



Repair. Regenerate. Restore.

Embodiment, Inc. and Zimmer Biomet Holdings, Inc.,
v.

LifeNet Health

Case No. IPR2025-00248
U.S. Patent 10,237,223

Case No. IPR2025-00249
U.S. Patent 11,318,227

Petitioners' Hearing Demonstratives

May 6, 2026

'223 Patent (IPR2025-00248)

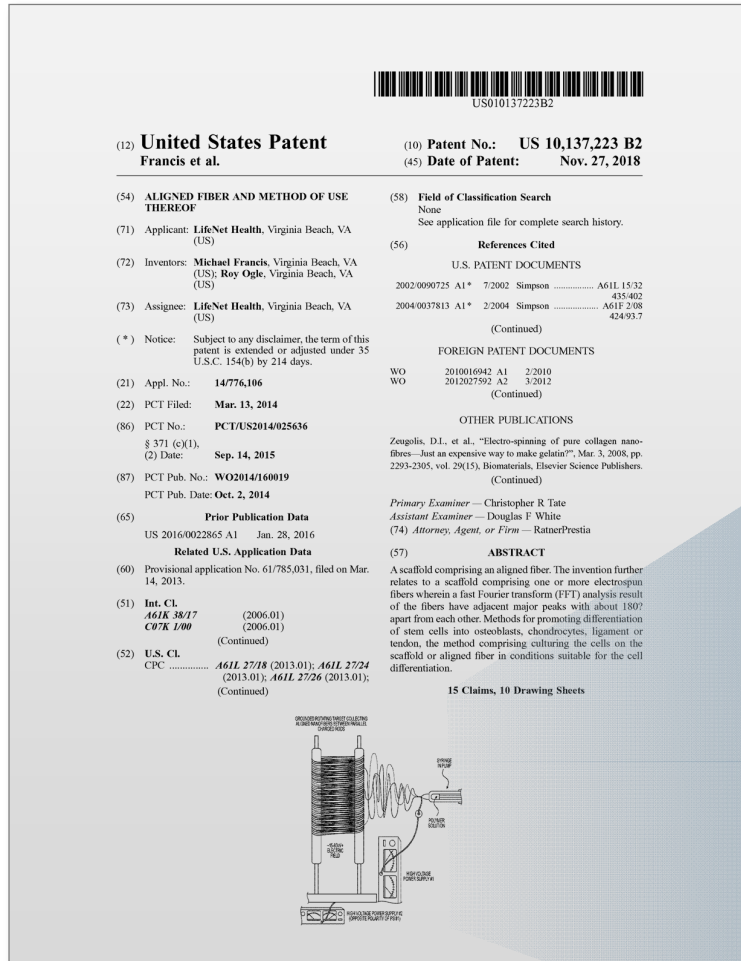
'223 Patent (IPR2025-00248)	Ground 1	Huang2 anticipates claims 1-3 and 5-12
	Ground 2	Huang2 in view of Kumbar renders claim 4 obvious
	Ground 3	Lee anticipates claims 1-12
	Ground 4	Lee renders claims 1-12 obvious

'227 Patent (IPR2025-00249)

'227 Patent (IPR2025-00249)	Ground 1	Claims 1-6 of the '227 patent would have been obvious in view of Huang2 when read in combination with Kuo
	Ground 2	Lee alone or in view of Kuo renders claims 1-6 obvious
	Ground 3	Huang2 anticipates claims 7-8

The '223 Patent

'223 Patent (Ex. 1001)



(12) United States Patent Francis et al.

(10) Patent No.: US 10,137,223 B2
(45) Date of Patent: Nov. 27, 2018

(54) ALIGNED FIBER AND METHOD OF USE THEREOF

(71) Applicant: LifeNet Health, Virginia Beach, VA
(US)

(72) Inventors: Michael Francis, Virginia Beach, VA
(US); Roy Ogle, Virginia Beach, VA
(US)

(73) Assignee: LifeNet Health, Virginia Beach, VA
(US)

(58) Field of Classification Search
None

See application file for complete search history.

(56) References Cited

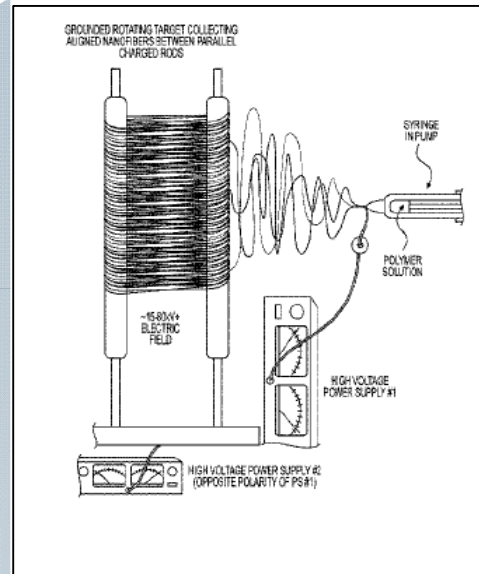
U.S. PATENT DOCUMENTS

2002/0090725 A1* 7/2002 Simpson A61L 15/32
435/402
2004/0037813 A1* 2/2004 Simpson A61F 2/08
424/93.7

(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.

(Ex. 1001) '223 Patent, cover and abstract



'223 Patent (Ex. 1001)

FIG. 6 depicts aligned heart base membrane fiber scaffold that allow dorsal root ganglia attachment and outgrowth.

FIG. 7 depicts aligned placenta and heart base membrane fiber scaffolds that allow dorsal root ganglia attachment and outgrowth.

FIG. 8 depicts exemplary FFT analysis results (a) of the fibers described herein and (b) a control sample.

(Ex. 1001) '223 patent at 1:51-57

fibers. A fast Fourier transform (FFT) was performed to indicate the degree of alignment as seen in FIG. 3 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.

(Ex. 1001) '223 patent at 21:26-31

'223 Patent (Ex. 1001)



US010137223B2

(12) **United States Patent**
Francis et al.

(10) **Patent No.:** US 10,137,223 B2
(45) **Date of Patent:** Nov. 27, 2018

(54) **ALIGNED FIBER AND METHOD OF USE THEREOF**

(71) Applicant: LifeNet Health, Virginia Beach, VA (US)

(72) Inventors: Michael Francis, Virginia Beach, VA (US); Roy Ogle, Virginia Beach, VA (US)

(73) Assignee: LifeNet Health, Virginia Beach, VA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 214 days.

(21) Appl. No.: 14/776,106

(22) PCT Filed: Mar. 13, 2014

(86) PCT No.: PCT/US2014/025636
§ 371 (c)(1),
(2) Date: Sep. 14, 2015

(87) PCT Pub. No.: WO2014/160019
PCT Pub. Date: Oct. 2, 2014

(65) **Prior Publication Data**
US 2016/0022865 A1 Jan. 28, 2016
Related U.S. Application Data
(60) Provisional application No. 61/785,031, filed on Mar. 14, 2013.

(51) **Int. Cl.**
A61K 38/17 (2006.01)
C97K 1/00 (2006.01)
(Continued)

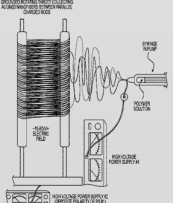
(52) **U.S. Cl.**
CPC A61L 27/18 (2013.01); A61L 27/24 (2013.01); A61L 27/26 (2013.01);
(Continued)

(58) **Field of Classification Search**
None
See application file for complete search history.

(56) **References Cited**
U.S. PATENT DOCUMENTS
2002/0090725 A1* 7/2002 Simpson A61L 15/32
2004/0037813 A1* 2/2004 Simpson A61F 2/08
424,93.7
(Continued)
FOREIGN PATENT DOCUMENTS
WO 2010016942 A1 2/2010
WO 2012027592 A2 3/2012
(Continued)
OTHER PUBLICATIONS
Zeugolis, D.L., et al., "Electro-spinning of pure collagen nano-fibres—just an expensive way to make gelatin?", Mar. 3, 2008, pp. 2293-2305, vol. 29(15), Biomaterials, Elsevier Science Publishers.
(Continued)
Primary Examiner—Christopher R Tate
Assistant Examiner—Douglas F White
(74) **Attorney, Agent, or Firm**—RatnerPrestia

(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.

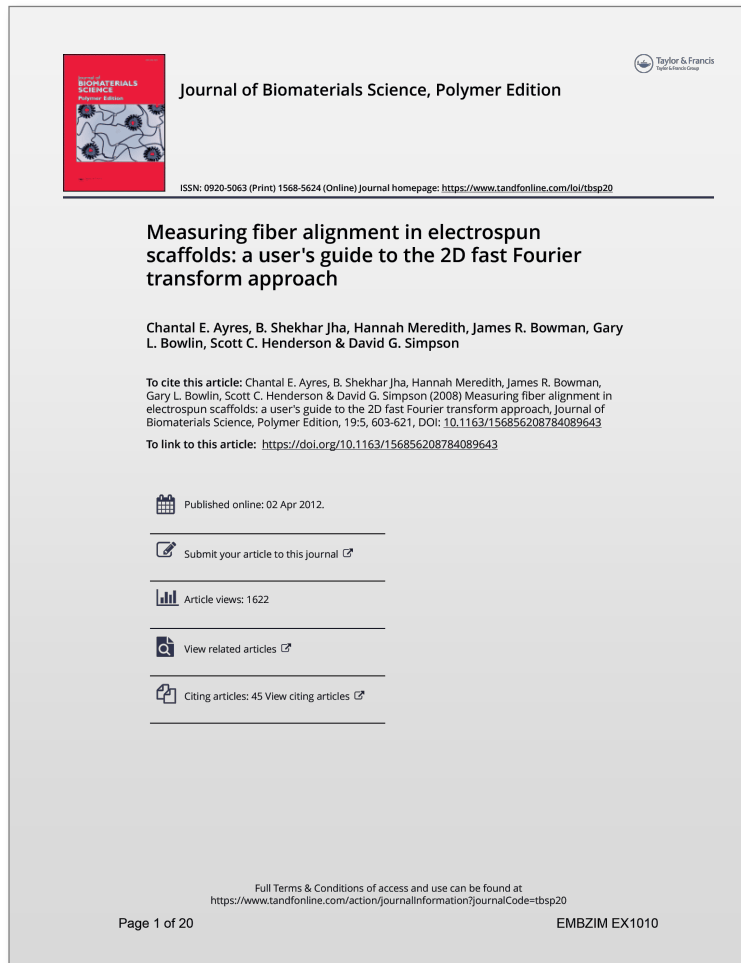
15 Claims, 10 Drawing Sheets



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 C E, Jha B S, Meredith H, Bowman J R, Bowlin G L, Henderson S C, Simpson D G. 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 FIG. 8.

(Ex. 1001) '223 Patent at 13:16-26

Ayres 2008 (Ex. 1010)



Measuring fiber alignment in electrospun scaffolds: a user's guide to the 2D fast Fourier transform approach

Chantal E. Ayres, B. Shekhar Jha, Hannah Meredith, James R. Bowman, Gary L. Bowlin, Scott C. Henderson & David G. Simpson

evidence that fiber alignment and overall scaffold anisotropy play central roles in defining the material properties of electrospun materials [10–12]. Anisotropic

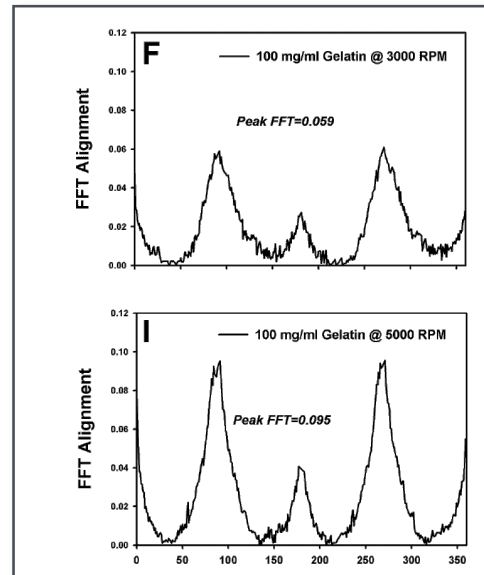
Morphological techniques and destructive materials testing strategies have been used to illustrate and detect anisotropy in electrospun materials. For example,

cells within an engineered tissue [13, 14]. Our goal is to develop non-invasive techniques that can be used to objectively measure fiber alignment and overall scaffold anisotropy.

its data acquisition to surface features and the sample is lost to the analysis. In this review, we describe how a two-dimensional fast Fourier transform (2D FFT), readily available in a number of programs designed for image analysis, can be coupled with a variety of optical imaging modalities to rapidly measure scaffold anisotropy [10, 12].

(Ex. 1010 at 603-604)

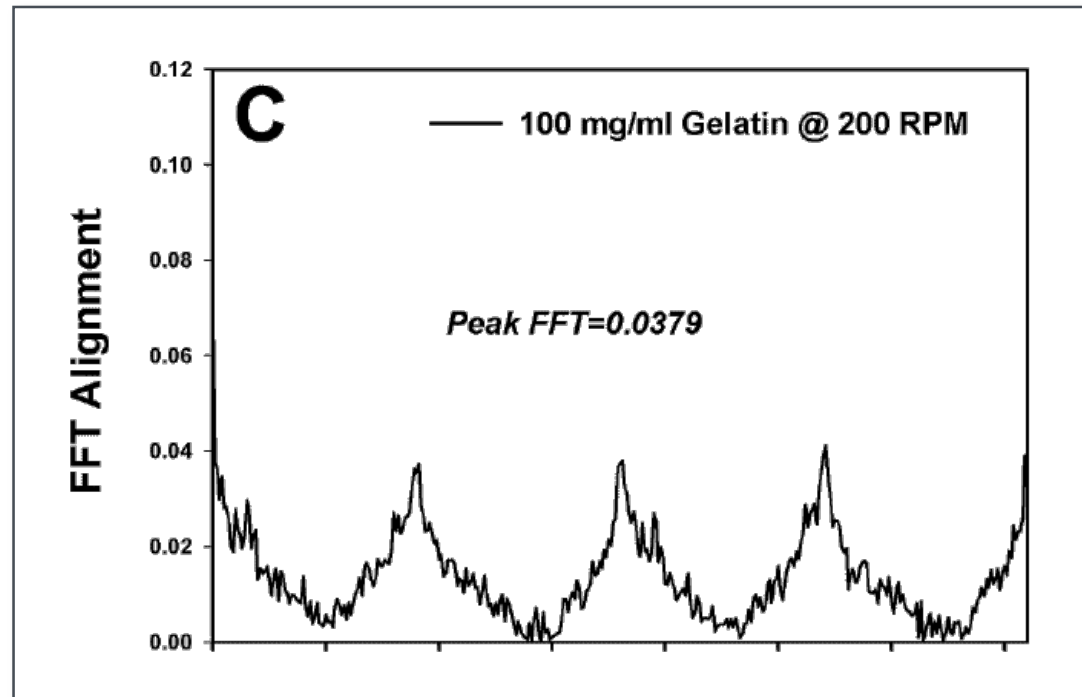
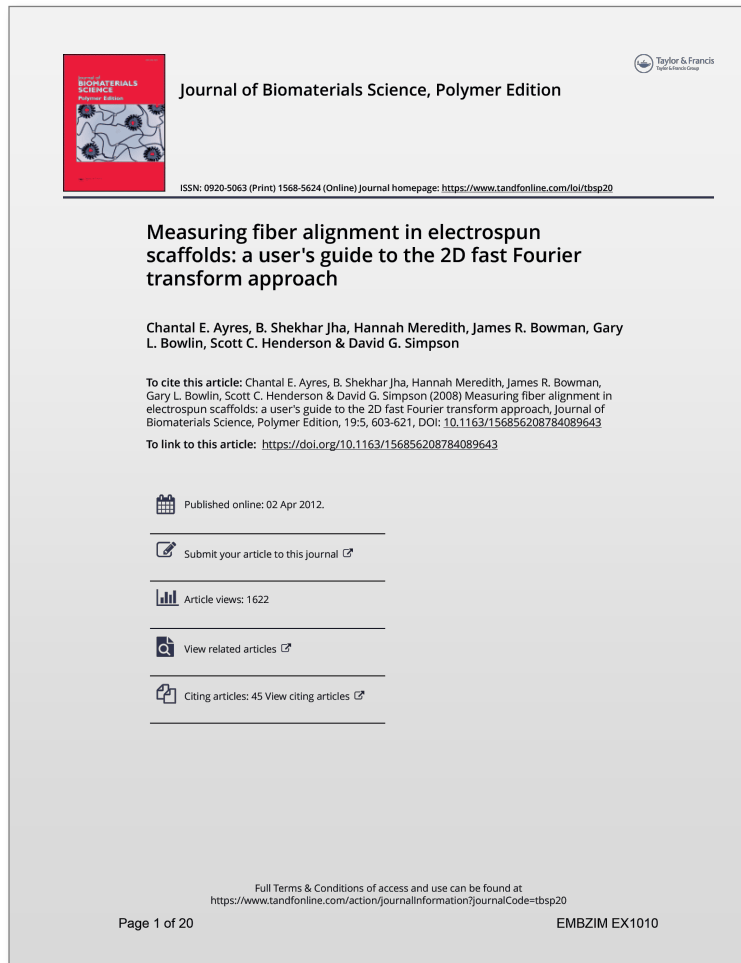
Ayres 2008 (Ex. 1010)



270° and two smaller peaks at 0 and 360° (Fig. 1F and I). The degree of alignment present in the original data image is reflected by the height and overall shape of the most prominent peaks present in the alignment plot. A high and narrow peak indicates a more uniform degree of fiber alignment, a broad peak, or a shoulder on the peak indicates that more than one axis of alignment may be present. The principal axis of fiber alignment is determined by the position of the alignment peaks, in the illustrated examples (Fig. 1F and I) the principle axis of alignment is in the vertical direction in the processed image.

(Ex. 1010 at 608, 610)

Ayres 2008 (Ex. 1010)



The 2D FFT alignment plot of a random matrix will exhibit 4 peaks that occur every 90°. Peaks of nearly uniform height are a hallmark of a scaffold composed of random elements. These peaks reflect low-frequency spatial information and

(Ex. 1010 at 608-609)

Ayres 2008 (Ex. 1010)



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Measuring fiber alignment in electrospun scaffolds: a user's guide to the 2D fast Fourier transform approach

Chantal E. Ayres, B. Shekhar Jha, Hannah Meredith, James R. Bowman, Gary L. Bowlin, Scott C. Henderson & David G. Simpson

To cite this article: Chantal E. Ayres, B. Shekhar Jha, Hannah Meredith, James R. Bowman, Gary L. Bowlin, Scott C. Henderson & David G. Simpson (2008) Measuring fiber alignment in electrospun scaffolds: a user's guide to the 2D fast Fourier transform approach, Journal of Biomaterials Science, Polymer Edition, 19:5, 603-621, DOI: 10.1163/156856208784089643

To link to this article: <https://doi.org/10.1163/156856208784089643>

Published online: 02 Apr 2012.

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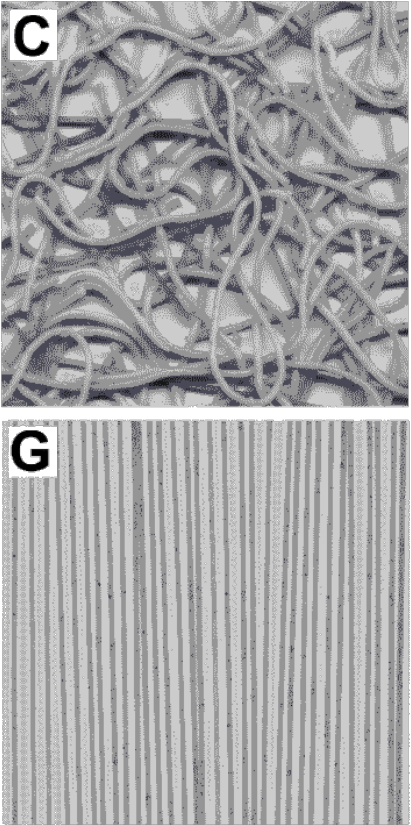
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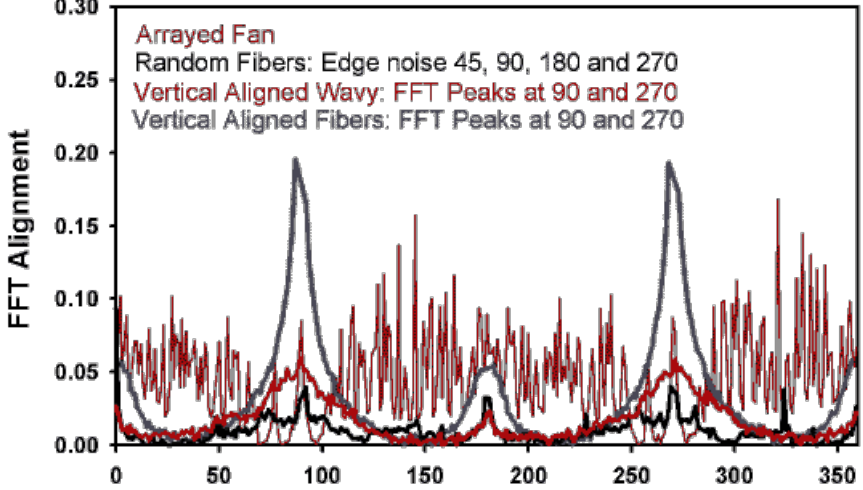
Page 1 of 20

EMBZIM EX1010



M

Determination of Fiber Orientation



Arrayed Fan
Random Fibers: Edge noise 45, 90, 180 and 270
Vertical Aligned Wavy: FFT Peaks at 90 and 270
Vertical Aligned Fibers: FFT Peaks at 90 and 270

FFT Alignment

Correction of Edge Effects

(Ex. 1010 at 614)

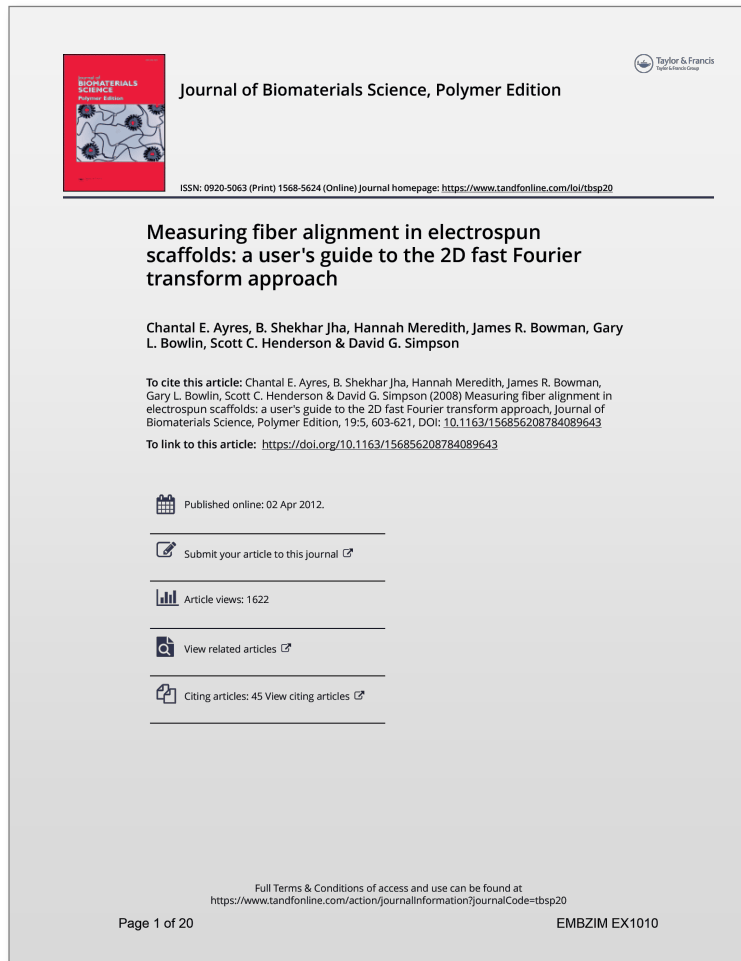
PR2025-00248, Paper 27 at 15; EX1219, ¶ 11
IPR2025-00249, Paper 26 at 12-13; EX1219, ¶ 11

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE


Repair. Regenerate. Restore.

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Ayres 2008 (Ex. 1010)



the plot has been rotated 90°, Fig. 3F). The 2D FFT alignment plot of this scaffold (Fig. 3M, vertical aligned wavy plot) contains distinct peaks at 90 and 270° (2D FFT alignment value of 0.06 units) and much smaller peaks at 180 and 360° (2D FFT alignment value of 0.02 units). The larger peaks report the principle axis of alignment while the smaller peaks correspond to background information, noise and edge effects (Fig. 3M).

(Ex. 1010 at 615)

'223 Patent (Ex. 1001)



US010137223B2

(12) **United States Patent**
Francis et al.

(10) **Patent No.:** US 10,137,223 B2
(45) **Date of Patent:** Nov. 27, 2018

(54) **ALIGNED FIBER AND METHOD OF USE THEREOF**

(71) Applicant: **LifeNet Health**, Virginia Beach, VA (US)

(72) Inventors: **Michael Francis**, Virginia Beach, VA (US); **Roy Ogle**, Virginia Beach, VA (US)

(73) Assignee: **LifeNet Health**, Virginia Beach, VA (US)

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(21) Appl. No.: **14/776,106**

(22) PCT Filed: **Mar. 13, 2014**

(86) PCT No.: **PCT/US2014/025636**
§ 371 (c)(1),
(2) Date: **Sep. 14, 2015**

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PCT Pub. Date: **Oct. 2, 2014**

(65) **Prior Publication Data**
US 2016/0022865 A1 Jan. 28, 2016

Related U.S. Application Data

(60) Provisional application No. 61/785,031, filed on Mar. 14, 2013.

(51) **Int. CL**
A61K 38/17 (2006.01)
C97K 1/00 (2006.01)
(Continued)

(52) **U.S. CL**
CPC **A61L 27/18** (2013.01); **A61L 27/24** (2013.01); **A61L 27/26** (2013.01);
(Continued)

(58) **Field of Classification Search**
None
See application file for complete search history.

(56) **References Cited**
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435:402
2004/0037813 A1* 2/2004 Simpson A61F 2/08
424:93.7
(Continued)

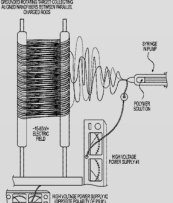
FOREIGN PATENT DOCUMENTS
WO 2010016942 A1 2/2010
WO 2012027592 A2 3/2012
(Continued)

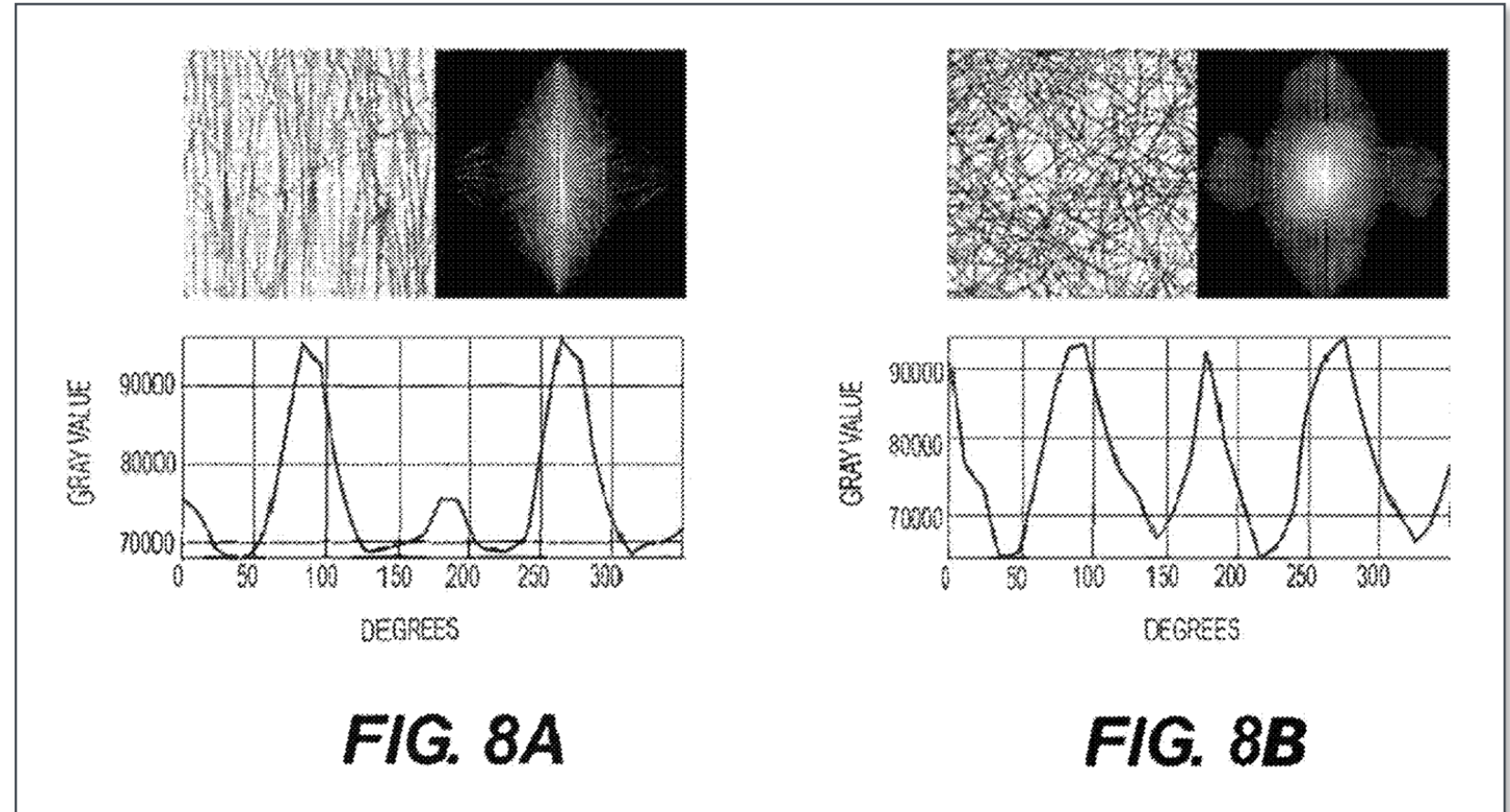
OTHER PUBLICATIONS
Zeugolis, D.I., et al., "Electro-spinning of pure collagen nano-fibres—Just an expensive way to make gelatin?", Mar. 3, 2008, pp. 2293-2305, vol. 29(15), *Biomaterials*, Elsevier Science Publishers.
(Continued)

Primary Examiner—Christopher R Tate
Assistant Examiner—Douglas F White
(74) **Attorney, Agent, or Firm**—RatnerPrestia

(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.

15 Claims, 10 Drawing Sheets





(Ex. 1001) '223 Patent, Figs 8a, 8b

Prosecution History (IPR2025-00248, Ex. 1002)

Appln. No.: 14/776,106 LNH-152US

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.

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Page 152 of 576 EMBZIM EX1002

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.

(IPR2025-00248, Ex. 1002 at 153)

Colloids and Surfaces B: Biointerfaces 82 (2011) 307–315

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb



Electrospun collagen–chitosan–TPU nanofibrous scaffolds for tissue engineered tubular grafts

Chen Huang^{a,b}, Rui Chen^{a,b}, Qinfei Ke^{a,b}, Yosry Morsi^c, Kuihua Zhang^{a,d}, Xiumei Mo^{a,*}

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^b College of Textiles, Donghua University, Shanghai 201620, China

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^d College of Biological Engineering and Chemical Engineering, Jiaxing College, Zhejiang 314001, China

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Collagen–chitosan–TPU

Nanofibrous scaffolds

Cell morphology

Tissue engineering

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1. Introduction

Autologous vein and artery segments have been claimed as the gold substitution for the repair of diseased vessels and peripheral nerve [1–3]. While vascular and nerve-related diseases can occur at any age in males and females, they become increasingly common as people get older. In those cases, the use of prosthetic vascular grafts can be offered as alternatives, as suitable autologous substitutions are probably not available. Tissue engineered grafts have been proposed as a promising solution, which involves the incorporation of isolated living cells from patients into three-dimensional scaffolds, followed by the transplantation of this scaffold back into the patient via surgery.

As a recently developed technology, tissue engineering is a multidisciplinary subject that combines genetic engineering of cells with chemical engineering to create artificial organs and tissues,

such as skin, bones, blood vessels and nerve conduits [4]. The main challenge for tissue engineered scaffolds is to design and fabricate customizable biodegradable matrices that can mimic the componential and structural aspects of extracellular matrices (ECM) [5]. Native ECM include the interstitial matrix and the basement membrane. Gels of polysaccharides and fibrous proteins (collagen in particular) fill the interstitial space and act as a compression buffer against the stress placed on the ECM. Basement membranes are sheet-like depositions of ECM on which various epithelial cells rest [6]. Based on these facts, this study selected collagen as the protein part and chitosan as the polysaccharide part to fabricate ideal tissue engineered scaffolds.

As the main protein of connective tissue in animals and the most abundant protein in mammals [7], collagen is widely used as biomaterials in wound dressing and medical fields. Chitosan, a massive natural polysaccharide derived from chitin, could be used to replace glycosaminoglycan, which is the main component of natural ECM [8]. Both collagen and chitosan possess good biocompatibility, appropriate biodegradability and commercial availability. Various studies have found that collagen–chitosan complex might be an excellent candidate for tissue engineering due to its good cell viability [8–10]. However, there remains a non-ignorable gap between

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Page 1 of 9

EMBZIM EX1008

Electrospun collagen–chitosan–TPU nanofibrous scaffolds for tissue engineered tubular grafts

Chen Huang^{a,b}, Rui Chen^{a,b}, Qinfei Ke^{a,b}, Yosry Morsi^c, Kuihua Zhang^{a,d}, Xiumei Mo^{a,*}

ARTICLE INFO

Article history:

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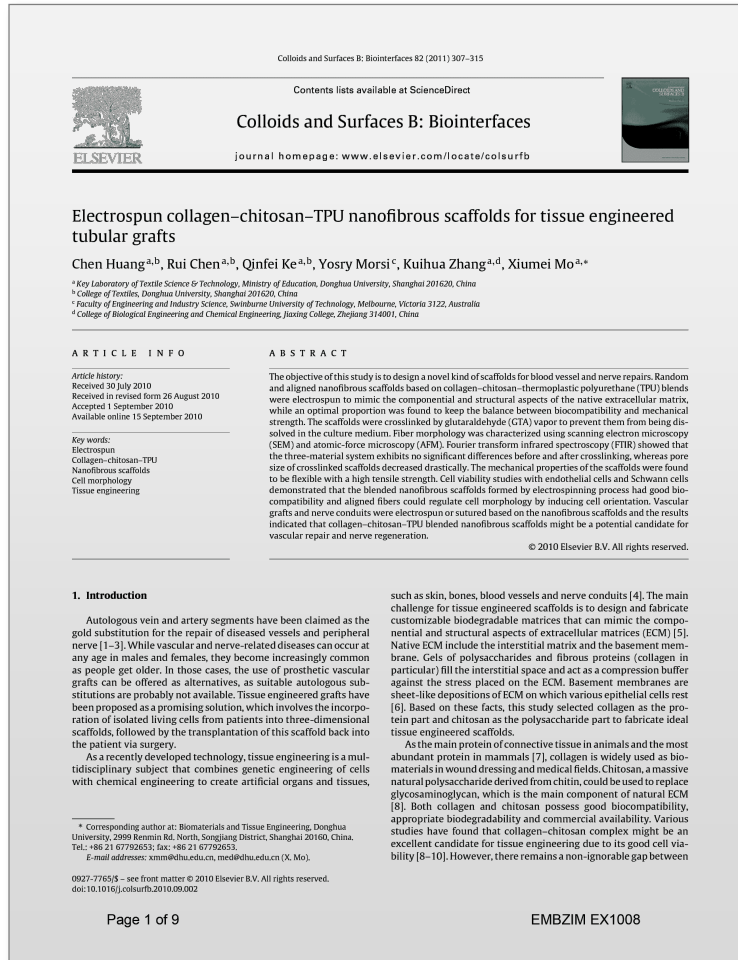
Accepted 1 September 2010

Available online 15 September 2010

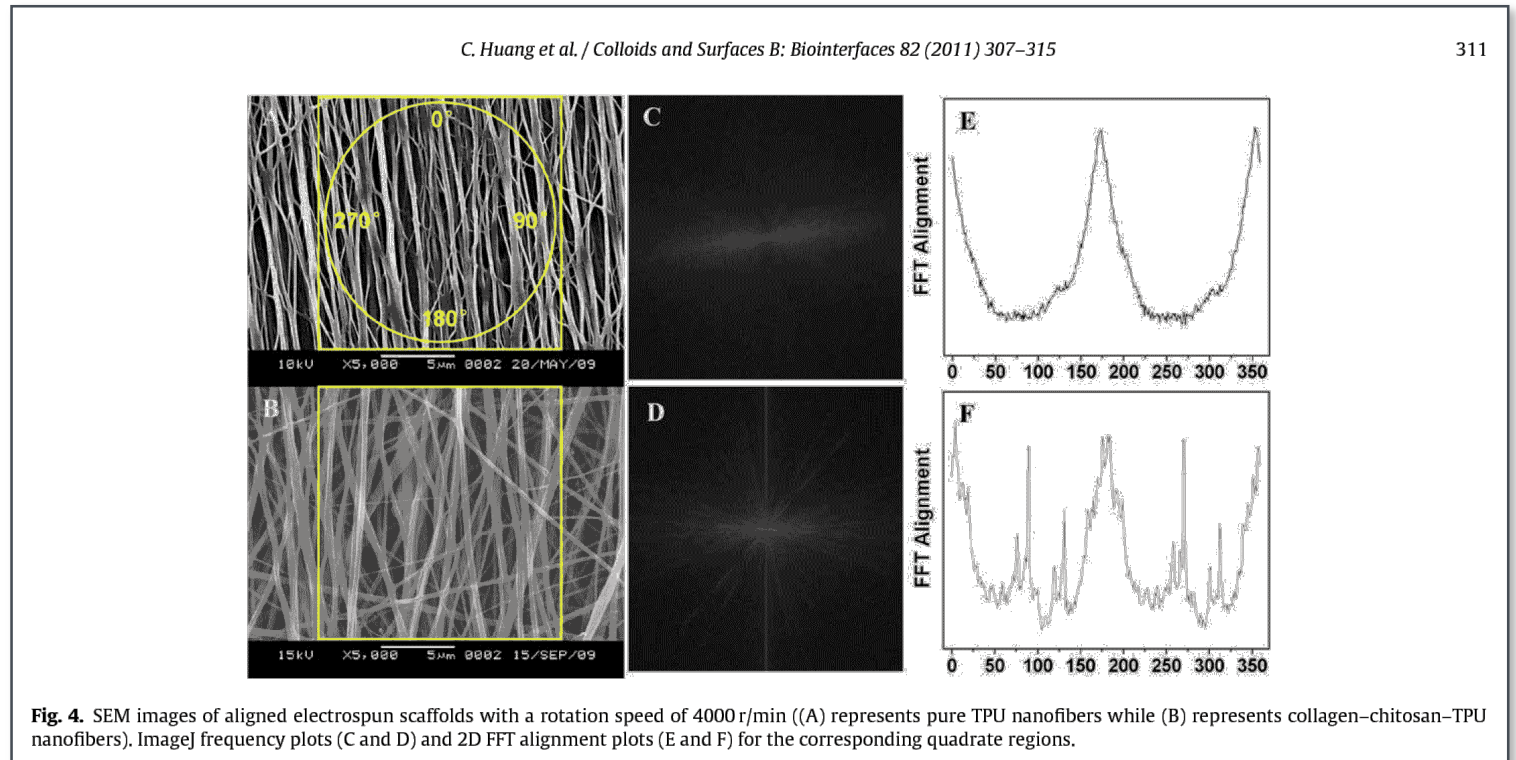
With the aim of measuring fiber alignment, a two-dimensional fast Fourier transform (2D FFT) approach described by Ayres et al. [25] was adapted. In brief, a quadrate region was captured from

(Ex. 1008 at 307, 309)

Huang1 (Ex. 1008)



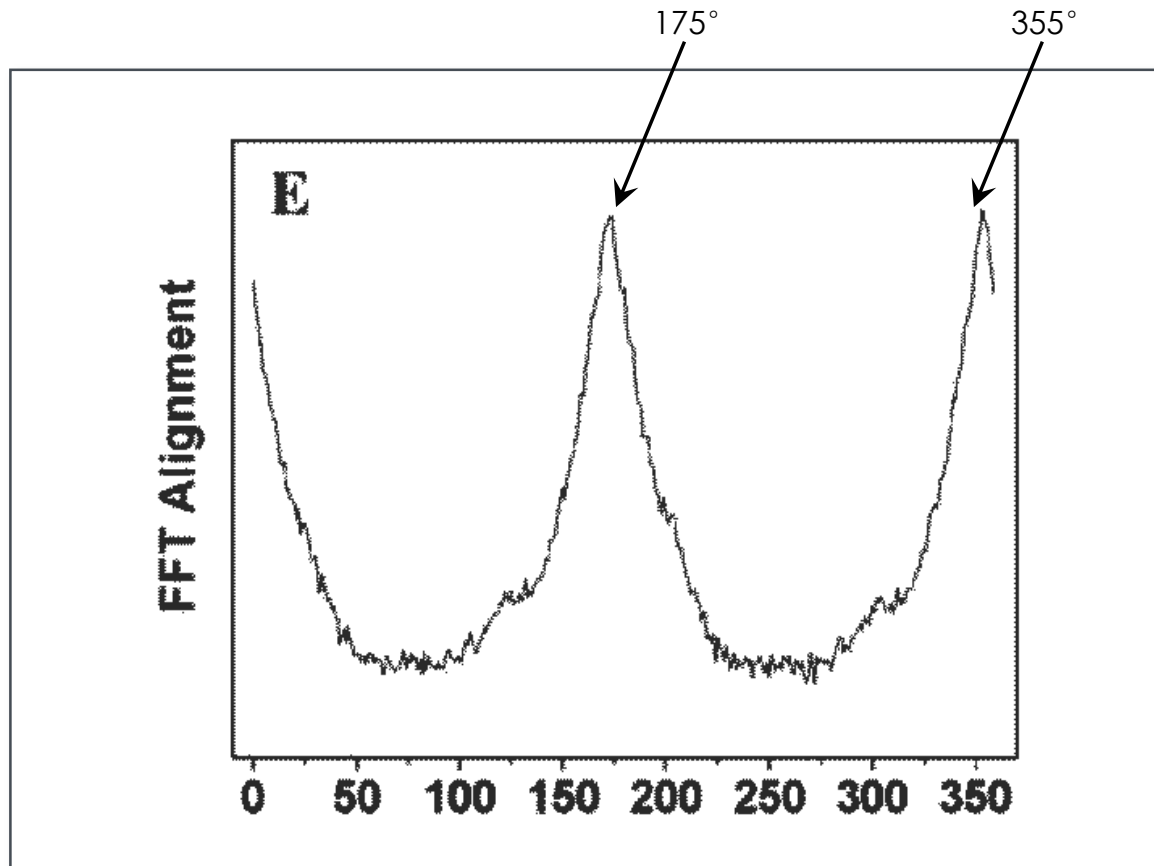
plots. The two peaks at 175° and 355° in Fig. 4E show that with a rotation speed of 4000 r/min, pure TPU nanofibers were highly arrayed along one direction, however, in the three-material based system (Fig. 4F), fiber orientation could not be regulated with such a high uniformity. Although two main peaks could also be found at 5° and 185°, the existence of other peaks indicates that some nanofibers are not well aligned. This phenomenon demonstrates



(Ex. 1008 at 309, 311, Fig. 4)

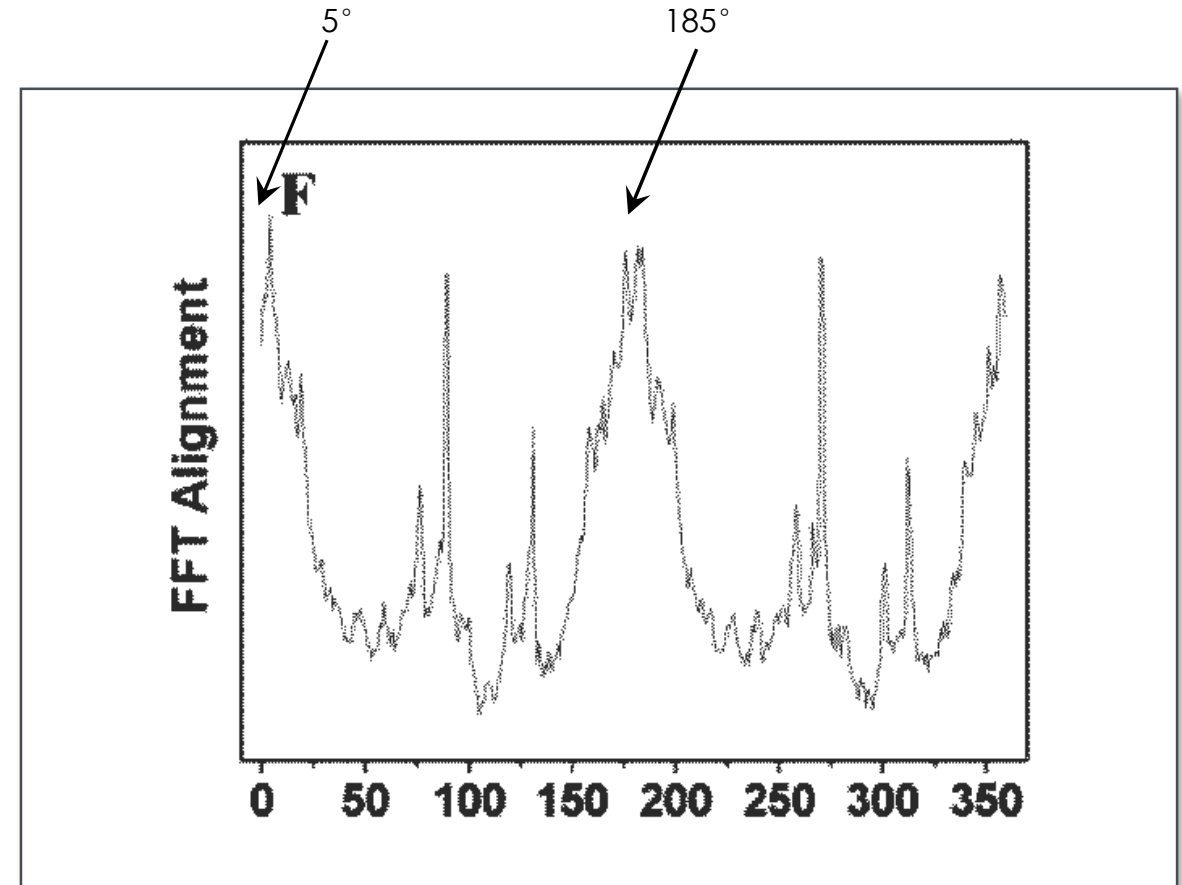
Huang1 (Ex. 1008)

Huang1 aligned plot



(Ex 1008 at 309, 311, Fig. 4E)

Huang1 Random Plot



(Ex 1008 at 309, 311, Fig. 4F)

Prosecution History (IPR2025-00248, Ex. 1002)

Application/Control Number: 14/776,106
Art Unit: 1653

Page 2

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

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Claim Rejections - 35 USC § 102

Huang discloses a scaffold comprising one or more electrospun fibers ...

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)).

(IPR2025-00248, Ex. 1002 at 210-211)

Prosecution History (Ex. 1002): Patent Owner's Response

Claims Rejections – 35 U.S.C. § 102

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

LNH-152US

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.

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EMBZIM EX1002

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.

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

(IPR2025-00248, Ex. 1002 at 237-238)

Prosecution History (Ex. 1002)

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

LNH-152US

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.

Page 5 of 7

Page 237 of 576

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Amendments to the Claims:

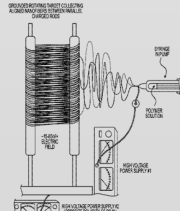
This list of claims replaces all prior versions and lists of claims in the application:

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.

(IPR2025-00248, Ex. 1002 at 234, 237-238)

Claim 1 of the '223 Patent

'223 Patent (Ex. 1001)

 US010137223B2	
(12) United States Patent Francis et al.	(10) Patent No.: US 10,137,223 B2 (45) Date of Patent: Nov. 27, 2018
(54) ALIGNED FIBER AND METHOD OF USE THEREOF	
(71) Applicant: LifeNet Health, Virginia Beach, VA (US)	
(72) Inventors: Michael Francis, Virginia Beach, VA (US); Roy Ogle, Virginia Beach, VA (US)	
(73) Assignee: LifeNet Health, Virginia Beach, VA (US)	
(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 214 days.	
(21) Appl. No.: 14/776,106	
(22) PCT Filed: Mar. 13, 2014	
(86) PCT No.: PCT/US2014/025636 § 371 (c)(1), (2) Date: Sep. 14, 2015	
(87) PCT Pub. No.: WO2014/160019 PCT Pub. Date: Oct. 2, 2014	
(65) Prior Publication Data US 2016/0022865 A1 Jan. 28, 2016 Related U.S. Application Data	
(60) Provisional application No. 61/785,031, filed on Mar. 14, 2013.	
(51) Int. Cl. A61K 38/17 (2006.01) C07K 1/00 (2006.01) (Continued)	
(52) U.S. CL. CPC A61L 27/18 (2013.01); A61L 27/24 (2013.01); A61L 27/26 (2013.01); (Continued)	
(58) Field of Classification Search None See application file for complete search history.	
(56) References Cited U.S. PATENT DOCUMENTS 2002/0090725 A1 * 7/2002 Simpson A61L 15/32 435-402 2004/0037813 A1 * 2/2004 Simpson A61F 2/08 424,937 (Continued) FOREIGN PATENT DOCUMENTS WO 2010016042 A1 2/2010 WO 2012027592 A2 3/2012 (Continued)	
OTHER PUBLICATIONS Zeugolis, D.I., et al., "Electro-spinning of pure collagen nano-fibres—Just an expensive way to make gelatin?", Mar. 3, 2008, pp. 2293-2305, vol. 29(15), Biomaterials, Elsevier Science Publishers. (Continued)	
<i>Primary Examiner</i>—Christopher R Tate <i>Assistant Examiner</i>—Douglas F White (74) <i>Attorney, Agent, or Firm</i>—RatnerPrestia	
(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.	
15 Claims, 10 Drawing Sheets	
	

1. 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.

(Ex. 1001) '223 Patent, Claim 1

Huang2 (Ex. 1005)

The involvement of integrin $\beta 1$ signaling in the migration and myofibroblastic differentiation of skin fibroblasts on anisotropic collagen-containing nanofibers

Chengyang Huang^{a,1}, Xiaoling Fu^{a,1}, Jie Liu^b, Yanmei Qi^b, Shaohua Li^{b,**}, Hongjun Wang^{a,*}

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Biomaterials 33 (2012) 1791–1800

Contents lists available at SciVerse ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

The involvement of integrin $\beta 1$ signaling in the migration and myofibroblastic differentiation of skin fibroblasts on anisotropic collagen-containing nanofibers

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ARTICLE INFO

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Anisotropic nanofibers
Normal human dermal fibroblasts
Fibroblast-to-myofibroblast differentiation
Integrin $\beta 1$ signaling

ABSTRACT

Utilization of nanofibrous matrices for skin wound repair holds great promise due to their morphological and dimensional similarity to native extracellular matrix (ECM). It becomes highly desired to understand how various nanofibrous matrices regulate skin cell behaviors and intracellular signaling pathways, important to tuning the functionality of tissue-engineered skin grafts and affecting the wound healing process. In this study, the phenotypic expressions of normal human dermal fibroblasts (NHDFs) on collagen-containing nanofibrous matrices with either isotropic (i.e., fibers collected randomly with no alignment) or anisotropic (i.e., fibers collected with alignment) fiber organizations were studied by immunostaining, migration assay and molecular analyses. Results showed that both nanofibrous matrices supported the attachment and growth of NHDFs similarly, while showing different cell morphology with distinct variation in focal adhesion formation and distribution. Anisotropic nanofibers significantly triggered the integrin $\beta 1$ signaling pathway in NHDFs as evidenced by an increase of active integrin $\beta 1$ (130 kD mature form) and phosphorylation of focal adhesion kinase (FAK) at Tyr-397. Anisotropic matrices also promoted the migration of NHDFs along the fibers, while neutralization of the integrin $\beta 1$ activity abolished this promotion. Moreover, the fibroblast-to-myofibroblast differentiation was greatly enhanced for the NHDFs cultured on anisotropic nanofibrous matrices over a period of 48 h. Inhibition of cellular integrin $\beta 1$ activity by neutralizing antibody eliminated this enhancement. These findings suggest the important role of integrin $\beta 1$ signaling pathway in regulating the nanofiber-induced fibroblast phenotypic alteration and providing insightful understanding of the possible application of collagen-containing nanofibrous matrices for skin regeneration.

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1. Introduction

Tissue-engineered skin grafts have proved to be effective in closing deep skin wounds despite their suboptimal structure, composition and function [1]. To better support and facilitate skin cells to form skin-like tissues, it is necessary for the temporary scaffolds not only to provide a surface for the cells to reside, but also to define a cell-friendly microenvironment that maximally recapitulates the native extracellular matrix (ECM). In this regard,

electrospun nanofibrous matrices characterized by ultrafine continuous nanoscale fibers, high surface-to-volume ratio, high porosity and variable pore-size distribution have great potential to mimic the skin ECM in both morphology and composition [2,3]. In recognition of the superiority of nanofibrous matrices, increasing efforts have been made to explore the possibility of creating tissue-engineered skin grafts using electrospun fibers. Clearly, nanofibrous matrices support the adhesion, spreading and proliferation of skin cells [4,5].

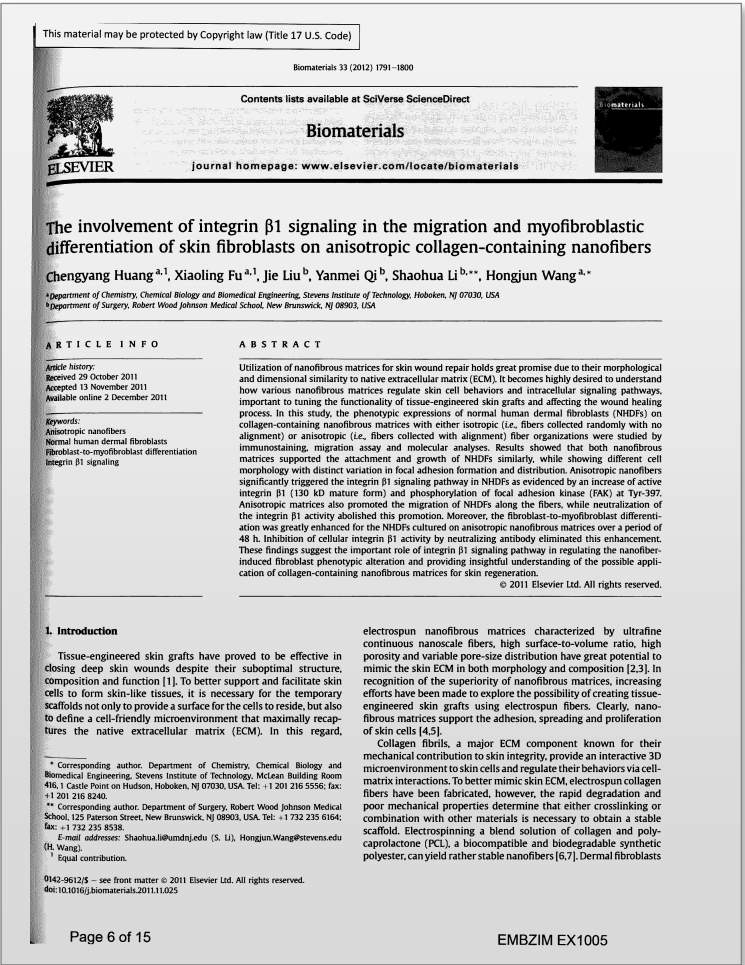
Collagen fibrils, a major ECM component known for their mechanical contribution to skin integrity, provide an interactive 3D microenvironment to skin cells and regulate their behaviors via cell-matrix interactions. To better mimic skin ECM, electrospun collagen fibers have been fabricated, however, the rapid degradation and poor mechanical properties determine that either crosslinking or combination with other materials is necessary to obtain a stable scaffold. Electrospinning a blend solution of collagen and polycaprolactone (PCL), a biocompatible and biodegradable synthetic polyester, can yield rather stable nanofibers [6,7]. Dermal fibroblasts

A B S T R A C T

process. In this study, the phenotypic expressions of normal human dermal fibroblasts (NHDFs) on collagen-containing nanofibrous matrices with either isotropic (i.e., fibers collected randomly with no alignment) or anisotropic (i.e., fibers collected with alignment) fiber organizations were studied by immunostaining, migration assay and molecular analyses. Results showed that both nanofibrous

normal human dermal fibroblasts (NHDFs) were seeded on collagen-containing PCL nanofibrous matrices with either isotropic nanofiber arrangement (i.e., randomly collected nanofibers with no alignment, abbreviated as “Iso”) or anisotropic nanofiber arrangement (i.e., collected nanofibers with alignment, abbreviated as “Aniso”). The elongated cell morphology and accelerated migration

(Ex. 1005 at 1791-1792)



substrate [21]. Considering that nanofiber organization could modulate cellular morphology and migration, we therefore hypothesize that the fiber alignment may also affect fibroblast-to-myofibroblast differentiation and integrins may be involved as well.

(Ex. 1005 at 1792)

nanofibers. NHDFs cultured on anisotropic PCL/collagen nanofibers showed elongated cell morphology, distinct distribution of focal adhesions, accelerated cell migration and promoted fibroblast-to-myofibroblast differentiation. The activation of the

(Ex. 1005 at 1799)

Huang2 (Ex. 1005): Testimony



Antonios Mikos

Q. Is it your opinion that Huang2 was able to get aligned electrospun fibers?

A. Yes.

(Ex. 1220) Mikos Tr. at 96:13-15



**Dr. David
G. Simpson**

Q. And sitting here today, you have no reason to disagree with Huang about --

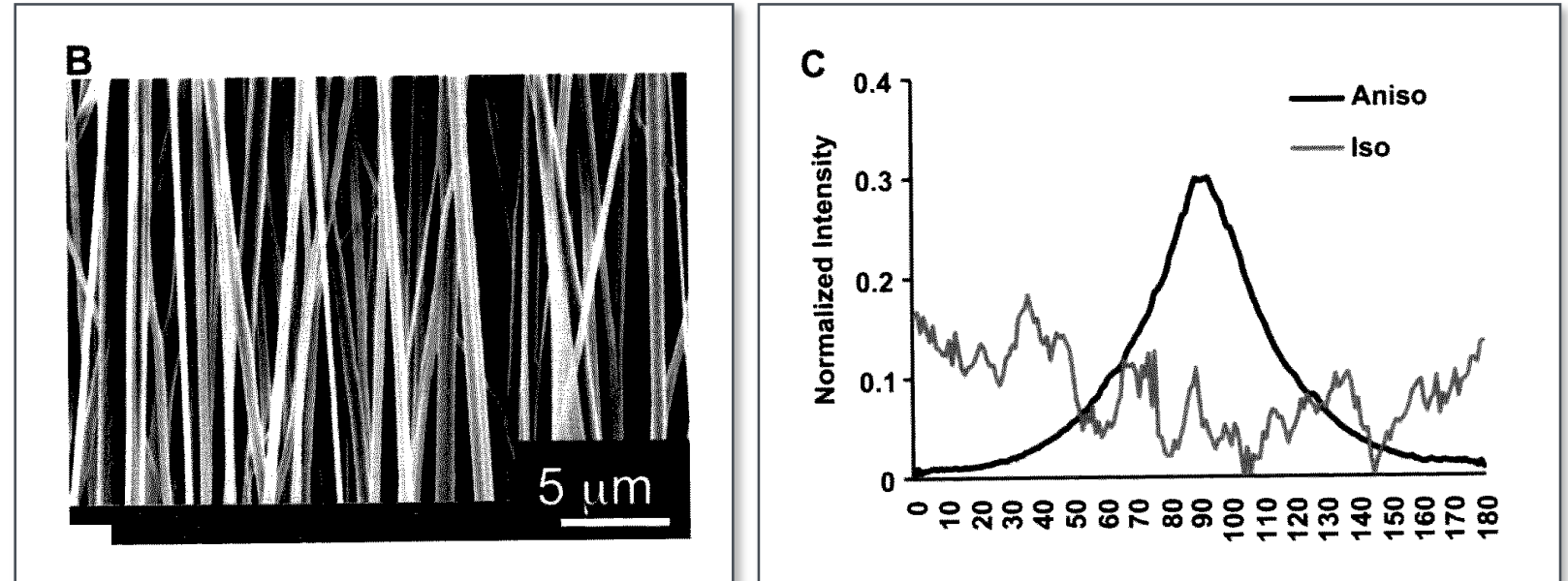
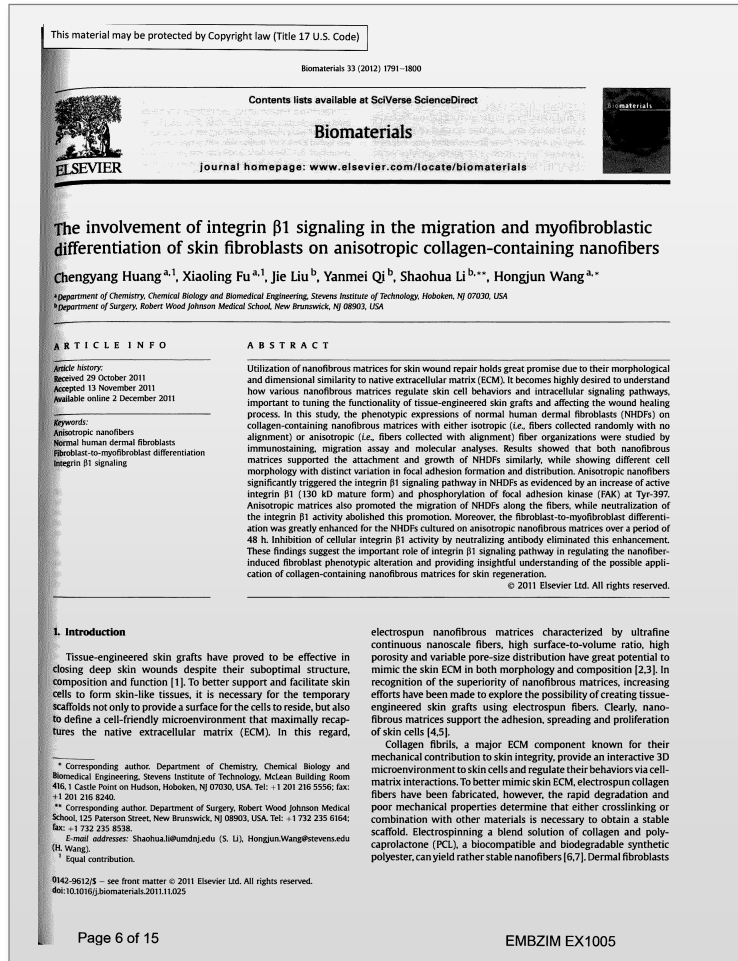
A. No.

Q. Okay.

A. He did anisotropic -- he calibrated this -- he actually went the extra mile and calibrated and **proved that there was alignment** in the scaffold by demonstrating that there were anisotropic mechanical properties. **That's the gold standard for knowing if something has mostly aligned elements**, because you're testing the entire scaffold at once.

(Ex. 2017) Simpson Tr. at 210:24-211:10

Huang2 (Ex. 1005)



a numerical value to fiber orientation. The narrow peak of the normalized intensity values in anisotropic matrices (Fig. 1C) indicated that PCL/Coll nanofibers aligned along a single axis (Fig. 1B). In contrast, the lack of significant peak for the fibers collected on a stationary ECM surface demonstrated no preferred fiber orientation in the isotropic matrices (Fig. 1C). The average fiber diameter for both

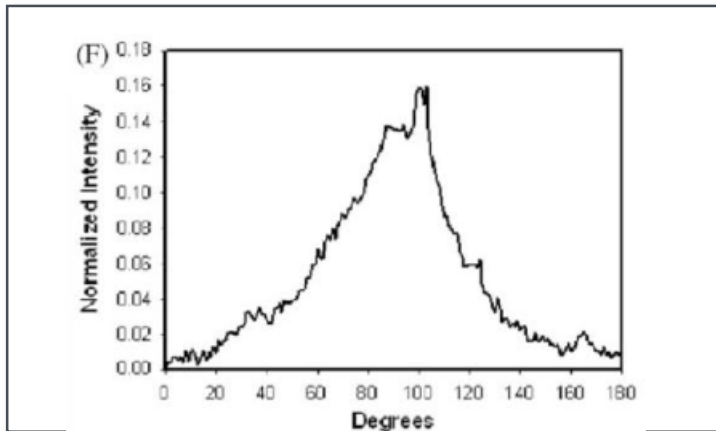
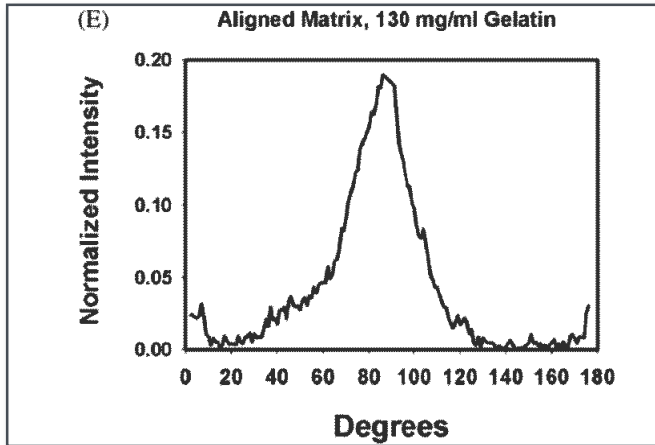
Fig. 1. Characterization of isotropic and anisotropic electrospun PCL/Coll nanofibrous matrices. SEM image of nanofibrous matrices with two types of fiber spatial organizations: isotropic (A) and anisotropic (B). (C) Normalized intensity plots against the angle of acquisition for isotropic and anisotropic nanofibrous matrices. (D) The measured average fiber diameter based on SEM images. Scale bar: 5 μ m.

(Ex. 1005 at 1793-1794)

Ayres 2006 (Ex. 1013) and Huang2 (Ex. 1005)

Ayres 2006 (Ex. 1013)

Figs. 1.E and 4.F



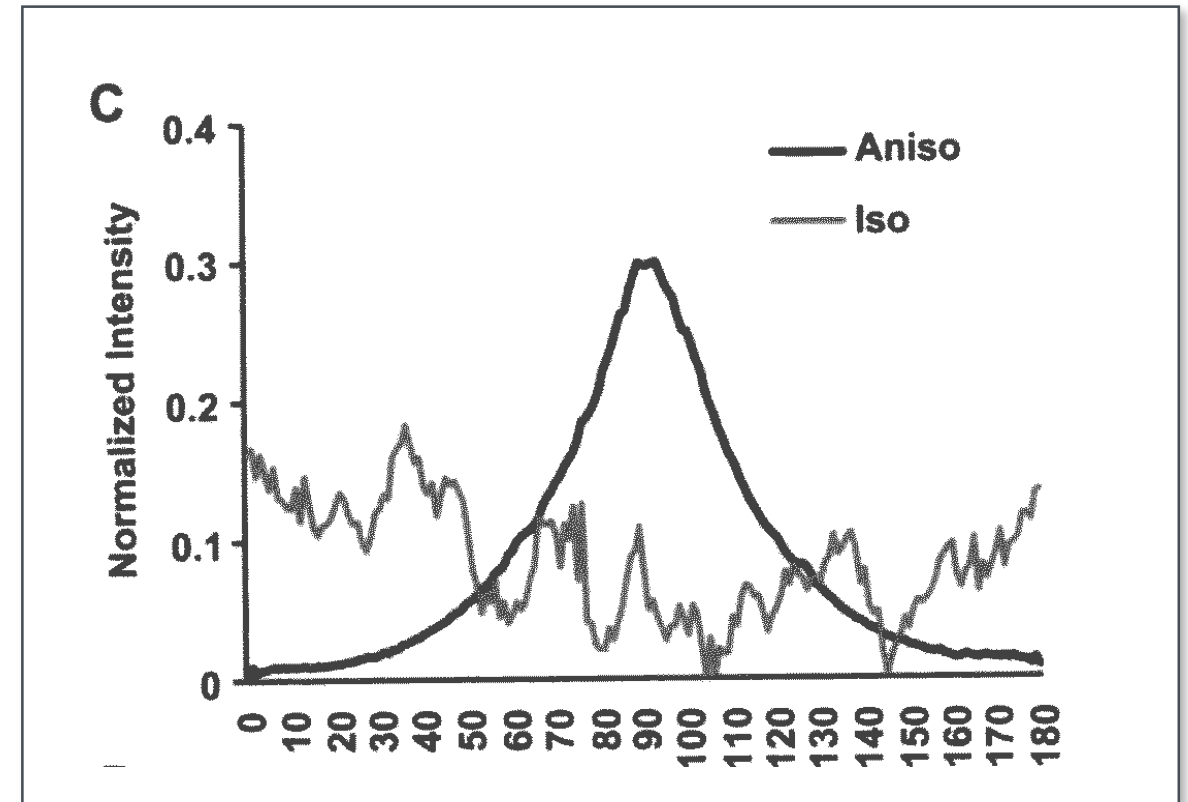
(Ex 1013 at 12 (Fig. 1.E) at 15 (Fig. 4.F))

” cross sectional
analysis of . . .
aligned (E) . . .
electrospun
scaffolds.”

”Note the
distinctive peak
produced by
the image
containing
aligned
information (f)”

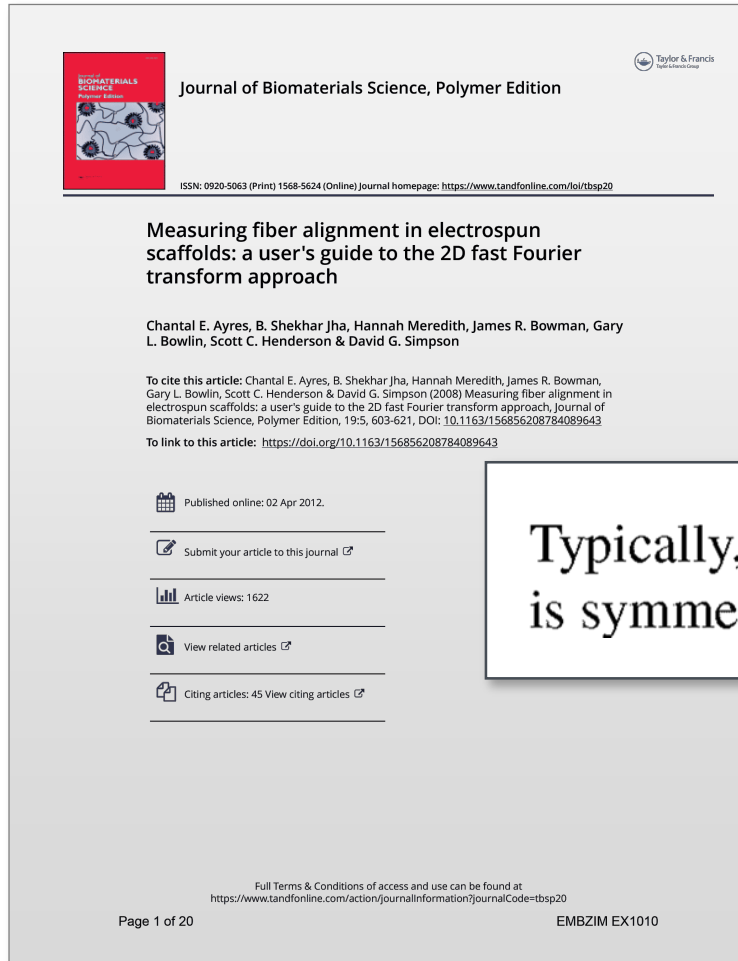
Huang 2 (Ex. 1005)

Fig. 1.C



(Ex 1005 at 1793, Fig. 1.C)

Ayres 2008 (Ex. 1010)



Typically, the data from this analysis is reported for 0 to 180°; the frequency plot is symmetric so a complete summation from 0 to 360° is not technically necessary.

(Ex. 1010 at 609)

Lee (Ex. 1011)

United States
Patent Application Publication
Lee et al.

Pub. No.: US 2010/0331980 A1
Pub. Date: Dec. 30, 2010

ALIGNED SCAFFOLDING SYSTEM FOR SKELETAL MUSCLE REGENERATION

Inventors: Sang Jin Lee, Winston-Salem, NC (US); James J. Yoo, Winston-Salem, NC (US); Anthony Atala, Winston-Salem, NC (US)

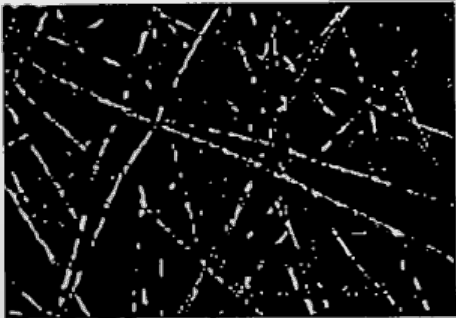
Correspondence Address: MYERS BIGEL SIBLEY & SAJOVEC, PO BOX 37428, RALEIGH, NC 27627 (US)

Appl. No.: 12/865,434
PCT Filed: Jan. 30, 2009
PCT No.: PCT/US09/00625
§ 371 (c)(1), (2), (4) Date: Aug. 11, 2010

Related U.S. Application Data
Provisional application No. 61/025,350, filed on Feb. 1, 2008.

Publication Classification
Int. Cl. A61F 2/00 (2006.01)
C12N 5/071 (2010.01)
B29C 47/00 (2006.01)
U.S. Cl. 623/14.13; 435/398; 435/377; 264/465

ABSTRACT
Provided herein are anisotropic muscle implants that have a biodegradable scaffold comprising a plurality of fibers oriented along a longitudinal axis. The implants may include mammalian muscle cells seeded and/or fused into myotubes on the scaffold. Methods of forming the muscle implants are provided, as are methods of treating a subject in need of skeletal muscle reconstruction.



(19) United States
(12) Patent Application Publication
Lee et al.

(10) Pub. No.: US 2010/0331980 A1
(43) Pub. Date: Dec. 30, 2010

(54) ALIGNED SCAFFOLDING SYSTEM FOR SKELETAL MUSCLE REGENERATION

Related U.S. Application Data

(60) Provisional application No. 61/025,350, filed on Feb. 1, 2008.

Publication Classification

(51) Int. Cl. A61F 2/08 (2006.01)
C12N 5/071 (2010.01)
B29C 47/00 (2006.01)
(52) U.S. Cl. 623/14.13; 435/398; 435/377; 264/465

(57) ABSTRACT

Provided herein are anisotropic muscle implants that have a biodegradable scaffold comprising a plurality of fibers oriented along a longitudinal axis. The implants may include mammalian muscle cells seeded and/or fused into myotubes on the scaffold. Methods of forming the muscle implants are provided, as are methods of treating a subject in need of skeletal muscle reconstruction.

(76) Inventors: Sang Jin Lee, Winston-Salem, NC (US); James J. Yoo, Winston-Salem, NC (US); Anthony Atala, Winston-Salem, NC (US)

Correspondence Address:
MYERS BIGEL SIBLEY & SAJOVEC
PO BOX 37428
RALEIGH, NC 27627 (US)

(21) Appl. No.: 12/865,434
(22) PCT Filed: Jan. 30, 2009
(86) PCT No.: PCT/US09/00625
§ 371 (c)(1), (2), (4) Date: Aug. 11, 2010

(Ex. 1011)

Lee (Ex. 1011)

Declaration of Dr. Mikos:

190. I disagree. Lee does not express any doubt regarding the alignment of its fibers. For example, Lee states that fiber morphology was determined with SEM (Ex. 1011 ¶ 65), fiber orientation was determined with ImageTool 3.0 (*id.* ¶ 66), cell alignment was assessed by SEM (*id.* ¶¶ 74, 77), and tensile testing was performed with fibers of different orientations (*id.* ¶¶ 67, 81). In the face of this information, a

(Ex. 2015), ¶190

Declaration of Dr. Simpson:

183. Lee determined fiber orientation. Specifically, “fiber morphologies of the electrospun PCL/collagen nanofiber meshes were examined by scanning electron microscopy (SEM; Model S-2260N, Hitachi Co., Ltd, Japan.)” (*Id.*, [0065].) Further, “the SEM images were analyzed with UTHSCSA ImageTool 3.0

191. Materials testing confirmed the enhanced strength of the fibers along the axis of alignment, indicating that they are aligned. (EX1011, Figure 2, [0025], [0067], Claim 8.)

(Ex. 1003), ¶¶183, 191

Lee (Ex. 1011): Testimony



Antonios Mikos

- Q.** Do you agree that Lee obtained aligned blended collagen/PCL fibers?
- A.** The ECM results that are presented in Lee's Exhibit 16 indicate aligned fibers. For example, when I see Figure 1D and 1H, that's my understanding that **these are aligned fibers** and, again, these fibers were formed with PCL/collagen blends 1 to 1.

(Ex. 1220) Mikos Tr. at 192:6-15 (objections omitted)

Arguments in Petitions

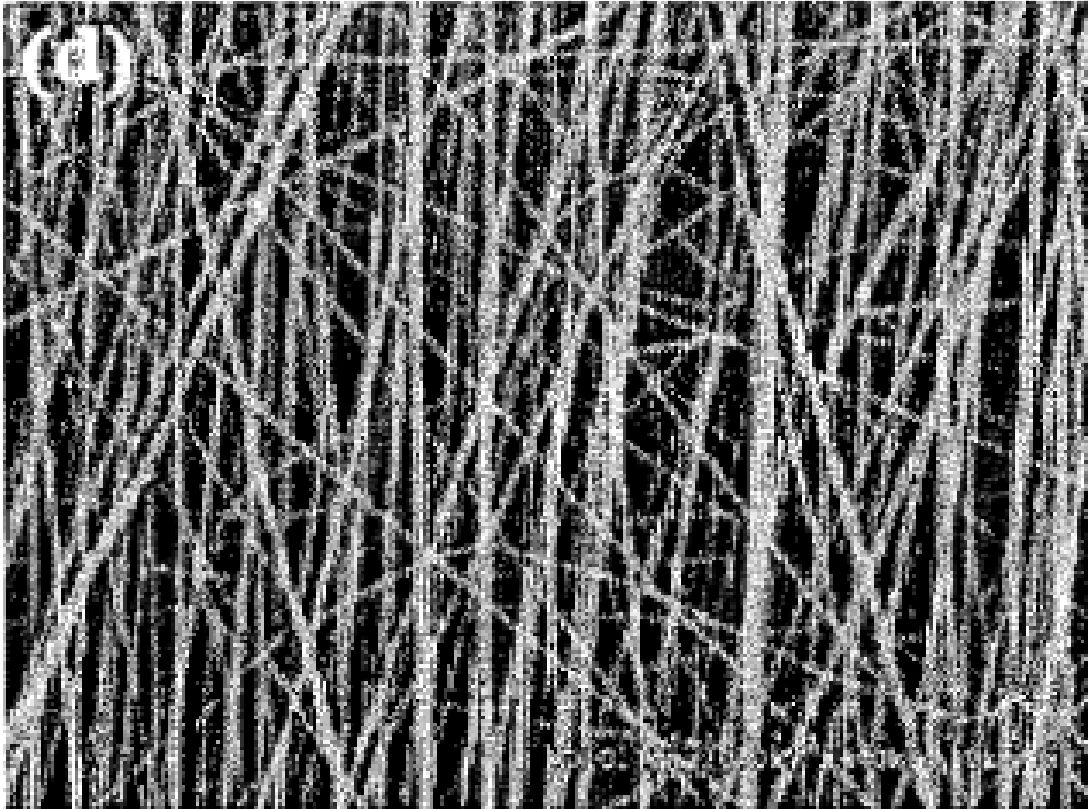
The Petitions state:

- “Lee by itself also discloses this element of the claim by describing aligned PCL/collagen fibers.” (’227 Petition at 47)
- “Lee discloses aligned PCL/collagen fibers that meet this element of the claim.” (’223 Petition at 49.)

Dr. Simpson (Ex. 1003, ¶¶ 181, 193) states:

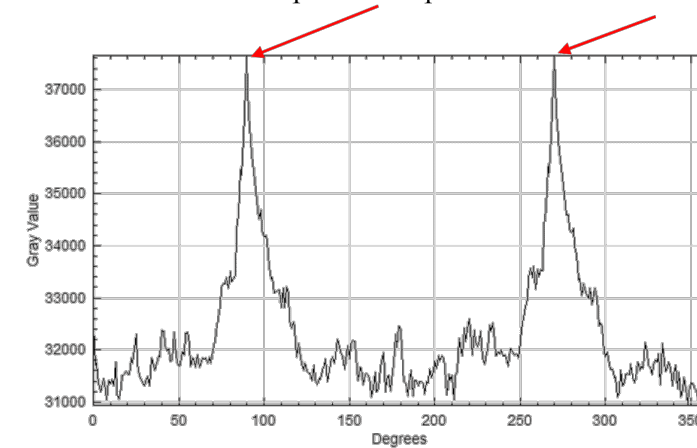
- ““an FFT analysis measures the degree of fiber alignment, and an FFT result showing fibers having adjacent major peaks with about 180° apart from each other only occurs when the fibers are aligned. (III.D, *supra*.) In Lee, the researchers specifically examined how the alignment and orientation of electrospun PCL/collagen nanofibers affected the performance and properties of the resulting electrospun scaffolds.”
- “Given that a POSA would have understood that Lee discloses all of the claim limitations, it is my opinion that Lee anticipates Claim 1.”

Dr. Simpson's Testing



(Ex. 1021 at Fig. 1.D)

188. I conducted my own analysis on Lee's Figure 1D using the FFT analysis described in the '223 patent. I report the data from 0° to 360°.



189. The graph generated from the FFT analysis demonstrates that the aligned fibers have a first major peak at about 90° and, because I reported all the peaks through 360°, a second major peak at about 270°, which shows that the peaks are 180° apart as recited in claim 1. The major peaks are annotated above with red arrows. This data confirms Lee's many statements that he had obtained aligned collagen fibers. (*E.g.*, Lee, Fig. 9, [0052].)

(Ex. 1003), ¶¶ 188-189

Inherency

time of filing of Lee. (EX1013, 4; EX1010, 607; EX1003, ¶186.) Further, it was well known at the time of filing of Lee that a FFT analysis could be performed to further confirm alignment. (See EX1010). This would show the fibers were oriented on the scaffold in Lee in an aligned manner.

To demonstrate this, Petitioners' expert, Dr. Simpson, ran an FFT analysis on Figure 1D from Lee's provisional, which has the same data as Figure 1D from Lee, and is clearer. (EX1003, ¶¶188-190.)

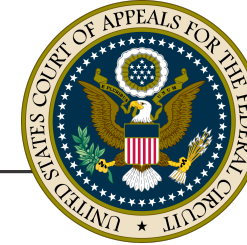
(Pet. at 52)

Dr. Simpson Declaration:

190. These properties are a natural and necessarily present property of the fibers that Lee discloses.

(Ex. 1003), ¶ 190

Federal Circuit:



“Thus, inherent anticipation requires ‘merely that the disclosure of the prior art is sufficient to show that the natural result flowing from the operation as taught in the prior art would result in the claimed product.’”

Arbutus Biopharma Corporation v. ModernaTX, Inc.,
65 F.4th 656, 662 (Fed. Cir. 2023)



“We do not see the bright line distinction between Petitioner's approach and the inherency standard that Patent Owner suggests because, as Petitioner asserts, the same evidence can satisfy both standards. Tr. 8:26–9:11.

Extrinsic evidence such as that Petitioner relies on here may be used to shed light on what a prior art reference means to one of ordinary skill in the art and that ‘such reliance on extrinsic evidence is proper in an inherency analysis.’” (citation omitted).

AMCOL Int'l Corp. v. Oil-Dri Corp. of Am., IPR2022-01107, 2023 WL 8264190, at *9-10 (P.T.A.B. Nov. 29, 2023)

Claim 1 of '223 and '227 patents	Patent Owner's Response	Claim construction excludes Huang2
	Patent Owner's Response	Dr. Simpson's testing is flawed

Claim construction

'223 Patent Claim Construction

USO10137223B2

(12) **United States Patent**
Francis et al.

(10) **Patent No.: US 10,137,223 B2**
(45) **Date of Patent:** Nov. 27, 2018

(54) **ALIGNED FIBER AND METHOD OF USE THEREOF**

(71) Applicant: **LifeNet Health**, Virginia Beach, VA
(US)

(72) Inventors: **Michael Francis**, Virginia Beach, VA
(US); **Roy Ogle**, Virginia Beach, VA
(US)

(73) Assignee: **LifeNet Health**, Virginia Beach, VA
(US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 214 days.

(21) Appl. No.: **14/776,106**

(22) PCT Filed: **Mar. 13, 2014**

(86) PCT No.: **PCT/US2014/025636**

§ 371 (c)(1),

(2) Date: **Sep. 14, 2015**

(87) PCT Pub. No.: **WO2014/160019**

PCT Pub. Date: **Oct. 2, 2014**

(65) **Prior Publication Data**

US 2016/0022865 A1 Jan. 28, 2016

Related U.S. Application Data

(60) Provisional application No. 61/785,031, filed on Mar. 14, 2013.

(51) **Int. Cl.**

A61K 38/17 (2006.01)

C07K 1/00 (2006.01)

(Continued)

(52) **U.S. CL.**

6A1L 27/18 (2013.01); **6A1L 27/24**

CPC **6A1L 27/26** (2013.01);

(Continued)

(58) **Field of Classification Search**

None

See application file for complete search history.

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(Continued)

Primary Examiner — Christopher R Tate

Assistant Examiner — Douglas F White

(74) Attorney, Agent, or Firm — Ratner Prestia

(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.

15 Claims, 10 Drawing Sheets

1. 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.

(Ex. 1001) '223 Patent, Claim 1

Claim 1 of the '227 Patent (Ex 1001)

		US011318227B2
(12) United States Patent Francis et al.	(10) Patent No.: US 11,318,227 B2 (45) Date of Patent: *May 3, 2022	
(54) ALIGNED FIBER AND METHOD OF USE THEREOF	(56) References Cited U.S. PATENT DOCUMENTS	
(71) Applicant: LifeNet Health , Virginia Beach, VA (US)	6,189,537 B1 2/2001 Wolfenburger 6,339,379 B1 10/2000 Wolfenburger 2002/0097225 A1 7/2002 Simpson et al. 2004/0037813 A1 2/2004 Simpson et al. 2007/0225631 A1 9/2007 Bowlin et al. 2008/0213389 A1 9/2008 Lelkes et al. 2009/0188443 A1 1/2009 Hashi et al. 2010/0233115 A1 9/2010 Patel et al. 2011/0236974 A1 9/2011 Ogbe et al. 2012/0221025 A1* 8/2012 Simpson ———— A61F 2/02 606:152	
(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 591 days.	2012/0273993 A1 11/2012 Shoseyov et al.	A61F 2/02 606:152
This patent is subject to a terminal disclaimer.	FOREIGN PATENT DOCUMENTS	
(21) Appl. No.: 16/045,159	WO 2010/016942 A1 2/2010 WO 2012/027592 A2 3/2012 WO WO 2012/078472 * 6/2012 WO 2013/03680 A1 3/2013 WO 2013/119873 A1 8/2013	
(22) Filed: Jul. 25, 2018	OTHER PUBLICATIONS	
(65) Prior Publication Data US 2019/0167842 A1 Jun. 6, 2019	Sharma et al., State-of-Art Functional Biomaterials for Tissue Engineering, Front. Mater., 2019, pp. 1-10.* Hutmacher et al., State of the art future directions of scaffold-based bone engineering from a biomaterials perspective, J Tissue Eng Regen Med 2007; 1: 245-260.* Dash and Kokkinnala, Poly-caprolactone based formulations for drug delivery and tissue engineering: A review, Journal of Controlled Release, vol. 158, Issue 1, Feb. 25, 2012, pp. 15-33.* Werner et al., State of the art and future perspectives of scaffold-based bone engineering from a biomaterials direction, J Tissue Eng Regen Med 2007; 1: 245-260.* Anderson et al., "Biodegradation and Biocompatibility of PLA and PLGA Microspheres", Advanced Drug Delivery Reviews, 1997, vol. 28, pp. 5-29. 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. Behravesh et al., "Synthetic Biodegradable Polymers for Orthopaedic Applications," Clinical Orthopaedics and Related Research, No. 367R, pp. S118-S125. Chick et al., "Microcrystalline poly(D,L-lactic-co-glycolic acid)/poly(ethylene glycol) blends for controlled Drug Delivery," Journal of Controlled Release, vol. 48 1997, pp. 259-268. Huang et al., "Electrospun Collagen-Chitosan-TPU Nanofibrous Scaffolds For Tissue Engineered tubular Grafts", Colloids and Surfaces B: Biointerfaces, vol. 82 2011, pp. 307-315. (Continued)	
(60) Provisional application No. 61/785,031, filed on Mar. 14, 2013.	Primary Examiner — Maria Marvich (74) Attorney, Agent, or Firm — RatnerPrestia	
(51) Int. Cl. A61F 2/08 (2006.01) A61L 27/24 (2006.01) A61L 27/26 (2006.01) A61K 38/17 (2006.01) A61L 27/38 (2006.01) A61L 27/18 (2006.01) A61L 27/36 (2006.01) A61L 27/86 (2006.01) D01D 5/00 (2006.01) C12N 50/79 (2010.01) U.S. CL. (52) A61T 27/38 (2013.01); A61L 27/24 (2013.01); A61L 27/26 (2013.01); A61L 27/3604 (2013.01); A61L 27/3683 (2013.01); A61L 27/38 (2013.01); A61L 27/56 (2013.01); A61L 27/56 (2013.01); D01D 5/0076 (2013.01); D01D 5/0092 (2013.01); A61F 2/08 (2013.01); A61F 2/340901 (2013.01); A61F 2/40012 (2013.01); C12N 2533/30 (2013.01); C12N 2533/34 (2013.01); C12N 2533/90 (2013.01)	(57) ABSTRACT A scaffold comprising an aligned fiber. Further, 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. Also, methods for promoting differentiation of stem cells into osteoblasts, chondrocytes, ligament or tendon, the method comprising seeding the cells on the scaffold or aligned fiber in conditions suitable for the cell differentiation.	
(58) Field of Classification Search None See application file for complete search history.	14 Claims, 10 Drawing Sheets	

“Major peaks” **not** recited in claim 1 of the '227 Patent

1. A method of treating a tissue defect in a subject comprising
implanting at the tissue defect a scaffold comprising crosslinked fibers comprising collagen, wherein a result of a fast Fourier transform (FFT) analysis of the fibers demonstrates that the fibers have major adjacent peaks that are about 180° apart from each other, and
applying the scaffold to the tissue defect to repair a tendon, a ligament or a nerve defect.

(Ex. 1001) '227 Patent, Claim 1

'223 Patent (Ex. 1001)

US010137223B2

(12) **United States Patent**
Francis et al.

(10) **Patent No.:** **US 10,137,223 B2**
(45) **Date of Patent:** **Nov. 27, 2018**

(54) **ALIGNED FIBER AND METHOD OF USE THEREOF**

(71) Applicant: **LifeNet Health**, Virginia Beach, VA (US)

(72) Inventors: **Michael Francis**, Virginia Beach, VA (US); **Roy Ogle**, Virginia Beach, VA (US)

(73) Assignee: **LifeNet Health**, Virginia Beach, VA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 214 days.

(21) Appl. No.: **14/776,106**

(22) PCT Filed: **Mar. 13, 2014**

(86) PCT No.: **PCT/US2014/025636**
§ 371 (c)(1),
(2) Date: **Sep. 14, 2015**

(87) PCT Pub. No.: **WO2014/160019**
PCT Pub. Date: **Oct. 2, 2014**

(65) **Prior Publication Data**
US 2016/0022865 A1 Jan. 28, 2016

Related U.S. Application Data

(60) Provisional application No. 61/785,031, filed on Mar. 14, 2013.

(51) Int. Cl. **A61K 38/17** (2006.01)
C07K 1/00 (2006.01)
(Continued)

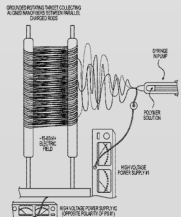
(52) U.S. Cl. **A61L 27/18** (2013.01); **A61L 27/24** (2013.01); **A61L 27/26** (2013.01);
CPC (Continued)

(58) **Field of Classification Search**
None
See application file for complete search history.

(56) **References Cited**
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OTHER PUBLICATIONS
Zeugolis, D.L., et al., "Electro-spinning of pure collagen nano-fibres—Just an expensive way to make gelatin?", Mar. 3, 2008, pp. 2293-2305, vol. 29(15), Biomaterials, Elsevier Science Publishers.
(Continued)
Primary Examiner—Christopher R Tate
Assistant Examiner—Douglas F White
(74) **Attorney, Agent, or Firm**—RatnerPrestia

(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.

15 Claims, 10 Drawing Sheets



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.

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 C E, Jha B S, Meredith H, Bowman J R, Bowlin G L, Henderson S C, Simpson D G. 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 FIG. 8.

(Ex. 1001) '223 Patent at 13:3-7; 13:15-26

Patent Owner's Claim Constructions



During Prosecution:

- No construction necessary

(IPR2025-00248, Ex. 1002 at 210-211, 237-238)



Patent Owner's Preliminary Response:

- No construction necessary
- Reference (Huang2) not have **two peaks** in 360°

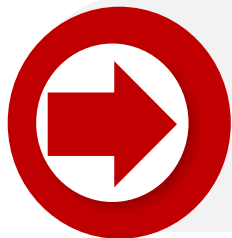
(IPR2025-00248, Paper 9 at 10, 14-15)



Patent Owner's Response:

- Must have **four peaks** in 360° field
- Two of the four peaks must be bigger than average

(IPR2025-00248, Paper 24 at 7-9; Paper 30 at 7)



Patent Owner's Sur-reply:

- Must use statistics
- Must distinguish noise from peaks

(IPR2025-00248, Paper 30 at 4-5)

Federal Circuit:



“Ultimately, for the reasons below: we conclude that Mylan’s proposed construction, albeit articulated differently, is correct because it “most naturally aligns with the patent’s description of the invention,” as further informed by the prosecution history.”

AstraZeneca AB v Mylan Pharmaceuticals Inc., 19 F.4th 1325, 1329 (Fed. Cir. 2001)

Federal Circuit:



“The inventor’s words that are used to describe the invention – the inventor’s lexicography – must be understood and interpreted by the court as they would be understood and interpreted by a person in that field of technology.”

“Such special meaning, however, must be sufficiently clear in the specification that any departure from common usage would be so understood by a person of experience in the field of the invention.”

Multiform Desiccants, Inc. v. Medzam Ltd., 133 F.3d 1473, 1477 (Fed. Cir. 1998)

Dr. Simpson's Reply Declaration

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

EMBODY, INC. AND ZIMMER BIOMET HOLDINGS, INC.,
Petitioner,

v.

LIFENET HEALTH,
Patent Owner.

Case IPR2025-00248
U.S. Patent No. 10,137,223

DECLARATION OF DAVID G. SIMPSON, PH.D.
IN SUPPORT OF REPLY TO PATENT-OWNER'S RESPONSE

6. It is my opinion, that identifying “major peaks” is a visual test that works by comparing the relative size of the peaks present and this is what is meant by examining whether peak heights are higher than an “average.” This is the test that I used in my published papers, including the Ayres 2008 paper, which is incorporated into the text of the '223 patent.

(Ex. 1219) Simpson Reply Declaration ¶ 6

Patent Owner's Multi-step Method to Fall Within Claim

- 1 select peaks in alignment plot
- 2 determine if peaks constitute noise or alignment peaks
- 3 identify the heights of those peaks
- 4 mathematically computing their arithmetic mean
- 5 comparing the arithmetic mean to each of the peak heights
- 6 calculate statistics to determine if differences constitute measurement error*
- 7 finally labeling the peaks that are higher than the arithmetic mean as "major peaks"

*improperly raised in Patent Owner's Sur-reply Brief (See IPR2025-00248, Paper 35)

Claim Construction

- Patent Owner relies entirely on Dr. Mikos for its claim construction
- ✗ Dr. Mikos did not conduct any testing in this case
- ✗ Dr. Mikos never performed the Ayres FFT analysis himself
- ✗ Dr. Mikos has no publications with an Ayres FFT alignment plot
 - One of Dr. Mikos's publication uses a different method for determining alignment, where there was no alignment plot. (See Ex. 1219, ¶24)

'223 Patent (Ex. 1001)



Does not identify FFT peaks or their values



Does not average peak values



Does not explain how alignment peaks are above average

Simpson Reply Declaration (Ex. 1219, ¶ 10)

Claim Construction: Testimony



Antonios Mikos

“[T]he Specification is silent regarding peak height and Dr. Mikos testifies that Figure 8B “is not very clear” such that peak height can only be “guesstimate[d].”

(Ex. 1220) Mikos Tr. 69:25-70:7; IPR2025-00248, Paper 30 at 4

Claim Construction

1

Patent Owner's calculation not in the patent

(Mikos Tr. (Ex. 1220) at 63:6-9, Simpson Reply Declaration (Ex. 1219), ¶10)

2

Patent Owner's calculation not in Ayres

(Mikos Tr. (Ex. 1220) at 86:3-5, Simpson Reply Declaration (Ex. 1219), ¶¶ 7,8)

3

Patent Owner's calculation not in any publication cited in record

(Mikos Tr. (Ex. 1220) at 94:20-95:7, Simpson Reply Declaration (Ex. 1219), ¶9)

'223 Patent, Fig. 8a

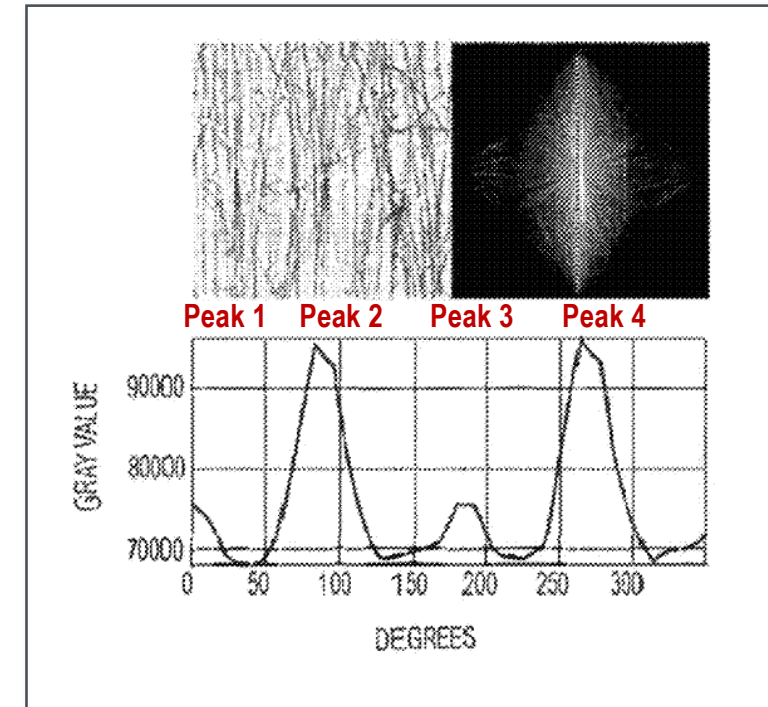
Declaration of Dr. Mikos:

specifically, Figure 8A depicts peaks at 0°, 90°, 180°, and 270°, but the peaks at 0° and 180° are much shorter than those at 90° and 270°. The peaks at 90° and 270° have heights of approximately 95,000 units, while the peaks at 0° and 180° have heights of approximately 75,000. Accordingly, the average height of the four peaks depicted in the result is 85,000 and the peaks at 90° and 270° are higher than that average.

(Ex. 2015), ¶ 56

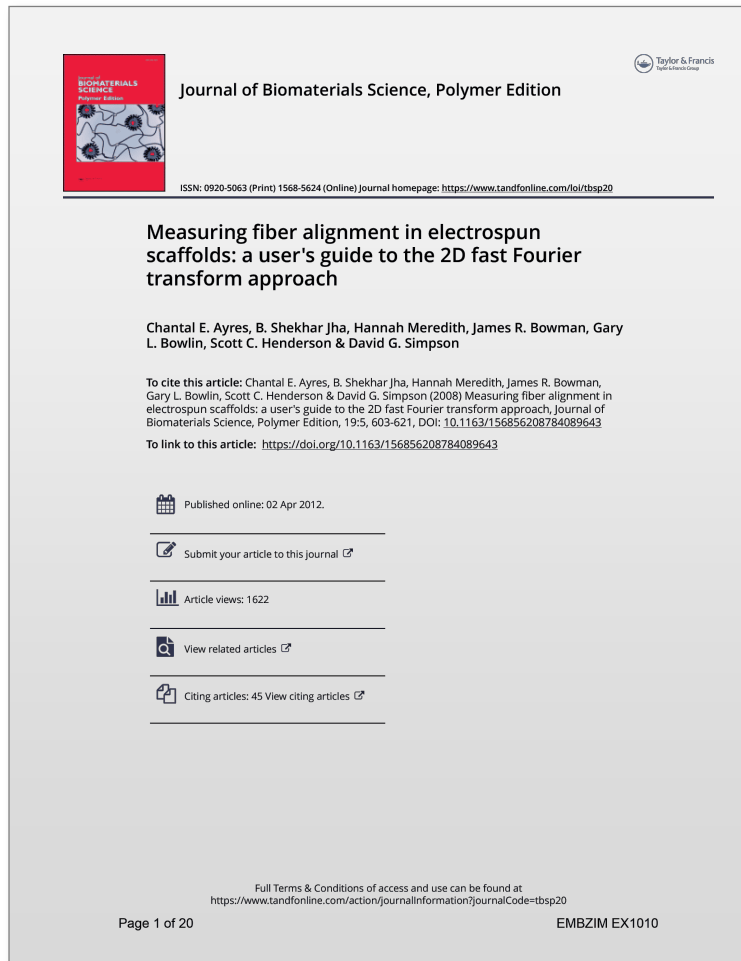
PEAKS	POSITION	VALUE	ABOVE/BELOW AVERAGE
1	0°	75,000	↓
2	90°	95,000	↑
3	180°	75,000	↓
4	270°	95,000	↑
		AVERAGE = 85,000	

Two peaks 180° apart
above average



(Ex. 1001) '223 Patent, Fig. 8a

Ayres 2008 (Ex. 1010)



the plot has been rotated 90°, Fig. 3F). The 2D FFT alignment plot of this scaffold (Fig. 3M, vertical aligned wavy plot) contains distinct peaks at 90 and 270° (2D FFT alignment value of 0.06 units) and much smaller peaks at 180 and 360° (2D FFT alignment value of 0.02 units). The larger peaks report the principle axis of alignment while the smaller peaks correspond to background information, noise and edge effects (Fig. 3M).

(Ex. 1010 at 615)

Federal Circuit:



“Admissions in the specification regarding the prior art are binding on the patentee for purposes of a later inquiry into obviousness. *See Constant v. Advanced Micro-Devices, Inc.*, 848 F.2d 1560, 1570 (Fed. Cir. 1988) (“A statement in the patent that something is in the prior art is binding on the applicant and patentee for determinations of anticipation and obviousness.”)

PharmaStem Therapeutics, Inc. v. ViaCell, Inc., 491 F.3d 1342, 1362 (Fed. Cir. 2007)

Ayres 2008 (Ex. 1010)



Journal of Biomaterials Science, Polymer Edition

ISSN: 0920-5063 (Print) 1568-5624 (Online) Journal homepage: <https://www.tandfonline.com/loi/tbsp20>

Measuring fiber alignment in electrospun scaffolds: a user's guide to the 2D fast Fourier transform approach

Chantal E. Ayres, B. Shekhar Jha, Hannah Meredith, James R. Bowman, Gary L. Bowlin, Scott C. Henderson & David G. Simpson

To cite this article: Chantal E. Ayres, B. Shekhar Jha, Hannah Meredith, James R. Bowman, Gary L. Bowlin, Scott C. Henderson & David G. Simpson (2008) Measuring fiber alignment in electrospun scaffolds: a user's guide to the 2D fast Fourier transform approach, Journal of Biomaterials Science, Polymer Edition, 19:5, 603-621, DOI: 10.1163/156856208784089643

To link to this article: <https://doi.org/10.1163/156856208784089643>

Published online: 02 Apr 2012.

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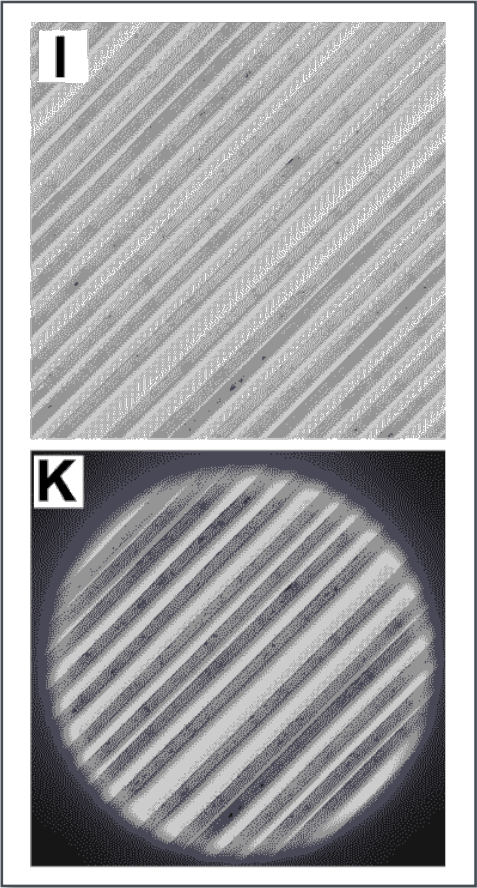
Article views: 1622

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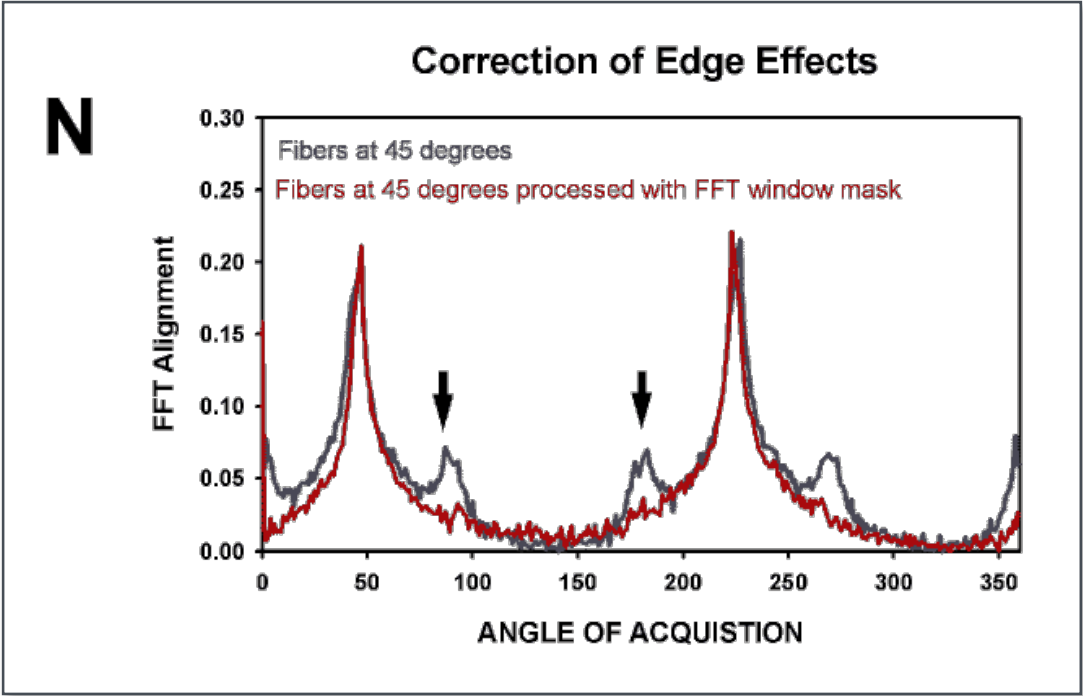
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Page 1 of 20EMBZIM EX1010



N

Correction of Edge Effects



Angle of Acquisition (degrees)	FFT Alignment (Black Curve)	FFT Alignment (Red Curve)
0	0.05	0.15
45	0.21	0.21
90	0.05	0.05
135	0.01	0.01
180	0.05	0.05
225	0.21	0.21
270	0.05	0.05
315	0.01	0.01
360	0.05	0.05

(Ex. 1010 at 614)

IPR2025-00248, Paper 27 at 15; EX1003, ¶ 49; EX1219, ¶ 11
IPR2025-00249, Paper 26 at 12-13; EX1003, ¶ 50; EX1219, ¶ 11

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

embody
Repair. Regenerate. Restore.

53

Claim Construction: Testimony



Antonios Mikos

- Q.** I guess my first question is: Do you agree with Dr. Simpson that the peaks identified by the arrows are -- in the blue trace are noise effects?
- A.** Again, if I see the blue line in isolation, what appears to be a peak that an arrow points to, I would not consider that noise. For me, the noise is that movement of the line up and down with equal probability going up and down. This, to me, at the arrow points appears to be a peak.

(Ex. 1220) Mikos Tr. at 82:19-83:7

Claim Construction: Testimony



Antonios Mikos

- Q.** Is it correct that the blue trace, the blue line, would fall within your definition of having two major peaks?
- A.** I would have to calculate how much is the average. And if the peaks at 45 degrees and 225 degrees are above the average, they will be classified as major peaks.
- Q.** Okay. And is it correct that under your analysis, the red trace would not have two major peaks?
- A.** If the heights at 45 degrees 15 and 225 degrees of the red trace are the same, these would not be classified as major peaks.
- Q.** But isn't it correct that it's -- that both the red and the blue are analyzing the same scaffold?
- A.** The scaffold is the same. The analysis is different.

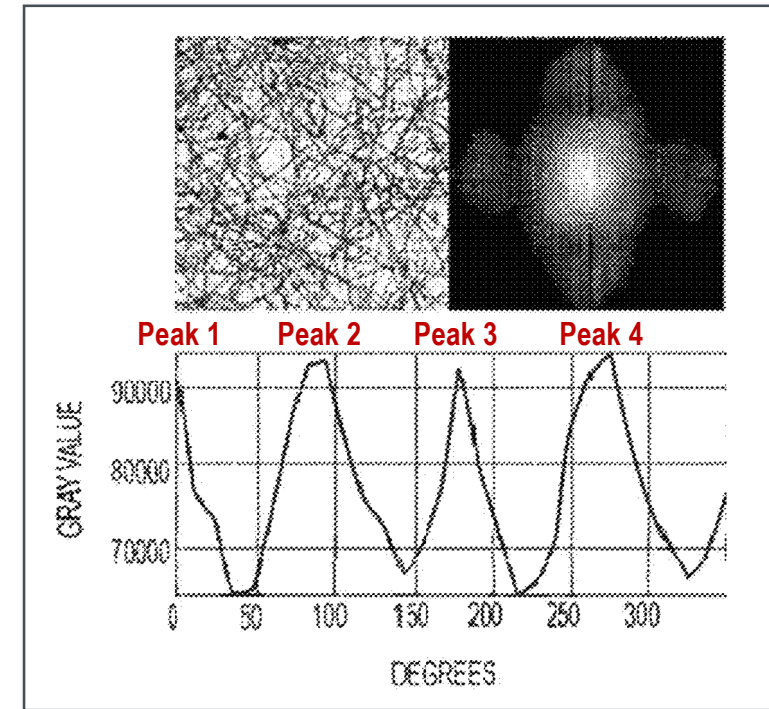
(Ex. 1220) Mikos Tr. at 83:21-84:23

'223 Patent, Fig. 8b

Dr. Simpson's measurement of Figure 8B
Two "major peaks" above the average 180° apart

PEAKS	POSITION	VALUE	ABOVE/BELOW AVERAGE
1	0°	90,440	↓
2	90°	93,630	↑
3	180°	92,570	↓
4	270°	94,600	↑
		AVERAGE = 92,810	

Two peaks 180° apart
above average



(Ex. 1001) '223 Patent, Fig. 8b

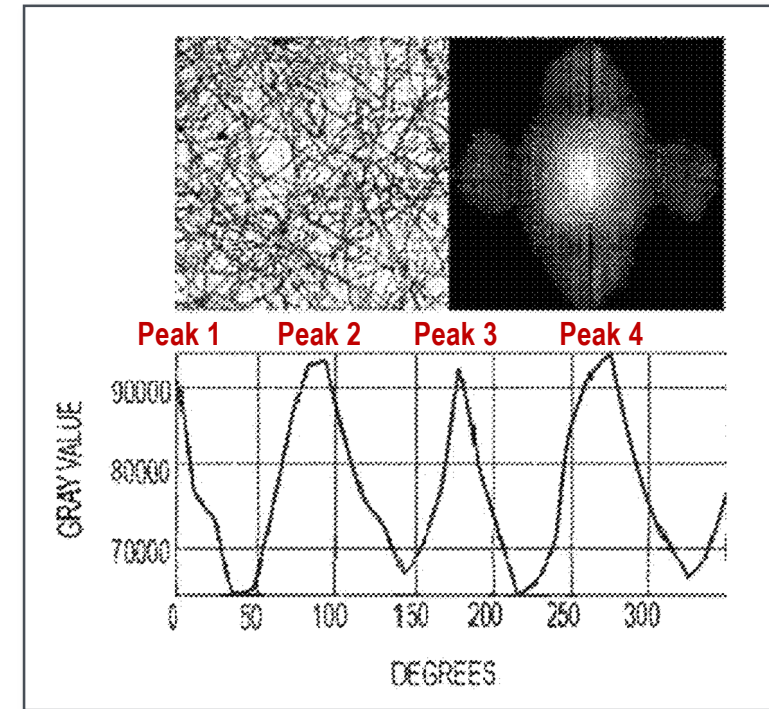
'223 Patent, Fig. 8b

Dr. Mikos's testimony concerning Figure 8B
Two "major peaks" above the average 180° apart

PEAKS	POSITION	VALUE	ABOVE/BELOW AVERAGE
1	0°	91,000 OR 93,000	↓
2	90°	94,000 OR 95,000	↑
3	180°	92,500	↓
4	270°	95,000	↑
		AVERAGE = 93,125 OR 93,875	

Two peaks 180° apart
above average

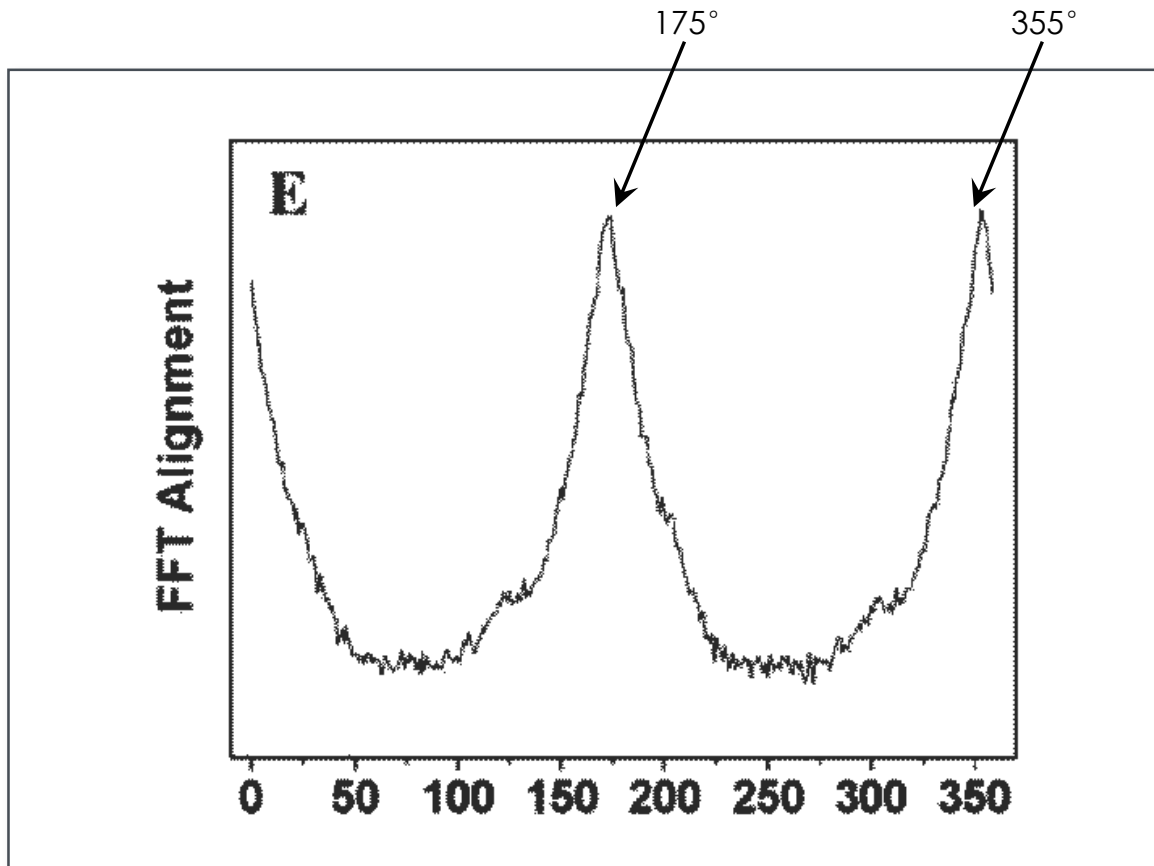
(Ex. 1220, Tr. 66:2-11, 68:25-69:23)



(Ex. 1001) '223 Patent, Fig. 8b

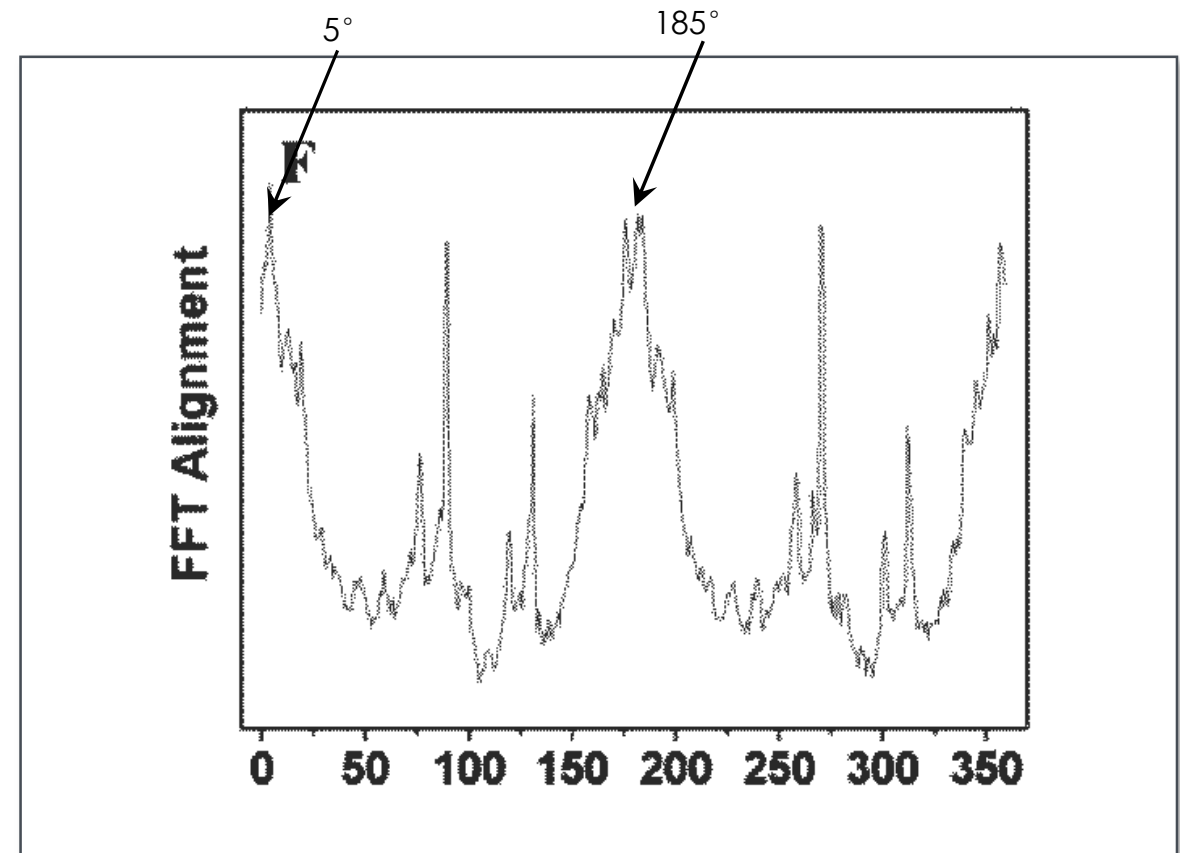
Prosecution History: Ex 1002 at 210-211, 234, 237-238

Huang1 (Ex. 1008) aligned plot



(Ex 1008 at 309, 311, Fig. 4E)

Huang1 (Ex. 1008) random Plot



(Ex 1008 at 309, 311, Fig. 4F)

Dr. Simpson's testing

Dr. Simpson's Testing (Ex. 1003)

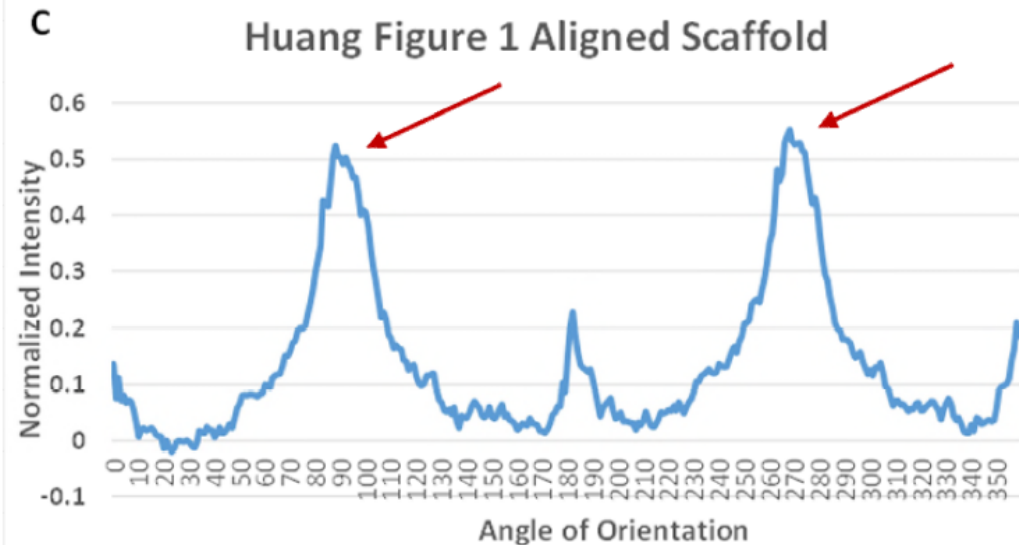


Figure 2. (A) Figure 1 from Huang aligned scaffold cropped and masked for 2DFFT. (B) 2DFFT spectrum of aligned scaffold, the image has been rotated to correct for the rotation intrinsic to the FFT analysis. (c) Normalized intensity values for aligned scaffold 0-360 degrees.

110. Finally, I conducted my own analysis of Figure 1B using the FFT analysis described in Huang2 and the '223 patent. I report the data from 0° to 360° and normalized the y-axis.

111. The graph generated from the FFT analysis demonstrates that the aligned fibers have a first major peak at about 90° and, because I reported all the peaks through 360°, a second major peak at about 270°, which shows that the peaks are 180° apart as recited in claim 1. The major peaks are annotated above with red arrows.

(Ex. 1003), ¶¶ 110-111

Federal Circuit:



“The specification of the ’593 patent is entirely silent on how to transmit user locations and maps from a server to a user's mobile device, suggesting that a person of ordinary skill in the art was more than capable of selecting between the known methods of accomplishing this.”

Uber Techs., Inc. v. X One, Inc., 957 F.3d 1334, 1339 (Fed. Cir. 2020)

Publicover argues that Venable and Kiderman's disclosures are too sparse But as the examiner and Board correctly found, Publicover's specification is just as sparse on how a system would identify this type of eye movement. Under the circumstances, we find this attorney argument as to the capabilities of a skilled artisan unpersuasive.

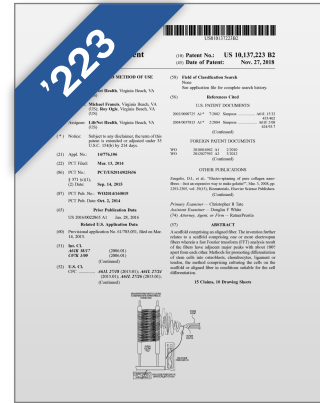
In re Publicover, 813 F. App'x 527, 532 (Fed. Cir. 2020)

“Patent Owner did not provide in its own specification ‘the type of detail [it] now argues is necessary in prior art references,’ which permits a ‘finding that one skilled in the art would have known how to implement the features of the references and would have concluded that the reference disclosures would have been enabling.’”

Donghee Am., Inc. v. Plastic Omnium Advanced Innovation & Rsch., No. IPR2017-01633, 2019 WL 80465, at *5 (P.T.A.B. Jan. 2, 2019) (quoting *In re Epstein*, 32 F.3d 1559, 1568 (Fed. Cir. 1994))

Dr. Simpson's Testing

PATENT OWNER'S CRITICISMS	NOT DISCLOSED IN THE '223 PATENT
Use Of Masking	Patent does not disclose whether a mask was used
File Type	Patent does not disclose file type used to store images or how many times images open and closed
Illumination	Patent does not disclose how even the illumination was used to capture the image
Placement Of Circular Projection	Patent does not disclose how the circular projection was applied
Normalization	Patent does not describe if the results were normalized or how it was performed
Software	Patent does not disclose version of software used



Dr. Simpson's Testing

PATENT OWNER STATES

“Whether masks are consistent with Ayres-2008 is irrelevant. Ex.1219 ¶38. Dr. Simpson claimed to follow *Huang2*. Ex.1003 ¶110.”

(Sur-reply (Paper 30) at 13, n.8.)

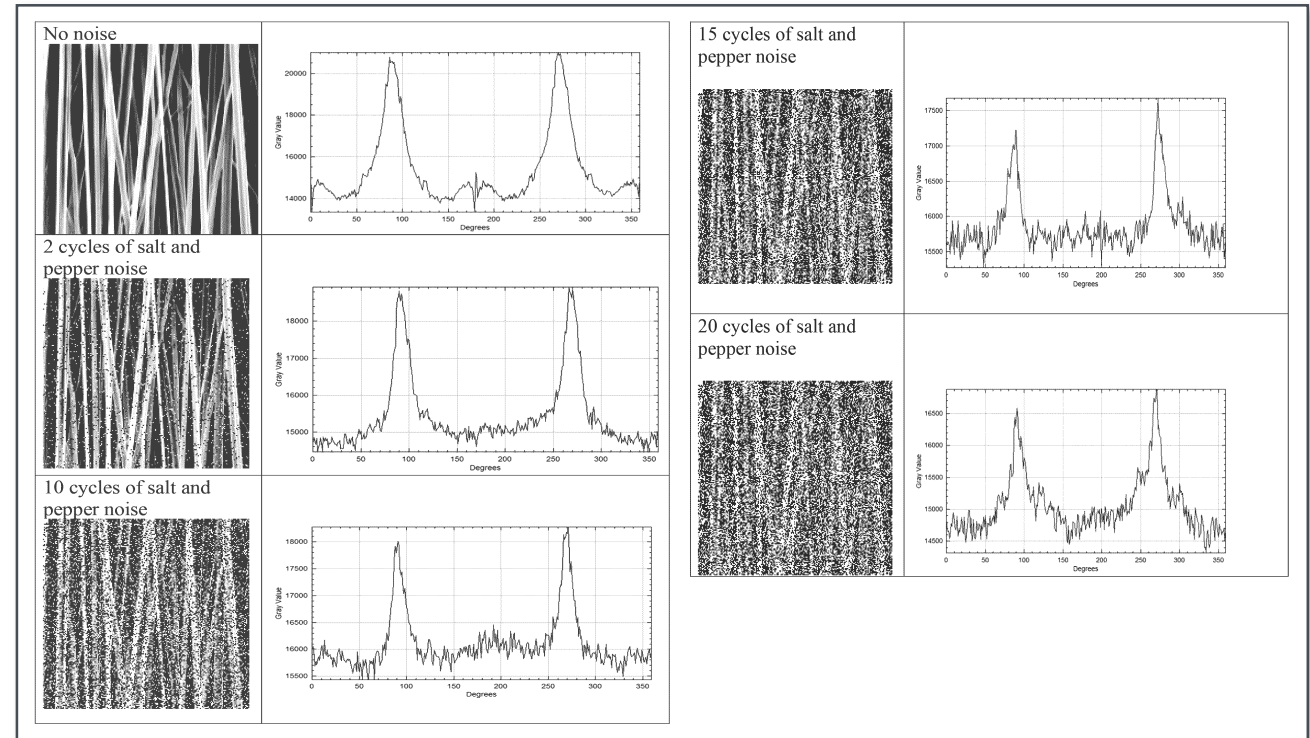
DR. SIMPSON STATED

“I conducted my own analysis of Figure 1B using the FFT analysis described in *Huang2* and the '223 patent.”

(Ex. 1003), ¶110

Dr. Simpson's Testing

- Dr. Simpson testified:
 - FFT “technique is pretty forgiving. And so even an imperfect image can be used”
 - Biomaterials would not have published Huang2 without an adequate image
(EX2017, 61:11-15, 142:10-18.)



(Ex. 1219), ¶ 39

Dr. Simpson's Testing and Huang2 (Ex. 1005)



Both show two major peaks when viewed over 360 degrees due to the symmetry of FFT



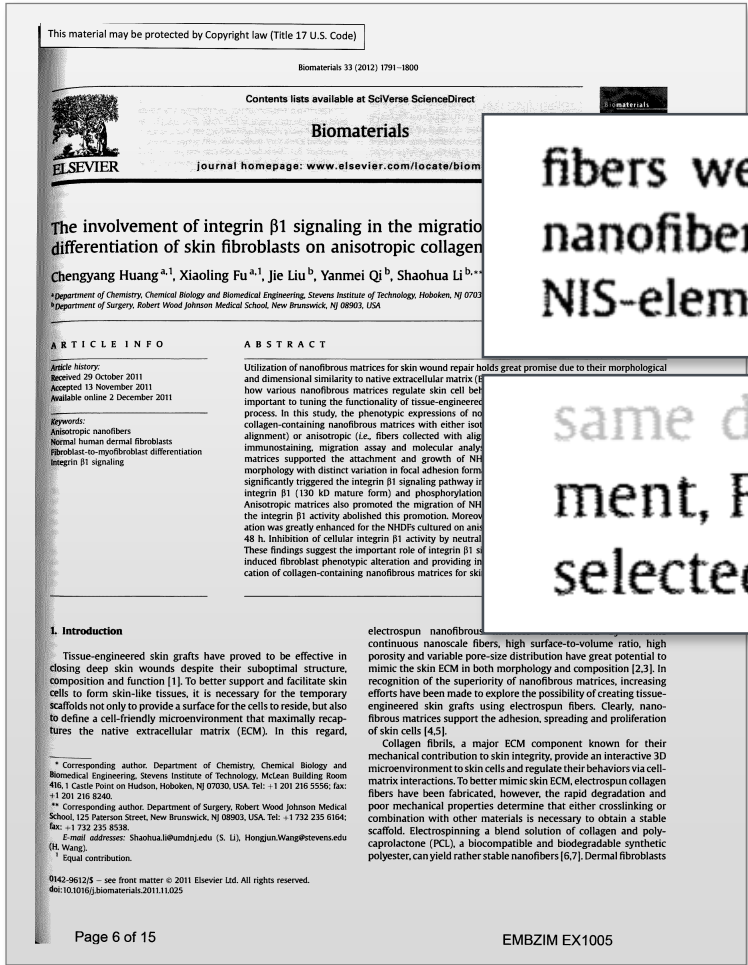
Both show the scaffolds prepared by Huang2 are aligned



Huang2 confirmed alignment by mechanical testing



Dr. Mikos admits that Huang2's fibers are aligned (EX1220 at 96:13-15)



fibers were examined with a LEO 982 FEG SEM. To determine the diameter of nanofibers, images of five randomly selected areas were captured and analyzed by NIS-elements BR 3.10 analysis software from Nikon. Tensile properties of the elec

same direction as the rotation. To better analyze the fiber alignment, FFT analysis was performed on the SEM images of randomly selected area (n = 5) to characterize the anisotropy and assign

(Ex. 1005 at 1791, 1794)

Claim 1 of the '227 Patent

Claim 1 of the '227 Patent (Ex 1001)

		US 1318227B2	
		US 1318227 B2	
		Francis et al.	
		(10) Patent No.: US 13,182,227 B2	
		(45) Date of Patent: *May 3, 2022	
		References Cited	
		U.S. PATENT DOCUMENTS	
(54) ALIGNED FIBER AND METHOD OF USE THEREOF	(56)		
(71) Applicant: LifeNet Health, Virginia Beach, VA (US)		6,189,537 B1	2/2001 Wolfenburger
(72) Inventors: Michael Francis, Virginia Beach, VA (US); Roy Ogle, Virginia Beach, VA (US)		6,305,379 B1	10/2001 Wolfenburger
(73) Assignee: LIFENET HEALTH		2,002,009,075 A1	7/2002 Simpson et al.
(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 591 days.		2,004,003,813 B1	2/2004 Simpson et al.
		2007,025,563 A1	9/2007 Simpson et al.
		2008,021,389 A1	9/2008 Lelkes et al.
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		2012,027,393 A1	11/2012 Shoseyov et al.
			606:152
		FOREIGN PATENT DOCUMENTS	
(1) Appl. No.: 16/045,159		WO	2010/016942 A1 2/2010
(2) Filed: Jul. 25, 2018		WO	2012/027,592 A2 3/2012
(25) Prior Publication Data		WO	2012/027,8472 * 6/2012
US 2019/0167842 A1 Jun. 6, 2019		WO	2013/033,680 A1 3/2013
		WO	2013/198,731 A1 8/2013
		OTHER PUBLICATIONS	
(63) Division of application No. 14/776,106, filed as application No. PCT/US2014/025636 on Mar. 13, 2014, now Pat. No. 10,137,223.		Sharma et al., State-of-Art Functional Biomaterials for Tissue Engineering, Front. Mater., 2019, pp. 1-10.*	
(64) Provisional application No. 61/785,031, filed on Mar. 14, 2013.		Hutmacher et al., State of the art and future directions of scaffold-based bone engineering from a biomaterials perspective, J Tissue Eng Regen Med 2007; 1: 245-260.*	
(51) Int. Cl.		Dash and Kokkinnala, Poly- ϵ -caprolactone based formulations for drug delivery and tissue engineering: A review, Journal of Controlled Release, vol. 158, Issue 1, Feb. 28, 2012, pp. 15-33.*	
A61F 2/08 (2006.01)		Werner et al., State of the art and future directions of scaffold-based bone engineering from a biomaterials perspective, J Tissue Eng Regen Med 2007; 1: 245-260.*	
A61L 27/24 (2006.01)		Anderson et al., "Biodegradation and Biocompatibility of PLA and PLA Microspheres", Advanced Drug Delivery Reviews, 1997, vol. 28, pp. 603-621.	
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A61L 38/17 (2006.01)		Behravesh et al., "Synthetic Biodegradable Polymers for Orthopaedic Applications," Clinical Orthopaedics and Related Research, No. 3678, pp. S118-S125.	
A61L 27/18 (2006.01)		Clark et al., "Microparticles of Poly(D,L-lactic-co-glycolic acid)/poly(ethylene glycol) Blends for Controlled Drug Delivery," Journal of Controlled Release, vol. 48 1997 pp. 259-268.	
A61L 27/36 (2006.01)		Huang et al., "Electrospun Collagen-Chitosan-TPU Nanofibrous Scaffolds For Tissue Engineered tubular Grafts", Colloids and Surfaces B: Biointerfaces, vol. 82 2011, pp. 307-315.	
A61L 27/56 (2006.01)		(Continued)	
D01D 5/00 (2006.01)		Primary Examiner — Maria Marvich	
C12N 5/079 (2010.01)		(74) Attorney, Agent, or Firm — Ratner/Prestia	
U.S. Cl.		(57) ABSTRACT	
(52) CPC		A scaffold comprising an aligned fiber. Further, 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. Also, 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.	
A61F 2/08 (2013.01); A61L 27/24 (2013.01); A61L 27/26 (2013.01); A61L 27/36 (2013.01); A61L 27/38 (2013.01); A61L 27/56 (2013.01); A61L 27/58 (2013.01); A61L 27/60 (2013.01); A61L 27/62 (2013.01); A61L 27/64 (2013.01); A61L 27/66 (2013.01); A61L 27/68 (2013.01); A61L 27/70 (2013.01); A61L 27/72 (2013.01); A61L 27/74 (2013.01); A61L 27/76 (2013.01); A61L 27/78 (2013.01); A61L 27/80 (2013.01); A61L 27/82 (2013.01); A61L 27/84 (2013.01); A61L 27/86 (2013.01); A61L 27/88 (2013.01); A61L 27/90 (2013.01); A61L 27/92 (2013.01); A61L 27/94 (2013.01); A61L 27/96 (2013.01); A61L 27/98 (2013.01); A61L 28/00 (2013.01); A61L 28/02 (2013.01); A61L 28/04 (2013.01); A61L 28/06 (2013.01); A61L 28/08 (2013.01); A61L 28/10 (2013.01); A61L 28/12 (2013.01); A61L 28/14 (2013.01); A61L 28/16 (2013.01); A61L 28/18 (2013.01); A61L 28/20 (2013.01); A61L 28/22 (2013.01); A61L 28/24 (2013.01); A61L 28/26 (2013.01); A61L 28/28 (2013.01); A61L 28/30 (2013.01); A61L 28/32 (2013.01); A61L 28/34 (2013.01); A61L 28/36 (2013.01); A61L 28/38 (2013.01); A61L 28/40 (2013.01); A61L 28/42 (2013.01); A61L 28/44 (2013.01); A61L 28/46 (2013.01); A61L 28/48 (2013.01); A61L 28/50 (2013.01); A61L 28/52 (2013.01); A61L 28/54 (2013.01); A61L 28/56 (2013.01); A61L 28/58 (2013.01); A61L 28/60 (2013.01); A61L 28/62 (2013.01); A61L 28/64 (2013.0			

1. A method of treating a tissue defect in a subject comprising
implanting at the tissue defect a scaffold comprising crosslinked fibers comprising collagen, wherein a result of a fast Fourier transform (FFT) analysis of the fibers demonstrates that the fibers have major adjacent peaks that are about 180° apart from each other, and
applying the scaffold to the tissue defect to repair a tendon, a ligament or a nerve defect.

(Ex. 1001) '227 Patent, Claim 1

Claim 1 of '227: Testimony



Antonios Mikos

A. Let me review those examples. I did not see in Examples 1 through 4 of the '227 patents any mention of an animal model for the tissue repair, and the tissue repair that you're referring to was the tendon, right?

Q. Correct.

A. Yes.

Q. And since you've just reviewed the -- the examples, in any of the examples of the '227 patent did the inventors actually treat a ligament injury?

A. Again, just reading Examples 1 through 4 of the '227 patent, I don't see in the examples any study involving an animal model for the repair of ligament.

(Ex. 1220) Mikos Tr. at 193:3-19

Petition at 29-30

- “Kuo, which is in the same field, discloses electrospun collagen/PCL scaffolds that are seeded with cells and formed into a desired shape to replace tissue including muscles, ligaments, tendons, and nerves, among others, to repair various tissue injuries.

(EX1018) Title, Abstract, [0005], [0052], [0113]

- Accordingly, a POSA would have been motivated and able to use a scaffold comprising Huang2's aligned fibers—as shown by FFT analysis—containing collagen, which may be cross-linked, to treat a tendon, ligament, or nerve defect as taught by Kuo.

Kuo (Ex. 1018)

(19) United States
(12) Patent Application Publication
Kuo et al.

(10) Pub. No.: US 2011/0293685 A1
(43) Pub. Date: Dec. 1, 2011

(54) **SCAFFOLDS FOR TISSUE ENGINEERING AND REGENERATIVE MEDICINE**

(75) **Inventors:** **Catherine K. Kuo**, Boston, MA (US); **Nathan Zamarripa**, Freeport, ME (US); **Amelia H. Thomas**, Medford, MA (US)

(73) Assignee: **TRUSTEES OF TUFTS COLLEGE**, Medford, MA (US)

(21) Appl. No.: 13/122,414

(22) PCT Filed: **Oct. 5, 2009**

(86) PCT No.: **PCT/US09/59547**

§ 371 (c)(1),
(2), (4) Date: **Aug. 22, 2011**

US 20110293685A1

Related U.S. Application Data

(60) Provisional application No. 61/102,440, filed on Oct. 3, 2008.

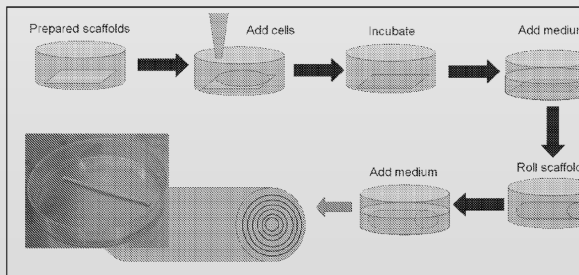
Publication Classification

(51) **Int. Cl.**
A61K 9/70 (2006.01)
A61P 17/00 (2006.01)
A61K 35/12 (2006.01)

(52) U.S. Cl. 424/422; 424/400; 424/93.7

(57) ABSTRACT

The methods and compositions described herein relate to novel 3-dimensional porous scaffolds useful for tissue regeneration, enhancement, or tissue repair. Electrospinning or other methods are used to create mats comprised of fibers that can be seeded with cells and subsequently rolled into a desired shape/form to replace a desired tissue.



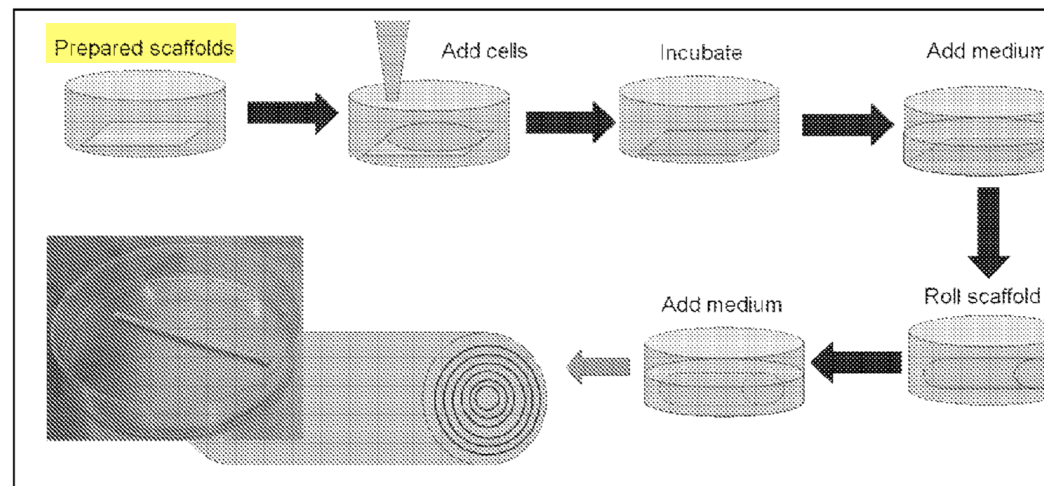
(19) **United States**

(12) **Patent Application Publication** (10) Pub. No.: US 2011/0293685 A1
Kuo et al. (43) Pub. Date: Dec. 1, 2011

(54) **SCAFFOLDS FOR TISSUE ENGINEERING AND REGENERATIVE MEDICINE**

(57) **ABSTRACT**

The methods and compositions described herein relate to novel 3-dimensional porous scaffolds useful for tissue regeneration, enhancement, or tissue repair. Electrospinning or other methods are used to create mats comprised of fibers that can be seeded with cells and subsequently rolled into a desired shape/form to replace a desired tissue.



(Ex. 1018)

Claim 1 of 227 Patent: Testimony



Antonios Mikos

- Q.** Isn't the first step of the schematic in Figure 1 to prepare a scaffold?
- A.** This is the first step but, as I said earlier, the schematic after the first arrow.
- Q.** So add cells is the second step after you prepared the scaffold, correct?
- A.** Yes.
- Q.** Okay. And is it correct that Figure 1 shows preparing the scaffold as a sheet?
- A.** That's my understanding. It looks like it's a two-dimensional scaffold that the cells are seeded onto.

(Ex. 1220) Mikos Tr. at 161:12-162:6

SUMMARY OF THE INVENTION

When seeded with cells and subsequently rolled into cylindrical form, these constructs can be used to form structures e.g., similar to fascicles of the muscle, tendon, nerve, or ligament.

[0022] Another aspect described herein is a method for replacing or enhancing a tissue, the method comprising: (a) forming a tissue construct by contacting a scaffold sheet of fibrous material with a cell; and rolling the scaffold sheet in a jelly-roll like manner to form a spirally wound tissue construct, (b) implanting the spirally wound tissue construct into a subject in need of tissue replacement or regeneration.

United States
Patent Application Publication
 Kuo et al.

(19)
 (12)

(10) Pub. No.: US 2011/0293685 A1
 (43) Pub. Date: Dec. 1, 2011

(54) SCAFFOLDS FOR TISSUE ENGINEERING AND REGENERATIVE MEDICINE

Related U.S. Application Data

(60) Provisional application No. 61/102,440, filed on Oct. 3, 2008.

(75) Inventors: **Catherine K. Kuo**, Boston, MA (US); **Nathan Zamarripa**, Freeport, ME (US); **Amelia H. Thomas**, Medford, MA (US)

Publication Classification

(51) **Int. Cl.**
A61K 9/70 (2006.01)
A61P 17/00 (2006.01)
A61K 35/12 (2006.01)

(52) **U.S. Cl.** **424/422; 424/400; 424/93.7**

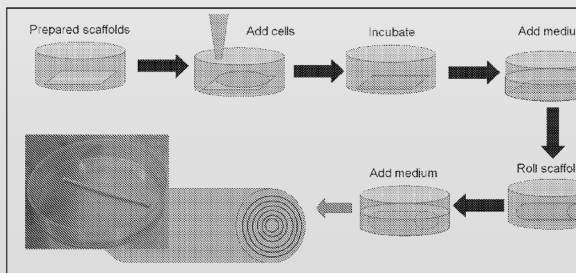
(21) Appl. No.: 13/122,414

(22) PCT Filed: **Oct. 5, 2009**

(86) PCT No.: **PCT/US09/59547**

§ 371 (c)(1),
(2), (4) Date: **Aug. 22, 2011**

The methods and compositions described herein relate to novel 3-dimensional porous scaffolds useful for tissue regeneration, enhancement, or tissue repair. Electrospinning or other methods are used to create mats comprised of fibers that can be seeded with cells and subsequently rolled into a desired shape/form to replace a desired tissue.



Kuo (Ex. 1018): Electrospinning

US 20110293685A1

(19) United States
(12) Patent Application Publication
Kuo et al.

(10) Pub. No.: US 2011/0293685 A1
(43) Pub. Date: Dec. 1, 2011

(54) SCAFFOLDS FOR TISSUE ENGINEERING AND REGENERATIVE MEDICINE

(75) Inventors: Catherine K. Kuo, Boston, MA (US); Nathan Zamarripa, Freeport, ME (US); Amelia H. Thomas, Medford, MA (US)

(73) Assignee: TRUSTEES OF TUFTS COLLEGE, Medford, MA (US)

(21) Appl. No.: 13/122,414
(22) PCT Filed: Oct. 5, 2009
(86) PCT No.: PCT/US09/59547
§ 371 (c)(1), (2), (4) Date: Aug. 22, 2011

Related U.S. Application Data
(60) Provisional application No. 61/102,440, filed on Oct. 3, 2008.

Publication Classification
(51) Int. Cl. A61K 9/70 (2006.01); A61P 17/00 (2006.01); A61K 35/12 (2006.01)
(52) U.S. Cl. 424/422; 424/400; 424/93.7
(57) ABSTRACT
The methods and compositions described herein relate to novel 3-dimensional porous scaffolds useful for tissue regeneration, enhancement, or tissue repair. Electrospinning or other methods are used to create mats comprised of fibers that can be seeded with cells and subsequently rolled into a desired shape/form to replace a desired tissue.

The diagram illustrates a cyclic process for preparing scaffolds. It starts with 'Prepared scaffolds' (a mesh cylinder), followed by 'Add cells' (a pipette adding liquid), 'Incubate' (a mesh cylinder), 'Add medium' (a pipette adding liquid), 'Roll scaffold' (a mesh cylinder being rolled), and finally 'Add medium' (a pipette adding liquid) to produce 'Prepared scaffolds' again. A detailed inset shows a cross-section of a scaffold with a central tube and a surrounding porous layer.

Production of Fibers

[0044] In one embodiment, the fibers are produced using electrospinning. Electrospinning can be performed by any means known in the art (see, for example, U.S. Pat. No. 6,110,590). Preferably, a steel capillary tube with a 1.0 mm

[0046] Electrospinning for the formation of fine fibers has been actively explored recently for applications such as high performance filters and biomaterial scaffolds for cell growth, vascular grafts, wound dressings or tissue engineering. Fibers

(Ex. 1018), ¶¶ [0044], [0046]

“Huang2 discloses that its scaffold is used for skin would repair.”

Dr. Mikos declaration (Ex. 2015, ¶ 115)

Claim 1 of the '227 Patent

Dr. Mikos (Ex. 2015, ¶ 116)

“[T]he scaffold in Huang2 is seeded with fibroblasts, which are incapable of repairing tendon, ligament, or nerve defects. Again, the Petition does not assert that a POSITA would have modified Huang2 to include alternate cell types.”

Petition at 30

Kuo further teaches that a wide range of cell types can be used “depending upon the intended function of the tissue engineered construct being produced.” (EX1018, [0058])

Dr. Simpson (Ex. 1003, ¶ 96)

“Kuo further teaches that a wide range of cell types can be used depending upon the intended function of the tissue engineered construct being produced.” (EX1018, [0058].)

Dr. Simpson (Ex. 1003, ¶ 102)

“And a POSA would have been able to make and use a scaffold comprising Huang2’s aligned fibers and seeded with various cell types using well-known established methods”

Claim 1 of '227 Patent: Testimony



Antonios Mikos

- Q.** For example, if you know sitting here today, in healing a tendon injury, is there a differentiation of fibroblast to myofibroblast?
- A.** It is one of the cellular processes that's important. It's not the only cellular process, but it's one of them.

(Ex. 1220) Mikos Tr. at 105:23-106:7

Repair of Tendon Defect with Dermal Fibroblast Engineered Tendon in a Porcine Model

TISSUE ENGINEERING
Volume 12, Number 4, 2006
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Repair of Tendon Defect with Dermal Fibroblast Engineered Tendon in a Porcine Model

WEI LIU, M.D., Ph.D.,* BIN CHEN, M.D.,* DAN DENG, M.D., FENG XU, M.D.,
LEI CUI, M.D., Ph.D., and YILIN CAO, M.D., Ph.D.

ABSTRACT

Harvesting autologous tenocytes for tendon engineering may cause secondary donor site defect. Dermal fibroblasts are an easily accessible cell source and do not require a large surgical site. This study aims to explore the possibility of tendon engineering using dermal fibroblasts. A total of 45 hybrid pigs were randomly divided into three groups: experimental group 1 (PGA) alone, experimental group 2 (PGA) with dermal fibroblast engineered tendon; control group 1 (PGA) alone, control group 2 (PGA) with tenocyte engineered tendon. At weeks 6, 14, and 26 for gross, histological, and mechanical analyses. Attachment of both dermal fibroblasts and tenocytes on PGA fibers and results showed that fibroblast and tenocyte engineered tendons were similar in gross view, histology, and tensile strength. At 6 weeks, parallel collagen fibers were observed in the middle in histology, with more cellular component. At weeks 14 and 26, both engineered tendons exhibited histology similar to natural tendon. Collagens became parallel throughout the tendon structure, and PGA fibers were degraded. Interestingly, dermal fibroblast and tenocyte engineered tendon collagen at 26 weeks, which remained observable in normal pig skin and in polarized microscopy, suggesting a possible phenotype change of myofibroblasts. Furthermore, both fibroblast and tenocyte engineered tendons shared similar tensile strength. At 6 weeks in control group 2, neo-tendon formed in peripheral area by host cells. A cord-like tissue was formed at weeks 14 and 26. Tissue was histologically disorganized and mechanically weaker than the natural tendon ($p < 0.05$). These results suggest that dermal fibroblasts may have the potential for tendon engineering.

Department of Plastic and Reconstructive Surgery, Shanghai Ninth People's Hospital, Shanghai Second Medical University, Shanghai, P. R. China.
*These two authors contributed equally to this work.

This work was presented previously at the 47th Annual Meeting of the Plastic Surgery Research Society, Massachusetts, and at the 49th Annual Meeting of the Orthopedic Surgery Research Society, Louisiana.

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Page 1 of 14

Compared to BMSCs, dermal fibroblasts share more common characteristics with tenocytes. Both cell types are terminal differentiated cells and originate from mesoderm. During *in vitro* culture, both exhibit similar cell morphology. Biochemically, both cells produce major extracellular matrix type I collagen, although dermal fibroblasts also produce type III collagen. Functionally, both cells form connective tissues to carry out tissue-supporting functions. In addition, dermal fibroblasts are an easily accessible cell source. Harvesting of a small piece of skin tissue will not cause major donor site defect. Furthermore, dermal fibroblasts are easily expanded during *in vitro* culture.

The result of this study demonstrated that tendons engineered by two different cell types exhibited similar features in their gross view, histology, and biomechanical property, indicating that dermal fibroblasts may replace tenocytes for tendon engineering. Importantly, the dermal fibroblast engineered tendon also resembled a natural tendon in its histological structure. In addition, at 26 weeks, its tensile strength reached 74% of natural tendon strength, similar to that of tenocyte engineered tendon (76%), indicating that dermal fibroblasts can also generate a functional tendon, at least from the point view of biomechanical property.

(Ex. 1205), pp. 775-776, 786-787

EMBZIM EX1205

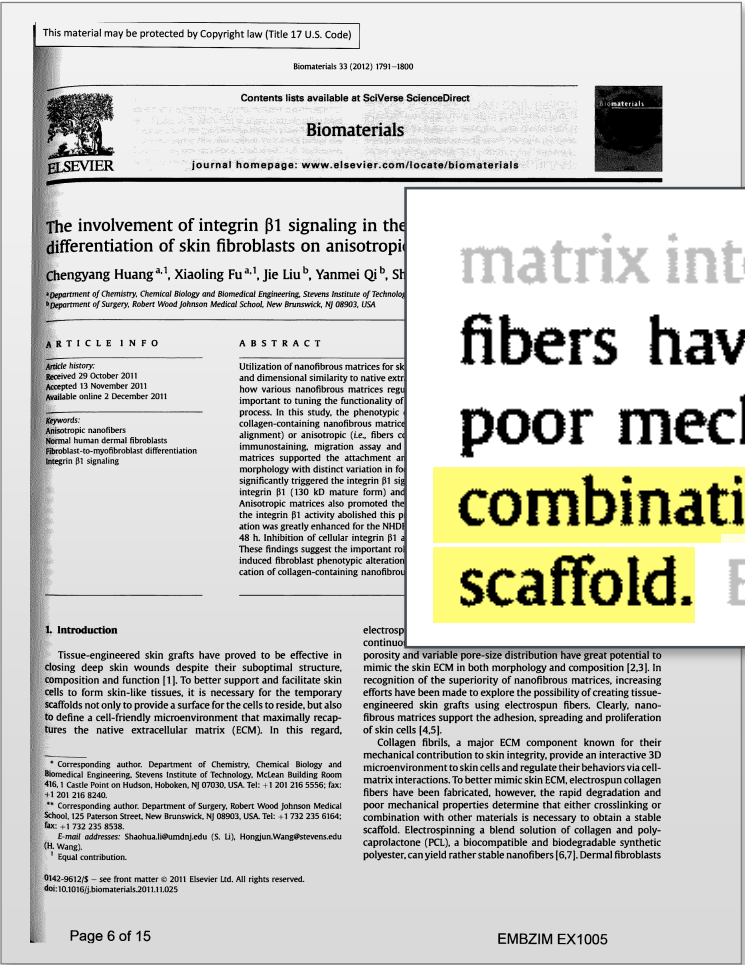
'227 Patent: crosslinking

 US011318227B2	
(12) United States Patent Francis et al.	(10) Patent No.: US 11,318,227 B2 (45) Date of Patent: *May 3, 2022
(54) ALIGNED FIBER AND METHOD OF USE THEREOF	(56) References Cited U.S. PATENT DOCUMENTS
(71) Applicant: LifeNet Health , Virginia Beach, VA (US)	6,189,537 B1 2/2001 Wolfburger 6,305,379 B1 10/2001 Wolfburger 2002/0090725 A1 7/2002 Simpson et al. 2004/0037813 A1 2/2004 Simpson et al. 2007/0225631 A1 9/2007 Bowlin et al. 2008/0213389 A1 9/2008 Lelkes et al. 2009/0186443 A1 1/2009 Hathi et al. 2010/0233115 A1 9/2010 Patel et al. 2011/0236974 A1 9/2011 Ogle et al. 2012/0221025 A1* 8/2012 Simpson A61F 2/02 606/152
(72) Inventors: Michael Francis , Virginia Beach, VA (US); Roy Ogle , Virginia Beach, VA (US)	
(73) Assignee: LIFENET HEALTH	
(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 591 days. This patent is subject to a terminal disclaimer.	2012/0273993 A1 11/2012 Shoseyov et al.
(21) Appl. No.: 16/045,159	FOREIGN PATENT DOCUMENTS WO 2010/016942 A1 2/2010 WO 2012/027592 A2 3/2012 WO 2012/078472 * 6/2012 WO 2013/03680 A1 3/2013 WO 2013/119873 A1 8/2013
(22) Filed: Jul. 25, 2018	OTHER PUBLICATIONS Sharma et al., State-of-Art Functional Biomaterials for Tissue Engineering, <i>Front. Mater.</i> , 2019, pp. 1-10.* Hutmacher et al., State of the art and future directions of scaffold-based bone engineering from a biomaterials perspective, <i>J Tissue Eng Regen Med</i> 2007, 1, 245-260.* Dash and Konkalmalla, Poly-ε-caprolactone based formulations for drug delivery and tissue engineering: A review, <i>Journal of Controlled Release</i> , vol. 158, Issue 1, Feb. 28, 2012, pp. 15-33.* Werner et al., State of the art and future directions of scaffold-based bone engineering from a biomaterials perspective, <i>J Tissue Eng Regen Med</i> 2007, 1, 245-260.* Anderson et al., "Biodegradation and Biocompatibility of PLA and PLGA Microspheres", <i>Advanced Drug Delivery Reviews</i> , 1997, vol. 28, pp. 5-24. Ayres et al., "Measuring Fiber Alignment in Electrospun Scaffolds: A User's Guide to the 2D Fast Fourier Transform Approach," <i>Journal of Biomaterials Science, Polymer Edition</i> , (2008), vol. 19, No. 5, pp. 603-621. Behavanh et al., "Synthetic Biodegradable Polymers for Orthopaedic Applications," <i>Clinical Orthopaedics and Related Research</i> , No. 3678, pp. S118-S125. Clark et al., "Microparticles of Poly(DL-lactic-co-glycolic acid)/poly(ethylene glycol) Blends for Controlled Drug Delivery," <i>Journal of Controlled Release</i> , vol. 48 1997 pp. 259-268. Huang et al., "Electrospun Collagen-Chitosan-TPC Nanofibrous Scaffolds For Tissue Engineered tubular Grafts", <i>Colloids and Surfaces B: Biointerfaces</i> , vol. 82 2011, pp. 307-315. (Continued)
(65) Prior Publication Data US 2019/0167842 A1 Jun. 6, 2019	
Related U.S. Application Data (62) Division of application No. 14/776,106, filed as application No. PCT/US2014/025636 on Mar. 13, 2014, now Pat. No. 10,137,223. (60) Provisional application No. 61/785,031, filed on Mar. 14, 2013.	
(51) Int. Cl. A61F 2/08 (2006.01) A61L 27/24 (2006.01) A61L 27/26 (2006.01) A61K 38/17 (2006.01) A61L 27/38 (2006.01) A61L 27/18 (2006.01) A61L 27/36 (2006.01) A61L 27/56 (2006.01) D01D 5/00 (2006.01) C12N 5/079 (2010.01)	
(52) U.S. CL. CPC A61L 27/18 (2013.01); A61L 27/24 (2013.01); A61L 27/26 (2013.01); A61L 27/36B4 (2013.01); A61L 27/36B3 (2013.01); A61L 27/38 (2013.01); A61L 27/38B (2013.01); A61L 27/56 (2013.01); C12N 5/0618 (2013.01); D01D 5/0076 (2013.01); D01D 5/0092 (2013.01); A61F 2/08 (2013.01); A61F 2/400101 (2013.01); A61L 2/40012 (2013.01); C12N 2533/30 (2013.01); C12N 2533/34 (2013.01); C12N 2533/30 (2013.01)	
(58) Field of Classification Search None See application file for complete search history.	ABSTRACT (57) A scaffold comprising an aligned fiber. Further, 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. Also, 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.
	14 Claims, 10 Drawing Sheets

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.

(Ex. 1001) '227 Patent 12:49-63

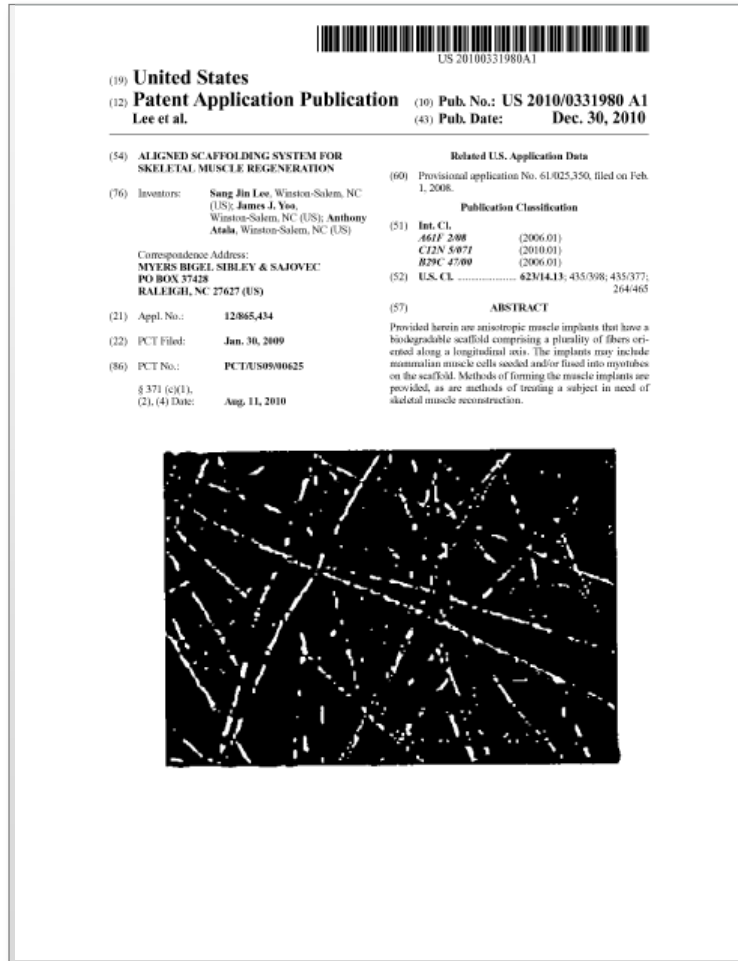
Huang2 (Ex. 1005): crosslinking



matrix interactions. To better mimic skin ECM, electrospun collagen fibers have been fabricated, however, the rapid degradation and poor mechanical properties determine that either crosslinking or combination with other materials is necessary to obtain a stable scaffold. Electrospinning a blend solution of collagen and poly-

(Ex. 1005 at 1791)

Lee (Ex. 1011)



different fiber orientations. After the fabrication process, the electrospun PCL/collagen nanofiber meshes were cross-linked under vapor of 2.5% glutaraldehyde solution for 6 hr to increase stability and strength of collagen. The degrees of cross-linking were measured by Ninhydrin assay. All electro-

(Ex. 1011), ¶ [0063]

Claim 1 of '227 Patent: Testimony



Dr. David Simpson

- Q.** Okay. Is cytotoxicity problematic for a tissue scaffold that would be implanted in a patient?
- A.** You would think so, but usually you do your best to eliminate most of that by incubating the material with blocking agents that absorb all the reactive sites.

(Ex. 2017) Simpson Tr. at 189:5-8

Dependent Claims of the '227 Patent

'227 Patent	Patent Owner does not raise additional arguments as to dependent claims 4-6, and 7-8
	Claims 4-6 are thus obvious if claim 1 is obvious, and claims 7-8 are anticipated if the Board finds that the prior art discloses major peaks FFT 180° apart
	Patent Owner raises additional defenses as to claims 2-3

Claims 2-3 of the '227 Patent

'227 Patent: Claims 2-3

	
US011318227B2	
(12) United States Patent Francis et al.	(10) Patent No.: US 11,318,227 B2 (45) Date of Patent: *May 3, 2022
(54) ALIGNED FIBER AND METHOD OF USE THEREOF	(56) References Cited U.S. PATENT DOCUMENTS
(71) Applicant: LifeNet Health , Virginia Beach, VA (US)	6,189,537 B1 2/2001 Wolfenburger 6,305,379 B1 10/2001 Wolfenburger 2002/0090725 A1 7/2002 Simpson et al. 2004/0037813 A1 2/2004 Simpson et al. 2007/0225631 A1 9/2007 Bowlin et al. 2008/0213389 A1 9/2008 Lelkes et al. 2009/0018643 A1 1/2009 Hathi et al. 2010/0233115 A1 9/2010 Patel et al. 2011/0236974 A1 9/2011 Ogile et al. 2012/0221025 A1* 8/2012 Simpson A61F 2/02 606/152
(72) Inventors: Michael Francis , Virginia Beach, VA (US); Roy Ogile , Virginia Beach, VA (US)	12/0273993 A1 11/2012 Shoseyov et al.
(73) Assignee: LIFENET HEALTH	FOREIGN PATENT DOCUMENTS
(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 591 days. This patent is subject to a terminal disclaimer.	WO 2010/016942 A1 2/2010 WO 2010/27592 A2 3/2012 WO 2010/278472 A 6/2012 WO 2010/36080 A1 3/2013 WO 2013/119873 A1 8/2013
(21) Appl. No.: 16/045,159	OTHER PUBLICATIONS
(22) Filed: Jul. 25, 2018	Sharma et al., State-of-Art Functional Biomaterials for Tissue Engineering, Front. Mater., 2019, pp. 1-10.* Hutmacher et al., State of the art and future directions of scaffold-based bone engineering from a biomaterials perspective, J Tissue Eng Regen Med 2007, 1: 245-260.* Dash and Konkamalla, Poly-ε-caprolactone based formulations for drug delivery and tissue engineering: A review, Journal of Controlled Release, vol. 158, Issue 1, Feb. 28, 2012, pp. 15-33.* Werner et al., State of the art and future directions of scaffold-based bone engineering from a biomaterials perspective, J Tissue Eng Regen Med 2007, 1: 245-260.* Anderson et al., "Biodegradation and Biocompatibility of PLA and PLGA Microspheres", Advanced Drug Delivery Reviews, 1997, vol. 28, pp. 5-24. 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. Behavanh et al., "Synthetic Biodegradable Polymers for Orthopedic Applications," Clinical Orthopaedics and Related Research, No. 3678, pp. S118-S125. Clark 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. Huang et al., "Electrospun Collagen-Chitosan-TPC Nanofibrous Scaffolds For Tissue Engineered tubular Grafts", Colloids and Surfaces B: Biointerfaces, vol. 82 2011, pp. 307-315. (Continued)
(65) Prior Publication Data US 2019/0167842 A1 Jun. 6, 2019	Primary Examiner — Maria Marvich (74) Attorney, Agent, or Firm — RatnerPreslia
Related U.S. Application Data (62) Division of application No. 14/776,106, filed as application No. PCT/US2014/025636 on Mar. 15, 2014, now Pat. No. 10,137,223. (60) Provisional application No. 61/785,031, filed on Mar. 14, 2013.	ABSTRACT (57) A scaffold comprising an aligned fiber. Further, 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. Also, 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.
(51) Int. Cl. <i>A61F 2/08</i> (2006.01) <i>A61L 27/24</i> (2006.01) <i>A61L 27/26</i> (2006.01) <i>A61K 38/17</i> (2006.01) <i>A61L 27/38</i> (2006.01) <i>A61L 27/18</i> (2006.01) <i>A61L 27/36</i> (2006.01) <i>A61L 27/56</i> (2006.01) <i>D01D 5/00</i> (2006.01) <i>C12N 5/079</i> (2010.01)	
(52) U.S. Cl. CPC <i>A61L 27/18</i> (2013.01); <i>A61L 27/24</i> (2013.01); <i>A61L 27/26</i> (2013.01); <i>A61L 27/3604</i> (2013.01); <i>A61L 27/3633</i> (2013.01); <i>A61L 27/38</i> (2013.01); <i>A61L 27/38B</i> (2013.01); <i>A61L 27/56</i> (2013.01); <i>C12N 5/0618</i> (2013.01); <i>D01D 5/0076</i> (2013.01); <i>D01D 5/0092</i> (2013.01); <i>A61F 2/08</i> (2013.01); <i>A61F 2/40101</i> (2013.01); <i>A61L 2/40012</i> (2013.01); <i>C12N 2533/30</i> (2013.01); <i>C12N 2533/54</i> (2013.01); <i>C12N 2533/90</i> (2013.01)	
(58) Field of Classification Search None See application file for complete search history.	14 Claims, 10 Drawing Sheets

1. A method of treating a tissue defect in a subject comprising implanting at the tissue defect a scaffold comprising crosslinked fibers comprising collagen, wherein a result of a fast Fourier transform (FFT) analysis of the fibers demonstrates that the fibers have major adjacent peaks that are about 180° apart from each other, and applying the scaffold to the tissue defect to repair a tendon, a ligament or a nerve defect.
2. The method of claim 1, wherein the scaffold is in the form of one or more elongated sheets.
3. The method of claim 1, wherein the scaffold is a composite of multiple layers of one or more sheets.

(Ex. 1001)

'227 Patent: Claims 2-3


US 20110293685A1

(19) **United States**
(12) **Patent Application Publication** (10) **Pub. No.:** US 2011/0293685 A1
Kuo et al. (43) **Pub. Date:** Dec. 1, 2011

(54) **SCAFFOLDS FOR TISSUE ENGINEERING AND REGENERATIVE MEDICINE**

(75) **Inventors:** Catherine K. Kuo, Boston, MA (US); Nathan Zamarripa, Freeport, ME (US); Amelia H. Thomas, Medford, MA (US)

(73) **Assignee:** TRUSTEES OF TUFTS COLLEGE, Medford, MA (US)

(21) **Appl. No.:** 13/122,414

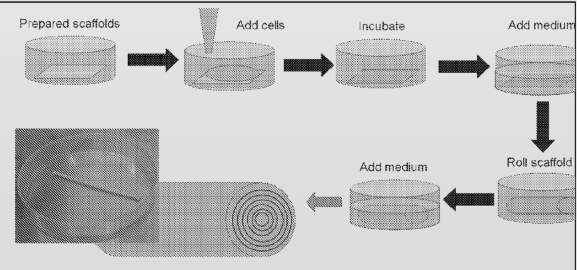
(22) **PCT Filed:** Oct. 5, 2009

(86) **PCT No.:** PCT/US09/59547

§ 371 (c)(1), (2), (4) Date: Aug. 22, 2011

Related U.S. Application Data
(60) Provisional application No. 61/102,440, filed on Oct. 3, 2008.

Publication Classification
(51) **Int. Cl.**
A61K 9/70 (2006.01)
A61P 17/00 (2006.01)
A61K 35/12 (2006.01)
(52) **U.S. Cl.** 424/422; 424/400; 424/93.7
(57) **ABSTRACT**
The methods and compositions described herein relate to novel 3-dimensional porous scaffolds useful for tissue regeneration, enhancement, or tissue repair. Electrospinning or other methods are used to create mats comprised of fibers that can be seeded with cells and subsequently rolled into a desired shape/form to replace a desired tissue.



[0024] As used herein, “scaffold” refers to a structure, comprising a biocompatible material, that provides a surface suitable for adherence of cells. A scaffold may further provide mechanical stability and support. A scaffold may be in a particular shape or form so as to influence or delimit a three-dimensional shape or form assumed by a population of proliferating cells. Such shapes or forms include, but are not limited to, films (e.g. a form with two-dimensions substantially greater than the third dimension), ribbons, cords, sheets, flat discs, cylinders, spheres, 3-dimensional amorphous shapes, etc.

[0049] A scaffold can be held together into any shape and/or size to be used as a construct for tissue regeneration, enhancement, or repair of a desired tissue. For example, in

(Ex. 1018)

Dependent Claims of the '223 Patent

'223 Patent	Patent Owner does not raise additional arguments as to depending claims 5-12
	These claims are thus anticipated if independent claim 1 is anticipated
	Patent Owner raises additional defenses as to claims 2, 3, 4, and 12 (but only to Lee)

Claims 2, 3, and 4 of the '223 Patent

Huang2 (Ex. 1005 at 1792) Starts With Type 1 Collagen, Just Like the '223 Patent

The '223 Patent discloses starting with type 1 collagen (Ex. 1001)


US010137223B2

(12) **United States Patent**
Francis et al.

(54) **ALIGNED FIBER AND METHOD OF USE THEREOF**

(71) Applicant: LifeNet Health, Virginia Beach, VA (US)

(72) Inventors: Michael Francis, Virginia Beach, VA (US); Roy Ogle, Virginia Beach, VA (US)

(73) Assignee: LifeNet Health, Virginia Beach, VA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35

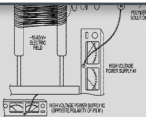
(10) Patent No.: US 10,137,223 B2

(45) Date of Patent: Nov. 27, 2018

(58) Field of Classification Search
None
See application file for complete search history.

(56) References Cited
U.S. PATENT DOCUMENTS
2002/0090725 A1* 7/2002 Simpson A61L 15/32 435-402
2004/0037813 A1* 2/2004 Simpson A61F 2/08 424/93.7
(Continued)
FOREIGN PATENT DOCUMENTS

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.



(Ex. 1001)
'223 Patent, 14:27-34

Huang2 (Ex. 1005 at 1792) starts with type 1 collagen

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Biomaterials 33 (2012) 1791–1800

Contents lists available at SciVerse ScienceDirect

 **Biomaterials**

Journal homepage: www.elsevier.com/locate/biomaterials

The involvement of integrin $\beta 1$ signaling in the migration and myofibroblastic differentiation of skin fibroblasts on anisotropic collagen-containing nanofibers

Chengyang Huang^{a,1}, Xiaoling Fu^{a,1}, Jie Liu^b, Yanmei Qi^b, Shaohua Li^{b,*}, Hongjun Wang^{a,*}

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^bDepartment of Surgery, Robert Wood Johnson Medical School, New Brunswick, NJ 08903, USA

2. Materials and methods

2.1. Materials

1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) was obtained from Oakwood products Inc (West Columbia, SC). Poly (epsilon-caprolactone) (PCL, Mw = 80,000) was purchased from Sigma–Aldrich (St. Louis, MO) and type I collagen (Coll) was obtained from Elastin Products Inc. (Owensville, MO). Fetal bovine serum (FBS) was purchased from American Type Culture Collection (ATCC, Manassas, VA). All other reagents and solutions were obtained from Invitrogen (Carlsbad, CA) except as indicated.

(Ex. 1005 at 1792)

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doi:10.1016/j.biomaterials.2011.11.025

Equal contribution.

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¹ Equal contribution.

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scaffold. Electrospinning a blend solution of collagen and polycaprolactone (PCL), a biocompatible and biodegradable synthetic polyester, can yield rather stable nanofibers [6,7]. Dermal fibroblasts

Page 6 of 15

EMBZIM EX1005

The '223 Patent



US010137223B2

(12) **United States Patent**
Francis et al.

(10) **Patent No.:** US 10,137,223 B2
(45) **Date of Patent:** Nov. 27, 2018

(54) **ALIGNED FIBER AND METHOD OF USE THEREOF**

(71) Applicant: **LifeNet Health**, Virginia Beach, VA (US)

(72) Inventors: **Michael Francis**, Virginia Beach, VA (US); **Roy Ogle**, Virginia Beach, VA (US)

(73) Assignee: **LifeNet Health**, Virginia Beach, VA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 214 days.

(21) Appl. No.: **14/776,106**

(22) PCT Filed: **Mar. 13, 2014**

(86) PCT No.: **PCT/US2014/025636**
§ 371 (c)(1),

(58) **Field of Classification Search**
None
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

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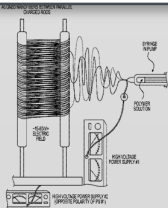
(Continued)

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Zengolis, D.I., et al., "Electro-spinning of pure collagen nano-

Example 1

1,1,1,3,3,3-hexafluoro-2-propanol (HFP) (CAS#920-66-1) was from Acros Organics (Geel, Belgium, acros.com).



(Ex. 1001) '223 Patent, 19:40-43

Huang2 (Ex. 1005 at 1792)

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Biomaterials 33 (2012) 1791–1800

Contents lists available at SciVerse ScienceDirect

Biomaterials

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journal homepage: www.elsevier.com/locate/biomaterials

The involvement of integrin $\beta 1$ signaling in the migration and myofibroblastic differentiation of skin fibroblasts on anisotropic collagen-containing nanofibers

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^aDepartment of Chemistry, Chemical Biology and Biomedical Engineering, Stevens Institute of Technology, Hoboken, NJ 07030, USA

^bDepartment of Surgery, Robert Wood Johnson Medical School, New Brunswick, NJ 08903, USA

2. Materials and methods

2.1. Materials

1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) was obtained from Oakwood products Inc (West Columbia, SC). Poly (epsilon-caprolactone) (PCL, Mw = 80,000) was purchased from Sigma–Aldrich (St. Louis, MO) and type I collagen (Coll) was obtained from Elastin Products Inc. (Owensville, MO). Fetal bovine serum (FBS) was purchased from American Type Culture Collection (ATCC, Manassas, VA). All other reagents and solutions were obtained from Invitrogen (Carlsbad, CA) except as indicated.

(Ex. 1005 at 1792)

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¹ Equal contribution.

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doi:10.1016/j.biomaterials.2011.11.025

fibers have been fabricated, however, the rapid degradation and poor mechanical properties determine that either crosslinking or combination with other materials is necessary to obtain a stable scaffold. Electrospinning a blend solution of collagen and polycaprolactone (PCL), a biocompatible and biodegradable synthetic polyester, can yield rather stable nanofibers [6,7]. Dermal fibroblasts

Page 6 of 15

EMBZIM EX1005

TISSUE ENGINEERING
Volume 12, Number 5, 2006
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Electrospinning of Polymeric Nanofibers for Tissue Engineering Applications: A Review

QUYNH P. PHAM,* UPMA SHARMA, Ph.D.,* and ANTONIOS G. MIKOS, Ph.D.

ABSTRACT

Interest in electrospinning has recently escalated due to the ability to produce materials with nanoscale properties. Electrospun fibers have been investigated as promising tissue engineering scaffolds since they mimic the nanoscale properties of native extracellular matrix. In this review, we examine electrospinning by providing a brief description of the theory behind the process, examining the effect of changing the process parameters on fiber morphology, and discussing the potential applications and impacts of electrospinning on the field of tissue engineering.

INTRODUCTION

ELECTROSPINNING HAS GAINED POPULARITY in the last 10 years due in large part to an increased interest in nanoscale properties and technologies. This technique allows for the production of polymer fibers with diameters varying from 3 nm to greater than 5 μm .¹ Potential applications of electrospinning include filtration membranes, catalytic nanofibers, fiber-based sensors, and tissue engineering scaffolds.¹⁻³

One attractive feature of electrospinning is the simplicity and inexpensive nature of the setup; the typical electrospinning setup consists of a syringe pump, a high voltage source, and a collector (Fig. 1). During the electrospinning process, a polymer solution is held at a needle tip by surface tension. The application of an electric field using the high-voltage source causes charge to be induced within the polymer, resulting in charge repulsion within the solution. This electrostatic force opposes the surface tension; eventually, the charge repulsion overcomes the surface tension, causing the initiation of a jet. As this jet travels, the solvent evaporates and an appropriate collector can be used to capture the polymer fiber. Figure 2A shows a scanning electron microscopy image of an electrospun polymer mesh.^{4,5} This approach has been used successfully to spin a number of synthetic and natural⁶⁻¹⁰ polymers into fibers many kilometers in length.^{3,5}

Recently, electrospinning has gained popularity with the tissue engineering community as a potential means of producing scaffolds. The objective of this review is to describe briefly the theory behind the technique, examine the effect of changing the process parameters on fiber morphology, and discuss the application and impact of electrospinning on the field of tissue engineering.

THEORY OF ELECTROSPINNING

The stable electrospinning jet was described in detail by Reneker and Chun as being composed of four regions: the base, the jet, the splay, and the collection.⁵ In the base region, the jet emerges from the needle to form a cone known as the Taylor cone. The shape of the base depends upon the surface tension of the liquid and the force of the electric field; jets can be ejected from surfaces that are essentially flat if the electric field is strong enough. Charging of the jet occurs at the base, with solutions of higher conductivity being more conducive to jet formation.⁵ Electric forces then accelerate and stretch the polymer jet, causing the diameter to decrease as its length increases. Additionally, solvents with high vapor pressures may begin to evaporate, causing a decrease in jet diameter and velocity.⁵ In the next region, Reneker and Chun hypothesized that radial charge repulsions cause the jet

Department of Bioengineering, Rice University, Houston, Texas.
*These two authors contributed equally to this work.

Electrospinning of Polymeric Nanofibers for Tissue Engineering Applications: A Review

QUYNH P. PHAM,* UPMA SHARMA, Ph.D.,* and ANTONIOS G. MIKOS, Ph.D.

A collagen nanofiber mesh scaffold may have the advantage of not only resembling the size scale but also the chemical and biological function of ECM. The spinning of collagen types I and III is feasible using 1,1,1,3,3,3 hexafluoro-2-propanol (HFP) as a solvent.^{10,91} Collagen fiber diameters of 100 nm (type I) and 250 nm (type III) were achieved; these fibers possessed the typical 67 nm banding pattern observed in native collagen.¹⁰ Culture of

10. Matthews, J.A., Wnek, G.E., Simpson, D.G., and Bowlin, G.L. Electrospinning of collagen nanofibers. Biomacromolecules **3**, 232, 2002.

(Ex. 1209 at 1205, 1208)

Claim 2 of the '223 Patent

Dr. Mikos (Ex. 2015, ¶ 110)

“Huang2 utilizes PCL as a polymer that is blended with collagen.”

“...degradation caused by the blending of PCL with collagen ...during storage.”

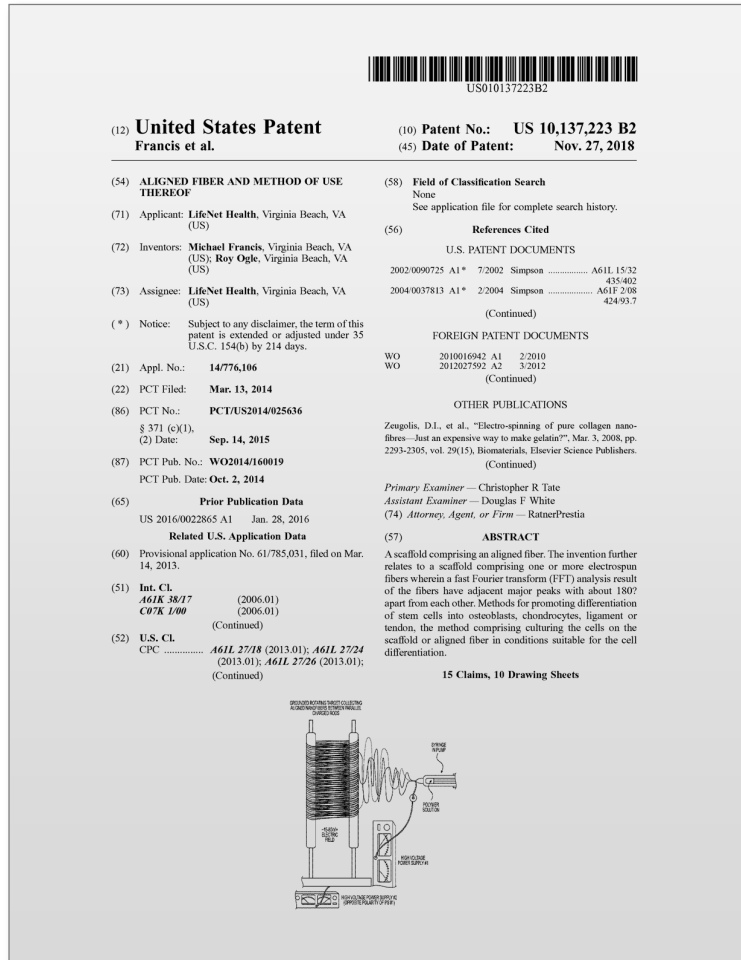
Dr. Simpson (Ex. 1219, ¶ 47)

“[T]he '223 patent suggests using blend of collagen and PCL without any explanation how to maintain the structure of collagen after electrospinning.”

“[c]laims do not require a triple helical structure after years of storage...”

“Dr. Mikos cited prior art demonstrating that PCL was stable for 3-4 years... (Ex. 2018, Abstract)”

Claim 3 of the '223 Patent (Ex. 1001)



3. The scaffold according to claim 1, wherein the fiber has less than about 8 wt % residual calcium.

(Ex. 1001) '223 Patent, Claim 3

- The '223 patent explains that calcium can be added (EX1001, 11:2-4)
- Huang2 does not use any calcium in its electrospinning and anticipates this claim (EX1003 at ¶¶ 119-22; EX1219 at ¶ 50)

Claim 3 of the '223 Patent

Dr. Mikos (Ex. 2015, ¶ 119)

“Huang2 discloses that it utilizes fetal bovine serum (“FBS”) in its cell culture medium. Ex. 1005, 1792. A POSITA would have readily understood FBS, naturally contains high concentrations of calcium, which is necessary for growth of the cells seeded on the scaffold.”

Dr. Simpson (Ex. 1219, ¶ 50)

“Further, my understanding of claim 3 is that it does not require the culturing of cells on the scaffold. Huang2 makes a scaffold without cells on it. (EX1005, 1792 (§2.2, Nanofibrous matrix preparation)). Thus, the fact that Huang2 subsequently cultures cells on it with FBS is irrelevant. (Id. (§2.3, Cell seeding and culture onto nanofibrous matrix).)”

'227 Prosecution History, Ex. 1002

Application No. 16/045,159
Amendment Filed: January 18, 2022
Response to the Final Office Action Dated July 19, 2021

LNH-152US1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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

AMENDMENT UNDER 37 C.F.R. § 1.116

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

Commissioner:

In response to the Final Office Action dated July 19, 2021, please amend the above-identified application as follows:

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

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

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

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

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

5531877_1 Page 1 of 8

Page 647 of 699 EMBZIM EX1002

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

Regarding growing or culturing cells, Applicant submits the invention disclosure provides that including cultured cells is an alternative feature. See, e.g., Applicant's Published Specification US 2019/0167842, paragraphs [0087]-[0093] and [0101]-[0103]. For example, the specification provides, at para [0091] (emphasis added):

The invention also relates to methods of promoting nerve repair using the aligned fiber, electrospun fiber, and/or scaffold described herein. In some embodiments, the nerve repair includes, but is not limited to, central and peripheral nerve repair. 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.

Similarly, in other portions of Applicant's specification, the invention *optionally* offers cell culturing, (1) including both cultured cells and scaffold, or (2) fiber, scaffold and/or implant without cultured cells, for example, at para [0101] (emphases added):

(Ex. 1002) '227 Prosecution History, p. 652

'223 Patent



US010137223B2

(12) **United States Patent**
Francis et al.

(10) **Patent No.:** US 10,137,223 B2
(45) **Date of Patent:** Nov. 27, 2018

(54) **ALIGNED FIBER AND METHOD OF USE THEREOF**

(71) Applicant: LifeNet Health, Virginia Beach, VA (US)

(72) Inventors: Michael Francis, Virginia Beach, VA (US); Roy Ogle, Virginia Beach, VA (US)

(73) Assignee: LifeNet Health, Virginia Beach, VA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 214 days.

(21) Appl. No.: 14/776,106

(22) PCT Filed: Mar. 13, 2014

(86) PCT No.: PCT/US2014/025636
§ 371 (c)(1),
(2) Date: Sep. 14, 2015

(87) PCT Pub. No.: WO2014/160019
PCT Pub. Date: Oct. 2, 2014

(65) **Prior Publication Data**
US 2016/0022865 A1 Jan. 28, 2016
Related U.S. Application Data
(60) Provisional application No. 61/785,031, filed on Mar. 14, 2013.

(51) **Int. Cl.**
A61K 38/17 (2006.01)
C97K 1/00 (2006.01)
(Continued)

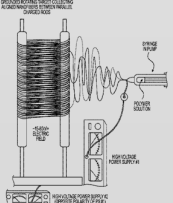
(52) **U.S. Cl.**
CPC A61L 27/18 (2013.01); A61L 27/24 (2013.01); A61L 27/26 (2013.01);
(Continued)

(58) **Field of Classification Search**
None
See application file for complete search history.

(56) **References Cited**
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(Continued)
Primary Examiner—Christopher R Tate
Assistant Examiner—Douglas F White
(74) **Attorney, Agent, or Firm**—RatnerPrestia

(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.

15 Claims, 10 Drawing Sheets



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.

(Ex. 1001) '223 Patent at 16:3-7

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).

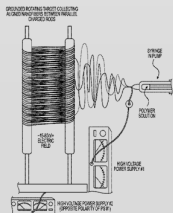
(Ex. 1001) '223 Patent at 18:32-40

Claim 4 of the '223 Patent

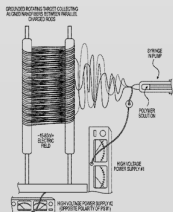
(12) United States Patent Francis et al.	(10) Patent No.: US 10,137,223 B2 (45) Date of Patent: Nov. 27, 2018
(54) ALIGNED FIBER AND METHOD OF USE THEREOF	(58) Field of Classification Search None See application file for complete search history.
(71) Applicant: LifeNet Health, Virginia Beach, VA (US)	(56) References Cited
(72) Inventors: Michael Francis, Virginia Beach, VA (US); Roy Ogle, Virginia Beach, VA (US)	U.S. PATENT DOCUMENTS 2002/0090725 A1 * 7/2002 Simpson A611 15/32 435-602 2004/0037813 A1 * 2/2004 Simpson A61F 2/08 42493.7
(73) Assignee: LifeNet Health, Virginia Beach, VA (US)	(Continued)
(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(d) by 214 days.	
(41) Appl. No.: 14/776,106	FOREIGN PATENT DOCUMENTS WO 2010016942 A1 * 2/2010 201027592 A2 3/2012 (Continued)
(22) PCT Filed: Mar. 13, 2014	OTHER PUBLICATIONS
(86) PCT No.: PCT/US2014/025366 3/71 (c)(1), (2) Date: Sep. 14, 2015	Zengolis, D.I., et al., "Electro-spinning of pure collagen nanofibers—Just aexpensive way to make gelatin?", Mar. 3, 2008, pp. 2293-2305, vol. 29(15), Biomaterials, Elsevier Science Publishers. (Continued)
(87) PCT Pub. No.: WO2014/016009 PCT Pub. Date: Oct. 2, 2014	Primary Examiner — Christopher R Tate Assistant Examiner — Douglas F White (74) Attorney, Agent, or Firm — Ratner/Prestia
(65) Prior Publication Data US 2016/0022865 A1 Jan. 28, 2016	
Related U.S. Application Data	
(60) Provisional application No. 61/785,031, filed on Mar. 14, 2013.	
(51) Int. Cl. A61B 38/47 C07K 1/00	(2006.01) (2006.01) (Continued)
(52) U.S. Cl. CPC	A61L 27/18 (2013.01); A61L 27/24 (2013.01); A61L 27/26 (2013.01); (Continued)

4. The scaffold according to claim 1, wherein the scaffold has an average porosity from about 60% to about 90%.

(Ex. 1001) '223 Patent, Claim 4



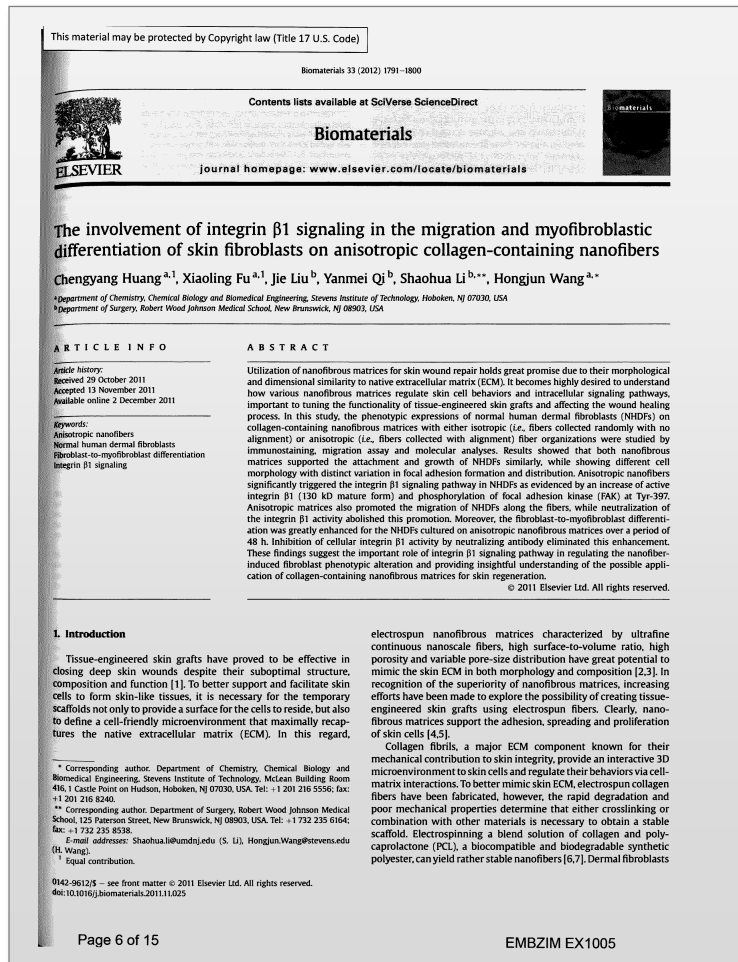
'223 Patent (Ex. 1001)

 US010137223B2			
(12) United States Patent Francis et al.		(10) Patent No.: US 10,137,223 B2 (45) Date of Patent: Nov. 27, 2018	
(54) ALIGNED FIBER AND METHOD OF USE THEREOF		(58) Field of Classification Search None See application file for complete search history.	
(71) Applicant: LifeNet Health, Virginia Beach, VA (US)		(56) References Cited U.S. PATENT DOCUMENTS 2002/0090725 A1* 7/2002 Simpson A61L 15/32 435:402 2004/0037813 A1* 2/2004 Simpson A61F 2/08 424:93.7 (Continued)	
(72) Inventors: Michael Francis, Virginia Beach, VA (US); Roy Ogle, Virginia Beach, VA (US)		FOREIGN PATENT DOCUMENTS WO 2010016942 A1 2/2010 WO 2013027592 A2 3/2012 (Continued)	
(73) Assignee: LifeNet Health, Virginia Beach, VA (US)		OTHER PUBLICATIONS Zeigolis, D.I., et al., "Electro-spinning of pure collagen nano-fibres—Just an expensive way to make gelatin?", Mar. 3, 2008, pp. 2293-2305, vol. 29(15), Biomaterials, Elsevier Science Publishers. (Continued)	
(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 214 days.			
(21) Appl. No.: 14/776,106			
(22) PCT Filed: Mar. 13, 2014			
(86) PCT No.: PCT/US2014/025636 § 371 (c)(1), (2) Date: Sep. 14, 2015			
(87) PCT Pub. No.: WO2014/160019 PCT Pub. Date: Oct. 2, 2014			
(65) Prior Publication Data US 2016/0022865 A1 Jan. 28, 2016 Related U.S. Application Data			
(60) Provisional application No. 61/785,031, filed on Mar. 14, 2013.			
(51) Int. Cl. A61K 38/17 (2006.01) C07K 1/00 (2006.01) (Continued)			
(52) U.S. Cl. CPC A61L 27/18 (2013.01); A61L 27/24 (2013.01); A61L 27/26 (2013.01); (Continued)			
		(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.	
		15 Claims, 10 Drawing Sheets	
			

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%.

(Ex. 1001) '223 Patent at 14:21-26

Huang2 (Ex. 1005)



cells to form skin-like tissues, it is necessary for the temporary scaffolds not only to provide a surface for the cells to reside, but also to define a cell-friendly microenvironment that maximally recaptures the native extracellular matrix (ECM). In this regard,

electrospun nanofibrous matrices characterized by ultrafine continuous nanoscale fibers, high surface-to-volume ratio, high porosity and variable pore-size distribution have great potential to mimic the skin ECM in both morphology and composition [2,3]. In

[3] Kumbhar SG, James R, Nukavarapu SP, Laurencin CT. Electrospun nanofiber scaffolds: engineering soft tissues. Biomed Mater 2008;3:034002.

(Ex. 1005 at 1791, 1799)

Kumbar (Ex. 1006)

Biomed. Mater. 3 (2008) 034002 (15pp)

Electrospun nanofiber scaffolds: engineering soft tissues

S G Kumbar¹, R James², S P Nukavarapu¹ and C T Laurencin^{1,2,3}

properties that can modulate cellular behavior. Electrospun nanofiber matrices show morphological similarities to the natural extra-cellular matrix (ECM), characterized by ultrafine continuous fibers, high surface-to-volume ratio, **high porosity** and variable pore-size distribution. Efforts have been made to modify nanofiber surfaces with several bioactive

Nanofiber diameter, surface morphology, mechanical properties, porosity and pore-size distribution greatly depend on these parameters selected for electrospinning. Various

(Rho *et al* 2006). Cross linking collagen nanofibers resulted in decrease of porosity **from 89% to 71%**. Relatively, less human

(Ex. 1006, pp. 2, 4, 8)



Claim 4: Porosity

Biomedical Materials

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Table 2. Percentage porosity, pore size distribution and contact angle for random and aligned electrospun scaffolds (*p < 0.05).

Electrospun scaffolds	Porosity (%)	Pore size (μm)	Contact angle (°)
Random PLGA	74.9 ± 10.3	8.3 ± 2.0*	99.2 ± 3.6*
Aligned PLGA	75.7 ± 3.2	3.5 ± 1.1*	112.4 ± 2.0*

Subramanian et al.
(Ex. 1211 at 7)

Table 1
Fiber diameter and porosity of PLGA/gelatin scaffolds.

Sample of PLGA/gelatin	Randomly-oriented fiber		Aligned fiber	
	Average diameter (nm)	Porosity (%)	Average diameter (nm)	Porosity (%)
10/0	1174 ± 350	83.55	594 ± 226	78.57
9/1	774 ± 181	82.98	560 ± 124	75.14
7/3	479 ± 307	78.41	568 ± 280	74.85

Meng et al.
(Ex. 1213 at 1207)

Materials Science and Engineering C 30 (2010) 1204–1210

Contents lists available at ScienceDirect

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Materials Science and Engineering C

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Electrospinning of PLGA/gelatin randomly-oriented and aligned nanofibers as potential scaffold in tissue engineering

Z.X. Meng^a, Y.S. Wang^{b,*}, C. Ma^a, W. Zheng^{a,*}, L. Li^a, Y.F. Zheng^{a,c}

^a Center for Biomedical Materials and Engineering, Harbin Engineering University, Harbin 150001, China

Table 1
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7/3	479 ± 307	78.41	568 ± 280	74.85

their growth into the corresponding tissue in vivo [3]. Among existing methods for fabricating tissue engineering scaffold, electrospinning technique has many advantages that can satisfy the particular requirements. Electrospinning is a simple and convenient method to produce an ultrafine fiber with a diameter ranging from nanometer to micron scales. The electrospun scaffold possesses reticular structure with high specific surface area, high porosity and interconnected pores,

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E-mail addresses: wangysong@ems.hrbmu.edu.cn (Y.S. Wang), zhengwei19@126.com (W. Zheng).

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doi:10.1016/j.msec.2010.06.018

nanofibers [4,11–13]. However, synthetic and natural polymer alone cannot meet all the requirements for tissue engineering. Cell affinity towards synthetic polymers is generally poor because of their low hydrophilicity and lacking of surface cell recognition sites. There is also the limitation of weak mechanical property for the natural polymers [14]. Therefore, it is desirable to prepare electrospun composite fibrous membranes including both the synthetic polymer for the backbone and the natural polymer for the cellular attachment, which might possess not only suitable mechanical properties but also the bioactive surface [15,16].

Poly(lactic-co-glycolic acid) is a FDA-approved biodegradable polymer which attracts much interests since it has controllable microstructure, degradation rate and mechanical properties for different applications [17,18]. However, the cell surface adherence on PLGA is poor because the PLGA material lacks of surface cell

Page 1 of 7

EMBZIM EX1213

Claim 4: Porosity



Antonios Mikos

Q. Let me ask you -- if you go down to the first complete paragraph on that same page, the third sentence, it says: "Porosity and pore size in electrospun scaffolds are mainly dependent on the fiber diameter and their packing density."

Sitting here today, do you agree with that statement that you published?

A. Yes. This is actually something that I also elaborated extensively in my expert report.

(Ex. 1220) Mikos Tr. at 148:9-19

2796

Biomacromolecules 2006, 7, 2796–2805

Electrospun Poly(ϵ -caprolactone) Microfiber and Multilayer Nanofiber/Microfiber Scaffolds: Characterization of Scaffolds and Measurement of Cellular Infiltration

Quynh P. Pham,[†] Upma Sharma,[†] and Antonios G. Mikos^{*}

Department of Bioengineering, Rice University, MS-142, P.

Received July 13, 2006; Revised Manuscript

The physical and spatial architectural geometries of electrospun tissue engineering strategies. In this work, poly(ϵ -caprolactone) microfibers ranging from 2 to 10 μm were individually electrospun to determine the effect of fiber diameter on scaffold properties. As fiber diameter increased, the average porosity increased (values ranging from 20 to 45 μm), while on both the larger pore sizes of the microfiber layers and the nanofiber scaffolds were fabricated by sequential electrospinning. These scaffolds (poly(ϵ -caprolactone) microfibers and poly(ϵ -caprolactone) nanofiber) different lengths of time, the thickness of the nanofiber layers could of microfiber scaffolds with varying thicknesses of nanofibers potential to affect rat marrow stromal cell attachment, spreading, not increase with increasing number of nanofibers, but the presence evidenced by stronger F-actin staining. Additionally, increasing the infiltration of cells into the scaffolds under both static and flow tested. The scaffold design presented in this study allows for cell same time providing nanofibers as a physical mimicry of extracellular matrix.

Introduction

Three-dimensional, porous polymer scaffolds are common in many tissue engineering strategies. A wide array of architectural configurations and geometries can be created using scaffold fabrication technologies such as rapid prototyping, melt extrusion, salt leaching, emulsion templating, phase separation, and electrospinning.^{1–7} Electrospinning is a process that can generate fiber mesh scaffolds with high porosities, large surface area-to-volume ratios, and variable fiber diameters.^{8,9} These features, along with the versatility and simplicity of the system, make electrospinning an attractive tool for the production of scaffolds.

The principle and theory behind electrospinning has been described in detail in the literature.^{10–12} By modulating a combination of solution and processing variables (e.g., the polymer solution concentration, flow rate, collector distance, and applied voltage), fibers with diameters up to 10 μm can be produced.¹³ Fiber diameter can have an effect on a variety of scaffold properties, such as porosity, pore size, and surface area.^{14,15} These properties, in turn, are important in determining the functionality of scaffolds in tissue engineering applications. The pore size and porosity are critical features of a scaffold that affect cell attachment, proliferation, migration, and/or differentiation.^{16–19} Surface topography and structural size-scale are additional parameters that have been shown to affect the orientation and biological function of cells.²⁰

The complexity of cell behavior leads to challenges in the design and fabrication of ideal scaffolds for tissue engineering applications.

Conclusion

The approach taken in this study was to exploit the extensive range of fiber diameters capable of being produced by electrospinning to generate scaffolds with unique properties. We demonstrated the ability to fabricate 3D microfiber scaffolds with controlled fiber diameters up to 10 μm . The average pore size of the scaffolds was found to be directly proportional to the fiber diameter (and thus pore size) has been suggested as a useful model for zonal cartilage tissue engineering.²² Scaffolds containing gradients in pore size and porosity have also been investigated as bone substitute materials that would allow vascularization and direct osteogenesis on one side while promoting osteochondral ossification on the other.^{16,23}

The primary focus in electrospinning has been on the production of nanofibers due to their resemblance scale-wise to native extracellular matrix (ECM).²⁴ Polymer meshes comprised of fibers with diameters as low as tens of nanometers, and exhibiting porosities as high as 90% and pore sizes as low as 100 nm, have been reported.^{24–27} Cells seeded onto nanofiber matrices tend to spread, attaching at multiple focal points, and in some cases extend filopodia along the length of the fiber.^{28,29} Several studies suggest the ability of attached cells to push against nanofibers and migrate into the nanofiber matrix;^{28,30,31} however, culture of cells on nanofiber matrices also results in a monolayer of cells and ECM, thus limiting their potential for 3D tissue engineering applications.^{32,33} It has been suggested that smaller fibers can inhibit cellular infiltration.²⁹ The reduced cellular infiltration into the depths of the scaffold has been attributed to the pore diameters being smaller than that of a cell.²⁶ For cell migration or infiltration to occur, the pore size of a scaffold should at least be the size of a cell;³² a value of 10 μm has been suggested as necessary for cellular infiltration.³⁴

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[†] Authors contributed equally to this work.

10.1021/bm060686g CCC: \$33.50 © 2006 American Chemical Society
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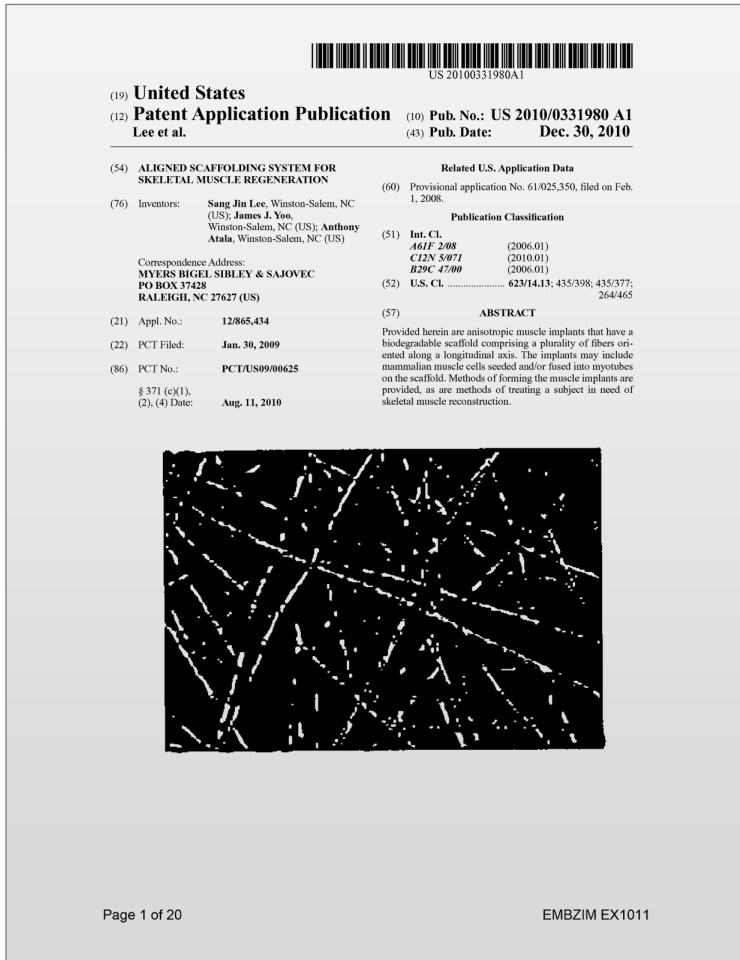
EMBZIM EX1215

Table 2. Average Fiber Diameters from the Top and Bottom of Microfiber PCL Scaffolds Fabricated Using the Conditions Described in Table 1^a

targeted fiber diameter (μm)	actual fiber diameter, top (μm)	actual fiber diameter, bottom (μm)
2	2.12 $\pm$ 0.45	2.34 $\pm$ 0.29
3	3.18 $\pm$ 0.35	3.05 $\pm$ 0.39
4	4.01 $\pm$ 0.49	3.95 $\pm$ 0.51
5	4.86 $\pm$ 0.56	4.96 $\pm$ 0.46
6	6.37 $\pm$ 0.55	6.13 $\pm$ 0.37
7	6.90 $\pm$ 0.84	6.78 $\pm$ 0.85
8	7.94 $\pm$ 0.37*	7.30 $\pm$ 0.46*
10	10.8 $\pm$ 0.81*	9.57 $\pm$ 0.72*

^a Measurements represent the mean and standard deviations of 25 fiber diameters on the top and bottom sides of the scaffolds. A “*” indicates a statistically significant difference ($p < 0.01$) between the top and bottom of the scaffolds.

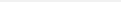
(Ex. 1215 at 2800, 2804)



[0055] The nano-scaled structure of the electrospun scaffolds can support cell adhesion and guide their behavior. Moreover, the composition, structure, and mechanical properties of biomaterials can be controlled (Lee et al. (2007) In vitro evaluation of electrospun nanofiber scaffolds for vascular graft application, *J. Biomed. Mater. Res. A*. in press; Stitzel et al. (2006) Controlled fabrication of a biological vascular substitute, *Biomaterials* 27, 1088-1094). Electrospun nanofiber scaffolds according to some embodiments have a three-dimensional architecture, **high porosity** with an interconnected pore structure, and a high surface-to-volume ratio, which provides a favorable environment for the cells to grow.

(Ex. 1011 at ¶ [0055])

High porosity, greater than 80% volume, provides adequate space for cell seeding, growth, and extracellular matrix (ECM) production.



US005514378A

[54] BIOCMPATIBLE POLYMER MEMBRANES AND METHODS OF PREPARATION OF THREE DIMENSIONAL MEMBRANE STRUCTURES	678407	9/1991	Switzerland	521/27
	9072604	11/1990	WIPO	424/426
OTHER PUBLICATIONS				

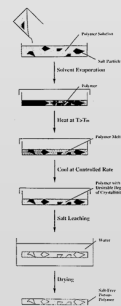
Vacanti, J. P. "Beyond Transplantation", *Arch. Surg.*, 123:545-549 (1988).

Primary Examiner—Peter F. Kulkosky
Attorney, Agent, or Firm—Arnall Golden & Gregory

[57] **ABSTRACT**

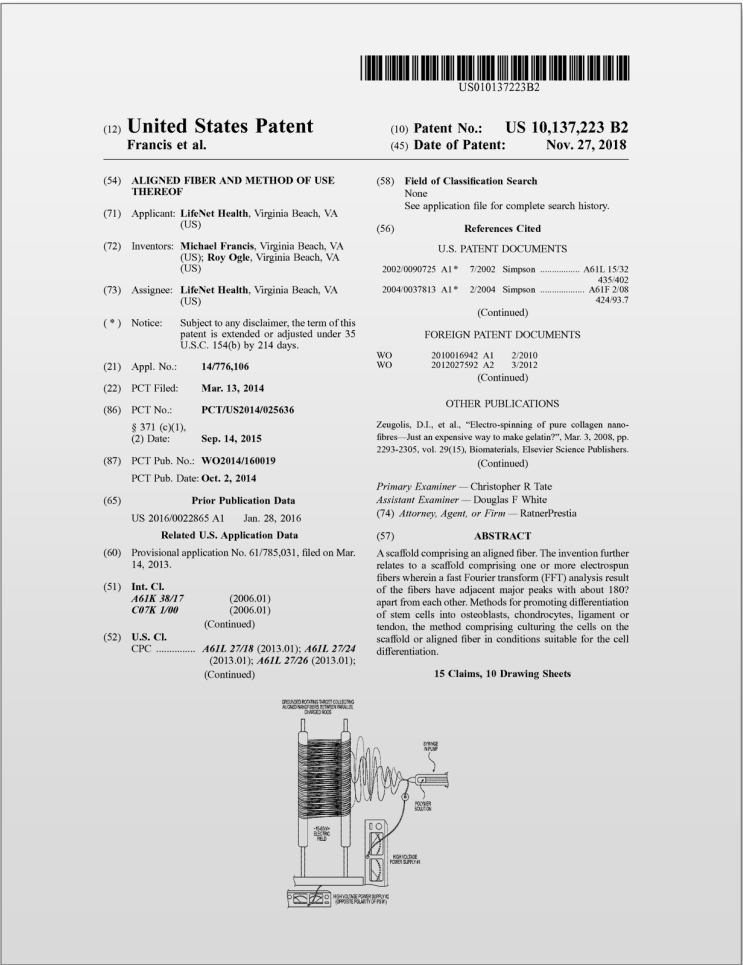
U.S. PATENT DOCUMENTS

9 Claims, 7 Drawing Sheets



Claim 12 of the '223 Patent

Lee Anticipates Claim 12



Claim 12 requires specific cells on the scaffold, such as “stem cells”

12. The method of claim 11, 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.

(Ex. 1001) '223 Patent, Claim 12

[0030] “Myoblasts” are a type of muscle stem cell, and are normally closely associated with myofibers during the course of their life cycle in the vertebrate organism. If the myofiber

(Ex. 1011 at ¶ [0030])

Dr. Simpson's Testing

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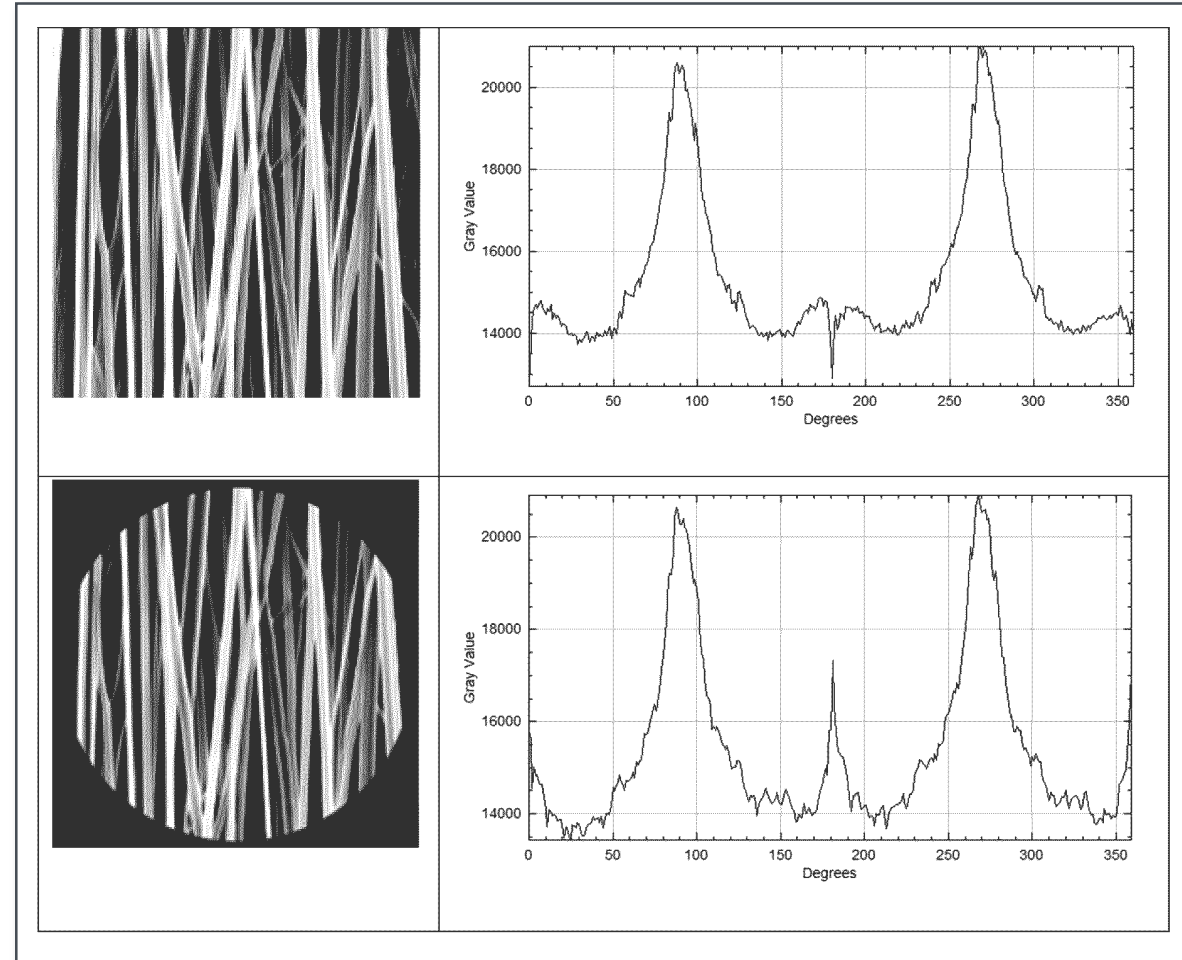
EMBODY, INC. AND ZIMMER BIOMET HOLDINGS, INC.,
Petitioner,

v.

LIFENET HEALTH,
Patent Owner.

Case IPR2025-00248
U.S. Patent No. 10,137,223

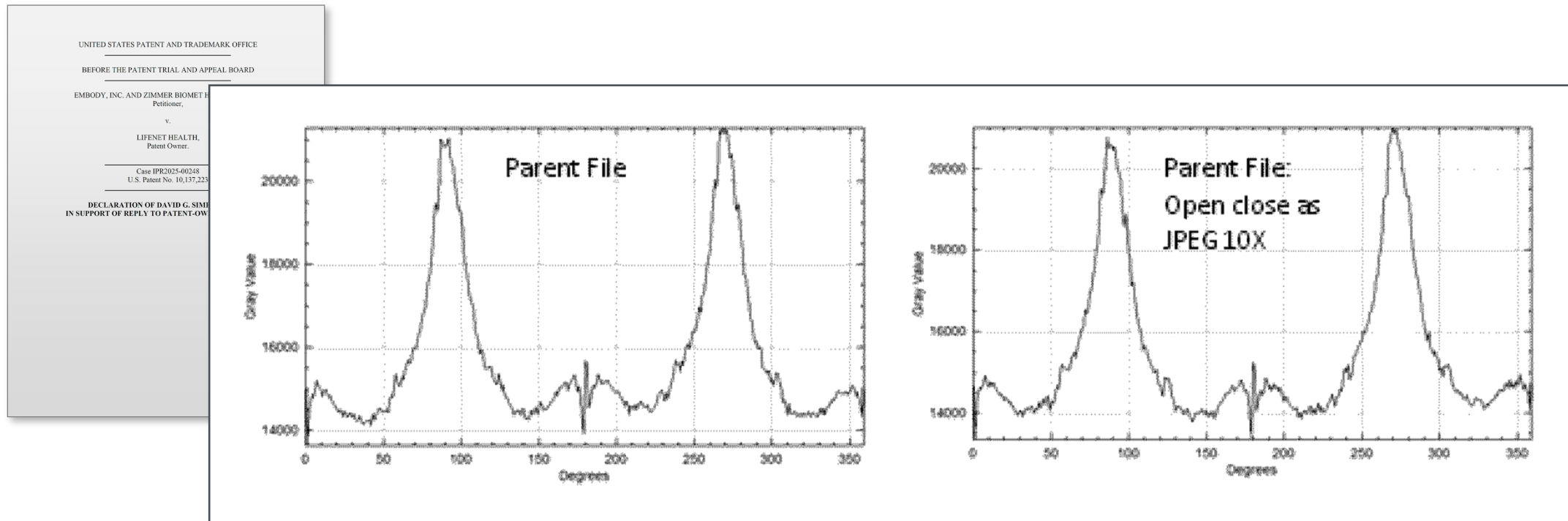
DECLARATION OF DAVID G. SIMPSON, PH.D.
IN SUPPORT OF REPLY TO PATENT-OWNER'S RESPONSE



(Ex. 1219) Simpson Reply Declaration ¶ 39

Dr. Simpson's Testing

- Dr. Simpson “opened that JPEG and saved it under a new filename, closed it, reopened the newly saved file, and repeated this open-save-close process ten times, each time saving over the immediately prior image”



(Ex. 1219, ¶ 32)

Dr. Simpson's Testing



Antonios Mikos

- Q.** Yeah. Does the patent show how the circular projection was applied in the ImageJ software?
- A.** The circular projection -- again, I didn't find any information about the circular projection as I was reading this patent now. The only thing that -- relative that I read was that they followed the FFT analysis that was described in the publication by Ayres.

(Ex. 1220) Mikos Tr. at 116:2-12 (objections omitted)

Dr. Simpson's Testing

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BEFORE THE PATENT TRIAL AND APPEAL BOARD

EMBODY, INC. AND ZIMMER BIOMET HOLDINGS, INC.,
Petitioner,

v.

LIFENET HEALTH,
Patent Owner.

Case IPR2025-00248
U.S. Patent No. 10,137,223

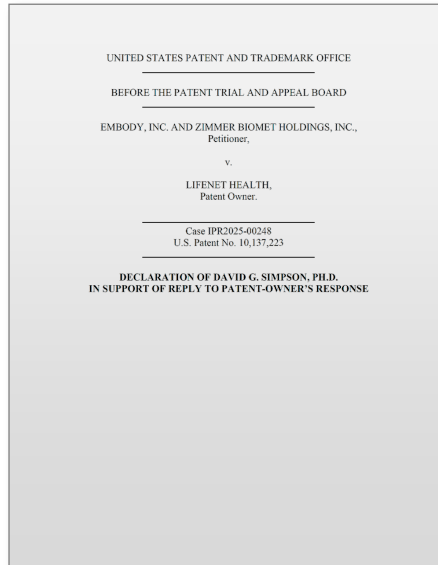
DECLARATION OF DAVID G. SIMPSON, PH.D.
IN SUPPORT OF REPLY TO PATENT-OWNER'S RESPONSE

29. Regarding the placement of the circular projection for the FFT analysis (EX2015, ¶94), I used the largest circle tangent to the edges of the image (EX2017, 165:9-11), as exactly described in Huang2 (EX1005, 1792) and Ayres 2008 (EX1010, 608 (Figure 1B), 609).

(Ex. 1219) Dr. Simpson Reply Declaration, ¶ 29

Dr. Simpson's Testing

- Dr. Simpson explained in his reply declaration:



37. Regarding normalization (EX2015, ¶101), normalization would not affect the number or location of the major peaks. Normalization is done to calibrate different alignment plots taken from different scaffolds so they can be compared on the same scale.

(Ex. 1219, ¶ 37)

Dr. Simpson's Testing



Dr. David Simpson

- Q.** Did you calibrate the angle information concerning the principal direction of fiber alignment to the version of the ImageJ software that you used?
- A.** I didn't have to. The software had been corrected.
- A.** No, all I can report is that it was the latest version that was available when this project started, and I downloaded it fresh from the NIH site.

(Ex. 2017) Simpson Tr. at 141:10-15. 161:18-21

Claim 1 of '227 Patent: Testimony



Antonios Mikos

Q. So if we can go back to Paragraph 63.

A. Exhibit 16 again.

Q. Yes. The – Lee patent publication

Q. So the fibers were -- were a blend of PCL and collagen and then they used crosslink -- crosslinking to increase the strength and stability of the collagen component of the blended PCL/collagen fibers. Is that right?

A. That's how I read the statement here.

(Ex. 1220) Mikos Tr. 186:16-21, 188:2-10