

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

REGENX SCIENCE, INC.
Petitioner,

v.

NEXTGEN BIOLOGICS, INC.
Patent Owner.

Case No: IPR2025-00621

U.S. Patent No. 11,660,376

**DECLARATION OF DR. STEPHEN F. BADYLAK
IN SUPPORT OF PETITION FOR *INTER PARTES* REVIEW OF
U.S. PATENT NO. 11,660,376**

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1002	Declaration of Stephen F. Badylak
1003	Stephen F. Badylak <i>Curriculum Vitae</i>
1004	Certified Prosecution History for U.S. Patent No. 11,660,376
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1010	Gilbert TW, Sellaro TL, Badylak SF. Decellularization of tissues and organs. <i>Biomaterials</i> . 2006 Jul;27(19):3675-83. doi: 10.1016/j.biomaterials.2006.02.014. Epub 2006 Mar 7. PMID: 16519932. (“Gilbert 2006”)
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1017	Ott, H., Matthiesen, T., Goh, SK. <i>et al.</i> Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. <i>Nat Med</i> 14, 213–221 (2008). https://doi.org/10.1038/nm1684 (“Ott”)
1018	Ota T, Gilbert TW, Schwartzman D, McTiernan CF, Kitajima T, Ito Y, Sawa Y, Badylak SF, Zenati MA. A fusion protein of hepatocyte growth factor enhances reconstruction of myocardium in a cardiac patch derived from porcine urinary bladder matrix. <i>J Thorac Cardiovasc Surg</i> . 2008 Nov;136(5):1309-17. doi: 10.1016/j.jtcvs.2008.07.008. Epub 2008 Sep 14. PMID: 19026821; PMCID: PMC2723859. (“Ota”)
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1022	WO 2008/109407 A2 (“Badylak 2008”)
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1025	McCusker C, Gardiner DM. The axolotl model for regeneration and aging research: a mini-review. <i>Gerontology.</i> 2011;57(6):565-71. doi: 10.1159/000323761. Epub 2011 Mar 2. PMID: 21372551. (“McCusker”)
1026	McGann CJ, Odelberg SJ, Keating MT. Mammalian myotube dedifferentiation induced by newt regeneration extract. <i>Proc Natl Acad Sci U S A.</i> 2001 Nov 20;98(24):13699-704. doi: 10.1073/pnas.221297398. PMID: 11717431; PMCID: PMC61104. (“McGann”)
1027	US 2003/0212024A1 (“Keating”)
1028	Stocum DL. Regenerative biology and engineering: strategies for tissue restoration. <i>Wound Repair Regen.</i> 1998 Jul-Aug;6(4):276-90. doi: 10.1046/j.1524-475x.1998.60404.x. PMID: 9824547. (“Stocum 1998”)
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1030	Lévesque M, Villiard E, Roy S. Skin wound healing in axolotls: a scarless process. <i>J Exp Zool B Mol Dev Evol.</i> 2010 Dec 15;314(8):684-97. doi: 10.1002/jez.b.21371. PMID: 20718005. (“Levesque 2010”)
1031	Seifert AW, Monaghan JR, Voss SR, Maden M. Skin regeneration in adult axolotls: a blueprint for scar-free healing in vertebrates. <i>PLoS One.</i>

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1032	Gilbert TW, Stolz DB, Biancaniello F, Simmons-Byrd A, Badylak SF. Production and characterization of ECM powder: implications for tissue engineering applications. <i>Biomaterials</i> . 2005 Apr;26(12):1431-5. doi: 10.1016/j.biomaterials.2004.04.042. PMID: 15482831. (“Gilbert 2005”)
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1036	Mazzitelli S, Luca G, Mancuso F, Calvitti M, Calafiore R, Nastruzzi C, Johnson S, Badylak SF. Production and characterization of engineered alginate-based microparticles containing ECM powder for cell/tissue engineering applications. <i>Acta Biomater</i> . 2011 Mar;7(3):1050-62. doi: 10.1016/j.actbio.2010.10.005. Epub 2010 Oct 13. PMID: 20950716. (“Mazzitelli”)
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1042	Shevchenko RV, Sibbons PD, Sharpe JR, James SE. Use of a novel porcine collagen paste as a dermal substitute in full-thickness wounds. <i>Wound Repair Regen</i> . 2008 Mar-Apr;16(2):198-207. doi: 10.1111/j.1524-475X.2008.00360.x. PMID: 18318805. (“Shevchenko”)
1043	Sill TJ, von Recum HA. Electrospinning: applications in drug delivery and tissue engineering. <i>Biomaterials</i> . 2008 May;29(13):1989-2006. doi: 10.1016/j.biomaterials.2008.01.011. Epub 2008 Feb 20. PMID: 18281090. (“Sill”)
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1045	Mase VJ Jr, Hsu JR, Wolf SE, Wenke JC, Baer DG, Owens J, Badylak SF, Walters TJ. Clinical application of an acellular biologic scaffold for surgical repair of a large, traumatic quadriceps femoris muscle defect. <i>Orthopedics</i> . 2010 Jul 13;33(7):511. doi: 10.3928/01477447-20100526-24. PMID: 20608620. (“Mase”)

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1051	Tu Q, Yang Z, Zhu Y, Xiong K, Maitz MF, Wang J, Zhao Y, Huang N, Jin J, Lei Y. Effect of tissue specificity on the performance of extracellular matrix in improving endothelialization of cardiovascular implants. <i>Tissue Eng Part A</i> . 2013 Jan;19(1-2):91-102. doi: 10.1089/ten.TEA.2011.0372. Epub 2012 Oct 4. PMID: 22924620; PMCID: PMC3530946. (“Tu”)
1052	Qi, Pengkai, Manfred F. Maitz, and Nan Huang. "Surface modification of cardiovascular materials and implants." <i>Surface and Coatings Technology</i> 233 (2013): 80-90. (“Qi”)
1053	Salvay DM, Rives CB, Zhang X, Chen F, Kaufman DB, Lowe WL Jr, Shea LD. Extracellular matrix protein-coated scaffolds promote the reversal of diabetes after extrahepatic islet transplantation. <i>Transplantation</i> . 2008 May 27;85(10):1456-64. doi:

Exhibit	Description
	10.1097/TP.0b013e31816fc0ea. PMID: 18497687; PMCID: PMC2597660. (“Salvay”)
1054	Sinani, Vladimir A., et al. "Collagen coating promotes biocompatibility of semiconductor nanoparticles in stratified LBL films." <i>Nano Letters</i> 3.9 (2003): 1177-1182. (“Sinani”)
1055	DeQuach, J. A., Mezzano, V., Miglani, A., Lange, S., Keller, G. M., Sheikh, F., & Christman, K. L. (2010). Simple and high yielding method for preparing tissue specific extracellular matrix coatings for cell culture. <i>PloS one</i> , 5(9), e13039. (“Dequach”)
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1058	Davis NF, McGuire BB, Callanan A, Flood HD, McGloughlin TM. Xenogenic extracellular matrices as potential biomaterials for interposition grafting in urological surgery. <i>J Urol</i> . 2010 Dec;184(6):2246-53. doi: 10.1016/j.juro.2010.07.038. Epub 2010 Oct 16. PMID: 20952029. (“Davis”)
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1060	Choi YC, Choi JS, Kim BS, Kim JD, Yoon HI, Cho YW. Decellularized extracellular matrix derived from porcine adipose tissue as a xenogeneic biomaterial for tissue engineering. <i>Tissue Eng Part C Methods</i> . 2012 Nov;18(11):866-76. doi: 10.1089/ten.TEC.2012.0009. Epub 2012 Jul 2. PMID: 22559904; PMCID: PMC3483066. (“Choi”)
1061	De Filippo RE, Yoo JJ, Atala A. Urethral replacement using cell seeded tubularized collagen matrices. <i>J Urol</i> . 2002 Oct;168(4 Pt 2):1789-92; discussion 1792-3. doi: 10.1097/01.ju.0000027662.69103.72. PMID: 12352360.
1062	Certified Prosecution History for U.S. Patent No. 11,617,790

I. INTRODUCTION

1. I have been retained by Quinn Emanuel Urquhart & Sullivan on behalf of RegenX Science, Inc. (“Petitioner”) to provide expert opinions on the invalidity of claims 1-3, 5-19, and 21-32 (the “Challenged Claims”) of U.S. Patent No. 11,660,376 (“’376 patent”) based on the state of knowledge regarding extracellular matrix-derived xenografts and the regenerative properties of urodeles as of the ’376 patent’s priority date.

2. My opinions in this declaration are based on the documents I cite along with my professional training, experience and knowledge that I have acquired working in the field of tissue engineering and regenerative medicine over the past 35 years. I am being compensated for my services as an expert at my standard consulting rate of \$525 per hour. My compensation is in no way contingent on the substance of my opinions or the outcome of this case.

3. My opinions and their underlying reasoning are explained in detail below.

II. BACKGROUND AND QUALIFICATIONS

4. I am a full Professor with tenure in the Department of Surgery at the University of Pittsburgh, with a secondary appointment in the Department of Bioengineering. I also serve as the Deputy Director of the McGowan Institute for Regenerative Medicine.

5. I earned my Doctor of Veterinary Medicine (D.V.M.) from Purdue University in 1976, followed by a Master of Science (M.S.) in Clinical Pathology in 1978. I obtained my Ph.D. in Anatomic Pathology from Purdue University in 1981 and my M.D. from Indiana University in 1985. My academic and professional career has focused on biomaterials, tissue engineering, and regenerative medicine, particularly in the study and application of extracellular matrix (ECM) scaffolds.

6. For over three decades, my research has been dedicated to the development and clinical application of biologic scaffolds for regenerative medicine. I have pioneered the use of ECM-based biomaterials derived from xenogeneic sources, particularly porcine small intestinal submucosa (SIS) and urinary bladder matrix (UBM), for tissue reconstruction with my first descriptions of these applications in the mid to late 1980's. My work has explored the mechanisms by which ECM scaffolds modulate the host response, and facilitates constructive tissue remodeling rather than fibrotic scarring. Through both preclinical and human clinical studies, I have investigated the role of ECM in musculoskeletal, cardiovascular, gastrointestinal, urogenital and other soft tissue repair, as well as its applications in nerve regeneration and whole-organ engineering.

7. Since joining the University of Pittsburgh in 2003, I have held various leadership roles within the McGowan Institute for Regenerative Medicine and the global Regenerative Medicine community. Before my tenure at the University of

Pittsburgh, I was a Senior Research Scientist in the Department of Biomedical Engineering at Purdue University and the Director of the Hillenbrand Biomedical Engineering Center. I have also served as an Adjunct Professor at Wake Forest University School of Medicine, where I collaborated on regenerative medicine initiatives.

8. Throughout my career, I have been actively involved in translational research (i.e., application to human medicine) and innovation. I was the founder and President of ACell, Inc., where I helped develop ECM-based technologies for wound healing and tissue repair, and I currently serve as the founder and Chief Scientific Officer of ECM-Therapeutics, Inc. My contributions to the field have been recognized with numerous awards, including the Jensen Tissue Engineering Award, the TERMIS Lifetime Achievement Award, and election as a Fellow of the National Academy of Inventors.

9. I have been a member of several professional organizations, including the Tissue Engineering and Regenerative Medicine International Society (TERMIS), the American Institute for Medical and Biological Engineering, and the Society for Biomaterials. My research has resulted in over 440 publications, including peer-reviewed scientific papers, book chapters, and patents related to ECM-based regenerative medicine. I hold multiple patents related to ECM-derived scaffolds and

their applications in tissue engineering, including methods for enhancing their regenerative properties.

10. In sum, I have over 35 years of academic and industry experience specializing in regenerative medicine, with particular focus on ECM derived scaffolds. My work continues to focus on the development and clinical translation of biomaterials for tissue engineering and regenerative medicine applications. I have included a copy of my *curriculum vitae* in support of my opinions. Ex. 1003, Badylak CV.

III. INFORMATION RELIED UPON

11. In addition to my general knowledge gained as a result of my education and experience, I have reviewed and considered, among other things, the prior art of record discussed herein.

12. The full list of information, in addition to my professional, academic and clinical experience, that I have relied upon in forming my opinions for this report is set forth throughout the report and in the Table of Exhibits.

13. Where my conclusions are based on certain assumptions that I have adopted, I have stated such assumptions in this Declaration.

IV. RELEVANT LEGAL STANDARD

14. In forming my opinions and considering the patentability of the claims of the '376 Patent, I am relying upon certain legal principles that counsel for Petitioner have explained to me, as listed below.

15. I understand that for an invention claimed in a patent to be found patentable, it must be, among other things, new and not obvious in light of what came before it. Patents and publications which predated the invention are generally referred to as “prior art.”

16. For patent invalidity under an *inter partes* review, I understand the burden is on the party asserting unpatentability to prove it by a preponderance of the evidence. I understand that “a preponderance of the evidence” is evidence sufficient to show that a fact is more likely than not.

A. Person of Ordinary Skill in the Art

17. I have been informed that the claims of a patent are judged from the perspective of a hypothetical construct involving “a person of ordinary skill in the art” (“POSA”). The “art” is the field of technology to which the patent is related. I understand that the purpose of using a POSA’s viewpoint is objectivity. Thus, I understand that the question of validity is viewed from the perspective of a POSA, and not from the perspective of (a) the inventor, (b) a layperson, or (c) a person of extraordinary skill in the art. I have been informed that the claims of the '376 patent

are interpreted as a POSA would have understood them at the relevant time period (i.e., when the patent application was filed or the earliest effective filing date).

18. Based on my review of the patent and prior art, a person of ordinary skill in the art (“POSA”) as of the earliest possible priority date of the ’376 patent (January 9, 2013) would have had at least a master’s degree in biochemistry, chemical engineering, pharmaceuticals, biomedical engineering or a related discipline with at least 3 years of experience in designing, formulating, engineering, or manufacturing biomaterial intended for implantation or bachelor’s degree in the same disciplines with a corresponding higher amount of experience.

B. Claim Construction

19. I understand the first step in determining whether a patent claim is valid is to properly construe the claims. I understand that each claim of a patent subject to *inter partes* review is to be interpreted in light of the language of the claim, the written description, the figures, and the prosecution history of the patent.

20. I further understand that in the absence of a specific definition within the specification or the reliance on a specific meaning in the prosecution history, the terms as used in the challenged claims of the ’376 Patent are to be afforded their plain and ordinary meaning at the time of the invention.

21. I understand that the “plain and ordinary meaning” of a term means the ordinary and customary meaning given to the term by those of ordinary skill in the

art at the time of the invention and that the ordinary and customary meaning of a term may be evidenced by a variety of sources, including the words of the claims, the specification, drawings, and prior art.

22. I understand that in construing claims all words in a claim must be considered in judging the patentability of that claim against the prior art.

23. I understand that extrinsic evidence may be consulted for the meaning of a claim term as long as it is not used to contradict claim meaning that is unambiguous in light of the intrinsic evidence. I also understand that in construing claim terms, the general meanings gleaned from reference sources must always be compared against the use of the terms in context, and the intrinsic record must always be consulted to identify which of the different possible dictionary meanings is most consistent with the use of the words by the inventor.

C. Presumption of Validity

24. I understand that a granted patent is presumed to be valid. I understand that the presumption of validity can be overcome in district court litigations if clear and convincing evidence is presented that proves the patent is invalid. I understand that the standard of proof for invalidating a patent in an *inter partes* review proceeding is a “preponderance of the evidence” (i.e., greater than 50% chance of likelihood), which is a lower standard than the clear and convincing evidence standard.

D. Obviousness

25. I understand that even if a patent claim is not anticipated, it is still invalid if the differences between the claimed subject matter and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person of ordinary skill in the pertinent art.

26. I understand that a person of ordinary skill in the art provides a reference point from which the prior art and claimed invention should be viewed. This reference point prevents one from using his or her own insight or hindsight in deciding whether a claim is obvious.

27. I also understand that an obviousness determination includes the consideration of various factors such as (1) the scope and content of the prior art, (2) the differences between the prior art and the asserted claims, (3) the level of ordinary skill in the pertinent art, and (4) the existence of secondary considerations or objective indicia of obviousness or non-obviousness.

28. I understand that an obviousness determination can be based on a single prior art reference or a combination of multiple prior art references or the knowledge of one of ordinary skill in the art. I understand that the prior art itself may provide a suggestion, motivation, or reason to combine or modify the teachings of the prior art, or that such a reason may come from other sources, such as the knowledge of a

person having ordinary skill in the art, common sense, and market forces. I understand that the following rationales may support a finding of obviousness:

- combining prior art elements according to known methods to yield predictable results;
- simple substitution of one known element for another to obtain predictable results;
- use of known technique to improve similar devices (methods, or products) in the same way;
- applying a known technique to a known device (method or product) ready for improvement to yield predictable results;
- “obvious to try” – choosing from a finite number of identified, predictable solutions, with a reasonable expectation of success;
- known work in one field of endeavor may prompt variations of it for use in either the same field or a different one based on design incentives or other market forces if the variations are predictable to one of ordinary skill in the art;
- some teaching, suggestion, or motivation in the prior art that would have led one of ordinary skill to modify the prior art reference or to combine prior art reference teachings to arrive at the claimed invention.

29. I understand that an obviousness determination requires a reasonable expectation of success in achieving the claimed invention.

30. I understand that the content of a patent or other printed publication (i.e., a reference) should be interpreted the way a person of ordinary skill in the art would have interpreted the reference as of the effective filing date of the patent application for the '376 Patent. I have assumed that the person of ordinary skill in the art is a hypothetical person who is presumed to be aware of all relevant art at the time of the invention. The person of ordinary skill is not an automaton, and may be able to fit together the teachings of multiple patents employing ordinary creativity and the common sense that familiar items may have obvious uses in another context or beyond their primary purposes.

31. I understand that if an alleged invention is otherwise rendered obvious in view of the prior art, it is not patentable simply because the inventors discover an inherent property or a result that naturally flows from its application or use.

32. I understand that secondary considerations or objective indicia of obviousness or non-obviousness may include (1) a long felt but unmet need in the prior art that was satisfied by the invention of the patent; (2) commercial success or lack of commercial success of processes covered by the patent; (3) unexpected results achieved by the invention; (4) praise of the invention by others skilled in the art; (5) the taking of licenses under the patent by others; (6) deliberate copying of

the invention; (7) teaching away; and (8) the simultaneous invention of the claimed subject matter. I also understand that there must be a relationship between any such secondary considerations and the invention. I further understand that contemporaneous and independent invention by others is a secondary consideration supporting an obviousness determination.

33. I understand that any secondary considerations or objective indicia must bear a nexus to the claimed invention. Where the offered secondary consideration actually results from something other than what is both claimed and novel in the claim, there is no nexus to the merits of the claimed invention. I further understand that the patentee bears the burden of demonstrating that the relevant commercial success is attributable to the claimed invention, as opposed to other economic and commercial factors unrelated to the technical quality of the patented subject matter.

34. I understand that whether a prior art reference "teaches away" from the claimed invention is a fact to be considered in determining obviousness. I understand that it is improper to combine references where the references teach away from their combination. However, I understand that the nature of the teaching is highly relevant and must be weighed in substance - a known or obvious composition does not become patentable simply because it has been described as somewhat inferior to some other product for the same use. Furthermore, I understand that the

prior art's mere disclosure of more than one alternative does not constitute a teaching away from any of these alternatives because such disclosure does not criticize, discredit, or otherwise discourage the solution claimed.

35. I understand that hindsight must not be used when comparing the prior art to the invention for obviousness.

V. THE '376 PATENT BACKGROUND

A. Overview

36. I understand that the '376 patent is directed to biomaterials, including tissue grafts and medical implants, for xenotransplantation comprising ECM components derived from a tissue of urodele and their methods of production.

37. According to the '376 patent, the claimed biomaterials are “the product of a process” comprising “obtaining a tissue sample from a urodele, where the tissue comprises ECM,” and “subjecting the tissue sample to a decellularization process that maintains the structural and functional integrity of the extracellular matrix...while removing sufficient cellular components of the sample to reduce or eliminate antigenicity and immunogenicity for xenograft purposes.” Ex.1001, 4:60-5:12.

38. The '376 patent discloses that the key feature of the '376 patent lies in “the inventor’s recognition that the growth factor profile, connective tissue matrix constituents and immunoprivileged status of urodele ECM ... render urodele-derive

tissue an ideal source for biological scaffolds for xenotransplantation.” *Id.*, 4:53-59. The ’376 patent discloses that the decellularization processes for the claimed biomaterials are described in the art and can be used to treat urodele tissue samples for producing the claimed biomaterials. *Id.*, 9:43-48 (“According to the invention, urodele tissue samples obtained for decellularization can be treated in the manner detailed in US 2008/0046095 or US 2010/0104539.”). Similarly, the ’376 patent discloses that “synthetic polymers and methods of incorporating or embedding cells into ... matrices are also known in the art.” *Id.*, 14:31-58.

39. The components of the urodele ECM according to the ’376 patent include “proteins such as collagen..., elastin, fibronectin, laminin, vitronectin, thrombospondins, osteopontin and tenascins, plus GAGs..., and growth factors such VEGF, BMP, TGF and FGF.” *Id.*, 5:43-59. The decellularized ECM of the ’376 patent can take on any variety of shapes, including a sheet, mesh, dry powder, reconstituted gel, or fraction. *Id.*, 6:43-8:15, 11:57-12:46. Examples 1–4 of the ’376 patent describe the decellularization and processing steps for urodele tissues. *Id.*, 17:5-19:28. Example 5 describes an in vitro characterization of decellularized urodele tissue. *Id.*, 19:30-20:24. Figures 1–6 present histological and biochemical analyses of native and processed axolotl dermal tissue and human amniotic membrane, including hematoxylin and eosin and Alcian Blue staining (Fig. 2), immunohistochemical detection of collagen IV and laminin (Fig. 3), histological

analysis of extracellular components of axolotl tissue (Figs. 1, 4, and 5), and ELISA analysis of DNA content in pre- and post-processed axolotl tissue (Fig. 6). *Id.*, Figures 1-6, 8:15-35.

40. I note however that the '376 patent provides no experimental data or specific examples demonstrating the use of the decellularized urodele tissues in xenotransplantation applications. It also does not disclose any new methods for manufacturing specific forms of ECM or for incorporating claimed additives or synthetic materials.

B. Challenged Claims

41. Independent claims 1 recites decellularized extracellular matrix (ECM) derived from a urodele, derived by obtaining a tissue sample from a urodele and decellularizing said tissue sample to reduce or eliminate antigenicity, making it suitable for use as a xenograft. Independent claim 31 recites a biomaterial comprising decellularized ECM derived by obtaining a tissue sample from a urodele and decellularizing said tissue sample to reduce or eliminate antigenicity making it suitable for use as a xenograft. Independent claim 32 recites a method of making a biomaterial comprising ECM derived by obtaining a tissue sample from a urodele and decellularizing said tissue sample to reduce or eliminate antigenicity, making it suitable for use as a xenograft.

42. For ease of reference, I have labeled the limitations of these claims as follows:

Claim 1	
1.p	Decellularized extracellular matrix (ECM) derived from a Urodele said decellularized ECM derived by:
1.a	a. obtaining a tissue sample from a urodele, which tissue sample comprises ECM; and
1.b	b. decellularizing said tissue sample to produce decellularized ECM by removing sufficient cellular components of the sample to reduce or eliminate antigenicity, making it suitable for use as a xenograft.
Claim 31	
31.p	A biomaterial comprising decellularized extracellular matrix (ECM) derived from a Urodele said decellularized ECM derived by:
31.a	a. obtaining a tissue sample from a urodele, which tissue sample comprises ECM; and
31.b	b. decellularizing said tissue sample to produce decellularized ECM by removing sufficient cellular components of the sample to reduce or eliminate antigenicity of the biomaterial making it suitable for use as a xenograft.
Claim 32	
32.p	32. A method of making a biomaterial comprising decellularized extracellular matrix (ECM) derived from a Urodele said decellularized ECM said method comprising:
32.a	a. obtaining a tissue sample from a urodele, which tissue sample comprises ECM; and
32.b	b. decellularizing said tissue sample to produce decellularized ECM by removing sufficient cellular components of the sample to reduce or eliminate antigenicity of the biomaterial making it suitable for use as a xenograft.

43. Dependent claims 2-3, 5-19, and 21-30 set forth additional limitations for the claimed biomaterials, including their physical forms and compositions, types and sources of ECM, method of production, and further processing steps.

C. Prosecution History

44. I understand that the '376 patent issued from U.S. Application No. 16/801,956 (“the '956 application”), filed on February 26, 2020. Below, I briefly describe my understanding of the prosecution history of the '376 patent.

45. On August 18, 2022, the Examiner issued a non-final office action rejecting the pending claims, among others, on the ground of non-statutory obviousness-type double patenting as being unpatentable over certain claims of U.S. Patent No. 10,617,790. Ex. 1004, PH, 205-211. In response, the applicant submitted a terminal disclaimer to the '790 patent. *Id.*, 236-240. On January 24, 2023, the Examiner issued a notice of allowance for the claims without making a further rejection. *Id.*, 249-256. The '376 patent issued on May 30, 2023. *Id.*, 276.

46. I understand that the primary references presented here were neither before the Examiner during prosecution, nor were they considered. I understand that while Van Dyke was disclosed during prosecution, it was not substantively evaluated by the Examiner.

D. Priority Date

47. I understand that the '376 patent claims priority to Provisional Application No. 61/750,555, which was filed on January 9, 2013. For purposes of this declaration, I have been asked by counsel for RegenX to assume that the '376 patent is entitled to the January 9, 2013 priority date.

VI. TECHNOLOGY BACKGROUND

48. I understand that the '376 patent describes tissue engineering approaches for wound healing and regeneration, focusing specifically on biomaterials derived from urodele ECM. I provide below an overview of ECM tissue grafts, the well-known regenerative properties of urodele tissues, and their conserved signaling pathways.

A. ECM Scaffolds

49. The ECM is an intricate network of structural and functional proteins, glycoproteins and proteoglycans secreted by the resident cells of a tissue or organ. Ex. 1005, Badylak 2004, 367-369; Ex. 1006, Badylak 2002, 377-380. The ECM provides support for tissue structure and maintenance of tissue architecture, and regulates essential processes such as signaling, adhesion, migration, proliferation, differentiation, immune response, and wound healing. *Id.*; *see also* Ex. 1007, Chan, S467-S469. The ECM is in a state of dynamic equilibrium with the cells, tissues and organs of which it is a part and it serves as an “information highway” between cells. Ex.1005, Badylak 2004, 367; Ex. 1006, Badylak 2002; Ex. 1007, Chan.

50. As I note in my paper, an advantage of utilizing the ECM in its native state as a scaffold for tissue repair is the presence of all signaling molecules, binding molecules and associated co-factors, including growth factors (and their inhibitors),

“in the relative amounts that exist in nature and perhaps most importantly, in their native three-dimensional ultrastructure.” Ex. 1006, Badylak 2002, 379.

51. Due to its critical role in cell-to-cell communication and its impact on tissue repair and reconstruction, ECM has been widely used as a model scaffold for tissue repair and reconstruction across nearly every body system. Ex. 1006, Badylak 2002, 379-380; Ex. 1005, Badylak 2004, 369; Ex. 1008, Badylak 2009, 2. While the native autologous ECM of the target tissue is intuitively the ideal scaffold material for in-vivo therapy or creation of an engineered replacement tissue, availability of autologous source tissue and/or the morbidity associated with harvest of such tissue is typically challenging. Ex. 1007, Chan, S467-468; Ex. 1009, Hoshiba, 1725. In addition the complex functions, composition, and ultrastructure make precise replication of the ECM virtually impossible. *Id.* One approach to overcome these challenges has been the use of ECM sourced from another human (i.e., cadaveric “allogeneic” ECM) or ECM sourced from another species: specifically animal—“xenogeneic” ECM—as a functional alternative. Ex.1007, Chan; Ex. 1006, Badylak 2002; Ex. 1008, Badylak 2009; Ex. 1005, Badylak 2004.

52. Xenogeneic ECM tissue grafts are derived from the ECM of species different from the recipient and are intended to be implanted into the recipient for a therapeutic, regenerative effect. The use of xenogeneic ECM as a functional tissue graft had been extensively researched and applied in medical practice and more

specifically in tissue engineering applications by early 2000s. Ex. 1006, Badylak 2002; Ex. 1005, Badylak 2004; Ex. 1008, Badylak 2009; Ex. 1007, Chan. Xenogeneic ECM grafts provide an abundance of source tissue and leverage the natural structural and functional properties of ECM, including its ability to support cell adhesion, migration, proliferation, and differentiation. Ex. 1006, Badylak 2002; Ex. 1005, Badylak 2004; Ex. 1008, Badylak 2009. Xenogeneic ECM scaffolds retain bioactivity across species, preserving structural and functional proteins such as collagen, laminin, and glycosaminoglycans, along with growth factors (i.e., “cytokines”) essential for tissue repair. Ex. 1006, Badylak 2002, 372; Ex. 1005, Badylak 2004; Ex. 1008, Badylak 2009. These retained components provide a bioactive environment that promotes vascularization and facilitates host cell integration, allowing the graft to meet the specific demands of the tissue being repaired. Ex. 1006, Badylak 2002, 372; Ex. 1007, Chan, S471.

53. Antigens present in xenogeneic tissues are recognized as foreign (i.e., “non-self”) by the recipient host and therefore induce an inflammatory response or an immune-mediated rejection of the tissue. Ex. 1010, Gilbert 2006, 3676; Ex. 1006, Badylak 2002, 373; Ex. 1009, Hoshiba, 1725. The large majority of these foreign antigens are present on or within the cellular component of tissues. Therefore, removal of the cells from tissues (i.e., decellularization) removes the source of inflammation and rejection. Because the composition of the ECM is “well-

conserved across species and readily tolerated even by xenogeneic recipients,” any adverse host immune response can be minimized if sufficient cellular and nucleic components are removed. Ex. 1009, Hoshiba, 1725. By January 2013, decellularization techniques were well developed and recognized. These methods efficiently remove cellular and nuclear materials while minimizing any adverse effect on the composition, biological activity, and mechanical integrity of the remaining ECM. Ex. 1010, Gilbert 2006; Ex. 1009, Hoshiba; Ex. 1008, Badylak 2009; Ex. 1005, Badylak 2004, Ex. 1006, Badylak 2002.

54. A typical method of processing a xenogeneic tissue to isolate the ECM includes rinsing in hypertonic or hypotonic saline solution, followed by treatment with incubation in a detergent, enzyme or chemical to lyse (break up) cells, additional rinsing to remove cell remnants, and terminal sterilization. Ex. 1005, Badylak 2004, 372; Ex. 1010, Gilbert 2006, 3676. These processing methods have been shown to eliminate intact cells (i.e. decellularize) and to degrade nucleic acid remnants while leaving the constituent growth factors biologically active. Ex. 1005, Badylak 2004, 372; Ex. 1010, Gilbert 2006; Ex. 1015, Freyman; Ex. 1023, Van Dyke. Importantly, once effectively decellularized, these xenogeneic scaffolds do not elicit an adverse innate or adaptive immune response. Ex. 1012, Hussey; Ex. 1013, Allman; Ex. 1014, Keane.

55. As acknowledged by the '376 patent and Patent Owner, decellularization and related processing methods for ECM scaffolds were well studied and had become standard practice for producing functional xenogeneic ECM scaffolds by January 2013. Ex.1001, 9:43-48 (“According to the invention, urodele tissue samples obtained for decellularization can be treated in the manner detailed in US 2008/0046095 or US 2010/0104539.”); *see also*, Ex. 1062, PH, 847 (“[R]emoving cells to produce a non-toxic graft is straight forward...[A]pplicant did not invent a new process of decellularization.”); Ex. 1010, Gilbert 2006, 3681; Ex. 1023, Van Dyke; Ex. 1015, Freyman.

56. By January 2013, xenogeneic ECM had been explored using tissues from a wide range of animal species, including dogs, rats, pigs, boars, monkeys, horses, and cows. Ex. 1006, Badylak 2002; Ex. 1008, Badylak 2009; Ex. 1009, Hoshiba. Remarkably, even ECM components from fish—an entirely different vertebrate class (non-mammalian, non-avian)—demonstrated good biocompatibility, low antigenicity, and cell growth potential, confirming that ECM from a fundamentally different species could still function effectively in xenograft applications. *See*, Ex. 1059, Hayashi.

57. Various forms of these grafts—e.g., powder, paste, gel, sheet, or mesh—likewise had been developed and tailored for specific applications in tissue repair and regeneration. *See, e.g.*, Ex. 1015, Freyman, 38:27-33, 46:1-17, 39:13-18,

46:15-17; 39:6-12; Ex. 1008, Badylak 2009. Moreover, processing techniques had been developed to further optimize these ECM grafts with improved physical and chemical characteristics, by, for example, incorporating agents such as growth factors, cytokines, gelling agent, anti-microbial agents and seeding cells. *Id.*; *see also*, Ex. 1016, Bader; Ex. 1017, Ott; Ex. 1018, Ota; Ex. 1019, Seif-Naraghi; Ex. 1020, Penolazzi; Ex. 1021, Villanola; Ex. 1022, Badylak 2008; Ex. 1023, Van Dyke.

58. By January 2013, ECM derived tissue xenografts had demonstrated safety and efficacy across a wide range of clinical and preclinical applications, with over 200,000 patients treated for musculoskeletal, cardiovascular, dermal, and integumentary tissue repair, among others. Ex. 1006, Badylak 2002, 367, 372. A summary table of xenogeneic ECM tissue graft applications known at the time is provided below.

Target tissues	Sources of matrices	Use	Ref.
Heart	Rat whole heart	Heart transplantation	[30]
Heart valves	Porcine aortic valves	Valvular heart disease	[31]
	Pig aortic valves	Bioprosthetic valves	[33]
	Porcine myocardial tissue	Injectable gel for cardiac tissue engineering	[35]
Blood vessels	Pig carotid arteries	Vascular graft	[29]
	Dog small intestinal submucosa	Vascular graft	[36]
	Porcine aortic blood vessel	Vascular graft	[37]
Lung	Rat whole lung	Lung transplantation	[40]
	Rat whole lung	Lung transplantation	[41]
	Mouse whole lung	Lung bioreactor system	[42]
Trachea	Yorkshire boar trachea	Airway replacement	[43]
Skin	Human dermal tissue	Abdominal wall, breast, ear, nose and throat/head and neck reconstruction, grafting	[44]
Nerve	Peripheral nerve	Peripheral nerve graft	[46]
	Rat peripheral nerve	Peripheral nerve graft	[47]
Cornea	Human cornea stroma	Cornea transplantation	[48]
	Porcine cornea	Cornea transplantation	[49,50]
Esophagus	Porcine aorta	Patch for esophageal injury	[51]
	Rat esophagus	Artificial esophagus	[52]
Liver	Rat homogenized liver	Hepatocyte culture	[53,54]
	Ischemic rat liver	Liver transplantation	[55]
Kidney	Rhesus monkey kidney	Scaffold for kidney tissue engineering	[56]
Bladder	Dog bladder submucosa	Bladder augmentation	[26]
	Porcine bladder	Scaffold for bladder tissue engineering	[57]
Cartilage	Porcine menisci	Scaffold for meniscal repair	[58]
Ligament	Porcine anterior cruciate ligament	Bone-anterior cruciate ligament-bone graft	[28]
Adipose tissue	Rat adipose tissue	Adipogenesis of mesenchymal stem cell	[59]
	Human adipose tissue	Adipose tissue regeneration	[60]
	Rat skeletal muscle	Adipose tissue regeneration	[61]
Vagina	Human amniotic membrane	Vaginal reconstruction	[62]
Decellularized small intestinal submucosa has been used for the reconstruction of integument, body wall, urinary bladder, rotator cuff, intestine, urethra, ureter and diaphragm			[38]

Ex. 1009, Hoshiba, Table 1; *see also* Ex. 1008, Badylak 2009, Table 1.

59. It was therefore well established that xenogeneic ECM tissue grafts were broadly applicable across species, with their suitability as xenografts not confined to specific animal species or tissue sources.

B. Regenerative Capacity of Urodele/Axolotl

60. Considered as the “divas of dedifferentiation,” the urodele amphibians, such as newts and axolotls, are distinguished by their exceptional ability to regenerate complex structures, including limbs, spinal cords, and even portions of the heart. Ex. 1024, Stocum 2002, 271; Ex. 1025, McCusker 2011, 566. Regeneration in urodeles depends on regulated molecular pathways, such as those

involving transforming growth factor-beta (TGF- β) and fibroblast growth factors (FGFs), which guide cellular dedifferentiation and proliferation. Ex. 1026, McGann, 13699; Ex. 1028, Stocum 1998, 276; Ex. 1027, Keating. This regenerative process involves dedifferentiation of mature cells to create a blastema, which consists of a mass of dedifferentiated cells (i.e., “stem cells”), that proliferate and redifferentiate to regenerate the damaged tissue. Ex. 1026, McGann; Ex. 1027, Keating, 13699; Ex. 1024, Stocum 2002, 271; Ex. 1029, Roy, 12-19; Ex. 1030, Levesque 2010, 684-695. Unlike mammals, whose response to injury often results in scar formation, urodele regeneration leads to the complete restoration of both form and function without scar tissue formation. Ex. 1030, Levesque 2010, 684; Ex. 1028, Stocum 1998, 278; Ex. 1031, Seifert, 1-2.

61. Due to their exceptional regenerative capacity, urodeles have been widely studied for potential applications in regenerative medicine. Ex. 1025, McCusker 2011, 566; Ex. 1024, Stocum 2002, 272. One of the most well-studied urodele species is axolotl (*Ambystoma mexicanum*), which by January 2013 had become a model organism for understanding the molecular and cellular mechanisms of regeneration. Ex. 1025, McCusker 2011, 566; Ex. 1029, Roy; Ex. 1030, Levesque 2010; Ex. 1031, Seifert. Axolotls exhibit rapid re-epithelialization of wounds, formation of blastema, and redevelopment of lost structures. *See*, Ex. 1030, Levesque 2010, 684–685; Ex. 1024, Stocum 2002, 271; Ex. 1029, Roy. Notably,

“the mechanisms of regeneration [in axolotl] involve many basic biological processes regulated by conserved signaling pathways,” and scientists “*expect[ed] to stimulate regeneration in a human by the same mechanisms used by the axolotl.*”

Ex. 1025, McCusker, 566; Ex. 1028, Stocum 1998, 277; Ex. 1029, Roy, 20. In fact, human fetal tissues during the first trimester of development exhibit many of the same regenerative properties as axolotls. *See*, Ex. 1030, Levesque 2010, 693-696; Ex. 1031, Seifert, E32875; Ex. 1028, Stocum 1998, 276-278, 280-284, 286-287; Ex. 1029, Roy, 13, 20; Ex. 1024, Stocum 2002, 271.

62. By January 2013, urodele’s unique ability to regenerate without scar formation and conserved molecular pathways generated significant interest in translating these processes for mammalian therapies. Ex. 1025, McCusker, 567; Ex. 1030, Levesque 2010, 685; Ex. 1024, Stocum 2002, 272.

63. For instance, McGann investigated the effect of regeneration extracts derived from newt¹ tissues on mammalian cells. Ex. 1026, McGann. McGann showed that newt regenerating extract was able to dedifferentiate the treated mammalian cells. *Id.* McGann further taught that because “proteins are required components of the dedifferentiation signal,” the factors present in the regenerating extract were “soluble extracellular proteins acting as ligands to activate receptors

¹ Newt is a type of urodele amphibian.

that transduce dedifferentiation signals through receptive cells.” *Id.*, 13704. Keating confirmed these experimental findings, further teaching that regenerating extracts can be prepared from “any animal that regenerates, for example, urodeles (newt or axolotl)” and can be used to “dedifferentiate tissue from, for example, humans.” *Id.*, [0026], [0027], [0129], [0215], Table C1 (listing “extracellular” components in the regenerating extract).

VII. KEY PRIOR ART

A. WO 2009/089110 (“Freyman”)

64. Freyman, published on January 2, 2009, is prior art under 35 U.S.C. § 102(b). Ex. 1015, Freyman. I understand that it was not submitted during prosecution, and was never addressed by the Examiner.

65. Freyman discloses methods for repairing and regenerating tissues by administering decellularized ECM into a site in need of treatment, such as the bone marrow cavity. Freyman states that “the decellularized extracellular matrix provides a microenvironment and contains important biological materials that promote the efficient and effective repair, regeneration and/or strengthening of cells, tissues or organs.” Ex. 1015, Freyman 13:22-25.

66. Freyman discloses that “suitable animal body tissue from which the decellularized extracellular matrix material of the present invention is produced includes body tissues originally from syngeneic, allogeneic, or *xenogeneic sources*.”

Ex. 1015, Freyman, 20:23-21:6. Freyman discloses that ECM may be derived from a wide range of animal sources, including “birds, reptiles, *amphibians* or marsupials.”

Ex. 1015, Freyman, 16:26-34. Freyman further lists animals including “non-primate (e.g., cows, pigs, horses, chickens, cats, dogs, rats, etc.) and primate (e.g., monkeys and humans).”

Ex. 1015, Freyman, 20:23-21:6. Freyman further discloses that the tissue source can be grouped into “four general categories: (1) epithelial tissue, (2) connective tissue, (3) muscle tissue, and (4) nerve tissue,” which include “myocardium, pericardium,...dermis, skin, muscle, nerves, spinal cord, pancreas, gut, intestines, peritoneum, submucosa, stomach, liver, and bladder.” Ex. 1015, Freyman, 21:13-28.

67. Freyman teaches the ECM decellularization methods. For example, Freyman discloses that “[d]ecellularization generally refers to the removal of all cells, cellular components, and other non-extracellular matrix components (e.g., serum, fat) while leaving intact an extracellular matrix (ECM) component.” Ex. 1015, Freyman, 34:12-39:33. The ECM “may be isolated from the conditioned body tissue using a physical technique, including but not limited to centrifugation, rinsing, agitation, freeze-thaw, sedimentation, dialysis, electrical stimulation, electromagnetic forces, hydrostatic or hydrodynamic forces, blasting with sound waves, and ultrasonication.” Ex. 1015, Freyman, 35:3-26. Freyman teaches that physical, chemical, and biological methods may be used for decellularization,

including the application of detergents and enzymatic solutions. Ex. 1015, Freyman, 34-39. It further discloses subjecting the ECM to “suitable chemical solutions,” including “basic solutions,” which “remov[es] the cellular and extracellular debris” and “reduces the likelihood of...eliciting an adverse immune response from the recipient upon injection or implantation.”

68. Freyman further discloses that the ECM “can be dried, concentrated, diluted, lyophilized, cryopreserved, electrically charged, sterilized, etc.,” “to facilitate administration, injection or implantation.” Ex. 1015, Freyman, 4:19-23. Freyman states that the ECM can be obtained in their native form “as an entire or partial structure, such as a sheet or a tubular member, such as a small intestine.” Ex. 1015, Freyman, 38:27-33. Freyman discloses that ECM scaffolds can be processed into various forms, including laminated sheets, slurry of small particles (i.e., paste), particles (i.e., powder), gel, lyophilized, and cryopreserved forms. Ex. 1015, Freyman, 38:27-33, 46:1-17, 39:13-18, 46:15-17; 39:6-12.

B. WO 2007/124127 (“Van Dyke”)

69. Van Dyke, published on November 1, 2007, is prior art under 35 U.S.C. § 102(b). Ex. 1023, Van Dyke. I understand that it was never substantively addressed by the Examiner.

70. Van Dyke teaches methods for producing bioscaffolds by decellularizing animal tissues, including amphibians, to remove cellular material

while preserving the beneficial components of ECM. Van Dyke discloses the tissue processing methods that increase porosity and pore size through chemical treatments such as oxidants, facilitating cell infiltration and remodeling while minimizing immunogenic reactions. Ex. 1023, Van Dyke, 7-17. Van Dyke teaches that the disclosed methods are suitable for various tissue types, including dense structures like tendons and ligaments, and are applicable across cardiovascular, musculoskeletal, and nerve tissue engineering. *Id.*, 4-17.

71. Van Dyke further discloses that “[p]roteins (such as growth factors) or other additives (such as antibiotics, anti-inflammatories, and modulators of immune response) may also be added to ... bioscaffold preparations at any time.” *Id.*, 9. “Various treatments may be applied to enhance adherence of cells to the substrate and/or to each other[,including...] application of various proteins...to the bioscaffold substrate or the growing construct.” *Id.* Bioscaffolds of Van Dyke “can be impregnated with growth factors such as nerve growth factor (NGF)...TGFβ...” *Id.*

C. Badylak 2004

72. Badylak 2004, titled “Xenogenic Extracellular Matrix as a Scaffold tissue reconstruction,” was published in 2004 in *Transplant Immunology*, and thus is prior art under 35 U.S.C. § 102(b). Ex. 1005, Badylak 2004. I understand that it was not submitted during prosecution, and was never addressed by the Examiner.

73. I am the author of Badylak 2004. This article was publicly available as of its publication date. I have reviewed the copy submitted as Exhibit 1005 and confirmed that it is a true and correct copy of my published article.

74. Badylak 2004 reviews the composition and characteristics of ECM based bioscaffolds and their widely successful use in tissue engineering applications by 2004. Badylak 2004 discloses that the ECM “plays a central role in mammalian development and physiology and the amino acid sequence and quaternary structure of many components of ECM such as collagen are highly conserved across species lines.” Ex. 1015, Badylak 2004, 367. Badylak 2004 states “[t]his high degree of sequence homology is one reason that xenogeneic ECM can function as a constructive scaffold in mammalian recipients rather than inciting a destructive inflammatory reaction.” *Id.*

75. Badylak 2004 teaches that the safety and efficacy of xenogeneic ECM bioscaffolds have been successful in numerous preclinical animal studies and in human clinical studies for repairing and reconstructing numerous body tissues, including musculoskeletal, cardiovascular, urogenital and integumentary structures. *Id.* Badylak 2004 further discloses that these scaffolds promoted “constructive remodeling instead of scar tissue,” while retaining “the structural and functional proteins that are part of” the native tissue from which they are derived, including

collagen, glycosaminoglycans, and other growth factors. *Id.*, Badylak 2004, 368-370.

76. Badylak 2004 discloses that the preparation of xenogeneic ECM biomaterials involves a decellularization process. *Id.*, 372-374. Badylak discloses that the decellularization process typically involves “rinsing in hypotonic saline, treatment with dilute peracetic acid or Triton X-100, and ammonium hydroxide to remove cellular material” while preserving the ECM’s bioactive components. *Id.*, 372. Badylak further teaches that sterilization methods such as gamma irradiation or ethylene oxide are used to ensure the scaffolds are safe for clinical use without compromising their biological properties. *Id.*, 372-373.

77. Badylak 2004 discloses specific applications of xenogeneic ECM scaffolds, including their use as vascular grafts, for urinary tract reconstruction, skin reconstitution, and orthopedic applications. *Id.*, 369-373. It further describes their use in arterial and venous grafts, lower urinary tract repair, musculoskeletal structures, myocardial patches, heart valve substitutes, dura mater grafts following intracranial surgery, hernia repair scaffolds, and as cartilage and bone substitutes. *Id.*

D. Badylak 2009

78. Badylak 2009, titled “Extracellular matrix as a biological scaffold material: structure and function” was published in 2009 in *Acta Biomaterialia*, and

thus is prior art under 35 U.S.C. § 102(b). Ex. 1008. I understand that it was not submitted during prosecution, and was never addressed by the Examiner.

79. I am the author of Badylak 2009. This article was publicly available as of its publication date. I have reviewed the copy submitted as Exhibit 1008 and confirmed that it is a true and correct copy of my published article.

80. Badylak 2009 discloses that ECM scaffolds are composed of “a variety of diverse molecules that are perfectly suited to support the cellular processes necessary for optimal function of the tissue and organ from which they are harvested.” *Id.*, 3. Badylak 2009 describes the xenogeneic ECM scaffolds derived from mammalian tissues, including small intestine submucosa (SIS) and urinary bladder matrix (UBM), for use in tissue engineering and regenerative medicine. *Id.*, 4-10.

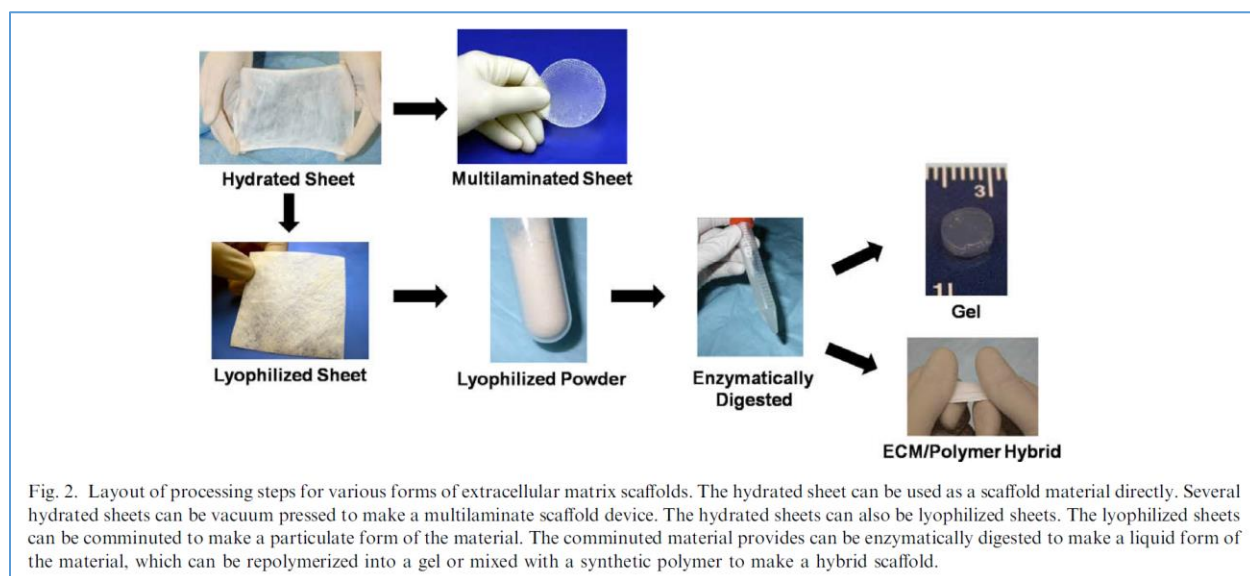
81. Badylak 2009 discloses that ECM scaffolds can be “derived from a variety of tissues, including heart valves, blood vessels, skin, nerves, skeletal muscle, tendons, ligaments, small intestinal submucosa (SIS), urinary bladder, and liver.” *Id.*, 2. Badylak 2009 discloses that ECM scaffolds “can be comminuted into an ECM powder or particulate form” with particle sizes ranging from 50 to 200 μm , which enables needle injection and manufacture of 3-D scaffolds. *Id.*, 6. Enzymatic digestion of ECM powders can produce gels that “can be delivered to a site of interest in its pre-gel liquid state more readily than a suspension of particles..., can be delivered by catheter or needle-based surgical techniques to irregularly shaped

anatomic sites..., and can also serve as a cell delivery vehicle when appropriate.”

Id., 6-7.

82. Badylak 2009 further discloses that the goal of “any decellularization protocol is to remove all cellular material without adversely affecting the composition, mechanical integrity and eventual biological activity of the remaining ECM.” *Id.*, 5. Badylak 2009 discloses that “[c]ommonly used methods of decellularization include a combination of physical and chemical treatments,” which include sonication, agitation, and detergent treatments. *Id.*

83. Badylak 2009 teaches the processing steps for various forms of ECM scaffolds, including hydrated sheet, multilaminated sheet, lyophilized sheet, lyophilized powder, gel, and ECM/polymer hybrid. Provided below is a summary diagram from Badylak 2009:



Ex. 1008, Badylak 2009, Figure 2.

E. McCusker

84. McCusker, titled “The Axolotl Model for Regeneration and Aging Research: A Mini-Review,” was published on March 2, 2011 in *Gerontology*, and thus is prior art under 35 U.S.C. § 102(b). Ex. 1025, McCusker. I understand that it was not submitted during prosecution, and was never addressed by the Examiner.

85. McCusker teaches that the Mexican axolotl (*Ambystoma mexicanum*) serves as a model for understanding regeneration, with mechanisms regulated by conserved signaling pathways. *Id.*, 565-566. McCusker states given that many of the biological processes and the signaling pathways are “highly conserved” across species, “it is likely that humans have the potential to regenerate structures in the same way as salamanders.” *Id.*, 565. McCusker further teaches “[t]he remarkable regenerative abilities of salamanders demonstrate what can reasonably be expected in enhancing human regenerative potential.” *Id.*, 565-567.

F. McGann

86. McGann, titled “Mammalian myotube dedifferentiation induced by newt regeneration extract,” was published on November 20, 2001 in *PNAS*, and thus is prior art under 35 U.S.C. § 102(b). Ex. 1026, McGann. I understand that it was not submitted during prosecution, and was never addressed by the Examiner.

87. Recognizing that “adult urodele (e.g., a newt or axolotl)...[possesses] remarkable regenerative capabilities...lacking in mammals and other vertebrates,”

McGann investigated the regenerative capabilities of regeneration extracts derived from newt tissues on mammalian cells. Ex. 1026, McGann. McGann disclosed that treating mammalian cells with newt regenerating extract reactivated the cell cycle, causing the cells to lose differentiation markers and divide into smaller, regenerative cells. *Id.*, 13699-13703. McGann teaches that the regenerating extract derived from newt likely contained “soluble extracellular proteins acting as ligands to activate receptors that transduce dedifferentiation signals through receptive cells.” *Id.*, 13704.

G. US 2003/0212024 (“Keating”)

88. Keating, published on November 13, 2003, is prior art under 35 U.S.C. § 102(b). Ex.1027, Keating. I understand that it was not submitted during prosecution, and was never addressed by the Examiner.

89. Keating describes a composition and an experimental method for promoting tissue regeneration in mammalian cells using regenerating newt limb extracts (“RNLE”). *See, e.g.*, Ex. 1027, Keating, [0010], [0017], [0026]-[0027], [0125]-[0130], [0342]-[0344]. Keating discloses that RNLE “induced the dedifferentiation of ... murine myotubes in culture” and that “mammalian (murine) myotubes are capable of dedifferentiating in response to dedifferentiation signals received from regenerating newt limbs.” *Id.*, [0027]. Keating teaches that these findings support the use of RNLE to “dedifferentiate tissue from, for example, humans.” *Id.*, [0027]. Keating further discloses that regenerating extracts can be

prepared from “any animal that regenerates, for example, urodeles (newt or axolotl),” and states that such extracts would likely include ECM components. *See, e.g., Id.*, [0129], [0215], Table C1.

H. Gilbert 2005

90. Gilbert 2005, entitled “Production and Characterization of ECM power: implications for tissue engineering applications” was published in 2005 in *Biomaterials*, and thus is prior art under 35 U.S.C. § 102(b). Ex. 1032, Gilbert 2005. I understand that it was not submitted during prosecution, and was never addressed by the Examiner.

91. Gilbert 2005 discloses methods for producing ECM powders with controlled particle sizes. Ex. 1032, Gilbert 2005. Two methods are disclosed: a snap freezing and grinding process, producing particles and a salt precipitation method followed by grinding. Ex. 1032, Gilbert 2005, 1432-1434. These methods produced an ECM powder with a mean particle size ranging from 158 µm to 190.6 µm. *Id.* Gilbert 2005 further discloses that the produced powder “has been successfully injected through a 22 gauge needle (410 µm inner diameter), which is a typical size used for intramuscular injection.” *Id.*

VIII. CLAIM CONSTRUCTION

92. In my analysis provided herein, I have applied the plain and ordinary meaning standard for all elements of the Challenged Claims.

IX. INVALIDITY UNDER 35 U.S.C. § 103

93. In my opinion, the Challenged Claims of the '376 patent are obvious.

94. As a preliminary matter, by the January 2013 priority date, the use of ECMs derived from xenogeneic sources had become standard, with xenogeneic ECM tissue grafts derived from various animal sources, including porcine, bovine, and equine, used in a variety of applications, such as urinary bladder repair, wound healing, cardiac tissue regeneration. Ex. 1005, Badylak 2004, 369-374; Ex. 1008, Badylak 2009, 1-8, Table 1.

95. By January 2013, over 200,000 patients had received such grafts, each tailored to specific applications using a variety of known ECM sources, additives, decellularization techniques, and processing methods. Ex. 1008, Badylak 2009, 1-8, Table 1; Ex. 1005, Badylak 2004, 369-374.

96. This extensive success of ECM xenografts taught that xenogeneic ECM tissue grafts could be successfully developed across species, with their suitability as xenografts not limited to specific animal sources. Based on the teachings of the prior art, a POSA would have understood that xenogeneic ECMs across a wide variety of species were successful because signaling pathways and ECM components are commonly conserved across species. Ex. 1005, Badylak 2004, 367; Ex. 1008, Badylak 2009, 4-5.

97. Additionally, the methods of decellularizing xenogeneic ECMs in order to minimize immune rejection were well known and were known to be effective when applied to ECMs derived from a wide variety of animal sources. Ex. , Badylak 2004, 367, 371-373; Ex. 1008, Badylak 2009, 4-10, Table 1. In fact, as noted above, as the applicant admitted during prosecution, she “did not invent a new process of decellularization” but relied on urodele ECM as “a new graft material [that] is immunoprivileged.” Ex. 1004, PH, 847.

98. Moreover, the remarkable regenerative properties of urodeles, and in particular axolotls (a type of salamander), were well known and the prior art had established that, like many other species, the signaling pathways used for their remarkable regenerative properties were conserved across species, including humans. Ex. 1025, McCusker, 565-566.

99. The prior art thus taught that “[t]he remarkable regenerative abilities of salamanders demonstrate what can reasonably be expected in enhancing human regenerative potential.” Ex. 1025, McCusker, 566. Given the teaching that xenogeneic ECMs from a wide variety of species were successful in combination with the teaching that axolotl’s regenerative signaling pathways are conserved across species, a POSA would have been motivated to select axolotl a source for xenogeneic ECM.

100. As further set out below, the Challenged Claims are therefore unpatentable as obvious in view of the prior art references. All claims are challenged based on Badylak 2004, Badylak 2009, Freyman, McCusker, McGann. Additional obviousness grounds based on Gilbert 2005 for claims directed to specific particle size of the ECM powder, and Van Dyke for claims covering additives to the claimed urodele ECM derived xenograft are further set forth below.

A. Ground I: Obviousness of Claims 1-3, 5-6, 9, 13-16, 21, 24, and 26-32 over Badylak 2004, Badylak 2009, Freyman, McCusker, McGann, and Keating (“Ground I References”)

1. Independent Claim 1

a) Limitation 1.p: Decellularized extracellular matrix (ECM) derived from a Urodele said decellularized ECM derived by:

101. In my opinion, the Ground I References render the limitation: “[d]ecellularized extracellular matrix (ECM) derived from a Urodele said decellularized ECM derived by” obvious.

102. As set forth in Section VI, ECM contains signaling molecules that are essential for tissue and organ morphogenesis, maintenance, and regeneration, making it an ideal natural scaffold for tissue graft applications. Xenogeneic ECM, in particular, had proven to be highly effective for various tissue reconstruction and repair applications because “[t]he molecules which constitute the extracellular matrix are ... conserved across species lines and are tolerated well ... by xenogeneic

recipients.” Ex. , Badylak 2009, 4-5. This “high degree of sequence homology,” along with routine decellularization techniques for removing immunogenic cellular components, allowed xenogeneic ECM to function as a constructive scaffold in recipients without eliciting a destructive immune response. Ex. 1005, Badylak 2004, 367.

103. By the time of the priority date, the use of xenogeneic ECM tissue grafts was well accepted and becoming increasingly common for therapeutic uses. For example, by 2009, over 200,000 patients had received such ECM xenografts, tailored to specific applications using known decellularization techniques, ECM sources, additives, and other processing methods. Ex. 1005, Badylak 2004, 367, 370-371; Ex. 1008, Badylak 2009, 4-10, Table 1. These xenogeneic ECM tissue grafts were derived from a wide variety of animal sources—including dog, rat, pig, boar, monkey, horse, and cow—had become standard practice in tissue regeneration and wound healing applications in humans. *Id.*; *see also, e.g.*, Ex. 1009, Hoshiba, Table 1. As noted above, even ECM components from fish had demonstrated sufficient biocompatibility and low immunogenicity suitable for xenogeneic applications. Ex. 1059, Hayashi.

104. The ability to use xenogeneic ECM from many different animal sources was based on the knowledge that the constituent components of ECM are conserved across species, thereby allowing xenogeneic sources to be used for treatment of

humans, among other animals. Ex. 1008, Badylak 2009, 4-5. Given this widespread adoption and cross-species compatibility of xenogeneic ECM, a POSA would have been motivated to develop ECM xenografts from any animal source with desirable biological properties.

105. In addition, it was well known before the priority date that various methods of decellularizing tissue could be applied to xenogeneic ECM in order to make it safe for use as a xenograft. Again, a POSA would have understood that decellularization methods were broadly applicable to ECM from a variety of different species. Ex. 1008, Badylak 2009; Ex. 1005, Badylak 2004; Ex. 1010, Gilbert 2010; Ex. 1009, Hoshiba.

106. Freyman embodies the knowledge that ECM sources from a variety of different species could be both efficacious and adequately decellularizing so as to not provoke an adverse immune response from the recipient organism. Freyman describes methods of treating damaged tissues by implanting decellularized ECM of body tissue into the site of damage. Ex. 1015, Freyman. Freyman teaches to decellularize the obtained tissue because it “can reduce or eliminate immune response associated with the cells as well as the cellular components,” making it an “ideal natural biomaterial[] for tissue repair and engineering.” Ex. 1015, Freyman, 34:13-19. Consistent with the state of the art, Freyman discloses that the ECM material can be derived from any suitable animal body tissue of xenogeneic origin.

Id., 20:23-21:6, 41:3-6. These include, but are not limited to, cows, pigs, horses, chickens, cats, dogs, rats, monkeys, and amphibians, which a POSA would know includes urodeles and, more specifically, axolotls. *Id.*

107. Prior to the priority date, urodele amphibians, particularly newts and axolotls, were well known for their extraordinary regenerative abilities. These species can regenerate entire limbs, spinal cords, and even portions of the heart. Ex. 1024, Stocum 2002, 271; Ex. 1025, McCusker 2011, 566. Before 2013, axolotl (*Ambystoma mexicanum*), in particular, was widely studied as a model organism for understanding the molecular and cellular mechanisms of regeneration. Ex.1025, McCusker 2011, 566; Ex. 1030, Levesque 2010; Ex. 1029, Roy; Ex. 1031, Seifert.

108. Notably, the mechanisms of regeneration in axolotl “involve many basic biological processes regulated by conserved signaling pathways,” and scientists “*expect[ed] to stimulate regeneration in a human by the same mechanisms used by the axolotl.*” Ex. 1025, McCusker 2011, 566; Ex. 1028, Stocum 1998, 277; Ex. 1029, Roy, 20 (“[B]ased on our knowledge that genes and their functions are highly conserved between species, the idea of using the axolotl... to uncover the mechanisms leading to ... regeneration in mammals is an excellent approach.”). Just as the conserved ECM components enabled the success of xenogeneic ECM grafts, the conserved regeneration signaling pathways in urodeles

suggested that their ECM could similarly facilitate tissue regeneration in other species without causing adverse reactions.

109. In fact, as noted above, xenogeneic urodele ECM components had already demonstrated success in achieving regeneration in mammalian cells by 2004, further supporting a POSA's motivation to select urodele as a source for xenogeneic ECM and to have reasonable expectation of success in doing so. Recognizing that "adult urodele (e.g., a newt or axolotl)...[possesses] remarkable regenerative capabilities...lacking in mammals and other vertebrates," McGann investigated the regenerative capabilities of regeneration extracts derived from newt tissues on mammalian cells. Ex. 1026, McGann. McGann showed that treating mammalian cells with newt regenerating extract reactivated the cell cycle, causing the cells to lose differentiation markers and divide into smaller, regenerative cells. *Id.*, 13699-13704. McGann teaches that the regenerating extract derived from newt likely contained "soluble extracellular proteins acting as ligands to activate receptors that transduce dedifferentiation signals through receptive cells."² This knowledge

² ECM components of the claimed biomaterials that may be present include proteins such as collagen and soluble growth factors such as "VEGF, BMP, TGF and FGF." Ex.1001, 5:57-65.

further confirmed that urodele ECM components could be successfully used in xenogeneic applications. *Id.*, 13704.

110. Keating further supports these findings. Keating describes a composition and an experimental method for promoting tissue regeneration in mammalian cells using regenerating newt limb extracts (“RNLE”). *See, e.g.*, Ex.1027, [0010], [0017], [0021]-[0027], [0122]-[0131], [0215], [0341]-[352]. Consistent with McGann, Keating discloses that RNLE “induced the dedifferentiation of ... murine myotubes in culture,” confirming that “mammalian (murine) myotubes are capable of dedifferentiating in response to dedifferentiation signals received from regenerating newt limbs.” *Id.*, [0027]. Keating teaches that these findings support the use of RNLE to “dedifferentiate tissue from, for example, humans.” *Id.*, [0027].

111. Notably, Keating discloses that regenerating extracts can be prepared from “any animal that regenerates, for example, urodeles (newt or axolotl),” and states that such extracts would likely include ECM components. *See, e.g., Id.*, [0129], [0215], Table C1 (listing “extracellular” proteins and growth factors in the regenerating extract). Keating further confirmed urodele ECM’s potential use as a xenograft source material.

112. The cumulative teachings of the Ground I References therefore would have motivated a POSA to select urodeles, and axolotl in particular, as a source of

xenogeneic ECM. First, Badylak 2004, Badylak 2009, and Freyman are all from the same field of endeavor—the use of xenogeneic ECMs. As described above, based on Badylak 2004, Badylak 2009, and Freyman, a POSA would have known that xenogeneic ECMs from a wide variety of sources had been successfully used in humans and that these varied xenogeneic ECMs were efficacious because the components of ECMs are conserved across species. *See* Ex.1015, 16:26-34, 20:23-21:6, 41:3-6; Ex.1025, 566; Ex.1005, 367, 370-371; Ex.1008, Table 1, 4-5; Ex.1009, Table 1. Indeed, Freyman expressly teaches that a variety of animals, including amphibians, can be the source of xenogeneic ECM. Ex.1015, 16:26-34; 20:23-21:6, 41:3-6. Likewise, as also described above, based on McCusker, a POSA would have known known of superior regenerative properties of urodeles, specifically axolotls, were well known and it had been shown that the signaling pathways involved in axolotl regeneration were conserved across species, including humans.

113. Moreover, as also explained above, the processes for decellularizing xenogeneic ECM in order to avoid immune-mediated inflammation and rejection in a recipient host organism was both well known and in fact had been shown to work across a wide xenogeneic ECM sources. Thus, the Ground I References make clear that selecting axolotl a source of xenogeneic ECM would involve a straightforward substitution of one known ECM source (e.g., porcine small intestinal submucosa

(SIS) or bovine pericardium) for another (axolotl ECM) to achieve a predictable result—a suitable xenograft.

114. A POSA would have had a reasonable expectation of success in using urodele, and in particular axolotl, as a source of xenogeneic ECM based on the teachings of the Ground I References. The fact that ECM from numerous different animal sources had been both successfully decellularized and used in applications would have given a POSA a reasonable expectation of success that other sources of xenogeneic ECM would similarly work. Moreover, the knowledge that regenerative pathways of axolotls are conserved across species would have further supported that reasonable expectation. In addition, the prior success of xenografts sourced from animals with less regenerative capacities (e.g., mammals) provided an expectation that urodele ECM with superior regenerative capacity would achieve comparative success.

115. Accordingly, the Ground I References render “[d]ecellularized extracellular matrix (ECM) derived from a Urodele said decellularized ECM derived by” obvious.

b) Limitation 1.a: obtaining a tissue sample from a urodele, which tissue sample comprises ECM; and

116. In my opinion, the Ground I References render the limitation: “obtaining a tissue sample from a urodele, which tissue sample comprises ECM” obvious.

117. As explained above in limitation 1.p, it would have been obvious to select urodele ECM, and the process of obtaining tissue samples from various animal sources for ECM extraction was standard practice in the field. *See, e.g.*, Ex. 1060 Choi; Ex. 1018, Ota; Ex. 1038, Freytes; Ex. 1057, Teebken. Freyman, Badylak 2004, and Badylak 2009 also explicitly discuss methods for decellularizing and preparing ECM from obtained animal tissues. Ex. 1015, Freyman, 34-48; Ex. 1008, Badylak 2009, 4-10; Ex. 1005, Badylak 2004.

118. For instance, Freyman discloses that “[t]he body tissue may be obtained at approved slaughterhouses from animals fit for human consumption or from herds of domesticated animals maintained for the purpose of providing tissues or organ.” Ex. 1015, Freyman, 20:30-21:2. Freyman further discloses that “the body tissue is handled in a sterile manner, and any further dissection of the body tissue is carried out under aseptic conditions.” *Id.* Badylak 2009 further discloses that “[t]he native tissue from which an ECM scaffold is prepared must be mechanically or physically separated from unwanted tissue structures.” Ex. 1008, Badylak 2009, 4.

119. Obtaining a tissue sample is the first step to making any tissue graft, and nothing in the ’376 patent discloses anything inventive or unique about obtaining a tissue sample. Extending this routine practice to urodeles would have been obvious.

c) Limitation 1.b: decellularizing said tissue sample to produce decellularized ECM by removing sufficient cellular components of the sample to reduce or

eliminate antigenicity, making it suitable for use as a xenograft.

120. In my opinion, the Ground I References render the limitation: “decellularizing said tissue sample to produce decellularized ECM by removing sufficient cellular components of the sample to reduce or eliminate antigenicity, making it suitable for use as a xenograft” obvious.

121. First, Badylak 2009 and Freyman reflect the general knowledge of a POSA that decellularization was routine and necessary to reduce or eliminate antigenicity of tissue xenografts. Badylak 2009 discloses “xenogeneic ... cellular antigens are recognized as foreign by the host and result in an adverse inflammatory response or overt immune-mediated rejection.” Consequently, “[t]he effective removal of antigenic epitopes associated with cell membranes and intracellular components of tissues and organs is necessary to minimize or avoid an adverse immunologic response by ... xenogeneic recipients of the ECM scaffold.” Ex. 1008, Badylak 2009, 4.

122. Freyman likewise discloses that “[d]ecellularization generally refers to the removal of all cells, cellular components, and other non-extracellular matrix components (*e.g.*, serum, fat) while leaving intact an extracellular matrix (ECM) component.” Ex. 1015, Freyman, 34:13-19. Decellularization “can reduce or eliminate immune response associated with the cells as well as the cellular components,” making decellularized tissues to be “ideal natural biomaterials for

tissue repair and engineering.” *Id.* Freyman describes methods of decellularizing a tissue. *See, e.g., id.*, 34:14-39:33. Freyman further teaches that the decellularized ECM material can be derived from any suitable animal body tissue of xenogeneic origin, including *amphibians* such as urodeles and axolotls, among others, and that the decellularization techniques disclosed by Freyman can be applied across this wide variety of animal sources. *Id.*, 20:23-21:6, 34:12-49:32.

123. Badylak 2004 further describes well-known decellularization processes for xenografts. Badylak 2004 discloses that xenogeneic scaffolds “are typically ... subjected to decellularization and terminal sterilization without significant loss of the biologic effects of the ECM.” Ex. 1005, Badylak 2004, 367. Badylak 2004 discloses that “[a] typical method of tissue processing would include extensive rinsing in hypotonic saline, treatment with dilute (0.1%) peracetic acid or incubation in Triton 100X and 0.1 ammonium hydroxide and a terminal sterilization step with either ethylene oxide, gamma irradiation or E-beam irradiation (2.0 Mrad).” *Id.*, Badylak 2004, 372.

124. Badylak 2004 states “[t]hese methods have been shown to eliminate all intact cells (i.e. decellularize) and to degrade any nucleic acid remnants to less than 300 base pairs while leaving the constituent growth factors such as bFGF and VEGF biologically active.” Ex. 1005, Badylak 2004, 372. Badylak 2004 thus describes successful decellularization methods that could be generally applied in xenograft

applications, providing a motivation to use such known methods with a reasonable expectation of success in producing a urodele ECM tissue xenograft. *Id.*

125. The '376 patent acknowledges that decellularization process was well known in the art by stating, “[t]he urodele tissue samples obtained for decellularization can be treated in the manner detailed in US 2008/0046095 or US 2010/0104539,” which describe placental tissue grafts and their preparation methods. Ex.1001, 9:43-61. During prosecution of a related patent in the same family with identical subject matter, Patent Owner further conceded that it “did not invent a new process of decellularization” and that “removing cells to produce [a] non-toxic graft is straightforward.” Ex. 1062, '790 patent PH, 847.

126. The Ground I References therefore render claim 1 obvious.

2. Claim 2

a) The decellularized ECM of claim 1, wherein said decellularizing comprises subjecting said tissue sample to an alkaline treatment.

127. In my opinion, the Ground I References render the limitation: “wherein said decellularizing comprises subjecting said tissue sample to an alkaline treatment” obvious.³

³ Claim 2 does not require any specific alkaline treatment. Nor could it, as there is not a single disclosure of any specific alkaline treatment in the patent.

128. A POSA would have also found the use of an alkaline treatment obvious in view of the routine and successful application of such treatments in tissue processing known at the time. For example, alkaline treatments, such as those using ammonium hydroxide, calcium hydroxide, and sodium hydroxide, were routinely used to solubilize cytoplasmic components and remove nucleic acids. *See, e.g.*, Ex. 1033, Mendoza-Novelo; Ex. 1023 Van Dyke, 14-15, and 25-26; Badylak 2004, 372; Ex. 1061, De Flippo.

129. For ECM xenografts specifically, Badylak 2004 teaches that “treatment with...ammonium hydroxide” was a standard step in the decellularization process. Ex. 1005, Badylak 2004, 372. Similarly, Freyman teaches the use of “basic solutions” to remove cell debris, reducing the likelihood of adverse immune responses upon implantation. Ex. 1015, Freyman, 38:6-11.

130. Given these disclosures and the well-established use of alkaline treatments in analogous xenograft applications, a POSA would have been motivated to use an alkaline treatment, such as ammonium hydroxide, in the decellularization process and would have expected success. Accordingly, the Ground I References render claim 2 obvious.

3. Claim 3

a) The decellularized ECM of claim 1, wherein said decellularized ECM is subjected to sterilization.

131. In my opinion, the Ground I References render the limitation: “wherein said decellularized ECM is subjected to sterilization” obvious.

132. Badylak 2004 discloses that xenogeneic ECM scaffolds are “typically... subjected to decellularization and terminal sterilization without significant loss of the biologic effects of the ECM.” Ex. 1005, Badylak 2004, 367. “A typical method of tissue processing would include ... a terminal sterilization step with either ethylene oxide, gamma irradiation or E-beam irradiation (2.0 Mrad).” Ex. 1005, Badylak 2004, 367.

133. These sterilization methods “have been shown to eliminate all intact cells (i.e. decellularize) and to degrade any nucleic acid remnants to less than 300 base pairs while leaving the constituent growth factors ... biologically active.” *Id.*, 372. Similarly, Badylak 2009 discloses that “[t]he native tissue from which an ECM scaffold is prepared must be mechanically or physically separated from unwanted tissue structures, decellularized, often disinfected and dehydrated or lyophilized and terminally sterilized.” Ex. 1008, Badylak 2009, 4.

134. Freyman also discloses “[t]he body tissue may be further processed after decellularization to facilitate administration, injection or implantation. For example[], the decellularized extracellular matrix can be ... sterilized...” Ex. 1015,

Freyman, 4:19-30. Freyman discloses that “the ECM is sterilized by irradiation, ultraviolet light exposure, ethanol incubation (70-100%), treatment with glutaraldehyde, peracetic acid (0.1-1 % in 4% ethanol), chloroform (0.5%), or antimycotic and antibacterial substances.” *Id.*, 38:21-26.

135. In addition to the explicit teachings of Badylak 2004, Badylak 2009, and Freyman, a POSA would have been motivated to sterilize xenogeneic tissue to ensure implantation safety and reduce complications, with a reasonable expectation of success. Sterilization eliminates pathogens and enhances biocompatibility by reducing immunogenic contaminants and had become a routine procedure for any xenogeneic applications by 2009. As discussed, based on these prior successes in xenograft applications and general concern about complications, a POSA would have been motivated to apply these known processes to urodele-derived ECM xenografts with a reasonable expectation of success. Accordingly, the Ground I References render claim 3 obvious.

4. Claim 5

a) The decellularized ECM of claim 1 wherein the ECM is derived from Axolotl tissue.

136. In my opinion, the Ground I References render the limitation: “wherein the ECM is derived from Axolotl tissue” obvious.

137. As discussed above for Limitation 1.p, it would have been obvious to select axolotl as the source for a xenogeneic ECM. For example, McCusker

describes the axolotl as a “model organism for identifying the relevant signaling pathways and factors” involved in regeneration. Ex. 1025, McCusker, 565. McCusker highlights its “remarkable regenerative abilities,” including its ability to “regenerate complex structures such as limbs and spinal cords.” *Id.*, 565–566. Notably, McCusker explains that “the mechanisms of regeneration involve many basic biological processes regulated by conserved signaling pathways,” teaching that “we can expect to stimulate regeneration in a human by the same mechanisms used by the axolotl.” *Id.* These disclosures would have motivated a POSA to investigate axolotl ECM for xenograft applications.

138. The use of axolotl ECM would also have been obvious to try, given the finite number of predictable solutions (e.g., axolotl and newt). For instance, “[t]he axolotl (*Ambystoma mexicanum*) and the newt (*Notophthalmus viridescens*) are the two most commonly used urodele amphibians in the laboratory and both possess unsurpassed abilities to regenerate body parts (limbs, jaws, tail, spinal cord, etc.) and heal their wounds without scars throughout their life.” Ex. 1029, Roy, 12-13.

139. A POSA aware of urodele amphibians’ regenerative potential and conserved pathways would have been motivated to explore axolotl tissue as one of two possible options. Keating further confirms that the regenerative properties of newt-derived ECM components also apply to axolotl extracts, which are similarly

described as suitable for “dedifferentiating tissue from, for example, humans.” Ex. 1027, Keating, [0027], [0129].

140. Accordingly, the Ground I References render claim 5 obvious.

5. Claim 6

a) The decellularized ECM of claim 1, wherein the ECM includes basement membrane.

141. In my opinion, the Ground I References render the limitation: “wherein the ECM includes basement membrane” obvious.

142. Freyman discloses that “the decellularized extracellular matrix may contain a significant portion of the original tissue mass retaining physical properties in regard to strength and elasticity and has components which ... comprise glycosaminoglycans and proteins closely associated with collagen such as *the basement membrane complex*, laminin, fibronectin, growth factors, and cytokines.” Ex. 1015, Freyman, 39:1-5.

143. The basement membrane was known to have benefits as an ECM source because it contains key matrix proteins, including fibronectin, collagen, and laminin, that “provide distinct mechanical and physical properties to the ECM and contribute to the population of ligands that interact with the resident cell populations.” Ex. 1005, Badylak 2004, 368.

144. For example, Type IV collagen is “an important component of the epithelial basement membrane that distinguishes this particular ECM scaffold from

most other ECM scaffold materials.” Ex. 1008, Badylak 2009, 3. It “functions as an anchoring fibril to protect the overlying keratinocytes from sheer stresses.” Ex. 1005, Badylak 2004, 368. Additionally, the affinity of Type IV collagen in the basement membrane for endothelial cells “is the reason for its use as a biocompatible coating for medical devices intended to have a blood interface.” *Id.* And the basement membrane can also “foster the formation of confluent cell populations that cover the surface.” Ex. 1008, Badylak 2009, 3. A number of ECM xenografts that include the basement membrane were successfully developed for these reasons. Ex. 1032, Gilbert 2005; Ex. 1038, Freytes; Ex. 1049, Brown; Ex. 1022, Badylak 2008.

145. Accordingly, based on the known characteristics of the basement membrane and prior successes in xenograft applications, a POSA would have been motivated to include a basement membrane in the claimed tissue graft with a reasonable expectation of success. The Ground I References render claim 6 obvious.

6. Claim 9

- a) Limitation 9.a: The decellularized ECM of claim 1, wherein the ECM is in a form selected from the group consisting of a sheet, a powder, a gel, a paste, or a mesh, and**

146. In my opinion, the Ground I References render the limitation: “wherein the ECM is in a form selected from the group consisting of a sheet, a powder, a gel, a paste, or a mesh” obvious—as they are tailored to various applications in tissue repair and regeneration known at the time.

147. As an preliminary matter, Freyman discloses that the ECM graft could be processed into many different forms, including “particles,” “a slurry of small particles” (i.e., paste), “a sheet,” “a gel,” “mesh,” and a “reconstituted” form. Ex. 1015, Freyman, 38:27-33, 46:1-17, 39:13-18, 46:15-17; 39:6-12. Moreover, as described above, Freyman teaches that its processing techniques are broadly applicable to a wide variety of animal sources for xenogeneic ECM. A POSA therefore would have been motivated to use these forms of ECM graft with a reasonable expectation of success.

148. For instance, the dry sheet is the most standard form of ECM grafts because the sheet form is inherent to the tissue from which they are derived. *See, e.g.*, Ex. 1008, Badylak 2009, Table 1 (listing over 20 commercially available ECM xenografts in a sheet form); *see also*, Ex. 1022, Badylak 2008, 3. Vacuum pressing can be used to laminate multiple sheets of ECM to increase the strength and/or design-in specific mechanical behavior. Ex. 1008, Badylak 2009, 6. A sheet form of ECM xenograft has been successfully used in a number of commercially approved tissue graft devices. Badylak 2009, Table 1 (listing over 20 commercially available ECM xenografts in a sheet form); Ex. 1035, Santucci, 192, 196; Ex. 1022, Badylak 2008; Ex. 1034, Grandi.

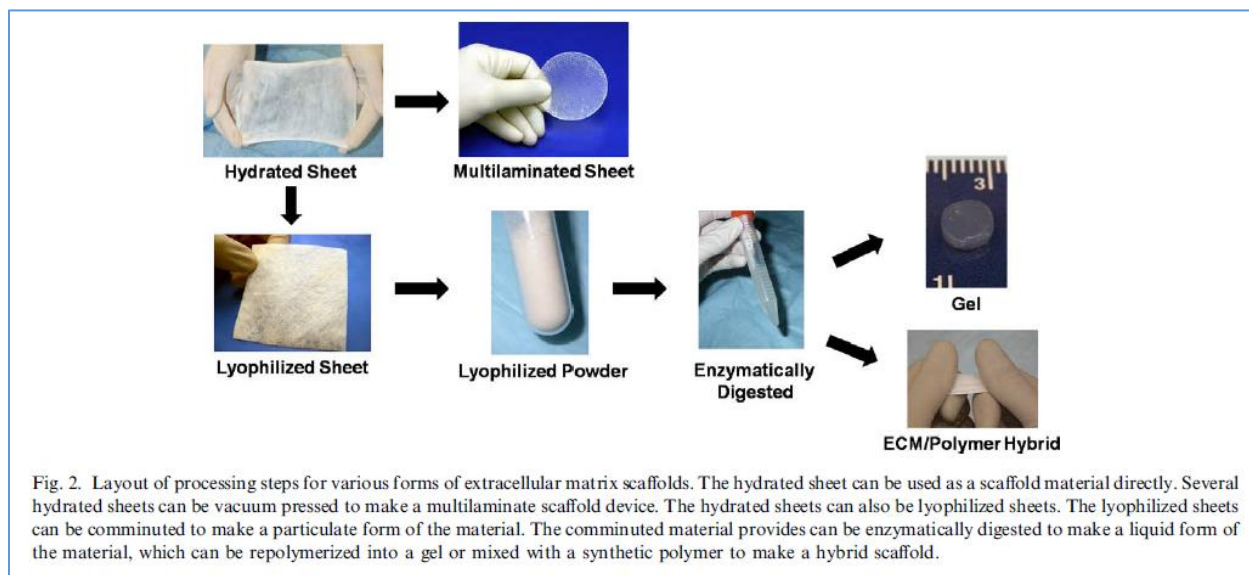
149. The lyophilized sheets of ECM can be comminuted into an ECM powder or particulate form. Ex. 1008, Badylak 2009, 6. ECM in powder or

particulate form offers advantages for “the delivery of the ECM as a suspension via minimally invasive techniques (e.g., needle injection) to the site of interest or for the manufacture of 3-D scaffolds by compaction methods.” *Id.*, 6. These particles “retain the ultrastructural, 3-D surface characteristics of the parent ECM sheet,” and particle sizes “ranging from 50 to 200 μm in diameter can be reproducibly manufactured.” *Id.*; *see also* Ex. 1032, Gilbert 2005. Powdered ECM’s scalability and suitability for minimally invasive delivery thus made it a practical choice for various clinical applications, with many successful xenograft uses known at the time. Powdered form of ECM xenograft has been successfully used in a number of applications. Ex. 1032, Gilbert 2005; Ex. 1022, Badylak 2008; Ex. 1036, Mazzitelli; Ex. 1037, Wood.

150. “A liquid or gel form of ECM can further expand the clinical utility of an ECM scaffold by allowing the delivery of the material via minimally invasive methods to sites of interest.” Ex. 1008, Badylak 2009, 6-7. The gel form can be delivered in its pre-gel liquid state more easily than a suspension of particles and applied via catheter or needle-based techniques. *Id.* An ECM gel can also function as a cell delivery vehicle, with rheological properties tailored to the tissue being repaired, while retaining the ECM graft’s bioactive properties. *Id.* A gel form of ECM scaffold can be produced, for example, by pepsin digestion followed by elevation of ionic strength, temperature and pH to physiological levels. *Id.* A gel

form of ECM xenografts has successfully used in a number of applications. Ex. 1038, Freytes; Ex. 1039, Wolf; Ex. 1040, Saldin; Ex. 1022, Badylak 2008.

151. Exemplary processing steps for the sheet, powder, and gel forms of ECM grafts are summarized below.



Ex. 1008, Badylak 2009, Fig. 2.

152. ECM paste offers another adaptable form for clinical use, combining the advantages of powdered and gel ECM. Like gel formulations, paste ECM enables minimally invasive delivery while providing better retention at the application site, particularly on irregular tissue surfaces. Paste ECM is typically composed of powdered ECM suspended in a biocompatible carrier and can conform to the target area without requiring additional fixation, facilitating controlled application in surgical and percutaneous procedures. *See, e.g.,* Ex. 1041, Furness; Ex. 1042,

Shevchenko. Paste based ECM xenografts have been successfully used in a number of applications. *Id.*

153. ECM can also be processed into a mesh. Ex. 1015, Freyman, 46:1-17. The ECM meshes mimic the mechanical properties of the native extracellular matrix while maintaining an ultrastructure to support cellular infiltration and tissue remodeling once implanted. Mesh structures were particularly useful in load-bearing applications, hernia repair, and other reconstructive procedures where tensile strength and durability were critical. ECM mesh can be produced by perforating the sheet or sheets of the decellularized ECM. Ex. 1015, Freyman, 46:1-17. ECM mesh has been successfully used in a number of applications. Ex. 1043, Sill; Ex. 1044, Badylak 1995; Ex. 1045, Mase.

154. Badylak 2009 and Freyman thus reflect the broader understanding in the field that ECM xenografts can be processed into the recited forms of ECM depending on the particular application of interest. Given the known successes of these ECM grafts, a POSA would have been motivated to apply the same processing techniques to produce the recited forms of urodele-derived ECM xenografts and would have reasonably expected comparable success. Accordingly, the Ground I References render the recited forms of ECM obvious.

b) Limitation 9.b: wherein the ECM is parietal ECM, mesothelial ECM, thoracic cavity ECM, abdominal

**cavity ECM, pericardium ECM or dermal tissue
ECM.**

155. In my opinion, the Ground I References render the limitation: “wherein the ECM is parietal ECM, mesothelial ECM, thoracic cavity ECM, abdominal cavity ECM, pericardium ECM or dermal tissue ECM” obvious.

156. Freyman discloses that decellularized ECM can be derived from various body tissues, including epithelial tissue, connective tissue, muscle tissue, and nerve tissue, and explicitly lists sources such as the skin, dermis, peritoneum, mesentery, submucosa, and serosa—tissues that correspond to dermal tissue ECM (e.g., skin and dermis), abdominal cavity ECM (e.g., peritoneum and mesentery), mesothelial ECM (serosa), and parietal ECM (e.g., peritoneum and mesentery). Ex. 1015, Freyman, 21:13-28.

157. Badylak 2009 similarly discloses that the ECM grafts can be derived from a variety of tissue types, including “heart valves, blood vessels, skin, nerves, skeletal muscle, tendons, ligaments, small intestinal submucosa (SIS), urinary bladder, and liver.” Ex. 1008, Badylak 2009, 2. Badylak 2009 further discloses a list of commercially available biological ECM materials, including those derived from pericardium ECM and dermal tissue ECM, as shown below.

Product	Company	Material	Processing	Form
AlloDerm	Lifecell	Human skin	Natural	Dry sheet
AlloPatch®	Musculoskeletal Transplant Foundation	Human fascia lata	Natural	Dry sheet
Axis™ dermis	Mentor	Human dermis	Natural	Dry sheet
Bard® Dermal Allograft	Bard	Cadaveric human dermis	Natural	Dry sheet
CuffPatch™	Arthrotek	Porcine small intestinal submucosa (SIS)	Cross-linked	Hydrated sheet
DurADAPT™	Pegasus Biologicals	Horse pericardium	Cross-linked	Dry sheet
Dura-Guard®	Synovis Surgical	Bovine pericardium	Cross-linked	Hydrated sheet
Durasis®	Cook SIS	Porcine small intestinal submucosa (SIS)	Natural	Dry sheet
Durepair®	TEI Biosciences	Fetal bovine skin	Natural	Dry sheet
FasLata®	Bard	Cadaveric fascia lata	Natural	Dry sheet
Graft Jacket®	Wright Medical Tech	Human skin	Natural	Dry sheet
Oasis®	Healthpoint	Porcine small intestinal submucosa (SIS)	Natural	Dry sheet
OrthADAPT™	Pegasus Biologicals	Horse pericardium	Cross-linked	Dry sheet
Pelvicol®	Bard	Porcine dermis	Cross-linked	Hydrated sheet
Peri-Guard®	Synovis Surgical	Bovine pericardium	Cross-linked	Dry sheet
Permacol™	Tissue Science Laboratories	Porcine skin	Cross-linked	Hydrated sheet
PriMatrix™	TEI Biosciences	Fetal bovine skin	Natural	Dry sheet
Restore™	DePuy	Porcine small intestinal submucosa (SIS)	Natural	Dry sheet
Stratasis®	Cook SIS	Porcine small intestinal submucosa (SIS)	Natural	Dry sheet
SurgiMend™	TEI Biosciences	Fetal bovine skin	Natural	Dry sheet
Surgisis®	Cook SIS	Porcine small intestinal submucosa (SIS)	Natural	Dry sheet
Suspend™	Mentor	Human fascia lata	Natural	Dry sheet
TissueMend®	TEI Biosciences	Fetal bovine skin	Natural	Dry sheet
Vascu-Guard®	Synovis Surgical	Bovine pericardium	Cross-linked	Dry sheet
Veritas®	Synovis Surgical	Bovine pericardium	Cross-linked	Hydrated sheet
Xelma™	Molnlycke	ECM protein, PGA, water		Gel
Xenform™	TEI Biosciences	Fetal bovine skin	Natural	Dry sheet
Zimmer Collagen Patch®	Tissue Science Laboratories	Porcine dermis	Cross-linked	Hydrated sheet

Id., Table 1.

158. Freyman and Badylak 2009 reflect that the recited ECM sources were commonly used and successfully commercialized for clinical xenograft applications. Based on this teaching, a POSA would have been motivated to obtain the ECM materials from the recited ECM in a urodele and would have expected comparable success. Accordingly, the Ground I References render claim 9 obvious.

7. Claim 13

a) The ECM of claim 1, wherein the ECM is a paste.

159. For the reasons set forth in Limitation 9.a, the Ground 1 References render the limitation: “wherein the ECM is a paste” obvious.

8. Claim 14

a) The ECM of claim 1, wherein the ECM is a gel.

160. For the reasons set forth in Limitation 9.a, the Ground 1 References render the limitation: “wherein the ECM is a gel” obvious.

9. Claim 15

a) The ECM of claim 14, wherein the gel comprises a synthetic polymer.

161. In my opinion, the Ground I References render the limitation: “wherein the gel comprises a synthetic polymer” obvious.

162. Badylak 2009 discloses that additional processing steps can be used to “tailor the functional properties of the device for specific applications.” Ex. 1008, Badylak 2009, 7. In addition, Badylak 2009 discloses that “[p]owdered forms of ECM scaffolds ... may be combined with synthetic polymers to create hybrid scaffolds.” Ex. 1008, Badylak 2009, 6, Fig. 2. As noted above, the powdered form of ECM scaffolds is used to form a gel. *See, e.g.*, Ex. 1008, Badylak 2009, Fig. 2.

163. Likewise, Freyman discloses that “polymeric materials can be used to achieve controlled or sustained release of the decellularized extracellular matrix material,” examples of which include “poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides, (PLG), ... poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glucosides) (PLGA), and polyorthoesters.” Ex. 1015, Freyman 43:24-44:3. A number of such synthetic polymers were used to

improve the physical and chemical characteristics of ECM gels, further providing a reasonable expectation of success. *See, e.g.,* Ex. 1046, Hong; Ex. 1019, Seif-Naraghi; Ex. 1048, Duan.

164. Indeed, the '376 patent confirms that the use of synthetic polymers for improving characteristics of an ECM gel was well-known in the art. It states “[s]ynthetic biocompatible and cyto-compatible material can be combined with the ECM,” to provide “a structural support for ... a gel of the ECM.” Ex.1001, 14:29-58. Exemplary synthetic polymers for these applications include “biodegradable polyester polymers made of polyglycolic acid,” “polyanhydrides,” “polyorthoesters,” and “polylactic acid,” all of which were “known in the art.” *Id.*

165. Accordingly, the Ground I References as well as the '376 patent confirm that the use of additional synthetic polymer agents for improving the mechanical and structural characteristics of a gel ECM was well established in the art. The Ground I References therefore render claim 15 obvious.

10. Claim 16

a) The ECM of claim 1, wherein the ECM is a mesh.

166. For the reasons set forth in Limitation 9.a, the Ground 1 References render the limitation: “wherein the ECM is a mesh” obvious.

11. Claim 21

a) A method for making a decellularized extracellular matrix (ECM) according to claim 1 derived from a

Urodele said decellularized ECM derived by:

a. obtaining a tissue sample from a urodele, which tissue sample comprises ECM; and

b. decellularizing said tissue sample to produce decellularized ECM by removing sufficient cellular components of the sample to reduce or eliminate antigenicity, making it suitable for use as a xenograft.

167. I understand that claim 21 depends from claim 1 and recites a method making the tissue graft of claim 1 using the same processing steps as claim 1. For the reasons set forth in claim 1, the Ground I References render the method of making the tissue graft of claim 1 obvious. The Ground I References therefore render claim 21 obvious.

12. Claim 24

a) The method of claim 21, further comprising sterilizing the decellularized ECM.

168. For the reasons set forth in claim 3, the Ground 1 References render the limitation: “further comprising sterilizing the decellularized ECM” obvious.

13. Claim 26

a) A method for making the ECM of claim 1, comprising extracellular matrix components derived from a Urodele, said method comprising:

a. obtaining a tissue sample from a urodele, which tissue sample comprises extracellular matrix; and

b. decellularizing said tissue sample to produce decellularized extracellular matrix by removing sufficient cellular components of the sample to reduce

or eliminate antigenicity, making it suitable for use as a xenograft.

169. I understand that claim 26 depends from claim 1 and recites a method of making the ECM of claim 1 comprising ECM components derived from a urodele using the same processing steps recited in claim 1. For the reasons set forth above in claim 1, the Ground I References render the method of making the ECM of claim 1 comprising urodele ECM components obvious. Accordingly, the Ground I References render claim 26 obvious.

14. Claim 27

- a) The method according to claim 26, wherein said decellularizing comprises subjecting said tissue sample to an alkaline treatment, and wherein said process further optionally comprises subjecting said sample to sterilization, and wherein the Urodele is an Axolotl.**

170. For the reasons set forth in claims 2, 3, and 5, the Ground I References render the limitation: “wherein said decellularizing comprises subjecting said tissue sample to an alkaline treatment, and wherein said process further optionally comprises subjecting said sample to sterilization, and wherein the Urodele is an Axolotl” obvious.

15. Claim 28

- a) A method for making the ECM of claim 27, comprising sterile, isolated, decellularized Urodele Extracellular Matrix (ECM) or a sterile Urodele fraction derived from the isolated, decellularized Urodele ECM, said method comprising:**

a. obtaining a tissue sample from a urodele, which tissue sample comprises extracellular matrix;

b. decellularizing and sterilizing said tissue sample, to produce the sterile decellularized extracellular matrix, by removing sufficient cellular components of the sample to reduce or eliminate antigenicity, making it suitable for use as a xenograft; and sterilizing said tissue.

171. I understand that claim 28 recites making the ECM of claims 1, 26, and 27 with the same processing steps and additionally requires sterilization of the tissue sample rather than making it optional. For the reasons set forth in claim 3, the Ground I References render such sterilization process of claim 28 obvious.

16. Claim 29

a) The method according to claim 28, wherein the ECM is a biocompatible sheet, mesh, gel, graft, tissue, device, or is a material coated with, impregnated with, encapsulated with or that is encapsulated by, or having attached thereto the isolated, decellularized Urodele ECM.

172. I understand that claim 29 depends from claim 28, and recites specific forms of ECM, which include “a biocompatible sheet, mesh, gel, graft, tissue, device, or ... a material coated with, impregnated with, encapsulated with or that is encapsulated by, or having attached thereto the isolated, decellularized Urodele ECM.” For the reasons set forth in Limitation 9.a, the Ground I References render such forms of ECM recited in claim 29, obvious.

17. Claim 30

- a) The method according to claim 28, wherein said decellularizing comprises subjecting said tissue sample to an alkaline treatment, wherein said process further comprises subjecting said sample to sterilization, wherein the Urodele is an Axolotl.**

173. For the reasons set forth in claims 2, 3, and 5, the Ground I References render “wherein said decellularizing comprises subjecting said tissue sample to an alkaline treatment, wherein said process further comprises subjecting said sample to sterilization, wherein the Urodele is an Axolotl” obvious.

18. Independent Claim 31

- a) Limitation 31.p: A biomaterial comprising decellularized extracellular matrix (ECM) derived from a Urodele said decellularized ECM derived by:**

174. For the reasons set forth in Limitation 1.p, the Ground I References render the limitation: “[a] biomaterial comprising decellularized extracellular matrix (ECM) derived from a Urodele said decellularized ECM derived by” obvious.

- b) Limitation 31.a: obtaining a tissue sample from a urodele, which tissue sample comprises ECM; and**

175. For the reasons set forth in Limitation 1.a, the Ground I References render the limitation: “obtaining a tissue sample from a urodele, which tissue sample comprises ECM” obvious.

- c) Limitation 31.b: decellularizing said tissue sample to produce decellularized ECM by removing sufficient cellular components of the sample to reduce or**

eliminate antigenicity of the biomaterial making it suitable for use as a xenograft.

176. For the reasons set forth in Limitation 1.b, the Ground I References render the limitation: “decellularizing said tissue sample to produce decellularized ECM by removing sufficient cellular components of the sample to reduce or eliminate antigenicity of the biomaterial making it suitable for use as a xenograft” obvious.

19. Independent Claim 32

- a) Limitation 32.p: A method of making a biomaterial comprising decellularized extracellular matrix (ECM) derived from a Urodele said decellularized ECM said method comprising:**

177. For the reasons set forth in claims 21 and Limitation 1.p, the Ground I References render the limitation: “[a] method of making a biomaterial comprising decellularized extracellular matrix (ECM) derived from a Urodele said decellularized ECM said method comprising:” obvious.

- b) Limitation 32.a: obtaining a tissue sample from a urodele, which tissue sample comprises ECM; and**

178. For the reasons set forth in Limitation 1.a, the Ground I References render the limitation: “obtaining a tissue sample from a urodele, which tissue sample comprises ECM” obvious.

- c) Limitation 32.b: decellularizing said tissue sample to produce decellularized ECM by removing sufficient cellular components of the sample to reduce or**

eliminate antigenicity of the biomaterial making it suitable for use as a xenograft.

179. For the reasons set forth in claim 21 and Limitation 1.b, the Ground I References render the limitation: “decellularizing said tissue sample to produce decellularized ECM by removing sufficient cellular components of the sample to reduce or eliminate antigenicity of the biomaterial making it suitable for use as a xenograft” obvious.

B. Ground II: Obviousness of Claims 9-11 and 23 over the Ground I References and Gilbert 2005

1. Claims 9

- a) The decellularized ECM of claim 1, wherein the ECM is in a form selected from the group consisting of a sheet, a powder, a gel, a paste, or a mesh, and wherein the ECM is parietal ECM, mesothelial ECM, thoracic cavity ECM, abdominal cavity ECM, pericardium ECM or dermal tissue ECM.**

2. Claim 10

- a) The ECM according to claim 9, wherein the powder has a particle size between 125 and 850 microns.**

3. Claim 11

- a) The ECM according to claim 10, wherein the powder has a particle size of less than 250 microns.**

180. Claims 10-11 depend from claims 1 and 9, which as discussed above, are rendered obvious by the Ground I References. Claim 10 additionally recites “wherein the powder has a particle size between 125 and 850 microns.” Claim 11

depends from claim 10 and additionally recites “wherein the powder has a particle size of less than 250 microns.” Thus, if it was obvious to make the decellularized ECM recited in claims 1 and 9 in an ECM power form having a particle size between 125 and 250 microns, then claims 9-11 are rendered obvious.

181. As an initial matter, I note that claims 9-11 do not require all of the particles in the recited powder to fall within the 125 and 250 microns range. Instead, the claims are directed to “*a* powder” having “*a* particle size” between 125 and 250 microns. Thus a POSA would need only find it obvious that at least *some* particles in the ECM powder are within the recited range. Based on the particle sizes taught in Gilbert 2005 and Badylak 2009, it would have been obvious to a POSA that at least some particles of Gilbert 2005 and Badylak 2009 would fall under the recited range.

182. Using the sonic sifting and laser diffraction techniques, one of the disclosed methods of Gilbert 2005 produced an average particle size, “based on an assumed maximum of 250 μm , [of] approximately 190.6 μm ...[and] 158.0 μm ” respectively, falling squarely within the claimed range between 125 and 250 microns. *Id.* A second method, using salt precipitation, produced a powder with a smaller mean particle size, but the majority still remained within the 125 to 250 micron range. Ex. 1032, Gilbert 2005, Fig. 2, 1431-1433.

183. Based on Gilbert 2005, a POSA would have been motivated to use a powder form of ECM because it “provides a great deal more flexibility in terms of delivery of the material to target sites.” *Id.*, 1434. Gilbert 2005 further discloses that the powder “has been successfully injected through a 22 gauge needle (410 mm inner diameter), which is a typical size used for intramuscular injection.” *Id.*, 1434. I also note that Gilbert 2005 discloses producing particulate forms of ECM using the same two methods described in the ’376 patent. *Compare* Ex. 1032, Gilbert 2005, 1431 *with* Ex.1001, 11:56-12:46.

184. Badylak 2009 additionally provides a motivation to use such powder form of ECM because it “allows for the delivery of the ECM as a suspension via minimally invasive techniques ... or for the manufacture of 3-D scaffolds by compaction methods,” retaining “the ultrastructural, 3-D surface characteristics of the parent ECM sheet.” Ex. 1008, Badylak 2009, 6. Badylak 2009 teaches that ECM “[p]article sizes ranging from 50 to 200 μ m in diameter can be reproducibly manufactured” and “suspensions made from a particulate (powdered) form of lyophilized urinary bladder matrix were successfully used as an injectable scaffold for the treatment of urinary incontinence in preclinical studies,” providing a reasonable expectation of success for the recited power form. *Id.*; *see also* Ex. 1037, Wood.

185. Accordingly, in my opinion, the Ground I References and Gilbert 2005 render claims 9-11 obvious.

4. Claim 23

- a) The method of claim 21, further comprising homogenizing the decellularized ECM to form a powder, and, optionally reconstituting the powder as a gel.**

186. The Ground I References and Gilbert 2005 render “further comprising homogenizing the decellularized ECM to form a powder, and, optionally reconstituting the powder as a gel” obvious.

187. Gilbert 2005 discloses two methods to homogenize and produce a powder form of ECM. Ex. 1032, Gilbert 2005, 1432. The first method “involved lyophilizing the disinfected material,” “chopping it into small sheets for immersion in liquid,” and then reducing to “small pieces with a Waring blender,” and then “restricting the collected powder size to less than 250 um” using a #60 screen. *Id.*

188. The second method involved “soaking the disinfected material in a 30% (w/v) NaCl solution for 5 min,” “snap fr[eezing] in liquid nitrogen to precipitate salt crystals,” “lyophilizing to remove residual water,” and comminuting as described in the first method. *Id.* The particles were then suspended in deionized water and centrifuged for 5 min at 1000 rpm three times to remove the NaCl, snap frozen, lyophilized again, and placed in a rotary knife mill to disaggregate the individual

particles. *Id.* As noted above, these methods produced ECM powder with a particle size falling within 125 and 250 microns.

189. A POSA would have been motivated to use these methods to produce a powder form of ECM because it “provides a great deal more flexibility in terms of delivery of the material to target sites.” *Id.* The powder produced from the disclosed methods “has been successfully injected through a 22 gauge needle (410 mm inner diameter), which is a typical size used for intramuscular injection.” *Id.*, 1434. I note that the same two methods of homogenizing and producing ECM powder are described in the ’376 patent. *Compare* Ex. 1032, Gilbert 2005, 1431 *with* Ex.1001, 11:56-12:46.

190. Accordingly, the Ground I References and Gilbert 2005 render claim 23 obvious.

C. Ground III: Obviousness of Claims 7, 8, 12, and 17-19, 22, and 25 over the Ground I References and Van Dyke

1. Claim 7

- a) The decellularized ECM of claim 1, wherein the ECM is infused with, coated with, or attached to an agent xenogenic to a Urodele that is a growth factor, a cytokine, a chemokine, a protein, a carbohydrate, a sugar, a steroid, an antimicrobial agent, a synthetic polymer, an adhesive, a drug or a cell.**

191. In my opinion, the Ground I References and Van Dyke render “wherein the ECM is infused with, coated with, or attached to an agent xenogenic to a Urodele

that is a growth factor, a cytokine, a chemokine, a protein, a carbohydrate, a sugar, a steroid, an antimicrobial agent, a synthetic polymer, an adhesive, a drug or a cell” obvious.

192. First, Freyman discloses that “[t]he ECM may ... comprise other biological materials ... includ[ing], but are not limited to, vascular endothelial growth factor, transforming growth factor..., small molecules such as statins. Ex. 1015, Freyman, 15:22-16:10. The ECM “may also contain therapeutic proteins that can be control-released locally,” and “drug-loaded microspheres, matrix-bound drug, coated microspheres or other implants.” *Id.* Freyman further discloses that “an antibacterial, an antifungal, or a sterilant or a combination thereof, may be included...to protect the transplant tissue matrix from contamination with environmental pathogens.” *Id.*, 38:21-23.

193. Van Dyke, which I note is directed to the same field of endeavor as Badylak 2004, Badylak 2009, and Freyman (xenogeneic ECMs), similarly discloses that “[p]roteins (such as growth factors) or other additives (such as antibiotics, anti-inflammatories, and modulators of immune response) may also be added to ... bioscaffold preparations at any time.” Ex. 1023, Van Dyke, 9.

194. It also notes that “[v]arious treatments may be applied to enhance adherence of cells to the substrate and/or to each other[,including...] application of various proteins...to the bioscaffold substrate or the growing construct...bioscaffold

can be impregnated with growth factors such as nerve growth factor (NGF)...TGF β ...” *Id.*

195. In my opinion, a POSA would have been motivated to include these additives to enhance the properties of ECM tissue grafts.

196. For instance, adding growth factors enhance the regenerative potential of native cells when the ECM is implanted. Similarly, incorporating antimicrobial provides additional protection against infection, reducing the risk of post-implantation complications. Moreover, incorporating anti-inflammatory agents can help mitigate the immune response, promoting better integration with host tissue. The successful and routine incorporation of such additives in various xenograft applications would have further provided a reasonable expectation of success for incorporating the recited agents into the ECM tissue graft. *See, e.g.,* Ex. 1036, Mazzitelli; Ex. 1019, Seif-Naraghi; Ex. 1018, Ota; Ex. 1046, Hong.

197. Accordingly, the Ground I References and Van Dyke render claim 7 obvious.

2. Claim 8

a) The decellularized ECM of claim 7, wherein the agent is a human cell.

198. The Ground I References and Van Dyke render “wherein the agent is a human cell” obvious.

199. For example, Van Dyke teaches that “[i]n preferred embodiments, improved bioscaffolds disclosed herein are...modified to allow seeding, infiltration, and attachment of the patient’s own cells prior to or after implantation.” Ex. 1023, Van Dyke, 7. Van Dyke further discloses that “the present invention is a method of seeding a bioscaffold with mammalian cells (e.g., human...) said seeding carried out either in vitro or in vivo wherein a bioscaffold produced as described herein is utilized for said seeding.” *Id.*, 4, 29-31.

200. Seeding human cells onto a decellularized xenograft before implantation can enhance functionality, potentially reduce immune rejection, promote integration with host tissue, and support remodeling and healing. Accordingly, a number of human cell seeded decellularized ECM grafts were successfully developed for vascular, osteogenic, and urethral applications. Ex. 1057, Teebken; Ex. 1058, Davis; Ex. 1020, Penolazzi; Ex. 1016, Bader; Ex. 1021, Villanola. Because cell seeded ECM grafts are capable of repairing larger and perhaps more complicated defects and have shown to be effective in prior studies, a POSA would have been motivated to seed the recited ECM graft with a human cell with a reasonable expectation of success.

201. Accordingly, the Ground I References and Van Dyke render claim 8 obvious.

3. Claim 12

a) **The decellularized ECM according to claim 1 contained within a package.**

202. The Ground I References and Van Dyke render “[t]he decellularized ECM according to claim 1 contained within a package” obvious.

203. For example, Van Dyke discloses that the bioscaffold containing ECM can be “freeze-dried or lyophilized after the oxidizing step, sterilized and (optionally) aseptically packaged for subsequent use.” Ex. 1023, Van Dyke, 4. Van Dyke further discloses that it can be aseptically packaged in a sterile container. *Id.*, 29.

204. A POSA would have recognized that packaging decellularized ECM in a sterile container, as described in Van Dyke, ensures product stability, maintains sterility, and facilitates storage and transport. Given that ECM is typically used in clinical or surgical settings, packaging it in a manner that preserves its integrity would have been an obvious and routine step. Thus, a POSA would have been motivated to follow Van Dyke’s teachings and apply them to the ECM of claim 1, rendering claim 12 obvious.

4. Claim 17

a) **The ECM according to claim 1 comprising sterile, isolated, decellularized Urodele extra cellular matrix (ECM), wherein the ECM is in the form of one or more sheets, wherein the ECM is parietal ECM, mesothelial ECM, thoracic cavity ECM, abdominal cavity ECM, pericardium ECM or dermal tissue ECM, and the sheet of ECM is infused with, coated**

with, or attached to an agent xenogeneic to a Urodele that is a growth factor, a cytokine, a chemokine, a protein, a carbohydrate, a sugar, a steroid, an antimicrobial agent, a synthetic polymer, an adhesive, or a drug.

205. For the reasons set forth in claims 3, 7 and 9, in my opinion, the Ground I References and Van Dyke render the limitation: “ECM...comprising sterile, isolated, decellularized Urodele extra cellular matrix (ECM), wherein the ECM is in the form of one or more sheets, wherein the ECM is parietal ECM, mesothelial ECM, thoracic cavity ECM, abdominal cavity ECM, pericardium ECM or dermal tissue ECM, and the sheet of ECM is infused with, coated with, or attached to an agent xenogeneic to a Urodele that is a growth factor, a cytokine, a chemokine, a protein, a carbohydrate, a sugar, a steroid, an antimicrobial agent, a synthetic polymer, an adhesive, or a drug” obvious.

5. Claim 18

a) The ECM of claim 17, wherein the agent is an anti-microbial agent.

206. I understand that claim 18 depends from claim 17 and additionally recites “wherein the agent is an anti-microbial agent.” For the reasons set forth in claim 7, in my opinion, the Ground I References and Van Dyke render “wherein the agent is an anti-microbial agent” obvious. In particular, Van Dyke discloses that the ECM can include “other additives ... such as *antibiotics*,” which is an anti-microbial agent. Ex. 1023, Van Dyke, 9. Likewise, Freyman discloses that “an

antibacterial, an *antifungal*, or a sterilant or a combination thereof, may be included...to protect the transplant tissue matrix from contamination with environmental pathogens.” Ex. 1015, Freyman, 38:21-23.

6. Claim 19

- a) The ECM of claim 17, wherein the agent is a growth factor.**

207. For the reasons set forth in claim 7, in my opinion, the Ground I References and Van Dyke render “wherein the agent is a growth factor” obvious.

7. Claim 22

- a) The method of claim 21, wherein the method further comprises performing the decellularization in a manner to retain structural and functional integrity of the decellularized ECM sufficient to permit the decellularized ECM to be useful as a matrix upon and within which cells can grow.**

208. The Ground I References and Van Dyke render the limitation: “wherein the method further comprises performing the decellularization in a manner to retain structural and functional integrity of the decellularized ECM sufficient to permit the decellularized ECM to be useful as a matrix upon and within which cells can grow” obvious.

209. As discussed in Section VII, Van Dyke discloses methods for producing bioscaffolds by decellularizing animal tissues, including amphibians, to remove cellular material while preserving the beneficial components of ECM. Ex. 1023, Van Dyke, 7-17. Specifically, the biomaterials disclosed in Van Dyke are

“decellularized to minimize inflammatory potential and host immune response,” while maintaining “optimum biomechanical integrity to withstand rehabilitation until complete remodeling has occurred.” *Id.*, 7.

210. For instance, Van Dyke discloses a decellularization method that includes “pre-treat[ing] the tissue with deionized (DI) water or other hypotonic solution to disrupt the cells,” “treat[ing] the disrupted tissue with an enzyme such as trypsin to break down cell adhesions,” “treat[ing] the tissue with detergent solution such as 0.5% tiron X-100 (a nonionic surfactant) in ammonium hydroxide,” and “wash[ing] residual reagents out of the bioscaffold with repeated washes of DI water.” Ex. 1023, Van Dyke, 14. Van Dyke notes that a POSA understood that any decellularization methods that “remove[] a substantial fraction of the cells while leaving the interstitial structure substantially intact” would work. *Id.*, 14-17. And “[i]t will be appreciated by those skilled in the art ... [a]n appropriate decellularization technique may be selected and parameters ... may be optimized in order to achieve a desired degree of decellularization.” *Id.*, 15.

211. In one example, Van Dyke discloses a tendon ECM scaffold prepared using a variation of the disclosed decellularization method, which enabled “cell infiltration and repopulation,” preserved the mechanical properties and biocompatibility of native tissue, and “did not exhibit any evidence of a gross inflammatory reaction...when implanted.” Ex. 1023, Van Dyke, 17-22. In another

example, Van Dyke discloses a decellularization method that “decrease[d] DNA content in a meniscus scaffold,” while “the extracellular matrix architecture of the meniscus remain[ed] intact,...providing an improved scaffold that minimizes immunologic response and encourages cellular in-growth.” *Id.*, 26-27.

212. Moreover, as noted in Limitation 1.b, Badylak 2004 further describes well-known decellularization processes for xenografts that allow preservation of structural and functional integrity of the decellularized ECM. Ex.1005, Badylak 2004. Badylak 2004 discloses that “[a] typical method of tissue processing would include extensive rinsing in hypotonic saline, treatment with dilute (0.1%) peracetic acid or incubation in Triton 100X and 0.1 ammonium hydroxide and a terminal sterilization step with either ethylene oxide, gamma irradiation or E-beam irradiation (2.0 Mrad).” *Id.*, Badylak 2004, 372. “These methods have been shown to eliminate all intact cells (i.e. decellularize) and to degrade any nucleic acid remnants to less than 300 base pairs while leaving the constituent growth factors such as bFGF and VEGF biologically active.” Ex. 1005, Badylak 2004, 372.

213. Based on these teachings, a POSA would have been motivated to apply decellularization in a way that preserves the structural and functional integrity of the ECM, so that it remains suitable as a matrix for cellular growth upon implantation and would have expected success. Accordingly, in my opinion, the Ground I References and Van Dyke render claim 22 obvious.

8. Claim 25

- a) The method of claim 21, further comprising attaching the decellularized ECM to an agent xenogenic to a Urodele.**

214. For the reasons set forth in claim 7, in my opinion, the Ground I References and Van Dyke render “attaching the decellularized ECM to an agent xenogenic to a Urodele” obvious.

X. NO SECONDARY CONSIDERATIONS

215. As noted above, I understand that certain objective factors called “secondary considerations” should also be considered when determining whether a claimed invention is non-obvious. I understand that these “secondary considerations” may be utilized to guard against a hindsight bias in consideration of the prior art, which include: (a) commercial success of a product due to the merits of the claimed invention; (b) a long-felt, but unsatisfied need for the invention; (c) failure of others to find the solution provided by the claimed invention; (d) copying of the invention by others; (e) unexpected results achieved by the invention; (f) praise of the invention by others skilled in the art; (g) lack of independent simultaneous invention within a comparatively short space of time; (h) teaching away from the invention in the prior art.

216. In my opinion, the secondary considerations here fail to show that the Challenged Claims of the '376 patent are non-obvious.

217. ***No Unexpected Results.*** As set out in Section X, the Challenged Claims do not require any particular levels of efficacy or immunogenicity. They only require removing sufficient cellular components to reduce antigenicity to make the material suitable for use as a xenograft. As discussed in Section VI, standard decellularization protocols were routinely used to reduce immunogenicity and antigenicity of ECM tissue grafts for xenograft applications across a wide range of animal sources. Thus, there was nothing unexpected about the immunoprivileged status of urodele ECM once it is decellularized. It was well within a POSA's knowledge as discussed above.

218. ***No Long-Felt, Unmet Need.*** There was no long-felt, unmet need. Any purported need for the claimed biomaterials would have been fulfilled long before January 2013 because other successful ECM xenografts existed as discussed above.

219. ***No Nexus.*** Patent Owner cannot establish nexus to the claimed biomaterials regimen of the '376 patent because the art disclosed all of the claimed elements.

220. For the foregoing reasons, in my opinion, there is a reasonable likelihood that the Challenged Claims are unpatentable.

XI. CONCLUDING STATEMENTS

221. This declaration, along with the attached appendices, is based on my present assessment of material and information currently available to me.

222. I hereby declare that all statements made herein of my own knowledge are true and that all statements made herein on information and belief are believed to be true. Further, these statements were made with the knowledge that willful false statements and the lie so made are punishable by fine, imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-identified patent.

2/19/2025

Date

A handwritten signature in blue ink that reads "Steve Badylak". The signature is written in a cursive style with a large, stylized "S" and "B".

Stephen F. Badylak, Ph.D.