

PATENT OWNER'S HEARING DEMONSTRATIVES

Embody, Inc. and Zimmer Biomet Holdings, Inc.

v.

LifeNet Health

IPR2025-00248 (Patent 10,137,223)

IPR2025-00249 (Patent 11,318,227)

May 6, 2026

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

INSTITUTED GROUNDS

I P R 2 0 2 5 - 0 0 2 4 8

Ground	Claim(s)	35 U.S.C. §	Reference(s)
1	1-12	§ 102	Huang2
2	4	§ 103	Huang2, Kumbar
3	1-12	§ 102	Lee
4	1-12	§ 103	Lee

I P R 2 0 2 5 - 0 0 2 4 9

Ground	Claim(s)	35 U.S.C. §	Reference(s)
1	1-6	§ 103	Huang2, Kuo
2	1-6	§ 103	Lee, Kuo
3	7-8	§ 102	Huang2

PETITIONER FAILS FOR MULTIPLE REASONS

Common Issues – All Independent Claims

- A. The Specification defines “major peaks”
- B. Huang2 & Lee lack “major peaks”
- C. Dr. Simpson’s testing warrants no weight

IPR2025-00248

- A. Claim 2: “native triple helical form”
- B. Claim 3: “residual calcium”
- C. Claim 4: “average porosity”
- D. Claim 12: “stem cells”

IPR2025-00249

- A. Claim 1: “crosslinked fibers”
- B. Claims 1 & 4-6: “to repair a tendon, a ligament or a nerve defect”
- C. Claims 2-3: “sheets”

CLAIM CONSTRUCTION – “MAJOR PEAKS”

U.S. Patent No. 10,137,223

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.**

U.S. Patent No. 11,318,227

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.

CLAIM CONSTRUCTION – “MAJOR PEAKS”

(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)

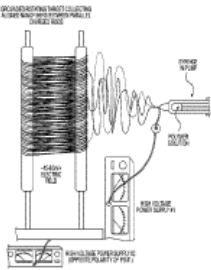
(52) **U.S. CL**
CPC **A61L 27/18** (2013.01); **A61L 27/24** (2013.01); **A61L 27/26** (2013.01);
(Continued)

(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
424:93.7
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WO 2010016942 A1 2/2010
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(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



Page 1 of 24

EMBZIM EX1001

13

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

Alnylam Pharms., Inc. v. Moderna, Inc., 138 F.4th 1326, 1333 (Fed. Cir. 2025)

- “the term to be defined ... is set off in quotation marks”
- “the sentence uses the term ‘refer to,’ ... which generally ‘conveys an intent for [that sentence] to be definitional”
- the patentee did not use “non-limiting terms” (“for example” or “e.g.”) that “contrast with the ‘refer to’ language”

McGuireWoods

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

-248 EX.1001, 13:1-7
-248 RESP. 6-9

6

CLAIM CONSTRUCTION – “MAJOR PEAKS”

Petitioner accepted this definition in the Petitions:

Petition for *Inter Partes* Review of U.S. Patent No. 10,137,223

[in the scaffold] have adjacent major peaks about 180° apart from each other.”

(*Id.*, 13:3-6.) According to the specification, “the major peaks . . . refer to peaks higher than the average peaks in the result.” (*Id.*, 13:6-7.)

Despite its obligation, Petitioner did not propose a different construction or argue this definition is unclear or inapplicable:

VIII. CLAIM CONSTRUCTION

For purposes of this Petition, Petitioners do not believe that any claims require construction. To the extent the Patent Owner identifies terms for construction, Petitioners reserve the right to respond.

CLAIM CONSTRUCTION – “MAJOR PEAKS”

Petitioner’s expert, Dr. Simpson, also accepted this definition.

Dr. David Simpson - October 07, 2025

89

1 you referenced the taller peaks as major peaks?

2 A. Yes.

3 Q. Would another way to describe a major

4 peak be that its height is higher than the average

5 heights of the peaks in the plot?

6 A. If a user so desired, yes.

7 Q. So in this

8 as you described it

9 A. Yes.

10 Q. Okay. All

11 610, please.

12 In the second

13 paragraph starts with

14 alignment."

15 You state

16 degrees of alignment

17 onset of distinctive

18 A. Yes.

19 Q. You identified

20 alignment value of

21 A. Yes.

22 Q. What scale is that 0.05 units reported

23 on?

24 A. That is alignment plot on Figure 1.

25 Q. And is that 0.05 -- is that an arbitrary

3 Q. Would another way to describe a major

4 peak be that its height is higher than the average

5 heights of the peaks in the plot?

6 A. If a user so desired, yes.

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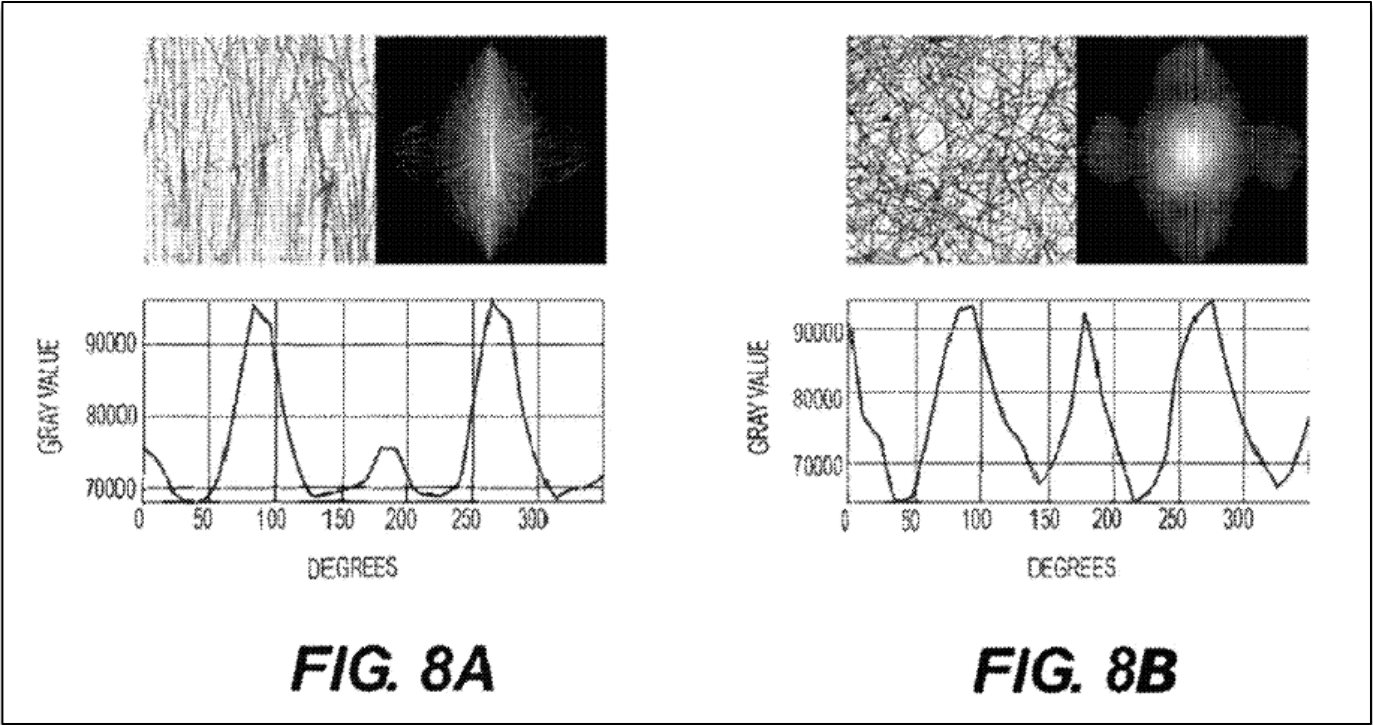
LifeNet Exhibit 2017

Embody, et al. v. LifeNet, IPR2025-00248, -00249

Page 89

CLAIM CONSTRUCTION – “MAJOR PEAKS”

This definition is consistent with the Specification’s embodiments:

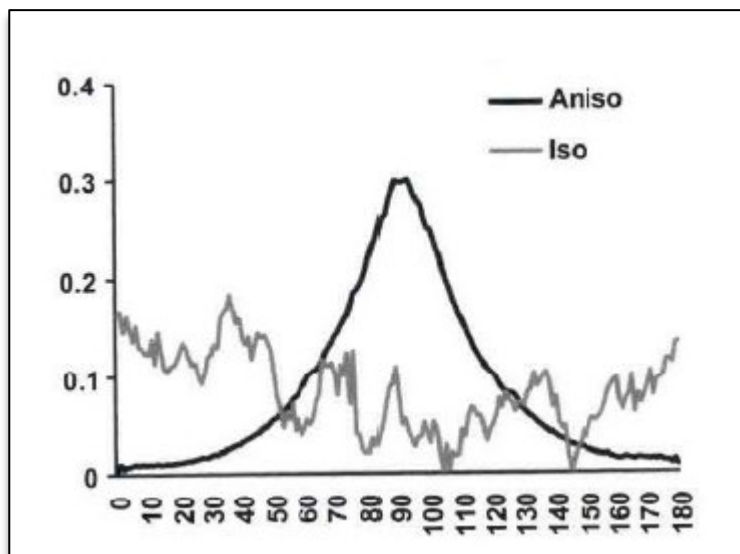


“major peaks”

no “major peaks”

CLAIM CONSTRUCTION – “MAJOR PEAKS”

Petitioner's construction has the same effect when applied to FFT results with a single peak.



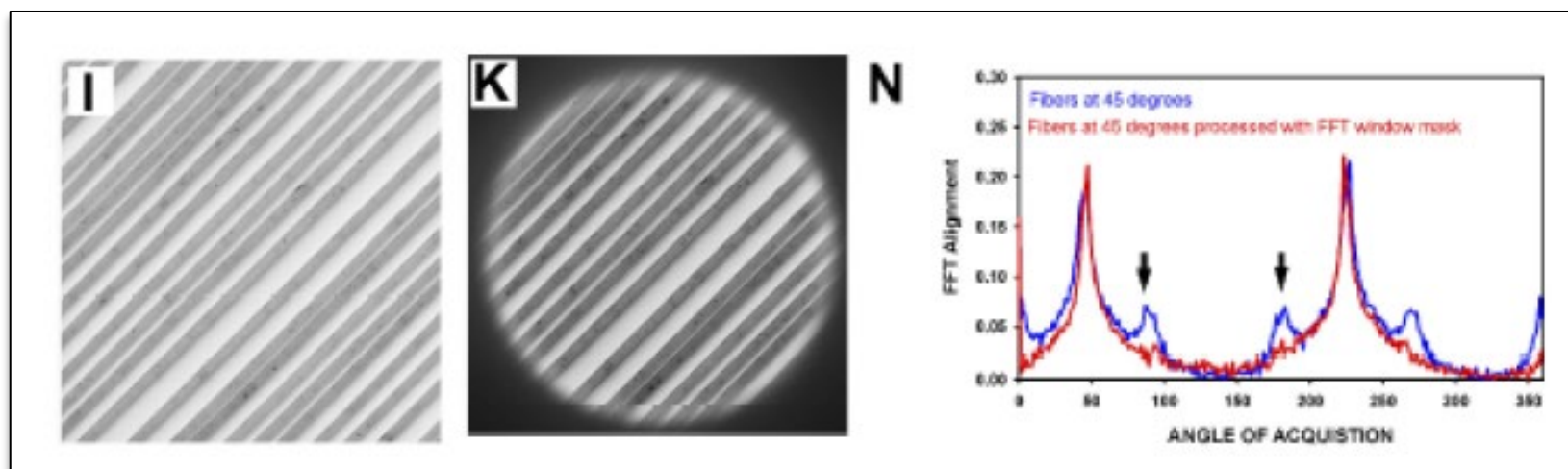
Instead, the '223 patent directs a POSA to Ayres2008, which it incorporates in its entirety. Ayres carries out a *visual assessment* of the relative sizes of the peaks to determine which peaks are “major” and thus indicate scaffold alignment. Ayres2008 explains that: “The 2D FFT alignment plots generated from sample scaffolds *containing aligned elements* exhibit asymmetrical peaks, two larger peaks at 90 and 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.” (EX1010, 610 (emphasis added); see also EX1220, 76:7-12.) “The overall 2D FFT alignment value for a scaffold *is determined by visually inspecting and selecting the maximal value from the peak of the alignment plot.*” (EX1010, 613 (emphasis added).) This is how a POSA would understand the phrase “major peaks” in the context of the '223 patent and is the correct claim construction.

This understanding was confirmed by Dr. Simpson, the senior author of Ayres2008, who testified that identifying “major peaks” is a visual comparison of relative peak height and that references to an “average” reflect this qualitative assessment, not a numerical calculation. (EX2017, 88:6–89:9; EX1219, ¶¶5-10.)

- 6 -

CLAIM CONSTRUCTION – “MAJOR PEAKS”

It is undisputed that FFT peaks reflect fiber alignment; noise and artifacts do not.

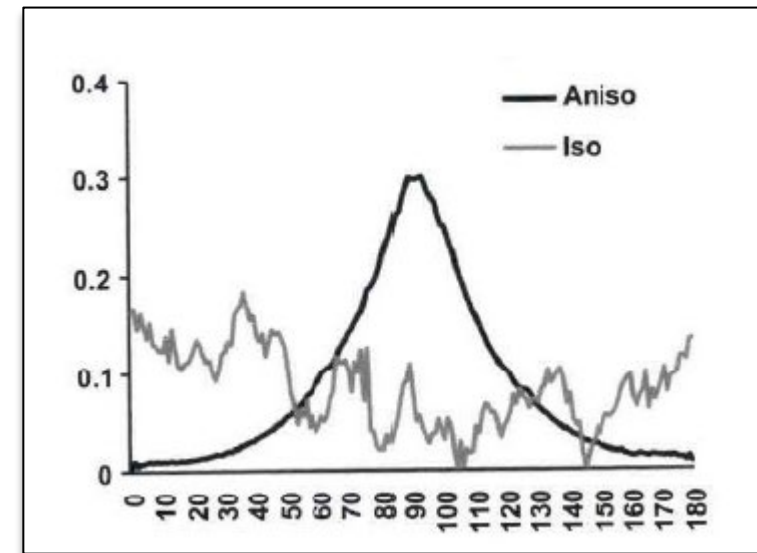


EX.1010, FIG.3, 613-615 (“EDGE EFFECTS AND BACKGROUND NOISE ... OBSCURE DATA”
AND CAN BE REMOVED WITH A MASK); -248 SUR-REPLY 7-10; -248 PET. 9

HUANG2 DOES NOT DISCLOSE “MAJOR PEAKS”

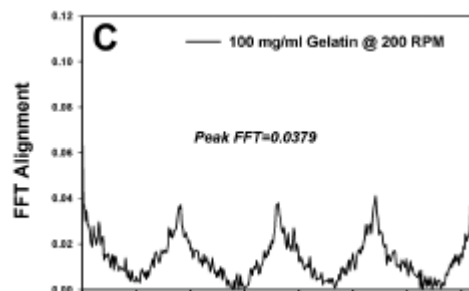
Huang2 lacks “major peaks” under either construction.

- The Specification’s definition:
 - “‘major peaks’ herein refer to peaks higher than the average of the peaks in the result”
- Petitioner’s construction:
 - “a visual assessment of the relative sizes of the peaks to determine which peaks are ‘major’”

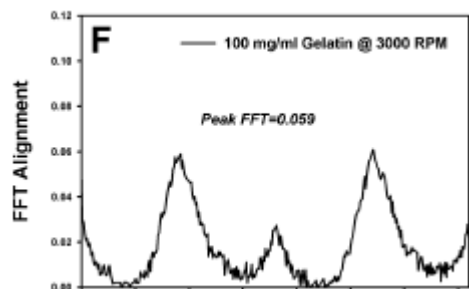


HUANG2 DOES NOT DISCLOSE “MAJOR PEAKS”

Dr. Simpson's testimony confirms this.



not “major peaks”



“major peaks”

10 And at approximately -- looks like about
 11 at approximately 90 degrees in C is a peak.
 12 I would not refer to that as a major
 13 peak because all the peaks in that particular plot
 14 look like they're essentially very similar in
 15 height.
 16 A major peak is evident in F, in that
 17 the peak that is present at about 180 degrees or
 18 so is very small.
 19 And there are adjacent peaks on either
 20 side of it, which are probably on the order of two
 21 and a half times taller. And the same description
 22 holds true for the final plot, where the major

Dr. David Simpson - October 07, 2025

88

1 BY MS. WIEKER:

2 Q. Okay. Are you familiar with the term
 3 "major peaks" in the context of a 2D FFT alignment
 4 plot?

5 A. I am.

how would you describe major peaks?

Panel C, F, and I, notice that the
 little bit of wiggle to it, there are
 downs in the trace.

at approximately -- looks like about
 tely 90 degrees in C is a peak.

ould not refer to that as a major
 all the peaks in that particular plot
 ey're essentially very similar in

major peak is evident in F, in that
 t is present at about 180 degrees or
 mall.

there are adjacent peaks on either
 which are probably on the order of two
 times taller. And the same description
 or the final plot, where the major
 gher than the other peaks that are
 - in the plot.

25 Q. So in describing Figures 1-F and 1-I,

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 Embody, et al. v. LifeNet, IPR2025-00248, -00249
 Page 88

DR. SIMPSON CANNOT SUPPLY HUANG2'S MISSING "MAJOR PEAKS"

Dr. Simpson's testing is entitled to no weight – it violates Board rules.

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.

37 C.F.R. § 42.65 – Expert testimony; tests and data

...

(b) If a party relies on a technical test or data from such a test, the party must provide an affidavit explaining: ...

(2) How the test was performed and the data was generated;

DR. SIMPSON CANNOT SUPPLY HUANG2'S MISSING "MAJOR PEAKS"

Dr. Simpson's testing is entitled to no weight – it conflicts with Huang2.

A. Different images

- i. Downloaded PDF vs. SEM images

B. Different FFT processes

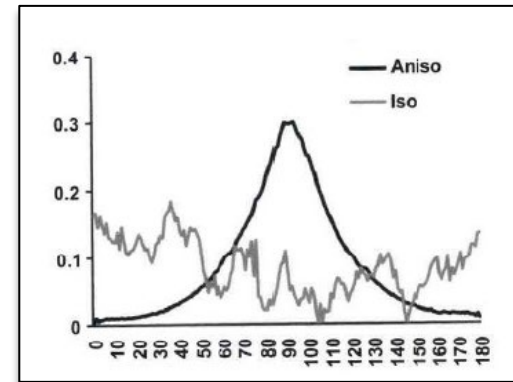
- i. $n=1$ vs. $n=5$
- ii. Cropped vs. not cropped
- iii. Mask vs. no mask
 - Dr. Simpson: *"I used a circular mask because I misread that sentence."*

C. Different FFT results despite normalization

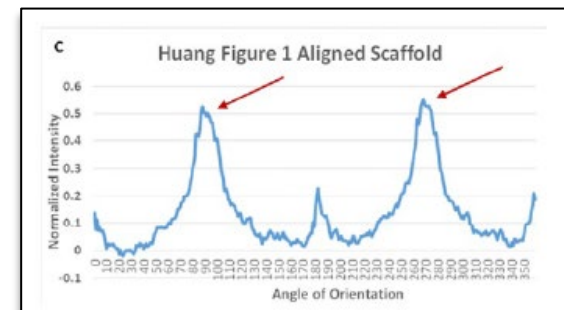
- i. $20-140^\circ$ peak vs. $0-180^\circ$ peak*
- ii. 0.55 vs. 0.3 normalized intensity units*
- iii. Minor peaks vs. no minor peaks

* These are undisputed on Reply

Huang2:



Dr. Simpson:



DR. SIMPSON CANNOT SUPPLY HUANG2'S MISSING LIMITATION

Testimony that conflicts with the reference should be disregarded.

Homeland Housewares, LLC v. Whirlpool Corp.,
865 F.3d 1372, 1378 (Fed. Cir. 2017)

- “[W]e must disregard the testimony of an expert that is plainly inconsistent with the record.”

Honeywell Int’l Inc. v. 3G Licensing, S.A.,
124 F.4th 1345, 1354 n.6 (Fed. Cir. 2025)

- “[W]e are not required to uncritically accept expert testimony that is flatly contradicted by the record.”

LEE DOES NOT DISCLOSE “MAJOR PEAKS”

Dr. Simpson agrees that Lee fails to disclose FFT at all; it necessarily lacks a FFT result with “major peaks.”

Dr. David Simpson - October 07, 2025

154

1 fashion. He just chose to use different criteria.

2 Q. So you agree that Lee did not disclose

3 using FFT to mea

4 A. No, he

5 alignment.

6 Q. I'm goi

7 be sure the reco

8 Does Le

9 alignment?

10 A. No.

11 Q. Okay.

12 MS. WIE

13 Exhibit 9.

14 (Simpso

15 identif

16 MS. WIE

17 you're ready

18 MR. LEV

19 BY MS. WIEKER:

20 Q. Dr. Sim

21 document?

22 A. I do no

23 Q. If you

24 the bottom, it s

25 compare this to your declaration where you list

6

7

8

9

10

Q. I'm going to ask this one more time to
be sure the record's clear here.
Does Lee disclose using FFT to measure
alignment?
A. No.

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Embody, et al. v. LifeNet, IPR2025-00248, -00249
Page 154

LEE DOES NOT DISCLOSE “MAJOR PEAKS”

The Petitions pled “aligned” fibers – which are not claimed.

3. “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”

Lee by itself also discloses this element of the claim by describing aligned

PCL/collagen fibers. (EX1003, §VI.B.1.c.) Indeed, in Lee, the investigators

degree of alignment, A POSA, however, would know that the degree of alignment could also be determined using ImageJ software to run an “FFT analysis” on the images published in Lee. (EX1003, ¶181.) An FFT analysis also measures the degree of orientation of fibers and can be used on a published SEM image. (EX1003, ¶181.) This application of ImageJ software was well-known at the time of filing of Lee. (EX1013; EX1010; EX1003, ¶181.)

Thus, if a POSA wanted to perform an FFT analysis on the SEM image in Lee, a POSA could do so using the ImageJ software, and would know how to perform the straightforward analysis on the very SEM image of the electrospun fibers shown in Lee. (EX1003, ¶¶181-87.) This would show the fibers were oriented on the scaffold in Lee in an aligned manner.

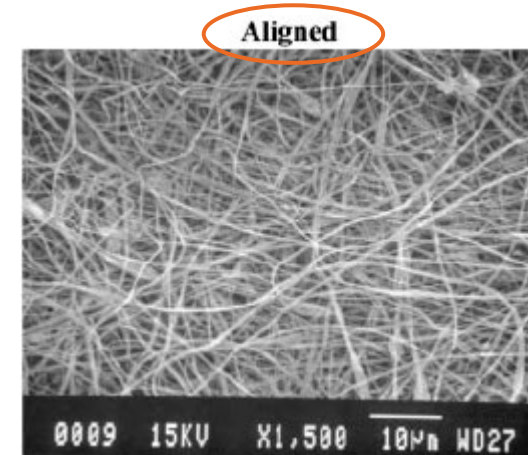
LEE DOES NOT DISCLOSE “MAJOR PEAKS”

The Petitions’ grounds based on Lee violate 35 U.S.C. § 312(a)(3) – and must fail.

With respect to these grounds, Petitioner does not clearly assert in the Petition that the fibers taught by Lee necessarily or inherently satisfy the FFT analysis requirement. Rather, Petitioner only asserts that “it was well known at the time of filing that an FFT analysis *could be* performed to further confirm alignment,” and “[t]his *would show* the fibers were oriented on the scaffold in Lee in an aligned manner.” Pet. 52 (emphasis added). Petitioner’s grounds based on Lee may ultimately fail on the merits due to this lack of specificity. See *Harmonic Inc. v. Avid Tech., Inc.*, 815 F.3d 1356, 1363 (Fed. Cir. 2016) (“In an IPR, the petitioner has the burden from the onset to show with particularity why the patent it challenges is unpatentable.”); 35 U.S.C. § 312(a)(3) (requiring that “the petition identifies in writing and with *particularity*, each claim challenged, the grounds on which the challenge to each claim is based, and the evidence that supports the grounds for the challenge to each claim”) (emphasis added).

Dr. Simpson:

produce a scaffold of randomly oriented fibers. The same procedure was used in an attempt to produce aligned fiber scaffolds; however the mandrel rotational speed was increased to 3000–4000 rpm. While results would indicate that truly aligned fibers were not created, scaffolds created with a high mandrel rotational speed were referred to as aligned scaffolds for the sake of simplicity in reporting results. Immediately after electrospinning, scaffolds were

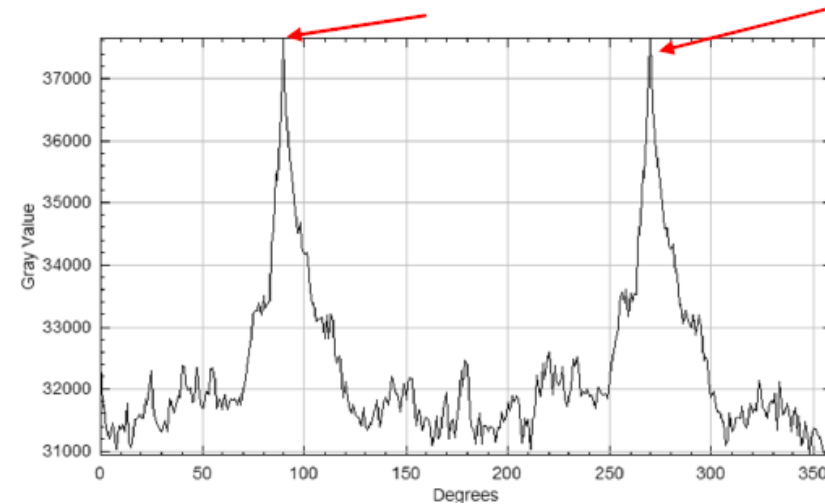
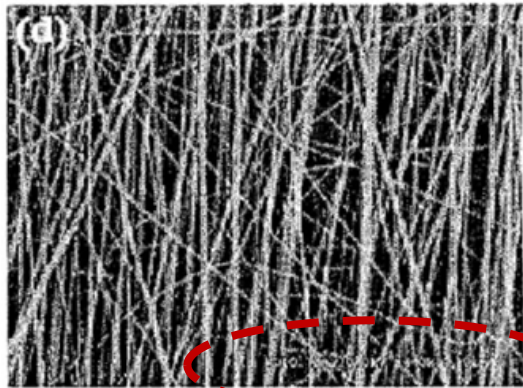


DR. SIMPSON CANNOT SUPPLY LEE'S MISSING LIMITATION

Dr. Simpson's testing is entitled to no weight:

- (1) it violates 37 C.F.R. § 42.65(b) and
- (2) it is based on an unacceptably noisy image.

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°.



DR. SIMPSON CANNOT SUPPLY LEE'S MISSING LIMITATION

Dr. Simpson himself indicates this type of image is unsuitable for FFT. His testing should not be credited.

Dr. Simpson:

For routine 2D FFT analysis of scaffold structure, objective resolving power is matched to average fiber diameter. If an objective with inadequate resolving power is used to capture data sets, individual fibers are not detected and the resulting image is composed largely of optical noise. In general, this type of optical image/data set is unsuitable for analysis. We note, however, that under

begins to be incorporated into the data set. Noise from either source (optical and/or electronic) can introduce artifacts into a data image and adversely impact the 2D FFT process.

As noted in the previous sections, edge effects and background noise (and detector noise) can “spill over” and obscure data in the 2D FFT frequency and alignment plots. This noise effect is quite pronounced in a model scaffold containing aligned

(signal or noise). We conclude from these experiments that optical (as seen in light scatter in widefield images) and electronic detector noise can degrade the absolute alignment value assigned to a scaffold by FFT analysis. However, the information that is present in a brightfield image allows the FFT approach to discern relative differences in alignment across different samples.

- 2 4 8 : C L A I M 2
(§ 1 0 2 : H U A N G 2 O R L E E)

-248 CLAIM 2

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.
2. The scaffold according to claim 1, wherein **the fibers comprise type I collagen in its native triple helical structure.**

-248 CLAIM 2

Huang2 and Lee are silent regarding native triple helical structure, and the Petition fails to show inherency:

Huang2 also discloses the “native triple helical structure” element of the claim. Huang2 discloses that “type I collagen (Coll) was obtained from Elastin Products Inc.” (*Id.*) Type I collagen was well-known and generally understood in the art to have a triple-helical form when it is in a monomer, as well as when it is in a polymer. (EX1017, 1, 9; EX1003, ¶117.) A POSA would have understood that, if the type 1 collagen purchased from a supplier were denatured and/or fragmented (i.e., not in a triple helix) it would be sold as “gelatin,” not “collagen.” (EX1003, ¶117.) In addition, Huang2 discloses that the 3:1 ratio of PCL/Collagen

-248 CLAIM 2

It is undisputed that HFIP/HFP, PCL, and FBS denature collagen.

108. In my opinion, Huang2's fibers do not necessarily possess collagen in its native triple helical form. It is not enough that Huang2 used type I collagen in its electrospinning process or did not use the term "gelatin." Pet. 33; Ex. 1003 ¶ 117. A POSITA would have readily understood that many compounds cause type I collagen to denature out of its native triple helix structure. For example, collagen is denatured out of its triple helical structure by low pH levels caused by the breakdown of various polymers, like PCL; by exposure to various solvents, like HFIP; and by the activity of various enzymes used for cell culture, like those present in fetal bovine serum.

ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

Biomaterials

Biomaterials 29 (2008) 2293–2305

www.elsevier.com/locate/biomaterials

Leading Opinion

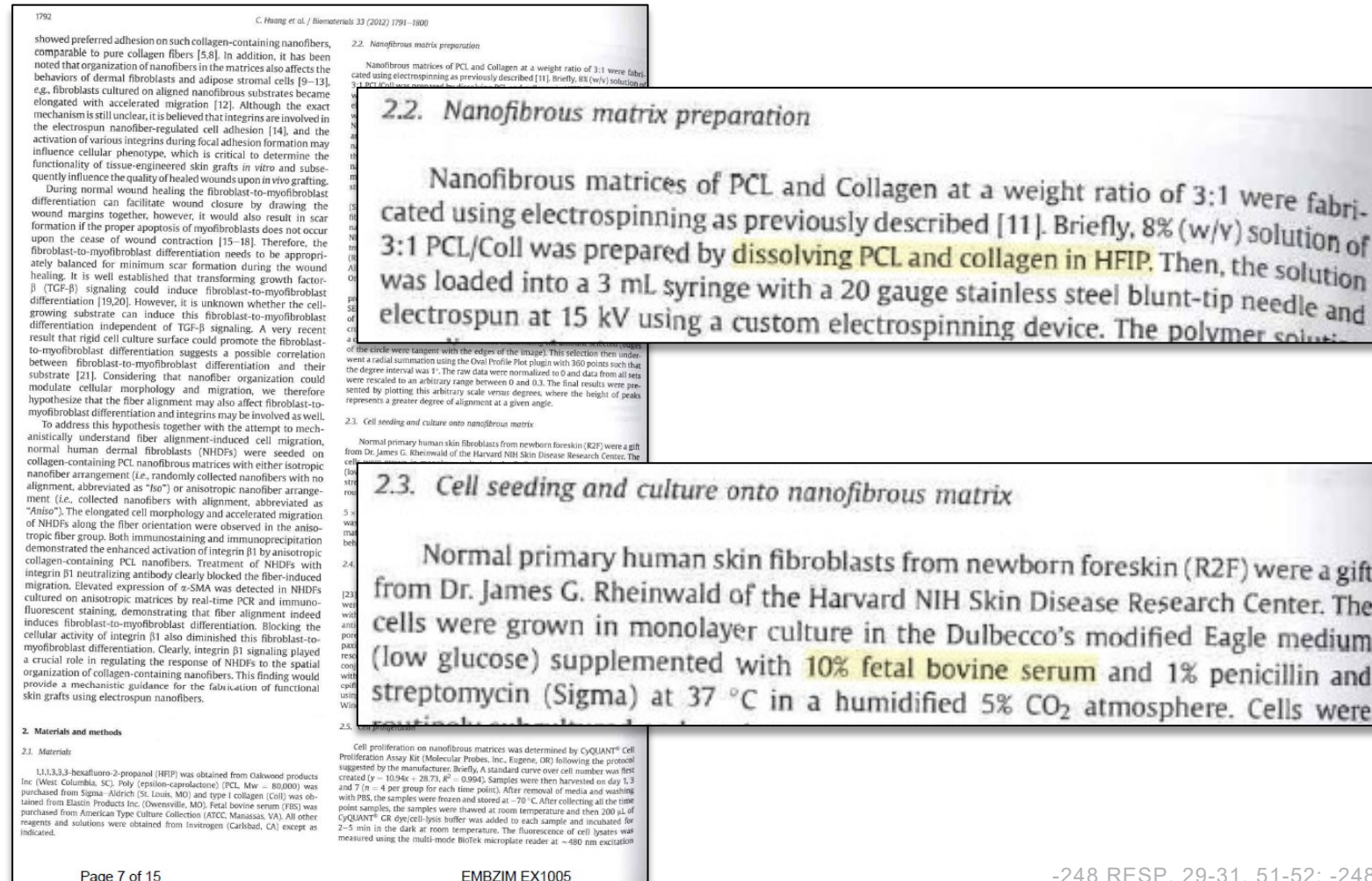
Electro-spinning of pure collagen nano-fibres — Just an expensive way to make gelatin?[☆]

Dimitrios I. Zeugolis^{a,b,c,1}, Shih T. Khew^d, Elijah S.Y. Yew^b, Andrew K. Ekaputra^b, Yen W. Tong^{b,d}, Lin-Yue L. Yung^d, Dietmar W. Hutmacher^{b,e,2}, Colin Sheppard^b, Michael Raghunath^{a,b,f,*}

Freeze-dried collagen dissolved in HFP and freeze-dried again (HFP-recovered collagen) also exhibited similar properties with those obtained from gelatin preparations, clearly indicating that fluoroalcohols are the major cause of denaturation. Taken together, this builds up the strongest evidence that electro-spinning of collagen or co-spinning of collagen-synthetic polymers out of fluoroalcohols results in the creation of gelatin, a protein derived from denatured collagen and is characterised by destroyed α -chains, disrupted triple-helical and fibrillar structure and lacking internal structure or configurational order

-248 CLAIM 2

It is undisputed that Huang2's seeded scaffolds are prepared with denaturing HFIP/HFP, PCL, FBS.



-248 CLAIM 2

It is undisputed that Lee's seeded scaffolds are prepared with denaturing HFIP/HFP, PCL, FBS.

US 20100331980A1

(19) United States
(12) Patent Application Publication (10) Pub. No.: US 2010/0331980 A1
Lee et al. (43) Pub. Date: Dec. 30, 2010

(54) ALIGNED SCAFFOLDING SYSTEM FOR SKELETAL MUSCLE REGENERATION

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

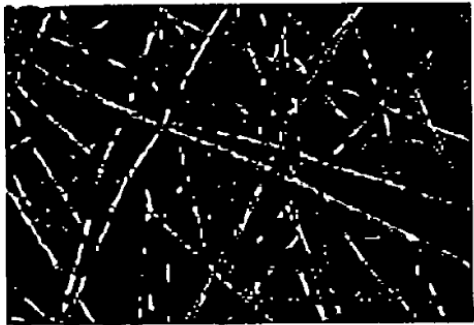
Correspondence Address:
MYERS BIGEL SIBLEY & SAJOVEC
PO BOX 37428
RALEIGH, NC 27627 (US)

(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

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.



Page 1 of 20 EMBZIM EX1011

[0063] Electrospinning. Electrospun nanofiber meshes were fabricated using a blend of PCL and collagen with a ratio of 1:1 in weight. Both PCL and collagen were dissolved in HFP at a total concentration of 5% (wt/vol). Briefly, the PCL/collagen blend solution was electrospun using a high

[0069] Isolated hSkMCs were grown in tissue culture dishes with skeletal muscle growth medium (SkGM-2, Lonza Group Ltd., Basel, Switzerland) containing the basal medium plus recombinant human epidermal growth factor (rhEGF), insulin, gentamicin, amphotericin, fetuin, dexamethasone, and bovine serum albumin supplemented with 5% fetal bovine serum (FBS) until 70% of the cells were confluent. Cell fusion was induced by replacing the growth medium with DMEM/F12 1:1 containing 2% horse serum, 1% penicillin/streptomycin. All reagents for cell culture were purchased from Invitrogen.

-248 CLAIM 2

“The mere fact that a certain thing may result from a given set of circumstances is not sufficient.” *PAR Pharm., Inc. v. TWI Pharms., Inc.*, 773 F.3d 1186, 1195 (Fed. Cir. 2014) (“probabilities or possibilities” insufficient). **Inherency** “is appropriate only when the reference discloses prior art that must necessarily include the unstated limitation.” *Transclean Corp. v. Bridgewood Servs., Inc.*, 290 F.3d 1364, 1373 (Fed. Cir. 2002). “Absent inevitability, inherency does not follow even from a very high likelihood that a prior art method will result in the claimed invention.” *In re Montgomery*, 677 F.3d 1375, 1384 (Fed. Cir. 2012).

- 2 4 8 : C L A I M 3
(§ 1 0 2 : H U A N G 2 O R L E E)

-248 CLAIM 3

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.

...

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

-248 CLAIM 3

Huang2 and Lee are silent regarding residual calcium levels, and the Petition fails to show inherency:

C. **Claim 3: “The scaffold according to claim 1, wherein the fiber has less than about 8 wt % residual calcium.”**

Huang2 discloses all the elements of this claim as arranged in the claim.

(EX1003, §VI.A.3.) As discussed in §X.A., *supra.*, Huang2 discloses all the

elements of claim 1. Further, Huang2 describes the materials used to prepare the nanofibers and does not indicate any calcium was used. (EX1003, ¶121.) In

“incorporate” or otherwise use calcium in the production of these fibers. (EX1003, §VI.A.3.; EX1001, 11:2-4.) Thus, the 3:1 PCL/Coll electrospun nanofibers used in Huang2 would contain less than about 8 wt % residual calcium. (EX1003, §VI.A.3.) Accordingly, Huang2 plainly discloses all the elements of claim 3 and anticipates this claim.

-248 CLAIM 3

It is undisputed that collagen scaffolds chelate calcium from FBS.

118. In my opinion, Huang2's fibers do not necessarily possess less than about 8 wt % residual calcium simply because Huang2 did not use calcium in the production of its fibers. Ex. 1003 ¶ 121; Pet. 34. A POSITA would have readily understood that residual calcium is necessarily imparted to electrospun fibers from a variety of conventional sources, including compounds utilized in both electrospinning and cell culture. For example, residual calcium levels are driven by the source of collagen and the presence of chemical cross-linkers, buffer solutions, or cell culture compounds.

119. As discussed regarding claim 2, Huang2 discloses that it utilizes fetal bovine serum ("FBS") in its cell culture medium. Ex. 1005, 1792. A POSITA would have readily understood that FBS naturally contains high concentrations of calcium, which is necessary for growth of the cells seeded on the scaffold. When collagen scaffolds are placed in a cell culture medium with FBS, like Huang2's, those scaffolds will chelate calcium present in the FBS. This will necessarily increase the fiber's residual calcium level. This is especially so when carboxyl terminated PCL is present in the scaffold, like in Huang2. Carboxyl groups are part of the aspartic acid and glutamic acid amino acid residues in collagen, and they are located at one end of the PCL chains. When placed in the cell culture medium containing FBS because the carboxyl is deprotonated at physiologic pH (exists as COO-) and, therefore, is negatively charged. That negative charge will chelate positively charged calcium.

-248 CLAIM 3

Dr. Mikos and Dr. Simpson agree that glutaraldehyde is “implicated in the development of calcification.”

173. Unlike Huang² above, Lee’s scaffold is also cross-linked with glutaraldehyde solution. Ex. 1011 ¶ 63. Glutaraldehyde is “implicated in the development of calcification.” Ex. 2020, 1594. Glutaraldehyde-crosslinked collagen, like Lee’s, mineralizes more effectively than un-crosslinked collagen because crosslinking enhances collagen’s mechanical stability, which is crucial for mineralization and calcification. This contrasts with the claimed scaffold, which seeks to minimize calcium to less than 8 wt %.

TISSUE ENGINEERING
Volume 13, Number 7, 2007
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DOI: 10.1089/ten.2006.0292

Cross-Linking Electrospun Type II Collagen Tissue Engineering Scaffolds with Carbodiimide in Ethanol

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fibrils.^{20–23} Glutaraldehyde, in particular, offers the advantages of being less expensive, reacting relatively quickly, cross-linking over varying distances, and reacting with a larger number of amino groups present in a protein molecule.²¹ It is the most commonly used and accepted agent for the cross-linking of biological tissues, but it has also been implicated in the development of calcification.^{21,24,25}

-248 CLAIM 3

It is undisputed that the Huang2 and Lee seeded scaffolds are prepared with calcium-contributing compounds.

Huang2: FBS

2.3. Cell seeding and culture onto nanofibrous matrix

Normal primary human skin fibroblasts from newborn foreskin (R2F) were a gift from Dr. James G. Rheinwald of the Harvard NIH Skin Disease Research Center. The cells were grown in monolayer culture in the Dulbecco's modified Eagle medium (low glucose) supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin (Sigma) at 37 °C in a humidified 5% CO₂ atmosphere. Cells were routinely subcultured and used at passages 5–7.

Lee: FBS and glutaraldehyde

[0069] Isolated hSkMCs were grown in tissue culture dishes with skeletal muscle growth medium (SkGM-2, Lonza Group Ltd., Basel, Switzerland) containing the basal medium plus recombinant human epidermal growth factor (rhEGF), insulin, gentamicin, amphotericin, fetuin, dexamethasone, and bovine serum albumin supplemented with 5% fetal bovine serum (FBS) until 70% of the cells were confluent. Cell fusion was induced by replacing the growth medium with DMEM/F12 1:1 containing 2% horse serum, 1% penicillin/streptomycin. All reagents for cell culture were purchased from Invitrogen.

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 electrospun nanofibers were stored under vacuum for 48 hr to remove residual solvent.

- 2 4 8 : C L A I M 4
(§ 1 0 2 : H U A N G 2 O R L E E)
(§ 1 0 3 : H U A N G 2 / K U M B A R)

-248 CLAIM 4

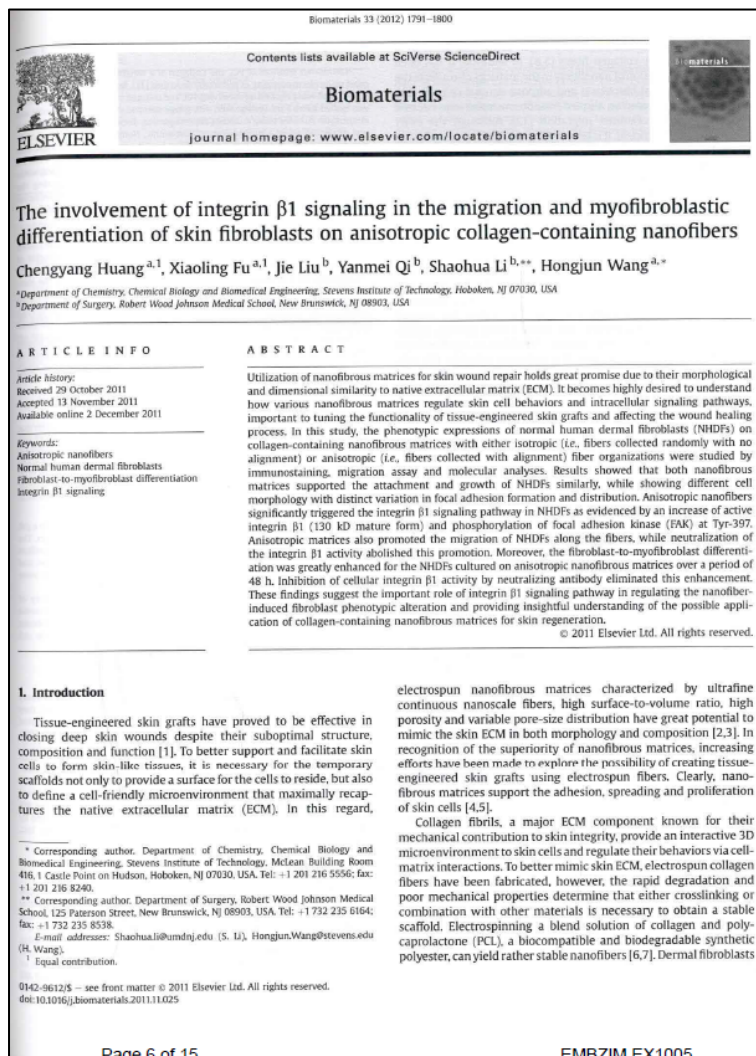
1. A scaffold comprising one or more electrospun fibers comprising collagen, wherein a fast Fourier transform (FFT) analysis result of the fibers have adjacent major peaks with about 180° apart from each other.

...

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

-248 CLAIM 4: § 102

Huang2 lacks the claimed 60% – 90% porosity range and does not anticipate.



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

-248 CLAIM 4: § 102

Lee lacks the claimed 60% – 90% porosity range and does not anticipate.

US 20100331980A1

(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**

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Winston-Salem, NC (US); **Anthony**
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(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**

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

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.

Page 1 of 20

EMBZim EX1011

[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.

-248 CLAIM 4: § 102

Mikos' porosities of non-collagen bone/cartilage scaffolds do not anticipate.

A. Different tissue

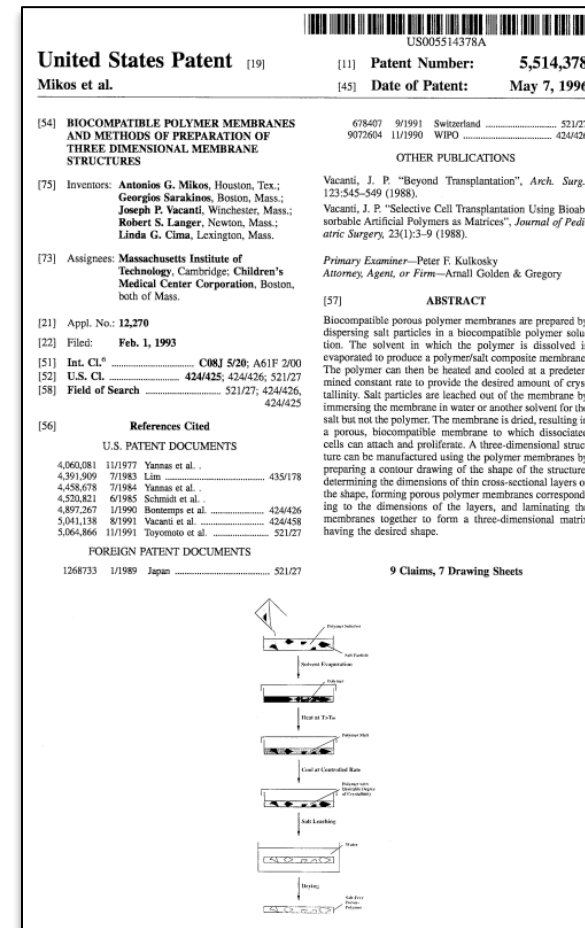
- i. Bone or cartilage vs. muscle

B. Different polymers

- i. No collagen vs. collagen/PCL blend

C. Different mechanisms

- i. Salt leaching vs. electrospinning
 - “[S]alt leaching ... allows an artisan to selectively and individually vary both porosity *and* pore size. But these features cannot be independently controlled through electrospinning.”



-248 CLAIM 4: § 103

It is undisputed that porosity “greatly depend[s]” on electrospinning parameters, which fundamentally differ for:

- Kumbar’s non-aligned, pure collagen scaffold vs.
- Huang2’s aligned, 3:1 PCL/collagen blended scaffold.

Various parameters that potentially affect electrospinning include polymer molecular weight, polymer solution properties, applied electrical potential, polymer solution flow rate, distance between spinneret and collector (working distance), motion of the grounded target and ambient parameters (temperature, humidity and air velocity). Nanofiber diameter, surface morphology, mechanical properties, porosity and pore-size distribution greatly depend on these parameters selected for electrospinning. Various

-248 CLAIM 4: § 103

The Petition did not propose modifying Huang2's electrospinning parameters to adjust porosity:

Further, both references are in the same field, involving using electrospun collagen-containing scaffolds for biomedical applications, such as in regenerative medicine (e.g., skin grafts). (E.g., EX1005, 1791; EX1006, 1; EX1003, ¶170.) Both note that “electrospun nanofibrous matrices . . . characterized by . . . high porosity and variable pore-size distribution” show morphological similarities to natural skin ECM. (EX1005, 1791; EX1006, 1, 6, 12; EX1003, §VI.B.) And Huang2's and Kumbar's discussion of the benefits of mimicking the porosity of the native skin ECM would have further motivated a POSA to combine the teachings of Kumbar modify Huang2's disclosed scaffolds to have porosity of 89% or 71%. (EX1003, § VI.B.; *see also* EX1006, 6-7.)

In addition, tailoring the porosity of electrospun fibers is well within the knowledge of a POSA, for example by using well-known methods including freeze-drying, salt leaching, and gas foaming. (EX1003, ¶171.) Such techniques have long been used on electrospun scaffolds, are well-understood, and allow a

- 2 4 8 : C L A I M 1 2
(§ 1 0 2 : L E E)

-248 CLAIM 12

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.

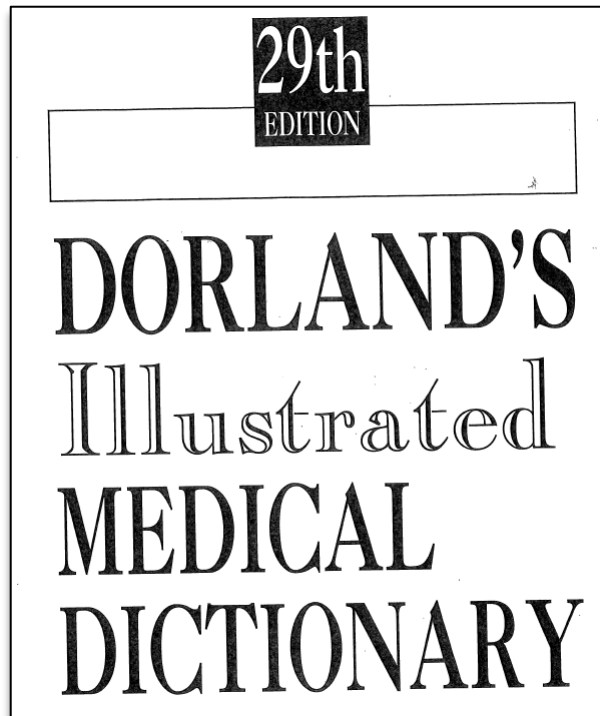
...

11. A method of cell culture comprising **culturing cells on the scaffold** according to claim 1 under conditions suitable for the cell culture.

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.

-248 CLAIM 12

Stem cells differentiate into different cell types.



stem c., 1. any precursor cell. 2. a blood cell progenitor or mother cell representing a slightly later stage than the blast cell; it has the capacity for both replication and differentiation, and has pluripotentiality, giving rise to precursors of various different blood cell lines, such as the proerythrocyte and myeloblast, which cannot self-replicate and must differentiate into more mature daughter cells. Called also *progenitor c.*, *hematopoietic stem c.*, and *colony-forming unit-spleen*.

-248 CLAIM 12

Lee seeds skeletal muscle cells including myoblasts.

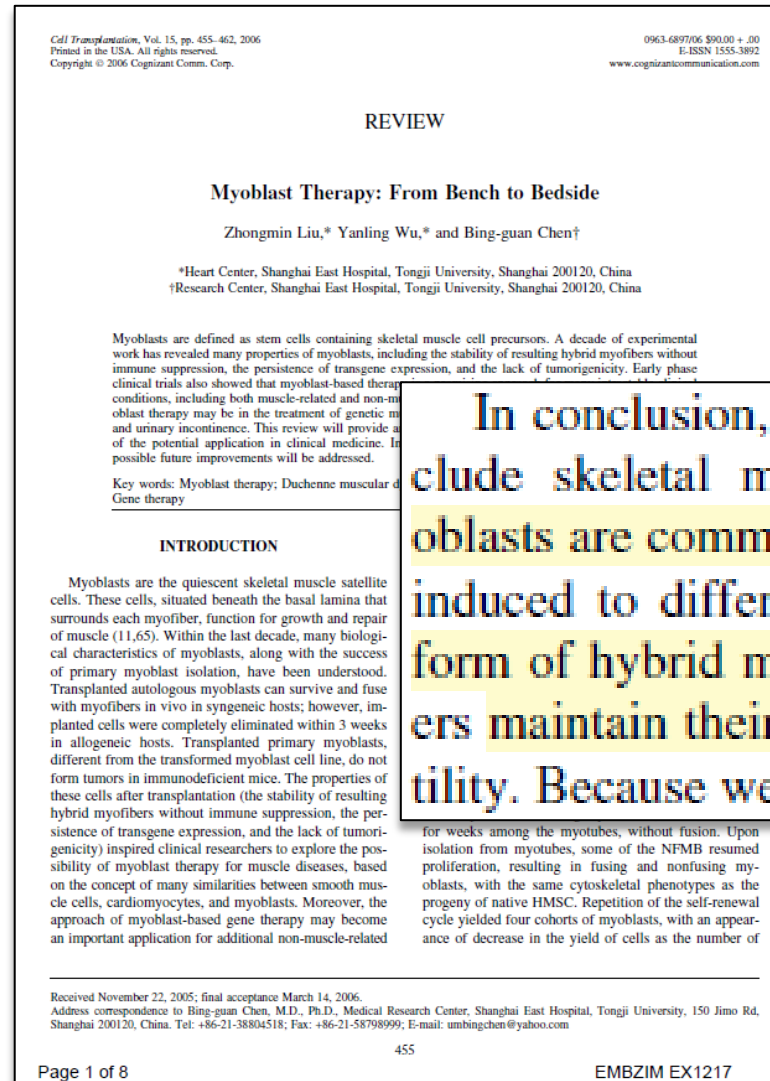
[0007] Further provided are methods of forming an anisotropic muscle implant, including the steps of: seeding skeletal muscle cells on a scaffold having oriented fibers; and then forming oriented myotubes from the seeded skeletal muscle cells. In some embodiments, the skeletal muscle cells include myoblasts. In some embodiments, fibers include natural poly-

Lee explains that myoblasts are – and remain – muscle. They do not differentiate into other cell types.

[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 is injured, the myoblasts are capable of dividing and repopulating it. Typically, after muscle injuries myofibers become necrotic and are removed by macrophages (Hurme et al. (1991) Healing of skeletal muscle injury: an ultrastructural and immunohistochemical study, *Med. Sci Sports Exerc.* 23, 801-810). This induces proliferation and fusion of myoblasts to form multinucleated and elongated myotubes, which self-assemble to form a more organized structure, namely muscle fibers (Campion (1984) The muscle satellite cell: a review, *Int. Rev. Cytol.* 87, 225-251).

-248 CLAIM 12

Petitioner's evidence confirms that myoblasts are "committed" muscle cells.



DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

- 249: CLAIM 1, NO “CROSSLINKED FIBERS”
(§ 103: HUANG2/KUO)

-249 CLAIM 1

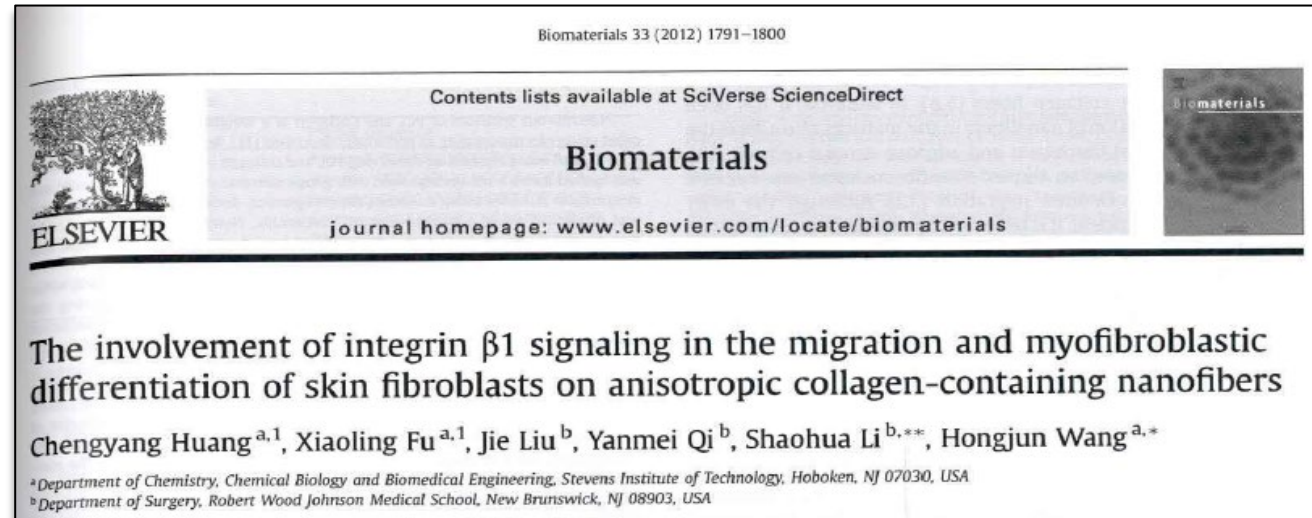
1. A method of treating a tissue defect in a subject comprising[:]

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.

-249 CLAIM 1

Huang2 discloses that it achieved stability by blending collagen with PCL, not by crosslinking.



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

-249 CLAIM 1

Huang2’s 3:1 PCL/collagen blend is more stable than crosslinked collagen because PCL degrades over 3-4 years while type I collagen degrades over weeks.



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The return of a forgotten polymer—Polycaprolactone in the 21st century

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ARTICLE INFO

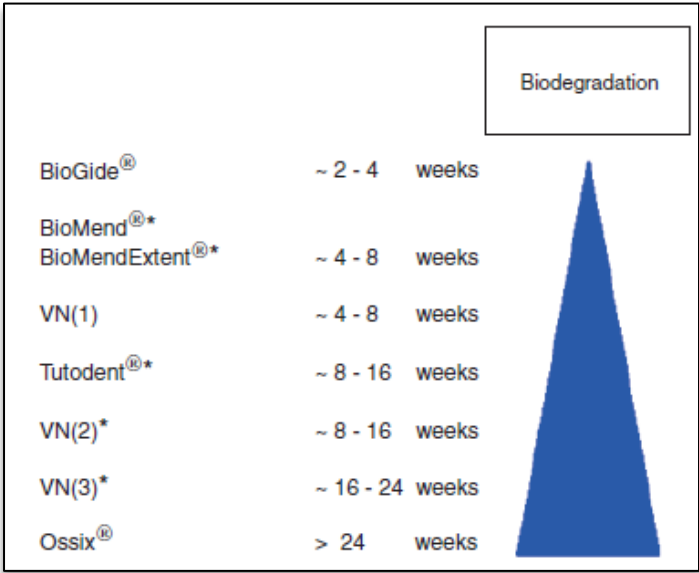
Article history:
Received 5 October 2009
Received in revised form 19 February 2010
Accepted 2 April 2010
Available online 7 April 2010

Keywords:
Polymer
Scaffold
Polycaprolactone
Tissue engineering
Bone
Resorbable

ABSTRACT

During the resorbable-polymer-boom of the 1970s and 1980s, polycaprolactone (PCL) was used extensively in the biomaterials field and a number of drug-delivery devices. Its popularity was soon superseded by faster resorbable polymers which had fewer perceived disadvantages associated with **long-term degradation (up to 3–4 years)** and intracellular resorption pathways; consequently, PCL was almost forgotten for most of two decades. Recently, a resurgence of interest has propelled PCL back into the biomaterials-arena. The superior rheological and viscoelastic properties over many of its aliphatic polyester counterparts renders PCL easy to manufacture and manipulate into a large range of implants and devices. Coupled with relatively inexpensive production routes and FDA approval, this provides a promising platform for the design and fabrication of **longer term degradable implants which** may be manipulated physically, chemically and biologically to possess tailorable degradation kinetics to suit a specific anatomical site. This review will discuss the application of PCL as a biomaterial over the last two decades focusing on the advantages which have propagated its return into the spotlight with a particular focus on medical devices, drug delivery and tissue engineering.

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Ex. 2021, Fig. 8: comparing degradation rates of various types of Type I collagen

-249 CLAIM 1

Petitioner presents insufficient reasoning why a POSITA would crosslink Huang2's stable fibers.

Meinel, as incorporated into Kuo, discloses that although “[c]ollagen is frequently used as a scaffold material because it is a major component of the cartilage matrix . . . its rapid degradation has been recognized as a limitation.” (EX1023, 380.) Meinel explains that crosslinking collagen scaffolds can address this issue by “decreas[ing] the rate of degradation of collagen by two orders of magnitude.” (EX1003, ¶112; EX1023, 380-81, 390.) And Meinel teaches that

...

Accordingly, for the reasons set forth above, a skilled artisan would have been motivated with a reasonable expectation of success to utilize Huang2's aligned fibers to create a scaffold that can treat a tissue defect, and would have been motivated to improve the stability of collagen fibers by chemically crosslinking, as taught by both Huang2 and Kuo.

-249 CLAIM 1

Meinel crosslinked pure collagen; it has no bearing on a more stable PCL/collagen blend.

Engineering Cartilage-Like Tissue Using Human Mesenchymal Stem Cells and Silk Protein Scaffolds

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Received 6 February 2004; accepted 20 April 2004

Published online 12 October 2004 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/bit.20252

Abstract: Human mesenchymal stem cells (hMSC) derived from bone marrow aspirates can form the basis for the in vitro cultivation of autologous tissue grafts and help alleviate the problems of immunorejection and disease transmission associated with the use of allografts. We explored the utility of hMSC cultured on protein scaffolds for tissue engineering of cartilage. hMSC were isolated, expanded in culture, characterized with respect to the expression of surface markers and ability for chondrogenic and osteogenic differentiation, and seeded on scaffolds. Four different scaffolds were tested, formed as a highly porous sponge made of: 1) collagen, 2) cross-linked collagen, 3) silk, and 4) RGD-coupled silk. Cell-seeded scaffolds were cultured for up to 4 weeks in either control medium (DMEM supplemented with 10% fetal bovine serum) or chondrogenic medium (control medium supplemented with chondrogenic factors). hMSC attachment, proliferation, and metabolic activity were markedly better on slowly degrading silk than on fast-degrading collagen scaffolds. In chondrogenic medium, hMSC formed cartilaginous tissues on all scaffolds, but the extent of chondrogenesis was substantially higher for hMSC cultured on silk as compared to collagen scaffolds. The deposition of glycosaminoglycan (GAG) and type II collagen and the expression of type II collagen mRNA were all higher for hMSC cultured on silk than on collagen scaffolds. Taken together, these results suggest that silk scaffolds are particularly suitable for tissue engineering of cartilage starting from hMSC, presumably due to their high porosity, slow biodegradation, and structural integrity. © 2004 Wiley Periodicals, Inc.

Keywords: tissue engineering; mesenchymal stem cells; cartilage scaffold; silk; in vitro

INTRODUCTION

Healthy joint cartilage can withstand an astonishing amount of repetitive physical stress. However, once injured, articular cartilage fails to self-repair even minor defects in its structure. At present, osteoarthritis accounts for half of all chronic conditions in persons over age 65 and it affects ~25% of the population over age 60 (Bone and Joint Decade, 2000, www.boneandjointdecade.org). Current treatment protocols are limited by incomplete integration between the graft and host tissue and the scarcity of suitable donor material (Britberg et al., 1994; Wakitani et al., 1994). The inability of cartilage for self-repair and the lack of protocols that can reproducibly regenerate durable articular surface provide the rationale for the development of new treatment options based on tissue-engineered cartilage repair (Lohmander, 2003).

Tissue engineering has a potential to provide functional substitutes of lost or damaged native tissues by an integrated use of cells, biomaterial scaffolds, and biochemical and physical regulatory factors (Langer and Vacanti, 1993). Engineered constructs can also serve as high-fidelity tissue models for controlled quantitative studies of cell function and tissue development (Freed and Vunjak-Novakovic, 2000). In particular, research in tissue engineering can help us understand the complex requirements for regeneration of joint cartilage (Lohmander, 2003). The acquired experience suggests that successful tissue engineering involves the recapitulation of certain embryonic events (Caplan, 2003). Central to the regeneration of mesenchymal tissues, including cartilage, is the availability of mesenchymal stem cells (MSCs), their recruitment into the repair site, and directed conversion into specialized tissues. Ideally, cartilage can be engineered starting from

Abstract: Human mesenchymal stem cells (hMSC) derived from bone marrow aspirates can form the basis for the in vitro cultivation of autologous tissue grafts and help alleviate the problems of immunorejection and disease transmission associated with the use of allografts. We explored the utility of hMSC cultured on protein scaffolds for tissue engineering of cartilage. hMSC were isolated, expanded in culture, characterized with respect to the expression of surface markers and ability for chondrogenic and osteogenic differentiation, and seeded on scaffolds. Four different scaffolds were tested, formed as a highly porous sponge made of: 1) collagen, 2) cross-linked collagen, 3) silk, and 4) RGD-coupled silk. Cell-seeded scaffolds were cultured for up to 4 weeks in either control medium (DMEM supplemented with 10% fetal bovine serum) or chondrogenic medium (control medium supplemented with chondrogenic factors). hMSC attachment, proliferation,

-249 CLAIM 1

Dr. Mikos explains that a POSITA would not crosslink Huang2's stable fibers due to, e.g., cost, complexity, reduced biocompatibility, and chemical toxicity.

67. Given the lower degradation rate of Huang2's PCL/collagen blend, a POSITA would not have further modified Huang2's blended fibers to additionally crosslink them. Doing so would not have been necessary, given the stability already achieved, and would not have imparted additional stability beyond that imparted by blending PCL with collagen. In addition to being unnecessary, a POSITA would not have crosslinked Huang2's blend because this would have introduced to Huang2's process additional cost, complexity, and the very real known drawbacks of crosslinking.

68. For example, cross-linking can both strip away the benefits of using collagen in the first place, such as enhanced biocompatibility and can introduce difficulties in removing the toxic cross-linking chemicals, which pose additional risks. Dr. Simpson himself has expressed these concerns, stating that "[c]ross-linking ... may compromise biocompatibility" and "be cytotoxic because of the

-249 CLAIM 1

“To avoid” known disadvantages, Dr. Simpson’s lab “electrospun blends of collagen with synthetic polymers.”

TISSUE ENGINEERING
Volume 13, Number 7, 2007
© Mary Ann Liebert, Inc.
DOI: 10.1089/ten.2006.0292

Cross-Linking Electrospun Type II Collagen Tissue Engineering Scaffolds with Carbodiimide in Ethanol

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DAVID D. BRAND, Ph.D.,⁴ DAVID G. SIMPSON, Ph.D.,² and GARY L. BOWLIN, Ph.D.¹

Cross-linking can be used to tailor the biomechanical characteristics (typical characteristics of the tissue designated for regeneration), but it may compromise biocompatibility. The requirement for tissue engineering scaffolds to not cause any prolonged immune responses has led to research and development of fixation techniques that may prove more biocompatible than popular chemical cross-linking agents, including glutaraldehyde, formaldehyde, and epoxy compounds, which have been shown to be cytotoxic because of the reactive moieties that are covalently coupled between neighboring collagen fibrils.^{20–23} Glutaraldehyde, in particular, offers the advantages of being less expensive, reacting relatively quickly, cross-linking over varying distances, and reacting with a larger number of amino groups present in a protein molecule.²¹ It is the most commonly used and accepted agent for the cross-linking of biological tissues, but it has also been implicated in the development of calcification.^{21,24,25}

However, typical protocols for cross-linking electrospun collagen have used glutaraldehyde, but even then, the scaffolds tend to gel (unless more-severe cross-linking protocols are followed) and lose their nanofibrous morphology, which is one of the most important features of the scaffold to begin with. To avoid this, our laboratory has previously electrospun blends of collagen with synthetic polymers,²⁶ which has proven successful in initial cell studies, but it is desirable to be able to work with collagen-only electrospun scaffolds for such applications as cartilage tissue engineering. Thus, a complementary, exogenous cross-linking agent is required if pure collagen electrospun scaffolds are to be used as tissue-engineering scaffolds.

- 249: CLAIM 1 AND 4-6, NO TENDON, LIGAMENT, NERVE REPAIR
(§ 103: HUANG2/KUO OR LEE/KUO)

-249 CLAIMS 1 AND 4-6

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.

4. ... wherein the tissue defect is ... the **nerve defect**.

5. ... wherein the tissue defect is ... the **ligament defect**.

6. ... wherein the tissue defect is ... the **tendon defect**.

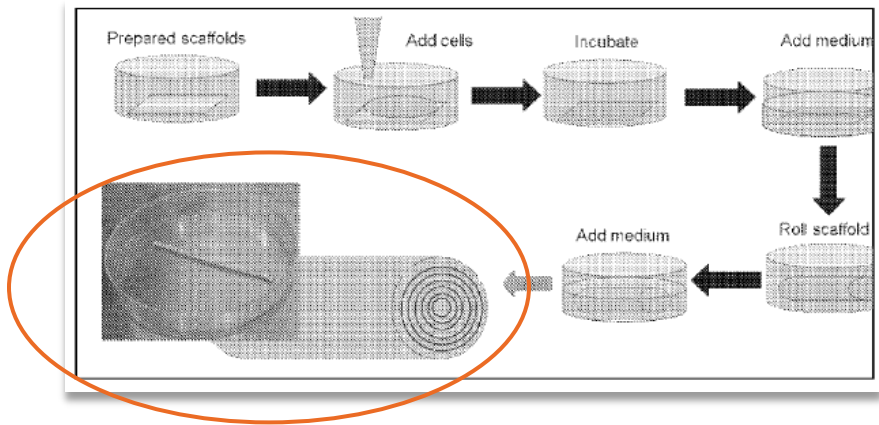
-249 CLAIM 1 AND 4-6

Treatment of tendon, ligament, or nerve would not have been obvious.

1. Huang2 and Lee's scaffolds lack the rolled structure of Kuo that is required for tendon, ligament, or nerve repair.
2. Huang2 and Lee's scaffolds present an inappropriate elastic modulus for tendon, ligament, or nerve repair.
3. Huang2 and Lee's scaffolds are seeded with cells that are incapable of tendon, ligament, or nerve repair.

-249 CLAIM 1 AND 4-6: ROLLED SCAFFOLDS

Kuo's scaffold is "rolled into cylindrical form." The Huang and Lee scaffolds are not.



[0034]. It is not clear why a POSITA would have chosen to use Huang2's scaffold in place of the scaffolds taught by Kuo for the repair of tendon, nerve, or ligament tissue when Huang2 does not teach the same rolled structure that mimics the natural tendon, nerve, or ligament tissue. Petitioner does not address Kuo's teaching regarding this rolled structure at all, let alone present any rationale to modify Huang2's scaffolds to mimic this rolled structure of natural tendon, nerve, or ligament issue, or otherwise explain why a POSITA would not seek to mimic that rolled structure when developing a scaffold to repair those specific types of tissues. Nonetheless

EX.1018 ¶5, FIG.1; DI 33-34, 40 (NOTING THAT KUO'S ROLLED STRUCTURE IS ABSENT IN LEE)
-249 RESP. 31-39, 55-62; -249 SUR-REPLY 18-24, 28-31

-249 CLAIM 1 AND 4-6 : ROLLED SCAFFOLDS

Dr. Simpson provides many reasons a POSITA would avoid rolling.

 US0957290B2	
(12) United States Patent Simpson et al.	(10) Patent No.: US 9,572,909 B2 (45) Date of Patent: Feb. 21, 2017
(54) ELECTROSPUN NERVE GUIDES FOR NERVE REGENERATION DESIGNED TO MODULATE NERVE ARCHITECTURE	(58) Field of Classification Search None See application file for complete search history.
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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 1227 days.	
(21) Appl. No.: 13/094,415	OTHER PUBLICATIONS Dedts et al. Biomaterials 2008 29:33-46.* Chew et al. Advanced Functional Materials 2007 17:1288-1296.* * cited by examiner
(22) PCT Filed: Sep. 14, 2010	Primary Examiner — Robert A Wax Assistant Examiner — Carlyne Helm
(86) PCT No.: PCT/US2010/048744 § 371 (c)(1), (2), (4) Date: May 17, 2012	(74) Attorney, Agent, or Firm — Whitham, Curtis & Cook, P.C.
(87) PCT Pub. No.: WO2011/032139 PCT Pub. Date: Mar. 17, 2011	
(65) Prior Publication Data US 2012/0221025 A1 Aug. 30, 2012	(57) ABSTRACT Nerve guides which are formed from three dimensional (3D) arrays of highly aligned electrospun fibers are provided. The electrospun fibers are oriented parallel to the long axis of the guide, and gaps and elongated spaces between the stacked fiber arrays provide channels for directed axonal growth. In some embodiments, the nerve guides also comprise high precision gradients of beneficial substances such as growth factors, which aid in nerve regeneration and growth along the guide.
(51) Int. Cl. A61L 27/18 (2006.01) A61F 2/02 (2006.01) A61B 17/11 (2006.01)	
(52) U.S. Cl. CPC A61L 27/18 (2013.01); A61B 17/1128 (2013.01); A61F 2/02 (2013.01); A61L 24/3032 (2013.01)	
11 Claims, 21 Drawing Sheets	
LifeNet Exhibit 2022 Embody, et al. v. LifeNet, IPR2025-00249 Page 1	

Other attempts at making nerve guides from electrospun (and other) types of fibers generally involve rolling sheets of material to form tubes. Unfortunately, the resulting tubes are hollow and thus fail to mimic the architecture of natural nerve growth. Further, a flat sheet that is rolled into a tube must, of necessity, have “seams” where the edges of the rolled sheets are surface exposed, e.g. on the wall of the hollow center of the tube and/or on the surface of the tube, resulting in discontinuities and possible weak connective points in the structure. In attempts to produce a more autologous position prior to injury. In turn, this type of regenerative materials patterns is designed to increase the probability that the regenerating axon returns to the distal target tissue (sensory and motor) that it innervated prior to injury. In addition, the seamless nature of the guide provides a far more uniform profile of pore spaces (i.e. the channels between the individual fibers) than a rolled sheet of material, reducing the tendency, of axons to cluster and grow as a jumbled disorganized mass along the seams, a pattern of growth in a regenerating nerve which is associated with reduced axon targeting. Because the air gap electrospinning process is very rapid it is possible to place substances of interest, e.g. cells, therapeutic agents, gradient threads (as described

-249 CLAIM 1 AND 4-6: ELASTIC MODULI

It is undisputed that elastic modulus must match the target tissue.

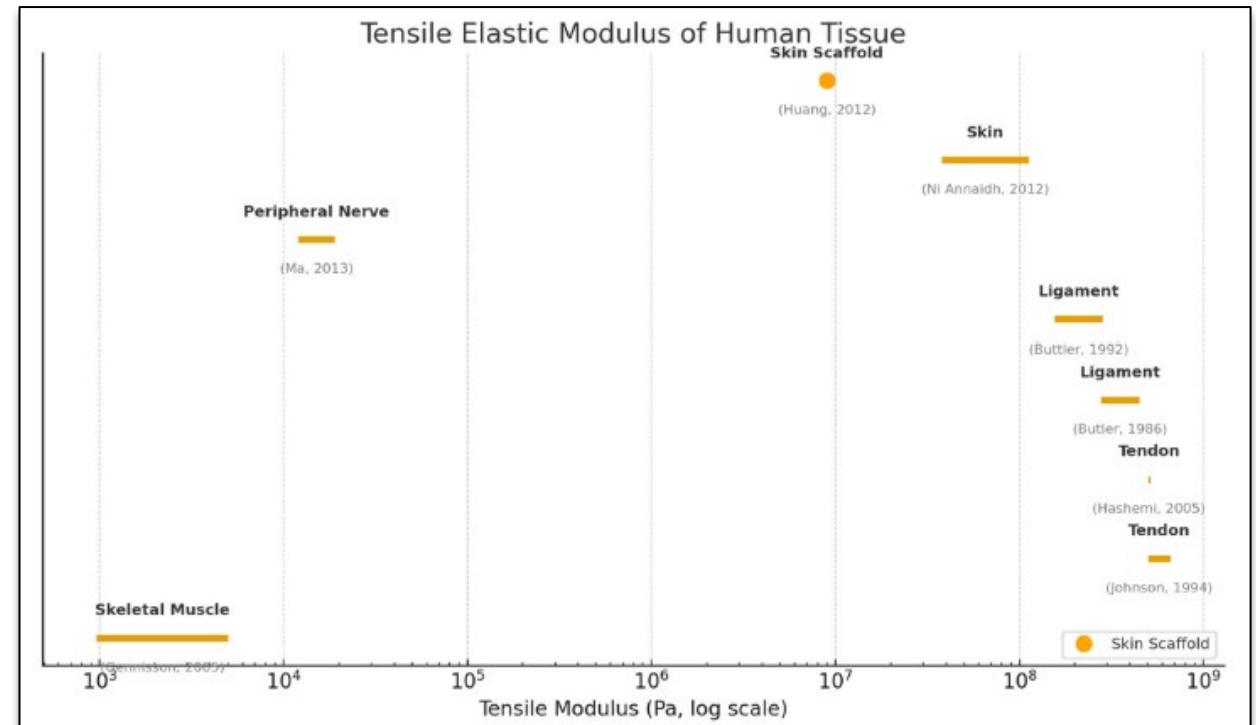
The elastic modulus of the Huang2 and Lee scaffolds are insufficient to repair tendon, ligament, or nerve.

Huang2's scaffold is:

- 73.5 times too compliant for tendon repair
- 49.75 times too compliant for ligament repair
- 472 times too stiff for nerve repair

Lee's scaffold is:

- 687,500 times too compliant for tendon repair
- 465,625 times too compliant for ligament repair
- 19 times too compliant for nerve repair



-249 CLAIM 1 AND 4-6: CELL SEEDING

It is undisputed that seeded cells must match the target tissue.

The cells seeded on the Huang2 and Lee scaffolds are insufficient to repair tendon, ligament, or nerve.

136. Every type of specifically differentiated cell type is associated with the growth or repair of an associated tissue type. Alternatively, stem cells and/or progenitor cells may be used for tissue repair of various tissue types because those cells further differentiate into specific cell types, as determined by the appropriate environmental conditions and the mechanical responsiveness of the scaffold material. Regardless, in all cases, the type of cell and the scaffold environment in which the cell is seeded dictates the precise tissue type that will be repaired or regenerated. Kuo recognizes this, stating that different cell types will be used “depending upon the intended function of the tissue engineered construct.” Ex. 1018 ¶ 58.

Cells

[0058] A number of different cell types or combinations thereof may be employed in the methods and compositions described herein, depending upon the intended function of the tissue engineered construct being produced. In one embodiment, the cells are eukaryotic cells. Exemplary cell types

-249 CLAIM 1 AND 4-6: CELL SEEDING

It is undisputed that “fibroblasts” are heterogenous.

Petitioner lacks any evidence that Huang2’s NHDFs can repair tendon, ligament, or nerve.

4. Heterogeneity in Fibroblast Function

4.1. Fibroblast Heterogeneity in Skin

There is growing evidence that fibroblasts are not one unified cell population but that there is much heterogeneity between different tissues and even within each tissue [34,42,43]. Because fibroblast heterogeneity has so far been best characterized in skin, skin fibroblasts will be discussed first. There are several ways skin fibroblasts are classified, for example, dermal fibroblasts are often defined as either reticular or papillary [44]. This classification is determined partly by the anatomical location, as well as the cell morphology. Reticular fibroblasts are typically more square shaped in contrast to papillary fibroblasts, which are more spindle shaped [45]. There are also differences in cell expression: reticular cells contain more α SMA-positive fibroblasts and express genes associated with cytoskeletal organization and cell motility. Papillary fibroblasts, in contrast, are more associated with immune system response genes [45]. Another recent discovery found that there are subpopulations of fibroblasts that have distinctly different effects on wound healing; for example, in the dorsal skin, paraxial mesoderm-derived *Engrailed-1* lineage negative cells have been showed to contribute to regenerative, scarless healing, while *Engrailed-1* lineage positive cells contribute to fibrosis [46,47].

-249 CLAIM 1 AND 4-6: OTHER STRUCTURAL CHARACTERISTICS

It is undisputed that structural characteristics of the Huang2 and Lee scaffolds preclude tendon, ligament, or nerve repair.

140. A POSITA would have known that skin tissue is regenerated at a notably different rate than tendon, ligament, or nerve tissue because different tissues secrete extracellular matrix (ECM) at different rates. Secreted ECM provides structural support to newly formed tissue and, therefore, the scaffold degradation rate must generally match the native rate of ECM secretion. Accordingly, a POSITA would have expected Huang2's and Kuo's scaffolds to employ different ratios of polymers to appropriately control the rate of degradation to match the appropriate rate of ECM secretion for each different tissue type. For example, a POSITA would have understood that the tissue regeneration rate of skin is much faster than that of muscle, nerve, ligament, or tendon because of the abundance of stem cells and the richness of blood supply that is present in skin as compared to muscle, nerve, ligament or tendon.

192. As also discussed above, successful tissue regeneration also requires a scaffold matched to that of the target tissue with respect to other mechanical properties such as degradation rate and stiffness. Here, a POSITA would not have expected success in using Lee's muscle scaffold to repair tendon, ligament, or nerve tissue in the absence of the appropriately matched scaffold mechanical characteristics. As above, a POSITA would have known that muscle tissue is regenerated at a notably different rate than tendon, ligament, or nerve tissue because different tissues secrete extracellular matrix (ECM) at different rates. Skeletal muscle regenerates at a rapid rate due to its high vascularity and abundance of stem cells. Tendons and ligaments are not as vascularized as skeletal muscle and regenerate more slowly by fibrosis (fibroblast proliferation and extracellular matrix deposition). Peripheral nerve regenerates the slowest by axonal sprouting, depending on nerve length.

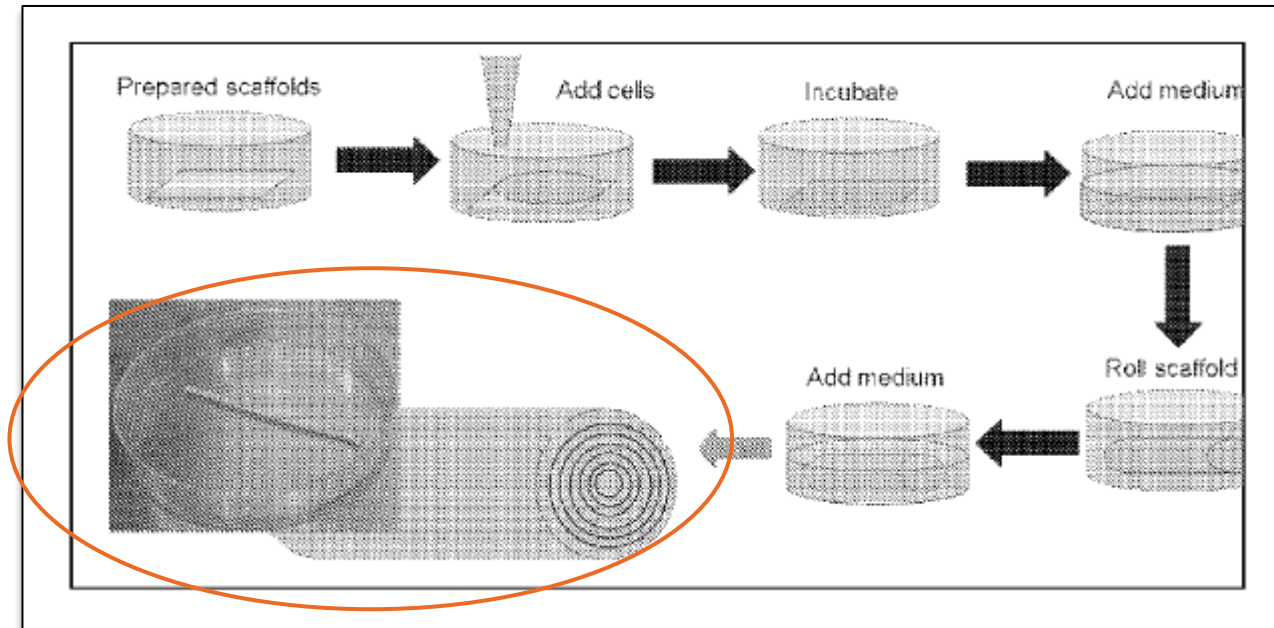
- 2 4 9 : C L A I M S 2 A N D 3
(§ 1 0 2 : H U A N G 2 / K U O O R L E E / K U O)

-249 CLAIMS 2-3

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**.

-249 CLAIMS 2-3

It is undisputed that Kuo teaches a scaffold that is rolled before being implanted.



[0034] Described herein are novel 3-dimensional porous scaffold compositions, that can be implanted into a subject for tissue regeneration, tissue enhancement, or tissue repair. Scaffold sheets comprised of fibers with diameters of micrometer or nanometer or other dimension and with fibers that are oriented at random, aligned, etc. are produced by e.g., electrospinning. The fibrous material may be comprised of natural materials, synthetic materials, flexible metal fibers, or any combination thereof. The compositions are seeded with cells and subsequently rolled into cylindrical form, to form structures e.g., that mimic that of fascicles of the muscle, tendon, nerve, or ligament. In addition, a plurality of compositions can be bundled together to form fascicular-like constructs that when cultured in vitro or implanted in vivo will support cell adhesion, growth, differentiation and/or regenerate tissues such as, for instance and without limitation muscle, tendon, ligament, nerve or other tissue.