

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

**DECLARATION OF DAVID G. SIMPSON, PH.D.
IN SUPPORT OF PETITION FOR *INTER PARTES* REVIEW OF
U.S. PATENT NO. 10,137,223**

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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. 10,137,223 (the “’223 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 1-12 of the ’223 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 '223 Patent as of March 14, 2013, which I have been informed is the earliest date to which the '223 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 Petitioners 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 '223 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 '223 patent;
- 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”);

- Kumbar et al., *Electrospun nanofiber scaffolds: engineering soft tissues*, Biomed. Mater. 3 (2008) (“Kumbar”);
- Lee et al., U.S. Patent Application No. US 2010/0331980 A1, Pub. Date December 30, 2010 (“Lee”); and
- 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 '223 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 '223 patent, Huang2, Kumbar, Lee, 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 1-12 are anticipated by Huang2.
2. Claim 4 is obvious in light of Huang2 in view of Kumbar.
3. Claims 1-12 are anticipated by Lee.
4. Claims 1-12 are obvious in light of Lee alone.

II. MY UNDERSTANDING OF THE LEGAL STANDARDS

18. In forming my opinions and considering the subject matter of the '223 Patent and its claim 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 '223 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

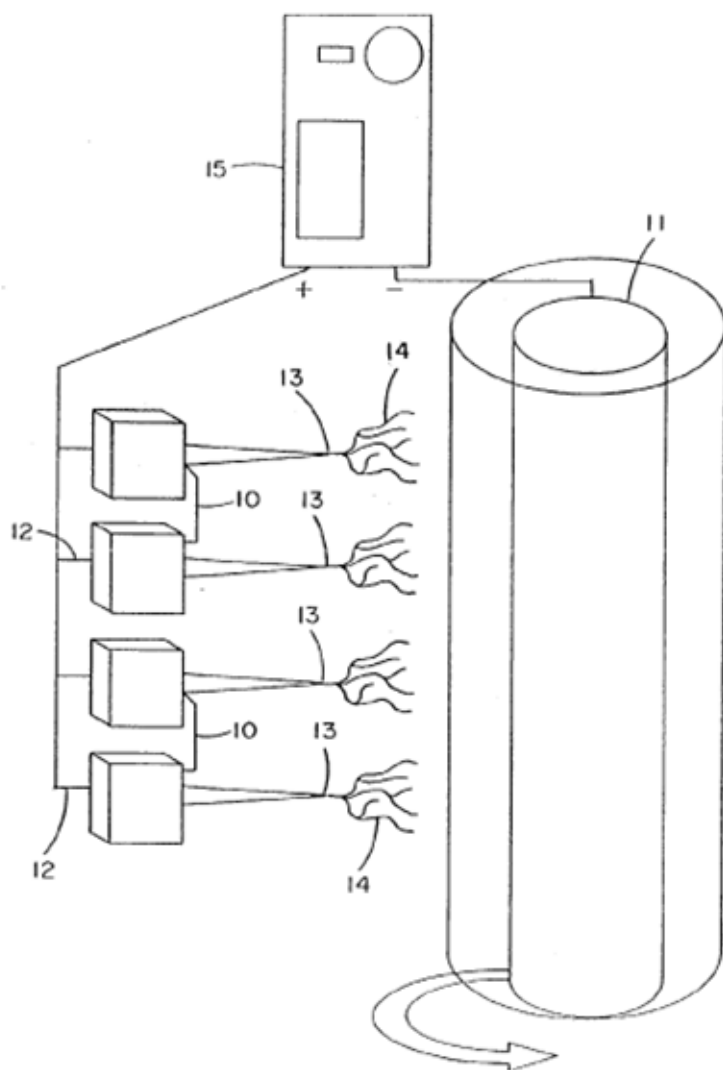
A. Electrospinning is A Proven Technique for Producing Nanofibers with Different Characteristics

30. As of March 2013, electrospinning was a well-established and widely used method for producing nanofibers. (EX1006, 1.) This technique was valued for its simplicity, reproducibility, and scalability. (EX1006, 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. (EX1006, 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]; EX1006, 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]; EX1006, 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. (EX1006, 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. (EX1006, 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 '223 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. (EX1006, 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. (EX1006, 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; EX1006, 6, 12.) As a result, electrospun nanofiber scaffolds are widely recognized as effective materials for promoting tissue regeneration. (EX1006, 12.) Electrospun scaffolds can also be composed of biodegradable

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

(EX1006, 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; EX1006, 7-8),
- tendon (EX1018, [0112]; EX1006, 10),
- blood vessels (EX1006, 8-10),
- muscle (EX1006, 11),
- ligament (EX1018, [0112]), and
- nerves (EX1006, 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 nerve 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. (EX1006, 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.)

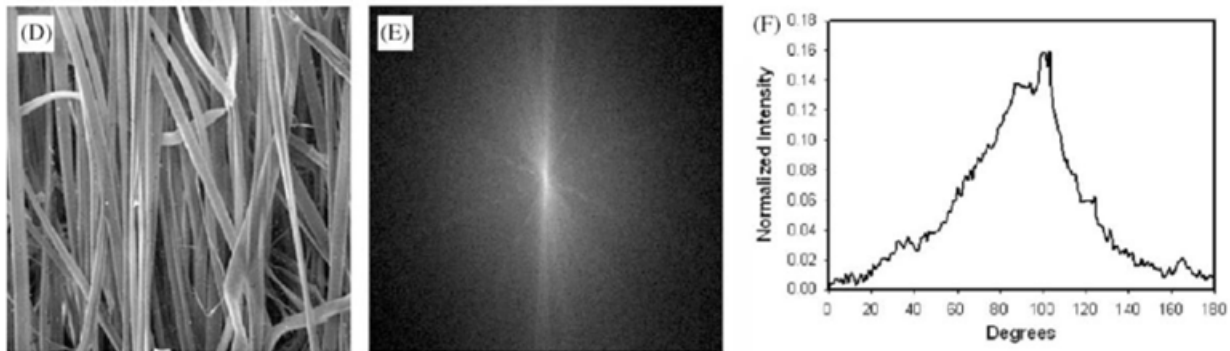
37. Given its properties, using aligned nanofiber scaffolds in tissue grafts had been described as “highly encouraging” and “promising.” (EX1006, 10; EX1019, 27.)

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, 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.)

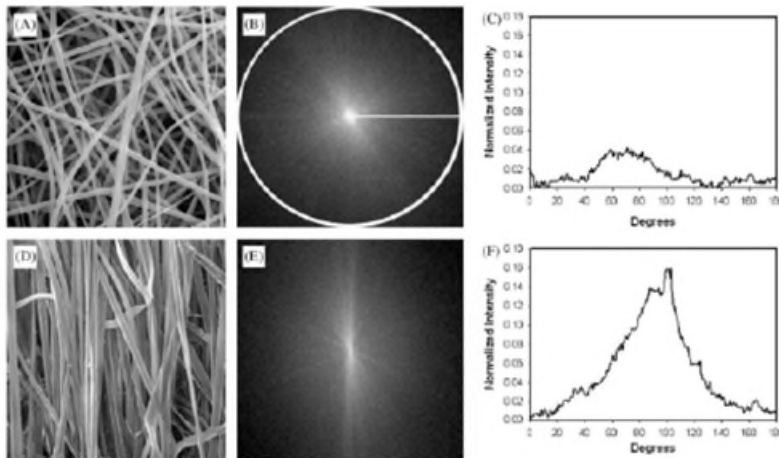
42. To further understand the fiber alignment, the 2D FFT output image can be processed into a 2D FFT alignment plot. (EX1013, 4; EX1010, 609.) This plot graphically represents the alignment of the fibers, providing a visual summary of their orientation (EX1013, 4; EX1010, 609.) The 2D FFT alignment 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.

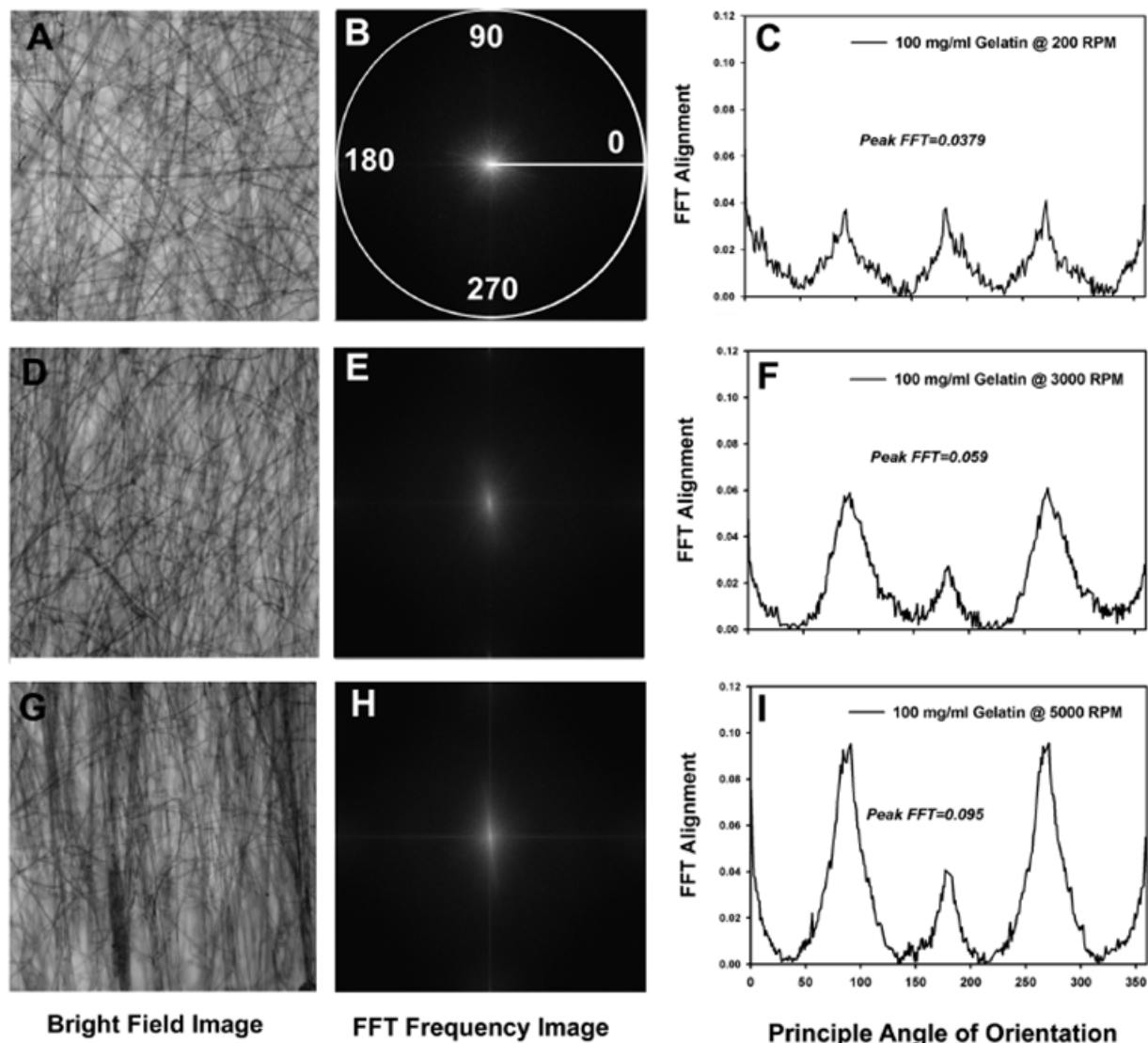


(EX1013, 12.)

46. When creating the 2D FFT alignment plot, it is sufficient to sum pixel intensities from 0° to 180°, which is the standard 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.



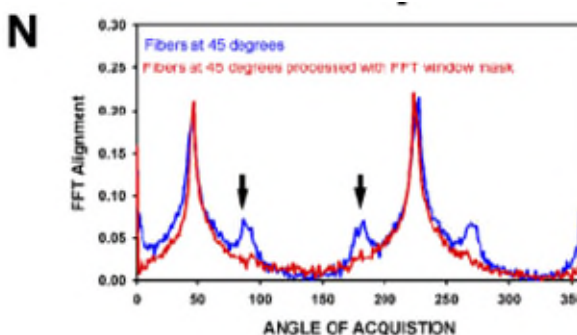
(EX1010, Figure 1.)

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

48. 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,

pp. 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.”

49. 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.



50. .
 (EX1010, 614.)

51. In addition to the FFT method, other techniques for measuring fiber alignment include laser scattering and the analysis of representative images.
 (EX1013, 2; EX1010, 1.) 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

52. 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*.)

53. 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. (EX1006, 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. (EX1006, 7.) Collagen is biodegradable, so it can be replaced by natural ECM over time. (EX1006, 10; EX1005, 1791.)

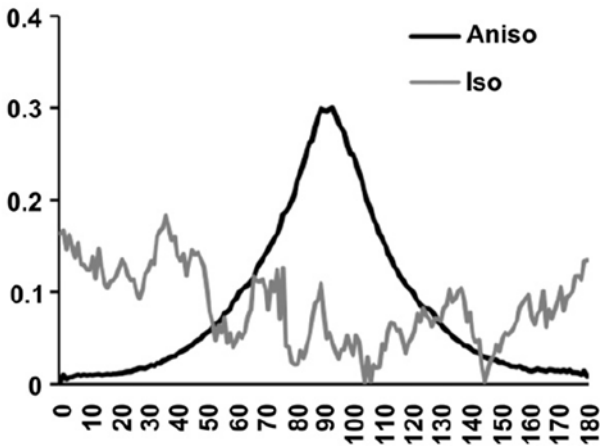
54. However, collagen alone degrades quickly and has poor mechanical properties, limiting its stability in electrospun scaffolds. (EX1005, 1791.) These limitations could be overcome using well-known methods, including combining the collagen with other materials. (EX1005, 1791.) For example, polycaprolactone-collagen 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]; EX1006, 6; EX1005, 1791.)

F. Previously Developed Aligned Collagen Scaffolds

55. 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; EX1011, Fig. 9(b), [0019]; EX1016; EX1015, 3778-81.)

56. 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.)

57. 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 '223 PATENT

58. According to the '223 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.*, 14:51-55.)

59. The examples in the specification, however, provide scaffolds made of either heart basement membrane (“HBM”) combined with polycaprolactone (“PCL”) or PLC alone. (*Id.*, 19:40-23:4 (Examples 1-4).) Many of the figures concern examples made of the same materials:

- “FIG. 6 depicts aligned heart base [sic] membrane fiber scaffold that allow dorsal root ganglia attachment and outgrowth.” (*Id.*, 1:51-52.)
- “FIG. 7 depicts aligned placenta and heart base membrane fiber scaffolds that allow dorsal root ganglia attachment and outgrowth.” (*Id.*, 1:50-55.)
- “FIGS 9A, 9B, and 9C depict exemplary scaffold prepared into an elongated roll by folding an elongated sheet (9A), an elongated roll by rolling and elongated sheet (9B), and a composite of multiple layers of elongated sheets (9C).” (*Id.*, 1:57-60.)

60. 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.*, 13:1-5.) The specification provides that “the major peaks . . . refer to peaks higher than the average peaks, in the result.” (*Id.*, 13:6-7.)

61. 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.*, 13:16-24.) As discussed above, I am senior author of the Ayres paper.

62. 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 ’223 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.” (EX1010, 609; *see also* ’223 patent, 12:16-26.)

63. FIG. 8 of the ’223 patent, reproduced below, provides the results of an FFT analysis of fibers prepared by the Applicants: “FIG. 8 depicts exemplary FFT analysis results (a) of the fibers described herein and (b) a control sample.” (EX1001, 1:55-56.) The specification further provides that “in some embodiments, FFT result of the fibers described herein may have adjacent major peaks with about 180° apart from each other as shown in FIG.8.” (*Id.*, 13:24-26.) 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 ’223 patent, as I discuss in more detail below (§V.A, *infra*).

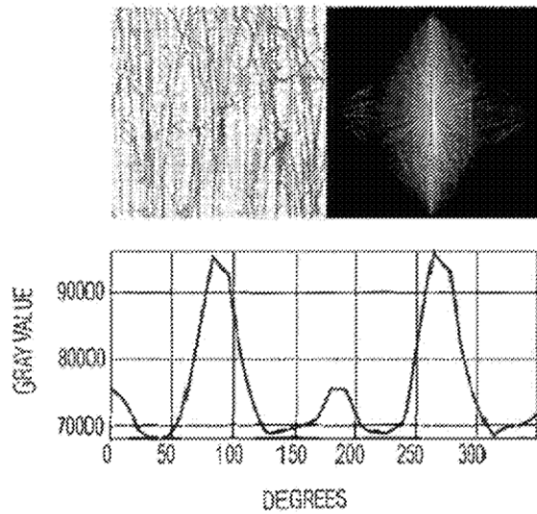


FIG. 8A

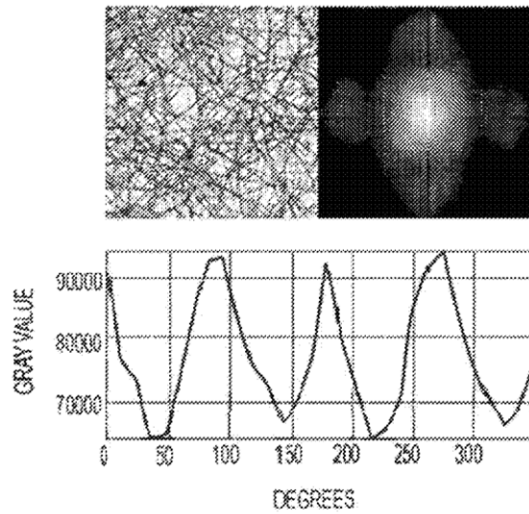


FIG. 8B

64. 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, 13:16-26; §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, typical of an aligned scaffold, there are no other major peaks in the 0°-180° range.

65. The y-axis of the '223 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.

66. 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°.

67. The specification also discloses additional materials used in scaffolds. These were all well-known in the prior art and are required by dependent claims 2-10. For example, the specification provides:

- The “scaffold of the present invention may comprise type I collagen.” (EX1001, 13:27-28.)
- The “electrospun fiber and/or scaffold” and may “have less than about 8 wt% . . . of residual calcium.” (*Id.*, 11:2-4; 13:47-50.)

- The scaffold can have a porosity between about 60% and 90%. (*Id.*, 14:21-26.)
- The scaffold may contain a biodegradable polymer (*Id.*, 14:51-55), such as polycaprolactone or “PCL” (*Id.*, 9:60-63; claim 6.)
- An “accessory polymer” can be used for the fibers in the scaffold as “a polymer that may be added to the electrospun fibers and have any effect on [the fibers’] physical, chemical, and/or biological properties (e.g., tensile strength, hydrophilicity, biocompatibility).” (EX1001, 12:7-26.)
- The scaffold may also comprise “a bioactive factor.” (*Id.*, 14:61-63.) A “bioactive factor” is defined as “a protein, carbohydrate or mineral that has an effect on a cellular activity.” (*Id.*, 12:43-45.)
- In some embodiments, the scaffold comprises what it terms a “extracellular matrix.” (*Id.*, 14:27-35.) In some embodiments, “the extracellular matrix may include, but is not limited to, collagen, glycosaminoglycans, osteocalcin, bone sialo protein, osteopontin, fibronectin, laminin, vitronectin, elastin, or mixtures thereof.” (*Id.*, 14:64-15:3.)

68. The specification also discloses growing and/or culturing cells on the scaffold using techniques that were well-known in the prior art. (*Id.*, 16:8-10.) For example, the specification discloses that:

- that “the invention further relates to methods of culturing cells the scaffold or aligned fiber described herein. In some embodiments, the cells are selected from the group consisting of stem cells, adipose derived stem cells, dental pulp stem cells, fibroblasts, and dorsal root ganglia.” (*Id.*, 16:3-7.)
- “‘Growing and/or culturing cells onto a scaffold’ includes traditional cell culture methods as well as placing on a surface in any setting, such as in natural or synthetic biocompatible matrices or tissues.” (*Id.*, 16:10-20.)
- In some embodiments, “the cells that are cultured on the scaffold are stem cells.” (*Id.*, 16:14-19.)
- “[T]he scaffold can be used in in vitro methods for supporting cell growth and proliferation as well as for increasing osteogenesis, chondrogenesis, or ligament/tendon genesis in the stem cells cultured on the scaffold.” (*Id.*, 16:15-19.)

- “[C]ells may be seeded onto an . . . scaffold . . . The cells seeded on the scaffold can be any cell such as but not limited to . . . progenitor cells, and stem cells.” (*Id.*, 19:15-20.)
- “Suitable biocompatible matrices include, but are not limited to, porous biocompatible scaffolds into which bone cells or progenitor cells may migrate.” (*Id.*, 9:4-6.) These techniques for growing different cell types on a scaffold, as discussed in detail below, were well-known and used in the art before the filing of the ’223 patent.

V. SUMMARY OF THE MOST RELEVANT PRIOR ART

A. Huang2

69. Huang2 published in print in February 2012, which is more than a year before the date of the first priority application of the ’223 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 ’223 patent.

70. 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. Top 5% journals have an impact factor of 6. Biomaterials has

an impact factor of 12. 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.

71. 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.

72. 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.

73. Huang2 concerns electrospun fibrous scaffolds comprising collagen and is directed to examining the effect of aligned fibers compared to unaligned fibers on the attachment and growth of normal human dermal fibroblasts (“NHDFs”), including fibroblast-to-myofibroblast differentiation. (EX1005, 1796-97.) Huang2 is aimed at “understand[ing] how various nanofibrous matrices

regulate skin cell behaviors and intracellular signaling pathways.” (EX1005, Abstract.) In order to do so, Huang2 created scaffolds of “stable” collagen blended fibers for engineering skin grafts. (EX1005, 1792.) In Huang2, “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*) were studied.” (EX1005, Abstract.) Huang2’s results demonstrate, among other things, that the fibroblast-to-myofibroblast differentiation was greatly enhanced for the NHDFs cultured on the aligned nanofibrous matrices comprising collagen. (EX1005, 1797, 1799.) That result was likely due to the impact of the alignment on the integrin $\beta 1$ signaling pathway. (EX1005, 1797, 1799.)¹

¹ “NHDFs cultured on anisotropic [aligned] PCL/collagen nano-fibers showed elongated cell morphology, distinct distribution of focal adhesions, accelerated cell migration and promoted fibroblast-to-myofibroblast differentiation.” (EX1005, 1799.) Huang2’s results suggests an important role of the integrin $\beta 1$ signaling pathway in regulating the nanofiber-induced fibroblast alteration, “and provid[e] insightful understanding of the possible application of collagen-containing nanofibrous matrices for skin regeneration.” (EX1005, abstract.)

74. Huang2 explains the importance of collagen in electrospinning fibers in tissue engineering. Huang2 teaches that “collagen fibrils, a major ECM component known for their mechanical contribution to skin integrity, provide an interactive 3D microenvironment to skin cells and regulate their behaviors via cell-matrix interactions.” (EX1005, 1791.) Huang2’s fibers comprise both collagen and PCL because collagen alone can be unstable. (*Id.*) Specifically, “nanofibrous matrices of PCL and Collagen at a weight ratio of 3:1 were fabricated using electrospinning previously described.” (EX1005, 1792.) Additionally, Huang2 teaches that “fibroblasts showed preferred adhesion on such collagen containing nanofibers, compared to pure collagen fibers.” (EX1005, 1791-92.)

75. Scanning electron microscope images of Huang2's fibers comprising collagen are shown in Figure 1:

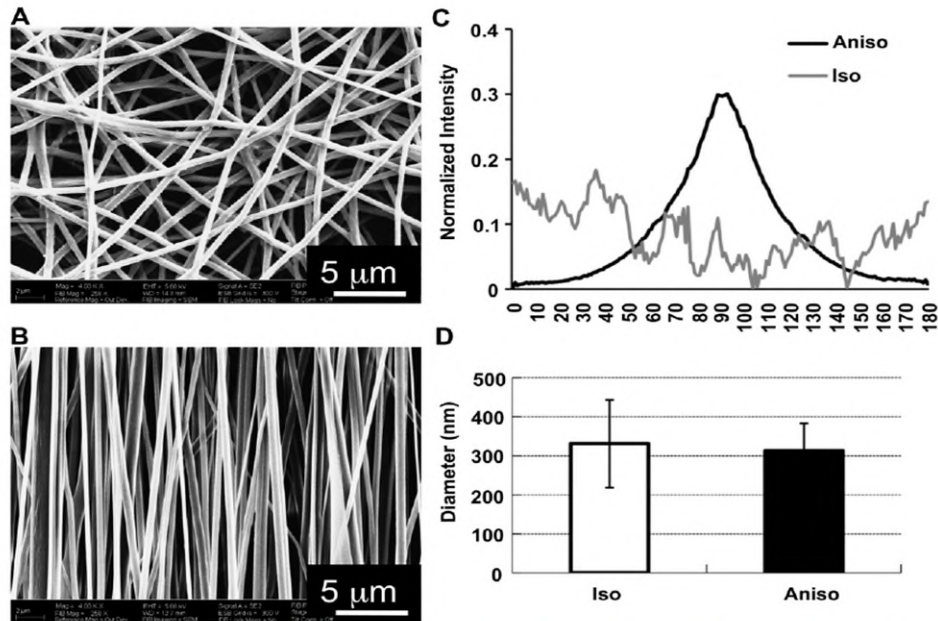


Fig. 1. Characterization of isotropic and anisotropic electrospun PCL/Coll nanofibrous matrices. SEM image of nanofibrous matrices with two types of fiber spatial organizations: isotropic (A) and anisotropic (B). (C) Normalized intensity plots against the angle of acquisition for isotropic and anisotropic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

(EX1005, Fig. 1.)

76. These images show the isotropic (unaligned) sample (Figure 1A) and the anisotropic (aligned) fiber sample (EX1005, Figure 1B). (EX1005, 1793.) For the aligned fiber sample, the major peak reports the direction of alignment with respect to the image.

77. 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.

78. 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.)

79. 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.) To support this point, Huang2 cites to Kumbar, discussed below.

80. 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.”).)

B. Kumbar

81. Kumbar published in August 8, 2008, which is more than a year before the date of the first priority application of the ’223 patent, which is March 14, 2013. (EX1006.) I have been informed that Kumbar was not disclosed during prosecution of the ’223 patent. As I discussed above, Huang2 relies on Kumbar in its discussion of the desirable properties of electrospun scaffolds. (EX1005, 1791.)

82. Kumbar discusses research efforts in “designing nanofiber matrices as scaffolds in the regeneration of various soft tissues including skin, blood vessel,

tendon/ligament, cardiac patch, nerve and skeletal muscle.” (EX1006, Abstract.)

Kumbar explains that “electrospun nanofiber matrices have gained a lot of attention, and are being explored as scaffolds in tissue engineering due to their properties that can modulate cellular behavior.” (*Id.*, Abstract.) Kumbar discloses the importance of using collagen in electrospun fibers. (*Id.*, 7.) For example, Kumbar explains that “[c]ollagen nanofibers play a dominant role in maintaining the biological and structural integrity of various tissues and organs, including bone, skin, tendon, blood vessels and cartilage.” (*Id.*, 7.)

83. Kumbar also teaches desirable mechanical properties of scaffolds in tissue engineering. (EX1006, 6.) Kumbar describes that “fiber alignment in nanofiber matrices significantly improves the mechanical properties.” (*Id.*)

84. Kumbar also discusses that “[n]anofiber matrices as tissue engineering scaffolds need to have interconnected highly porous structures to facilitate cellular migration and transport of nutrients, and metabolic wastes to allow the formation of new tissue.” (EX1006, 6.) With respect to “[e]lectrospun nanofiber skin grafts,” Kumbar discusses scaffolds of collagen nanofibers having a porosity of 71% to 89%, depending on the degree of crosslinking. (*Id.*, 7.)

C. Lee

85. 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 '223 patent, which is March 14, 2013. (EX1011.) I have been informed that Lee was not cited as prior art during the prosecution of the '223 patent.

86. 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].) Lee provides that its “[s]caffold” refers to an array of natural or synthetic matrix molecules to which cells or fibers can attach,” which may include various components and molecules. (*Id.*, [0044].)

87. 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.” (EX1011, [0052].) Lee discloses that when PCL and collagen are used together, “the mesh can be improved biocompatibility, mechanical properties, and processability,” and further explains that “in some embodiments the biomechanical properties of PCL collagen scaffolds are similar to that of native tissue.” (EX1011, [0052].)

88. 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.*) Lee obtained different levels of fiber alignment by using different rotation speeds for the drum. (EX1011, [0011].)

89. 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 along the axis of the alignment. (EX1011, [0081].)

90. Lee also teaches that “[e]lectrospun nanofiber scaffolds [that]...have a three-dimensional architecture, *high porosity* with an interconnected pore structure,

and a high surface-to-volume ratio, which provides a favorable environment for the cells to grow.” (EX1011, [0055] (emphasis added).) Lee explains that to achieve the desired “composition, structure, and mechanical properties,” its “biodegradable scaffold can be ‘shaped’ using methods such as...leaching,” “nucleation,” and “laser shaping.” (EX1011, [0055]-[0056].) Lee provides that the salt leaching process involves spreading “a solution containing two materials...into a shape that resembles the final form” and “dissolv[ing] away one of the components, resulting in pore formation (see U.S. Pat. No. 5,514,378 [Mikos]).” (*Id.*, [0056] (citing Mikos (EX1014)), [0021] (incorporating all cited patents by reference).) Lee incorporates Mikos by reference, which describes the salt-leaching process in greater detail. In addition, Mikos teaches that “[H]igh porosity, greater than 80% volume, provides adequate space for cell seeding, growth, and extracellular matrix (ECM) production.” (EX1014, 13:15-17.)

91. 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] (emphasis added).)

92. 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].)

93. Lee teaches that “[s]keletal muscle cells may be provided by isolating cells from skeletal muscle tissue harvested from the skeletal muscle of an organism, differentiating *stem* or progenitor cells (e.g., embryonic or amniotic fluid stem cells), etc.” (EX1011, [0040] (emphasis added).) Lee also discloses that “[s]keleton muscle cells include . . . myoblasts.” Lee defines “myoblasts” as “a type of *muscle stem cell* . . . [that] are normally closely associated with myofibers during the course of their life cycle” (*Id.*, [0030] (emphasis added).)

VI. MY ANALYSIS OF THE CLAIMS

A. Point 1: Huang2 Anticipates Claims 1-12

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

94. In my opinion, Huang2 teaches all the elements of Claim 1.

(a) “A scaffold comprising”

95. Huang2 teaches a scaffold.

96. Huang2 teaches “scaffolds [that] not only . . . provide a surface for cells to reside, but also . . . define a cell-friendly microenvironment that maximally recaptures the native extracellular matrix (ECM).” (EX1005, 1791.) Huang2’s scaffolds comprise “nanofibrous matrices of PCL and Collagen” of anisotropic (aligned) nanofibers, [and] are formed by electrospinning a solution of polycaprolactone (PCL) and collagen using a rotating mandrel. (EX1005, 1792.)

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

(b) “one or more electrospun fibers comprising collagen”

98. Huang2 teaches a scaffold comprising electrospun fibers comprising collagen.

99. Huang2 teaches the creation of electrospun nanofibers containing collagen. (EX1005, 1792.) Huang2 discloses electrospinning “[n]anofibrous

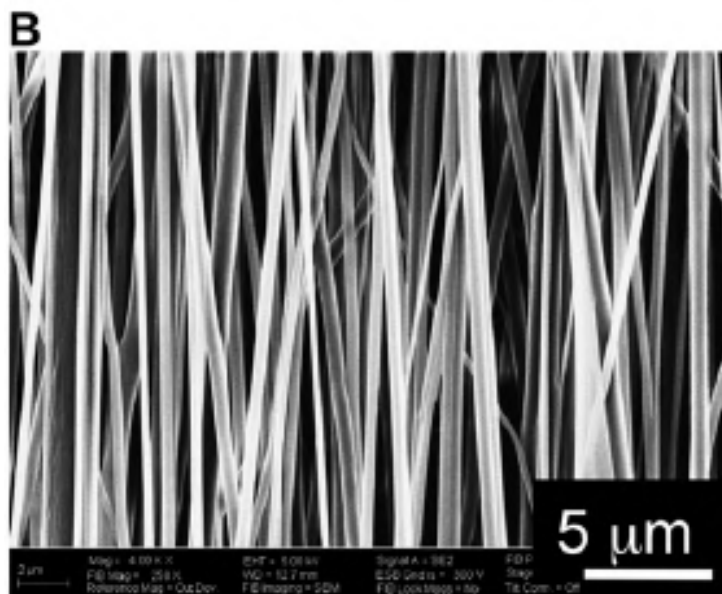
matrices of PCL and *Collagen*” from an “8% (w/v) solution of 3:1 PCL/Coll” with an electrospinning device. (EX1005, 1792.) Huang2 further teaches that “[e]lectrospinning a blend solution of collagen and polycaprolactone (PCL) . . . can yield rather stable nanofibers.” (*Id.*, 1791.)

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

(c) “wherein a fast Fourier transform (FFT) analysis result of the fibers have adjacent major peaks with about 180° apart from each other.”

101. Huang2 teaches a scaffold wherein a fast Fourier transform (FFT) analysis result of the fibers have adjacent major peaks with about 180° apart from each other.

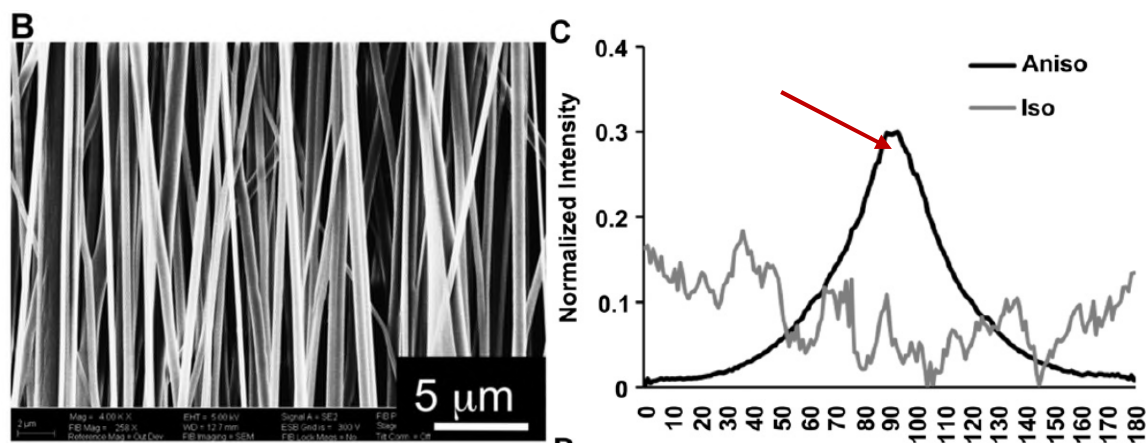
102. Huang2 discloses a scaffold comprising PCL/collagen nanofibers that are visually highly aligned with major peaks that are about 180° apart by FFT analysis. Huang2 Figure 1B (below) has “anisotropic” fibers, which as set forth above (*Supra* [references section]), means that the scaffold has “collected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



103. 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).)

104. Huang2 uses the same ImageJ software to perform the FFT analysis as used in the ’223 patent, which produces data from 0 to 360 degrees. (EX1005, 1792, 1794, 1800 (citing Ayres 2006 (EX1013) for the FFT analysis procedure); EX1001, 13:16-23, 21:27-31 (citing Ayres 2008 (EX1010) for the same).) 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).)

105. In Huang2’s Figure 1C above, the dark line shows the FFT result of the anisotropic (aligned) fibers of Figure 1B. 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.* (emphasis added).)

106. As typical in the field, 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°. (*Id.*, §V.A; EX1010, 609.) That is because of the intrinsic symmetry of an FFT. (EX1010, 609.)

107. 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 along each radius for 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.)

108. 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.)

109. 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.

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

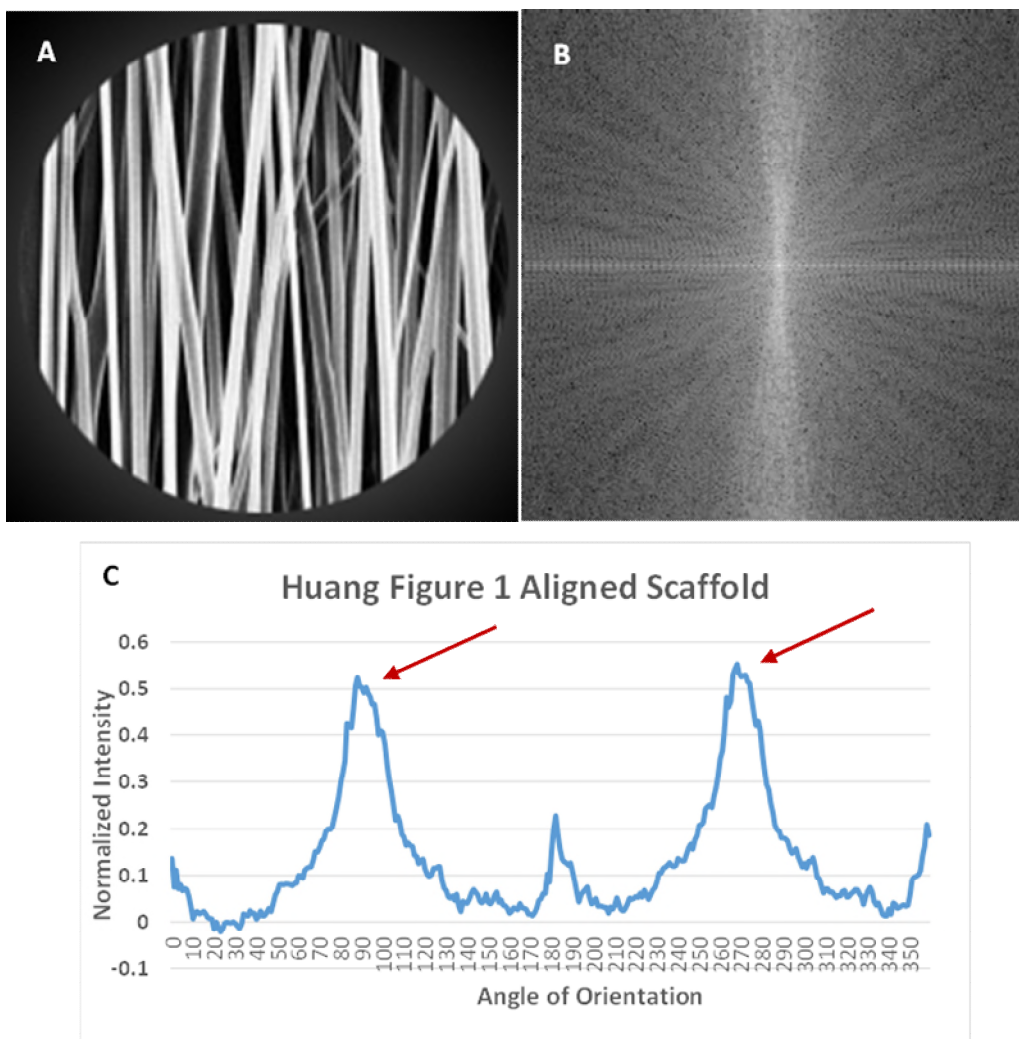


Figure 2. (A) Figure 1 from Huang aligned scaffold cropped and masked for 2DFFT. (B) 2DFFT spectrum of aligned scaffold, the image has been rotated to correct for the rotation intrinsic to the FFT analysis. (c) Normalized intensity values for aligned scaffold 0-360 degrees.

111. The graph generated from the FFT analysis demonstrates that the aligned fibers have a first major peak at about 90° and, because I reported all the peaks through 360°, a second major peak at about 270°, which shows that the peaks

are 180° apart as recited in claim 1. The major peaks are annotated above with **red** arrows.

112. Materials testing confirmed the enhanced tensile properties along the axis of the fibers, indicating that they are aligned. (EX1005, 1794.)

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

114. Given that a POSA would have understood that Huang2 discloses all of the claim limitations it is my opinion that Huang2 anticipates Claim 1.

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

115. In my opinion, Huang2 teaches all the elements of Claim 2.

116. As discussed above, Huang2’s PCL/Collagen fibers have aligned fibers with major peaks about 180° apart, which discloses the scaffold according to Claim 1. (See §VI.A.1, *supra*.)

117. Huang2 also teaches the “native triple helical structure” element of the claim. Huang2 teaches that “type I collagen (Coll) was obtained from Elastin Products Inc.” (EX1005, 1792.) Type I collagen was well-known and generally understood in the art to have a triple-helical form when it is in a monomer, as well as a polymer. (EX1017, 9.) A POSA would have understood that, if the type 1 collagen purchased from a supplier were denatured and/or fragmented (i.e., not in a

triple helix) it would be sold as “gelatin,” not “collagen.” In addition, Huang2 teaches that the 3:1 ratio of PCL/Collagen was prepared by dissolving PCL and collagen in 1,1,1,3,3,3-hexafluoro-2-propanol or “HFIP.” (EX1005, 1792.) HFIP was known to stabilize collagen’s triple helix structure. (EX1015, 3782.) For example, the prior art demonstrated that collagen purchased from Elastin Products maintained its native triple helical structure when electrospun using HFIP as the solvent. (EX1015, 2778-79.) Moreover, HFIP is also the same solvent used in the ’223 patent. (EX1001, 19:40-55 (example 1), 22:1-23 (example 2), 22:25-48 (example 3), 22:50-23:4 (example 4).)

118. Given the above, a POSA would have understood that Huang2 discloses Claim 2’s limitations. Thus, it is my opinion that Huang2 anticipates Claim 2.

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

119. In my opinion, Huang2 teaches all the elements of Claim 3.

120. As discussed above, Huang2’s PCL/Collagen fibers have aligned fibers with major peaks about 180° apart, which discloses the scaffold according to Claim 1. (See §VI.A.1, *supra*.)

121. Huang2’s nanofibers have less than 8% by weight residual calcium. Indeed, Huang2 describes the materials used to prepare the nanofibers. (EX1005,

1792.) The 3:1 ratio of PCL/Collagen was prepared by dissolving PCL and collagen in 1,1,1,3,3,3-hexafluoro-2-propanol or “HFIP.” (EX1005, 1792.) Thus, Huang2 did not incorporate or otherwise use calcium in the production of its fibers at all. (EX1001, 11:2-4.) Therefore, the 3:1 PCL/Coll electrospun nanofibers used in Huang2 would necessarily contain less than about 8 wt % residual calcium.

122. Given the above, a POSA would have understood that Huang2 discloses Claim 3’s limitations. Thus, it is my opinion that Huang2 anticipates Claim 3.

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

123. In my opinion, Huang2 teaches all the elements of Claim 4.

124. As discussed above, Huang2’s PCL/Collagen fibers have aligned fibers with major peaks about 180° apart, which discloses the scaffold according to Claim 1. (*See §VI.A.1, supra.*)

125. A POSA would have also understood that Huang2 discloses a scaffold with an average porosity from about 60% to about 90%. A POSA’s knowledge is shown by Kumbar, a review article dated years earlier, that summarizes the point of view of a POSA concerning the desirable properties of a scaffold. Kumbar discusses the benefits of “high porosity” electrospun scaffolds and specifically describes electrospun scaffolds of collagen nanofibers having a

porosity of 71% to 89%, depending on the degree of crosslinking. (EX1006, 1, 6-7.) In my opinion and experience, a POSA would have been in possession of this knowledge. In this regard, Huang2 specifically cites Kumbar's review for its discussion of the benefits of "high porosity" electrospun scaffolds. (EX1005, 1791.) Huang2 explains that a goal of tissue-engineered skin grafts is to "define a cell-friendly microenvironment that maximally recaptures the native extracellular matrix (ECM)." (EX1005, 1791.) And the results of Huang2 show integrin activation and FAK signaling. Huang2 further explains that electrospun scaffolds with a "*high porosity*" have a "great potential to mimic the skin ECM in both morphology and composition." (EX1005, 1791 (Introduction) (emphasis added) (citing Kumbar).) Thus, Huang2 teaches "high porosity" scaffolds, which a POSA would have known includes the percentages of porosity that are within the range of 60% to 90% porosity.

126. Given the above, a POSA would have understood that Huang2 discloses Claim 4's limitations. Thus, it is my opinion that Huang2 anticipates Claim 4.

5. **Claim 5: “The scaffold according to claim 1, wherein the scaffold comprises collagen type I, bone matrix, adipose extracellular matrix, heart basement membrane extract, heart basement membrane extracellular matrix, placenta basement membrane extract, placenta basement membrane extracellular matrix, brain-derived extracellular matrix, polycaprolactone, an accessory polymer, or the mixture thereof.”**

127. In my opinion, Huang2 teaches all the required elements necessary to anticipate Claim 5.

128. As discussed above, Huang2’s PCL/Collagen fibers have aligned fibers with major peaks about 180° apart, which discloses the scaffold according to Claim 1. (*See* §VI.A.1, *supra*.)

129. Huang2’s scaffold comprises at least collagen type I, polycaprolactone, and the mixture thereof. For example, Huang2 discloses “Poly (epsilon-caprolactone) (PCL, Mw=80,000) . . . and type I collagen (Coll)” and fabrication of “[n]anofibrous matrices of PCL and Collagen.” (EX1005, 1792.)

130. Huang2 also discloses an “accessory polymer.” The ’223 patent specifically teaches that the use of polycaprolactone or “PCL” as an accessory polymer. (EX1001, 12:8-14.) The ’223 patent describes that an “accessory polymer” affects the “physical, chemical, and/or biological properties” of the scaffold, such as “tensile strength, hydrophilicity, biocompatibility.” (EX1001, 12:8-14.) Likewise, Huang2 discloses the use of polycaprolactone or “PCL” to

improve the stability and mechanical properties of the fibers, explaining that “[e]lectrospinning a blend solution of collagen and polycaprolactone (PCL), a biocompatible and biodegradable synthetic polyester, can yield rather stable nanofibers,” thereby avoiding drawbacks of pure collagen fibers. (*Id.*, 1791-92 (observing that pure collagen fibers degrade rapidly and have poor mechanical properties).)

131. Given the above, a POSA would have understood that Huang2 discloses Claim 5’s limitations. Thus, it is my opinion that Huang2 anticipates Claim 5.

6. **Claim 6: “The scaffold according to claim 5, wherein the accessory polymer is selected from the group consisting of polycaprolactone, poly(glycolic acid), poly(lactic acid), polydioxanone, poly(lactide-co-glycolide) copolymers, polyesters polysaccharides, polyhydroxyalkanoates, starch, polylactic acid, cellulose, proteins, agar, silks, alginate, collagen/gelatin, carrageenan, elastin, pectin, resilin, konjac, adhesives, gums, polyamino acids, polysaccharides, soy, zein, wheat gluten, casein, chitin/chitosan, serum albumin, hyaluronic acid, lipids/surfactants, xanthan, acetoglycerides, waxes, surfactants, dextran, emulsan, gelatin, polyphenols, levan, lignin, curd, inulin, tannin, polygalactosamine, humic acid, shellac, pullulan, poly-gamma-glutamic acid, elastin, natural rubber, yeast glucans, synthetic polymers from natural fats and oils.”**

132. In my opinion, Huang2 teaches all required elements necessary to anticipate Claim 6.

133. As discussed above, Huang2's PCL/Collagen fibers have aligned fibers with major peaks about 180° apart, which discloses the scaffold according to Claim 5. (*See* §VI.A.5, *supra*.)

134. Huang2's scaffold uses polycaprolactone as an accessory polymer. (*See* §VI.A.5, *supra*.) Indeed, Huang2 specifically uses polycaprolactone (PCL) for its blended collagen-PCL fibers. (EX1005, 1792.) Further, the '223 patent specification identifies "polycaprolactone" as an exemplary "accessory polymer." (EX1001, 12:7-26.)

135. Given the above, a POSA would have understood that Huang2 discloses Claim 6's limitations. Thus, it is my opinion that Huang2 anticipates Claim 6.

7. Claim 7: "The scaffold according to claim 1, wherein the scaffold comprises a bioactive factor."

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

137. As discussed above, Huang2's PCL/Collagen fibers have aligned fibers with major peaks about 180° apart, which discloses the scaffold according to Claim 1. (*See* §VI.A.1, *supra*.)

138. Huang2's scaffold comprises a "bioactive factor." The '223 patent specification provides that a "bioactive factor" is "protein, carbohydrate, or mineral that has any effect on a cellular activity," and lists "collagen" as an exemplary

“bioactive factor.” (EX1001, 12:44-67.) Huang2 discloses a scaffold comprising *collagen*. (EX1005, 1792 (disclosing “type I collagen (Coll)” and fabrication of “[n]anofibrous matrices of PCL and Collagen”).)

139. Additionally, Huang2 discloses how collagen affects cellular activity. Indeed, Huang2 studies how the alignment of collagen containing fibers affects the proliferation of fibroblasts (normal human dermal fibroblasts) on bioengineered scaffolds: “In this study, the phenotypic expressions of normal human dermal fibroblasts (NHDFs) on collagen-containing nanofibrous matrices with either isotropic (i.e., fibers collected randomly with no alignment) or anisotropic (i.e., fibers collected with alignment) fiber organizations were studied by immunostaining, migration assay and molecular analyses.” (EX1005, Abstract.) Huang2 discloses that “[c]ollagen fibrils, a major ECM [extracellular matrix] 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.) Similarly, Huang2 explains that “[t]o 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).” (*Id.*) Thus, collagen is a “bioactive factor.”

140. Given the above, a POSA would have understood that Huang2 discloses Claim 7's limitations. Thus, it is my opinion that Huang2 anticipates Claim 7.

8. **Claim 8: "The scaffold according to claim 7, wherein the bioactive factor is selected from the group consisting of an osteogenic growth factor, collagen, glycosaminoglycans, osteonectin, bone sialo protein, an osteoinductive factor, a chondrogenic factor, a cytokine, a mitogenic factor, a chemotactic factor, a transforming growth factor (TGF), a fibroblast growth factor (FGF), an angiogenic factor, an insulin-like growth factor (IGF), a platelet-derived growth factor (PDGF), an epidermal growth factor (EGF), a vascular endothelial growth factor (VEGF), a nerve growth factor (NGF), a neurotrophin, a bone morphogenetic protein (BMP), osteogenin, osteopontin, osteocalcin, cementum attachment protein, erythropoietin, thrombopoietin, tumor necrosis factor (TNF), an interferon, a colony stimulating factor (CSF), stem cell derived factor-1 (SDF-1), and an interleukin."**

141. In my opinion, Huang2 teaches all the required elements necessary to anticipate Claim 8.

142. As discussed above, Huang2's PCL/Collagen fibers have aligned fibers with major peaks about 180° apart, which discloses the scaffold according to Claim 7. (*See §VI.A.7, supra.*)

143. Huang2's scaffold uses collagen as a bioactive factor. (*See §VI.A.7, supra.*) Claim 8 requires that the bioactive factor is selected from a list including collagen.

144. 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.

9. Claim 9: "The scaffold according to claim 1, wherein the scaffold comprises an extracellular matrix component."

145. In my opinion, Huang2 teaches all the elements of Claim 9.

146. As discussed above, Huang2's PCL/Collagen fibers have aligned fibers with major peaks about 180° apart, which discloses the scaffold according to Claim 1. (*See §VI.A.1, supra.*)

147. Huang2's scaffold comprises an extracellular matrix component. The '223 patent discusses that the "extracellular matrix component" that can be part of the "aligned fiber, electrospun fiber, and/or scaffold" "include . . . *collagen*" (EX1001, 14:64-15:3 (emphasis added).) Huang2 discloses the use of collagen in its scaffold. (EX1005, 1792 (disclosing fabrication of "[n]anofibrous matrices of PCL and Collagen"), 1794 ("Using the established electrospinning condition as described in Materials and Methods, PCL/Coll nanofibers [were] collected on a rotating mandrel or on a stationary surface had distinct spatial arrangements.").)

148. Huang2 also teaches that collagen is an extracellular matrix component. For example, Huang2 teaches 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.) Likewise, Huang2 teaches that, “[t]o better mimic skin ECM, electrospun collagen fibers have been fabricated.” (EX1005, 1791.)

149. Given the above, a POSA would have understood that Huang2 discloses Claim 9’s limitations. Thus, it is my opinion that Huang2 anticipates Claim 9.

10. Claim 10: “The scaffold according to claim 9, wherein the extracellular matrix component is selected from the group consisting of collagen, glycosaminoglycans, osteocalcin, osteonectin, bone sialo protein, osteopontin, fibronectin, laminin, and mixtures thereof.”

150. In my opinion, Huang2 teaches all required elements necessary to anticipate Claim 10.

151. As discussed above, Huang2’s PCL/Collagen fibers have aligned fibers with major peaks about 180° apart, which discloses the scaffold according to Claim 9. (*See* §VI.A.9, *supra*.)

152. Huang2’s scaffold comprises collagen. (*See* §VI.A.9.) Claim 10 requires that the extracellular matrix component is selected from a list including collagen.

153. Given the above, a POSA would have understood that Huang2 discloses Claim 10's limitations. Thus, it is my opinion that Huang2 anticipates Claim 10.

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

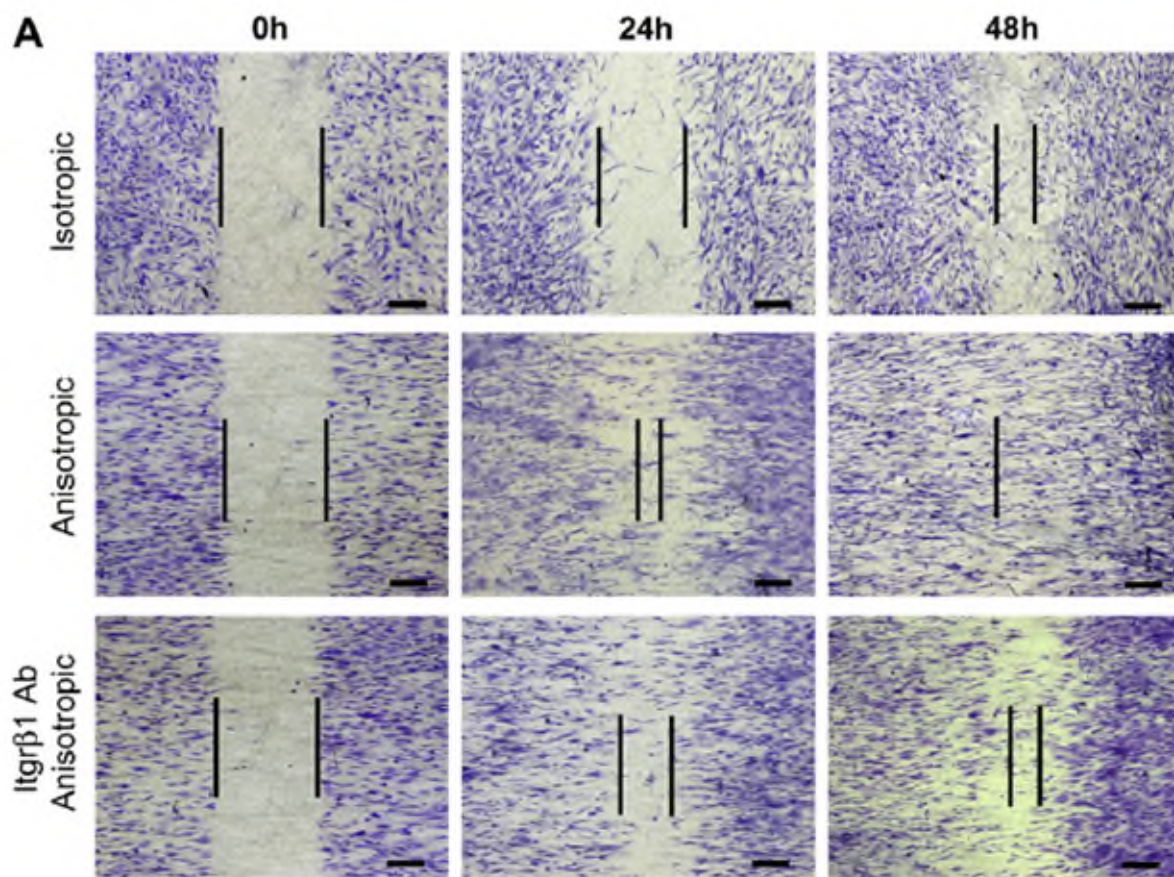
154. In my opinion, Huang2 teaches all the elements of Claim 11.

155. As discussed above, Huang2's PCL/Collagen fibers have aligned fibers with major peaks about 180° apart, which discloses the scaffold according to Claim 1. (*See §VI.A.1, supra.*)

156. Huang2 discloses a method of cell culture comprising culturing cells on its scaffold under conditions suitable for cell culture. For instance, Huang2 teaches culturing fibroblasts under suitable conditions such that the cells grow. 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.) These cells attached, proliferated, and migrated. (EX1005, 1797.) Huang2 further teaches 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.)

157. 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.)



158. Given the above, a POSA would have understood that Huang2 discloses Claim 11's limitations. Thus, it is my opinion that Huang2 anticipates Claim 11.

12. Claim 12: "The method of claim 11, wherein the cells are selected from the group consisting of stem cells, adipose derived stem cells, dental pulp stem cells, fibroblasts, and dorsal root ganglia"

159. In my opinion, Huang2 teaches all the required elements necessary to anticipate Claim 12.

160. As discussed above, Huang2 discloses a method of cell culture comprising culturing cells on its scaffold, which has PCL/Collagen aligned fibers with major peaks about 180° apart, under conditions suitable for cell culture. (See §VI.A.1, *supra*.)

161. Huang2's discloses culturing fibroblasts on its scaffold. Claim 12 requires culturing a specific type of cell from a list that includes fibroblasts. Huang2 specifically discloses culturing fibroblasts on its scaffolds, noting that the "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.)

162. Given the above, a POSA would have understood that Huang2 discloses Claim 12's limitations. Thus, it is my opinion that Huang2 anticipates Claim 12.

B. Point 2: Claim 4 Is Obvious Over Huang2 In View of Kumbar

163. It is my opinion that Huang2 in view of Kumbar further renders claim 4 obvious.

164. Claim 4 recites "[t]he scaffold according to claim 1, wherein the scaffold has an average porosity from about 60% to about 90%."

165. As discussed above, Huang2 discloses a scaffold of electrospun fibers that include collagen that have aligned fibers with major peaks about 180° apart, as required by claim 1 of the '223 patent. (*See* §VI.A.1, *supra*.)

166. Regarding the porosity, Huang2 explains that a goal of tissue-engineered skin grafts is to "define a cell-friendly microenvironment that maximally recaptures the native extracellular matrix (ECM). In this regard, electrospun nanofibrous matrices characterized by ultrafine continuous nanoscale fibers, high surface-to-volume ratio, *high porosity*, and variable pore-size distribution, have great potential to mimic the skin ECM in both morphology and composition [2, 3]." (EX1005, 1791)(emphasis added).) Huang2 cites Kumbar in for this teaching. Specifically, reference [3] is a citation to Kumbar.

167. Kumbar teaches methods of designing electrospun nanofiber matrix scaffolds “to provide cells with the necessary chemical cues and a more *in vivo* like environment” for use “in the regeneration of various soft tissues including skin[.]” (EX1006, 1 (Abstract).) Kumbar teaches that a “high porosity” is desirable because it more closely approximates the natural extra-cellular matrix or “ECM.” (*Id.*)

168. In its discussion of electrospun nanofiber skin grafts, Kumbar teaches that “collagen nanofibers play a dominant role in maintaining the biological and structural integrity of various tissues and organs, including bone, skin, tendon, blood vessels and cartilage.” (EX1006, 7.) Kumbar discloses that the scaffold of electrospun collagen type I fibers had a porosity of 89%. Kumbar also teaches that it can be useful to cross-link collagen type I nanofibers and that such cross-linked fibers were similar in strength to certain commercially available wound care products. (*Id.*) When these collagen fibers were cross-linked, the porosity decreased to 71%. (*Id.*) Therefore, with respect to fibers comprising collagen in electrospun nanofiber skin grafts, Kumbar discloses porosity of samples at 89% or 71%, if crosslinked. (*Id.*, 7.) Both of these high porosity values are within the claim 4’s range.

169. Thus, a POSA who desired to make aligned collagen blended fibers (as taught by Huang²) with “high porosity” (as also taught by Huang²) would have

been motivated to look at the reference cited by Huang2 in support of the “high porosity.” Because Huang2 cites Kumbar for this point, a POSA would have been motivated to combine Huang2 and Kumbar. As discussed above, Kumbar teaches the desirability of high porosity and further discloses ranges of porosity within the scope of claim 4, including collagen fiber scaffolds that have a porosity of 89% or 71%. Thus, a POSA would have been motivated to make Huang2’s scaffold with a porosity between 71% and 90%.

170. A POSA would have had additional motivations to combine Huang2 and Kumbar to obtain Huang2’s scaffold with a porosity between 71% and 90%. For example, both Huang2 and Kumbar are in the same field: the use of electrospun collagen-containing scaffolds for biomedical applications, such as in regenerative medicine (e.g., skin grafts). (§§V.A-B.) Further, both references also note that “electrospun nanofibrous matrices . . . characterized by . . . high porosity and variable pore-size distribution” show morphological similarities to natural skin ECM. (EX1005, 1791; EX1006, 1, 6, 12.) Moreover, Huang2’s and Kumbar’s discussion of the benefits of mimicking the porosity of the native skin ECM would further motivate a POSA to combine the teachings of Kumbar modify Huang2’s disclosed scaffolds to have porosity of 89% or 71%. (See Kumbar, 6-7.)

171. A POSA would have had an expectation of success in combining Huang2 and Kumbar to achieve a scaffold with a porosity from about 60% to about

90%. Tailoring the porosity of electrospun fibers is well within the knowledge of a POSA, for example by using well-known methods including leaching, nucleation, and laser shaping. (EX1011, [0055]-[0056].) Such techniques have long been used on electrospun scaffolds, are well-understood, and allow a POSA to achieve the predictable result of a desired porosity (e.g., about 60-90%) with a reasonable expectation of success. (EX1011, [0055]-[0056].)

172. Accordingly, it is my opinion that Huang² in view of Kumbar renders obvious all of the elements of claim 4.

C. Point 3: Lee Anticipates Claims 1-12

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

173. In my opinion, Lee teaches all the elements of Claim 1.

(a) “A scaffold comprising:

174. Lee teaches a scaffold.

175. Lee discusses extensively the use and preparation of scaffolds, demonstrating that scaffolds were well-known in the art. (*Id.*, [0005], [0044]-[0059].) For example, Lee teaches that:

- “[Fibers] include natural polymers (e.g., collagen, elastin, etc.) and/or “synthetic polymers (e.g., polycaprolactone (PCL)).” (EX1011, [0005].)
- A scaffold “refers to an array of natural or synthetic matrix of molecules to which cells or fibers can attach.” (EX1011, [0044].)
- “[A] composite scaffolding system including PCL and collagen has been developed that possess excellent cell compatibility as well as maintains structural integrity (Lee et al. (2008).” (*Id.*, [0050]; *see also id.* [0055] (discussing additional disclosures of scaffolds in the prior art).)
- Scaffolds can be biodegradable and made of biodegradable materials, which include PCL and collagen. (*Id.*, [0045]-0046].)
- “A ‘biodegradable scaffold,’ ‘biodegradable mesh’ or ‘biodegradable matrix’ is a scaffold having materials capable of being degraded and/or absorbed in a subject’s body.” (*Id.*, [0045].)

176. Therefore, a POSA would have understood that Lee discloses this claim limitation.

(b) “one or more electrospun fibers comprising collagen”

177. Lee teaches a scaffold comprising electrospun fibers comprising collagen.

178. As discussed above, Lee discloses that collagen and fibrin materials have been used for scaffolding systems. (§V.C, *supra.*) Additionally, Lee disclosed the below about electrospun collagen scaffolds:

- Lee noted that collagen “biomaterials are limited by the tissue size that may be formed.” (EX1011, [0052].) Thus, Lee taught that, in some embodiments, “aligned electrospun mesh included a mixture or blend of PCL and/or collagen is combined with primary cultured human skeletal muscle cells for skeletal muscle cells for skeletal regeneration.” (*Id.*, [0052].)
- Lee discloses that “PCL-collagen composite scaffolds according to some embodiments have preferable mechanical properties . . .” (*Id.*) As discussed above, Lee describes “a mesh” as a scaffold. (*See* §V.C, *supra.*)
- Lee also uses collagen type 1 in the preparation of the scaffold. (EX1011, [0062].)
- Further, Lee discloses that “biodegradable scaffolds are formed by the electrospinning technique.” (*Id.*, [0054].) As described above, biodegradable materials used in Lee’s biodegradable scaffolds include collagen. (*See* §V.C, *supra.*)

- Lee teaches that “electrospinning is known in the art and has been used in the fabrication of tissue engineered scaffolds.” (EX1011, [0054].)
- Lee discloses that the electrospun fibers in some embodiments are nanofibers that “have an average diameter from 10, 100 or 200 nm to 400, 500, 750 or 1000 nanometers.” (*Id.*)
- Lee also teaches that “electrospun nanofiber meshes were fabricated using a blend of PCL and collagen with a ratio of 1:1 in weight.” (*Id.*, [0063].)

179. Therefore, a POSA would have understood that Lee discloses this claim limitation.

(c) “wherein a fast Fourier transform (FFT) analysis result of the fibers have adjacent major peaks with about 180° apart from each other”

180. Lee teaches a scaffold wherein a fast Fourier transform (FFT) analysis result of the fibers have adjacent major peaks with about 180° apart from each other.

181. 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.C., *supra*.)

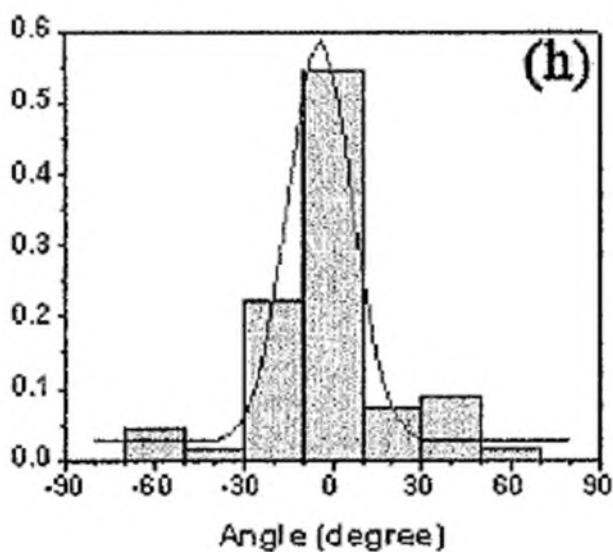
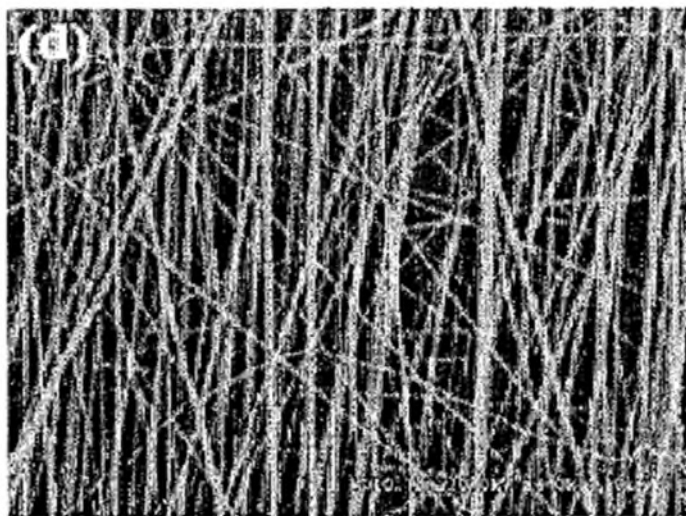
182. Lee discloses “PCL/collagen nanofiber meshes with different fiber angles . . . produced by electrospinning at various rotation rates of 0 (static), 800, 1500, and 2350 rpm. Progressive increase in fiber orientation was observed as the rotation rate increased (FIG. 1 b). (EX1011, [0080].) Lee further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

183. Lee determined fiber orientation. Specifically, “fiber morphologies of the electrospun PCL/collagen nanofiber meshes were examined by scanning electron microscopy (SEM; Model S-2260N, Hitachi Co., Ltd, Japan.).” (*Id.*, [0065].) Further, “the SEM images were analyzed with UTHSCSA ImageTool 3.0 (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).) In Lee’s histogram, 0 degrees represents that a single fiber is aligned, and

the further away from 0 degrees, the less aligned that the individual fibers are.

(EX1011, Fig. 9, [0088].)

184. 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 scaffold in both Lee and its provisional are the same. (*E.g., compare* Lee, [0062]-[0095] (materials and methods, results, discussion, conclusion *with* Lee Provisional, 14-24 (substantively identical).) The image analysis from Figures 1D and 1H reveals that Lee demonstrates highly aligned fibers. (*Id.*, [0012].)

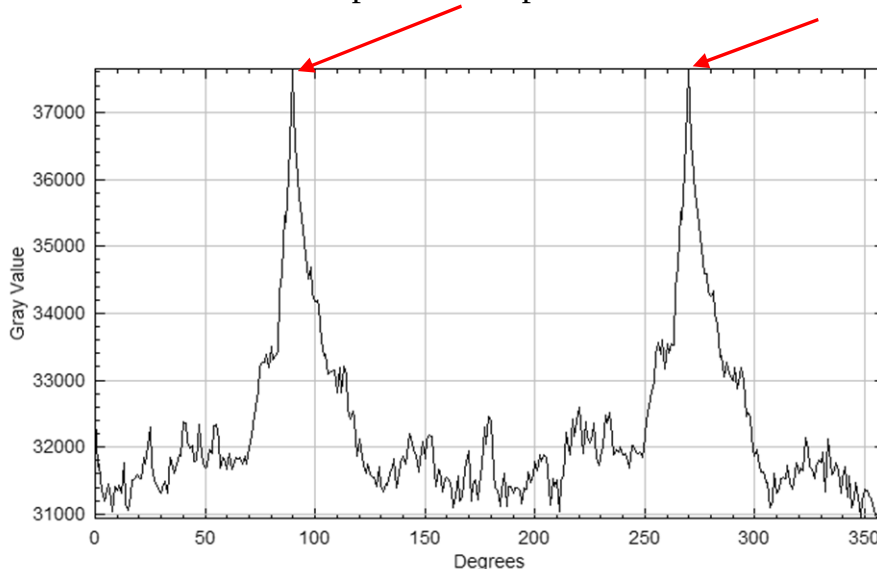
185. Lee used ImageTool 3.0, freeware provided by the University of Texas Health Sciences Center in San Antonio, TX, USA, to measure the degree of fiber alignment.

186. A POSA would have understood that the degree of alignment could also have been determined using the method discussed in the '223 patent and detailed in Ayres 2008 (EX1010). (EX1001, 13:16-24; §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.*)

187. 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 along each radius for 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.)

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



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

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

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

192. Therefore, a POSA would have understood that Lee discloses this claim limitation.

193. Given that a POSA would have understood that Lee discloses all of the claim limitations, it is my opinion that Lee anticipates Claim 1.

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

194. In my opinion, Lee teaches all the elements of Claim 2.

195. As discussed above, Lee discloses PCL/Collagen fibers that have aligned fibers with major peaks about 180° apart, which discloses the scaffold according to Claim 1. (*See* §VI.C.1, *supra*.)

196. Lee also teaches the “native triple helical structure” element of the claim. Lee teaches that it obtained “[c]ollagen type I derived from calf skin,” which “was purchased from Elastin Products Company.” (EX1011, [0062].) Type I collagen was well-known and generally understood in the art to have a triple-helical form when it is in a monomer, as well as a polymer. (EX1017, 9.) A POSA

would have understood that, if the type 1 collagen purchased from a supplier were denatured and/or fragmented (i.e., not in a triple helix) it would be sold as “gelatin,” not “collagen.” Additionally, Lee teaches that its scaffold was prepared by dissolving PCL and collagen in 1,1,1,3,3,3-hexafluoro-2-propanol or “HFIP.” (EX1011, [0062].) (EX1005, 1792.) HFIP was known to stabilize collagen’s triple helix structure. (EX1015, 3782.) For example, the prior art demonstrated that collagen purchased from Elastin Products maintained its native triple helical structure when electrospun using HFIP as the solvent. (EX1015, 2778-79.) Moreover, HFIP is also the same solvent used in the ’223 patent. (EX1001, 19:40-55 (example 1), 22:1-23 (example 2), 22:25-48 (example 3), 22:50-23:4 (example 4).)

197. Given the above, a POSA would have understood that Lee discloses Claim 2’s limitations. Thus, it is my opinion that Lee anticipates Claim 2.

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

198. In my opinion, Lee teaches all the elements of Claim 3.

199. As discussed above, Lee discloses PCL/Collagen fibers that have aligned fibers with major peaks about 180° apart, which discloses the scaffold according to Claim 1. (*See §VI.C.1, supra.*)

200. Lee discloses nanofibers that have less than 8% by weight residual calcium. Lee discloses fabricating “electrospun nanofiber meshes using a blend of PCL and collagen with a ratio of 1:1 in weight.” (EX1011, [0063].) Lee lists “[c]ollagen type I derived from calf skin,” “poly(μ -caprolactone),” “1,1,1,3,3,3-hexafluoro-2-propanol (HFP),” and a “2.5% glutaraldehyde solution” crosslinker materials used to prepare its electrospun nanofiber scaffolds. (EX1011, [0062]-[0063].) Thus, Lee did not incorporate or otherwise use calcium in the production of its fibers at all. (EX1001, 11:2-4.) Therefore, the 3:1 PCL/Coll electrospun nanofibers used in Lee would necessarily contain less than about 8 wt % residual calcium.

201. Given the above, a POSA would have understood that Lee discloses Claim 3’s limitations. Thus, it is my opinion that Lee anticipates Claim 3.

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

202. In my opinion, Lee teaches all the elements of Claim 4.

203. As discussed above, Lee discloses PCL/Collagen fibers that have aligned fibers with major peaks about 180° apart, which discloses the scaffold according to Claim 1. (*See* §VI.C.1, *supra*.)

204. Lee discloses the porosity element of this claim. Lee teaches “[e]lectrospun nanofiber scaffolds [that]...have a three-dimensional architecture,

high porosity with an interconnected pore structure, and a high surface-to-volume ratio, which provides a favorable environment for the cells to grow.” (EX1011, [0055] (emphasis added).) Lee explains that to achieve the desired “composition, structure, and mechanical properties,” its “biodegradable scaffold can be ‘shaped’ using methods such as...leaching,” “nucleation,” and “laser shaping.” (EX1011, [0055]-[0056].) Lee provides that the salt leaching process involves spreading “a solution containing two materials...into a shape that resembles the final form” and “dissolv[ing] away one of the components, resulting in pore formation (see U.S. Pat. No. 5,514,378 [Mikos]).” (*Id.*, [0056] (citing Mikos (EX1014)), [0021] (incorporating all cited patents by reference).) Lee incorporates Mikos by reference, which describes the salt-leaching process in greater detail. In addition, Mikos teaches that “[H]igh porosity, greater than 80% volume, provides adequate space for cell seeding, growth, and extracellular matrix (ECM) production.” (EX1014, 13:15-17.) Thus, Lee, by itself and through its incorporation of Mikos, discloses high porosity values that are within the range of porosity recited by claim 4.

205. Given the above, a POSA would have understood that Lee discloses Claim 4’s limitations. Thus, it is my opinion that Lee anticipates Claim 4.

5. **Claim 5: “The scaffold according to claim 1, wherein the scaffold comprises collagen type I, bone matrix, adipose extracellular matrix, heart basement membrane extract, heart basement membrane extracellular matrix, placenta basement membrane extract, placenta basement membrane extracellular matrix, brain-derived extracellular matrix, polycaprolactone, an accessory polymer, or the mixture thereof.”**

206. In my opinion, Lee teaches all the required elements necessary to anticipate Claim 5.

207. As discussed above, Lee discloses PCL/Collagen fibers that have aligned fibers with major peaks about 180° apart, which discloses the scaffold according to Claim 1. (*See* §VI.C.1, *supra*.)

208. Lee discloses a scaffold comprising at least collagen type I, polycaprolactone, and the mixture thereof. (EX1011, [0062] (listing “Collagen type I derived from calf skin” and “Poly(μ-caprolactone)” as components in Lee’s electrospinning solution), [0063] (“Electrospun nanofiber meshes were fabricated using a blend of PCL and collagen.”); *see also* Lee [0005]-[0007] (disclosing “a biodegradable scaffold comprising a plurality of fibers...[that] include natural polymers (e.g., collagen, elastin, etc.) and/or synthetic polymers (e.g., polycaprolactone (PCL)...).”.)

209. Lee also discloses an “accessory polymer.” The ’223 patent specifically teaches that the use of polycaprolactone or “PCL” as an accessory

polymer. (EX1001, 12:8-14.) The '223 patent describes that an “accessory polymer” affects the “physical, chemical, and/or biological properties” of the scaffold, such as “tensile strength, hydrophilicity, biocompatibility.” (EX1001, 12:8-14.) Likewise, Lee discloses that “PCL-collagen composite scaffolds . . . have preferable biomechanical properties, *e.g.*, yield tensile strength, elasticity, and compliance. PCL is [a] biodegradable synthetic polymer that supports high mechanical properties, especially, high elongation, but has lower biocompatibility compared to natural polymer such as collagen and elastin. Collagen has a good biocompatibility, but poor mechanical properties and processability. When PCL and collagen are combined together, the mesh can be improved biocompatibility, mechanical properties, and processability.” (EX1011, [0052]; *see also* Lee, [0050].)

210. Given the above, a POSA would have understood that Lee discloses Claim 5’s limitations. Thus, it is my opinion that Lee anticipates Claim 5.

6. **Claim 6: “The scaffold according to claim 5, wherein the accessory polymer is selected from the group consisting of polycaprolactone, poly(glycolic acid), poly(lactic acid), polydioxanone, poly(lactide-co-glycolide) copolymers, polyesters polysaccharides, polyhydroxyalkanoates, starch, polylactic acid, cellulose, proteins, agar, silks, alginate, collagen / gelatin, carrageenan, elastin, pectin, resilin, konjac, adhesives, gums, polyamino acids, polysaccharides, soy, zein, wheat gluten, casein, chitin / chitosan, serum albumin, hyaluronic acid, lipids / surfactants, xanthan, acetoglycerides, waxes, surfactants, dextran, emulsan, gelatin, polyphenols, levan, lignin, curd, inulin, tannin, polygalactosamine, humic acid, shellac, pullulan, poly-gamma-glutamic acid, inulin, natural rubber, yeast glucans, synthetic polymers from natural fats and oils.”**

211. In my opinion, Lee teaches all the required elements necessary to anticipate Claim 6.

212. As discussed above, Lee discloses PCL/Collagen fibers that have aligned fibers with major peaks about 180° apart, which discloses the scaffold according to Claim 5. (*See §VI.C.5, supra.*)

213. Lee discloses a scaffold that uses polycaprolactone as an accessory polymer. (*See §VI.C.5, supra.*) Indeed, Lee specifically uses polycaprolactone (PCL) for its blended collagen-PCL fibers. (EX1011, [0052]; *see also* Lee, [0050].) Further, the '223 patent specification identifies “polycaprolactone” as an exemplary “accessory polymer.” (EX1001, 12:7-26.)

214. Given the above, a POSA would have understood that Lee discloses Claim 6's limitations. Thus, it is my opinion that Lee anticipates Claim 6.

7. Claim 7: The scaffold according to claim 1, wherein the scaffold comprises a bioactive factor.”

215. In my opinion, Lee teaches all the elements of Claim 7.

216. As discussed above, Lee discloses PCL/Collagen fibers that have aligned fibers with major peaks about 180° apart, which discloses the scaffold according to Claim 1. (*See* §VI.C.1, *supra*.)

217. Lee discloses a scaffold comprising a “bioactive factor.” The ’223 patent specification provides that a “bioactive factor” is a “protein, carbohydrate, or mineral that has any effect on a cellular activity,” and lists “collagen” as an exemplary “bioactive factor.” (EX1001, 12:44-67.) Lee teaches that its electrospun scaffolds may be formed with natural polymers such as collagen for biocompatibility, cell adhesion, proliferation, and differentiation. (EX1011, [0095], [0005]-[0007], [0052]; *see also id.* [0062]-[0063] (“Electrospun nanofiber meshes were fabricated using a blend of PCL and collagen.”).) Lee further teaches that its “biodegradable scaffold can be treated with additives or drugs prior to implantation (before or after it is seeded with cells), e.g., to promote the formation of new tissue after implantation.” (EX1011, [0057].) Additionally, Lee teaches that additives “can be added to the biodegradable scaffold to promote graft healing and the formation of new tissue.” (EX1011, [0057].) Those additives include “growth factors, cytokines, extracellular matrix components, and/or other bioactive

materials” and “will in general[] be selected according to the tissue or organ being reconstructed or augmented, to ensure that appropriate new tissue is formed in the engrafted organ or tissue.” (*Id.*)

218. As described above, collagen is a bioactive factor. (EX1011, [0052] (“Collagen has a good biocompatibility.”).) Elastin and fibrin, as well as growth factors, cytokines, extracellular matrix components, and/or other bioactive materials are also bioactive factors. They are proteins, carbohydrates, or minerals that have effects on a cellular activity. (EX1001, 12:44-67.)

219. Given the above, a POSA would have understood that Lee discloses Claim 7’s limitations. Thus, it is my opinion that Lee anticipates Claim 7.

8. **Claim 8: “The scaffold according to claim 7, wherein the bioactive factor is selected from the group consisting of an osteogenic growth factor, collagen, glycosaminoglycans, osteonectin, bone sialo protein, an osteoinductive factor, a chondrogenic factor, a cytokine, a mitogenic factor, a chemotactic factor, a transforming growth factor (TGF), a fibroblast growth factor (FGF), an angiogenic factor, an insulin-like growth factor (IGF), a platelet-derived growth factor (PDGF), an epidermal growth factor (EGF), a vascular endothelial growth factor (VEGF), a nerve growth factor (NGF), a neurotrophin, a bone morphogenetic protein (BMP), osteogenin, osteopontin, osteocalcin, cementum attachment protein, erythropoietin, thrombopoietin, tumor necrosis factor (TNF), an interferon, a colony stimulating factor (CSF), stem cell derived factor-1 (SDF-1), and an interleukin.”**

220. In my opinion, Lee teaches all the required elements necessary to anticipate Claim 8.

221. As discussed above, Lee discloses PCL/Collagen fibers that have aligned fibers with major peaks about 180° apart, which discloses the scaffold according to Claim 7. (*See §VI.C.7, supra.*)

222. The scaffold that Lee discloses comprises collagen as a bioactive factor. (*See §VI.C.7, supra.*) Claim 8 requires that the bioactive factor is selected from a list including collagen.

223. Therefore, a POSA would have understood that Lee discloses this claim limitation.

224. Given the above, a POSA would have understood that Lee discloses Claim 8's limitations. Thus, it is my opinion that Lee anticipates Claim 8.

9. Claim 9: "The scaffold according to claim 1, wherein the scaffold comprises an extracellular matrix component."

225. In my opinion, Lee teaches all the elements of Claim 9.

226. As discussed above, Lee discloses PCL/Collagen fibers that have aligned fibers with major peaks about 180° apart, which discloses the scaffold according to Claim 1. (*See §VI.C.1, supra.*)

227. The scaffold that Lee discloses comprises an extracellular matrix component. The '223 describes "extracellular matrix components" that can be part of the "aligned fiber, electrospun fiber, and/or scaffold" as "includ[ing], but not limited to, *collagen*, glycosaminoglycans, osteocalcin, osteonectin, bone sialo protein, osteopontin, fibronectin, laminin, and mixtures thereof." (EX1001, 14:64-15:3 (emphasis added).) Lee discloses that its "[s]caffolds may include *extracellular matrix components*, such as elastin, elastin-like or elastin-mimetic peptides, fibrin, proteoglycans, commercially available matrix or matrix-substitutes..., collagen of any type, synthetic fibers or fibrils, or bioactive hydrogels" or be made of "fibers...include[ing] extracellular matrix molecules or components, such as elastin, elastic strands or peptides, fibrin, collagen, proteoglycans, hyaluronan or hyaluronan oligomers, synthetic fibers or fibrils, or

bioactive hydrogels, microparticles, beads, liposomes, or vesicles.” (EX1011, [0044] (emphasis added).) And Lee explains that “extracellular matrix components . . . can be added to the biodegradable scaffold to promote graft healing and the formation of new tissue.” (*Id.*, [0057].)

228. Given the above, a POSA would have understood that Lee discloses Claim 9’s limitations. Thus, it is my opinion that Lee anticipates Claim 9.

10. Claim 10: “The scaffold according to claim 9, wherein the extracellular matrix component is selected from the group consisting of collagen, glycosaminoglycans, osteocalcin, osteonectin, bone sialo protein, osteopontin, fibronectin, laminin, and mixtures thereof.”

229. In my opinion, Lee teaches all the required elements necessary to anticipate Claim 10.

230. As discussed above, Lee discloses PCL/Collagen fibers that have aligned fibers with major peaks about 180° apart, which discloses the scaffold according to Claim 1. (*See* §VI.C.1, *supra.*)

231. Lee discloses a scaffold that comprises at least collagen or proteoglycan. Claim 10 requires that the extracellular matrix component is selected from a list including collagen and proteoglycan. Specifically, Lee discloses “[s]caffolds [that] may include extracellular matrix molecules or components, such as . . . proteoglycan . . . [or] collagen of any type,” and mixtures thereof. (EX1011, [0044].)

232. Given the above, a POSA would have understood that Lee discloses Claim 10's limitations. Thus, it is my opinion that Lee anticipates Claim 10.

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

233. In my opinion, Lee teaches all the elements of Claim 11.

234. As discussed above, Lee discloses PCL/Collagen fibers that have aligned fibers with major peaks about 180° apart, which discloses the scaffold according to Claim 1. (*See* §VI.C.1, *supra*.)

235. Lee discloses a method of cell culture comprising culturing cells on the scaffold under conditions suitable for cell culture. Specifically, Lee discloses:

- "[S]eeding...skeletal muscle cells on a scaffold comprising oriented [collagen-containing] fibers" and "growing said cells on said scaffold in a growth medium," which "is a high serum medium that promotes the expansion of cells with or without minimal differentiation."
(EX1011, Claims 16, 18, 20, [0037], [0007].)
- "Seeding of cells onto the biodegradable scaffold may be performed according to standard methods [describing standard seeding methods.]").) (EX1011, [0058]-[0059].)

- “Lee notes that “the muscle cells readily adhered and proliferated for up to 7 days in culture, indicating that the nanofiber meshes were conducive to cell adhesion and proliferation.” (EX1011, [0083].)
- “For cell culture studies, the electrospun PCL/collagen nanofiber meshes were placed into 6-well plates, gas sterilized by ethylene oxide...at 30° C. for 36 hr, and incubated at 37° C. for 30 min in culture medium prior to cell seeding.”). (EX1011, [0064].)
- A figure that depicts human skeleton muscle cell adhesion and proliferation on the electrospun PCL/collagen nanofibers. (EX1011, Fig. 4, [0014].)

236. Given the above, a POSA would have understood that Lee discloses Claim 11’s limitations. Thus, it is my opinion that Lee anticipates Claim 11.

12. Claim 12: “The method of claim 11, wherein the cells are selected from the group consisting of stem cells, adipose derived stem cells, dental pulp stem cells, fibroblasts, and dorsal root ganglia”

237. In my opinion, Lee teaches all the required elements necessary to anticipate Claim 12.

238. As discussed above, Lee discloses a method of cell culture comprising culturing cells on the scaffold that has PCL/Collagen aligned fibers with major

peaks about 180° apart, under conditions suitable for cell culture. (See §VI.C.11, *supra*.)

239. Lee discloses culturing the particular cell types required by claim 12 on the scaffold. Claim 12 requires that the cells that are cultured on the scaffold include “stem cells.” Lee teaches that “[s]keletal muscle cells may be provided by isolating cells from skeletal muscle tissue harvested from the skeletal muscle of an organism, differentiating *stem* or progenitor cells (e.g., embryonic or amniotic fluid stem cells), etc.” (EX1011, [0040] (emphasis added).) Lee also discloses that “[s]keleton muscle cells include . . . myoblasts.” Lee defines “myoblasts” as “a type of *muscle stem cell* . . . [that] are normally closely associated with myofibers during the course of their life cycle” (*Id.*, [0030] (emphasis added).)

240. Given the above, a POSA would have understood that Lee discloses Claim 12’s limitations. Thus, it is my opinion that Lee anticipates Claim 12.

VII. CLAIMS 1-12 ARE OBVIOUS IN VIEW OF LEE ALONE

241. In my opinion, Lee discloses each and every element of Claims 1-12, including the above element, and anticipates each of these claims. (§VI.C., *supra*.) Additionally, in my opinion, for claim 1’s required element that “wherein a fast Fourier transform (FFT) analysis result of the fibers have adjacent major peaks with about 180° apart from each other,” the POSA would have been motivated to run an FFT analysis on Lee’s aligned scaffold, and expected success in doing so.

Such an FFT analysis would have obtained major peaks that are about 180° apart from each other, as required by the claim element.

242. As I discussed above, in Lee, the investigators investigated the impact of alignment and the orientation of electrospun PCL/collagen nanofibers on the performance and properties of electrospun scaffolds. (EX1011, [0060], [0061], [0080]; *see* §V.C., *supra*.) For example, Lee discloses that “fiber morphologies of the electrospun PCL/collagen nanofiber meshes were examined by scanning electron microscopy (SEM; Model S-2260N, Hitachi Co., Ltd, Japan).” (EX1011, [0065].) Lee concluded that the fibers obtained were aligned through 1) mechanical testing and 2) calculating the angles of alignment for the fibers. (*Id.*, [0065], [0067], [0081]; Fig. 1H.)

243. As I discussed above, (*see* §VI.C.1c., *supra*), to determine fiber orientation in Lee, Fig 1H, Lee’s “SEM images were analyzed with UTHSCSA ImageTool 3.0 (Freeware provided by the University of Texas Heal Sciences Center, San Antonio, Tex., USA).” (*Id.*, [0066] (emphasis added).) Additionally, by March 2013, it was well within the ordinary knowledge of a POSA that “FFT analysis” and ImageJ software could be run on Lee’s SEM images to confirm the degree of alignment. (§III.D., *supra*.) At that time, 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 with a well-known tool. And a POSA would have more than a reasonable expectation of success of successfully running an FFT on Lee's SEM images. Indeed, I was able to run such an analysis. (See §VI.C.1.3, *supra*.)

244. My FFT analysis shows clear adjacent major peaks at around 90° and 270°, which are 180° apart from each other, and thus showing that Lee's collagen/PCL fibers fall within the scope of claim 1. (¶¶188-190, *supra*.)

245. Thus, in my opinion, claims 1-12, which require “wherein a fast Fourier transform (FFT) analysis result of the fibers have adjacent major peaks with about 180° apart from each other,” would have been obvious based on Lee alone, when viewed from the perspective of a POSA. (See §VI.C.1-12 (EX1011 discloses all elements of claims 1-12), *supra*.)

VIII. CONCLUSION

246. For the reasons set forth above, it is my opinion that Claims 1-12 of the '223 Patent are unpatentable in view of the prior art based on the following points:

1. Claims 1-12 are anticipated by Huang2.
2. Claim 4 is obvious in light of Huang2 in view of Kumbar.
3. Claims 1-12 are anticipated by Lee.
4. Claims 1-12 are obvious over Lee alone.

247. In short, as discussed above, Huang2 discloses each and every limitation of Claims 1-12. Specifically, Huang2 discloses:

- An electrospun scaffold;
- Comprising type 1 collagen in its native triple helical structure and polycaprolactone;
- Wherein a fast Fourier transform (FFT) analysis result of the fibers have adjacent major peaks with about 180° apart from each other;
- Comprising less than 8% by weight of residual calcium;
- With high porosity;
- And culturing fibroblasts on that scaffold.

248. In short, as discussed above, Huang2 in view of Kumbar would have motivated the POSA to make a scaffold like Huang2's scaffold with a high porosity like the 71% and 89% porosity scaffolds disclosed in Kumbar to be high porosity, and expected success in doing so. Indeed, Huang2 cites Kumbar in its discussion of high porosity.

249. In short, as discussed above, Lee discloses each and every limitation of Claims 1-12. Specifically, Lee discloses:

- An electrospun scaffold;
- Comprising type 1 collagen in its native triple helical structure and polycaprolactone;
- Wherein a fast Fourier transform (FFT) analysis result of the fibers have adjacent major peaks with about 180° apart from each other;
- Comprising less than 8% by weight of residual calcium;
- With high porosity;
- And culturing stem cells on that scaffold.

250. In short, as discussed above, a POSA would have been motivated to run an FFT analysis on Lee's aligned scaffold to confirm the alignment using a well-known tool, and expected success in being able to run an FFT analysis. That FFT analysis would have obtained major peaks that are about 180° apart from each other, as required by the claims.

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

252. 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.

253. 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. G. Simpson', written over a horizontal line.

David G. Simpson, Ph.D.

Appendix A

Exhibit No.	Description
1001	U.S. Patent No. 10,137,223 to Francis et al. (“the ’223 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”)
1014	U.S. Pat. No. 5,514,378 to Mikos (“Mikos”)
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”)
1017	Shoulders & Raines, <i>Collagen Structure and Stability</i> , 78 Annu. Rev. Biochem. 929 (2009)
1018	Kuo et al., U.S. Patent Application No. US 2011/0293685 A1, Pub. Date Dec. 1, 2011 (“Kuo”)
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