

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

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

v.

LIFENET HEALTH,
Patent Owner.

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

**DECLARATION OF DAVID G. SIMPSON, PH.D.
IN SUPPORT OF PETITION FOR *INTER PARTES* REVIEW OF
U.S. PATENT NO. 11,318,227**

TABLE OF CONTENTS

	Page
I. Introduction.....	4
A. Engagement	4
B. My Qualifications	5
C. Summary of Opinions.....	8
1. Claims 1-6 are obvious in light of Huang2 in view of Kuo...10	
2. Claims 1-6 are obvious in light of Lee alone or in view of Kuo. 10	
3. Claims 7-8 are anticipated by Huang2. 10	
II. My Understanding of the Legal Standards	10
A. Anticipation	10
B. Obviousness	10
C. Level of Ordinary Skill in the Art	12
D. Claim Construction.....	13
III. Technical Background	14
A. Electrospinning is A Proven Technique for Producing Nanofibers with Different Characteristics.....14	
B. Electrospun Fiber Scaffolds Provide an Ideal Solution for Tissue Grafting.....17	
C. Enhancing Biological Performance of Microfiber Scaffolds Through Fiber Alignment.....18	
D. Determining Fiber Alignment Through FFT Analysis.....19	
E. Collagen Enhances Biological Properties of Nanofiber Scaffolds...26	
F. Previously Developed Aligned Collagen Scaffolds	27
IV. The Subject Matter of the '227 Patent	28
V. Summary of the Most Relevant Prior Art.....	34
A. Kuo.....	34

B.	Lee.....	35
C.	Huang2.....	38
VI.	My Analysis of the Claims	44
A.	Ground 1: Claims 1-6 are Obvious in light of Huang2 in view of Kuo.....	44
1.	Claim 1	48
(a)	“A method of treating a tissue defect in a subject comprising implanting at the tissue defect a scaffold”...48	
(b)	“a scaffold comprising crosslinked fibers comprising collagen”	51
(c)	“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”	53
(d)	“and applying the scaffold to the tissue defect to repair a tendon, a ligament or a nerve defect”	59
2.	Claim 2: “The method of claim 1, wherein the scaffold is in the form of one or more elongated sheets.”	62
3.	Claim 3: “The method of claim 1, wherein the scaffold is a composite of multiple layers of one or more sheets.”	63
4.	Claim 4: “The method of claim 1, wherein the tissue defect is a the nerve defect.”	65
5.	Claim 5: “The method of claim 1, wherein the tissue defect is a the ligament defect.”	66
6.	Claim 6: “The method of claim 1, wherein the tissue defect is a the tendon defect.”	67
B.	Ground 2: Claims 1-6 are Obvious in Light of Lee Alone or in View of Kuo	68
1.	Claim 1	68
(a)	“A method of treating a tissue defect in a subject comprising implanting at the tissue defect a scaffold”...68	
(b)	“a scaffold comprising crosslinked fibers comprising collagen”	70

	(c)	“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”	70
	(d)	“and applying the scaffold to the tissue defect to repair a tendon, a ligament or a nerve defect”	76
2.	Claim 2:	“The method of claim 1, wherein the scaffold is in the form of one or more elongated sheets.”	80
3.	Claim 3:	“The method of claim 1, wherein the scaffold is a composite of multiple layers of one or more sheets.”	81
4.	Claim 4:	“The method of claim 1, wherein the tissue defect is a the nerve defect.”	83
5.	Claim 5:	“The method of claim 1, wherein the tissue defect is a the ligament defect.”	84
6.	Claim 6:	“The method of claim 1, wherein the tissue defect is a the tendon defect.”	86
C.	Ground 3:	Claims 7-8 are Anticipated by Huang2	88
1.	Claim 7		88
	(a)	“A method of cell culture comprising placing cells on a scaffold”	88
	(b)	“comprising aligned fibers wherein a result of a FFT analysis of the fibers demonstrates that the fibers have major adjacent peaks that are about 180° apart from each other”	88
	(c)	“and growing the cells in conditions suitable for the cell culture”	94
2.	Claim 8:	The method of claim 7, wherein the cells are stem cells, adipose derived stem cells, dental pulp stem cells, fibroblasts, and/or dorsal root ganglia.....	96
VII.	Conclusion		96
1.	Claims 1-6 are obvious in light of Huang2 in view of Kuo...		97
2.	Claims 1-6 are obvious in light of Lee alone or in view of Kuo.		97
3.	Claims 7-8 are anticipated by Huang2.		97

I, David G. Simpson, Ph.D., declare as follows:

I. INTRODUCTION

A. Engagement

1. I have been retained by Hogan Lovells US LLP on behalf of Embody, Inc. and Zimmer Biomet Holdings, Inc. (“Petitioners”), as an independent technical expert in this proceeding before the Patent Trial and Appeal Board (“PTAB” or “Board”). I understand that Petitioners are requesting that the Board institute an inter partes review (“IPR”) proceeding of U.S. Patent No. 11,318,227 (the “’227 Patent”) (EX1001), currently assigned to LifeNet Health (“Patent Owner” or “PO”).

2. I have been retained as a technical expert by Petitioners to study and evaluate claims 1-8 of the ’227 Patent (“the challenged claims”), and the technology underlying the subject matter recited in these claims. I have been asked to provide my opinion regarding whether or not a person of ordinary skill in the art (“POSA”) in the subject matter of the claims would find the challenged claims anticipated or obvious in view of certain publications.

3. This declaration sets forth the opinions I have formed, the conclusions I have reached, and the bases for each.

4. I am not and have never been an employee of Petitioners. I am being compensated at my standard hourly consulting rate of \$150 per hour. No part of

my compensation depends on the outcome of this proceeding, and I have no other interest in this proceeding.

B. My Qualifications

5. All of my opinions stated in this declaration are based on my own personal knowledge and professional judgment. In forming my opinions, I have relied on my decades of experience as a biologist, inventor, founder, consultant, and my career as an academic research scientist in the electrospinning, cellular scaffold, and tissue engineering spaces.

6. I am very familiar with the technology described in the '227 Patent as of March 14, 2013, which I have been informed is the earliest date to which the '227 patent can claim priority.

7. From 1995-1997, I was a post doc studying the function of the extracellular matrix in the regulation of heart muscle cell structure and the role of the integrins in such regulation. Since 2000, I have been a tenured Associate Professor in the Department of Anatomy and Neurobiology of the Virginia Commonwealth University School of Medicine in Richmond, Virginia. In this role, I perform research on electrospinning and the use of electrospun fibrous scaffolds. My formal teaching responsibilities include giving anatomy lectures to medical students and Directing the Anatomy and Neuroanatomy courses in my

Institutions Dental School. At the graduate level I have trained several M.S. and Ph.D. students and have served numerous graduate committees.

8. I understand that a copy of my current curriculum vitae, which details my education and professional and academic experience, is being submitted by Petitioner as Exhibit 1004. The following provides an overview of some of my experiences that are relevant to the matters set forth in this declaration.

9. My professional career has focused on electrospinning techniques, methods, compositions, and uses. I have explored in depth how the distribution of fibers modulates the biological and material properties of tissue engineering scaffolds.

10. I have authored over sixty papers, the majority of which concern the electrospinning of natural proteins, including collagen, and a variety of synthetic polymers. (EX1004, 1-5.) These papers have been cited over 19,000 times. My h-index score through Google Scholar is 61; my most cited paper was the first publication to report the electrospinning of collagen (>3000 citations). Of the remaining electrospinning publications, two of which exceed 1,400 citations and twelve others exceed three hundred citations. I have also published more than twenty book chapters, more than five of which concern electrospinning and the use of electrospun fibrous scaffolds.

11. I also adapted, characterized and was the first to publish the 2D FFT method as a reliable and non-destructive technique to measure fiber alignment in electrospun scaffolds. (EX1013; EX1010.) The '227 patent relies on this method. (EX1001, Fig. 8, 13:15-26, Claims 1-12.)

12. My work has resulted in 9 US Patents that concern electrospinning, several International Patents and three start-up companies through the VCU Intellectual Property Foundation. A subset of my patents are below:

- US Patent # 7,615,373 Electrospun Collagen and Tissue Engineering. Simpson DG, et al.,
- US Patent # 7,374,774 Electroprocessed material made by simultaneously electroprocessing a natural protein polymer and two synthetic polymers. Bowlin GL, Wnek G, Simpson DG.
- US Patent #7,759,082 Electroprocessed Fibrin-based Matrices and Tissues. Simpson DG, et al.
- US Patent # 8,586,345 Electroprocessed Collagen and Tissue Engineering. Simpson DG, et al
- US Patent # 10,046,081 Electrospun dextran fibers & devices formed therefrom. Simpson, DG et al.

- US Patent # 9,572,909 Electrospun dextran fibers & devices formed therefrom. Simpson, DG et al.
- US Patent # 9,572,909 Electrospun Nerve Guides, Simpson DG et al.

13. My patent portfolio includes the very first patent directed at the process and products associated with electrospun collagen, as well as various blends of collagen, other natural proteins, and synthetic polymers.

14. A subset of my patent portfolio has been successfully transitioned to St. Teresa Medical, Inc., a Virginia Commonwealth University start-up company devoted to producing a high-performance hemostatic bandage. The first hemostatic bandage based on my work completed three clinical trials, and was approved for use in New Zealand. That product is at present awaiting regulatory approvals in the United States.

C. Summary of Opinions

15. The opinions contained in my Declaration are based on my professional experience and knowledge, as well as the documents that I reviewed in connection with my analysis. Specifically, in forming my opinions expressed in this Declaration, I reviewed the following documents and materials:

- The '227 patent;
- Kuo et al., U.S. Patent Application No. US 2011/0293685 A1, Pub.

Date Dec. 1, 2011 (“Kuo”);

- Lee et al., U.S. Patent Application No. US 2010/0331980 A1, Pub. Date December 30, 2010 (“Lee”);
- Huang et al., *The involvement of integrin $\beta 1$ signaling in the migration and myofibroblastic differentiation of skin fibroblasts on anisotropic collagen-containing nanofibers*, 33 Biomaterials 1791-1800 (Feb. 2012) (“Huang2”);
- The materials in Appendix A (Table of Exhibits).

16. My opinions are also based on my appreciation of how the person of ordinary skill would have understood the claims at issue and the specification of the '227 patent as of March 14, 2013, which counsel asked me to assume as the relevant time period for my analysis. My opinions reflect how one of ordinary skill in the art would have understood the '227 patent, Kuo, Lee, Huang2, and the state of the art as of March 14, 2013.

17. Based on my experience, expertise, and review of the materials set forth in my Declaration, I conclude the following points:

1. Claims 1-6 are obvious in light of Huang2 in view of Kuo.
2. Claims 1-6 are obvious in light of Lee alone or in view of Kuo.
3. Claims 7-8 are anticipated by Huang2.

II. MY UNDERSTANDING OF THE LEGAL STANDARDS

18. In forming my opinions and considering the subject matter of the '227 Patent and its claims in the light of the prior art, I am relying on certain legal principles that counsel in this case explained to me. My understanding of these concepts is summarized below.

19. I understand that earlier publications and patents may act to render a patent unpatentable for one of two reasons: (1) anticipation, and (2) obviousness.

A. Anticipation

20. It is my understanding that the claims of a patent are anticipated under 35 U.S.C. §102 by a prior art reference if each and every element of the claim is found either explicitly or inherently in the reference, as arranged in the claim. I understand that inherency requires a showing that the missing descriptive matter in the claim is necessarily present in the allegedly anticipating reference, and that it would have been so recognized by a person of ordinary skill in the art ("POSA").

B. Obviousness

21. I understand that a claim is unpatentable under 35 U.S.C. § 103 as obvious if the claimed subject matter as a whole would have been obvious to a

POSA at the time of the alleged invention. I also understand that an obviousness analysis considers the scope and content of the prior art, the differences between the claimed subject matter and the prior art, and the level of ordinary skill in the art at the time of the invention.

22. I understand that a patent claim composed of several elements is not proved obvious merely by demonstrating that each of its elements was known in the prior art. It has been explained to me that three requirements must be satisfied for a combination of prior art references to render obvious a claimed invention. First, the cited art must teach or suggest all the requirements of the invention as recited in the claims. However, I understand that a claim is obvious if a missing feature is a natural result of the combination of prior art elements.

23. Second, the cited art, coupled with the knowledge generally available in the art at the time of the invention, must contain some suggestion or incentive that would have motivated the ordinary skilled artisan to modify a reference or to combine references. I understand that it is impermissible to use hindsight to arrive at the claimed invention. My understanding is that to preclude hindsight in the obviousness analysis, which I understand to be improper, there must be evidence at the time of the invention of some reason to make the variation or combination. I understand that there is no requirement that the prior art contain an express suggestion to combine known elements to achieve the claimed invention, but a

suggestion to combine known elements to achieve the claimed invention may come from the prior art, as filtered through the knowledge of one skilled in the art.

24. Third, the proposed modification of the cited art must have had a reasonable expectation of success, determined from the vantage point of the ordinary skilled artisan at the time the invention was made.

25. I understand that, in an obviousness analysis, only analogous art may be considered. Art is considered analogous if it is from the same field of endeavor as the patent. If it is not from the same field of endeavor, it may be considered analogous if it is reasonably pertinent to the particular problem with which the patent is involved. A reference is reasonably pertinent if, though in a different field, it would have commended itself to an inventor's attention in considering the problem the inventor is addressing.

26. I understand that in evaluating whether patent claims are obvious, objective indicia of non-obviousness can be considered, such as, but not limited to, commercial success, long-felt but unsolved needs, praise, unexpected results, industry acceptance, failure of others, and skepticism by experts.

C. Level of Ordinary Skill in the Art

27. I have been informed that the person of ordinary skill is a hypothetical person presumed to be aware of all of the pertinent prior art. I have been asked to

consider what the ordinary level of skill in the art is based on my review of the patent, the knowledge in the art, and my experience in the field.

28. In my opinion, the person of ordinary skill for the purposes of the '227 patent would be a Ph.D. student with a minimum of two years of experience in their faculty advisor's laboratory. At this stage of graduate training, a Ph.D. student is expected to establish and design an independent research project in the pursuit of their degree. This level of expertise was long expected of graduate students in my laboratory and the laboratories of my principal collaborators. At the relevant time, a POSA would have understood the following technologies and techniques: electrospinning fibers, including for tissue engineering, tissue engineering techniques, and using FFT analysis and performing such analysis. My opinions discussed herein would not be changed due to a modestly lesser or greater level of experience. All of my opinions in this Declaration are from the perspective of one of ordinary skill in the art, as I have defined it here, at the relevant time, i.e., March 14, 2013. During this period, I possessed at least the qualifications of the person of ordinary skill in the art, as defined above.

D. Claim Construction

29. I have been informed by counsel that terms used in patent claims are typically given their ordinary and customary meaning in their technological field. However, when claim terms have no standard meaning in the art, I understand that

the terms must be interpreted in light of the patent claims, the patent's disclosure (referred to as the "specification"), and the "prosecution history" (the record of the back-and-forth between the patent applicant and the United States Patent and Trademark Office during the process of obtaining allowance of the patent's claims). I have applied this understanding in my analysis below. No reasonable interpretation of the claim terms would alter my conclusions.

III. TECHNICAL BACKGROUND

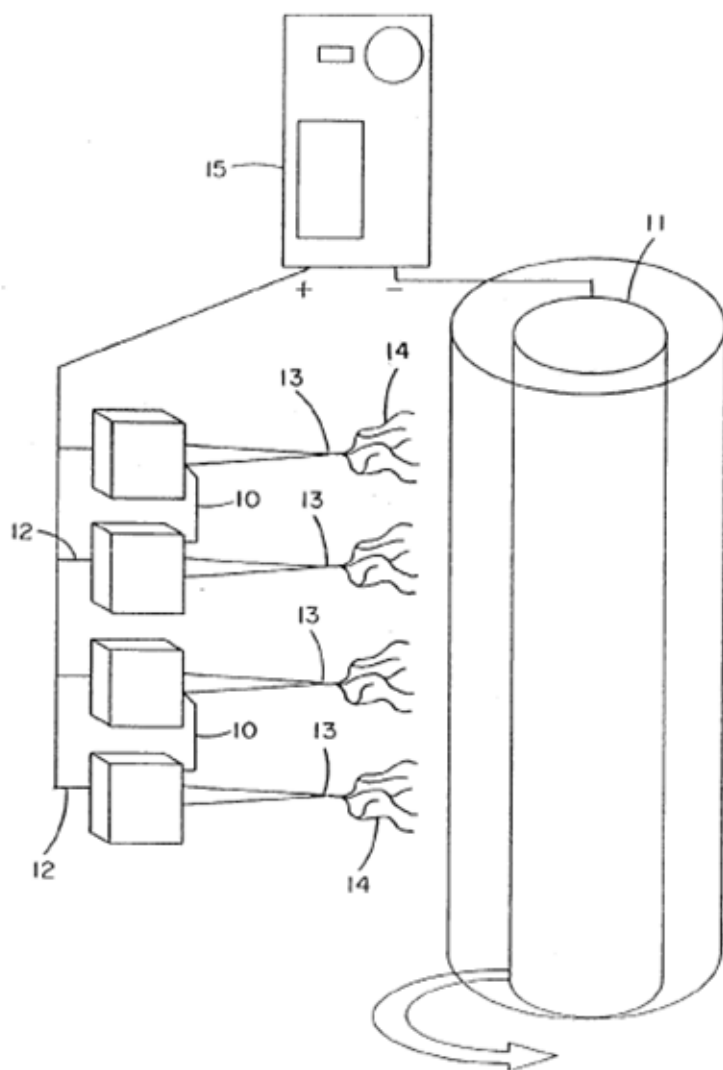
A. Electrospinning is A Proven Technique for Producing Nanofibers with Different Characteristics

30. As of March 2013, electrospinning was a well-established and widely used method for producing nanofibers. (EX1005, 1.) This technique was valued for its simplicity, reproducibility, and scalability. (EX1005, 2.)

31. Electrospinning is illustrated in the figure below, which is Figure 3 from my U.S. Patent Application No. 2004/0037813. (EX1009, [0156].) That patent application was published in 2004, eventually granted as U.S. Patent No. 7,615,373, and explicitly discusses electrospinning collagen. (*Id.*) Electrospinning works by using a high electric potential to eject a charged polymer solution from a syringe. (EX1005, 1-2.) First, a charge is applied to the tip of the syringe containing the charged polymer solution. (*Id.*) In the figure below, each syringe 10 is filled with a polymer solution and a fine wire 12 is placed in the solution to

charge the solution in each pipette tip 13 to a high voltage. (EX1009, [0156].)

Once the repulsive force between the charge and the polymer solution overcomes surface tension, a jet of the polymer solution is expelled from the syringe and moves toward a collector. (EX1009, [0156]; EX1005, 1-2.) As the jet of solvent and polymer is ejected from the syringe and travels towards the collecting target, the solvent evaporates. And as the jet dries, the polymer molecules are brought into proximity with one another and dry together to form a continuous fiber, and are deposited on the grounded collector. (EX1009, [0156]; EX1005, 1-2.) The dry fibers accumulate on the grounded collecting target 11, producing a fibrous polymer scaffold.



(EX1009, Fig. 3.)

32. The properties of the resulting fibrous scaffold depend on several factors, such as the polymer's molecular weight, the solution composition, flow rate, charge, the distance between the syringe and the collector, and the motion and charge of the collector. (EX1005, 3-5.) For example, a rotating collector, called a mandrel, can be used. (EX1013, 1.) Altering its rotation speed can significantly

change the properties of the scaffold. (EX1013, 1.) A rotating collector can also be used to create aligned fibers. (EX1005, 3; EX1005, 1792; EX1009, [0176].) In my experience, by March 2013, there were many other ways to make aligned fibers. The device described in the '227 patent is not the only way to obtain aligned fibers, as shown by the aligned fibers disclosed in Huang2.

B. Electrospun Fiber Scaffolds Provide an Ideal Solution for Tissue Grafting

33. The extracellular matrix (“ECM”) is the scaffolding in human tissues that provides support for cells and an environment for cells to interact with the surroundings. (EX1005, 6.) The ECM is important for cell adhesion, proliferation, migration, differentiation, and shape. (*Id.*) It is primarily composed of collagen. (*Id.*) Electrospun nanofiber scaffolds share several key characteristics with the natural ECM, such as ultrafine, continuous fibers with a high surface-to-volume ratio, high porosity, and variable size distribution. (EX1005, 1; EX1008, 308.)

34. Because electrospun scaffolds have so many similarities to the natural ECM, electrospun scaffolds are well-suited for tissue regeneration and repair. (EX1008, 308; EX1005, 6, 12.) As a result, electrospun nanofiber scaffolds are widely recognized as effective materials for promoting tissue regeneration. (EX1005, 12.) Electrospun scaffolds can also be composed of biodegradable

materials, so when implanted, they can be replaced by natural ECM over time.

(EX1005, 8, 10.)

35. By March 2013, electrospinning had been successfully applied in the development of grafts for a variety of tissues, including:

- skin (EX1005, 1791; EX1005, 7-8),
- tendon (EX1018, [0112]; EX1005, 10),
- blood vessels (EX1005, 8-10),
- muscle (EX1005, 11),
- ligament (EX1018, [0112]), and
- nerves (EX1005, 10-11).

C. Enhancing Biological Performance of Microfiber Scaffolds Through Fiber Alignment

36. The native ECMs found in tissues or organs are often made of naturally aligned fibers. (EX1018, [0112].) By March 2013, it was known that muscle, blood vessel, ligament, tendon, and the epineurium of nerves had ECMs that were naturally composed of aligned collagen fibers. (EX1019, 3, 27.) For this reason, the effects of fiber alignment were well studied in electrospun materials, and it was known that fiber alignment plays a role in defining the mechanical and biological material properties of electrospun materials. (EX1010, 604.) For example, it was published that fiber alignment can be used to manipulate a

scaffold's mechanical properties and that aligning collagen in nanofiber scaffolds enhances cell elongation, regulates cell shape, and enhances cell migration along the axis of alignment, especially in skin grafts and nerve grafts, which was a sought-after quality of these grafts. (EX1005, 6-7; EX1019, 28-29.) There were many examples of aligned scaffolds used in tissue engineering for applications to neural tissue, vascular tissue, skeletal muscle, bone, cartilage, tendon, and ligament. (EX1019, 27-31.)

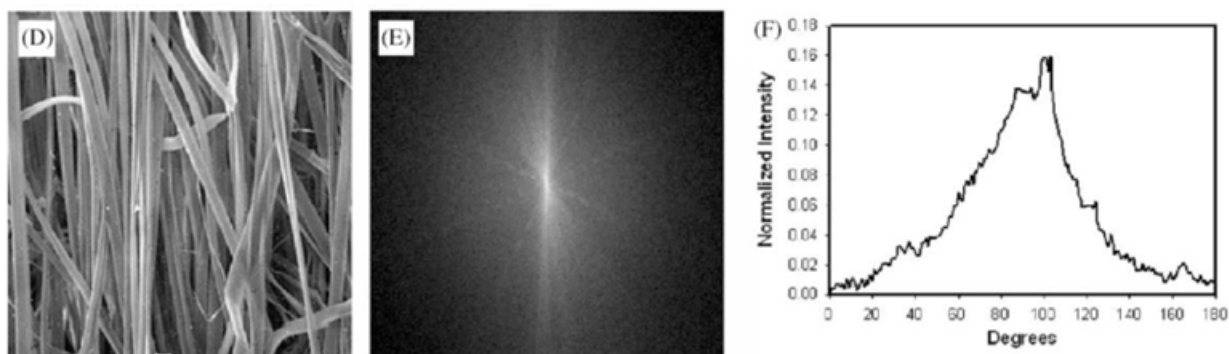
37. Given its properties, using aligned nanofiber scaffolds in tissue grafts had been described as “highly encouraging” and “promising.” (EX1005, 10; EX1019, 27.)

D. Determining Fiber Alignment Through FFT Analysis

38. As discussed above, the alignment of fibers within a fibrous scaffold is a factor in determining its mechanical and structural properties. To quantify fiber alignment, I adapted the mathematical method called the fast Fourier transform (2D FFT method) method for use as a non-destructive measure of fiber alignment in fibrous scaffolds. (EX1013, 2.)

39. The FFT analysis involves (1) capturing data images of electrospun scaffolds using a microscope, then (2) applying the 2D FFT method to create a 2D FFT output image, and finally (3) processing the 2D FFT output to create a 2D alignment plot. (EX1013, 2.) The below excerpt from Figure 1 from my 2006

methods paper shows (from left to right) a data image, the corresponding 2D FFT output image, and the corresponding 2D FFT alignment plot.



(EX1013, 12.)

40. To do the FFT analysis, first a data image of a fiber array is captured with a microscope. Then the data image is processed by the 2D FFT method to convert it from a spatial representation (“real” space) to a 2D FFT output image, which is a frequency-based representation (“frequency” space). Specifically, to convert the data image into the 2D FFT output image, the 2D FFT method “maps” the frequency at which pixels change across the original data image. So called low frequency signals in the 2D FFT output image correspond to areas in the original data image where pixel values are relatively unchanged over wide areas, indicating minimal variation between adjacent pixels. These low-frequency components are typically associated with the background and the general structure of the image, providing a baseline for identifying more detailed features. (EX1010, 609.) In contrast, high-frequency signals, which are mapped toward the periphery of the

frequency plot, represent regions with significant variation in pixel intensity, often indicating edges, details, and noise. (EX1010, 609.) Notably, the 2D FFT output image is symmetric. (EX1013, 3-4; EX1010, 609.)

41. When the fibers in the original image are randomly arrayed, the 2D FFT output image appears as a symmetrical circular “sunburst” shape. This symmetry indicates that changes in pixel intensities are uniformly distributed in all directions, suggesting a lack of preferential alignment. (EX1013, 3-4.) When the fibers in the original image are aligned, the 2D FFT output image shows an elliptical shape. This reflects the directional orientation of the fibers, with pixel intensities concentrated along a particular axis. (EX1013, 3-4.)

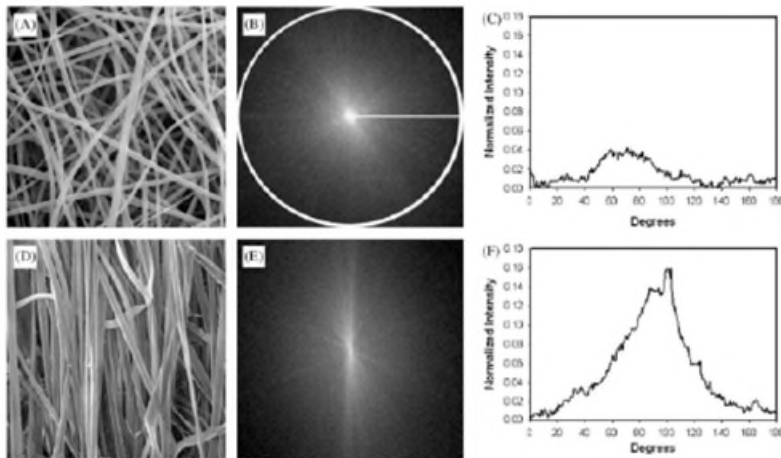
42. To further understand the fiber alignment, the 2D FFT output image can be processed into a 2D FFT alignment plot. (EX1013, 4; EX1010, 609.) This plot graphically represents the alignment of the fibers, providing a visual summary of their orientation (EX1013, 4; EX1010, 609.) The 2D FFT alignment plot illustrates the degree of fiber alignment, with the shape of its peaks indicating the extent of alignment in the original scaffold (EX1013, 4; EX1010, 610.)

43. To generate this plot, a circular projection is applied to the FFT output image, and pixel intensities are summed along each radius of that circle in a specified degree increment. The summed intensities are then plotted as a function

of the corresponding angle, resulting in the 2D FFT alignment plot. (EX1013, 4; EX1010, 609.)

44. The 2D FFT alignment plot exploits the accumulation of pixels in a particular direction in the 2D FFT output image to determine alignment. Recall in the 2D FFT output image of a random structure, the pixel map of the image has no preferential accumulation of pixels in any direction, but in the 2D FFT output image of an aligned structure, there is a preferential accumulation of pixels.

45. Below is Figure 1 from my 2006 methods paper, which demonstrates (from left to right) the original image, the 2D FFT output image, and 2D FFT alignment plot of randomly arrayed (top) and aligned (bottom) fibers.



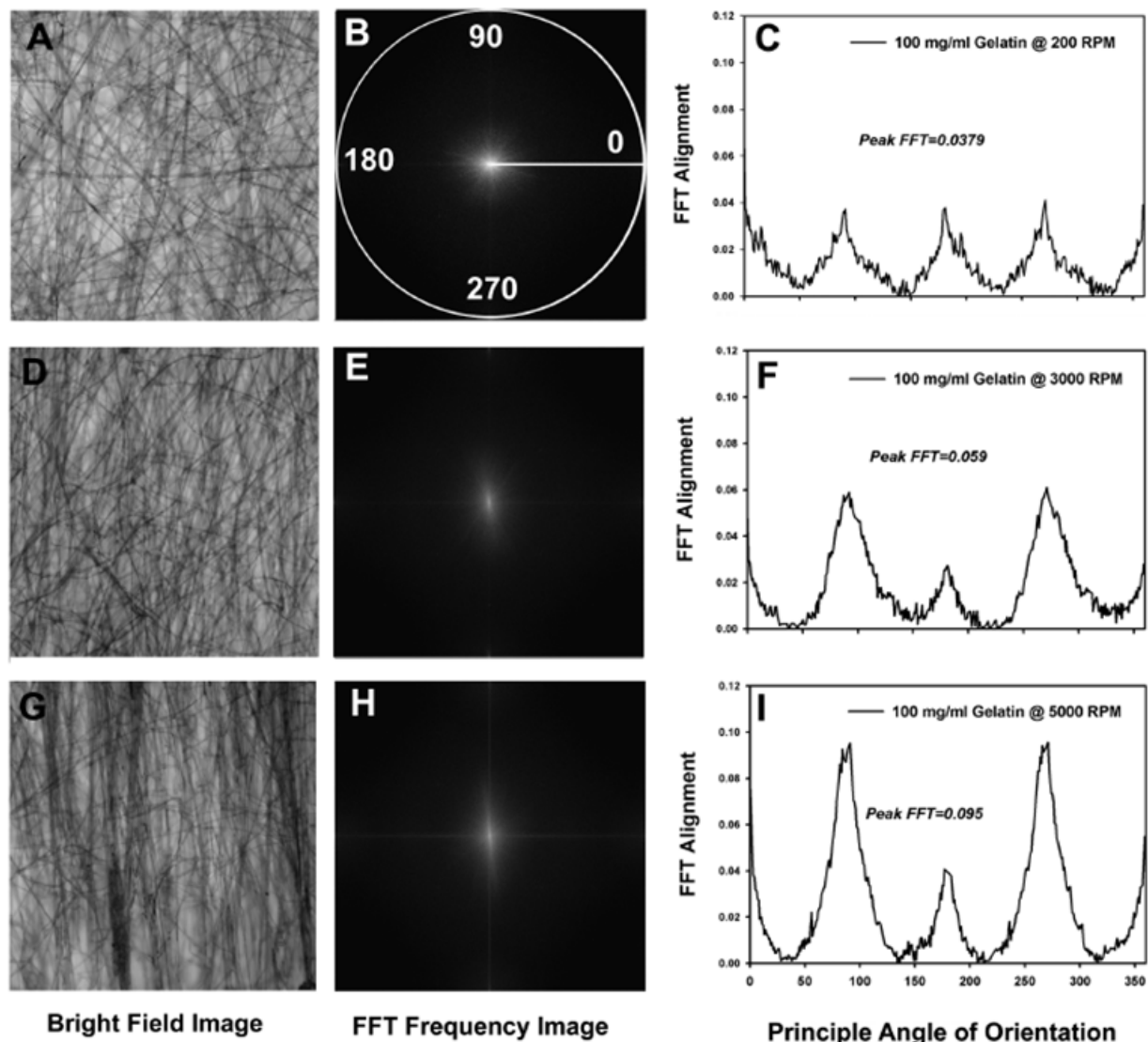
(EX1013, 12.)

46. I note that the location of the first peak depends on where the scientist taking the FFT chooses to orient the fibers. Typically, the scientist chooses to

orient the fibers such that the first peak appears at 90° or 180° , but any orientation is valid.

47. When creating the 2D FFT alignment plot, it is sufficient to sum pixel intensities from 0° to 180° , which is the typical reporting practice. (EX1013, 4; EX1010, 609.) That is because the 2D FFT output image is symmetric about the horizontal axis, so a pixel summation to 360° is unnecessary. (EX1013, 4.) As a way of further explanation, the fact that the 2D FFT output image is symmetric about the horizontal axis (i.e., that the pixel intensity at 1° is the same as 181° , 2° is the same as 182° , etc.), ensures that the peaks in the 2D FFT alignment plot repeat between 0° – 180° and 180° – 360° . (EX1010, 609.) Figure 1 from my 2008 methods paper (below) demonstrates the repeated regions in the 2D FFT alignment plot. Thus, each peak between 0° to 180° has a corresponding peak from 180° to

360° that is 180° away from the original peak.



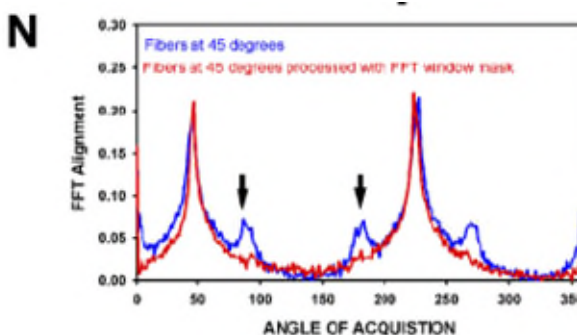
(EX1010, Figure 1.)

48. The narrower the peak in the 2D FFT alignment plot, the higher the degree of alignment in the original image.

49. To reduce edge noise in a 2D FFT alignment plot, a circular mask can be overlaid onto the data image before applying the 2D FFT method. (EX1010,

614–15). Then the analysis focuses specifically on the circular cutout. (EX1010, pp. 614–15.) A technique, known as a “window mask,” is used to create the circular cutout. (*Id.*). Below is Figure 3N from my 2008 paper, which demonstrates the noise reduction achieved using this technique, which I refer to as “masking.”

50. One weakness in the FFT analysis concerns the notion that the 2D FFT method computes the edges of the original image as an edge. That leads to artifacts in the 2D FFT alignment plot. The use of a circular mask reduces these artifacts in the 2D FFT alignment plot.



51. .
(EX1010, 614.)

52. In addition to the FFT method, other techniques for measuring fiber alignment include laser scattering and the analysis of representative images. (EX1013, 2; EX1010, 604.) These methods provide alternative approaches but are not as intuitive as some methods and certainly require specialized equipment and knowledge in refraction.

E. Collagen Enhances Biological Properties of Nanofiber Scaffolds

53. Prior to March 2013, I published on, and patented, the use of collagen in electrospun scaffolds. (§I.B., *supra*.) Indeed, my publication about the use of electrospun collagen was the first paper on the subject and is highly cited by other studies, it by far represents my most cited publication (>3000). (§I.B., *supra*.)

54. Collagen is the most common component of the ECM, making it an ideal material for biomedical applications due to its natural compatibility with human tissues. (EX1005, 6-7; EX1009, [0004], [0007], [0013].) Furthermore, collagen facilitates cell attachment, enhancing its effectiveness in tissue engineering. (EX1018, [0056], [0067].) When incorporated into nanofiber scaffolds, collagen significantly boosts cell proliferation, particularly in skin grafts. (EX1005, 7.) Collagen is biodegradable, so it can be replaced by natural ECM over time. (EX1005, 10; EX1005, 1791.)

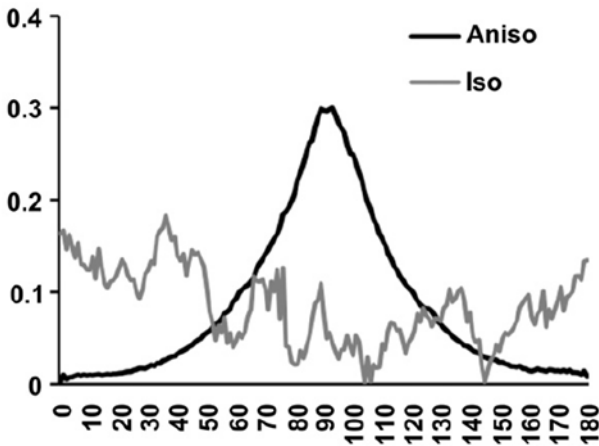
55. However, collagen alone degrades quickly and has poor mechanical properties, limiting its stability in electrospun scaffolds. (EX1005, 1791.) These limitations could be overcome using well-known methods, including combining the collagen with other materials. (EX1005, 1791.) For example, polycaprolactone-collagen composite scaffolds were known to have enhanced biomechanical properties, such as strength, elasticity, and compliance. (EX1011, [0052].) Moreover, crosslinking, a chemical technique that creates additional

bonds between atoms, was another common method to improve collagen's stability and strength in fibrous scaffolds. (EX1009, [0011], [0136], [0137]; EX1005, 6; EX1005, 1791.)

F. Previously Developed Aligned Collagen Scaffolds

56. Incorporating aligned collagen fibers into fibrous scaffolds provides significant advantages for tissue healing and regeneration. Consequently, by March 2013, many research groups had developed scaffolds with aligned collagen fibers. This was well-documented in the literature. (EX1005, 1793; EX1016; Lee, Fig. 9(b), [0019]; EX1009, [0300], [0370]; Hackett, 3716-17; EX1015, 3778-3781.)

57. Several of these scaffolds have been analyzed using the FFT technique that I developed to characterize their alignment. FFT analysis revealed a single major peak in their 2D FFT alignment plots from 0° to 180°, indicating consistent and highly uniform fiber alignment. (EX1005, 1793; Fu, 2012.) Below is a figure showing one such 2D FFT alignment plot of a fibrous scaffold comprising collagen.



(EX1005, 1792-93.)

58. Since the 2D FFT output image is symmetric about the horizontal axis and the peaks in the 2D FFT alignment plot repeat between 0°–180° and 180°–360°, these scaffolds exhibited adjacent major peaks 180° apart.

IV. THE SUBJECT MATTER OF THE '227 PATENT

59. According to the '227 patent specification, the invention “relates to a scaffold comprising one or more electrospun fibers.” (EX1001, Abstract.) The specification concerns electrospinning and electrospray techniques. It discusses that collagen can be electrospun to make a fibrous scaffold. (*Id.*, 15:8-15.)

60. The examples in the specification, however, provide scaffolds made of either heart basement membrane (“HBM”) combined with polycaprolactone (“PCL”) or PLC alone. (*Id.*, 19:60-23:27 (Examples 1-4).) Many of the figures concern examples made of the same materials:

- “FIG. 6 depicts aligned heart base membrane fiber scaffold that allow dorsal root ganglia attachment and outgrowth.” (*Id.*, 2:4-6.)
- FIG. 7 depicts “aligned placenta and heart base membrane fiber scaffolds that allow dorsal root ganglia attachment and outgrowth.” (*Id.*, 2:7-9.)
- FIGS 9A, 9B, and 9C depict exemplary scaffold prepared into an elongated roll by folding an elongated sheet (9A), an elongated roll by rolling an elongated sheet (9B), and a composite of multiple layers of elongated sheets (9C). (*Id.*, 2:13-19.)

61. The specification states that, in some embodiments, the fibers of the scaffold are aligned such that “a fast Fourier transform (FFT) analysis result of the fibers [in the scaffold] have adjacent major peaks about 180° apart from each other.” (*Id.*, 13:25-28.) The specification provides that “[t]he major peaks . . . refer to peaks higher than the average peaks in the result.” (*Id.*, 13:28-29.)

62. The specification provides that “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., and Simpson D.G, Biomater Sci Polym Ed. 2008; 19(5): 603-21, which is incorporated by reference in

its entirety herein.” (*Id.*, 13:40-46.) As discussed above, I am one of the authors of the Ayres paper.

63. As described in *Ayres*, and discussed above (§III.D, *supra*) in an FFT analysis, peaks can be measured from 0° to 360°. It is sufficient, however, to only report the FFT analysis over a 180° interval, which, for convenience is often from 0° to 180°. (§III.D, *supra*.) This is because the plot produced by the FFT analysis repeats peaks every 180°. (§III.D., *supra*.) As *Ayres* explains, which the ’227 patent incorporates in its entirety, “[t]ypically, the [FFT] 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.” (*Ayres*, 2008, 609; *see also* ’227 patent, 13:40-46.)

64. FIG. 8 of the ’227 patent, reproduced below, provides the results of an FFT analysis of fibers prepared by the Applicants: FIG. 8 depicts exemplary FFT analysis results of (a) the fibers described herein and (b) a control sample. (EX1001, 2:10-12.) The specification further provides that “[i]n 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.8A.” (*Id.*, 13:46-49.) Thus, in my opinion, aligned fibers in the prior art with major peaks corresponding to those shown in FIG.8 would anticipate the claims of the ’227 patent, as I discuss in more detail below (§V.A., *infra*).

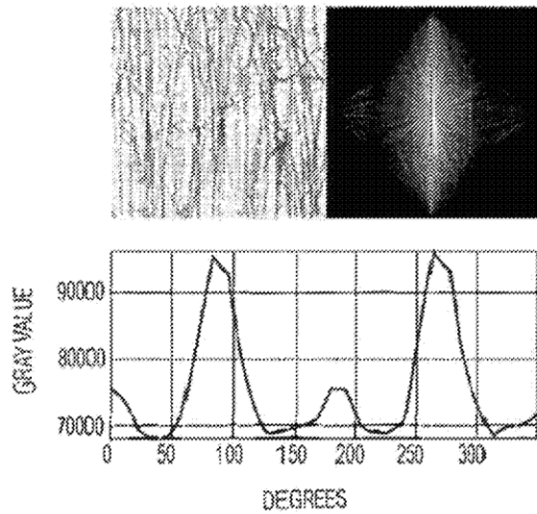


FIG. 8A

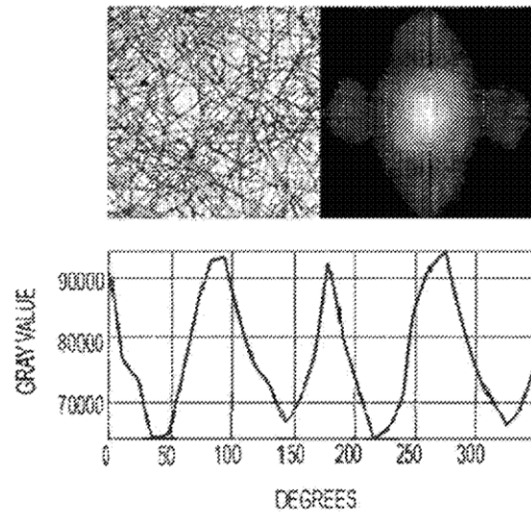


FIG. 8B

65. In FIG. 8A, the FFT analysis concerns *aligned* fibers that only have major peaks at about 90° and 270°. These major peaks are about 180° apart from one another (270° minus 90°=180°). As I discussed above, and according to Ayres, which the specification incorporates for the performance of the FFT method, the plot in FIGS. 8A would only need to be reported through 180°. (EX1010, 609; EX1001, 13:38-49; §III.D., *supra*.) This is because the plot produced by the FFT analysis repeats peaks every 180°. (§III.D, *supra*.) Therefore, even if FIG. 8A only reported data from 0° to 180°, a person of ordinary skill would have understood that the sample in FIG. 8A would necessarily have a second major peak at about 270°, and no other major peaks, because of the symmetry discussed in Ayres above and because there are no other major peaks in the 0°-180° range.

66. The y-axis of the '227 patent's 2D FFT alignment plots range from 70,000 to 90,000 because of the brightness of the data images. Because the y-axis of the 2D FFT alignment plot depends on the brightness of the image, the 2D FFT alignment plots are often normalized, which makes it easier to compare different samples.

67. In contrast, the control sample, in FIG. 8B, has repeating peaks at about 0°, 90°, 180°, and 270° that are all the same height. These peaks arise from the constantly changing fiber directions, artifacts associated with edges of the data image, and the large white spaces observed in the background of the data images. Seeing that the peaks are uniform in height and shape instantly communicates there is no preferential orientation to the data in the image. Thus, these fibers are not aligned, as shown in FIG. 8B. Like above, and according to *Ayres*, the graph in 8B would only need to be reported to 180°.

68. The '227 patent provides that “[i]n some embodiments, the aligned fiber and/or electrospun fiber in the scaffold is crosslinked.” (EX1001, 15:16-17.) The '227 patent explains that the crosslinking may be obtained “by any conventional chemical crosslinking method.” (EX1001, 12:52-54.)

69. The '227 patent provides that the invention “relates to methods of promoting nerve repair using the aligned fiber, electrospun fiber, and/or scaffold described herein.” (*Id.*, 16:53-55.) Regarding implanting the scaffold, the

specification provides that “[t]he ‘tissue’ defect may be treated by applying an elongated sheet described herein to surround the defect, or by applying an elongated roll implant described herein between the tissue defect or gap.”

(EX1001, 19:9-12.) Further, the ’227 patent specification provides that “[o]ptionally, the elongated sheet may be applied around the tissue defect, and/or the elongated roll implant may be applied to facilitate surgery and healing.” (EX1001, 19:15-17.) The ’227 patent only discusses implanting the scaffold to treat an injury at lines 1-29 at column 19.

70. The ’227 patent specification also provides methods for culturing cells “on the scaffold or aligned fiber.” (*Id.*, 16:26-27.) The “method may further comprise culturing dorsal root ganglia and/or stem cells on the aligned fiber, electrospun fiber, and/or scaffold prior to implanting.” (*Id.*, 16:60-62.) The specification provides that “growing and/or culturing cells on a scaffold” includes traditional cell culture methods as well as placing on a surface any setting, such as in natural or synthetic biocompatible matrices or tissues.” (*Id.*, 16:31-36.) The ’227 patent provides methods of cell differentiation, including osteoinductivity (*Id.*, 17:6-17), chondroinductivity (*Id.*, 17:17-27), and promoting ligament/tendon differentiation (*Id.*, 17:28-38), using “methods comprising culturing cells on an aligned fiber, electrospun fiber, and/or scaffold produced or described herein” (*Id.*, 17:28-31).

V. SUMMARY OF THE MOST RELEVANT PRIOR ART

A. Kuo

71. Kuo published on December 1, 2011, which is more than a year before the date of the first priority application of the '227 patent, which is March 14, 2013. (EX1018.) I have been informed that Kuo was not cited during prosecution of the '227 patent.

72. Kuo is titled “Scaffolds For Tissue Engineering And Regenerative Medicine.” (EX1018, Title.) Kuo teaches electrospun collagen/PCL scaffolds for “tissue repair” that are seeded with cells and formed into a desired shape to replace tissue including muscles, *ligaments, tendons, and nerves*, among others. (EX1018, Abstract, [0005], [0052], [0113] (emphasis added).) The scaffolds disclosed in Kuo are created by electrospun micro or nano fibers with “fiber orientation that is random, *aligned*, or any combination thereof.” (EX1018, [0005] (emphasis added).) Figure 3 “depict[s] an exemplary method for making random or aligned fibers in a scaffold mat.” (EX1018, [0030].) Figure 4 “is a set of micrographs depicting FD-SEM images of *aligned* nanofiber scaffolds.” (EX1018, [0031] (emphasis added).) Kuo teaches how “[a]ligned nanofiber scaffolds were seeded with progenitor [stem] cells and subsequently rolled with the leading edge parallel to the axis of alignment.” (EX1018, [0112].) Kuo discloses that “[t]his aligned nanofiber scaffold locally mimicked the native microstructure of tendon/ligament

(aligned collagen fibrils), globally mimicked a fascicle or bundle in tendon/ligament tissue, and resulted in cell seeding throughout the construct.” (*Id.*) Kuo also teaches that the electrospun fibers may be comprised of natural materials or synthetic materials or a combination of both and can include collagen. (EX1018, [0005], [0014].)

73. Kuo discloses that stem cells that can seed the scaffold are “selected from the group consisting of stem cells, osteoblasts, myoblasts, neuroblasts, fibroblasts, glioblasts, germ cells hepatocytes, chondrocytes, keratinocytes, smooth muscle cells, cardiac muscle cells, connective tissue cells, epithelial cells, endothelial cells, hormone-secreting cells, neurons, tenocytes, skeletal myocytes, and skeletal myoblasts.” (EX1018, [0016]; [0058]-[0066].)

B. Lee

74. Lee is U.S. Patent Application No. US 2010/0331980 A1, which published on December 30, 2010, which is more than a year before the date of the first priority application of the '227 patent, which is March 14, 2013. (EX1011.) I have been informed that Lee was not cited as prior art during the prosecution of the '227 patent.

75. Lee discloses that the reconstruction of skeletal muscle tissue due to a traumatic injury is difficult because of the lack of “functional muscle tissue substitutes.” (EX1011, [0003].) To solve this problem, Lee discloses engineered

muscle tissue, including “a biodegradable scaffold comprising a plurality of fibers oriented along a longitudinal axis of the fibers” and “methods of forming a biodegradable scaffold, including the steps of electrospinning a solution comprising a biodegradable scaffold material onto a grounded mandrel to form a plurality of fibers oriented along a longitudinal axis; and crosslinking said fibers to form the scaffold.” (EX1011, [0005]-[0006].)

76. Lee further teaches using fibers for the scaffold that are natural—such as collagen—or synthetic—such as PCL—or blends of natural and synthetic fibers between 2:1 and 1:2 by weight. (EX1011, [0005].) Lee discloses Type 1 collagen and electrospun nanofiber meshes “using a blend of PCL and collagen with a ratio of 1:1 in weight.” (EX1011, [0062-63].) Lee uses the HFIP solvent. (EX1011, [0062].) Lee teaches that “PCL-collagen composite scaffolds according to some embodiments have preferable biomechanical properties, e.g., yield tensile strength, elasticity and compliance.” (*Id.*, [0052].) Lee also discloses that “[a]fter 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.” (EX1011, [0063].)

77. Further, to approximate skeletal muscle, Lee made “nanofiber meshes that are aligned in a unidirectional manner to facilitate myotube formation.”

(EX1011, [0090].) Lee explains that it was known that “aligned nanofibrous structures can be obtained using a variety of collecting mandrel systems, including rotating drum.” (*Id.*, [0091].) Lee obtained different levels of fiber alignment by using different rotation speeds for the drum. (EX1011, [0011].)

78. Lee undertook materials testing to demonstrate alignment of the fibers. (EX1011, [0025], [0066].) The results demonstrate that the fibers are aligned. (EX1011, Fig. 2; Claim 8.) Indeed, Lee teaches that “[f]iber alignment had a profound effect on the mechanical properties of electrospun PCL/collagen nanofiber meshes,” including increased “tensile strength for the aligned nanofiber meshes.” (EX1011, [0081].) The mechanical properties are improved along the axis of alignment. (EX1011, [0081].)

79. Lee teaches that the aligned fibers aided in cell growth. First, Lee confirms that the fibers were aligned:

- “The myotubes that formed on the oriented nanofiber meshes showed unidirectionally organized myotubes that are consistently aligned along the longitudinal axis of nanofibers, which is in contrast to the randomly oriented nanofiber meshes (FIGS. 5c, f).” (EX1011, [0084].)
- While the “myotubes formed on the randomly oriented nanofiber meshes . . . did not show a global cellular organization,” Lee states

that the “myotubes on the aligned nanofiber meshes organized within close proximity to the direction of the nanofiber direction and formed globally aligned myotubes (FIG. 6d).” (EX1011, [0085].)

80. Then, Lee teaches that “[a]ligned nanofibers greatly influenced muscle cell organization and enhanced myotube formation. The myotubes formed on the aligned nanofiber meshes were highly organized and were significantly longer than that of the myotubes on the randomly oriented meshes. These findings indicate that the electrospun PCL/collagen nanofiber meshes are able to guide and orient skeletal muscle cells into organized structures that mimic native tissues.” (EX1011, [0094].)

C. Huang2

81. Huang2 published in print in February 2012, which is more than a year before the date of the first priority application of the '227 patent, which is March 14, 2013. (EX1005.) I have been informed by counsel that Huang2 was not cited as prior art during the prosecution of the '227 patent.

82. I understand that Huang2 was published in print in February 2012. The table of contents (EX1005, PDF p. 3) states that Huang2 was published on February Volume 33, Issue 6 of the scientific journal called Biomaterials. I am familiar with Biomaterials and understand that it is published regularly. It has one of the highest impact factors in the field, meaning that it is one of the most cited

journals in the art. Huang2 has an ISSN 0142-9612, which further confirms that it was published in February 2012. (EX1005, PDF p. 1.) Moreover, the National Library of Medicine received and filed a copy of Huang2 by February 21, 2012 (EX1005, PDF p. 1.) I understand that the National Library of Medicine is a large library in Bethesda Maryland and is an institute within the National Institutes of Health.

83. Biomaterials is a well-known peer-reviewed journal that a POSA would have looked at, and the National Library of Medicine is a well-known library with items indexed and catalogued by subject matter. A POSA would have had access to Huang2 with reasonable diligence.

84. Additionally, I understand that Huang2 was published online on Science Direct as of December 2, 2011, as confirmed by metadata. (EX1020.) A POSA would have been aware of Science Direct, which was, and is, indexed and catalogued by subject matter. A POSA would have had access to Huang2 online with reasonable diligence.

85. Huang2 is a different prior art reference from a different research group than Huang1.

86. Huang2 discloses the use of skin grafts in treating tissue injuries. (EX1005, 1791.) Huang2 discloses fibers comprising collagen and teaches the effect of *aligned* fibers compared to *non-aligned* fibers on the attachment and

growths of normal human dermal fibroblasts (“NHDFs”), including fibroblast-to-myofibroblast differentiation. (EX1005, 1796-97.) Huang2 is directed at “understand[ing] how various nanofibrous matrices regulate skin cell behaviors and intracellular signaling pathways.” (EX1005, Abstract.) Huang2 discloses “the phenotypic expressions of normal human dermal fibroblasts (NHDFs) on collagen-containing matrices with either isotropic (i.e., fibers collected randomly with *no alignment*) or anisotropic (i.e., fiber collected with *alignment*).” (EX1005, Abstract (emphasis added).) Huang2 teaches that, among other things, the fibroblast-to-myofibroblast differentiation was greatly enhanced for the NHDFs cultured on the anisotropic or aligned nanofibrous matrices comprising collagen, likely due to the impact of the alignment on the integrin $\beta 1$ signaling pathway. (EX1005, 1797, 1799, Abstract.)

87. Huang2 teaches the importance of collagen in electrospinning fibers in tissue engineering. Huang2 discloses that “[c]ollagen 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.” (EX1005, 1791.) Huang2 teaches that its fibers comprise both collagen and PCL because collagen alone can be unstable. (*Id.*) Huang2 states that alternatively “crosslinking” can be used to “obtain a stable scaffold.” (EX1005, 1791.) Huang2 discloses that “nanofibrous matrices of PCL and

Collagen at a weight ratio of 3:1 were fabricated using electrospinning previously described.” (EX1005, 1792.) Additionally, Huang2 discloses that “fibroblasts showed preferred adhesion on such collagen-containing nanofibers, as compared to pure collagen fibers.” (EX1005, 1791-1792.)

88. Figure 1 from Huang2 (below) discloses scanning electron microscope images of Huang2’s fibers comprising collagen.

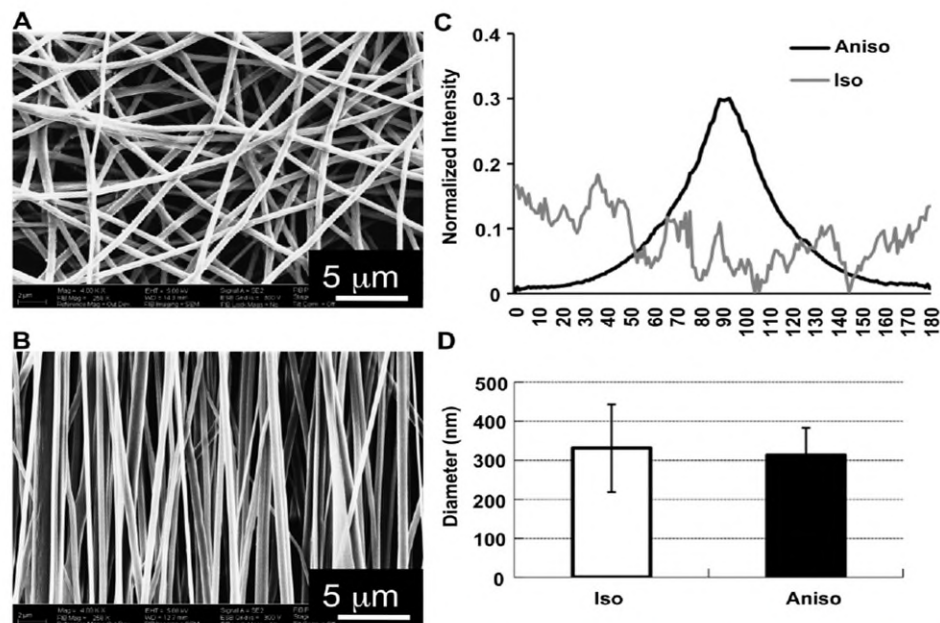


Fig. 1. Characterization of isotropic and anisotropic electrospun PCL/Coll nanofibrous matrices. SEM image of nanofibrous matrices with two types of fiber spatial organizations: isotropic (A) and anisotropic (B). (C) Normalized intensity plots against the angle of acquisition for isotropic and anisotropic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

(EX1005, Fig. 1.)

89. This figure discloses the isotropic (unaligned) sample (Figure 1A) and the anisotropic (aligned) fiber sample (EX1005, Figure 1B). (EX1005, 1793.) Huang2 discloses that the anisotropic PCL/collagen nanofibers performed better in

promoting certain properties useful in cell growth and wound healing. (EX1005, 1797.)

90. To determine the alignment of the fibers used in its scaffolds, Huang² performed FFT analyses. Figure 1C is the 2D FFT alignment plot of both the aligned and unaligned scaffolds. (EX1005, 1793.) The aligned sample, referred to as “Aniso,” has one major peak at about 90° and no other major peaks present. (EX1005, 1793.) The unaligned sample, which is referred to as “Iso,” has no major peaks that are about 180° degrees apart. Based on these 2D alignment plots, and as discussed in detail above, because the plot produced by the FFT analysis repeats peaks every 180°, a person of ordinary skill would have known that if the plot was extended to 360°, a second peak for the Aniso (aligned) sample would necessarily be at the position of about 270°. (§III.D., *supra.*) Further, for the same reason, no other major peaks would be present between 180° and 360° degrees for the Aniso (aligned) sample. (See §III.D., *supra.*) Therefore, Figure 1C in Huang² for the Aniso (aligned) sample demonstrates aligned fibers containing collagen that have major peaks 180° apart from each other. In contrast, for the Iso sample, the FFT readout does not show a single major peak between 0° and 180°. Therefore, the Iso (unaligned) sample does not have major peaks 180° degrees apart, which would be expected for an unaligned sample.

91. In my opinion and experience, the FFT method that Huang2 used—involving ImageJ with the Oval Profile Plot plugin—produced data from 0° to 360°. Thus, it is likely that Huang2 collected data from 0° to 360°, but because the data repeated, the authors chose to show only 0° to 180° in Figure 1C, based on the custom in the field, and as Ayres teaches (EX1010, 609.)

92. Additionally, when explaining the importance of tissue engineering skin grafts, Huang2 teaches that “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 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.” (EX1005, 1791.)

93. Huang2 also undertook materials testing, which confirmed that the fibers were aligned. (EX1005, 1794 (“Compared to the [random] isotropic matrices, the freestanding [aligned] anisotropic matrices showed higher ultimate stress . . . and Young’s modulus . . . in the alignment.”).)

VI. MY ANALYSIS OF THE CLAIMS

A. Ground 1: Claims 1-6 are Obvious in light of Huang2 in view of Kuo.

94. In my opinion, Claims 1-6 of the '227 patent would have been obvious in light of Huang2 in view of Kuo.

95. Claim 1 of the '227 patent requires a scaffold with aligned fibers containing collagen that have peaks 180° apart, as measured by FFT. Huang2 discloses such a scaffold. Additionally, the other requirements of claim 1—using that scaffold to treat a tissue injury—is the primary purpose of tissue engineering. Thus, multiple references, including Kuo, disclose using nanofiber scaffolds to treat injuries. Similarly, many references disclose crosslinking collagen fibers to improve their stability and structural integrity, including both Huang2 and Kuo.

96. Moreover, a POSA would have been motivated to combine Huang2 and Kuo because both are in the same field of art, and both seek to solve the same problems:

- Huang2 provides that researchers sought to create a stable scaffold “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).” (EX1005, 1791.)

- Huang² teaches that a goal of electrospinning scaffolds for biomedical applications is to “maximally recapture[] the native extracellular matrix” “[t]o better support and facilitate skin cells to form skin-like tissues” when treating a wound to the skin. (EX1005, 1791.)
- Huang² was published in Biomaterials, one of the most influential journals in the art.
- Kuo, which is in the same field, teaches 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].)
- Kuo discloses the benefits of “mimic[ing] the native microstructure” of damaged tissue with engineered electrospun fiber scaffolds to support cell growth and stem cell differentiation, such that various injuries can be repaired and tissues regenerated, including injuries to muscle, tendon, ligament or other connective tissue.” (EX1018, [0003]-[0004], [0006], 0112].)
- Indeed, Kuo teaches that “[i]n one embodiment, the compositions are used for muscle, tendon, ligament or nerve repair, replacement or

regeneration strategies.” (EX1018, [0067].) 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].)

97. Thus, a POSA would have been motivated and expected success in creating a scaffold comprising Huang2’s aligned fibers—as shown by FFT analysis—containing collagen, which may be crosslinked, such that they can be used to treat a tendon, ligament, or nerve defect as taught by Kuo.

98. Additionally, Huang2 specifically discloses how aligned fibers are beneficial for cell growth, which provides further motivation to combine the two references. Likewise, Kuo teaches that aligned fibers are desirable to mimic the native structure of a tendon/ligament. (EX1018, [0112].)

99. The POSA’s knowledge of the composition of tendon, ligament, and nerve tissues would have further motivated the POSA to use Huang2’s scaffold to repair a tendon, ligament, or nerve defect. It was well-known that tendon, ligament, and nerve tissues are naturally aligned. (EX1019, 3.) And it was believed in the art that aligned scaffolds that mimic the native tissues could be used to regenerate tendon, ligament, and nerve tissues. (EX1018, [0006], [0034], [0112], Beachley, 27.) Thus, aligned scaffolds were being investigated to heal those tissues. (EX1019, 27.)

100. Indeed, a POSA would have been well aware that aligned nanofiber scaffolds are well-suited for the regeneration of ligaments and tendons because they mimic the tightly packed aligned type 1 collagen that are a major component of these tissues. (EX1019, 32.) Further, a POSA would have known that tendon and ligament scaffolds have to withstand large forces, and must be mechanically strong along the axis of alignment. (EX1019, 3, 32.) Thus, the promising mechanical testing data in Huang2 that demonstrated improved strength along the axis of alignment would have further motivated a POSA to use Huang2's scaffold to treat tendon and ligament tissue defects.

101. Moreover, a POSA would have been motivated to use aligned fiber to treat nerve tissue defects because aligned fibers can promote alignment and elongation in regenerating axons. (EX1019, 3, 28.) Thus, the promising biological testing data in Huang2 that demonstrated that fibroblasts grown on the aligned scaffold oriented and migrated along the aligned nanofibers (EX1005, 1794) would have further motivated a POSA to use Huang2's scaffold to treat nerve tissue defects, as described by Kuo (EX1018, [0067]).

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 described in both Huang2 and Kuo to achieve predictable results with a reasonable expectation of success. Indeed, that is

particularly true because both Huang2 and Kuo successfully created scaffolds containing fibers containing blends of collagen and PCL, and both Huang2 and Kuo reported obtaining aligned fibers. (§§V.A, V.C, which are both incorporated by reference herein.) The composition and properties of Huang2's scaffold discussed above also would have contributed to the expectation of success.

103. I incorporate this analysis into my analysis for all of Claims 1-6 below.

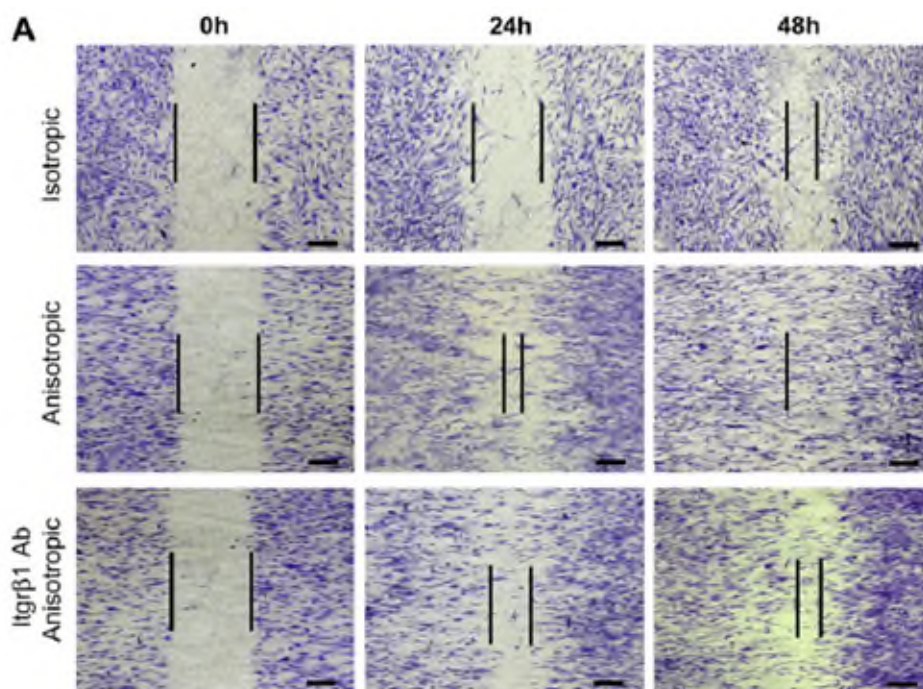
1. Claim 1

104. Claim 1 of the '227 patent recites "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." In my opinion, this claim would have been obvious in light of Huang2 in view of Kuo.

(a) "A method of treating a tissue defect in a subject comprising implanting at the tissue defect a scaffold"

105. Huang2 discloses "[a] method of treating a tissue defect in a subject comprising implanting at the tissue defect a scaffold."

- Huang2's scaffolds are designed to treat a tissue defect in a subject, i.e., a wound in the skin. (EX1005, 1791.)
- Huang2 discusses implanting the scaffold in a subject and discloses that “during normal wound healing the fibroblast-to-myofibroblast differentiation can facilitate wound closure by drawing the wound margins together, however, it would also result in scar formation if the proper apoptosis of myofibroblasts does not occur upon the cease of wound contraction. Therefore, the fibroblast-to-myofibroblast differentiation needs to be appropriately balanced for minimum scar formation during the wound healing.” (EX1005, 1792.)
- Huang2 further teaches a “wound healing assay” wherein nanofibrous matrices are seeded with fibroblasts for wound treatment, (*Id.*, 1793, 1796), as shown in Figure 6A:



(EX1005, 1797, Figure 6A.)

- This figure shows that the cells grown on the aligned fibers became aligned, too. (EX1005, 1794.) These cells also proliferated and migrated at enhanced rates relative to the random fibers. (EX1005, 1797.) Moreover, the cells expressed an elongated cell shape that was in parallel with the scaffold and migrated along the direction of the fibers. (EX1005, 1792.).
- One of the goals of Huang2 is to understand “how various nanofibrous matrices regulate skin cell behaviors and intracellular signaling pathways.” (EX1005, abstract.) This is to fine tune “the functionality

of tissue-engineered skin grafts and *affecting the wound healing process.*” (*Id.*)

106. For the above reasons, Huang2 discloses this element.

107. Further, as set forth in §VI.A.1.d., *infra*, which is incorporated herein, Kuo also discloses implanting an electrospun scaffold at a tissue defect to treat an injury. (EX1018, Title, Abstract, [0005-0006], [0022-0023], [0034], [0052], [0067], [0112-0113].)

108. For the reasons set forth above, it is my opinion that a skilled artisan would have been motivated to, and had a reasonable expectation of success of, utilizing Huang2’s aligned fibers to treat the tissue defects described in Kuo.

(b) “a scaffold comprising crosslinked fibers comprising collagen”

109. Huang2 discloses “a scaffold comprising crosslinked fibers comprising collagen.” Indeed, Huang2 discloses the creation of electrospun nanofibers containing collagen. (EX1005, 1792.) Huang2 discloses electrospinning “[n]anofibrous matrices of PCL and *Collagen*” from an “8% (w/v) solution of 3:1 PCL/Coll” with an electrospinning device. (EX1005, 1792.) Huang2 further discloses that “[e]lectrospinning a blend solution of collagen and polycaprolactone (PCL) . . . can yield rather stable nanofibers.” (EX1005, 1791.)

Additionally, Huang² states that alternatively “crosslinking” can be used to “obtain a stable scaffold.” (EX1005, 1791.)

110. Kuo also discloses “a scaffold comprising crosslinked fibers comprising collagen.” (EX1018, [0013-0014], [0040] (incorporating by reference Meinel (EX1023)).) The scaffolds described in Kuo are created by electrospun micro or nano fibers with “fiber orientation that is random, **aligned**, or any combination thereof.” (EX1018, [0005] (emphasis added).) To fabricate the scaffold, Kuo electrospun a blend of “PCL:collagen type I” and then seeded the scaffold with mesenchymal [stem] progenitor cells. (EX1018, [0112]-[0115].)

111. Kuo also discloses the crosslinking element of the claim. Kuo specifically incorporates by reference a paper by Meinel, et al., 2004, *Biotechnol. Bioeng.* 88:379-391, for the teaching of how to prepare a scaffold. (*Id.*, [0040].)

112. Meinel, as incorporated into Kuo, teaches 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 discloses that crosslinking collagen scaffolds can address this issue by “decreas[ing] the rate of degradation of collagen by two orders of magnitude.” (EX1023, 380-81, 390.) And Meinel teaches that collagen scaffolds can be crosslinked with specific reagents. (EX1023, 380-81.) Meinel specifically teaches at pages 380-81 that “[t]o reduce the rate of degradation, in some

experiments the collagen scaffolds were crosslinked using the ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride/N-hydroxy succinimide (EDC/NHS) chemistry previously established for collagen films and fibers. . . .” (EX1023, 380-81.)

113. The teaching in Kuo is similar to the teaching of crosslinking described in the specification of the ’227 patent. In the ’227 patent, the specification only mentions crosslinking in lines 49-63 of column 12, stating that “some embodiments of the present invention *may* further comprise crosslinking the electrospun fibers.” (EX1001, 12:49-63; *see also* 14:17 (emphasis added).)

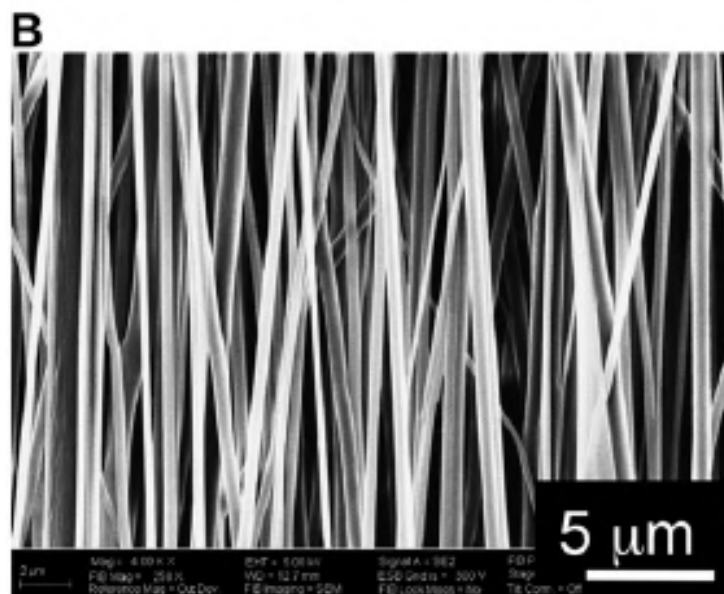
114. For the reasons set forth above, it is my opinion that a skilled artisan would have been motivated to, and had a reasonable expectation of success of, utilizing Huang2’s aligned fibers to create a scaffold that can treat a tissue defect, and would have been motivated to improve the stability of collagen fibers by chemically crosslinking, as taught by both Huang2 and Kuo.

(c) **“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”**

115. Huang2 teaches this claim element.

116. Huang2 performed an FFT analysis on its aligned fibers and discloses that the fibers have major adjacent peaks that are about 180° apart from each other.

117. Huang2 discloses a scaffold comprising PCL/collagen nanofibers that are visually highly aligned with major peaks that are about 180° apart by FFT analysis. Huang2 Figure 1B (below) has “anisotropic” fibers, which as set forth above (§V.C. , *supra*), means that the scaffold has “collected . . . fibers with *alignment*.” (EX1005, 1792 & 1793 Fig. 1B.)



118. Huang2 itself states that it discloses fibers “aligned in the same direction.” (EX1005, 1794.) Additionally, Huang2 confirmed the alignment of its anisotropic PCL/Coll fibers through the use of FFT analysis. Indeed, Huang2 teaches that “[t]o better analyze the fiber alignment, an FFT analysis was performed on [Huang2’s] SEM [(scanning electron microscope) images . . . to characterize the anisotropy [alignment] and assign a numerical value to fiber orientation.” (*Id.*; *see also id.*, 1792 (describing FFT analysis using ImageJ).)

119. Huang2 uses the same ImageJ software to perform the FFT analysis as used in the '227 patent. (EX1005, 1792, 1794, 1800 (citing Ayres 2006 for the FFT analysis procedure); EX1001, 13:38-46 (citing Ayres for the same), 21:40-45.) Huang2 provides its FFT analysis of the aligned (anisotropic) fibers imaged in Figure 1B in Figure 1C, plotting the peaks from 0°-180°:

(Figures 1B, 1C (annotated).)

121. Figure 1C reports its results out to 180°. As such, and as explained above, Huang2's FFT analysis would necessarily show a second major peak at around 270° (which is 180° from the 90° peak) if the scale were extended to 0°-360°. (§V.D., *supra*; EX1010, 609.) That is because of the intrinsic symmetry of an FFT. (EX1010, 609.)

122. Specifically, to generate the 2D FFT alignment plot, like in Figure 1(c), first the microscopy image (like in Figure 1B) is captured. (EX1010, 608.) Second, the FFT is applied, converting the fiber image from spatial representation ("real" space) to a frequency-based representation ("frequency" space). (EX1010, 608.) This is called the FFT output image. Importantly, based on the mathematical properties of the FFT function, the FFT output image is symmetrical about the horizontal axis. (EX1010, 609.) Third, to generate the 2D FFT plot from the FFT output, a circular projection is applied to the FFT output image, and pixel intensities are summed in a specified degree increment. Finally, the summed intensities are then plotted as a function of the corresponding angle, resulting in the 2D FFT alignment plot. (EX1010, 609.)

123. Because the FFT output image is symmetric about the horizontal axis, it is unnecessary to generate a 2D FFT plot from 0° to 360°. (EX1013, 4.) As a way of further explanation, the fact that the FFT output image is symmetric about the horizontal axis (i.e. that the pixel intensity at 1° is the same as 181°, 2° is the

same as 182° , etc.), means that the 2D FFT alignment plot repeats peaks every 180° . (EX1010, 609.)

124. Given the above, a POSA would have understood that, in the FFT analysis result of Huang2's scaffold, there would necessarily be major peaks at 90° , and because of the symmetry, at 270° . Because these are the only major peaks, a POSA would have understood that the FFT analysis result of Huang2's scaffold necessarily had adjacent major peaks that are about 180° apart from each other.

125. Finally, I conducted my own analysis of Figure 1B using the FFT analysis and ImageJ software described in Huang2 and the '227 patent. I report the data from 0° to 360° .

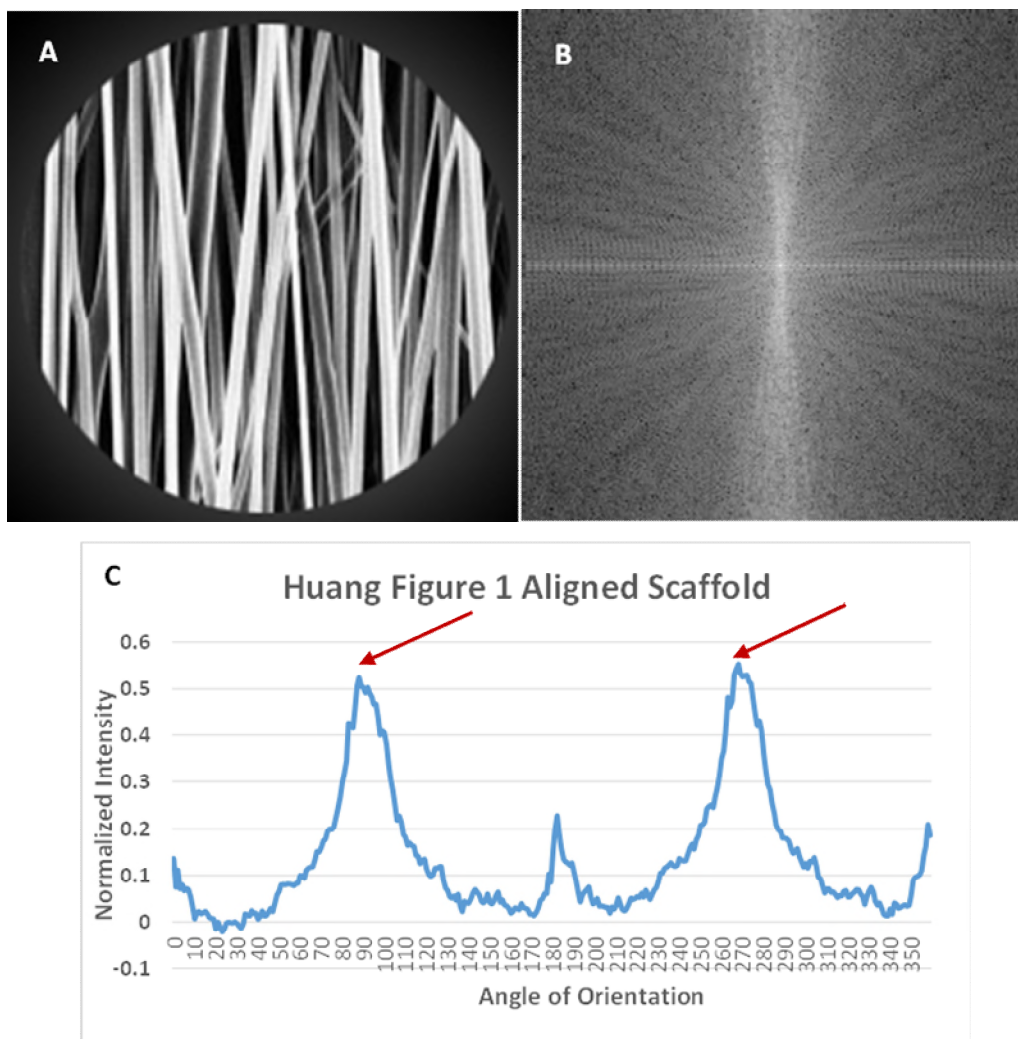


Figure 2. (A) Figure 1 from Huang aligned scaffold cropped and masked for 2DFFT. (B) 2DFFT spectrum of aligned scaffold, the image has been rotated to correct for the rotation intrinsic to the FFT analysis. (c) Normalized intensity values for aligned scaffold 0-360 degrees.

126. 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.

127. Materials testing confirmed the enhanced strength of the fibers along the axis of alignment, indicating that they are aligned. (EX1005, 1794.)

128. Therefore, a POSA would have understood that Huang2 discloses this claim limitation.

(d) “and applying the scaffold to the tissue defect to repair a tendon, a ligament or a nerve defect”

129. Huang2 discusses using the scaffolds of aligned fibers comprising collagen to repair a tissue injury, i.e., a wound. Huang2 does not specifically disclose using his scaffolds to repair a tendon, ligament, or nerve defect. This claim element, however, would have been obvious in view of Huang2 over Kuo, because Kuo teaches using an engineered scaffold comprising collagen fibers to repair multiple tissue injuries, including, tendon, ligament, and nerve.

130. Kuo discloses electrospun collagen/PCL scaffolds for “tissue repair” that are seeded with cells and formed into a desired shape to replace tissue including muscles, ligaments, tendons, and nerves, among others. (EX1018, Title, Abstract, [0005], [0052], [0113].) Kuo specifically teaches a method for “replacing or enhancing a tissue” that comprises “(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, wherein the spirally wound tissue construct replaces a tissue.” (EX1018, [0022].) Kuo further discloses that the tissue to be replaced or regenerated is “selected from the group consisting of a muscle, a nerve, a ligament, a tendon, or another tissue.” (EX1018, [0023].)

131. As I discussed above, a POSA would have been motivated to use Huang2’s aligned fibers, as shown by FFT analysis, which are stated to improve cell growth and differentiation, to fabricate a scaffold and then use that scaffold to treat the type of tissue defects discussed in Kuo, i.e. a tendon, ligament, or a nerve defect. Kuo itself discloses the desirability of using aligned fibers in its spirally wound tissue construct. (EX1018, [0112].)

132. Kuo teaches a POSA that methods of forming scaffolds “are known to the skilled artisans” and cites to various references regarding fabricating the scaffold. (EX1018, [0040].) Kuo could not cite Huang2 because the application was filed before Huang2 was published. Additionally, as I discuss above, there would be more than a reasonable expectation of success in making this combination as both Huang2 and Kuo successfully created a scaffold of aligned fibers comprising collagen.

133. Further, as discussed above, the POSA's knowledge of the composition of tendon, ligament, and nerve tissues would have further motivated the POSA to use Huang2's scaffold to repair a tendon, ligament, or nerve defect. It was well-known that tendon, ligament, and nerve tissues are naturally aligned. (EX1019, 3.) And it was believed in the art that aligned scaffolds that mimic the native tissues could be used to regenerate tendon, ligament, and nerve tissues. (EX1018, [0006], [0034,], [0112], Beachley, 27.) Thus, aligned scaffolds were being investigated to heal those tissues. (EX1019, 27.)

134. Indeed, a POSA would have been well aware that aligned nanofiber scaffolds are well-suited for the regeneration of ligaments and tendons because they mimic the tightly packed aligned type 1 collagen that are a major component of these tissues. (EX1019, 32.) Further, a POSA would have known that tendon and ligament scaffolds have to withstand large forces, and must be mechanically strong along the axis of alignment. (EX1019, 3, 32.) Thus, the promising mechanical testing data in Huang2 that demonstrated improved strength along the axis of alignment would have further motivated a POSA to use Huang2's scaffold to treat tendon and ligament tissue defects.

135. Moreover, a POSA would have been motivated to use aligned fiber to treat nerve tissue defects because aligned fibers can promote alignment and elongation in regenerating axons. (EX1019, 3, 28.) Thus, the promising biological

testing data in Huang2 that demonstrated that fibroblasts grown on the aligned scaffold oriented and migrated along the aligned nanofibers (EX1005, 1794) would have further motivated a POSA to use Huang2's scaffold to treat nerve tissue defects, as described by Kuo (EX1018, [0067]). Indeed, in Huang2, cells attached, proliferated, and migrated along the aligned fibers. (EX1005, 1792, 1797.)

136. Accordingly, it is my opinion that Huang2 in view of Kuo renders obvious claim 1.

2. Claim 2: “The method of claim 1, wherein the scaffold is in the form of one or more elongated sheets.”

137. In my opinion, Claim 2 is obvious in light of Huang2 in view of Kuo.

138. Claim 1 is obvious for the reasons discussed above in §VI.A.1., *supra*, which are incorporated herein.

139. Additionally, Kuo discloses a scaffold in the form of one or more elongated sheets, as required by claim 2. Specifically, Kuo discloses “a composition comprising: a porous scaffold *sheet* of fibrous material; and living cells deposited thereupon.” (EX1018, [0007], [0020], [0022] (emphasis added.)

140. Furthermore, based on my experience, electrospinning naturally results in sheets. (*E.g.*, EX1009, [0152].)

141. For the reasons set forth above, a POSA would have been motivated with a reasonable expectation of success to use Huang2's aligned fibers in scaffolds

in the form of one or more elongated sheets as disclosed by Kuo. (*See* §VI.A.1., *supra*.)

142. Accordingly, it is my opinion that Huang2 in view of Kuo renders obvious claim 2.

3. Claim 3: “The method of claim 1, wherein the scaffold is a composite of multiple layers of one or more sheets.”

143. In my opinion, Claim 3 is obvious in light of Huang2 in view of Kuo.

144. Claim 1 is obvious for the reasons discussed above in § VI.A.1., *supra*, which are incorporated herein.

145. Additionally, Kuo discloses a scaffold that is a composite of multiple layers of one or more sheets, as required by claim 3. Specifically, Kuo discloses “a composition comprising: a porous scaffold sheet of fibrous material; and living cells deposited thereupon.” (EX1018, [0007], [0020], [0022].) Further, Kuo teaches that there can be multiple “sheets” in the scaffold, which can be arranged in any configuration or shape:

- “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 . . . sheets” (EX1018, [0024].)

- “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.” (EX1018, [0049].)
- “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.” (EX1018, [0034].)
- “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.” (*Id.*)

146. For the reasons set forth above, a POSA would have been motivated with a reasonable expectation of success to use Huang2’s aligned fibers in scaffolds in the form of multiple layers of one or more sheets as disclosed by Kuo. (*See* §VI.A.1., *supra.*) Indeed, in addition to the above, the use of multi-layered sheets for an implant was well within the level of skill of a POSA, having been used extensively in the scientific literature. (*E.g.*, EX1022.)

147. Accordingly, it is my opinion that Huang2 in view of Kuo renders obvious claim 3.

4. Claim 4: “The method of claim 1, wherein the tissue defect is a the nerve defect.”

148. In my opinion, Claim 4 is obvious in light of Huang² in view of Kuo.

149. Claim 1 is obvious for the reasons discussed above in §VI.A.1., *supra*, which are incorporated herein.

150. Additionally, Kuo specifically discloses using the method wherein the tissue defect is a nerve defect, as required by Claim 4. Specifically, Kuo discloses using an engineering scaffold to treat a nerve defect: “Another aspect described herein is a method for replacing or enhancing a tissue [T]he tissue is selected from the group consisting of a muscle, a nerve, a ligament, a tendon, or another tissue.” (EX1018, [0022], *see also* [0034].) Kuo further disclose that “scaffolds can be formed that replace/repair muscle, ligament, tendon, connective tissue, nerves, [and] nerve channels” (*Id.*, [0052].)

151. For the reasons set forth above, a POSA would have been motivated with a reasonable expectation of success to use Huang²’s aligned fibers in scaffolds to treat a nerve defect, as disclosed by Kuo. (*See* §VI.A.1., *supra*.) For example, the structure and function of nerve tissue require that the cells grow in an aligned manner, and the native tissue are aligned. (¶¶133-35, *supra*.) Along those lines, Kuo teaches the desirability of using aligned fibers to mimic native tissues such as nerves. (EX1018, [0006], [0112]; ¶¶133-35, *supra*.) Moreover, a POSA would

have known that nerves are composed of aligned fibers that induce cell growth along the axis of alignment, like Huang2's scaffolds. (EX1019, 3, 28; EX1005, 1794; ¶¶133-35, *supra*.)

152. Accordingly, it is my opinion that Huang2 in view of Kuo renders obvious claim 4.

5. Claim 5: “The method of claim 1, wherein the tissue defect is a the ligament defect.”

153. In my opinion, Claim 5 is obvious in light of Huang2 in view of Kuo.

154. Claim 1 is obvious for the reasons discussed above in §VI.A.1., *supra*, which are incorporated herein.

155. Additionally, Kuo specifically discloses using the method wherein the tissue defect is a ligament defect, as required by Claim 5. Specifically, Kuo discloses using an engineered scaffold to treat a ligament injury. (EX1018, [0006], [0034], [0067], [0112].)

156. For the reasons set forth above, a POSA would have been motivated with a reasonable expectation of success to use Huang2's aligned fibers in scaffolds to treat a ligament defect, as disclosed by Kuo. (See §VI.A.1., *supra*.) For example, the structure and function of ligament tissue require that the cells grow in an aligned manner with strength along the axis of alignment and the native tissue are aligned, like the fibers in Huang2's scaffolds. (¶¶133-34, *supra*; EX1005,

1794.) Along those lines, Kuo teaches the desirability of using aligned fibers to mimic native tissues such as ligament. (EX1018, [0006], [0112]; ¶¶133-34, *supra*.)

157. Claim 5 is thus obvious over Huang2 in view of Kuo.

6. Claim 6: “The method of claim 1, wherein the tissue defect is a the tendon defect.”

158. In my opinion, Claim 6 is obvious in light of Huang2 in view of Kuo.

159. Claim 1 is obvious for the reasons discussed above in §VI.A.1., *supra*, which are incorporated herein.

160. Additionally, Kuo specifically discloses using the method wherein the tissue defect is a tendon defect, as required by Claim 6. (EX1018, [0006], [0034], [0067], [0112].)

161. For the reasons set forth above, a POSA would have been motivated with a reasonable expectation of success to use Huang2’s aligned fibers in scaffolds to treat a tendon defect, as disclosed by Kuo. (See §VI.A.1., *supra*.) For example, the structure and function of tendon tissue require that the cells grow in an aligned manner with strength along the axis of alignment and the native tissue are aligned, like the fibers in Huang2’s scaffolds. (¶¶133-34, *supra*; EX1005, 1794.) Along those lines, Kuo teaches the desirability of using aligned fibers to mimic native tissues such as tendon. (EX1018, [0006], [0112]; ¶¶133-34, *supra*.)

162. Claim 6 is thus obvious over Huang2 in view of Kuo

B. Ground 2: Claims 1-6 are Obvious in Light of Lee Alone or in View of Kuo

163. In my opinion, Claims 1-6 of the '227 patent would have been obvious in light of Lee alone or in view of Kuo.

164. Lee discloses all of the elements of claim 1, except Lee uses the scaffolds for the treatment of a muscle injury. It would have been obvious to use the scaffolds fabricated in Lee with aligned crosslinked collagen fibers to treat a tendon, nerve, or ligament injury to a POSA, particularly if viewed in combination with Kuo.

165. I incorporate this analysis into my analysis for all of Claims 1-6 below.

1. Claim 1

(a) “A method of treating a tissue defect in a subject comprising implanting at the tissue defect a scaffold”

166. Lee discloses “[a] method of treating a tissue defect in a subject comprising implanting at the tissue defect a scaffold.”

167. Specifically, Lee discloses using an electrospun scaffold to treat a tissue defect, i.e., an injury to muscle tissue. Lee discloses that the reconstruction of skeletal muscle tissue due to a traumatic injury is difficult because of the lack of “functional muscle tissue substitutes.” (EX1011, 0003.) To solve this problem, Lee discloses engineered muscle tissue, including “a *biodegradable scaffold*

comprising a plurality of fibers oriented along a longitudinal axis of the fibers” and “methods of forming a biodegradable scaffold, including the steps of electrospinning a solution comprising a biodegradable scaffold material onto a grounded mandrel to form a plurality of fibers oriented along a longitudinal axis; and crosslinking said fibers to form the scaffold.” (EX1011, [0005]-[0006] (emphasis added).) Lee further teaches that treating a muscle injury includes reconstructing skeletal muscle tissue (e.g., where such tissue has been damaged or lost by, e.g., injury or disease) by *implanting an anisotropic scaffold* (with or without muscle cells) into a subject in need thereof. Scaffolds may be *implanted*, e.g., *at or adjacent to the site of injury*, and/or at another site in the body of a subject that would impart a benefit to the subject, as would be appreciated by one of skill in the art.” (EX1011, 0028 (emphasis added).)

168. For the above reasons, Lee discloses this element.

169. Further, as set forth in §VI.B.d., *infra*, which is incorporated herein, Kuo also discloses implanting an electrospun scaffold at a tissue defect to treat an injury. (EX1018, Title, Abstract, [0005-0006], [0022-0023], [0034], [0052], [0067], [0112-0113].)

170. For the reasons set forth above, it is my opinion that a skilled artisan would have been motivated to, and had a reasonable expectation of success of, utilizing Lee’s aligned fibers to treat the tissue defects described in Lee and Kuo.

(b) “a scaffold comprising crosslinked fibers comprising collagen”

171. Lee discloses “a scaffold comprising crosslinked fibers comprising collagen.”

172. As discussed above, Lee discloses “a biodegradable scaffold comprising a plurality of fibers” and “crosslinking said fibers to form the scaffold.” (EX1011, [0005]-[0006] (emphasis added).) Lee also discloses creating a scaffold that contains collagen, as required by this claim element, stating that they had electrospun a “blend of PCL and collagen with a ratio of 1:1 in weight.” (EX1011, [0063].)

173. Lee also specifically teaches how the fabricated fibers were crosslinked, providing that the “electrospun PCL/collagen nanofiber meshes were crosslinked under vapor of 2.5% glutaraldehyde solution for 6 hr to increase stability and strength of collagen.” (EX1011, [0063].)

174. For the reasons set forth above, it is my opinion that a skilled artisan would have been motivated to, and had a reasonable expectation of success of, utilizing Lee’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 Lee and Kuo.

(c) “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”

175. Lee teaches this claim element.

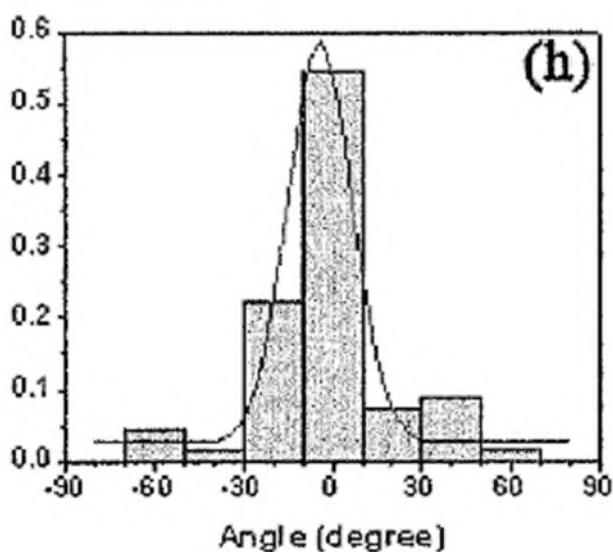
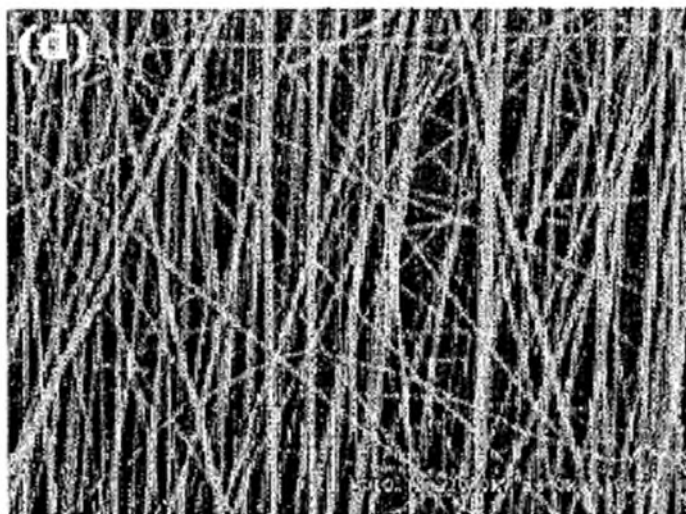
176. In my opinion, Lee discloses aligned PCL/collagen fibers that meet this element of the claim. As I discussed above, an FFT analysis measures the degree of fiber alignment, and an FFT result showing fibers having adjacent major peaks with about 180° apart from each other only occurs when the fibers 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. (EX1011, [0060]; *see* §V.B., *supra.*)

177. Lee discloses “PCL/collagen nanofiber meshes with different fiber angles . . . produced by electrospinning at various rotation rates of 0 (static), 800, 1500, and 2350 rpm. Progressive increase in fiber orientation was observed as the rotation rate increased (FIG. 1 b). (EX1011, [0080].) Lee further discloses that “fiber orientations were induced by electrospinning onto a collector at different rotation rates.” (*Id.*, [0061].)

178. 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 (Freeware provided by the University of Texas Health Sciences Center, San Antonio, Tex., USA) to determine the average fiber diameters and *fiber orientations* within the nanofiber meshes In fiber angles of the nanofibers, histograms were plotted over the entire +90° to -90 ° range” (*Id.*, [0066] (emphasis added).)

179. The most highly aligned collagen-containing fibers in Lee's study are presented in Figure 1D, with the corresponding histogram shown in Figure 1H:



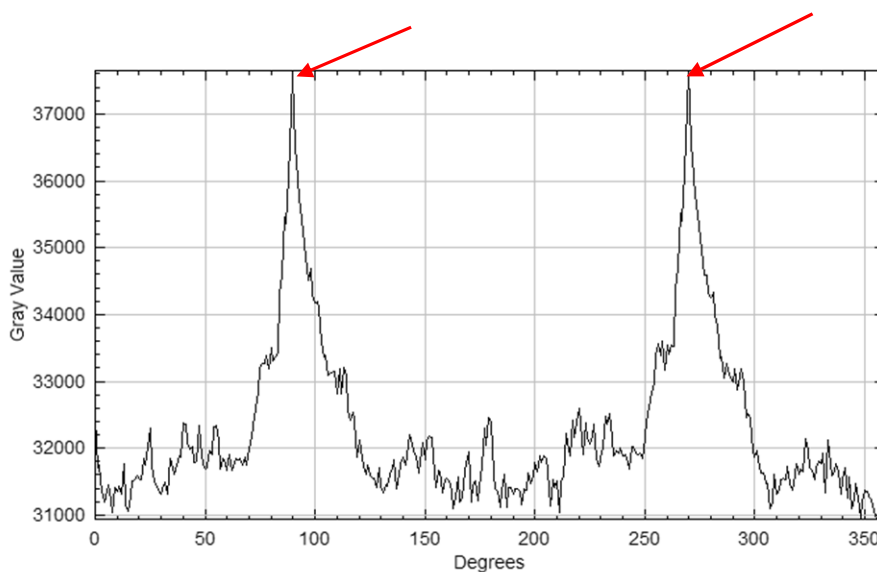
(EX1021, Figs. 1D, 1H.) I used the images from Lee's provisional patent application (Provisional Application No. 61/025,350) for my analysis because they are the same subject matter as the images in Lee, but they are higher quality images. The image analysis from Figures 1D and 1H reveals that Lee demonstrates highly aligned fibers. (*Id.*, [0012].)

180. Lee used ImageTool 3.0, freeware provided by the University of Texas Health Sciences Center in San Antonio, TX, USA, to measure the degree of fiber alignment.

181. A POSA would have understood that the degree of alignment could also have been determined using the method discussed in the '227 patent and detailed in Ayres 2008. (EX1001, 13:39-46; §III.D., *supra*.) That method uses imageJ software to run an "FFT analysis." (§III.D., *supra*.) An FFT analysis also measures the degree of orientation of fibers using a SEM image. This application of ImageJ software was well known at the time of filing of Lee. (§III.D., *supra*.)

182. Specifically, to generate a 2D FFT alignment plot, first the microscopy image (like in Lee's Figure 1D) is captured. (EX1010, 608.) Second, the FFT is applied, converting the fiber image from spatial representation ("real" space) to a frequency-based representation ("frequency" space). (EX1010, 608.) This is called the FFT output image. Third, to generate the 2D FFT plot from the FFT output, a circular projection is applied to the FFT output image, and pixel intensities are summed for each degree in a specified degree increment. Finally, the summed intensities are then plotted as a function of the corresponding angle, resulting in the 2D FFT alignment plot. (EX1010, 609.)

183. I conducted my own analysis on Lee's Figure 1D using the FFT analysis described in the '227 patent. I report the data from 0° to 360°.



184. 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].)

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

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

187. Therefore, a POSA would have understood that Lee discloses this claim limitation. Indeed, at the very least, in my opinion, this element would have

been obvious in view of Lee alone because Ayres 2008 (EX1010) had been published for years and was an established tool that was well-within the routine skill of a POSA at the time. Thus, a POSA would have been motivated to confirm the alignment of Lee's fibers with a well-known established tool. Further, a POSA would have more than a reasonable expectation of success of successfully running an FFT on Lee's SEM images, as demonstrated by my above success in running an FFT analysis.

(d) “and applying the scaffold to the tissue defect to repair a tendon, a ligament or a nerve defect”

188. While Lee discloses using his scaffolds of aligned fibers comprising collagen to repair a tissue injury, i.e., a muscle injury, Lee does not expressly disclose using his scaffolds to repair a tendon, ligament, or nerve defect. In my opinion, this claim element, however, would have been obvious in view of Lee alone. As of March 2013, it would have been well-understood by a POSA that Lee's scaffold comprising crosslinked aligned collagen fibers, which was used to treat a muscle injury, could also have been used to treat a tendon or ligament injury given the similarities between the tissue, thus rendering this claim element obvious. Indeed, Kuo even uses the same scaffold for muscles, tendon, nerve, and ligament. (EX1018, [0005].)

189. Moreover, in my opinion it also would have been obvious when Lee is viewed in combination with Kuo. That is because Kuo teaches using an engineered scaffold comprising crosslinked aligned collagen fibers to repair multiple tissue injuries, including, tendon, ligament, and nerve. (EX1018, [0005], [0022-23], [0052]; *see also* §V.A., *supra*, which is incorporated herein.)

190. A POSA would have been motivated to combine Lee and Kuo because:

- Both are in the same field of art. They both concern tissue engineering using biodegradable scaffolds. (§§V.A-B, which is incorporated herein.)
- Both seek to solve the same problem, which is using a bioengineered scaffolds containing crosslinked collagen fibers to treat a tissue injury—muscle in Lee, ligament, nerve, and tendon in Kuo.
- Both teach that aligned fibers are desirable to promote cell growth. (See Lee, [0003], [0005]-[0006], [0090], [0081], [0094]; EX1018, [0003-0006], [0052], [0058], [0067], [0112], [0113].)

191. Underscoring that a POSA would have looked to combine Lee and Kuo is the fact that Kuo cites a paper by one of the co-inventors of Lee. Specifically, Kuo cites Lanza & Atala, *Methods of Tissue Engineering* Academic Press; 1st edition October 2001, for the proposition that appropriate growth

conditions for cells “are well known in the art.” (EX1018, [0060].) Anthony Atala, one of the authors of this Tissue Engineering textbook, is the co-inventor of Lee. He is a leader in the field, and is well-known and well-respected.

192. A POSA would have been motivated to use Lee’s aligned fibers, as shown by FFT analysis, which are stated to improve cell growth and differentiation, to fabricate a scaffold and then use that scaffold to treat the type of tissue defects discussed in Kuo, i.e., a tendon, ligament, or a nerve defect. That is because Lee specifically explains how the aligned fibers benefit cell growth and improve the mechanical properties of the scaffold (EX1011, [0084-85], [0094-0095].) Lee even demonstrates that aligned fibers enhance mechanical properties. (EX1011, Figure 2, [0025], [0067], Claim8.) That also provides further motivation to combine the two references, because Kuo contains a similar teaching. (See Kuo, [112].)

193. A POSA would have had a reasonable expectation of success to use Lee’s aligned fibers, as shown by FFT analysis, which are stated to improve cell growth and differentiation, to fabricate a scaffold and then use that scaffold to treat the type of tissue defects discussed in Kuo, i.e. a tendon, ligament, or a nerve defect. That is because Lee and Kuo describe well-known established methods to achieve predictable results with a reasonable expectation of success. Indeed, both Lee and Kuo both disclose successfully creating scaffolds containing fibers

containing blends of collagen and PCL, and both Lee and Kuo reported obtaining aligned fibers. (§§V.A, V.C, which are both incorporated by reference herein.) Lee even shows that its aligned fibers have enhanced mechanical properties. (EX1011, Figure 2, [0025], [0067], Claim 8.)

194. Further, as discussed above, the POSA's knowledge of the composition of tendon, ligament, and nerve tissues would have further motivated the POSA to use Lee's aligned collagen scaffold to repair a tendon, ligament, or nerve defect. It was well-known that muscle, tendon, ligament, and nerve tissues are naturally aligned. (EX1019, 3.) And it was believed in the art that aligned scaffolds that mimic the native tissues could be used to regenerate tissues. (EX1019, 27; *see* Lee [0004], [0090], [0094].) Thus, aligned scaffolds were being investigated to heal muscle, tendon, ligament, and nerve tissues. (EX1019, 27.)

195. Indeed, a POSA would have been well aware that aligned nanofiber scaffolds are well-suited for the regeneration of ligaments and tendons because they mimic the tightly packed aligned type 1 collagen that are a major component of these tissues. (EX1019, 32.) Further, a POSA would have known that muscle, tendon, and ligament scaffolds have to withstand large forces, and must be mechanically strong along the axis of alignment. (EX1019, 3, 32.) Thus, the promising mechanical testing data in Lee that showed increased strength along the

axis of alignment would have further motivated a POSA to use Lee's aligned scaffold to treat tendon and ligament tissue defects.

196. Moreover, a POSA would have been motivated to use aligned fiber to treat nerve tissue defects because aligned fibers can promote alignment and elongation in regenerating axons. (EX1019, 3, 28.) Thus, the promising biological testing data in Lee that demonstrated that cells grown on the aligned scaffold oriented along the aligned nanofibers (EX1011, [0094]) would have further motivated a POSA to use Lee's scaffold to treat nerve tissue defects.

197. Additionally, it would have been obvious in view of Lee alone to treat a tendon or ligament, because a POSA would have understood that a scaffold used to treat a muscle could be used to treat a tendon or ligament given the related composition, structure, and function of the native tissue, such as the alignment of the fibers and the strength along the axis of alignment.

198. Accordingly, it is my opinion that Lee in view of Kuo renders obvious claim 1.

2. Claim 2: "The method of claim 1, wherein the scaffold is in the form of one or more elongated sheets."

199. In my opinion, Claim 2 is obvious in light of Lee alone or in view of Kuo.

200. Claim 1 is obvious for the reasons discussed above in §VI.B.1., *supra*, which are incorporated herein.

201. Additionally, Kuo discloses a scaffold in the form of one or more elongated sheets, as required by claim 2. Specifically, Kuo discloses “a composition comprising: a porous scaffold *sheet* of fibrous material; and living cells deposited thereupon.” (EX1018, [0007], [0020], [0022] (emphasis added.)

202. For the reasons set forth above, a POSA would have been motivated with a reasonable expectation of success to use Lee’s aligned fibers in scaffolds in the form of one or more elongated sheets as disclosed by Kuo. (See §VI.B.1.d., *supra*.)

203. Furthermore, based on my experience, electrospinning naturally results in sheets. (*E.g.*, EX1009, [0152].)

204. Accordingly, it is my opinion that Lee in view of Kuo renders obvious claim 2.

3. Claim 3: “The method of claim 1, wherein the scaffold is a composite of multiple layers of one or more sheets.”

205. In my opinion, Claim 3 is obvious in light of Lee in view of Kuo.

206. Claim 1 is obvious for the reasons discussed above in §VI.B.1.a., *supra*, which are incorporated herein.

207. Additionally, Kuo discloses a scaffold that is a composite of multiple layers of one or more sheets, as required by claim 3. Specifically, Kuo discloses “a composition comprising: a porous scaffold sheet of fibrous material; and living cells deposited thereupon.” (EX1018, [0007], [0020], [0022].) Further, Kuo teaches that there can be multiple “sheets” in the scaffold, which can be arranged in any configuration or shape:

- “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 . . . sheets” (EX1018, [0024].)
- “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.” (EX1018, [0049].)
- “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.” (EX1018, [0034].)
- “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.” (*Id.*)

208. Lee additionally discloses that “a polymeric matrix can be attached to one or more polymeric matrixes to form a multilayer polymeric matrix structure.” (EX1011, [0056].)

209. For the reasons set forth above, a POSA would have been motivated with a reasonable expectation of success to use Lee’s aligned fibers in scaffolds in the form of multiple layers of one or more sheets as disclosed by Kuo. (See §VI.B.1.d., *supra*.) Indeed, in addition to the above, the use of multi-layered sheets for an implant was well within the level of skill of a POSA, having been used extensively in the scientific literature. (*E.g.*, EX1022.)

210. Accordingly, it is my opinion that Lee in view of Kuo renders obvious claim 3.

4. Claim 4: “The method of claim 1, wherein the tissue defect is a the nerve defect.”

211. In my opinion, Claim 4 is obvious in light of Lee in view of Kuo.

212. Claim 1 is obvious for the reasons discussed above in §VI.B.1., *supra*, which are incorporated herein.

213. Additionally, Kuo specifically discloses using the method wherein the tissue defect is a nerve defect, as required by Claim 4. Specifically, Kuo discloses using an engineering scaffold to treat a nerve defect: “Another aspect described herein is a method for replacing or enhancing a tissue [T]he tissue is selected

from the group consisting of a muscle, a nerve, a ligament, a tendon, or another tissue.” (EX1018, [0022], *see also* [0034].) Kuo further disclose that “scaffolds can be formed that replace/repair muscle, ligament, tendon, connective tissue, nerves, [and] nerve channels” For example, the structure and function of nerve tissue require that the cells grow in an aligned manner, and the native tissue are aligned. (¶¶194-96, *supra*.) Along those lines, Kuo teaches the desirability of using aligned fibers to mimic native tissues such as nerves. (EX1018, [0006], [0112]; ¶¶194-96, *supra*.) Moreover, a POSA would have known that nerves are composed of aligned fibers that induce cell growth along the axis of alignment, like Lee’s scaffolds. (EX1019, 3, 28; Lee, [0094]; ¶¶194-96, *supra*.)

214. For the reasons set forth above, a POSA would have been motivated with a reasonable expectation of success to use Lee’s aligned fibers to fabricate a scaffold to treat a nerve defect, as disclosed by Kuo. (*See* §VI.B.1.d., *supra*.)

215. Accordingly, it is my opinion that Lee in view of Kuo renders obvious claim 4.

5. Claim 5: “The method of claim 1, wherein the tissue defect is a the ligament defect.”

216. In my opinion, Claim 5 is obvious in light of Lee in view of Kuo.

217. Claim 1 is obvious for the reasons discussed above in §VI.B.1., *supra*, which are incorporated herein.

218. Further, in my opinion, Lee alone renders the additional element of this claim obvious, i.e. “where the tissue defect is a the ligament defect.” It would have been well-understood by a POSA as of March 2013 that the teaching of Lee of using a scaffold comprising cross-linked aligned collagen fibers to treat a muscle injury could also be used to treat a ligament injury ,given the similarities between the tissues thus rending this claim element obvious. (§VI.B.1.d, *supra.*) Both muscle and ligament are naturally aligned and require strength along the axis of alignment. (*Id.*)

219. Additionally, Kuo discloses the additional elements of claim 5. Kuo specifically teaches using an engineered scaffold to treat a ligament injury. (EX1018, [0006], [0034], [0067], [0112].)

220. For the reasons set forth above, a POSA would have been motivated with a reasonable expectation of success to use Lee’s aligned fibers to fabricate a scaffold to treat a ligament injury, as disclosed by Kuo. (*See* §VI.B.1.d., *supra.*)

221. Additionally, it would have been obvious in view of Lee alone to treat a tendon or ligament, because a POSA would have understood that a scaffold used to treat a muscle could be used to treat a tendon or ligament. This is particularly true where the structure and function of ligament tissue require that the cells grow in an aligned manner with strength along the axis of alignment and the native tissue are aligned, like the fibers in Lee’s scaffolds. (¶¶194-96, *supra*; Lee, Figure 2,

[0025], [0067], Claim 8.) Along those lines, Kuo teaches the desirability of using aligned fibers to mimic native tissues such as ligament. (EX1018, [0006], [0112]; EX1003, ¶¶194-96.)

222. Accordingly, it is my opinion that Lee in view of Kuo renders obvious claim 5.

6. Claim 6: “The method of claim 1, wherein the tissue defect is a the tendon defect.”

223. In my opinion, Claim 6 is obvious in light of Lee in view of Kuo.

224. Claim 1 is obvious for the reasons discussed above in §VI.B.1., *supra*, which are incorporated herein.

225. Further, in my opinion, Lee alone renders the additional element of this claim obvious, i.e. “where the tissue defect is a the tendon defect.” It would have been well-understood by a POSA as of March 2013 that the teaching of Lee of using a scaffold comprising cross-linked aligned collagen fibers to treat a muscle injury could also be used to treat a ligament injury given the similarities between the tissues thus rendering this claim element obvious. (§VI.B.1.d, *supra*.) Both muscle and ligament are naturally aligned and require strength along the axis of alignment. (*Id.*)

226. Additionally, Kuo discloses the additional elements of claim 6. Kuo specifically teaches using an engineered scaffold to treat a tendon injury. (EX1018, [0006], [0034], [0067], [0112].)

227. For the reasons set forth above, a POSA would have been motivated with a reasonable expectation of success to use Lee's aligned fibers to fabricate a scaffold to treat a tendon injury, as disclosed by Kuo. (See §VI.B.1.d., *supra*.)

228. Additionally, it would have been obvious in view of Lee alone to treat a tendon or ligament, because a POSA would have understood that a scaffold used to treat a muscle could be used to treat a tendon or ligament. For example, the structure and function of tendon tissue require that the cells grow in an aligned manner, have strength along the axis of alignment, and the native tissue are aligned. (¶¶194-96, *supra*.) Along those lines, Kuo teaches the desirability of using aligned fibers to mimic native tissues such as tendons. (EX1018, [0006], [0112]; ¶¶194-96, *supra*.) Moreover, a POSA also would have also known that tendons are composed of aligned type 1 collagen and require strength along the axis of alignment, like the aligned fibers in Lee's scaffolds. (EX1019, 3, 32; Lee, Figure 2, [0025], [0067], Claim 8; ¶¶194-96, *supra*.)

229. Accordingly, it is my opinion that Lee in view of Kuo renders obvious claim 6.

C. Ground 3: Claims 7-8 are Anticipated by Huang2

1. Claim 7

230. In my opinion, Huang2 teaches all the elements of Claim 7.

(a) “A method of cell culture comprising placing cells on a scaffold”

231. Huang2 teaches a method of cell culture comprising placing cells on a scaffold comprising aligned fibers.

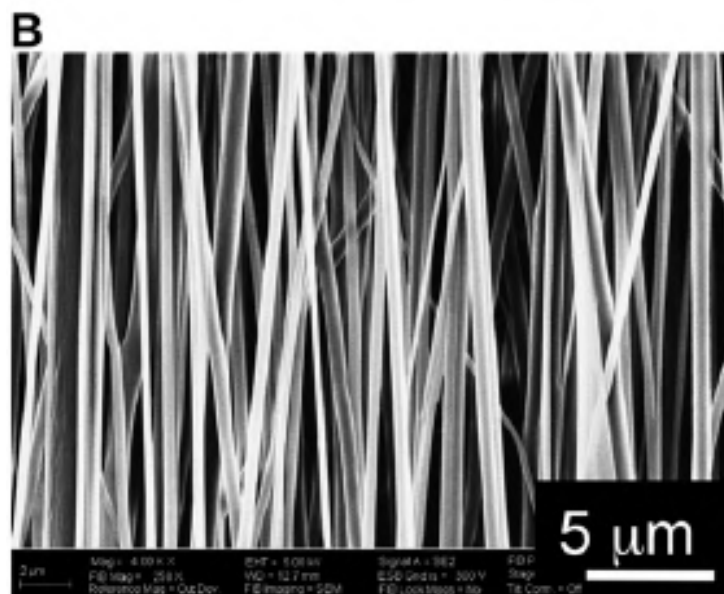
232. Huang2 discloses placing cells, fibroblasts, on a scaffold containing both aligned and unaligned fibers to determine how the alignment affects cell growth and behavior on the scaffold. (EX1005, 1791.) Indeed, Huang2 discloses that “[n]ormal human dermal fibroblasts (NHDFs) were seeded on collagen-containing 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”).” (*Id.*, 1792.)

(b) “comprising aligned fibers wherein a result of a FFT analysis of the fibers demonstrates that the fibers have major adjacent peaks that are about 180° apart from each other”

233. Huang2 teaches this claim element.

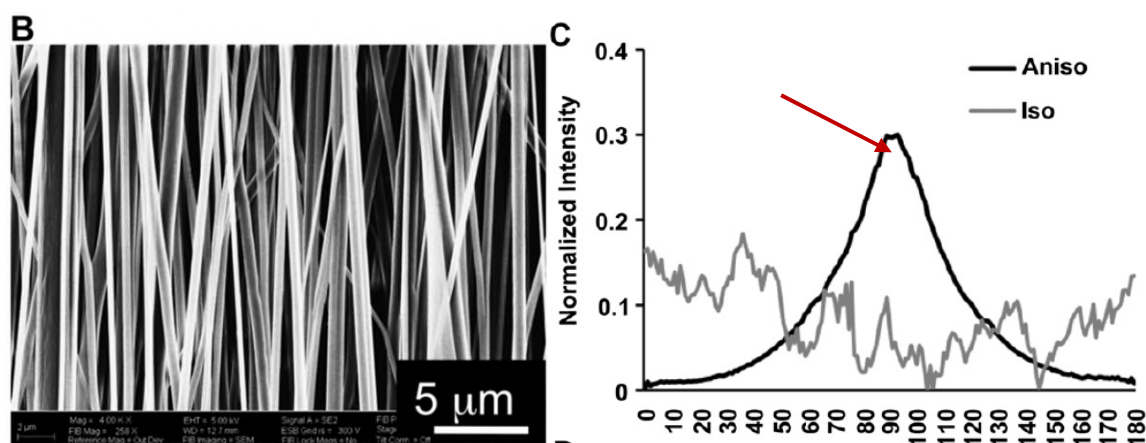
234. Huang2 performed an FFT analysis on its aligned fibers and discloses that the fibers have major adjacent peaks that are about 180° apart from each other.

235. Huang2 discloses a scaffold comprising PCL/collagen nanofibers that are highly aligned with major peaks that are about 180° apart. Huang2 Figure 1B (below) has “anisotropic” fibers, which as set forth above (§V.C. , *supra*), means that the scaffold has “collected fibers with *alignment*.” (EX1005, 1792 & 1793 Fig. 1B.)



236. Huang2 itself states that it discloses fibers “aligned in the same direction.” (EX1005, 1794.) Additionally, Huang2 confirmed the alignment of its anisotropic PCL/Coll fibers through the use of FFT analysis. Indeed, Huang2 teaches that “[t]o better analyze the fiber alignment, FFT analysis was performed on [Huang2’s] SEM [(scanning electron microscope)] images . . . to characterize the anisotropy [alignment] and assign a numerical value to fiber orientation.” (*Id.*; see also *id.*, 1792 (describing FFT analysis using ImageJ).)

237. Huang2 uses the same ImageJ software to perform the FFT analysis as used in the '227 patent. (EX1005, 1792, 1794, 1800 (citing Ayres 2006 (EX1013) for the FFT analysis procedure); EX1001, 13:38-46 (citing Ayres 2008 (EX1010) for the same), 21:40-45.) Huang2 provides its FFT analysis of the aligned (anisotropic) fibers imaged in Figure 1B in Figure 1C, plotting the peaks from 0°-180°:



(Figures 1B, 1C (annotated).)

238. In Huang2's Figure 1C above, the dark line shows the FFT result of the anisotropic (aligned) fibers of Figure 1B. (*Id.*) The FFT of the aligned fibers has a single “major peak” around about 90° (annotated with a **red** arrow). (*Id.*) Consistent with my analysis, Huang2 discloses that “[t]he narrow peak of the normalized intensity values in anisotropic [aligned] matrices (Figure 1C) indicated that *PCL/Coll nanofibers aligned along a single axis* (Fig. 1B).” (*Id.*, 1794 (emphasis added).)

239. Figure 1C reports its results out to 180°. As such, and as explained above, Huang2's FFT analysis would necessarily show a second major peak at around 270° (which is 180° from the 90° peak) if the scale were extended to 0°-360°. (§V.D., *supra*; EX1010, 609.) That is because of the intrinsic symmetry to an FFT. (EX1010, 609.)

240. Specifically, to generate the 2D FFT alignment plot, like in Figure 1(c), first the microscopy image (like in Figure 1B) is captured. (EX1010, 608.) Second, the FFT is applied, converting the fiber image from spatial representation ("real" space) to a frequency-based representation ("frequency" space). (EX1010, 608.) This is called the FFT output image. Importantly, based on the mathematical properties of the FFT function, the FFT output image is symmetrical about the horizontal axis. (EX1010, 609.) Third, to generate the 2D FFT plot from the FFT output, a circular projection is applied to the FFT output image, and pixel intensities are summed for each degree in a specified degree increment. Finally, the summed intensities are then plotted as a function of the corresponding angle, resulting in the 2D FFT alignment plot. (EX1010, 609.)

241. Because the FFT output image is symmetric about the horizontal axis, it is unnecessary to generate a 2D FFT plot from 0° to 360°. (EX1013, 4.) As a way of further explanation, the fact that the FFT output image is symmetric about the horizontal axis (i.e., that the pixel intensity at 1° is the same as 181°, 2° is the

same as 182°, etc.), means that the 2D FFT alignment plot repeats peaks every 180°. (EX1010, 609.)

242. Given the above, a POSA would have understood that, in the FFT analysis result of Huang2's scaffold, there would necessarily be major peaks at 90°, and because of the symmetry, at 270°. Because these are the only major peaks, a POSA would have understood that the FFT analysis result of Huang2's scaffold necessarily had adjacent major peaks that are about 180° apart from each other.

243. Finally, I conducted my own analysis of Figure 1B using the FFT analysis described in Huang2 and the '227 patent. I report the data from 0° to 360° and normalized the y-axis.

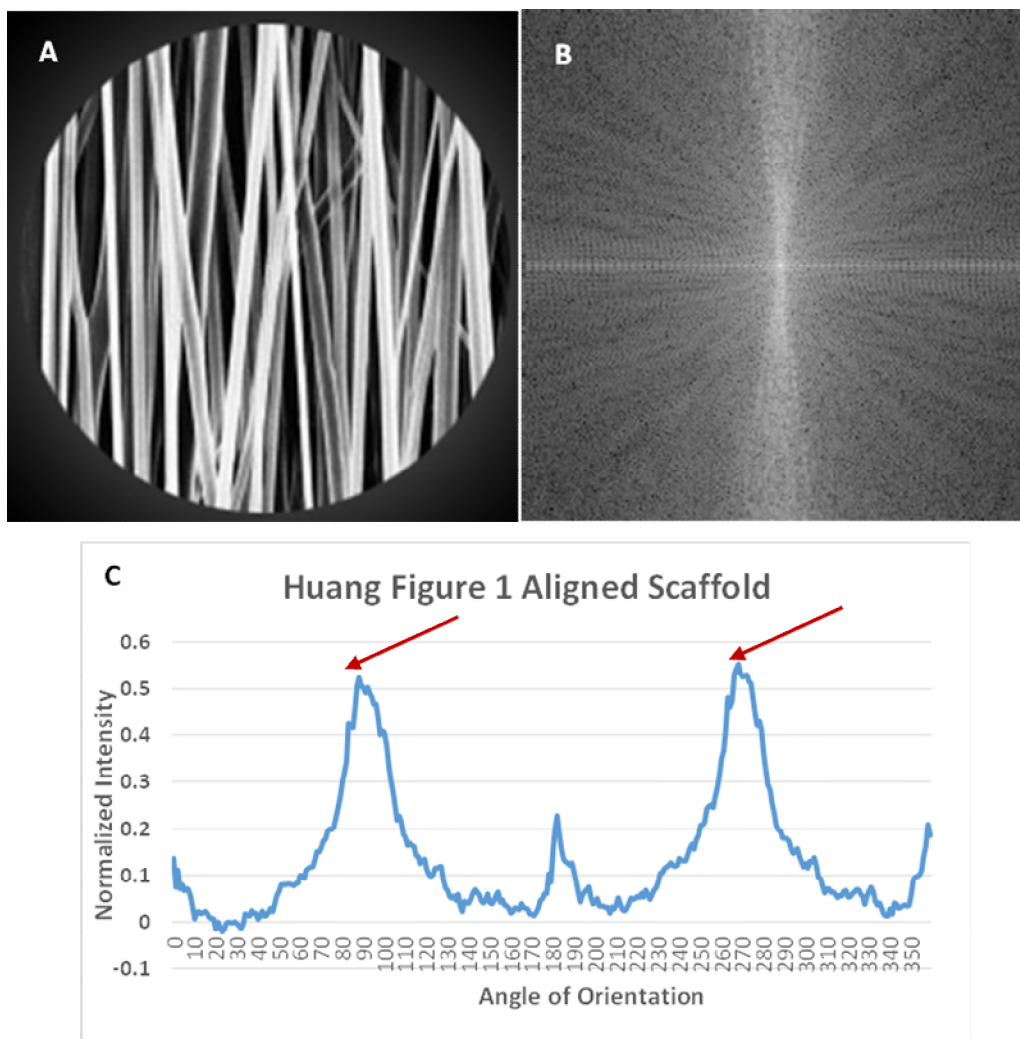


Figure 2. (A) Figure 1 from Huang aligned scaffold cropped and masked for 2DFFT. (B) 2DFFT spectrum of aligned scaffold, the image has been rotated to correct for the rotation intrinsic to the FFT analysis. (c) Normalized intensity values for aligned scaffold 0-360 degrees.

244. 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.

245. Materials testing confirmed the enhanced strength of the fibers along the axis of alignment, indicating that they are aligned. (EX1005, 1794.)

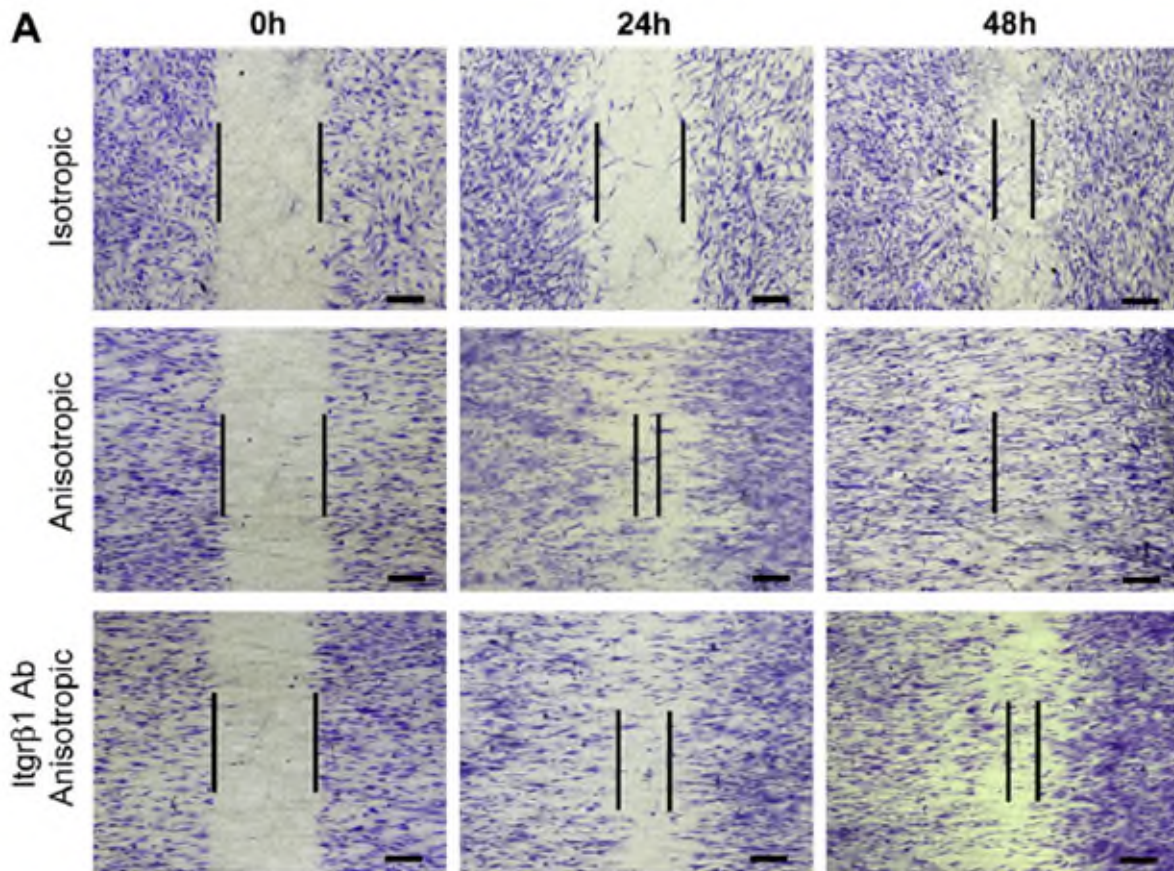
246. Therefore, a POSA would have understood that Huang2 discloses this claim limitation.

(c) “and growing the cells in conditions suitable for the cell culture”

247. Huang2 discloses a method of growing the cells in conditions suitable for the cell culture.

248. Huang2 teaches culturing fibroblasts under suitable conditions such that the cells grow. Huang2 teaches that “fibroblasts were seeded on the nanofibrous matrices at a density of 5×10^3 cells/cm² and allowed to attach for 60 min before fully supplemented media was added. The cells were cultured on the isotropic or anisotropic nanofibrous matrices for up to 7 days.” (EX1005, 1792.) Huang2 further discloses that “[i]n order to evaluate the effects of various fiber organizations (i.e., isotropic vs. anisotropic) on [normal human dermal fibroblast] migration, an in vitro wound healing assay was performed by culturing cells on various matrices with ‘wound gaps’ created by an insert (Fig. 6A).” (*Id.*, 1796.)

249. As seen in Huang2's Figure 6 (below), Huang2 discloses culturing the NHDFs for 48 hours and compares the differences between cell growth observed on anisotropic (aligned) and isotropic (randomly oriented) scaffolds. (EX1005, 1799, Figs. 2-7.)



250. Given the above, a POSA would have understood that Huang2 discloses this limitation.

251. Thus, it is my opinion that Huang2 anticipates Claim 7.

2. Claim 8: The method of claim 7, wherein the cells are stem cells, adipose derived stem cells, dental pulp stem cells, fibroblasts, and/or dorsal root ganglia.

252. In my opinion, Huang2 teaches all the elements of Claim 8.

253. As discussed above, Huang2 discloses all of the elements of Claim 7.

(See §VI.C.1., *supra*.)

254. Additionally, Huang2 discloses culturing fibroblasts. Specifically, Huang2 discloses that “fibroblasts were seeded on the nanofibrous matrices at a density of 5×10^3 cells/cm² and allowed to attach for 60 min before fully supplemented media was added. The cells were cultured on the isotropic or anisotropic nanofibrous matrices for up to 7 days.” (EX1005, 1792; *see also* §VI.C.1.c., *supra*.)

255. Given the above, a POSA would have understood that Huang2 discloses Claim 8’s limitations. Thus, it is my opinion that Huang2 anticipates Claim 8.

VII. CONCLUSION

256. For the reasons set forth above, it is my opinion that Claims 1-8 of the ’227 Patent are unpatentable in view of the prior art.

257. For the reasons set forth above, it is my opinion that Claims 1-8 of the ’227 Patent are unpatentable in view of the prior art based on the following points:

1. Claims 1-6 are obvious in light of Huang2 in view of Kuo.
2. Claims 1-6 are obvious in light of Lee alone or in view of Kuo.
3. Claims 7-8 are anticipated by Huang2.

258. In short, as discussed above, Huang2 in view of Kuo would have motivated the POSA to use a scaffold like Huang2's scaffold to treat a nerve, ligament, or tendon, and expected success in doing so.

259. In short, as discussed above, Lee in view of Kuo would have motivated the POSA to use a scaffold like Lee's scaffold to treat a nerve, ligament, or tendon, and expected success in doing so. Indeed, a POSA would have been motivated to treat a nerve, ligament, or tendon with such a scaffold in view of Lee alone, and expected success in doing so.

260. In short, as discussed above, Huang2 discloses each and every limitation of Claims 7-8. Specifically, Huang2 discloses culturing fibroblasts on an aligned scaffold.

261. The above summaries do not limit my opinions, which are provided in detail in §VI.

262. In signing this declaration, I recognize that it will be filed as evidence in a contested case before the Patent Trial and Appeal Board of the United States Patent and Trademark Office. I also recognize that I may be subject to cross-examination in the case and that cross-examination will take place in the United

States. If cross-examination is required of me, I will appear for cross-examination within the United States during the time allotted for cross-examination.

263. I reserve the right to supplement my opinions in the future to respond to any arguments that the Patent Owner raises and to consider new information as it becomes available to me.

I declare under penalty of perjury under the laws of the United States of America that the foregoing is true and correct.

Date:

11/29/24

A handwritten signature in black ink, appearing to read 'D. Simpson', written over a horizontal line.

David G. Simpson, Ph.D.

Appendix A

Exhibit No.	Description
1001	U.S. Patent No. 11,318,227 to Francis et al. (“the ’227 patent”)
1004	CV of David G. Simpson, Ph.D.
1005	Huang et al., <i>The involvement of integrin $\beta 1$ signaling in the migration and myofibroblastic differentiation of skin fibroblasts on anisotropic collagen-containing nanofibers</i> , 33 Biomaterials 1791-1800 (Feb. 2012) (“Huang2”) (available online Dec. 2, 2011)
1006	Kumbar et al., <i>Electrospun nanofiber scaffolds: engineering soft tissues</i> , Biomed. Mater. 3 (2008) (“Kumbar”)
1008	Huang et al., <i>Electrospun collagen-chitosan-TPU nanofibrous scaffolds for tissue engineered tubular grafts</i> , 82 Colloids and Surfaces B: Biointerfaces 307-315 (2011) (“Huang1”)
1009	U.S. Patent App. Pub. No. 2004/0037813 to Simpson et al. (“Simpson”)
1010	Ayres et al., <i>Measuring fiber alignment in electrospun scaffolds: a user’s guide to the 2D fast Fourier transform approach</i> , 19 J. Biomater. Sci. Polymer. 603-621 (2008) (“Ayres 2008”)
1011	Lee et al., U.S. Patent Application No. US 2010/0331980 A1, Pub. Date December 1, 2011 (“Lee”)
1013	Ayres et al., <i>Modulation of Anisotropy in Electrospun Tissue-Engineering Scaffolds: Analysis of Fiber Alignment by the Fast Fourier Transform</i> , 27(32) Biomaterials 5524-5534 (2006) (“Ayres 2006”)
1015	Jose et al, <i>Fabrication and Characterization of Aligned Nanofibrous PLGA/Collagen Blends as Bone Tissue Scaffolds</i> , 50 Polymer 3778-85 (2009) (“Jose”)
1016	Fu et al, <i>Spatial Arrangement of Polycaprolactone/Collagen Nanofiber Scaffolds Regulates the Wound Healing Related Behaviors of Human Adipose Stromal Cells</i> , 18(5) Tissue Engineering, 631-42 (2012) (“Fu”)

1018	Kuo et al., U.S. Patent Application No. US 2011/0293685 A1, Pub. Date Dec. 1, 2011 (“Kuo”)
1019	Beachley et al, <i>Highly Aligned Polymer Nanofiber Structures: Fabrication and Applications in Tissue Engineering</i> (2011) in Vol. 246 Biomedical Applications of Polymeric Nanofibers (Jayakumar & Nair eds. 2011)
1020	Declaration of Emma Xu
1021	U.S. Provisional Patent App. No. 61/0255,35 (“Lee Provisional”)
1022	McClure et al, <i>Tri-Layered Vascular Grafts Composed of Polycaprolactone, Elastin, Collagen, and Silk: Optimization of Graft Properties</i> , 10 J. Mechanical Behavior Biomedical Materials 48 (2012)
1023	Meinel et al., <i>Engineering Cartilage-Like Tissue Using Human Mesenchymal Stem Cells and Silk Protein Scaffolds</i> , 88(3) Biotechnology Bioengineering 379 (2004)