the HBME. Nidogen was also present. Tandem mass spec sequencing analysis provided further confirmation of the identity of these polypeptides and identified the presence of several non-basement membrane cytoskeletal proteins, including titin protein, myosin 7 and actinin.

[0013] Figure 2 depicts the attachment of cells to the cell support composition of the present invention. 20,000 of each cell type was allowed to attach to a surface coating of 10 $\mu\text{G}/\text{cm}^2$ for 1 hour at 37°C in serum free media. The Table in this figure shows the ratio of attachment to the cell support compositions of the present invention relative to MATRIGEL™. For stem cells and fibroblasts, the cell support compositions of the present invention promote roughly twice the level of attachment as compared to MATRIGEL™.

[0014] Figure 3 depicts the growth and maintenance of plasticity of pluripotent stem cells cultured on the cell support compositions of the present invention and on MATRIGEL™. Either the cell support compositions of the present invention or MATRIGEL™ was coated at $17 \mu\text{G}/\text{cm}^2$ were tested for the ability to support the growth and prevent the unwanted differentiation of embryonic and induced pluripotent stem cells in serum-free, chemically defined medium. Mouse embryonic stem cells (D3), mouse induced pluripotent stem cells (W5) or human induced pluripotent stem cells were plated and passaged 2 times on the indicated substrates. Proliferation rates and colony morphology was comparable. When the human induced pluripotent stem cells were stained at second passage using a live cell marker for pluripotency (Tra-1-60, stain alive, Stemgent) the green FITC signal indicates the cells were still in a pluripotent state.

Detailed Description of the Invention

[0015] The invention relates to a cell support composition comprising a basement membrane extract isolated from human cardiac or smooth muscle tissues. The compositions comprise molecules from extracellular matrices including, but not limited laminin, collagen, perlecan, and nidogen/entactin. In some embodiments, the compositions may further comprise additional heparan sulfate proteoglycans. The cell support compositions can be obtained from any mammal, such as but not limited to dogs, cats, horses, pigs, cows, non-human and human primates. It is understood that the components of the cell support compositions of the present invention will be species-specific base on the source of the tissue. For example, if the cell surface compositions of the present invention are extracted from human cardiac basement membrane, then the core components of the composition, *e.g.*, laminin, will be human laminin.

[0016] If the tissue from which the compositions are extracted is human tissue, the human tissue can, but need not, be obtained from certified tissue banks. The starting material, regardless of the animal source, can also be screened for bioburden, endotoxins and/or the presence of known pathogens. If the material is obtained from a tissue bank, it is likely that the tissue bank would perform the screening process to ensure safety and efficacy. Accordingly, the methods of preparing the cell support compositions of the present invention as disclosed below may or may not include steps of screening the material for bioburden, endotoxins and/or known pathogens. The screening steps may be performed on the starting material or the screening steps may be performed after extraction of the cell support compositions.

[0017] The compositions of the present invention comprise laminin type 2 and laminin type 10. In some embodiments, total laminin is present in the cell support compositions of the present invention in an amount from about 5% to about 50% by weight. As used herein, laminin type 2 is well understood in the art and is understood to mean a laminin molecule with an alpha 2 chain, a beta 1 chain and a gamma 1 chain. Laminin 10 is also well understood in the art and is understood to mean a laminin molecule with an alpha 5 chain, a beta 1 chain and a gamma 1 chain. In some embodiments, the compositions of the present invention also comprise one or more additional types of laminins, with these additional types of laminins containing an alpha 2, 3, 4 or 5 chain. Examples of laminins containing alpha chains 2, 3, 4 or 5 include but are not limited to, laminin type 2, laminin type 4, laminin type 5, laminin type 6, laminin type 7, laminin type 8, laminin type 9, laminin type 10, laminin type 11, laminin type 12, laminin type 13, laminin type 14, and laminin type 15. The molecular structure of laminin present in the compositions can be elucidated by using monoclonal antibodies specific for one of the chains the laminin type to isolate the laminin, which can then be analyzed using native gels or chromatography followed by mass spectroscopy and/or SDS-PAGE. One of skill in the art would easily be able to identify the laminin types based on well-known procedures in the art.

[0018] The compositions of the present invention do not contain any detectable levels of laminin type 1. In one embodiment, the compositions of the present invention lack detectable levels of laminin type 3. In another embodiment, the compositions of the present invention lack detectable levels of both laminin type 1 and laminin type 3. As used herein, laminin type 1 is well understood in the art and is understood to mean a laminin molecule with an alpha 1 chain, a beta 1 chain and a gamma 1 chain. Laminin type 3 is also well known in the art and is understood to mean a laminin molecule with an alpha 1 chain, a beta 2 chain and a gamma 1 chain. In another embodiment, the compositions of the present invention lack any detectable levels of any laminin that normally contains an alpha 1 chain.

[0019] In yet another embodiment, the compositions of the present invention do not contain any detectable levels of laminin type 5. In still another embodiment, the compositions of the present invention lack detectable levels of both laminin type 1 and laminin type 5. In still another embodiment, the compositions of the present invention lack detectable levels of both laminin type 3 and laminin type 5. In still another embodiment, the compositions of the present invention lack detectable levels of laminin type 1, laminin type 3 and laminin type 5. As used herein, laminin type 5 is well understood in the art and is understood to mean a laminin molecule with an alpha 3 chain, a beta 3 chain and a

gamma 2 chain. In another embodiment, the compositions of the present invention lack any detectable levels of any type of laminin that normally contains a beta 3 chain and/or a gamma 2 chain.

[0020] The compositions of the present invention comprise at least one type of collagen. In one embodiment, the composition comprises type IV collagen. In some embodiments, total collagen is present in the cell support compositions of the present invention in an amount from about 5% to about 50% by weight. In another embodiment, the composition comprises type I collagen. In yet another embodiment, the composition comprises type III collagen. In another embodiment, the composition comprises type XIII collagen. In another embodiment, the composition comprises type XVII collagen. In yet another embodiment, collagen type II is absent from the cell support compositions 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. The type IV collagen was identified by its unique molecular mass (180 kD) and reaction with multiple monospecific antibodies. 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. Nephrol.*, 7:96-104 (1993), which is incorporated by reference.

[0021] The compositions of the present invention comprise perlecan. Perlecan is a well known protein in the art that is also known as basement membrane-specific heparan sulfate proteoglycan core protein. In some embodiments, perlecan is present in the cell support compositions of the present invention in an amount from about 5% to about 60% by weight. Perlecan is essential for normal vascularization, critical for normal heart development and critical for regulating vascular responses to injuries.

[0022] The compositions of the present invention comprise entactin, which is also known as nidogen 1 protein. In some embodiments, entactin is present in the cell support compositions of the present invention in an amount from about 5% to about 50% by weight.

[0023] In some embodiments, the compositions of the present invention further comprise titin protein, which is also known as connectin protein. In some embodiments, the amount of titin protein, by weight, present in the cell support compositions of the present invention can be up to about 15%.

[0024] In some embodiments, the compositions of the present invention further comprise myosin 7 protein. In some embodiments, the amount of myosin protein, by weight, present in the cell support compositions of the present invention can be up to about 15%.

[0025] In some embodiments, the compositions of the present invention further comprise actinin protein. In some embodiments, the amount of actinin protein, by weight, present in the cell support compositions of the present invention can be up to about 7.5%. In still additional embodiments, the compositions of the present invention may comprise one or more of titin, myosin 7 and actinin, including all three molecules.

[0026] In select embodiments the compositions optionally comprise signaling molecules. In one embodiment, one or more signaling molecules are naturally present in cardiac or smooth muscle, such that the extract composition contains one or more signaling molecules normally present in the natural tissue, *i.e.*, “endogenous signaling molecules.” In another embodiment, one or more signaling molecules can be added to the extract such that the one or more signaling molecules present in the composition are not normally present in the natural tissue, *i.e.*, exogenous signaling molecules. In yet another embodiment, additional levels of one or more signaling molecules that are normally present in the natural tissue can be added to the extract such that the levels of a particular signaling molecule may be higher in the composition than would normally be after the extraction process, *i.e.*, “additional endogenous signaling molecules.” The exogenous signaling molecules or the additional endogenous signaling molecules added to the extracts of the invention need not be from the same animal source as the source of the extracted tissue, *e.g.*, mouse VEGF may be added to human-derived cardiac basement membrane extract. In addition, the exogenous signaling molecules and the additional endogenous signaling molecules added to the extract can be recombinant or isolated from an organism, cell or tissue. As used herein, the term “natural tissue” is used to mean heart muscle or smooth muscle that is either present in an organism or that has been physically removed from the organism, but prior to any processing steps, *e.g.*, enzyme digestion. Tissue that is removed and frozen and has not been subjected to any processing steps, *e.g.*, enzyme digestion and the like, is considered to be “natural tissue” for the purposes of the present invention.

[0027] As used herein, a “signaling molecule” is a molecule that either initiates an intracellular signal or signaling pathway or is an intermediate molecule in the signaling or pathway process. A “signal” is a stimulus that creates a cellular response thereto. Signaling molecules can include any molecule that either initiates or participates in the signaling pathway. For example, signaling molecules include but are not limited to growth factors, cytokines and chemokines that may or may not be present in the natural tissue.

[0028] Examples of signaling molecules include but are not limited to fibroblast growth factors (FGF), *e.g.*, FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, FGF7, FGF8, FGF9, FGF10, FGF11, FGF12, FGF13, FGF14, FGF15, FGF16, FGF17, FGF18, FGF19, FGF20, FGF21, FGF22, FGF23, epidermal growth factor (EGF), nerve growth factor (NGF), platelet derived growth factor (PDGF-AA, AB and BB), insulin-like growth factors, *e.g.*, IGF-1 and 2, transforming growth factor beta, *e.g.*, TGF-beta 1,2 or 3, vascular endothelial cell growth factor, *e.g.*, VEGF-A, VEGF-B, VEGF-C, VEGF-D, placental growth factor, tumor necrosis factor alpha (TNF-alpha), TNF-beta, bone morphogenetic factors *e.g.*, BMP1, BMP2, BMP3, BMP4, BMP5, BMP6, BMP7, BMP8, BMP9, BMP10, BMP11, BMP12, BMP13, BMP14, and BMP15, Leukemia inhibitory factor (LIF-1), endostatin, angiostatin, thrombospondin, osteogenic protein-1, osteonectin, somatomedin-like peptide, osteocalcin, interferons (IFN), such as but not limited to IFN Type I, IFN Type II and IFN Type III, examples of all of which include but are not limited to IFN-alpha, IFN, beta, IFN-gamma and IFN-omega, interleukins (IL), *e.g.*, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-32, IL-33 and IL-35 and stromal-derived factor (SDF-1) to name a few.

[0029] The compositions of the present invention may or may not be polymerized in any setting used. The cell support compositions of the present invention are liquid at temperatures of between about 0°C and about 8°C at normal atmospheric pressure. The compositions, however, will polymerize within about 2-10 hours when the temperature is raised to at least about 22°C or within about 20 minutes to about 1 hours at 37°C. As used herein, the term “polymerize” or “gelation” when used in conjunction with the cell support compositions of the present invention means that the compositions form a gel. The term gel is well understood in the art and, in general, means a cross-linked network of molecules dispersed in a liquid that generally exhibits little to no flow. As used herein, the terms “gelation” and “polymerization” are interchangeable when used in conjunction with the cell support compositions of the present invention.

[0030] In some embodiments, the cell support compositions further comprise materials that are added to the compositions. The additional materials may be natural or synthetic, or a combination thereof. Examples of natural materials include, but are not limited to, amino acids, peptides, polypeptides, proteins, carbohydrates, lipids, nucleic acids, glycoproteins, glycosaminoglycans, and proteoglycans. Examples of synthetic materials include, but are not limited to, polymers such as poly (lactic acid) (PLA), polyglycolic acid (PGA), copolymers of PLA and PGA, polycaprolactone, poly (ethylene-

co-vinyl acetate), (EVOH), poly (vinyl acetate) (PVA), polyethylene glycol (PEG) and poly (ethylene oxide) (PEO).

[0031] The cell support compositions of the present invention can be used in cell culturing techniques. As used herein, cell culture refers to the maintenance of cells in an artificial environment, commonly referred to as an *in vitro* environment. The term cell culture is a generic term and may be used to encompass the cultivation not only of individual cells, but also of tissues, organs, organ systems or whole organisms. The cells used in the culture methods disclosed herein can be any prokaryotic or eukaryotic cell. The cell type used in the culture methods disclosed herein need not be from the same species from which the cell support compositions derive. In addition, the cells may be from an established cell line, or they may be primary cells or genetically engineered cells.

[0032] The cell support compositions of the present invention can be used in *in vitro* methods for supporting cell growth and proliferation as well as for sustaining or maintaining the plasticity of stem cells in culture. Accordingly, the invention provides methods of maintaining the plasticity of stem cells with the methods comprising culturing stem cells on a cell culture surface that comprises the cell support compositions of the present invention. In one embodiment, the cell support composition is polymerized prior to culturing the stem cells on the cell surface.

[0033] As used herein, a stem cell is used as it is in the art and means a cell that has the ability to divide and give rise to one daughter cell that may be at least partially differentiated and to another daughter cell that retains the developmental potential of the mother cell. As used herein, stem cells can be adult stem cells (ASCs), embryonic stem cells (ESCs), committed progenitor cells, and/or induced pluripotent stem cells (iPSCs).

[0034] The terms “cell” and “cell line” may be used interchangeably herein. In one embodiment, stem cells that are attached to cell culture surfaces coated with the cell support compositions of the present invention and are able to maintain their state of plasticity after attachment. Specifically, cells attached on surfaces coated the cell support compositions of the present invention can maintain their state of plasticity for up to at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 passages or even more. As used herein, “state of plasticity” is used to mean the development potential that the cells have, such as, but not limited to, pluripotent, totipotent, multipotent or unipotent cells. Maintaining a state of plasticity indicates that cell daughter cells, after division, are equally as plastic as the parent cells, *e.g.*, the daughter cells are pluripotent like the

pluripotent parent cell. In another embodiment, the cells that are attached to surfaces coated with the cell support compositions of the present invention are able to maintain at least a partial state of plasticity after attachment. Specifically, the cells attached on surfaces coated with the cell support compositions of the present invention can maintain at least a partial state of plasticity for up to at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 passages or even more. As used herein, "partial state of plasticity" is used to mean the development potential that the daughter cells have, such as, but not limited to, pluripotent, totipotent or unipotent cells. Maintaining at least a partial state of plasticity indicates that cell daughter cells, after division, are not as plastic as the parent cells but nonetheless are still in an undifferentiated state, *e.g.*, the daughter cells are multipotent whereas the parent cell was considered to be pluripotent.

[0035] The terms pluripotent, totipotent, multipotent and unipotent are used as they are in the art. Namely, a pluripotent cell is a cell that, depending on environment, has the potential to develop into any type of mature cell found in the organism from which it derived, except germ cells. A totipotent cell is a cell that, depending on environment, has the potential to develop into any type of mature cell found in the organism from which it derived, including germ cells. A multipotent cell is a cell that, depending on environment, has the potential to develop into several different types of mature cell found in the organism from which it derived, usually 2 or more. One example of a multipotent cell is a hematopoietic stem cell. A unipotent cell is a cell that, depending on environment, has the potential to develop into one type of mature cell found in the organism from which it derived. Unipotent cells are not completely differentiated cells, but may be partially differentiated or completely undifferentiated.

[0036] The cell support compositions of the present invention can be used in methods for supporting cell growth and proliferation as well as for sustaining or maintaining the state of differentiation of differentiated or partially differentiated cells, with the methods comprising culturing the differentiated or partially differentiated cells on a cell culture surface that comprises the cell support compositions of the present invention. In one embodiment, the cell support composition is polymerized prior to culturing the differentiated or partially differentiated cells on the cell surface. In one embodiment, the differentiated or partially differentiated cells that are attached to cell culture surfaces coated with the cell support compositions of the present invention and are able to maintain their state of differentiation after attachment. Specifically, differentiated or partially differentiated cells attached on surfaces coated the cell support compositions of the present invention can maintain their state of differentiation for up to at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85,

90, 95 or 100 passages or even more. As used herein, “state of differentiation” is used to mean the development state of the cells after division. Maintaining a state of differentiation means that the daughter cells are at least as differentiated, and perhaps more so, than the parent cell. The differentiated or partially differentiated cells may or may not divide when cultured on surfaces coated with the cell support compositions of the present invention.

[0037] In one embodiment, neuronal precursors are seeded onto cell culture surfaces comprising the cell support compositions of the present invention. After adhesion, the cells will extend neurites and express neuron-specific genes in the process of neuronal differentiation. In another embodiment, endothelial cells are seeded are seeded onto cell culture surfaces comprising the cell support compositions of the present invention. After adhesion, the cells will associate to form branching tubular structures with lumens resembling blood vessels. These processes may or may not be inhibited in the presence of excess free peptides derived from the laminin binding sites like IKVAV.

[0038] In one embodiment, neuronal precursors are seeded onto cell culture surfaces comprising the cell support compositions of the present invention. After adhesion, the cells will extend neurites and express neuron-specific genes in the process of neuronal differentiation. In another embodiment, endothelial cells are seeded are seeded onto cell culture surfaces comprising the cell support compositions of the present invention. After adhesion, the cells will associate to form branching tubular structures with lumens resembling blood vessels. These processes may or may not be inhibited in the presence of excess free peptides derived from the laminin binding sites like IKVAV.

[0039] The cells may be from an established cell line, or they may be primary cells or genetically engineered cells. For example, neovascularization can be stimulated by angiogenic and growth-promoting factors, administered as peptides, proteins or as gene therapy. Angiogenic agents can be incorporated into the cell support compositions by culturing on the cell support compositions (or adding genetically engineered cells to unpolymerized cell support compositions) genetically engineered cells that are engineered to produce, for example, VEGF. Alternatively, where neovascularization is not desired, antiangiogenic agents can be incorporated into the cell support compositions by culturing on the cell support compositions (or adding genetically engineered cells to unpolymerized cell support compositions) genetically engineered cells that are engineered to produce, for example, angiostatin.

[0040] The term “culture surface” as used herein means a surface on which cells or tissue can be cultured. The culture surface may, for example, be glass or plastic, or polymers such as alginate. Other

surfaces include but are not limited to, biopolymers such as PLAGA (polylactic acid glycolic acid copolymer), PLA (polylactic acid), PCL (polycaprolactone), PDO (polydione), PEO (polyester), metals such as but not limited to, stainless steel or titanium, glass, other forms of plastic besides polystyrene, such as but not limited to polycarbonate, polyallomer, polyethylene, and any composition of calcium phosphate crystal including but not limited to hyaluronic acid. Culture surfaces include, but are not limited to, single and multiwall culture plates, chambered and multi-chambered culture slides, cover-slips, cups, flasks, tubes, bottles, roller bottles, spinner bottles, perfusion chambers, bioreactors, fermenters and the like.

[0041] In one embodiment, the cell support compositions of the present invention can be fabricated into a scaffold, for cell culture or tissue engineering applications. For example, the cell support compositions of the present invention can be fabricated into nanofibers and mesh through electrospinning technologies as described in US2010/0120115, which is incorporated by reference. The term nanofiber as used herein means a fiber comprising a diameter of about 1000 nanometers or less. Once fabricated into a scaffold, the cell support compositions of the present invention can be placed into cell culture environment as disclosed herein.

[0042] The cell support compositions of the present invention can be used in *in vivo* methods as well. For example, in one embodiment of the present invention, the cell support composition can be used as a tissue regenerative composition for repair or replacement of tissues in an *in vivo* setting. Thus the present invention provides therapeutic methods, with the methods comprising administering the cell support compositions of the present invention to a subject in need of such therapy. The cell support compositions can be used in a variety of tissues or organs, including but not limited to, cardiac tissue, bone tissue, ligament tissue, tendon tissue, skin, muscle tissue, vasculature, liver tissue, lung tissue, and the like. The tissue to which the cell support compositions can be applied may be diseased, normal, damaged or even dead. The tissues or organs to which the cell support compositions can be applied would include all compartments or subdivisions of the tissue or organ. For example, cardiac tissue includes, but is not limited to, diseased, damaged, or missing heart tissue including myocardium, epicardium, endocardium, pericardium. For example, the cell support compositions of the present invention can be fluidized or maintained as a fluid, powdered, or pulverized and applied to or injected into or adjacent to diseased or defective cardiac tissue to promote tissue repair.

[0043] As used herein, the term “administer” and “administering” are used to mean introducing at least one compound or composition into a subject. When administration is for the purpose of treatment, the substance is provided at, or after the diagnosis of an abnormal condition, such as an infarction.

[0044] The cell support compositions may also be coadministered with other compounds or compositions. As used herein, the term “coadminister” is used to mean that each of at least two compounds are administered during a time frame wherein the respective periods of biological activity overlap. Thus the term includes sequential as well as coextensive administration of the compositions of the present invention. If more than one substance is coadministered, the routes of administration of the two or more substances need not be the same. The scope of the invention is not limited by the identity of the substance which may be coadministered with the compositions of the present invention.

[0045] For embodiments in which the cell support compositions are used *in vivo*, the cell support compositions can further comprise one or more therapeutic molecules including, without limitation, any pharmaceutical or drug. Examples of pharmaceuticals include, but are not limited to, anesthetics, hypnotics, sedatives and sleep inducers, antipsychotics, antidepressants, antiallergics, antianginals, antiarthritics, antiasthmatics, antidiabetics, antidiarrheal drugs, anticonvulsants, antigout drugs, antihistamines, antipruritics, emetics, antiemetics, antispasmodics, appetite suppressants, neuroactive substances, neurotransmitter agonists, antagonists, receptor blockers and reuptake modulators, beta-adrenergic blockers, calcium channel blockers, disulfuram and disulfuram-like drugs, muscle relaxants, analgesics, antipyretics, stimulants, anticholinesterase agents, parasympathomimetic agents, hormones, anticoagulants, antithrombotics, thrombolytics, immunoglobulins, immunosuppressants, hormone agonists/antagonists, vitamins, antimicrobial agents, antineoplastics, antacids, digestants, laxatives, cathartics, antiseptics, diuretics, disinfectants, fungicides, ectoparasitocides, antiparasitics, heavy metals, heavy metal antagonists, chelating agents, gases and vapors, alkaloids, salts, ions, autacoids, digitalis, cardiac glycosides, antiarrhythmics, antihypertensives, vasodilators, vasoconstrictors, antimuscarinics, ganglionic stimulating agents, ganglionic blocking agents, neuromuscular blocking agents, adrenergic nerve inhibitors, anti-oxidants, vitamins, cosmetics, anti-inflammatories, wound care products, antithrombogenic agents, antitumoral agents, antiangiogenic agents, anesthetics, antigenic agents, wound healing agents, plant extracts, growth factors, emollients, humectants, rejection/anti-rejection drugs, spermicides, conditioners, antibacterial agents, antifungal agents, antiviral agents, antibiotics, tranquilizers, cholesterol-reducing drugs, antitussives, histamine-blocking drugs, monoamine oxidase

inhibitor. All substances listed by the U.S. Pharmacopeia are also included within the substances of the present invention.

[0046] The cell support compositions of the present invention may or may not further comprise objects. Examples of objects include, but are not limited to, tablets, vesicles, liposomes, capsules, nanoparticles, and other structures that could enclose molecules. In some embodiments, the additional objects comprise vesicles, liposomes, capsules, or other enclosures that contain compounds that are released at a time such as at the time of implantation or upon later stimulation or interaction. In one illustrative embodiment, transfection agents such as liposomes contain desired nucleotide sequences that can be incorporated into cells that are located in or on the cell support compositions.

[0047] The cell support compositions of the present invention may or may not further comprise additional amino acids, proteins or peptides that are not necessarily signaling molecules. Examples include, but are not limited to, structural proteins, enzymes, and peptide hormones. These additional proteins and peptides compounds can serve a variety of functions. In some embodiments, the matrix may contain peptides containing a sequence that suppresses enzyme activity through competition for the active site. In other applications, antigenic agents that promote an immune response and invoke immunity can be incorporated into a construct.

[0048] The cell support compositions of the present invention may or may not further comprise additional nucleic acids. Examples of nucleic acids include, but are not limited to deoxyribonucleic acid (DNA), ent-DNA, oligonucleotides, aptamers, and ribonucleic acid (RNA). Embodiments involving DNA include, but are not limited to, cDNA sequences, natural DNA sequences from any source, and sense or anti-sense oligonucleotides. For example, DNA can be naked (e.g., U.S. Pat. Nos. 5,580,859; 5,910,488) or complexed or encapsulated (e.g., U.S. Pat. Nos. 5,908,777; 5,787,567). DNA can be present in vectors of any kind, for example in a viral or plasmid vector. In some embodiments, nucleic acids used will serve to promote or to inhibit the expression of genes in cells inside and/or outside the present composition. The nucleic acids can be in any form that is effective to enhance uptake into cells.

[0049] The present invention also provides methods of preparing basement membrane extracts that has been isolated from cardiac or smooth muscle tissue from an organism. As used herein, isolated means that the material has been removed from its native environment, regardless of the level of impurities that may or may not be present. For example, cardiac basement membrane has been isolated when it has been removed or extracted from cardiac tissue. As used herein, the isolated

material can be placed back into a setting that resembles, substantially resembles or is identical to the original environment from which the material was isolated. For example, the isolated cardiac basement membrane extract may be inserted or introduced back into cardiac tissue of another organism. Any degree of purification or concentration greater than that which occurs in the natural setting of the compound, including, but not limited to, (1) purification from other associated structures or compounds or (2) association with structures or compounds to which the material is not normally associated is included in the term purified.

[0050] The following examples are illustrative and are not intended to limit the scope of the invention described herein.

Examples

[0051] *Example 1: Process for isolating basement membrane components from cardiac muscle*

[0052] Methods for preparing the isolated human basement membrane extract (HBME) can be conducted at a temperature of about 0°C, 4°C, 15°C, 24°C, 27°C, 30°C, or 37°C. The solutions used for the isolation process can be chilled or warmed to the appropriate temperature before starting the process.

[0053] Frozen, thinly sliced or minced pieces of cardiac tissue were washed or not washed with a buffer such as phosphate or potassium or calcium chloride at a concentration such as 0.4M to 0.05M for a period of time such as 1,2,3,4,...24 hour. After washing ice cold 3.0 M, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0 NaCl or KCl buffer is added to tissue including protease inhibitors such as PMSF, Aprotinin, leupeptin, pepstatin A, NEM, phenanthroline, Benzamidine, AEBSF, Bestatin, E-64 (volume to weight 2:1, 3:1, 4:1, 5:1, 6:1, 7:1) and homogenized.

[0054] The homogenized slurry was then centrifuged and the supernatant was poured off and the pellets collected in a tripour beaker. Another aliquot of 3.0 M, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0NaCl or KCl buffer was added as above and the pellets were washed and homogenized. After homogenization, the slurry was again centrifuged. These washes and homogenizations were repeated 3-5 times.

[0055] After repeated washing of the processed tissue, 2 M Urea Buffer was added at a concentration of 1 ml/gm starting weight of tissue. The mixture was stirred overnight. The mixture was then

centrifuged and decanted. The supernatant was saved for later use. The pellet was recovered and homogenized again in 2 M urea buffer (half the volume as the first use of the Urea Buffer) stirred at least 2 hours.

[0056] The mixture was centrifuged again and the supernatant was recovered and added to the first volume of supernatant recovered above. The mixture was dialysed against a buffer such as phosphate, TEA, Bicine, MOPS, Tris, Hepes, TAPSO, PIPES, MES, or other Good's buffers. The last buffer used can be DMEM, DMEM/F12, HEPES, Hanks or other cell culture media. Antibiotics can be added to the extract and stored at 4°C or long term at -80°C. The concentration of the mixture is determined by dialyzing the mixture against dH₂O and lyophilizing in tared containers and weighing when dry.

[0057] *Example 2: Coating Cell Culture Surfaces with Isolated HMBE*

[0058] The protein needed to coat the plate at each concentration is calculated at about 10-20 µg/cm². Other supplemental purified matrix molecules are coated in the range of 5-20 µg/cm². MATRIGEL™ is coated at 17.5µg /cm² according to the WiCell protocol and the cell support composition product may be used at this concentration or less for some applications or at higher concentration for other applications.

[0059] For dry coating, 100µl of the cell support composition was pipetted into each well of plate at the desired concentration and allowed to dry uncovered in biosafety cabinet. After coating, the plates were washed very gently with 1-2 volumes of a typical cell culture buffer such as culture media, PBS or Hanks balanced salt solution.

[0060] After washing, 200µl of sterile buffer containing 2% albumin can be added to each well and incubated for 15 min room temperature. After incubation, the albumin mixture is removed and media is added to wash the plate.

[0061] *Example 3: Cell Attachment Assay*

[0062] Plates were coated at various concentrations of the cell support compositions of the present invention (see Figure 2) to test cell attachment using 3T3, BHK and adipose stem cells. Cells were allowed to attach to plates coated with the indicated amounts of the cell support compositions of the present invention for 60 minutes in the absence of serum. After attachment, the plates were gently

rinsed to remove unattached cells and attached cells were counted. Similar results were observed with human mesenchymal stem cells.

[0063] *Example 4: Differentiation Assays of Attached Cells*

[0064] Adipose derived stem cells are isolated using well known procedures and are plated at a density of about 5,000 cells/100 mm dish. The dishes have been coated with isolated the cell support compositions of the present invention, and the cells are cultured for a few days after plating. After successive rounds of cell division, some clones are picked with a cloning ring and transferred to wells in a 48 well plate, where the wells have also been coated with isolated the cell support compositions of the present invention. These cells are cultured for several weeks, changing the medium twice weekly, until they are about 80% to about 90% confluent (at 37°C, in about 5% CO₂ in 2/3 F₁₂ medium (with 20% fetal bovine serum) and 1/3 standard medium). Thereafter, each culture is transferred to a 35 mm dish (coated with the cell support compositions of the present invention) and grown, and then retransferred to a 100 mm dish (coated with isolated the cell support compositions of the present invention) and grown until close to confluence. Following this, one cell population is frozen, and the remaining populations were plated on 12 well plates, at 1000 cells/well.

[0065] The cells are cultured for more than 15 passages in medium and on surfaces coated with the cell support compositions of the present invention and monitored for signs of differentiation. The undifferentiated state of each clone remains true after successive rounds of division.

[0066] Populations of the clones are then established on culture surfaces not coated with the cell support compositions of the present invention and then exposed to adipogenic, chondrogenic, myogenic, and osteogenic medium, or other medium or factors known to promote differentiation of the stem cells. The clones are able to differentiate into bone, fat, cartilage, and muscle when exposed to the respective media as determined by phenotypic assays of protein expression and visual inspection of cell types.

[0067] *Example 5: SDS-PAGE Analysis of HMBE*

[0068] An SDS-PAGE was performed on the cell support compositions of the present invention using standard procedures. The gel showed that the cell support compositions of the present invention is an aggregate of macromolecules purified from human cardiac tissue. These polypeptides range in size

between 350 and 100 kilodaltons (kDa) and cross-react with antibodies against mouse and human laminin, type IV collagen and heparan sulfate proteoglycan.

[0069] *Example 6: Process for isolating basement membrane components from smooth muscle*

[0070] Frozen, thinly sliced or minced pieces of smooth muscle tissue are washed with buffers such as Phosphate or potassium or Calcium Chloride then added to cold 3.4M NaCl or KCl buffer (3X volume to weight) including protease inhibitors such as PMSF, Aprotinin, leupeptin, pepstatin A, NEM, phenanthroline, Benzamidine, AEBSF, Bestatin, E-64 and homogenized.

[0071] The homogenized slurry is then centrifuged and the supernatant is poured off and the pellets collected. Another aliquot of 3.4M NaCl buffer is added as above and the pellets are homogenized. After homogenization, the slurry is again centrifuged. These homogenizations are repeated 4-5 times.

[0072] After repeated washing by homogenization of the processed tissue, 2 M Urea Buffer is added at a concentration of 1 ml/gm starting weight of tissue. The mixture is stirred overnight. The mixture is then centrifuged and decanted. The supernatant is saved for later use. The pellet is recovered and homogenized again in 2 M urea buffer (half the volume as the first use of the Urea Buffer) stirred at least 2 hours.

[0073] The mixture is centrifuged again and the supernatant is recovered and added to the first volume of supernatant recovered above. Antibiotics can be added to the combined supernatant and stored at 4°C or long term at -80°C.

[0074] *Example 7 – Direct Injection of the Cell Support Composition into a Subject*

[0075] The cell support composition as prepared according to Example 1 is prepared for injection into damaged cardiac tissue. A procedure is administered to mice to induce myocardial infarction as disclosed in Salto-Tellez, M. *et al.*, *Cardiovascular Path.*, 13(2):91-97 (2004). Specifically, a coronary artery is ligated to cause infarction. After reperfusion of the vessel, a cell support composition of the present invention is injected into the damaged cardiac tissue.

[0076] The injections can be single injections or multiple injections over time. A single injection of the cell support composition of the present invention improves heart function as measured by normal parameters, such as ejection volume, end systolic volume, stroke volume and echocardiography. In as