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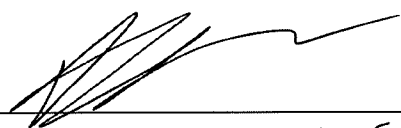
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First Named Inventor	: Ryanne Early	Confirmation No.: TBD
Title	: DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE	Dated: February 26, 2020

INFORMATION DISCLOSURE STATEMENT

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

Sir:

The Applicant encloses Form PTO-1449, which lists all the previously cited publications in parent US Application No. 14/759,895, filed July 8, 2015. Copies of the publications can be found in the image file wrapper of parent US Application No. 14/759,895.

The Applicant respectfully requests that this Information Disclosure Statement be officially entered into the file and that appropriate notification be made that all the publications were considered by the Examiner.

Respectfully submitted,

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DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE**BACKGROUND OF THE INVENTION**

[0001] Tissue engineering efforts are ongoing to produce methods and materials for replacing biological functions, typically repairing or replacing whole tissues or portions thereof. In this regard, wound treatment and skin repair are areas of predominant focus, as the loss of skin integrity due to illness or injury can lead to chronic, life threatening complications.

[0002] Wound healing involves complex interactions between cells, growth factors, and extracellular matrix (ECM) components to reconstitute tissue following injury. The wound healing process in adult mammalian tissue has been well characterized and can be broken down into three stages - inflammation, proliferation, and remodeling.

[0003] Typically, in response to an incision or trauma the body conveys blood, blood products, and proteins into the void (also referred to as the cavity or negative space) formed at the wound. During early inflammation, a wound exudate begins to form under the influence of inflammatory mediators and as a result of vasodilation. Fibrin and fibronectin present in clotting blood provide a scaffold over which cells such as keratinocytes, platelets and leukocytes migrate to the wound site. Bacteria and debris are phagocytosed and removed, and growth factors are released that stimulate the migration and division of fibroblasts.

[0004] The subsequent stage of wound healing involves new tissue formation as fibrous connective tissue, termed granulation tissue (composed of fibroblasts, macrophages and neovasculature) replaces the fibrin clot. New blood vessels are formed during this stage, and fibroblasts proliferate and produce a provisional ECM by excreting collagen and fibronectin. Nearly all mammalian cells require adhesion to a surface in order to proliferate and function properly. The ECM fulfills this function. Initially, the provisional ECM contains of a network of Type III collagen, a weaker form of collagen that is rapidly produced. This is later replaced by the stronger Type I collagen (which contributes to scar formation). At the same time, re-epithelialization of the epidermis occurs. During this process, epithelial cells proliferate and migrate over the newly forming tissue as proteases such as metalloproteinases (MMPs) and collagenases at the leading edge of the migrating cells help to invade the clot. These enzymes in addition to growth factor signaling (cell-cell interactions) and cell-ECM interactions (signal

transduction from interactions between cells, integrins (cell surface receptors), laminin, collagen, fibronectin, and other ECM proteins) stimulate cell migration into the wound and ECM degradation.

[0005] Finally, in the remodeling phase, collagen is remodeled and realigned along tension lines and cells that are no longer needed are removed by apoptosis. Wound contraction occurs as fibroblasts transform into myofibroblasts through their interactions with ECM proteins and growth factors. Myofibroblasts then interact with collagen, vitronectin, and other ECM proteins to contract the wound. As the remodeling phase proceeds, fibronectin and hyaluronic acid are replaced by collagen bundles that lend strength to the tissue.

[0006] By applying biological, chemical and engineering principles to tissue repair and regeneration, tissue engineers have developed transplantable products for use in promoting the tissue repair and regeneration processes. The ability to restore biomechanical function of damaged tissue presents a true challenge. In response, both synthetic and biological scaffold products have been developed that mimic (to some extent) tissue structure and mechanical behavior to promote tissue repair. Such products serve as a temporary replacement, both mechanically and functionally, for damaged, diseased or absent tissue.

[0007] Ideally, transplantable scaffold products should support cell adhesion, proliferation and differentiation and act as an interim synthetic extracellular matrix (ECM) for cells prior to the formation of new tissue. Scaffold materials should be biocompatible, biodegradable and exhibit low antigenicity. The implant should degrade at a rate roughly equal to that of the new tissue formation. Once implanted, the scaffold must have the mechanical properties necessary to temporarily offer structural support until the new tissue has formed. Additionally, scaffold products must be porous, providing an appropriate path for nutrient transmission and tissue ingrowth. Tissue scaffolds also should promote fast healing and facilitate the development or regeneration of new tissue that resembles normal host tissue in both appearance and function. To this end, implanted scaffold products should offer (i) bioactive stimulation, e.g., protein and molecular signaling, to encourage cell migration, proliferation and differentiation, and (ii) mechanical or structural support for these processes.

[0008] Today, the development of synthetic scaffolds is an area of active research. Synthetic scaffolds have been manufactured from chemical compounds such as fibrous polymers, gelatin, apatite, and polymer/ceramic composites, polylactic acid, collagen. These scaffolds are designed

to mimic the structure of the naturally occurring ECM and have shown some success in bone tissue engineering.

[0009] In addition to synthetic scaffold products, biological scaffolds obtained from mammalian tissues are commercially available for use as allografts (transplanted cell or tissue from a donor of the same species) or xenografts (transplanted cells or tissue from a donor of a different species). Biological scaffolds are composed of mammalian ECM harvested from, for example, dermis, urinary bladder, small intestine, mesothelium, pericardium, bone or amniotic membrane of various mammals including human (either live donor or cadaver), porcine, bovine and equine. These commercially available products are commonly used for the repair and reconstruction of injured or missing tissues and organs such as soft tissue, tendons, cardiac tissue, neural tissue, chronic wounds, dura mater, bone and cornea.

[0010] Biological scaffold products may comprise skin cells in addition to extracellular matrices produced by tissue and subjected to a decellularization process. They are contacted with a wound site to give mechanical support for cell migration and proliferation as part of the wound healing process. In addition, factors such as growth factors or other proteins also may be provided that promote the wound healing process. The mechanical and material properties of biological scaffolds and the host tissue response to these biomaterials are believed to be influenced by the three dimensional configuration of the material and production processing methods. It further is believed that growth factors, surface topology and the distribution of surface ligands and modulation of the host innate immune response all contribute to the eventual functional performance of biological scaffolds for tissue repair or reconstruction. Tottey *et al.*, *Biomaterials* 32: 128-36 (2011).

[0011] In transplantation the use of human amniotic membrane (AM) has particular advantages, due to the structure of the relatively thick basement membrane, associated devitalized amniotic epithelial cells and stroma, and corresponding growth factor profile and structural protein composition. Meller *et al.*, *Dtsch Arztebl Int'l* 108: 243-8 (2011). For example, AM contains epidermal growth factor (EGF) and keratinocyte growth factor (KGF), which are important growth factors for promoting wound healing. In addition, laminin and type VII collagen present in the AM elicit an epitheliotropic effect. AM also is thought to reduce the expression of various growth factors and pro-inflammatory cytokines while releasing anti-inflammatory cytokines such as IL-10, IL-1 receptor antagonists, thus modulating the

inflammatory response favorably for wound healing. AM is immunoprivileged, moreover, likely by virtue of low MHC I expression, and so rejection of AM tissue is uncommon. These characteristics make AM an ideal substrate, for instance, with respect to ocular surface reconstruction, in pelvic reconstruction, and in the treatment of ulcers, among other wound-healing applications.

[0012] The use of conventional tissue scaffold products is not without drawbacks, however. Tissue harvesting from human donors can produce undesirable consequences such as donor site morbidity or infection associated with removal of skin for donation. Disease transmission risk and intersample variation are additional drawbacks associated with biological scaffold products. In addition, it may be difficult to obtain sufficient tissue components necessary to cover large areas of damaged tissue. Furthermore, conventional biological and synthetic materials can be costly, not effective in many instances, and limited in availability.

[0013] Accordingly, an abiding exists need for suitable tissue substrate biomaterial for use in transplantation to promote tissue regeneration while restoring functionality. Both the research industry and the medical transplant community would benefit from such a product that is readily available, does not impose additional complications to a donor or recipient (such as requiring an additional surgery to harvest the substrate), and exhibits all or some of the inherent material functionality reflective of the physiochemical, electrochemical, and biochemical properties of natural tissue.

SUMMARY OF THE INVENTION

[0014] The biomaterial of the present invention is obtained from tissue of a urodele. “Urodele” here denotes a salamander of the order Urodela, also known as the order Caudata, in the class Amphibia. In terms of phylogeny the relevant families include *Ambystomatidae*, *Cryptobranchidae*, *Amphiumidae*, *Proteidae*, and *Sirenidae*. See Wiens *et al.*, *Syst. Biol.* 54: 91-110 (2005), the contents of which are incorporated here by reference in their entirety. Accordingly, the urodele category includes, for example, the Pacific Giant Salamander (*Andrias davidianus*), the Tiger Salamander (*Ambystoma tigrinum*), and the Mexican Axolotl (*Ambystoma mexicanum*). The present inventor has recognized that the skin and ECM of urodeles possess desirable characteristics analogous to those of AM, making them an ideal source of biomaterial for xenotransplantation.

Urodele ECM and tissue regeneration

[0015] As amphibians, most urodeles begin life as aquatic animals in a larval state and undergo metamorphosis from a juvenile form with gills to an adult, terrestrial, air-breathing form with lungs. During metamorphosis, a urodele's physical features are altered in preparation for life on land. These alterations include caudal fin resorption, thickening of the skin, the development of dermal glands and resorption of gills. Sexual maturity also occurs during this time in most urodeles. Some families of urodeles are "neotenic," which means that individuals with such families can exhibit juvenile features, such as gills and fins, even after reaching sexually maturity. Indeed, neotenic urodeles often retain their aquatic (juvenile) form for the duration of their lives. Thus, the Mexican Axolotl normally remains in the neotenic state throughout its adult life although, under certain circumstances, it can undergo metamorphosis and transform into a terrestrial form.

[0016] Axolotls also are known for their ability to regenerate amputated body parts, which typically results in the complete restoration of both the structure and function of the damaged limb or organ. Aquatic axolotls undergo rapid re-epithelialization during wound healing and limb regeneration, both of which are scar-less processes. Similarly, metamorphic terrestrial axolotls retain several larval skin features and also exhibit scar-free wound healing, albeit at a slower rate than their aquatic, pre-metamorphic counterpart.

[0017] The healing process in axolotls varies from that observed in adult mammals. The axolotl process more closely resembles the scar-free healing process of fetal and embryonic wounds. Thus, such wounds likewise exhibit re-epithelialization and basement membrane reformation that occur at a faster rate (is "enhanced") than do the corresponding events in postnatal mammals.

[0018] Moreover, the cutaneous and subcutaneous structures of an axolotl resemble that of the amniotic/chorionic interface, in the sense that axolotl skin is composed of fused ectoderm and mesoderm. Axolotl skin also is rich in growth factors and antimicrobial peptides, similar to the AM. Furthermore, axolotl ECM is immunoprivileged and contains collagen III and tissue inhibitors of metalloproteinases (TIMPs), *inter alia*, also in resemblance to AM.

[0019] Of particular importance for transplantation purposes, more generally, is the immunologically privileged state of the human neonate (*e.g.*, fetal dermis) and the AM, a state mirrored by axolotl ECM, whereby immogenicity is rarely manifested. By virtue of the reduced

immune response and the generally decreased inflammatory response as compared to adult humans, neonatal and axolotl skin healing alike are not characterized by accelerated tissue resorption, as is observed in adult human wound healing. Rather, the growth factor profile, enzymatic activity, structural composition and immunomodulating effect of urodele and neonate tissues alike favor an appropriately staged removal of structural scaffolding and tissue growth into the resulting negative void space, in addition to enhanced re-epithelialization, during wound healing. This results in an optimal wound healing environment and process. Also, the high concentration of antimicrobial peptides present in AM and urodele tissue further contributes to the favorable environment and enhanced re-epithelialization observed during wound healing.

[0020] A key aspect of the present invention is the inventor's recognition that the growth factor profile, connective tissue matrix constituents, and immunoprivileged status of urodele ECM and accompanying cutaneous tissue, plus the presence of antimicrobial peptides therein, render urodele-derived tissue an ideal source for biological scaffolds for xenotransplantation.

[0021] In accordance with the invention, therefore, a biological scaffold biomaterial is provided that is the product of a process comprising (A) obtaining a tissue sample from a urodele, where the tissue comprises ECM, inclusive of the basement membrane, and (B) subjecting the tissue sample to a decellularization process that maintains the structural and functional integrity of the extracellular matrix, by virtue of retaining its fibrous and non-fibrous proteins, glycoaminoglycans (GAGs) and proteoglycans, while removing sufficient cellular components of the sample to reduce or eliminate antigenicity and immunogenicity for xenograft purposes. Also provided is methodology for using the urodele-derived biomaterial to enhance restoration of skin homeostasis, to reduce the severity, duration and associated damage caused by post-surgical inflammation, and to promote progression of natural healing and regeneration processes. In addition, biomaterial of the invention promotes the formation of remodeled tissue that is comparable in quality, function and compliance to undamaged human tissue.

Decellularization

[0022] The biomaterial of the invention is produced by decellularizing a tissue sample obtained from a urodele. The primary constituent of the resulting urodele biomaterial is ECM, possibly with devitalized epithelial cells, which can retain moisture and otherwise protect the wound-healing environment.

[0023] Urodele skin is one example of an appropriate starting material for the present invention. Thus, the starting material that is subjected to decellularization can comprise urodele dermis and basement membrane, with or without epidermis. Even upon decellularization, moreover, the biomaterial of the invention can comprise, with the ECM, adjacent epithelial cells that may be rendered non-viable by the process. Alternatively, non-cutaneous urodele tissues can serve as the starting material of the invention, particularly those comprising a basement membrane or epithelial tissues that form the lining of various body cavities, *i.e.*, parietal mesothelial tissues found, for example in the thoracic cavity, the abdominal cavity, and pericardium. Tissues that contain substantial amounts of fibrous connective tissue, such as cartilage, tendon, bone, dura mater and fascia, also are illustrative of appropriate starting materials of the present invention.

[0024] Effected via any conventional decellularization methodology, urodele tissue decellularization is performed to remove immunogenic cellular antigens that can induce an inflammatory response or immune-mediated tissue rejection, while preserving the structural integrity and composition of the associated ECM. Generally, ECM structural components, many if not all of which remain intact following decellularization, are well-tolerated by xenogeneic recipients. ECM components that may be present in the final biomaterial of the invention include proteins such as collagen (*e.g.*, fibrous collagen I and collagen III, as well non-fibrous collagen IV, collagen V and collagen VII), elastin, fibronectin, laminin, vitronectin, thrombospondins, osteopontin and tenascins, plus GAGs (*e.g.*, the proteoglycans, decoran and versican and sulfated GAGs, *e.g.*, heparin sulfate, keratan sulfate, dermatan sulfate and chondroitin sulfate) and growth factors such VEGF, BMP, TGF and FGF. For some indications the post-decellularization material comprises at least collagen IV, laminin, sulfated GAGs and one or more growth factors in amounts that approximate pre-decellularization levels when viewed via histological and immunohistological staining.

[0025] Suitable techniques for decellularizing tissues, pursuant to the invention, include physical methods such as freezing, direct pressure application, sonication, and agitation. In addition or in the alternative, chemical methods can be employed, such as alkaline and acid treatments, application of detergents (including amphoteric, cationic, anionic and non-ionic detergents), organic solvents, hypotonic or hypertonic solutions and chelating agents. Enzymatic approaches including protease digestion and treatment with one or more nucleases also may be

used to decellularize urodele tissue. In addition or alternatively, the urodele tissue is subjected to cleaning, sterilization, disinfection, antibiotic treatment and/or viral inactivation.

[0026] According to one aspect of the invention, a biomaterial is provided. The material is produced by the process that includes (A) obtaining a tissue sample from a urodele, which tissue sample comprises extracellular matrix, and (B) decellularizing the sample to retain structural and functional integrity while removing sufficient cellular components of the sample to reduce or eliminate antigenicity of the biomaterial as a xenograft. In some embodiments, decellularizing comprises subjecting said tissue sample to an alkaline treatment. In embodiments, the process can further comprise subjecting said sample to sterilization. In embodiments, the process can further comprise devitalizing cells.

[0027] According to one aspect of the invention, a tissue graft is provided. The graft includes extracellular matrix components derived from a urodele. In embodiments, the extracellular matrix components are substantially free of components that induce an immune response when implanted as a xenograft. In embodiments, the extracellular matrix components are non-toxic.

[0028] According to one aspect of the invention, a decellularized Urodele ECM is provided. In any of the embodiments, the decellularized ECM can be derived from Axolotl tissue. In any of the embodiments, the decellularized ECM can include basement membrane. In any of the embodiments, the decellularized ECM can be infused with, coated with, combined with or attached, covalently or non-covalently, to an agent xenogenic to a Urodele. In any of the embodiments, the agent can be any one or any combination of 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 and/or a human agent (i.e., an agent found in a human, isolated, synthetically or recombinantly produced). Further such agents forming part of the invention are described in more detail below. In some embodiments, the agent is a cell, optionally a human cell. In some embodiments, the agent is a progenitor cell, optionally a human progenitor cell. Further such cells forming part of the invention are described in more detail below. In any of the embodiments, the decellularized ECM can take on any variety of shapes, as the material can be formed, laminated, homogenized, gelled, etc. In some embodiments, the ECM is a sheet. The sheet optionally can include perforations. The sheet optionally can include a backing and/or an adhesive. The backing may be biodegradable or may be non-biodegradable. In some

embodiments, the decellularized ECM is a dry powder. In some embodiments the decellularized ECM is a reconstituted gel. In any of the embodiments, the ECM can be sterile.

[0029] According to one aspect of the invention, a package is provided. The package contains sterile, decellularized Urodele ECM or a Urodele fraction derived from the sterile, decellularized Urodele ECM. The package can contain any of the ECMs described above. For example, the package can include a sheet, a dry powder or a reconstituted gel of decellularized Urodele ECM. The package can contain any product, for example any implant, that comprises sterile, decellularized Urodele ECM or a Urodele fraction derived from the sterile, decellularized Urodele ECM. Such an implant may be made in whole or only in minor part of the ECM of the invention.

[0030] The invention also provides a sterile medical implant comprising decellularized Urodele ECM or a Urodele fraction derived from the decellularized Urodele ECM. Examples of such medical implants include a biocompatible sheet, mesh, gel, graft, plug, tissue or device. Devices include, for example, coated stents, bone replacements, joint replacements, implantable hardware and the like. The implant can be fabricated entirely or in part from the ECM. The implant also can encapsulate, can be infused with, coated with, impregnated with, laminated with, or covalently or non-covalently attached to the ECM of the invention.

[0031] The invention also provides a material, the material being coated with, impregnated with, encapsulating, or having attached thereto isolated, decellularized Urodele ECM or a Urodele fraction derived from the isolated, decellularized Urodele ECM. The material can be natural or synthetic. Examples are metals, plastics, ceramics and fibers.

[0032] According to one aspect of the invention, a tissue culture system is provided. The system comprises (a) an isolated Urodele decellularized ECM, (b) tissue culture medium, and (c) cells xenogenic to the Urodele. The cells may be from animal, and in some embodiments, the cell is a mammalian cell. The cell can be any type of cell capable of culture. In embodiments, the cell is a human cell, optionally a progenitor cell.

[0033] According to one aspect of the invention, a conditioned tissue culture medium is provided. The medium, which can be any commonly used in liquid tissue culture, is conditioned with isolated Urodele decellularized ECM or a Urodele fraction derived from isolated, decellularized Urodele ECM. Numerous liquid tissue culture media are commercially available and well known to those of ordinary skill in the art.

[0034] According to one aspect of the invention, a device is provided. The device is at least two sheets of isolated Urodele decellularized ECM laminated to one another. The sheets can be from the same or different tissue. The sheets can an orientation and can be oriented in the same direction or oriented at angles to one another. The sheets can further comprise any agent xenogenic to the Urodele, which agent may be coated on, infused or impregnated within, or otherwise attached to one or more of the laminated sheets.

[0035] In any embodiment described above involving a sheet, the sheet may further comprise a backing and/or adhesive.

[0036] According to one aspect of the invention, a product is provided. The product is prepared by isolating Urodele ECM from a Urodele, decellularizing the ECM, and sterilizing the decellularized ECM. The preparation of the device can further involve any one or more of the following steps (presented in no particular order): forming the ECM into a shape, homogenizing the ECM, laminating the ECM to a material, combining agents with the ECM such as by coating, impregnating, or otherwise attaching the agent to the ECM, and so on.

[0037] According to one aspect of the invention, a method of preparing a biologic material is provided. The method involves (A) obtaining a tissue sample from a urodele, which tissue sample comprises extracellular matrix, and (B) decellularizing the sample to remove sufficient cellular components of the sample to reduce or eliminate antigenicity of the biomaterial as a xenograft. In embodiments, the method can further involve performing the decellularization in a manner to retain structural and functional integrity of the ECM sufficient to permit the ECM to be useful as a matrix upon and within which cells can grow. In any of the embodiments, the method can further involve homogenizing the ECM to form a particulate or powder. In some embodiments, the method can further involve reconstituting the powder as a gel. In any of the embodiments, the method can further involve sterilizing the ECM. In any of the embodiments, the method can further involve attaching the ECM to an agent xenogenic to a Urodele.

[0038] According to another aspect of the invention, the materials such as implants, devices, sheets, gels and powders can be used in methods for treating subjects, where the materials are applied to wounds, surgical beds, and to internal and external tissues, generally, to prevent adhesion, provide tissue support, for example for suturing tissue, for treating a hernia or as a tissue plug, for treating burns and derm-abrasion, as well as other conditions described below.

[0039] In any of the embodiments above, the ECM is or can be isolated.

BRIEF DESCRIPTION OF THE FIGURES

[0040] FIGURE 1 shows native axolotl tissue samples prepared for histological examination, to identify EC elements.

[0041] FIGURE 2 hematoxylin and eosin (H&E) and Alcian Blue staining of native axolotl dermal tissue and human amniotic membrane (40x magnification).

[0042] FIGURE 3 shows immunohistochemical staining via species-specific collagen IV and laminin antibodies of native axolotl dermal tissue and human amniotic membrane tissue, at 40x magnification.

[0043] FIGURE 4 depicts axolotl skin and human amniotic membrane samples prepared for histological examination, to identify EC elements.

[0044] FIGURE 5 illustrates a histological evaluation of H&E-stained, paired native and post-processed sections of axolotl dermal tissue.

[0045] FIGURE 6 presents ELISA data for DNA content in pre- and post-processed axotol tissue, with comparison data from human amniotic membrane tissue.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Definitions

[0046] “Antimicrobial polypeptides” (or “AMPs”) means small peptides of variable length, sequence and structure with broad spectrum activity against a wide range of microorganisms including bacteria, viruses, fungi, protozoa, parasites, prions, and tumor/cancer cells. (See, e.g., Zaiou, J Mol Med, 2007; 85:317; herein incorporated by reference in its entirety). AMPs have broad-spectrum of rapid onset of killing activities, with potentially low levels of induced resistance and concomitant broad anti-inflammatory effects. Anti-microbial polypeptides include defensins, such as α -defensins (e.g., neutrophil defensin 1, defensin alpha 1, neutrophil defensin 3, neutrophil defensin 4, defensin 5, defensin 6), β -defensins (e.g., beta-defensin 1, beta-defensin 2, beta-defensin 103, beta-defensin 107, beta-defensin 110, beta-defensin 136), and 0-defensins. Anti-microbial polypeptides include cathelicidins such as hCAP18.

[0047] “Biocompatible” means that a composition and its normal degradation products *in vivo* are substantially non-toxic and non-carcinogenic in a subject within useful, practical and/or acceptable tolerances.

[0048] “Cytocompatible” means that a composition can sustain the viability and growth of a population of cells.

[0049] “Decellularized ECM” means extra cellular matrix sufficiently free of cellular components to eliminate or reduce antigenicity of the extra cellular matrix to an extent where the matrix would be considered non-toxic as a xenograft.

[0050] “Isolated” when used in connection with the ECM of the invention means separated from other Urodele tissue.

[0051] “Non-toxic” means that a composition, when implanted in a subject, causes little or no adverse reaction or substantial harm to cells and tissues in the body, and does not cause a substantial adverse reaction or substantial harm to cells and tissues in the body, for instance, the composition does not cause necrosis, an infection, or a substantial immune response resulting in harm to tissues from the implanted or applied composition.

[0052] “Progenitor cell” means a cell that can differentiate under certain conditions into a more-differentiated cell type. Non-limiting examples of progenitor cells include stem cells that may be totipotent, pluripotent, multipotent stem cells, or referred to as progenitor cells. Additional non-limiting examples of progenitor cells include perivascular stem cells, blastema cells, and multilineage progenitor cells.

[0053] “Retain structural and functional integrity” used in connection with the ECM of the invention means retaining sufficient structure and function to permit and support the use of the matrix as a substrate for the growth of cells *in vivo* or *in vitro*.

[0054] “Subject” means an animal. In some embodiments the animal is a mammal. The mammal can be a dog, cat, a horse, a cow, a goat, a sheep, a pig or a non-human primate. In any embodiment the mammal can be a human.

[0055] “Treatment” or “treating” means administration or application to a subject by any suitable dosage means, regimen and route of a composition with the object of achieving a desirable clinical/medical end-point, such as assisting in wound healing, tissue closure, bulking tissue, preventing tissue adhesion, providing structural support to tissue, providing a protective barrier, correcting a defect, etc.

[0056] “Urodele fraction derived from decellularized Urodele ECM” means an extract or isolate of decellularized Urodele ECM maintaining sufficient characteristics of a Urodele in terms of chemical structure and/or relative chemical concentrations of two (or three, or four, or

five or more) chemical entities in the extract or isolate to distinguish the extract as obtained from a Urodele by any one or more of electron microscopy, HPLC, immunohistochemistry, and the like.

General Preparative Methodology

[0057] 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. Thus, tissue samples may be subjected to cleaning and chemical decontamination. In this manner, a tissue sample is washed for approximately 10 to 30 minutes in a sterile basin containing 18% NaCl (hyperisotonic saline) solution that is at or near room temperature. Visible cellular debris, such as epithelial cells adjacent to the tissue basement membrane, is gently scrubbed away using a sterile sponge to expose the basement membrane. Using a blunt instrument, a cell scraper or sterile gauze, any residual debris or contamination also is removed. Other techniques including, but not limited to, freezing the membrane, physical removal using a cell scraper, or exposing the cells to nonionic detergents, anionic detergents, and nucleases also may be used to remove cells.

[0058] In one embodiment, urodele tissue is decellularized using alkaline treatment.

[0059] The tissue is placed into a sterile container, such as a Nalgene jar, for the next step of chemical decontamination. Thus, each container is aseptically filled with 18% saline solution and sealed (or closed with a top). The containers then are placed on a rocker platform and agitated for between 30 and 90 minutes, which further cleans the tissue of contaminants.

[0060] In a sterile environment using sterile forceps, the tissue is gently removed from the container containing the 18% hyperisotonic saline solution and placed into an empty container. This empty container with the tissue is then aseptically filled with a pre-mixed antibiotic solution. Preferably, the premixed antibiotic solution is comprised of a cocktail of antibiotics, such as Streptomycin Sulfate and Gentamicin Sulfate. Other antibiotics, such as Polymixin B Sulfate and Bacitracin, or similar antibiotics available now or in the future, are suitable as well. It is preferred that the antibiotic solution be at room temperature when added so that it does not change the temperature of or otherwise damage the tissue. This container containing the tissue and antibiotics is then sealed or closed and placed on a rocker platform and agitated for, preferably, between 60 and 90 minutes. Such rocking or agitation of the tissue within the antibiotic solution further cleans the tissue of contaminants and bacteria.

[0061] In a sterile environment, the container is opened and, using sterile forceps, the tissue is gently removed and placed in a sterile basin containing sterile water or normal saline (0.9% saline solution). The tissue is allowed to soak in place in the sterile water/normal saline solution for at least 10 to 15 minutes. The tissue may be slightly agitated to facilitate removal of the antibiotic solution and any other contaminants from the tissue.

[0062] In some cases, the present invention involves treating urodele tissue using a chemical sterilization methodology, as illustrated the Tutaplast® and Allowash® procedures, optionally in combination with mechanical processes that gently agitate chemical agents, as in the BioCleanse® system. Thus, urodele tissue is subjected to oxidative and/or alkaline treatments as well as osmotic treatment to break down cell walls, to inactivate pathogens, and to remove bacteria. In addition, tissue may be subjected to delipidization, solvent dehydration (to permit room temperature storage of tissue without damaging the collagen structure) and/or low-dose gamma irradiation to ensure sterility of the final product.

[0063] Efficient cell removal upon decellularization can be verified by various known means, including histological analyses to detect nuclear and cytoplasmic structures, immunohistochemical or immunofluorescent assaying for indicative intracellular proteins, and DNA detection. The nature of desirable components in the final urodele-derived scaffold biomaterial varies depending on the clinical indication being treated. Once a particular indication is identified, the knowledgeable clinician can determine which components in the urodele tissue sample should be retained in the final scaffold product, and standard methodology can be employed to ensure that the desired components are present following decellularization.

[0064] Samples may be viewed histologically before, during, and/or after decellularization to monitor the process and to confirm that the desired degree of cellular component removal is reached. For instance, tissues can be analyzed for cytoskeletal content to gauge sufficient decellularization. Intracellular protein content also may be assayed to determine if decellularization is sufficient. In addition, the tissue sample thickness and chemical makeup may be monitored to determine when sufficient decellularization has been achieved. Periodic monitoring during processing allows for a real time response to the observed tissue properties.

[0065] In some cases, a sufficiently decellularized tissue comprises no more than 50 ng dsDNA per mg ECM dry weight. Alternatively, for some indications, a sufficiently

decellularized tissue lacks visible nuclear material in a tissue section stained with 4',6-diamidino-2-phenylindole (DAPI) or haematoxylin and eosin (H&E).

[0066] In scenarios where removal of an adjacent epithelial cell layer is required, the presence or absence of epithelial cells remaining in the sample can be evaluated using techniques known in the art. For example, after removal of the epithelial cell layer, a representative tissue sample from the processing lot is placed onto a standard microscope examination slide. The tissue sample is then stained using Eosin Y Stain and evaluated as described below. The sample is then covered and allowed to stand. Once an adequate amount of time has passed to allow for staining, visual observation is done under magnification. The presence of cells and cellular material will appear darker than the areas which have been de-epithelialized.

[0067] Once cellular removal has progressed sufficiently, conventional methods are employed to confirm the retention of desired structural and functional properties of the remaining ECM scaffold. The specific structural testing that should be conducted depends on the intended clinical application of the final scaffold product. In some cases, the urodele tissue starting material may be monitored before, during, and after decellularization to ensure that the desired structural components and configuration are maintained in the final product.

[0068] One method for determining whether the desired ECM components are present involves staining parallel tissue sections and examining them histologically to determine whether the desired constituents and structural orientation of the urodele tissue have been preserved. For instance, urodele tissue can be stained with H&E and immunoperoxidase stain for laminin to assess preservation of ECM and laminin. In general, the three-dimensional configuration of ECM components remaining in the final biomaterial scaffold product should approximate that of pre-decellularized material when viewed via histological staining. Another component one can assay for is AMPs, as the ECM of the invention is rich in AMPs.

[0069] Accordingly, the urodele-derived biomaterial of the invention comprises ECM components useful for directing enhanced re-epithelialization and promoting efficient tissue regeneration or wound healing. The inventive biomaterial also serves as a matrix and reservoir for bioactive peptides such as growth factors, adhesion proteins and AMPs. Accordingly, the biomaterial functions effectively as a biological scaffold for tissue regeneration, providing both the necessary bioactive stimulation and structural support. The product can be used as is, cut into smaller pieces or shapes, laminated to itself or other materials, pre-punctured to provide

openings for securing attachments, formed into desired three dimensional shapes, as well as other formats, discussed in more detail below.

Powders and Gels

[0070] In embodiments, the scaffold can be further processed into small grains or a powder. The fine particles can be hydrated in water, saline or a suitable buffer or medium to produce a paste or gel. This fine material, paste or gel produced from it may be used for a multitude of purposes, described in greater detail below.

[0071] Although numerous methods exist, two exemplary methods may be used to produce a particulate form of the scaffold. The first method involved lyophilizing the disinfected material and then immersing the sample in liquid nitrogen. The snap frozen material is then reduced to small pieces with a blender so that the particles are small enough to be placed in a rotary knife mill, such as a Wiley mill. A #60 screen can be used to restrict the collected powder size to a desired size, for example less than 250 μ m. A Sonic sifter or other classification device can be used to remove larger particles and/or to obtain a particle size distribution within a desired range. A second method is similar to the previous method except the sample is first soaked in a 30% (w/v) NaCl solution for 5 min. The material is then snap frozen in liquid nitrogen to precipitate salt crystals, and lyophilized to remove residual water. This material is then comminuted as described in above. By precipitating NaCl within the sample, it is expected that the embedded salt crystals would cause the material to fracture into more uniformly sized particles. The particles are then suspended in deionized water and centrifuged for 5 min at 1000 rpm three times to remove the NaCl. The suspension is snap frozen and lyophilized again. Finally, the powder is placed in a rotary knife mill to disaggregate the individual particles.

[0072] The powder can be hydrated to create a gel, with or without other gelling materials to supplement gelling.

[0073] The powder, paste or gel can be applied without further processing to treat a subject. It can be sprayed, painted, injected or otherwise applied to a wound or surgical site. The gel can be shaped. The powder, paste or gel also can be placed inside a “bag”, such as a polymeric synthetic material or a ECM sheet as described herein to produce a larger three-dimensional structure, such as an orthopedic implant for cartilage repair (e.g., knee or TMJ cartilage repair) or an implant for breast reconstruction or augmentation. In such a case, a bag of a desirable size and shape is formed from sheets of ECM material or other biocompatible polymeric material,

and then the bag or cover can be filled with the tissue-derived powder or gel described herein. The shape of the device or implant can vary with its intended use. The bag may be molded into a useful shape by any useful molding technique, such as the shape of cartilage for the ear, nose, knee, TMJ, rib, etc., prior to filling the molded bag with the scaffold material described herein. In one example, a biodegradable polymeric matrix (e.g., PEUU or PEEUU) is sprayed or electrodeposited onto a mold. The resultant molded cover can then be filled with the material. Heat, for example, may be used to seal the cover.

Additives

[0074] In another embodiment, at least one agent xenogenic to a Urodele is added to the ECM or Urodele fraction thereof before it is implanted in the subject, otherwise administered to the subject or used in cell culture. Generally, the agents include any agent useful in cell culture or as a therapeutic or therapeutic adjuvant. The agents can be coated on, infused into or otherwise covalently or non-covalently attached to or incorporated onto or into the ECM of the invention. The agents also can be otherwise combined with a product that contains the ECM, for example, as by mixing powders of the agent and ECM together. Each agent may be used alone with the ECM of the invention or in combination with other agents. Non-limiting examples of such agents include antimicrobial agents, growth factors, cytokines, chemokines, emollients, retinoids, steroids, and cells, including but not limited to the subject's own cells.

[0075] In certain non-limiting embodiments, the agent is a growth factor. Non-limiting examples of growth factors include basic fibroblast growth factor (bFGF), acidic fibroblast growth factor (aFGF), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), insulin-like growth factors 1 and 2 (IGF-1 and IGF-2), platelet derived growth factor (PDGF), stromal derived factor 1 alpha (SDF-1 alpha), nerve growth factor (NGF), ciliary neurotrophic factor (CNTF), neurotrophin-3, neurotrophin-4, neurotrophin-5, pleiotrophin protein (neurite growth-promoting factor 1), midkine protein (neurite growth-promoting factor 2), brain-derived neurotrophic factor (BDNF), tumor angiogenesis factor (TAF), corticotrophin releasing factor (CRF), transforming growth factors .alpha. and .beta. (TGF-.alpha. and TGF-.beta.), interleukin-8 (IL-8), granulocyte-macrophage colony stimulating factor (GM-CSF), interleukins, and interferons. Commercial preparations of various growth factors, including neurotrophic and angiogenic factors, are available from R & D Systems, Minneapolis, Minn.;

Biovision, Inc, Mountain View, Calif.; ProSpec-Tany TechnoGene Ltd., Rehovot, Israel; and Cell Sciences.RTM., Canton, Mass.

[0076] In certain non-limiting embodiments, the therapeutic agent is an antimicrobial agent, such as, without limitation, an anti-microbial peptide, isoniazid, ethambutol, pyrazinamide, streptomycin, clofazimine, rifabutin, fluoroquinolones, ofloxacin, sparfloxacin, rifampin, azithromycin, clarithromycin, dapsone, tetracycline, erythromycin, ciprofloxacin, doxycycline, ampicillin, amphotericin B, ketoconazole, fluconazole, pyrimethamine, sulfadiazine, clindamycin, lincomycin, pentamidine, atovaquone, paromomycin, diclazaril, acyclovir, trifluorouridine, foscarnet, penicillin, gentamicin, ganciclovir, iatroconazole, miconazole, Zn-pyrithione, and silver salts such as chloride, bromide, iodide and periodate.

[0077] In certain non-limiting embodiments, the therapeutic agent is an anti-inflammatory agent, such as, without limitation, an NSAID, such as salicylic acid, indomethacin, sodium indomethacin trihydrate, salicylamide, naproxen, colchicine, fenoprofen, sulindac, diflunisal, diclofenac, indoprofen, sodium salicylamide; an anti-inflammatory cytokine; an anti-inflammatory protein; a steroidal anti-inflammatory agent; or an anti-clotting agents, such as heparin.

[0078] Other drugs that may promote wound healing and/or tissue regeneration may also be included.

[0079] The agent may be dispersed within the scaffold by any useful method, e.g., by adsorption and/or absorption. For example, the therapeutic agent may be dissolved in a solvent (e.g., DMSO) and added to the scaffolding. In another embodiment, the agent is mixed with a carrier polymer (e.g., polylactic-glycolic acid microparticles, agarose, a poly(ester urethane) urea elastomer (PEUU) or a poly(ether ester urethane) urea elastomer (PEEUU)), which is subsequently dispersed within or applied to the scaffold. By blending the agent with a carrier polymer or elastomeric polymer, the rate of release of the therapeutic agent may be controlled by the rate of polymer degradation and/or by release from the polymer by diffusion or otherwise. Likewise, a therapeutic agent may be provided in any dissolvable matrix for extended release, as are known in the pharmaceutical arts, including sugar or polysaccharide matrices. The agent also may be included with the powdered ECM and gelled with the powdered ECM. The agent may be covalently attached to the ECM of the invention. The foregoing are meant to be non-limiting examples.

Extracts

[0080] In addition to the decellularized ECM in its native state or ground as a particulate or powder, the invention also provides extracts and isolates of the same. As mentioned above, the Urodele ECM is loaded with antimicrobial peptides, growth promoting factors, collagen and laminins, and Urodele fractions of the ECM are useful according to the invention.

[0081] Extraction buffers are well known in the art. One such buffer is 4 M guanidine and 2 M urea each prepared in 50 mM Tris-HCl, pH 7.4. The powder form of the ECM can be suspended in the relevant extraction buffer (e.g., 25% w/v) containing phenylmethyl sulphonyl fluoride, N-ethylmaleimide, and benzamidine (protease inhibitors) each at 1 mM and vigorously stirred for 24 hours at 4° C. The extraction mixture can then be centrifuged and the supernatant collected. The insoluble material can be washed in the extraction buffer, centrifuged, and the wash combined with the original supernatant. The supernatant can be dialyzed against deionized water. The dialysate can then be centrifuged to remove any insoluble material and the supernatant used immediately or lyophilized for long term storage. Such an isolate will contain growth factors in concentrations specific to Urodeles.

[0082] In another aspect, the extraction is done by conditioning medium. A method of making Urodele tissue-specific extract by taking the powdered ECM, forming a solution thereby generating a supernatant and a particulate, wherein the supernatant is an extract and isolating the extract from the particulate. One also could grow cells on the ECM, and isolate the supernatant after a period of time of cell growth.

Synthetic Materials

[0083] Synthetic biocompatible and cyto-compatible material can be combined with the ECM, such as, for example, (a) a structural support for a sheet or a gel of the ECM, (b) a structural support for shaping the ECM, (c) a coating for the ECM (or a coating containing the particulate ECM), a supplemental gelling agent, or (d) a sustained release material for the particulate ECM or an isolate thereof. Such polymers have been known to be applied to other ECM materials as a backing sheet, including materials that are themselves biodegradable. Suitable synthetic material for a matrix can be biocompatible to preclude migration and immunological complications, and can be able to support cell growth and differentiated cell function. Some are resorbable, allowing for a completely natural tissue replacement. Some can be configurable into a variety of shapes and have sufficient strength to prevent collapse upon implantation. Studies indicate that

the biodegradable polyester polymers made of polyglycolic acid fulfill all of these criteria (Vacanti, et al. J. Ped. Surg. 23:3-9 (1988); Cima, et al. Biotechnol. Bioeng. 38:145 (1991); Vacanti, et al. Plast. Reconstr. Surg. 88:753-9 (1991)). Other synthetic biodegradable support matrices include synthetic polymers such as polyanhydrides, polyorthoesters, and polylactic acid. Further examples of synthetic polymers and methods of incorporating or embedding cells into these matrices are also known in the art. See e.g., U.S. Pat. Nos. 4,298,002 and 5,308,7.

[0084] As a non-limiting example, the powder may be formulated with tri-block copolymers. See international published application WO2012131104 and WO2012131106, each of which is incorporated herein by reference in its entirety. Other examples include poloxamers, which are nonionic triblock copolymers composed of a central hydrophobic chain of polyoxypropylene (poly(propylene oxide)) flanked by two hydrophilic chains of polyoxyethylene (poly(ethylene oxide)). Poloxamers are also known by the trade name Pluronic (BASF). Certain poloxamers are useful as sustained release materials for pharmaceuticals.

[0085] Particles of the invention also may be encapsulated into a polymer, hydrogel and/or surgical sealant. As a non-limiting example, the polymer, hydrogel or surgical sealant may be PLGA, ethylene vinyl acetate (EVAc), poloxamer, GELSITE® (Nanotherapeutics, Inc. Alachua, Fla.), HYLENEX® (Halozyme Therapeutics, San Diego Calif.), surgical sealants such as fibrinogen polymers (Ethicon Inc. Cornelia, Ga.), TISSELL® (Baxter International, Inc Deerfield, Ill.), PEG-based sealants, and COSEAL® (Baxter International, Inc Deerfield, Ill.). In another embodiment, the particle may be encapsulated into any polymer known in the art which may form a gel when injected into a subject. As another non-limiting example, the particle may be encapsulated into a polymer matrix which may be biodegradable. Additional examples of polymers for controlled release and/or targeted delivery may also include at least one controlled release coating. Controlled release coatings include, but are not limited to, OPADRY®, polyvinylpyrrolidone/vinyl acetate copolymer, polyvinylpyrrolidone, hydroxypropyl methylcellulose, hydroxypropyl cellulose, hydroxyethyl cellulose, EUDRAGIT RL®, EUDRAGIT RS® and cellulose derivatives such as ethylcellulose aqueous dispersions (AQUACOAT® and SURELEASE®).

Uses

[0086] The decellularized ECMs described herein are useful for growing cells, tissues, organs in virtually any *in vivo*, *ex vivo*, or *in vitro* use. The ECMs can be used as a substrate to facilitate

the growth and/or differentiation of cells. In vitro, the ECMs are useful as a cell growth substrate to support the growth in culture of cells, including virtually any type of cells or cell-lines, including stem cells, progenitor cells or differentiated cells. In one embodiment, the cells are cancer cells. In one embodiment, the cancer cells form nodules when grown on the ECMs. Cells on the substrate also may be grown into tissue, organ or body part precursors, or even mature tissues or structures. Cells grown on ECMs may be used for implantation, for wound dressings, for *in vitro* drug testing, for modeling differentiation, etc. The cells may be matched in tissue cell type to the ECM or unmatched. The cells are xenogenic.

[0087] The ECM of the invention is useful *in vivo* as a cell growth scaffold for tissue growth for any useful purpose, including repair, replacement or augmentation of tissue in a subject in either humans or animals. For example, the materials are useful in repair and/or replacement of tissue lost or damaged during trauma or surgery, for example in loss of tissue after tumor removal. The materials are useful for structural repair, such as inguinal hernia repair, parastomal reinforcement, soft tissue reinforcement, surgical staple-line reinforcement during, for example, bariatric surgery or lung resection, umbilical hernia grafts, Peyronie's repair grafts, incision grafts and fistula plugs. The materials are useful for wound dressings, such as for burns, graft and split-thickness graft coverings, ulcers including decubitus ulcers and dermal abrasion procedures. The materials are useful for cosmetic purposes, for example in breast, lip or buttock augmentation. An aspect of the invention particularly appealing for anti-adhesion surgical uses is the properties of the basement membrane, which inhibit or prevent adhesion. The presence of the AMPs make the ECM of the invention particularly well suited for the foregoing applications.

[0088] As mentioned above, the materials described herein can be molded or contained within a structure to form desired shapes, such as, for cartilage repair or replacement by seeding the material with, e.g., chondrocytes and/or chondroprogenitor cells. The materials can be ground into a powder and used to reconstitute and/or form gels, as cell culture additives, as a powder, spray, liquid, suspension or coating for application to (a) a wound, (b) an implant, (c) a wound dressing, etc.

[0089] In one embodiment, for example, adipose stem cells are propagated in the cell growth scaffolds described herein. Adipose stem cells are of mesodermal origin. They typically are pluripotent, and have the capacity to develop into mesodermal tissues, such as: mature adipose tissue; bone; heart, including, without limitation, pericardium, epicardium, epimyocardium,

myocardium, pericardium, and valve tissue; dermal connective tissue; hemangial tissues; muscle tissues; urogenital tissues; pleural and peritoneal tissues; viscera; mesodermal glandular tissues; and stromal tissues. The cells not only can differentiate into mature (fully differentiated) cells, they also can differentiate into an appropriate precursor cell (for example and without limitation, preadipocytes, premyocytes, preosteocytes). Also, depending on the culture conditions, the cells can also exhibit developmental phenotypes such as embryonic, fetal, hematopoietic, neurogenic, or neuralgiagenic developmental phenotypes.

[0090] In one embodiment, a subject's own cells are dispersed within the matrix. For example, in the production of cartilaginous tissue, chondrocytes and/or chondroprogenitor cells can be dispersed within the matrix and optionally grown ex vivo prior to implantation. Likewise, skin cells of a subject can be dispersed within the scaffolding prior to implantation on a damaged skin surface of a subject, such as a burn or abrasion.

[0091] When used as a gel, a non-limiting example is injecting the gel into a subject at a desirable site, such as in a wound. In one instance, the gel can be injected in a bone breakage or in a hole drilled in bone to facilitate repair and/or adhesion of structures, such as replacement ligaments, to the bone. In another use, finely comminuted particles can be sprayed onto a surface of a subject, such as in the case of large surface abrasions or burns. The scaffold can also be sprayed onto skin sutures to inhibit scarring. The ECM of the invention can be place or sutured in place inside the body at a surgical site such as mentioned above. All of these treatments are embraced by the present invention.

[0092] Urodele decellularized ECM can be used also for sustained delivery of therapeutic molecules, proteins or metabolites, to a site in a host. See, for example, U.S. 2004/0181240, which describes an amniotic membrane covering for a tissue surface which may prevent adhesions, exclude bacteria or inhibit bacterial activity, or to promote healing or growth of tissue, and U.S. patent No. 4,361,552, which pertains to the preparation of cross-linked amnion membranes and their use in methods for treating burns and wounds. The ECMs of the invention can be used in the same manner.

Pharmaceutical Formulations

[0093] Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable

for administration to any other animal, e.g., to non-human animals, *e.g.* non-human mammals. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and/or perform such modification with merely ordinary, if any, experimentation.

[0094] The pharmaceutical compositions described herein may be prepared by any method known in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with an excipient and/or one or more other accessory ingredients, and then, if necessary and/or desirable, dividing, shaping and/or packaging the product into a desired single- or multi-use configuration.

[0095] The ECM in accordance with the invention may be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. For example, the composition may comprise between 0.1% and 100% (w/w) of the ECM. When other active agents are included, relative amounts of agents combined with the ECM of the invention will be known to those of ordinary skill in the art, similar to those amounts used in combination with ECM as formulated in the prior art. Relative amounts also may vary, depending upon the identity, size, and/or condition of the subject being treated and further depending upon the route by which the ECM is to be administered.

[0096] Pharmaceutical formulations may additionally comprise a pharmaceutically acceptable excipient, which, as used herein, includes, but is not limited to, any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, and the like, as suited to the particular dosage form desired. Various excipients for formulating pharmaceutical compositions and techniques for preparing the composition are known in the art. See Remington: THE SCIENCE AND PRACTICE OF PHARMACY (21st Ed.), A. R. Gennaro, Lippincott, Williams & Wilkins (Baltimore, Md., 2006); incorporated herein by reference in its entirety.

EXAMPLES

Example 1. Processing axolotl dermis

Axolotl dermis samples can be decellularized by preparing excised samples from healthy or healing axolotl dermal tissue and then subjecting the samples to hypo/hyperosmotic soaks for cell lysis, solvent dehydration, and oven drying. Specific processing of these grafts includes storage in 15-26% NaCl, multiple hypo/hyperosmotic soaks (utilizing NaCl solutions and water), and then solvent dehydration using ethanol, and then evaporation of the solvent either with air drying or oven drying at 37° C.

Histological examination of native axolotl dermal tissue was performed to identify the presence of the notable ECM elements, such as the basement membrane. See FIG 1 and FIG. 4. Comparative histological and immunohistochemical analysis of native axolotl dermal tissue and human amniotic membrane was performed to compare the ECM structure and constituents, and to assess relative concentration and distribution of critical constituents. See FIG. 2, FIG. 3, and FIG. 4. Figure 3 shows immunohistochemical staining via species-specific collagen IV and laminin antibodies of native axolotl dermal tissue and human amniotic membrane tissue, at 40x magnification. Figure 2 shows H&E and Alcian Blue staining (40x) of native axolotl dermal tissue and human amniotic membrane, and it demonstrates the comparable histoarchitecture and presence of sulfated glycosaminoglycans in both tissues. Histological evaluation with hematoxylin and eosin-stained, paired native and post-processed sections of axolotl dermal tissue (see FIG. 5) showed post-process preservation of the extracellular matrix histoarchitecture and the absence of cells or any significant concentration of cellular debris.

Example 2. Splitting and lamination of acellular dehydrated axolotl dermis.

Decellularized dehydrated axolotl dermis can be split, via a mechanical splitter, to isolate heterogeneous matrix into homogenous sections. Isolated sections of desired thickness then can be rehydrated and lyophilized to obtain multilayered laminate structures of desired orientation with facial surface features. More specifically, dual-sided basement membrane structure, with interior open porous matrix obtained from the reticular dermis region of the dermal matrix, can be constructed to obtain desired facial surface properties. Alternatively, isolated native section can be used in native form for desired clinical outcome. For example, open porous homogenous matrix of the reticular dermis can be used to obtain augmentation of soft tissue structures.

A laminated custom construct with sulfated gags on both facial surface and collagen IV and laminin could be obtained for desirable dual-surface, anti-adhesion and antimicrobial properties for clinical benefit. In addition, multilayer structures could be constructed to prolong in vivo durability of the graft.

Example 3. Preparation of solubilized acellular dehydrated axolotl dermis, pericardium, fascia lata, periosteum, peritoneum, or dura mater

Decellularized dehydrated axolotl native or isolated section of acellular urodele connective tissue matrix can be prepared by sectioning decellularized soft tissue structures into 1 cm² sections and homogenizing the sections in a Warring blender (~100 grams of tissue) in aqueous 1M glacier acetic acid for 30-60 seconds. Preparation of sponge can be obtained by the addition of varying volumes of water followed then neutralization and lypoholization of the slurry in a mold of desired geometric shape. The resultant porosity will correlate to the volume of water added to the matrix. Additionally, a selected range of bioactive extracts can be added to the slurry prior to neutralization, including particulated or small protein constituents extracted from digested human or urodele mineralized and nonmineralized connective tissues, such as demineralized bone matrix, elastin, or bone morphogenic proteins, which can be covalently loaded into constructs. Extracts will be covalently bound with collagen fibers after neutralization and return to physiological condition where fibrillogenesis will occur. Subsequent release of bioactive constituents will occur during proteolytic degradation in vivo and ensure molecules are not consume or exposed during acute inflammation in vivo. Alternatively, aqueous NaCl can be added to the slurry, prior to neutralization, to obtain a sustained, low viscosity solution for injection, which is stable at room temperature. Injection of slurry through ion-selective membrane will remove salt ions and permit for fibrillogenesis to occur post injection and formation of three-dimensional matrix.

Example 4. Preparation of sterilized particulated or powder form of mineralized and non-mineralized decellularized and dehydrated urodele connective tissue matrix

Following decellularization of sections of mineralized collagen urodele connective tissue, one can perform a demineralization process, similar to that employed by Urist, and solvent or lyophilization dehydration, cryomilling of sectioned acellular demineralized, mineralized, or non-mineralized urodele connective tissue extracellular matrix, thereby to obtain particulated or

powder form of the ECM with preserved histoarchitecture and function. The final particle size distribution can be varied depending on duration and sieving, post-cyromilling, between 125 and 850 microns. Low-dose cold gamma irradiation or e-beam irradiation (<25 Kgy) can be employed to sterilize acellular ECM sheets, particulate or powder and custom engineered constructs

Example 5. *In vitro* characterization of acellular mineralized and non-mineralized urodele healthy or healing connective tissue matrix

Through a series of *in vitro* analyses one can verify decellularization and preservation of native or custom engineered functional and structural properties of decellularized extracellular matrix constructs and/or particulate, including multilayer laminated constructs such as a dual-sided basement membrane sheet matrix or isolated native homogenous open porous matrix or solubilized, lyophilized and loaded ECM-derived collagen sponge. DNA content, as a marker for cell debris, can be employed to assess decellularization quantitatively, using a single, ethanol-based extraction technique with a fluorometric dye, Quant-iT PicoGreen (Molecular Probes, USA), in a ratio of 170 μ L working solution to 30 μ L samples/standards in a 96-well plate. Paired native and post-processed analysis and comparison to commercially available tissue ECM's can be performed to verify acceptability. See Figure 6.

ELISA analysis for quantification of bioactive constituent and native and post process proteolytic resorption profiles can be performed. Upon digestion with collagenase (232 – 262 mg/unit activity), normalized-weight-to-surface-area sections of decellularized and dehydrated urodele ECM tissue or constructs (Sigma, USA), in a pH 7.6 buffer (50 mM Tris-HCl, 200 mM CaCl₂, 50 mM NaCl) for 24 hours at 37°C, can be analyzed at various time points to construct a relative resorption curve of pre- and post-process tissue to verify preservation of histoarchitecture. Solubilized collagen following digestion can be assessed, using a Sircol kit (Biocolor Ltd., UK), in 100 μ L aliquots of acid/salt-washed digests. Specifically, levels of BMP 2/4 and TGF- β 1 growth factors or sulfated gags can be assessed, following digestion, by means of commercially available ELISA kits (R&D Systems, Minneapolis, MN). Protein content in 1:10 dilution digests can be measured via a standard Bradford absorbance assay.

Microscopic evaluation of samples can be performed using fixation in 4% paraformaldehyde and paraffin embedding, sectioning at 5 μ m, and routine histological staining (Histoserv, Inc., USA). Longitudinal cross sections can be stained with hematoxylin and eosin.

Images can be acquired and analyzed using standard brightfield techniques on an Olympus IM inverted microscope. Samples can analyzed using scanning electron microscopy after dehydration in a graded ethanol series (15%, 30%, 50%, 70%, 95%, and 100%), critical-point drying in CO₂, and sputter coating with gold. Samples can be visualized in an FEI Quanta 600 FEG scanning electron microscope, and representative images of scaffold ultrastructure can be acquired.

Direct cell contact methodology (ISO10993, Part 5), for qualitative cell viability assessment at 24 hours, can be conducted for cytotoxicity testing and at extended time points (48 and 72 hours) to gauge cell proliferation and adhesion efficiency. A manual count of non-adherent cells in a hemocytometer, following transfer and trypsinization of the culture wells, can be conducted. A CellTiter 96 assay can performed to quantify viable cells after four days. A live/dead cell staining kit can used as well to visualize scaffolds via fluorescence microscopy at 24 hours and day four, thereby to verify biocompatibility.

What is claimed:

1. Decellularized extracellular matrix (ECM) 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 of the biomaterial making it suitable for use as a xenograft.
2. The decellularized ECM of claim 1, wherein said decellularizing comprises subjecting said tissue sample to an alkaline treatment.
3. The decellularized ECM of claim 1, wherein said decellularized ECM is subjected to sterilization.
4. The decellularized ECM of claim 3 further comprising devitalized cells.
5. The decellularized ECM of claim 1 wherein the ECM is derived from Axolotl tissue.
6. The decellularized ECM of claim 1, wherein the ECM includes basement membrane.
7. 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 human agent.
8. The decellularized ECM of claim 7, wherein the agent is a cell, optionally a human cell

9. 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, a sheet, or a mesh, and wherein the ECM is parietal ECM, mesothelial ECM, thoracic cavity ECM, abdominal cavity ECM, pericardium ECM or dermal tissue ECM.

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

11. A tissue culture system comprising (a) a decellularized ECM according to claim 1, (b) tissue culture medium, and (c) cells xenogenic to a Urodele.

12. The tissue culture system of claim 11, wherein the cells are human cells.

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

14. The method of claim 13, 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.

15. The method of claim 13, further comprising homogenizing the decellularized ECM to form a powder, and, optionally reconstituting the powder as a gel.

16. The method of claim 13, further comprising sterilizing the decellularized ECM.

17. The method of claim 13, further comprising attaching the decellularized ECM to an agent xenogenic to a Urodele.

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

19. The ECM according to claim 9, wherein the powder has a particle size of less than 250 microns.

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

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

22. The ECM of claim 21, wherein the gel comprises a synthetic polymer.

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

24. The ECM according to claim 1 comprising sterile, isolated, decellularized Urodele extra cellular matrix (ECM), wherein the ECM is a sheet, 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.

25. The ECM of claim 24, wherein the agent is an anti-microbial agent.

26. The ECM of claim 24, wherein the agent is a growth factor.

27. The ECM of claim 1, wherein the sheet is at least two sheets of isolated Urodele decellularized ECM laminated to one another.

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

29. The method according to claim 28, 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.

30. A method for making the ECM of claim 29, 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 of the biomaterial, making it suitable for use as a xenograft; and sterilizing said tissue.

31. The method according to claim 30, 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.

32. The method according to claim 30, 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.

33. A method for making the material coated with, impregnated with, encapsulating, or having attached thereto isolated, decellularized Urodele ECM of claim 1, wherein said isolated, decellularized Urodele ECM is produced by a process comprising:

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

34. A biomaterial comprising decellularized extracellular matrix (ECM) 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 of the biomaterial making it suitable for use as a xenograft.

35. A method of making a biomaterial comprising decellularized extracellular matrix (ECM) derived from a Urodele said decellularized ECM said method comprising:

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

ABSTRACT

The growth factor profile, connective tissue matrix constituents, and immunoprivileged status of urodele extracellular matrix (ECM) and accompanying cutaneous tissue, plus the presence of antimicrobial peptides there, render urodele-derived tissue an ideal source for biological scaffolds for xenotransplantation. In particular, a biological scaffold biomaterial can be obtained by a process that entails (A) obtaining a tissue sample from a urodele, where the tissue comprises ECM, inclusive of the basement membrane, and (B) subjecting the tissue sample to a decellularization process that maintains the structural and functional integrity of the extracellular matrix, by virtue of retaining its fibrous and on-fibrous proteins, glycoaminoglycans (GAGs) and proteoglycans, while removing sufficient cellular components of the sample to reduce or eliminate antigenicity and immunogenicity for xenograft purposes. The resultant urodele-derived biomaterial can be used to enhance restoration of skin homeostasis, to reduce the severity, durations and associated damage caused by post-surgical inflammation, and to promote progression of natural healing and regeneration processes. In addition, the biomaterial promotes the formation of remodeled tissue that is comparable in quality, function, and compliance to undamaged human tissue.

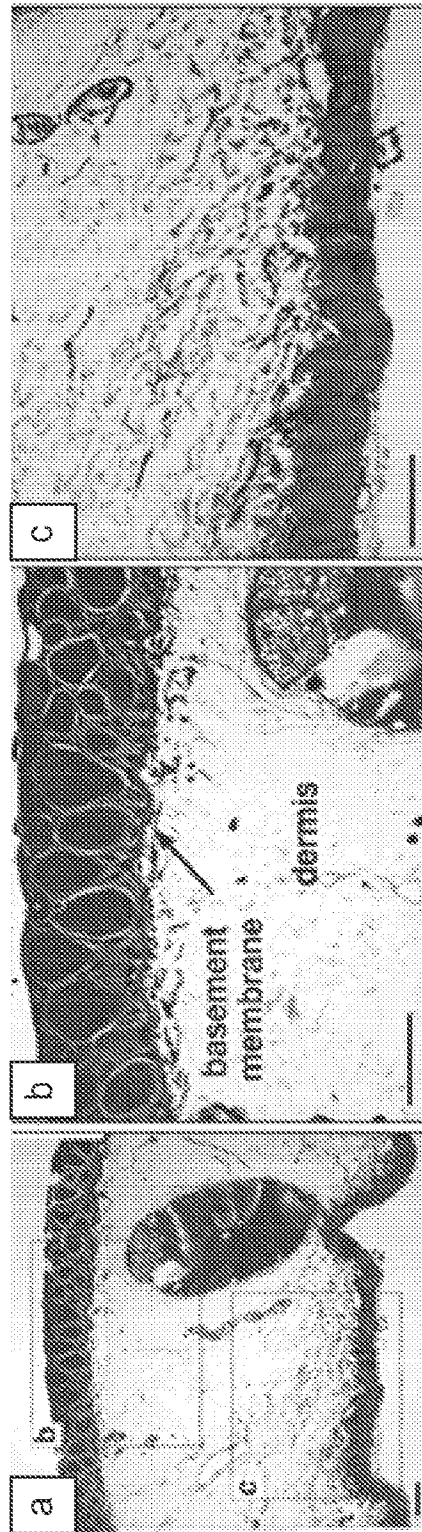


FIGURE 1

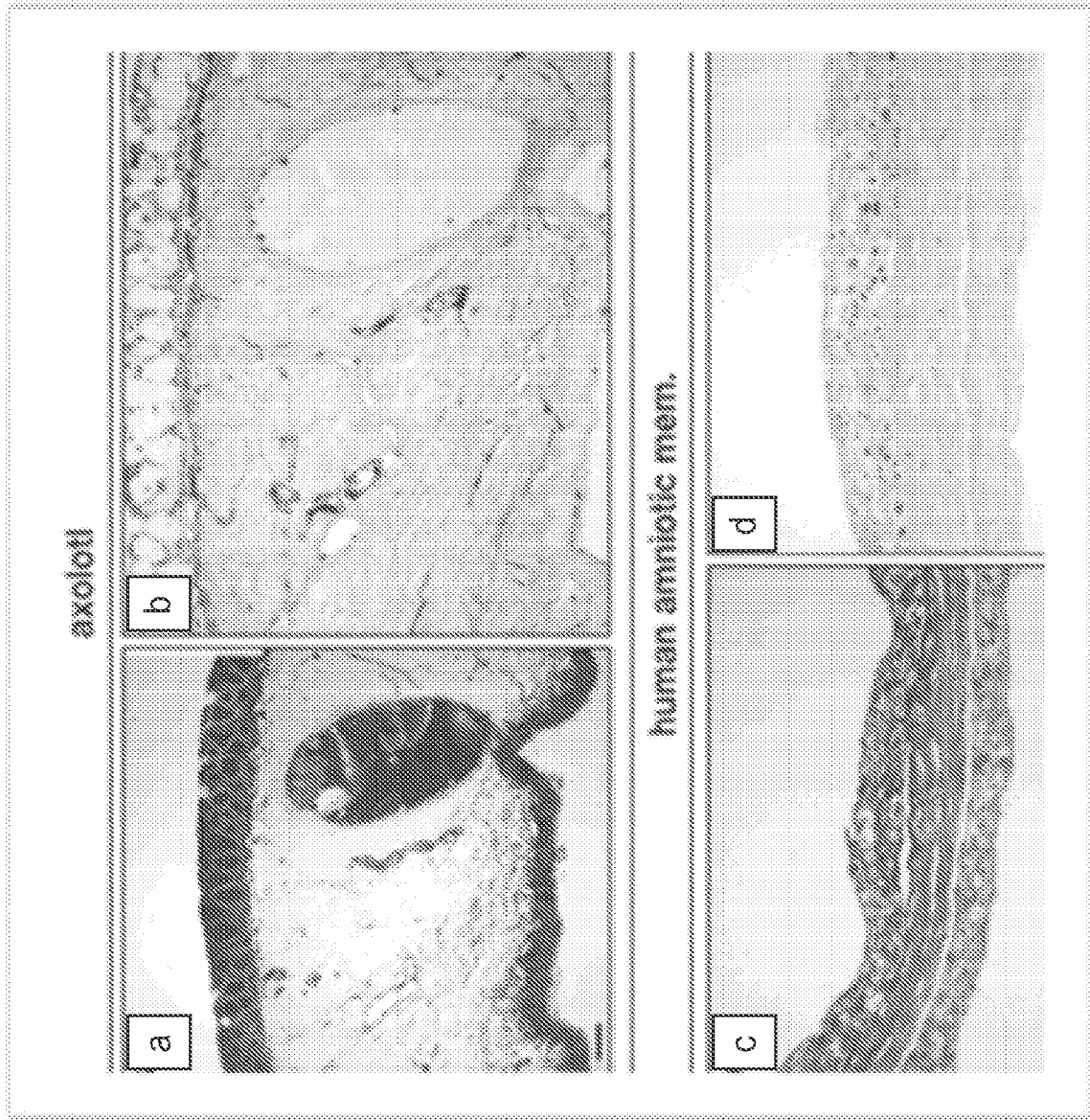


FIGURE 2

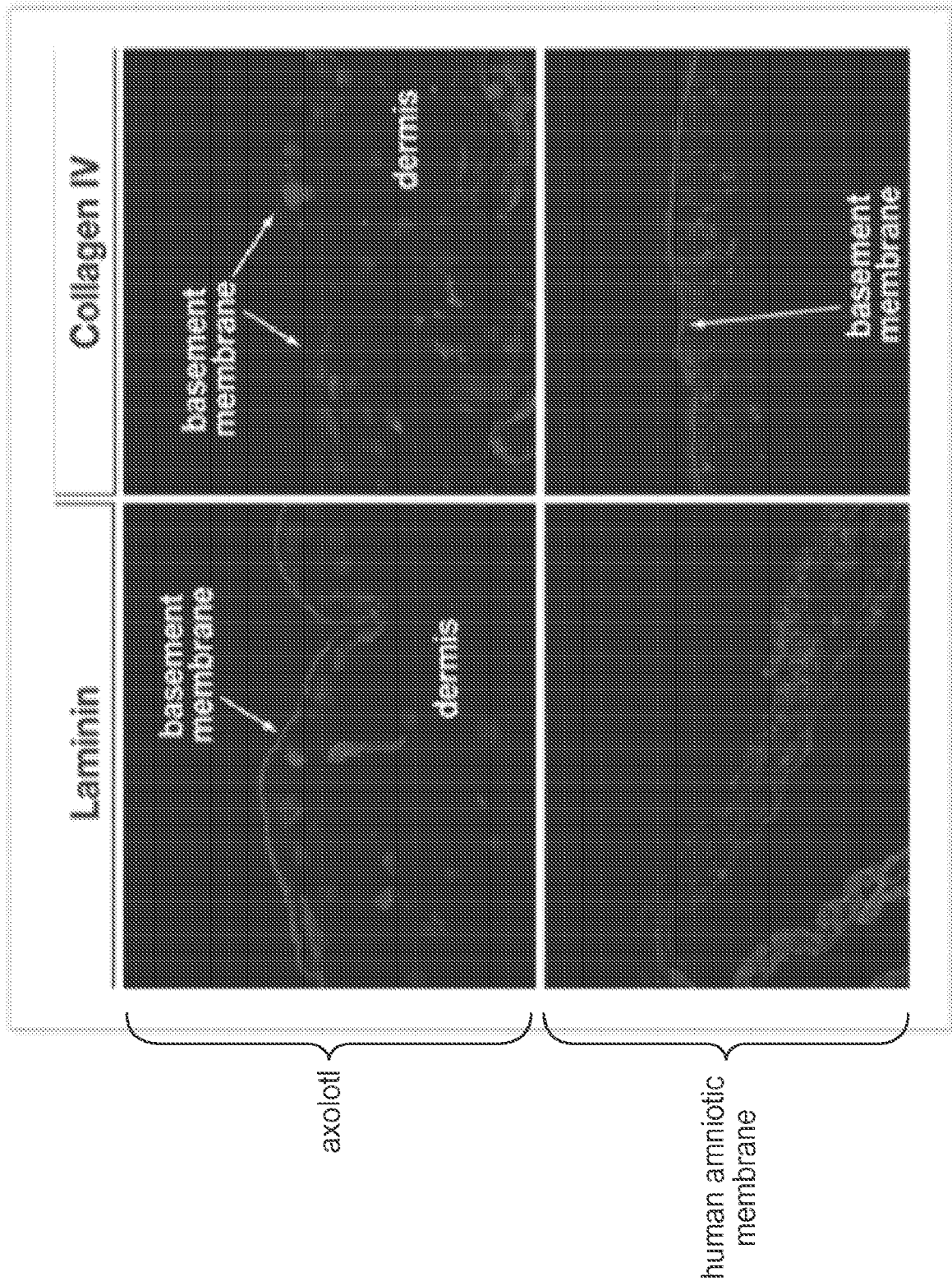


FIGURE 3

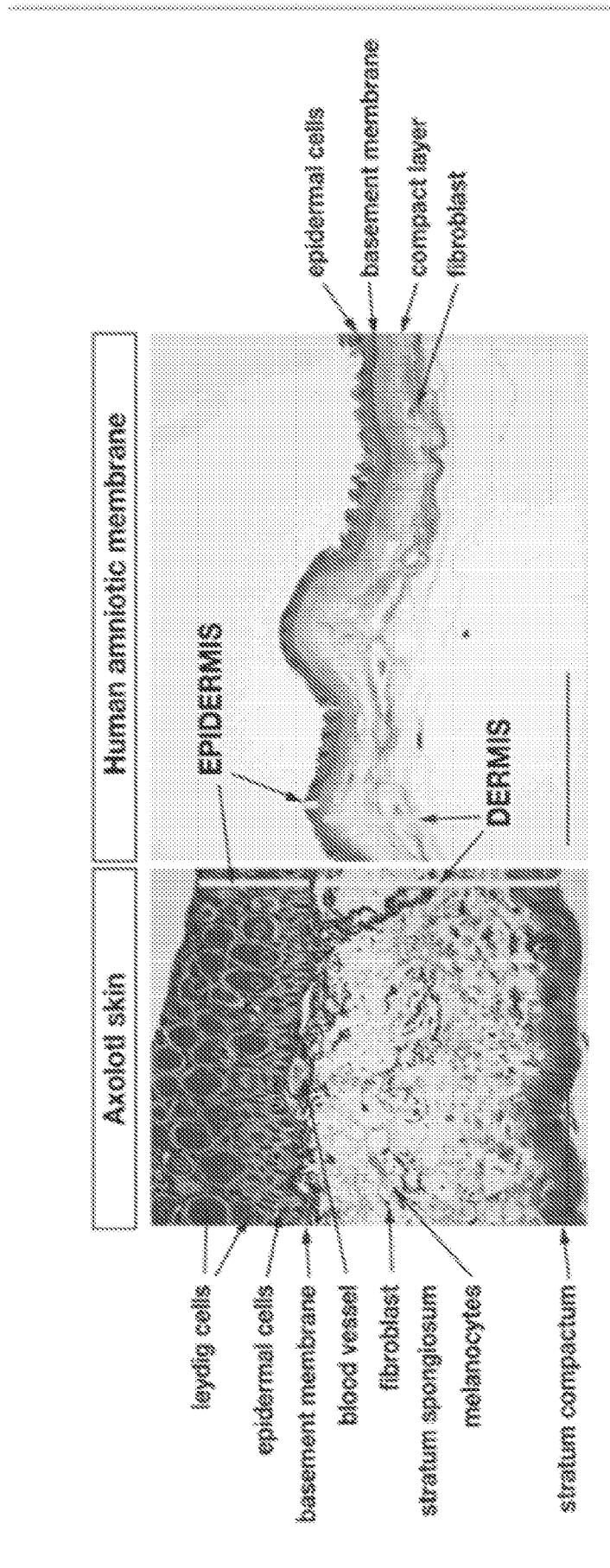


FIGURE 4

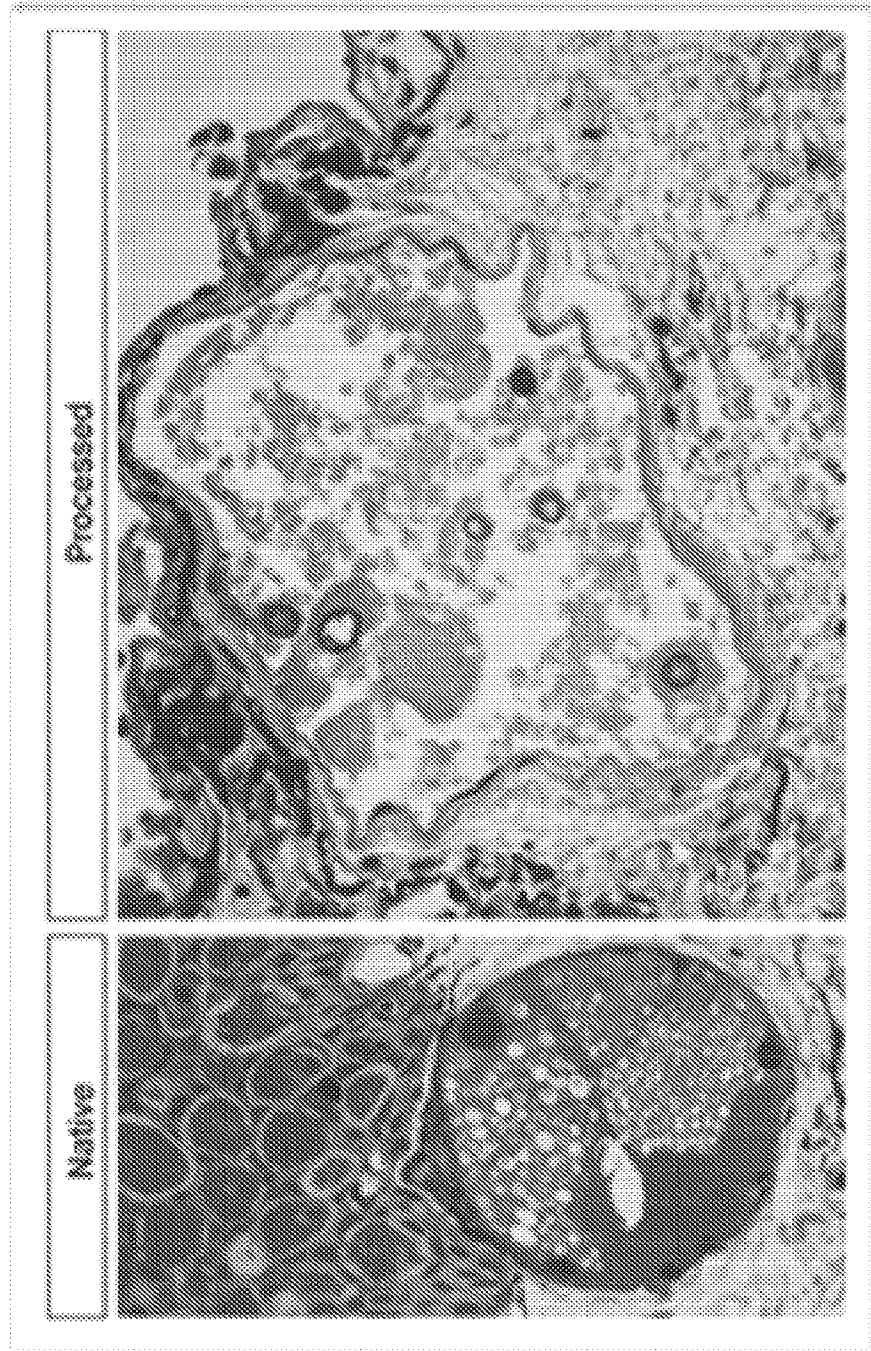


FIGURE 5

	Epoch Plate Reader	Avg. Duplicate	dsDNA conc. ng/ul	dsDNA conc. ug/ul	Percent Reduction
	Sample ID	260/280			
Paired native and post decellurization urodele connective tissue Residual DNA Analysis	A-Native	2	1702.8	1.7028	89.8%
	A-Native	2	1977.5	1.9775	92.8%
	A-Native	2	1618.3	1.6183	92.7%
	A-P1	1.96	173.7	0.1737	
	A-P2	1.95	141.7	0.1417	
	A-P3	1.97	117.9	0.1179	
Commercially available Amniotic Membrane Products residual Dna					
	BioDlogic (BioD)	2	1.34	0.00134	
	Biotissue (Amniograft)	1.5	27.81	0.02781	
	Mimedx (Amniofix)	1.26	104.6	0.1046	
	Seed Biotech (Aril)	1.75	3.16	0.00316	

FIGURE 6

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US PATENT DOCUMENTS							
EXAMINER INITIAL*		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE
	AA	2006/153797 A1 (corr. to CN '039)	07/13/06	Bortolotto et al.			
	AB	6,485,969 B1	11/26/02	Asem et al.			
	AC	4,298,002	11/03/81	Ronel et al.			
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	AE	2004/0181240 A1	09/16/04	Tseng et al.			
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	AG	2010/0104539 A1	04/29/10	Daniel et al.			
	AH						
	AI						
	AJ						
	AK						
FOREIGN PATENT DOCUMENTS							
		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION
							YES NO
	AL	2009-510091 A (corr. to WO '686)	03/12/09	JP			X
	AM	2007/038686 A2 (corr. to JP '091)	04/05/07	WO			
	AN	2004-521935 A (corr to. WO '720)	04/22/04	JP			X
	AO	02/078720 A2 (corr. to JP '935)	10/10/02	WO			
	AP	2007-532153 A (corr. to WO '219)	11/15/07	JP			X
OTHER PUBLICATIONS (Including Author, Title, Date, Pertinent Pages, Etc.)							
	AR	Notice for Reasons of Refusal from counterpart Japanese Patent Appln. No. 2018-224475 dated February 3, 2020, and its English translation.					
	AS	Second Office Action from counterpart Chinese Patent Appln No. 201480013359.0 dated May 22, 2019, along with its English translation.					
	AT	Duan et al., "Preliminary study on porous scaffold prepared with decellularized artery" Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi, 2010 Sep., 24(9), pages 1052-1057, Abstract only.					
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FOREIGN PATENT DOCUMENTS							
		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION
							YES NO
	AL	2005/097219 A1 (corr. to JP '153)	10/20/05	WO			
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OTHER PUBLICATIONS (Including Author, Title, Date, Pertinent Pages, Etc.)							
	AR	Meng et al., "An experimental study of extracellular matrix of conjunctiva", <u>Journal of Injuries and Occupational Diseases of the Eye with Ophthalmic Surgeries</u> , 2007 May; Vol.1, pages 26-28.					
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	AL	102227225 A	10/26/11	CN			X
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	AN	03/082201 A2	10/09/03	WO			
	AO	2005/009498 A2	02/03/05	WO			
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	AT	Extended European Search Report from counterpart European Appln. No. 14797892.1 mailed September 28, 2016					
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	AL	2012/131104	10/04/12	WO			
	AM	2012/131106	10/04/12	WO			
	AN						
	AO						
	AP						
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	AP						
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	AR	Meller et al., "Amniotic Membrane Transplantation in the Human Eye", <u>Deutsches Arzteblatt International</u> , 2011 April, 108(14): 243-248.					
	AS	Totter et al., "The effect of source animal age upon extracellular matrix scaffold properties", <u>Biomaterials</u> , 2011 January, 32(1): 128-136.					
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	AR	Wiens et al., "Ontogeny discombobulates phylogeny: paedomorphosis and higher-level salamander relationships", <u>Systematic Biology</u> , 2005 Feb., 54(1): 91-110.					
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Electronic Patent Application Fee Transmittal				
Application Number:				
Filing Date:				
Title of Invention:		DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE		
First Named Inventor/Applicant Name:		Ryanne Early		
Filer:		Sima Singadia Kulkarni/Stephanie De marco		
Attorney Docket Number:		SYG-19-1252US-CON2		
Filed as Small Entity				
Filing Fees for Utility under 35 USC 111(a)				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
UTILITY FILING FEE (ELECTRONIC FILING)	4011	1	75	75
UTILITY SEARCH FEE	2111	1	330	330
UTILITY EXAMINATION FEE	2311	1	380	380
Pages:				
Claims:				
CLAIMS IN EXCESS OF 20	2202	15	50	750
Miscellaneous-Filing:				
Petition:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				1535

Electronic Acknowledgement Receipt

EFS ID:	38697881
Application Number:	16801956
International Application Number:	
Confirmation Number:	9621
Title of Invention:	DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE
First Named Inventor/Applicant Name:	Ryanne Early
Customer Number:	35811
Filer:	Sima Singadia Kulkarni/Stephanie De marco
Filer Authorized By:	Sima Singadia Kulkarni
Attorney Docket Number:	SYG-19-1252US-CON2
Receipt Date:	26-FEB-2020
Filing Date:	
Time Stamp:	15:52:55
Application Type:	Utility under 35 USC 111(a)

Payment information:

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Payment Type	DA
Payment was successfully received in RAM	\$ 1535
RAM confirmation Number	E20202PF53083760
Deposit Account	
Authorized User	

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Warnings:					
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2	Application Data Sheet	SYG_19_1252US_CON2_ADS.pdf	1334345	no	8
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			9a5373e6931a4ba359e9ed057024572695e751e7		
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			3ef0641cd4b63c3dcf0373dca8ee3c4fd182b673		
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6		SYG_19_1252US_CON2_Appln.pdf	164063	yes	33
			be116e41e3287c8cf3bffde08be7a5e1323c7ccb		
	Multipart Description/PDF files in .zip description				
	Document Description		Start	End	

	Specification	1	27
	Claims	28	32
	Abstract	33	33

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First Named Inventor: Ryanne Early

For: DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE

We submit herewith:

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x Application Data Sheet
 — Hard Copy of Sequence Listing with computer readable form and Statement
x Information Disclosure Statement with Form PTO-1449 with references from Parent Appln. No. 14/759,895

The filing fee has been calculated as shown below:

For	Number Filed	Number Extra
Basic Fee (electronically filed)	---	---
Search Fee	---	---
Examination Fee	---	---
Total Claims	35 Minus 20 =	15
Independent Claims	3 Minus 3 =	0
Application Size Fee	If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$400 (\$200 for small entity) for each additional 50 sheets or fraction thereof.	
Multiple Dependent Claim(s) Present		
Surcharge for late filing of oath or declaration		

SMALL ENTITY	
Rate	Fee
\$75.00	\$75.00
\$330.00	\$330.00
\$380.00	\$380.00
x 50.00 =	\$750.00
x 230.00 =	\$
\$200.00	\$
\$410.00	\$
\$ 80.00	\$

OTHER THAN SMALL ENTITY	
Rate	Fee
\$300.00	\$
\$660.00	\$
\$760.00	\$
x 100.00 =	\$
x 460.00 =	\$
\$400.00	\$
\$820.00	\$
\$160.00	\$

OR

TOTAL FEE \$1,535.00 OR \$_____

x Please charge my Deposit Account No. 50-2719 in the amount of \$1,535.00.

x In regard to this communication, the Commissioner is hereby authorized to charge payment of any additional filing fees required under 37 CFR §1.16 and any additional patent application processing fees under 37 CFR §1.17 or credit any overpayment to Deposit Account No. 50-2719.

x During the pendency of this application, the Commissioner is hereby authorized to charge payment of any filing fees for presentation of extra claims under 37 CFR §1.16 and any patent application processing fees under 37 CFR §1.17 or credit any overpayment to Deposit Account No. 50-2719.

Respectfully submitted,

/sima singadia kulkarni/

Date: February 26, 2020
SSK/sh
(215) 656-2478

Sima Singadia Kulkarni, Reg. No. 43,732

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	SYG-19-1252US-CON2
		Application Number	
Title of Invention	DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE		
The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76. This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.			

Secrecy Order 37 CFR 5.2:

☐	Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)
--------------------------	---

Inventor Information:

Inventor	1				Remove	
Legal Name						
Prefix	Given Name	Middle Name	Family Name	Suffix		
	Ryanne		Early			
Residence Information (Select One) • US Residency Non US Residency Active US Military Service						
City	Fleming Island	State/Province	FL	Country of Residence	US	
Mailing Address of Inventor:						
Address 1	1909 Hickory Trace Drive					
Address 2						
City	Fleming Island	State/Province	FL			
Postal Code	32003	Country	US			
All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the Add button.						

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below. For further information see 37 CFR 1.33(a).			
☐ An Address is being provided for the correspondence information of this application.			
Customer Number	035811		
Email Address	pto.phil@us.dlapiper.com	Add Email	Remove Email

Application Information:

Title of the Invention	DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE		
Attorney Docket Number	SYG-19-1252US-CON2	Small Entity Status Claimed	☒
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	6	Suggested Figure for Publication (if any)	

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	SYG-19-1252US-CON2
		Application Number	
Title of Invention	DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE		

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not be** the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32). Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	☒ Customer Number	☐ US Patent Practitioner	☐ Limited Recognition (37 CFR 11.9)
Customer Number	035811		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.

When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status		Remove	
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
	Continuation of	14/759895	2015-07-08

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	SYG-19-1252US-CON2
		Application Number	
Title of Invention	DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE		
Prior Application Status	Expired	Remove	
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
14/759895	a 371 of international	PCT/US2014/010890	2014-01-09
Prior Application Status	Expired	Remove	
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
PCT/US2014/010890	Claims benefit of provisional	61/750555	2013-01-09
Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the Add button.			Add

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)ⁱ the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

			Remove
Application Number	Country ⁱ	Filing Date (YYYY-MM-DD)	Access Code ⁱ (if applicable)
Additional Foreign Priority Data may be generated within this form by selecting the Add button.			Add

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.

NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	SYG-19-1252US-CON2
		Application Number	
Title of Invention	DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE		

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	SYG-19-1252US-CON2
		Application Number	
Title of Invention	DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE		

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant	1	Remove
If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section. Clear		
☒ Assignee	Legal Representative under 35 U.S.C. 117	Joint Inventor
Person to whom the inventor is obligated to assign.		Person who shows sufficient proprietary interest
If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:		
Name of the Deceased or Legally Incapacitated Inventor:		
If the Applicant is an Organization check here. ☒		
Organization Name	SE Professional Testing & Consulting Services, Inc.	
Mailing Address Information For Applicant:		
Address 1	1909 Hickory Trace Drive	
Address 2		
City	Fleming Island	State/Province
Country	US	Postal Code
Phone Number		Fax Number
Email Address		
Additional Applicant Data may be generated within this form by selecting the Add button. Add		

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	SYG-19-1252US-CON2
		Application Number	
Title of Invention	DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE		

Assignee	1			
Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.				
				Remove
If the Assignee or Non-Applicant Assignee is an Organization check here.				☐
Prefix	Given Name	Middle Name	Family Name	Suffix
Mailing Address Information For Assignee including Non-Applicant Assignee:				
Address 1				
Address 2				
City		State/Province		
Country ⁱ		Postal Code		
Phone Number		Fax Number		
Email Address				
Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button.				Add

Signature:

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). However, if this Application Data Sheet is submitted with the **INITIAL** filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/sima singadia kulkarni/		Date (YYYY-MM-DD)	2020-02-26
First Name	Sima Singadia	Last Name	Kulkarni	Registration Number
				43732
Additional Signature may be generated within this form by selecting the Add button.				Add

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	SYG-19-1252US-CON2
		Application Number	
Title of Invention	DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE		

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

- 1 The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.
- 2 A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
- 3 A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
- 4 A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
- 5 A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
- 6 A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
- 7 A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
- 8 A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.
- 9 A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Doc Code: Oath

Document Description: Oath or declaration filed

PTO/AIA/08 (11-15)

Approved for use through 11/30/2020. OMB 0651-0032

U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)	Attorney Docket Number	RE-US1
	First Named Inventor	RYANNE EARLY
	COMPLETE IF KNOWN	
	Application Number	14/759895
	Filing Date	01/09/2014
	Art Unit	1651
	Examiner Name	RUTH DAVIS A.
☐ Declaration Submitted With Initial Filing OR ☒ Declaration Submitted After Initial Filing (surcharge (37 CFR 1.16(f)) required)		

DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE

(Title of the Invention)

As a below named inventor, I hereby declare that:

This declaration is directed to:

☐ The attached application,

OR

☒ United States Application Number or PCT International application number 14/759895

filed on 01/09/2014.

The above-identified application was made or authorized to be made by me.

I believe I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

Direct all correspondence to: ☐ The address associated with Customer Number: OR ☒ Correspondence address below

Name

GERARD H. BENCEN, ESQ.

Address

10 E. UNIVERSITY AVENUE

City

GAINESVILLE

State

FLORIDA

Zip

32608

Country

UNITED STATES

Telephone

352-214-0385

Email

GERARD@PATENT-ARTS.COM

[Page 1 of 2]

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 21 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

RegenX Science Exhibit 1004
Page 64

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

DECLARATION — Utility or Design Patent Application**WARNING:**

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available. Petitioner/applicant is advised that documents which form the record of a patent application (such as the PTO/SB/01) are placed into the Privacy Act system of records DEPARTMENT OF COMMERCE, COMMERCE-PAT-7, System name: *Patent Application Files*. Documents not retained in an application file (such as the PTO-2038) are placed into the Privacy Act system of COMMERCE/PAT-TM-10, System name: *Deposit Accounts and Electronic Funds Transfer Profiles*.

LEGAL NAME OF SOLE OR FIRST INVENTOR:

(E.g., Given Name (first and middle if any) and Family Name or Surname)

RYANNE EARLY

Inventor's Signature

Date (Optional)

Residence: City

State

Country

Dallas

TX

USA

Mailing Address

5614 SWISS AVE

City

State

Zip

Country

Dallas

TX

75214

USA

☐

Additional inventors are being named on the

Supplemental sheet(s) PTO/AIA/10 attached hereto



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY,DOCKET,NO	TOT CLAIMS	IND CLAIMS
16/801,956	02/26/2020		1535	SYG-19-1252US-CON2	35	3

CONFIRMATION NO. 9621

FILING RECEIPT



0000000115359873

35811
IP GROUP OF DLA PIPER LLP (US)
ONE LIBERTY PLACE
1650 MARKET ST, SUITE 5000
PHILADELPHIA, PA 19103

Date Mailed: 03/13/2020

Receipt is acknowledged of this non-provisional utility patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF FIRST INVENTOR, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection.

Please verify the accuracy of the data presented on this receipt. If an error is noted on this Filing Receipt, please submit a written request for a corrected Filing Receipt, including a properly marked-up ADS showing the changes with strike-through for deletions and underlining for additions. If you received a "Notice to File Missing Parts" or other Notice requiring a response for this application, please submit any request for correction to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections provided that the request is grantable.

Inventor(s)

Ryanne Early, Fleming Island, FL;

Applicant(s)

ISE Professional Testing & Consulting Services, Inc., Fleming Island, FL;

Power of Attorney:

Thomas Christenbury--31750	William Bartow--54981
Paul Taufer--35703	Lisa Lint--60856
Darius Gambino--41472	Deborah Grove--61091
Sima Kulkarni--43732	Priya Narahari--71526
Richard Cruz--52783	Joseph Wolfe--73173

Domestic Priority data as claimed by applicant

This application is a CON of 14/759,895 07/08/2015
which is a 371 of PCT/US2014/010890 01/09/2014
which claims benefit of 61/750,555 01/09/2013

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see <http://www.uspto.gov> for more information.) - None.

Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 03/11/2020

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 16/801,956**

Projected Publication Date: 06/18/2020

Non-Publication Request: No

Early Publication Request: No

**** SMALL ENTITY ****

Title

DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE

Preliminary Class

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

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Title 37, Code of Federal Regulations, 5.11 & 5.15

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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875						Application or Docket Number 16/801,956				
APPLICATION AS FILED - PART I										
(Column 1)		(Column 2)		SMALL ENTITY		OR OTHER THAN SMALL ENTITY				
FOR	NUMBER FILED	NUMBER EXTRA	RATE(\$)	FEE(\$)		RATE(\$)	FEE(\$)			
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	75		N/A				
SEARCH FEE (37 CFR 1.16(k), (l), or (m))	N/A	N/A	N/A	330		N/A				
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	380		N/A				
TOTAL CLAIMS (37 CFR 1.16(j))	35 minus 20 =	*	x 50 =	750	OR					
INDEPENDENT CLAIMS (37 CFR 1.16(h))	3 minus 3 =	*	x 230 =	0.00						
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			0.00						
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				0.00						
* If the difference in column 1 is less than zero, enter "0" in column 2.				TOTAL		TOTAL				
				1535						
APPLICATION AS AMENDED - PART II										
(Column 1)		(Column 2)		(Column 3)		SMALL ENTITY		OR OTHER THAN SMALL ENTITY		
AMENDMENT A		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE(\$)	ADDITIONAL FEE(\$)		RATE(\$)	ADDITIONAL FEE(\$)
	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))							OR		
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	
AMENDMENT B		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE(\$)	ADDITIONAL FEE(\$)		RATE(\$)	ADDITIONAL FEE(\$)
	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))							OR		
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	
* If the entry in column 1 is less than the entry in column 2, write "0" in column 3. ** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20". *** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3". The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.										

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Application	Document	Mailroom Date	Attorney Docket No.
16801956	APP.FILE.REC	03/13/2020	SYG-19-1252US-CON2

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/801,956	02/26/2020	Ryanne Early	SYG-19-1252US-CON2

CONFIRMATION NO. 9621

PUBLICATION NOTICE



OC000000117851199

35811
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ONE LIBERTY PLACE
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PHILADELPHIA, PA 19103

Title: DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE

Publication No. US-2020-0188557-A1

Publication Date: 06/18/2020

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

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In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently <https://portal.uspto.gov/pair/PublicPair>. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

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Application	Document	Mailroom Date	Attorney Docket No.
16801956	NTC.PUB	06/18/2020	SYG-19-1252US-CON2

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Form PTO-1449 US DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE				ATTY. DOCKET NO. SYG-19-1252US-CON2		SERIAL NO. 16/801,956	
LIST OF PUBLICATIONS CITED BY APPLICANT <i>(Use several sheets if necessary)</i>				APPLICANT ISE Professional Testing & Consulting Services, Inc.			
				FILING DATE February 26, 2020		GROUP TBD	
US PATENT DOCUMENTS							
EXAMINER INITIAL*		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE
	AA	2009/0142836 A1	06/04/09	Wang et al.			
	AB	2007/0248638 A1	10/25/07	Van Dyke et al.			
	AC						
	AD						
	AE						
	AF						
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	AH						
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	AJ						
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FOREIGN PATENT DOCUMENTS							
		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION
							YES NO
	AL						
	AM						
	AN						
	AO						
	AP						
OTHER PUBLICATIONS (Including Author, Title, Date, Pertinent Pages, Etc.)							
	AR	Notice of Reasons for Refusal from corresponding Korean Patent Appln. No. 10-2015-7021367 dated June 29, 2020, and its English translation.					
	AS						
	AT						
EXAMINER				DATE CONSIDERED			
*EXAMINER: Initial if publication considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.							

**PATENT TERM ADJUSTMENT
STATEMENT UNDER 37 CFR 1.704(d)**

Application Number	16/801,956
Filing Date	February 26, 2020
First Named Inventor	Ryanne Early
Art Unit	TBD
Examiner Name	TBD
Practitioner Docket No.	SYG-19-1252US-CON2

APPLICANT HEREBY STATES THE FOLLOWING (please review 37 CFR 1.704(d) before filing this form):

☒ Each item of information contained in the information disclosure statement was first cited in any communication from a patent office in a counterpart foreign or international application or from the Office, and this communication was not received by any individual designated in 37 CFR 1.56(c) more than thirty days prior to the filing of the information disclosure statement.

AND/OR

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- The present form (PTO/SB/133) should be filed concurrently with the information disclosure statement to derive benefit under 37 CFR 1.704(d).

Signature	/sima singadia kulkarni/	Date	July 27, 2020
Typed or Printed Name	Sima Singadia Kulkarni	Practitioner Registration Number	43,732
Note: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required in accordance with 37 CFR 1.33 and 11.18. Please see 37 CFR 1.4(d) for the form of the signature. If necessary, submit multiple forms for more than one signature, see below*.			
☐	*Total of _____ forms are submitted.		

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8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
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Sending number : 9-5-2020-044161374
 Dispatched date : 2020.06.29.
 Submission due date : 2020.08.29.

YOUR INVENTION PARTNER

KIPO

Notification of Reason for Refusal

Applicant.	Name.	AIESeul PeuRoPeSyeoNeol TeSeuTing AenDeu KeonSeolTing SeoBiSiJeu, InKoPeoReITiDeu(patent client number: 520150476737)
	Address.	Secure Information
Agent.	Name.	AJU INTERANTIONAL LAW & PATENT GROUP
	Address.	Secure Information
Inventor.	Name.	EolRi, RalAen
	Address.	Secure Information
Application No.	10-2015-7021367	
Title of Invention	The biomaterial which is cell removed from the non- mammalia tissue.	

1. Because of having the reason for refusal like the examination result next about this application and notifying of this according to the article 63 of Patent Act in case it has the opinion or the correction is needed or it wants opinion (the reply, and the Written Reply) to submit the Amendment [form of attached document No.9 of Enforcement Regulation of Patent Act]. 2. In the case to extend the submission due date (2020.08. 29.), the due date of submission of the can be extended through the request for an extension of designated period to 4 month. In this case, request for the extension has to do by unit of 1 month and 2 month or greater is summed up in the need in the range that does not exceed 4 month and it can request the extension. The Written Substantiation describing the proprietary is additionally attached in the time for to postponing the designated period to the generation (the guideline reference of the lower part) of the inevitable proprietary in excess of 4 month and request for the extension has to be applied.

[Examination result]

☐ Subject of examination claim:

Claim 1-33.

☐ The law articles in connection with the part in which it has the reason for refusal of this application.

The part related law articles in which it has the sequence number reason for refusal.

1

Claim 1, claim 2, and claim 5 to claim 7, claim 11, claim 28, claim 29.

The article 29(1)(2) of Patent Act.



2

Claim preceding clause.

The article 29(2) of Patent Act.

3

Claim 1 to claim 4, claim 7 to claim 15, claim 17 to claim 19, claim 21, and claim 24.

The article 42(4)(2) of Patent Act.

[The detailed reason for refusal]

1. Claim 1 of the patent claim of this application, claim 2, and claim 5 to claim 7, claim 11, claim 28, the invention in claim 29 may be the invention inserted in the publication distributed in the Republic of Korea or abroad before the filing of the patent application like the lower part. And since it is the invention in which air is possible to use through the telecommunication circuit it corresponds to under Article 29(1)(2) of Patent Act and patent cannot be received.

– Follows –

Cited invention 1: the Akira Satoh et al., the ZOOLOGICAL SCIENCE (2012), the Vol. 29, and the pp.191–197.

a. As the biomaterial which is cell removed as the biomaterial in which claim 1 invention is cell removed, it is manufactured by the method including the step it removes the step and (B) sample obtaining the tissue sample including the extracellular matrix from (A) Urodela Amphibia with cell in order to maintain the structural functional integrity, of removing enough cellular component of sample and reducing the antigenicity of the biomaterial as the xenograft or removing. But the method is disclosed the collagen sheet obtains from the hole rattle (axolotl) (the sub-concept of the water dog neck). Therefore incubates the cell corresponding to (b) and removes the nucleus and obtains the cell-free ECM sheet of separating the skin corresponded to 'cited invention 1' to (a) both invention is identical.

b. As to claim 2 invention is claim 1 invention, comprises the step that the removal of a cell step performs the alkali treatment to the tissue sample. But (the left column reference of the p.192) is disclosed to process in the phosphate buffered saline containing EDTA in 'cited invention 1'. Therefore both invention is identical.

c. The tissue graft in which claim 5 invention includes the extracellular matrix compositional component originating from the Urodela Amphibia is claimed. But hole rattle origin collagen sheet (the left column 5 line of the p.192, and the left column 39 of the p.194 through 42 line reference) are disclosed in 'cited invention 1'. Therefore both invention is identical.

d. Claim 6 invention is isolated and the Urodela Amphibia ECM which is cell removed is claimed. But hole rattle origin collagen sheet (the left column 5 line of the p.192, and the left column 39 of the p.194 through 42 line reference) including the step of removing the nucleus



in 'cited invention 1' and obtaining the cell-free ECM sheet are disclosed. Therefore both invention is identical.

e. As to claim 7 invention is claim 6 invention, it is isolated. But ECM originates from the hole rotle organization hole rotle origin collagen sheet (the left column 5 line of the p.192, and the left column 39 of the p.194 through 42 line reference) are disclosed in 'cited invention 1'. Therefore both invention is identical.

f. As to claim 11 invention is claim 6 invention, ECM is sheet. But hole rotle origin collagen sheet (the left column 5 line of the p.192, and the left column 39 of the p.194 through 42 line reference) are disclosed in 'cited invention 1'. Therefore both invention is identical.

g. As the method in which claim 28 invention manufactures the biological material, the manufacturing method including the step of removing the cell of sample to remove enough cellular component of (B) sample obtaining the tissue sample including the extracellular matrix from (A) Urodela Amphibia and step and reducing the antigenicity of the biomaterial as the xenograft or removing of the biological material is claimed. But the manufacturing method is disclosed the collagen sheet obtains from the hole rotle (axolotl) (the sub-concept of the water dog neck). Therefore incubates the cell corresponding to (b) and removes the nucleus and obtains the cell-free ECM sheet of separating the skin corresponded to 'cited invention 1' to (a) both invention is identical.

h. As to claim 29 invention is claim 28 invention, the step of performing the removal of a cell to the mode which is useful as the substrate growing in the inside but it is enough maintains the structural functional integrity of ECM further is included and or the cell has ECM in the upper part. But the step is presented. Therefore incubates and removes the nucleus in 'cited invention 1' and obtains the cell-free ECM both invention is identical.

2. The invention described in claim preceding clause of the patent claim of this application cannot receive patent like the lower part according to the article 29(2) of Patent Act since a person having ordinary skill in the art to which the invention pertains easily can invent before the application.

– Follows –

Cited invention 2:US2009-0142836 A(2009.06. 04.)

Cited invention 3:US2007-0248638 A(2007.10. 25.)

 The simplicity of the combination between cited invention.

: the collagen sheet in which it is about the biomaterial in which the application removes the cell from the Urodela Amphibia for the xenogeneic transplantation of the tissue sample obtained and but it is separated from the hole rotle in 'cited invention 1' and it gets rid of the cell are presented. And disclosed is the point in which but the cell in which the



application is the devitalization is added or the cell has the difference in the point in which the additive is added and but the cell which becomes as the biomaterial formed in 'cited invention 2' into the extracellular matrix with the devitalization is added. And in 'cited invention 3', it is disclosed as the bio scaffold which decellularizes the organization and made to add the protein or the other additives. And it corresponds to the technology which generally it uses in this technical field to add the material the additional function in the decellularization biomaterial and improve the effect of the tissue regeneration. Therefore the effect due to the addition of the composition dissimilar if it is the normal technical engineer is easily predicted and it can assemble in 'cited invention 1'.

a. As shown in it describes in the reason for refusal '1. a. my paper.' the or Turner all configurations written in claim 1, 2, 5 through 7, 11, and 28 and 29 invention are in 'cited invention 1' or it is the extent of drawing easily from 'cited invention 1' and no more. Therefore the normal technical engineer claim 1, 2, 5 through 7, 11, and 28 and 29 invention easily can invent from the cited invention 1.

b. As to claim 3 invention is claim 1 invention, the step of performing sterilization to sample further is included and the step has. But it corresponds to the technology which generally it uses in this technical field to perform the sterilization of the material for the transplant. Therefore if it is the normal technical engineer it is easy as necessary it can adopt and the effect is predictable.

c. Disclosed is the point in which claim 8 invention ECM includes the base film as to claim 6 invention and which is isolated and but obtains hole rattle origin collagen sheet (the left column 5 line of the p.192, and the left column 39 of the p.194 through 42 line reference) in 'cited invention 1'. And if it is the normal technical engineer it is easy as necessary it can adopt as the basic optional matter to include the base film when separating skin and the effect is predictable.

d. As to claim 13 invention is claim 6 invention, ECM is the dry powder.

It corresponds to the technology known in this technical field to manufacture ECM with the dry powder shape. Therefore if it is the normal technical engineer it can adopt easily according to the need and purpose.

e. As to claim 14 invention is claim 6 invention, it is the gel in which ECM is re-organized.

It corresponds to the technology which generally it uses in this technical field to after ECM is manufactured with the powder melt in the water phase and make with the gel form. Therefore if it is the normal technical engineer it is easy as necessary it can adopt and the effect is predictable.

f. Disclosed is hole rattle origin collagen sheet (the left column 5 line of the p.192, and the left column 39 of the p.194 through 42 line reference) in 'cited invention 1' the apparatus including at least 2 sheet of ECM which is cell removed the isolated Urodela Amphibia in which claim 26 invention is laminated is claimed. And if it is the normal technical engineer so that the transplant man with underdeveloped genital organs to the utmost copy the organization, it is easy as necessary multiple sheets can be adopted as the basic optional matter to use and the effect is predictable.



g. As to claim 30 invention is claim 29 invention, the step of homogenizing ECM which is cell removed and forming the powder further is included and the step has.

It corresponds to the technology known in this technical field to manufacture ECM with the dry powder shape. Therefore if it is the normal technical engineer it can adopt easily according to the need and purpose.

h. As to claim 31 invention is claim 30 invention, the step of reconstructing the powder as the gel further is included and the step has.

It corresponds to the technology which generally it uses in this technical field to after ECM is manufactured with the powder melt in the water phase and make with the gel form. Therefore if it is the normal technical engineer it is easy as necessary it can adopt and the effect is predictable.

i. As to claim 32 invention is claim 29 invention, the step of sterilizing ECM which is cell removed further is included and the step has. But it corresponds to the technology which generally it uses in this technical field to perform the sterilization of the material for the transplant. Therefore if it is the normal technical engineer it is easy as necessary it can adopt and the effect is predictable.

?

j. As to claim 4 invention is claim 1 invention, it further includes the cell which becomes with the devitalization (paragraph number [0068] reference) is disclosed in 'cited invention 2' to hold the cell in which the cell matrix is the devitalization as the extracellular matrix organization engineering structure. Therefore in order to improve the organization regeneration effect if claim 4 invention is the normal technical engineer, it is easy as necessary it can adopt and the effect is predictable.

?

k. As to claim 9 invention is claim 6 invention, it is adhered to the agent in which ECM is the heterogeneity about the growth factor, cytokine, chemokine, protein, carbohydrate, per, steroid, antimicrobial agent, synthetic polymer, adhesive, drug and/or the human agent phosphorus, and the Urodela Amphibia as injection, and the coating. But (paragraph number [0047] reference) is disclosed as the bio scaffold using the organization which becomes in 'cited invention 3' with decellularization to add the protein (growth factor) or other additives (the antibiotic, and the anti-inflammatory agent and immune response modifier) in the scaffold and it corresponds to the technology in which it is generally used to add the additive in order to enhance the regeneration effect of the scaffold. Therefore if it is the normal technical engineer it is easy as necessary it can adopt and the effect is predictable.



l. Disclosed is claim 10 invention the agent as to claim 6 invention, is the cell, and the point which the selectively is the human cell and but seeds the mammalian cell in 'cited invention 3' in the scaffold. And it corresponds to the notification technique to seed the cell in order to improve the organization regeneration effect. Therefore if it is the normal technical engineer it is easy as necessary it can adopt and the effect is predictable.

m. As to claim 12 invention is claim 11 invention, sheet comprises the hole. But (claim 1 reference) is disclosed in 'cited invention 3' that the bio scaffold has the pore. Therefore if it is the normal technical engineer it is easy as necessary it can adopt and the effect is predictable.

n. Disclosed is hole rattle origin collagen sheet (the left column 5 line of the p.192, and the left column 39 of the p.194 through 42 line reference) in 'cited invention 1' the package containing the Urodela Amphibia fraction originating from the Urodela Amphibia ECM which is cell removed is claimed claim 16 invention is isolated of sterilization and it is isolated of the Urodela Amphibia ECM or the sterilization which is cell removed. And in 'cited invention 3', the step (claim 7 reference) sterilizing the scaffold begins after the washing process. And the sterilization step corresponds to the well-known usage technique of this technical field. Therefore if it is the normal technical engineer it is easy as necessary it can adopt and the effect is predictable.

As to the one. claim 17 invention is claim 16 invention, it is the sheet of the Urodela Amphibia ECM which is cell removed. But hole rattle origin collagen sheet (the left column 5 line of the p.192, and the left column 39 of the p.194 through 42 line reference) are disclosed in 'cited invention 1'. Therefore if it is the normal technical engineer it is easy as necessary it can adopt and the effect is predictable.

As to the you. claim 18 invention is claim 17 invention, it is the dry powder.

It corresponds to the technology known in this technical field to manufacture ECM with the dry powder shape. Therefore if it is the normal technical engineer it can adopt easily according to the need and purpose.

More, as to claim 19 invention is claim 17 invention, the Urodela Amphibia fraction is the gel

It corresponds to the technology which generally it uses in this technical field to after ECM is manufactured with the powder melt in the water phase and make with the gel form. Therefore if it is the normal technical engineer it is easy as necessary it can adopt and the effect is predictable.

Disclosed is hole rattle origin collagen sheet (the left column 5 line of the p.192, and the left column 39 of the p.194 through 42 line reference) in the Urodela Amphibia ECM which claim 20 invention is isolated of sterilization and is cell removed. Or 'cited invention 1' the medical implant including the Urodela Amphibia fraction of the sterilization originating from the Urodela Amphibia ECM which is cell removed of sterilization is claimed it is isolated. And in 'cited invention 3', the step (claim 7 reference) sterilizing the scaffold begins after the



washing process. And the sterilization step corresponds to the well-known usage technique of this technical field. Therefore if it is the normal technical engineer it is easy as necessary it can adopt and the effect is predictable.

It is far and claim 21 invention the implant is the bio compatibility sheet, mesh, gel, implanted piece, organization or the apparatus as to claim 19 invention. But the apparatus is disclosed in 'cited invention 1' to be the hole rattle origin collagen sheet. Therefore if the form of the implant is the normal technical engineer it is easy as necessary it can adopt and the effect is predictable.

Disclosed is hole rattle origin collagen sheet (the left column 5 line of the p.192, and the left column 39 of the p.194 through 42 line reference) in 'cited invention 1' the burr. claim 22 invention is isolated and it is isolated from the Urodela Amphibia ECM or the above of being cell removed and it is coated on the Urodela Amphibia fraction originating from the Urodela Amphibia ECM which is cell removed or it is permeated. And in 'cited invention 3', it is disclosed (the paragraph number [0017], and [0018] and [0047] reference) that the bio scaffold in which the mammalian cell is seeded is dipped to the growth factor or the culture medium can be provided. Therefore if the above-mentioned configuration of limiting is the normal technical engineer it can adopt easily in consideration of the effect of the tissue regeneration and the effect is predictable.

The west. claim 23 invention it claims. But hole rattle origin collagen sheet (the left column 5 line of the p.192, and the left column 39 of the p.194 through 42 line reference) are disclosed in 'cited invention 1' and it is disclosed (the paragraph number [0017], and [0018] and [0047] reference) that the bio scaffold in which the mammalian cell is seeded in 'cited invention 3' is dipped to the growth factor or the culture medium can be provided. Therefore if the above-mentioned configuration of limiting is the normal technical engineer it can adopt easily in consideration of the effect of the tissue regeneration and the effect is predictable the tissue culture system including ECM which is cell removed the isolated Urodela Amphibia, (b) tissue culture media, and the cell which is the heterogeneity about (c) Urodela Amphibia.

It is frozen and claim 24 invention the cell is the human cell as to claim 22 invention. But (paragraph number [0018] reference) is disclosed in 'cited invention 3' to seed the mammalian cell in the bio scaffold. Therefore if it is the normal technical engineer it is easy as necessary it can adopt and the effect is predictable.

Disclosed is hole rattle origin collagen sheet (the left column 5 line of the p.192, and the left column 39 of the p.194 through 42 line reference) in ECM which is cell removed the Urodela Amphibia in which the base. claim 25 invention is isolated or 'cited invention 1' the tissue culture media which carries the Urodela Amphibia fraction originating from the Urodela Amphibia ECM which is cell removed making as a condition is claimed it is isolated. And in 'cited invention 3', it is disclosed (the paragraph number [0017], and [0018] and [0047] reference) that the bio scaffold in which the mammalian cell is seeded is dipped to the growth factor or the culture medium can be provided. Therefore if the above-mentioned



configuration of limiting is the normal technical engineer it can adopt easily in consideration of the effect of the tissue regeneration and the effect is predictable.

Disclosed is hole rotte origin collagen sheet (the left column 5 line of the p.192, and the left column 39 of the p.194 through 42 line reference) in 'cited invention 1' the wife. claim 27 invention isolates the Urodela Amphibia ECM from the Urodela Amphibia and the cell of ECM is removed. And in 'cited invention 3', the step (claim 7 reference) sterilizing the scaffold begins after the washing process. And the sterilization step corresponds to the well-known usage technique of this technical field. Therefore if it is the normal technical engineer it is easy as necessary it can adopt and the effect is predictable.

It is large and claim 33 invention further includes the step of adhering to ECM which is cell removed in the agent which is the heterogeniety about the Urodela Amphibia as to claim 29 invention and the step has. But (paragraph number [0047] reference) is disclosed as the bio scaffold using the organization which becomes in 'cited invention 3' with decellularization to add the protein (growth factor) or other additives (the antibiotic, and the anti-inflammatory agent and immune response modifier) in the scaffold and it corresponds to the technology in which it is generally used to add the additive in order to enhance the regeneration effect of the scaffold. Therefore if it is the normal technical engineer it is easy as necessary it can adopt and the effect is predictable.

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As to the tert. claim 15 invention is one invention among claim 6 to 13 invention, ECM is sterilized. But it corresponds to the well-known usage technique of this technical field to sterilize ECM of the organization regeneration use. Therefore if it is the normal technical engineer it is easy as necessary it can adopt.

?

3. The application cannot be granted for a patent since it cannot satisfy the requisite according to the article 42(4)(2) of Patent Act due to deficiency in the description of claim 1 of the patent claim to claim 4, claim 7 to claim 15, claim 17 to claim 19, claim 21, and claim 24 as pointed out below.

– Follows –

a. Claim 1 describes as "the enough cellular component of sample is removed". But the range that "it is sufficient" limits is vague. Therefore claim 1 and claim 2 quoting claim 1 to the claims in which claim 4 tries to be protected are clear and the range is not written.

b. Claim 7 to claim 15 describes the termination of claim as "isolated ECM". But the cited termination of claim is written as "urodela Amphibia ECM". Therefore it is vague whether the invention to claim is the what. Therefore the claims in which claims try to be protected is clear and the invention is not written.



c. Claim 9 describes as "the ~ drug and/or the human agent phosphorus". But it is vague whether because "and" means all configurations mentioned above the c is included and it is mixed and it is vague whether it is the what because "human agent phosphorus" means. Therefore the claims in which claim 9 tries to be protected is clear and the concisely is not written.

d. Claim 10 describes as "selectively, the human cell phosphorus" and "agent". But it has the meaning that "selectively" limits among this and it is the configuration vague and the in which there is no material about "agent" and which "above" indicates object is vague whether the object is the what in used claim. Therefore the claims in which claim 10 tries to be protected is clear and the configuration is not written.

e. Claim 17 quotes the same claim as claim 17 to "as for claim 17" and the configuration is vague. Therefore claim 17, claim 18 quoting claim 17 and the claims in which claim 19 tries to be protected are clear and the configuration is not written.

f. Claim 21 describes as "implant". But the in which there is no material about "implant" and which "above" indicates object is vague whether the object is the what in cited claim. Therefore the claims in which claim 21 tries to be protected is clear and the concisely is not written.

g. As to claim 24, the termination of claim is written as the material in which the cited termination of "tissue culture system" or claim is adhered. Therefore it is vague whether the invention to claim is the what. Therefore the claims in which claim 24 tries to be protected is clear and the invention is not written.

※ List of reference.

In claim of this application, ECM is described as the abbreviation. But it does with desirable to once describe the full name of ECM.

[Appendix]

The appendix 1 Akira Satoh et al., the ZOOLOGICAL SCIENCE (2012), the Vol. 29, and the pp.191-197 appendix 2 US2009-0142836 A (2009.06. 04.) one copy appendix. 3 US2007-0248638 A (2007.10. 25.) one copy. end.

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출 원 번 호 10-2015-7021367
출 원 일 자 2015.08.06.
발 명 의 명 칭 비-포유류 조직으로부터의 세포제거된 생체재료

1. 이 출원에 대한 심사결과 다음과 같은 거절이유가 있어 특허법 제63조에 따라 이를 통지 하오니 의견이 있거나 보정이 필요할 경우에는 상기 제출기일(2020.08.29.)까지 의견(답변, 소명)서[특허법시행규칙 별지 제24호서식] 또는/및 보정서[특허법시행규칙 별지 제9호서식] 를 제출하여 주시기 바랍니다.

2. 상기 제출기일(2020.08.29.)을 연장하려는 경우에는 지정기간연장신청을 통해 그 제출기 일을 4개월까지 연장할 수 있습니다. 이 경우 연장신청은 1개월 단위로 해야 하며, 필요 시 4개월을 초과하지 않는 범위에서 2개월 이상을 일괄하여 연장신청할 수 있습니다. 불가피한 사유의 발생(하단의 안내참조)으로 4개월을 초과하여 지정기간을 연장받고자 하는 때에는 그 사유를 기재한 소명서를 추가로 첨부해서 연장신청을 해야 합니다.

[심사결과]

☐ 심사 대상 청구항 : 제1-33항

☐ 이 출원의 거절이유가 있는 부분과 관련 법조항

순번	거절이유가 있는 부분	관련 법조항
1	청구항 제1항, 제2항, 제5항 내지 제7항, 제11항, 제28항, 제29항	특허법 제29조제1항제2호
2	청구항 전항	특허법 제29조제2항
3	청구항 제1항 내지 제4항, 제7항 내지 제15항, 제17항 내지 제19항, 제21항, 제24항	특허법 제42조제4항제2호

[구체적인 거절이유]

1. 이 출원의 특허청구범위의 청구항 제1항, 제2항, 제5항 내지 제7항, 제11항, 제28항, 제29항에 기재된 발명은 아래와 같이 그 출원 전에 국내 또는 국외에서 반포된 간행물에 게재된 발명이거나 전기통신회선을 통하여 공중이 이용가능하게 된 발명이므로 특허법 제29조제1항제2호에 해당되어 특허를 받을 수 없습니다.

- 아 래 -

인용발명1: Akira Satoh et al., *ZOOLOGICAL SCIENCE*(2012), Vol. 29, pp.191-197

가. 청구항 1 발명은 세포제거된 생체재료에 관한 것으로, 세포제거된 생체재료로서, (A) 유미목 양서류로부터 세포외 기질을 포함하는 조직 샘플을 얻는 단계, 및 (B) 샘플을 구조적 및 기능적 완전성을 유지하도록 세포 제거하는 한편 샘플의 충분한 세포 구성성분을 제거하여 이중이식편으로서 생체재료의 항원성을 감소 또는 제거하는 단계를 포함하는 방법에 의해 제조되는 것을 특징으로 하고 있으나,

‘인용발명1’에도 (a)에 대응되는 피부를 분리하는 단계; (b)에 대응되는 세포를 인큐베이션하고 핵을 제거하여 무세포 ECM 시트를 얻는 단계(p. 192의 우측컬럼 1-5째 줄, p. 194의 좌측컬럼 39-42 참조) 및 콜라겐 시트는 아홀로틀(axolotl)(도롱뇽목의 하위 개념)로부터 얻는 점이 개시되어 있으므로, 양 발명은 동일합니다.

나. 청구항 2 발명은 청구항 1 발명에 있어서, 세포제거 단계는 조직 샘플에 알칼리 처리를 실시하는 단계를 포함하는 것을 특징으로 하고 있으나,

‘인용발명1’에도 EDTA를 함유하는 인산완충식염수에서 처리하는 점(p.192의 좌측 컬럼 참조)이 개시되어 있으므로, 양 발명은 동일합니다.

다. 청구항 5 발명은 유미목 양서류로부터 유래된 세포외 기질 구성성분을 포함하는 조직 이식편을 청구하고 있으나,

‘인용발명1’에도 아홀로틀 유래 콜라겐 시트(p.192의 좌측컬럼 5째줄, p.194의 좌측컬럼 39 내지 42째줄 참조)가 개시되어 있으므로, 양 발명은 동일합니다.

라. 청구항 6 발명은 단리되고, 세포제거 된 유미목 양서류 ECM을 청구하고 있으나,

‘인용발명1’에도 핵을 제거하여 무세포 ECM 시트를 얻는 단계를 포함한 아홀로틀 유래 콜라겐 시트(p.192의 좌측컬럼 5째줄, p.194의 좌측컬럼 39 내지 42째줄 참조)가 개시되어 있으므로, 양 발명은 동일합니다.

마. 청구항 7 발명은 청구항 6 발명에 있어서, ECM은 아홀로틀 조직으로부터 유래된,

단리된 것을 특징으로 하고 있으나,

‘인용발명1’에도 아홀로틀 유래 콜라겐 시트(p.192의 좌측컬럼 5째줄, p.194의 좌측컬럼 39 내지 42째줄 참조)가 개시되어 있으므로, 양 발명은 동일합니다.

바. 청구항 11 발명은 청구항 6 발명에 있어서, ECM은 시트인 것을 특징으로 하고 있으나,

‘인용발명1’에도 아홀로틀 유래 콜라겐 시트(p.192의 좌측컬럼 5째줄, p.194의 좌측컬럼 39 내지 42째줄 참조)가 개시되어 있으므로, 양 발명은 동일합니다.

사. 청구항 28 발명은 생물학적 재료를 제조하는 방법으로서, (A) 유미목 양서류로부터 세포외 기질을 포함하는 조직 샘플을 얻는 단계, 및 (B) 샘플의 충분한 세포 구성성분을 제거하도록 샘플의 세포를 제거하여 이중이식편으로서 생체재료의 항원성을 감소 또는 제거하는 단계를 포함하는, 생물학적 재료의 제조방법을 청구하고 있으나,

‘인용발명1’에도 (a)에 대응되는 피부를 분리하는 단계; (b)에 대응되는 세포를 인큐베이션하고 핵을 제거하여 무세포 ECM 시트를 얻는 단계(p. 192의 우측컬럼 1-5째 줄, p. 194의 좌측컬럼 39-42 참조) 및 콜라겐 시트는 아홀로틀(axolotl)(도롱뇽목의 하위 개념)로부터 얻는 점이 개시되어 있으므로, 양 발명은 동일합니다.

아. 청구항 29 발명은 청구항 28 발명에 있어서, ECM이 세포가 그 위에서 또는 그 안에서 성장할 수 있는 기질로서 유용하게 되는데 충분하게 ECM의 구조적 및 기능적 완전성을 유지하는 방식으로 상기 세포제거를 수행하는 단계를 더 포함하는 것을 특징으로 하고 있으나,

‘인용발명1’에도 인큐베이션하고 핵을 제거하여 무세포 ECM을 얻는 단계가 개시되어 있으므로, 양 발명은 동일합니다.

2. 이 출원의 특허청구범위의 청구항 전항에 기재된 발명은 아래와 같이 그 출원 전에 이 발명이 속하는 기술분야에서 통상의 지식을 가진 자가 용이하게 발명할 수 있는 것이므로 특허법 제29조제2항에 따라 특허를 받을 수 없습니다.

- 아 래 -

인용발명2: 미국공개특허공보 제2009-0142836호 (2009.06.04.)

인용발명3: 미국공개특허공보 제2007-0248638호 (2007.10.25.)

☞ 인용발명 간 결합의 용이성

: 출원발명은 이중이식을 위한 유미목 양서류로부터 얻은 조직 샘플에서 세포를 제거한 생체재료에 관한 것이나, ‘인용발명1’에도 아홀로틀로부터 분리되어 세포가 제거된 콜라겐 시트가 제시되어 있고, 다만 출원발명은 탈활력화된 세포가 부가되거나, 첨가제가 부가되는

점에서 차이가 있으나, ‘인용발명2’에도 세포외 매트릭스로 형성된 생체재료에 관한 것으로, 탈활력화된 세포가 부가된 점이 개시되어 있고, ‘인용발명3’에는 조직을 탈세포화하여 제작한 바이오 스캐폴드에 관한 것으로, 단백질 또는 기타 첨가제를 부가한 점이 개시되어 있는 바, 탈세포화 생체 재료에 부가적인 기능을 하는 재료를 첨가하여 조직 재생의 효과를 높이는 것은 이 기술분야에서 일반적으로 사용하는 기술에 해당하므로, 통상의 기술자라면 다른 조성물의 첨가로 인한 효과를 쉽게 예측하여 ‘인용발명1’에 조합할 수 있습니다.

가. 상기 거절이유 ‘1. 가. 내지 아.’에 기재한 바와 같이, 청구항 1, 2, 5 내지 7, 11, 28 및 29 발명에 기재된 모든 구성은 ‘인용발명 1’에 나타나있거나, ‘인용발명 1’로부터 쉽게 도출할 수 있는 정도에 불과하므로, 청구항 1, 2, 5 내지 7, 11, 28 및 29 발명은 통상의 기술자가 인용발명 1로부터 쉽게 발명할 수 있습니다.

나. 청구항 3 발명은 청구항 1 발명에 있어서, 샘플에 멸균화를 실시하는 단계를 더 포함하는 것을 특징으로 하고 있으나,

이식용 재료의 멸균화를 실시하는 것은 이 기술 분야에서 일반적으로 사용하는 기술에 해당하므로, 통상의 기술자라면 필요에 따라 쉽게 채택할 수 있으며, 그 효과도 예측 가능합니다.

다. 청구항 8 발명은 청구항 6 발명에 있어서, ECM은 기저막을 포함하는, 단리된 것을 특징으로 하고 있으나,

‘인용발명1’에도 아홀로를 유래 콜라겐 시트(p.192의 좌측컬럼 5째줄, p.194의 좌측컬럼 39 내지 42째줄 참조)를 얻는 점이 개시되어 있고, 피부를 분리할 때 기저막을 포함하는 것은 단순 선택적 사항으로, 통상의 기술자라면 필요에 따라 쉽게 채택할 수 있으며, 그 효과도 예측 가능합니다.

라. 청구항 13 발명은 청구항 6 발명에 있어서, ECM은 건조 분말인 것을 특징으로 하고 있으나,

ECM을 건조 분말 형태로 제조하는 것은 이 기술 분야에서 공지된 기술에 해당하므로, 통상의 기술자라면 필요와 목적에 따라 쉽게 채택할 수 있습니다.

마. 청구항 14 발명은 청구항 6 발명에 있어서, ECM은 재구성된 겔인 것을 특징으로 하고 있으나,

ECM을 분말로 제조한 후 수용액 상에 녹여 겔 형태로 만드는 것은 이 기술분야에서 일반적으로 사용하는 기술에 해당하므로, 통상의 기술자라면 필요에 따라 쉽게 채택할 수 있으며, 그 효과도 예측 가능합니다.

바. 청구항 26 발명은 서로에 대해 적층된 단리 된 유미목 양서류 세포 제거된 ECM의

적어도 2개 시트를 포함하는 장치를 청구하고 있으나,

‘인용발명1’에도 아플로를 유래 콜라겐 시트(p.192의 좌측컬럼 5째줄, p.194의 좌측컬럼 39 내지 42째줄 참조)가 개시되어 있고, 복수의 시트를 사용하는 것은 단순 선택적 사항으로, 통상의 기술자라면 이식하고자 하는 조직을 최대한 모사하기 위해 필요에 따라 쉽게 채택할 수 있으며, 그 효과도 예측 가능합니다.

사. 청구항 30 발명은 청구항 29 발명에 있어서, 세포제거된 ECM을 균질화하여 분말을 형성하는 단계를 더 포함하는 것을 특징으로 하고 있으나,

ECM을 건조 분말 형태로 제조하는 것은 이 기술 분야에서 공지된 기술에 해당하므로, 통상의 기술자라면 필요와 목적에 따라 쉽게 채택할 수 있습니다.

아. 청구항 31 발명은 청구항 30 발명에 있어서, 겔로서 분말을 재구성하는 단계를 더 포함하는 것을 특징으로 하고 있으나,

ECM을 분말로 제조한 후 수용액 상에 녹여 겔 형태로 만드는 것은 이 기술분야에서 일반적으로 사용하는 기술에 해당하므로, 통상의 기술자라면 필요에 따라 쉽게 채택할 수 있으며, 그 효과도 예측 가능합니다.

자. 청구항 32 발명은 청구항 29 발명에 있어서, 세포제거된 ECM을 멸균화하는 단계를 더 포함하는 것을 특징으로 하고 있으나,

이식용 재료의 멸균화를 실시하는 것은 이 기술 분야에서 일반적으로 사용하는 기술에 해당하므로, 통상의 기술자라면 필요에 따라 쉽게 채택할 수 있으며, 그 효과도 예측 가능합니다.

【따라서 청구항 1 내지 3, 5 내지 8, 11, 13, 14, 26 및 30 내지 32 발명은 통상의 기술자라면 ‘인용발명1’로부터 쉽게 발명할 수 있습니다.】

차. 청구항 4 발명은 청구항 1 발명에 있어서, 탈활력화된 세포를 더 포함하는, 세포제거된 것을 특징으로 하고 있으나,

‘인용발명2’에도 세포외 매트릭스 조직 공학 구조물에 관한 것으로, 세포 매트릭스는 탈활력화된 세포를 보유하는 점(문단번호 [0068] 참조)이 개시되어 있으므로, 청구항 4 발명은 통상의 기술자라면 조직 재생 효과를 높이기 위해 필요에 따라 쉽게 채택할 수 있으며, 그 효과도 예측 가능합니다.

【따라서 청구항 4 발명은 통상의 기술자라면 ‘인용발명1 및 2’의 단순 결합으로부터 쉽게 발명할 수 있습니다.】

카. 청구항 9 발명은 청구항 6 발명에 있어서, ECM은 성장인자, 사이토카인, 케모카인, 단백질, 탄수화물, 당, 스테로이드, 항균제, 합성 중합체, 접착제, 약물 및/또는 인간 작용제인, 유미목 양서류에 대해 이종성인 작용제에 주입, 코팅 또는 부착되는 것을 특징으로 하고 있으나,

‘인용발명3’에도 탈세포화된 조직을 이용한 바이오 스캐폴드에 관한 것으로, 스캐폴드에 단백질(성장 인자) 또는 기타 첨가제(항생제, 항염증제 및 면역 반응 조절제)를 첨가하는 점(문단번호 [0047] 참조)이 개시되어 있고, 스캐폴드의 재생 효과를 높이기 위해 첨가제를 부가하는 것은 일반적으로 사용되는 기술에 해당하므로, 통상의 기술자라면 필요에 따라 쉽게 채택할 수 있으며, 그 효과도 예측 가능합니다.

타. 청구항 10 발명은 청구항 6 발명에 있어서, 작용제는 세포, 선택적으로 인간 세포인 것을 특징으로 하고 있으나,

‘인용발명3’에도 포유동물 세포를 스캐폴드에 시딩하는 점이 개시되어 있고, 조직 재생 효과를 높이기 위해 세포를 시딩하는 것은 공지기술에 해당하므로, 통상의 기술자라면 필요에 따라 쉽게 채택할 수 있으며, 그 효과도 예측 가능합니다.

파. 청구항 12 발명은 청구항 11 발명에 있어서, 시트는 천공을 포함하는 것을 특징으로 하고 있으나,

‘인용발명3’에도 바이오 스캐폴드가 기공을 가지는 점(청구항 1 참조)이 개시되어 있으므로, 통상의 기술자라면 필요에 따라 쉽게 채택할 수 있으며, 그 효과도 예측 가능합니다.

하. 청구항 16 발명은 멸균의, 단리되고, 세포제거된 유미목 양서류 ECM, 또는 멸균의, 단리되고, 세포제거된 유미목 양서류 ECM으로부터 유래된 유미목 양서류 분획을 함유하는 패키지를 청구하고 있으나,

‘인용발명1’에도 아홀로틀 유래 콜라겐 시트(p.192의 좌측컬럼 5째줄, p.194의 좌측컬럼 39 내지 42째줄 참조)가 개시되어 있고, ‘인용발명3’에는 세척단계 후에 스캐폴드를 멸균하는 단계(청구항 7 참조)가 개시되어 있는바, 멸균 단계는 이 기술분야의 주지관용 기술에 해당하므로, 통상의 기술자라면 필요에 따라 쉽게 채택할 수 있으며, 그 효과도 예측 가능합니다.

거. 청구항 17 발명은 청구항 16 발명에 있어서, 세포제거 된 유미목 양서류 ECM의 시트인 것을 특징으로 하고 있으나,

‘인용발명1’에도 아홀로틀 유래 콜라겐 시트(p.192의 좌측컬럼 5째줄, p.194의 좌측컬럼 39 내지 42째줄 참조)가 개시되어 있으므로, 통상의 기술자라면 필요에 따라 쉽게 채택할 수 있으며, 그 효과도 예측 가능합니다.

너. 청구항 18 발명은 청구항 17 발명에 있어서, 건조 분말인 것을 특징으로 하고 있으나,

ECM을 건조 분말 형태로 제조하는 것은 이 기술 분야에서 공지된 기술에 해당하므로, 통상의 기술자라면 필요와 목적에 따라 쉽게 채택할 수 있습니다.

더. 청구항 19 발명은 청구항 17 발명에 있어서, 유미목 양서류 분획은 겔인 것을 특징으로 하고 있으나,

ECM을 분말로 제조한 후 수용액 상에 녹여 겔 형태로 만드는 것은 이 기술분야에서 일반적으로 사용하는 기술에 해당하므로, 통상의 기술자라면 필요에 따라 쉽게 채택할 수 있으며, 그 효과도 예측 가능합니다.

러. 청구항 20 발명은 멸균의, 단리되고, 세포제거된 유미목 양서류 ECM, 또는 단리되고, 세포제거된 유미목 양서류 ECM으로부터 유래된 멸균의 유미목 양서류 분획을 포함하는, 멸균의 의료용 이식물을 청구하고 있으나,

‘인용발명1’에도 아플로틀 유래 콜라겐 시트(p.192의 좌측컬럼 5째줄, p.194의 좌측컬럼 39 내지 42째줄 참조)가 개시되어 있고, ‘인용발명3’에는 세척단계 후에 스케폴드를 멸균하는 단계(청구항 7 참조)가 개시되어 있는바, 멸균 단계는 이 기술분야의 주지관용 기술에 해당하므로, 통상의 기술자라면 필요에 따라 쉽게 채택할 수 있으며, 그 효과도 예측 가능합니다.

머. 청구항 21 발명은 청구항 19 발명에 있어서, 이식물은 생체적합성 시트, 메쉬, 겔, 이식편, 조직 또는 장치인 것을 특징으로 하고 있으나,

‘인용발명1’에도 아플로틀 유래 콜라겐 시트인 점이 개시되어 있으므로, 이식물의 형태는 통상의 기술자라면 필요에 따라 쉽게 채택할 수 있으며, 그 효과도 예측 가능합니다.

버. 청구항 22 발명은 단리되고, 세포제거된 유미목 양서류 ECM 또는 상기 단리되고, 세포제거된 유미목 양서류 ECM으로부터 유래된 유미목 양서류 분획에 코팅되거나, 침윤되거나, 캡슐화되거나 또는 부착된 재료를 청구하고 있으나,

‘인용발명1’에도 아플로틀 유래 콜라겐 시트(p.192의 좌측컬럼 5째줄, p.194의 좌측컬럼 39 내지 42째줄 참조)가 개시되어 있고, ‘인용발명3’에는 포유동물세포가 시딩된 바이오 스케폴드는 성장 인자로 함침되거나 배양배지가 제공될 수 있음이 개시되어 있으므로(문단번호 [0017], [0018] 및 [0047] 참조), 상기 한정하는 구성은 통상의 기술자라면 조직 재생의 효과를 고려하여 쉽게 채택할 수 있으며, 그 효과도 예측 가능합니다.

서. 청구항 23 발명은 단리된 유미목 양서류 세포제거된 ECM, (b) 조직 배양 배지, 및 (c) 유미목 양서류에 대해 이종성인 세포를 포함하는, 조직 배양 시스템을 청구하고 있으나,

‘인용발명1’에도 아플로를 유래 콜라겐 시트(p.192의 좌측컬럼 5째줄, p.194의 좌측컬럼 39 내지 42째줄 참조)가 개시되어 있고, ‘인용발명3’에는 포유동물세포가 시딩된 바이오 스캐폴드는 성장 인자로 함침되거나 배양배지가 제공될 수 있음이 개시되어 있으므로(문단번호 [0017], [0018] 및 [0047] 참조), 상기 한정하는 구성은 통상의 기술자라면 조직 재생의 효과를 고려하여 쉽게 채택할 수 있으며, 그 효과도 예측 가능합니다.

어. 청구항 24 발명은 청구항 22 발명에 있어서, 세포는 인간 세포인 것을 특징으로 하고 있으나,

‘인용발명3’에도 포유동물세포를 바이오 스캐폴드에 시딩하는 점(문단번호 [0018] 참조)이 개시되어 있으므로, 통상의 기술자라면 필요에 따라 쉽게 채택할 수 있으며, 그 효과도 예측 가능합니다.

저. 청구항 25 발명은 단리된 유미목 양서류 세포제거된 ECM 또는 단리되고, 세포제거된 유미목 양서류 ECM으로부터 유래된 유미목 양서류 분획을 지니는 조건화된 조직 배양 배지를 청구하고 있으나,

‘인용발명1’에도 아플로를 유래 콜라겐 시트(p.192의 좌측컬럼 5째줄, p.194의 좌측컬럼 39 내지 42째줄 참조)가 개시되어 있고, ‘인용발명3’에는 포유동물세포가 시딩된 바이오 스캐폴드는 성장 인자로 함침되거나 배양배지가 제공될 수 있음이 개시되어 있으므로(문단번호 [0017], [0018] 및 [0047] 참조), 상기 한정하는 구성은 통상의 기술자라면 조직 재생의 효과를 고려하여 쉽게 채택할 수 있으며, 그 효과도 예측 가능합니다.

처. 청구항 27 발명은 유미목 양서류로부터 유미목 양서류 ECM을 단리시키고, ECM의 세포를 제거하며, 세포제거된 ECM을 멸균시킴으로써 제조된 생성물을 청구하고 있으나,

‘인용발명1’에도 아플로를 유래 콜라겐 시트(p.192의 좌측컬럼 5째줄, p.194의 좌측컬럼 39 내지 42째줄 참조)가 개시되어 있고, ‘인용발명3’에는 세척단계 후에 스캐폴드를 멸균하는 단계(청구항 7 참조)가 개시되어 있는바, 멸균 단계는 이 기술분야의 주지관용 기술에 해당하므로, 통상의 기술자라면 필요에 따라 쉽게 채택할 수 있으며, 그 효과도 예측 가능합니다.

커. 청구항 33 발명은 청구항 29 발명에 있어서, 유미목 양서류에 대해 이종성인 작용제에 세포제거된 ECM을 부착하는 단계를 더 포함하는 것을 특징으로 하고 있으나,

‘인용발명3’에도 탈세포화된 조직을 이용한 바이오 스캐폴드에 관한 것으로, 스캐폴드에 단백질(성장 인자) 또는 기타 첨가제(항생제, 항염증제 및 면역 반응 조절제)를 첨가하는 점(문단번호 [0047] 참조)이 개시되어 있고, 스캐폴드의 재생 효과를 높이기 위해 첨가제를

부가하는 것은 일반적으로 사용되는 기술에 해당하므로, 통상의 기술자라면 필요에 따라 쉽게 채택할 수 있으며, 그 효과도 예측 가능합니다.

《따라서 청구항 9, 10, 12, 16 내지 25, 27, 33 발명은 통상의 기술자라면 ‘인용발명1 3’의 단순 결합으로부터 쉽게 발명할 수 있습니다.》

터. 청구항 15 발명은 청구항 6 내지 13 발명 중 어느 한 발명에 있어서, ECM은 멸균된 것을 특징으로 하고 있으나,

조직 재생 용도의 ECM을 멸균하는 것은 이 기술분야의 주지관용 기술에 해당하므로, 통상의 기술자라면 필요에 따라 쉽게 채택할 수 있습니다.

《따라서 청구항 15 발명은 통상의 기술자라면 ‘인용발명1’로부터 또는 ‘인용발명1 및 3’의 단순 결합으로부터 쉽게 발명할 수 있습니다.》

3. 이 출원은 특허청구범위의 청구항 제1항 내지 제4항, 제7항 내지 제15항, 제17항 내지 제19항, 제21항, 제24항의 기재가 아래에 지적한 바와 같이 불비하여 특허법 제42조제4항제2호에 따른 요건을 충족하지 못하므로 특허를 받을 수 없습니다.

- 아 래 -

가. 제1항은 “샘플의 충분한 세포 구성성분을 제거하여”로 기재하고 있으나, “충분한”이 한정하는 범위가 불명확하므로, 제1항 및 제1항을 인용하는 제2항 내지 제4항은 보호받고자하는 청구범위가 명확하고 간결하게 기재되어 있지 않습니다.

나. 제7항 내지 제15항은 청구항의 종결어를 “단리된 ECM”으로 기재하고 있으나, 인용되는 항의 종결어는 “유미목 양서류 ECM”으로 기재되어 있으므로, 청구하고자하는 발명이 무엇인지 불명확하므로, 상기항들은 보호받고자하는 청구범위가 명확하고 간결하게 기재되어 있지 않습니다.

다. 제9항은 “~약물 및/또는 인간 작용제인”으로 기재하고 있으나, “및”이 의미하는 바가 상기 기재된 모든 구성을 다 포함하여 혼합되는 것인지 불명확하며, “인간 작용제인”이 의미하는 바가 무엇인지 불명확하므로, 제9항은 보호받고자하는 청구범위가 명확하고 간결하게 기재되어 있지 않습니다.

라. 제10항은 “선택적으로 인간 세포인” 및 “상기 작용제”로 기재하고 있으나, “선택적으로”는 이중 한정하는 의미를 지녀 구성을 불명확하며, 인용되는 항에는 “작용제”에 대한 기재가 없어 “상기”가 지시하는 대상이 무엇인지 불명확하므로, 제10항은 보호받고자하는 청구범위가 명확하고 간결하게 기재되어 있지 않습니다.

마. 제17항은 “제17항에 있어서”로 제17항과 동일한 항을 인용하고 있어 구성이

불명확하므로, 제17항 및 제17항을 인용하는 제18항 및 제19항은 보호받고자하는 청구범위가 명확하고 간결하게 기재되어 있지 않습니다.

바. 제21항은 “상기 이식물”로 기재하고 있으나, 인용되는 항에는 “이식물”에 대한 기재가 없어 “상기”가 지시하는 대상이 무엇인지 불명확하므로, 제21항은 보호받고자하는 청구범위가 명확하고 간결하게 기재되어 있지 않습니다.

사. 제24항은 청구항의 종결어가 “조직 배양 시스템”이나 인용되는 항의 종결어는 ~ 부착된 재료로 기재되어 있으므로, 청구하고자하는 발명이 무엇인지 불명확하므로, 제24항은 보호받고자하는 청구범위가 명확하고 간결하게 기재되어 있지 않습니다.

※참고사항

- 이 출원의 청구항에는 ECM을 약자로만 기재하고 있으나, ECM의 전체 이름을 한번 기재하는 것이 바람직합니다.

[첨 부]

첨부1 Akira Satoh et al., ZOOLOGICAL SCIENCE(2012), Vol. 29, pp.191-197

첨부2 미국공개특허공보 제2009-0142836호 (2009.06.04.) 1부.

첨부3 미국공개특허공보 제2007-0248638호 (2007.10.25.) 1부. 끝.

2020.06.29.

특허청

화학생명기술심사국

의료기술심사과

심사관 이수희



<< 안내 >>

1. 특허법 제47조제2항에 따라 특허출원서에 최초로 첨부된 명세서 또는 도면에 기재된 사항의 범위 안에서 명세서 또는 도면을 보정할 수 있음을 알려드립니다.
 2. 출원일이 2013년 7월 1일 이후인 출원에 대해서는, 하나의 의견제출통지에 따른 지정기간 내에 보정서를 2회 이상 제출한 경우에는, 마지막 보정 이외의 모든 보정은 취하간주되오니 주의하시기 바랍니다.
 3. 보정료 납부안내
 - o 명세서 또는 도면을 보정하기 위하여 명세서등 보정서를 전자문서로 제출할 경우 매건 4,000원, 서면으로 제출할 경우 매건 14,000원의 보정료를 납부하여야 합니다.
 - o 보정료는 접수번호를 부여받아 이를 납부자번호로 "특허료등의 징수규칙" 별지 제1호서식에 기재하여, 접수번호를 부여받은 날의 다음 날까지 납부하여야 합니다. 다만, 납부일이 공휴일(토요일·휴무일을 포함한다)에 해당하는 경우에는 그날 이후의 첫 번째 근무일까지 납부하여야 합니다.
 - o 보정료는 국고수납은행(대부분의 시중은행)에 납부하거나, 인터넷지로(www.giro.or.kr)로 납부할 수 있습니다. 다만, 보정서를 우편으로 제출하는 경우에는 보정료에 상응하는 통상환을 동봉하여 제출하시면 특허청에서 납부해드립니다.
 4. 지정기간연장 안내 : 연장가능기간(4개월)을 초과하여 지정기간을 연장하고자 소명서를 첨부하여 지정기간연장신청서를 제출한 경우 심사관은 아래의 사유에 해당되는지를 판단하여 지정기간연장의 승인 여부 및 연장할 수 있는 기간을 정하여 통지합니다.
【4개월을 초과하여 지정기간연장을 할 수 있는 사유】
 - ① 기간만료 전 1개월 이내에 최초로 대리인을 선임하거나 선임된 대리인 모두를 해임·변경한 경우
 - ② 기간만료 전 1개월 이내에 출원인변경신청서를 제출한 경우
 - ③ 기간만료 전 2개월 이내에 외국특허청의 심사결과를 받은 경우로서 동 심사결과를 보정서에 반영하고자 하는 경우(이 경우 신청서 제출 시 해당 심사결과 사본 및 그 기초가 된 청구범위 사본도 같이 제출해야 함)
 - ④ 의견제출통지서의 송달이 1개월 이상 지연된 경우(1개월 추가 연장 가능)
 - ⑤ 원출원 또는 분할출원이 심판이나 소송에 계류 중인 경우
 - ⑥ 거절이유와 관련된 시험 및 결과측정에 기간이 더 필요한 경우
 - ⑦ 기타 불가피하게 기간연장이 필요하다고 인정되는 경우단, 제3자가 심사청구한 때에는 ①~⑤의 경우라도 불승인
 5. 온라인 영상면담 및 보정안 리뷰(심층면담) 서비스 안내
 - o 심사관에게 방문면담을 신청할 경우 특허청(대전)에서 출원발명에 관한 상담 서비스를 받을 수 있고, 영상면담을 신청할 경우에는 특허청(대전) 방문 없이도 상담이 가능합니다(영상면담 장소: 특허청 서울사무소와 강원, 경남, 경북, 광주, 부산, 울산, 인천, 전남지역지식재산센터).
 - o 보정안 리뷰를 신청할 경우 심층면담을 통해 거절이유 해소 여부 등 보정안에 대한 보다 체계적인 사전검토 서비스를 받을 수 있습니다(특허로(www.patent.go.kr) - 출원신청 메뉴).
 6. 출원인의 명세서 작성 및 보정에 이해를 돕고자 아래 사이트에서 명세서작성사례 및 기술분야별 보정매뉴얼 등을 제공하고 있습니다. 인터넷 접속이 어려운 경우, 담당 심사관에게 연락주시면 명세서작성사례 등을 우편으로 송부해드리겠습니다.
 - o 특허로(www.patent.go.kr)-명세서작성관련 유용한 Tip
- ※ 「특허로」 고객센터에서 알람서비스를 신청하시면 담당 심사관 지정 알람, 마감기한 알람 등 각종 민원처리 사항을 이메일과 휴대폰(SMS)로 받아 보실 수 있습니다.
- ※ 위에서 안내한 후속 절차 또는 신청 서식 등에 관한 사항은 특허고객상담센터 ☎1544-8080로, 이 통지서 결정 내용에 대한 사항은 특허청 ☎042-481-5128(담당심사관 이수희)로 문의하시기 바랍니다.
- ※ 특허청 및 특허청 직원은 법령에서 정한 법정 수수료 외에 출원인 또는 대리인으로부터 일체의 금품·향응 및 편의제공을 요구하거나 받지 않습니다. 이러한 요구나 사례를 인지 또는 목격하였을 경우, 특허청 홈페이지(<https://www.kipo.go.kr>) 공직비리신고센터(특허청-민원/참여-국민신고-공직비리신고센터)로 신고하여 주시기 바랍니다.
- ※ (특허청) 우 35208 대전광역시 서구 청사로 189, 4동 (둔산동, 정부대전청사)

Electronic Acknowledgement Receipt

EFS ID:	40111864
Application Number:	16801956
International Application Number:	
Confirmation Number:	9621
Title of Invention:	DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE
First Named Inventor/Applicant Name:	Ryanne Early
Customer Number:	35811
Filer:	Sima Singadia Kulkarni/Stephanie De marco
Filer Authorized By:	Sima Singadia Kulkarni
Attorney Docket Number:	SYG-19-1252US-CON2
Receipt Date:	27-JUL-2020
Filing Date:	26-FEB-2020
Time Stamp:	14:38:19
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	SYG_19_1252US_CON2_SIDS. pdf	4627 ac9ad2880704f7fa4c80ed6d55de785d51a df8d7	no	1

Warnings:

Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	SYG_19_1252US_CON2_1449. pdf	135214 f54c63f1de19bc10f9ee09076b3d83909873 235c	no	1
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
3	Patent Term Adjustment Stmt Under 37CFR1.704(d)	SYG_19_1252US_CON2_IDS_C ertification.pdf	258768 d24b554fd84d1591f3d4820ed8cc73b354d d0d61	no	2
Warnings:					
Information:					
4	Non Patent Literature	20200629_KROA.pdf	5847425 e85f0487a0a296b51e58b09d8beab5a6d5d de009	no	20
Warnings:					
Information:					
Total Files Size (in bytes):			6246034		
This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503. <u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application. <u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course. <u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.					

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Art Unit	: TBD	Customer No.	035811
Examiner	: TBD		
Appl. No.	: 16/801,956	Docket No.:	SYG-19-1252US-CON2
Appl. Filed	: February 26, 2020		
First Named Inventor	: Ryanne Early	Confirmation No.:	9621
Title	: DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE	Dated:	July 27, 2020

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

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

Sir:

The Applicant encloses Form PTO-1449 together with a copy of the Notice of Reasons for Refusal from corresponding Korean Patent Application No. 10-2015-7021367 dated June 29, 2020, and its English translation. The document is submitted under 37 CFR §1.56.

Applicant certifies that each item of information contained in the Information Disclosure Statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months ago.

A Patent Term Statement under 37 CFR 1.704(d) is attached herewith.

The Applicant respectfully requests that this Information Disclosure Statement be officially entered into the file and that appropriate notification be made that all the publications were considered by the Examiner.

Respectfully submitted,

/sima singadia kulkarni/

Sima Singadia Kulkarni
Reg. No. 43,732

SSK/sh
(215) 656-2478

PATENT ASSIGNMENT COVER SHEET

Electronic Version v1.1
 Stylesheet Version v1.2

EPAS ID: PAT6338753

SUBMISSION TYPE:	NEW ASSIGNMENT
NATURE OF CONVEYANCE:	ASSIGNMENT
CONVEYING PARTY DATA	
Name	Execution Date
SYNOGEN MANAGEMENT GROUP, INC.	10/05/2020
RECEIVING PARTY DATA	
Name:	JONELLE TOOTHMAN
Street Address:	13709 PROGRESS BLVD., BOX 11
City:	ALACHUA
State/Country:	FLORIDA
Postal Code:	32615
PROPERTY NUMBERS Total: 3	
Property Type	Number
Patent Number:	10617790
Application Number:	16801956
Application Number:	61750555
CORRESPONDENCE DATA	
Fax Number:	
<i>Correspondence will be sent to the e-mail address first; if that is unsuccessful, it will be sent using a fax number, if provided; if that is unsuccessful, it will be sent via US Mail.</i>	
Phone:	352-214-0385
Email:	GERARD@PATENT-ARTS.COM
Correspondent Name:	GERARD BENCEN
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ATTORNEY DOCKET NUMBER:	RE-1US
NAME OF SUBMITTER:	GERARD H. BENCEN
SIGNATURE:	/GERARD H. BENCEN/
DATE SIGNED:	10/07/2020
This document serves as an Oath/Declaration (37 CFR 1.63).	
Total Attachments: 3	
source=SYN_NGNPATENT_ASSIGNMENT_not#page1.tif	
source=SYN_NGNPATENT_ASSIGNMENT_not#page2.tif	
source=SYN_NGNPATENT_ASSIGNMENT_not#page3.tif	

ASSIGNMENT

WHEREAS, Synogen Management Group, Inc., a corporation of Florida, with its principal place of business located at 101 SE 2nd Place, Suite 201 B, Gainesville, Florida 32601 ("Assignor"), is the owner of the entire right, title and interest in and to U.S. Provisional Patent Application No. 61/750,555, PCT International Patent Application No. PCT/US2014/010890, together with the patents and patent applications including but not limited to those listed in Schedule A, and of the invention(s) therein described; and

WHEREAS, NeXtGen Biologics, Inc., a corporation of Delaware, with its principal place of business located at 13709 Progress Blvd, Box 11, Alachua, FL, 32615 ("Assignee"), is desirous of acquiring the entire right, title and interest in U.S. Provisional Patent Application No. 61/750,555, PCT International Patent Application No. PCT/US2014/010890, together with the patents and patent applications including but not limited to those listed in Schedule A, and of the invention(s) therein described;

NOW, THEREFORE, in consideration of One Dollar (\$1.00) and of other good and valuable consideration, receipt of which is acknowledged, the undersigned, intending to be legally bound, does hereby sell, assign and transfer to the Assignee the entire right, title and interest, for the United States of America, its territories and possessions, and for all foreign countries, in said U.S. Provisional Patent Application No. 61/750,555, PCT International Patent Application No. PCT/US2014/010890, all divisions and continuations thereof, all rights to claim priority based thereon, all rights to file foreign applications on said inventions, and all letters patents and reissues thereof, issuing for said inventions and any improvements thereon in the United States of America and in any and all foreign countries including the patents and patent applications listed in Schedule A.

It is agreed that the undersigned shall be legally bound, upon request of the Assignee, or its successors or assigns or a legal representative thereof, to execute all instruments proper to patent the inventions in the United States of America and foreign countries in the name of the Assignee, and to execute all instruments proper to carry out the intent of this instrument.

The rights and property herein conveyed by the undersigned are free and clear of any encumbrance.

[Signature Page Follows]

EXECUTED under seal on October 5, 2020.

Synogen Management Group, Inc.

By: Jamie M. Grooms

Name: Jamie M. Grooms

Title: Director

State of Florida

County of Hillsborough

On October 5, 2020, before me, the undersigned, personally appeared Jamie Grooms, known to me or satisfactorily proven to be the person(s) who executed the within instrument, and who acknowledged herself/himself to be the Director of Synogen Management Group, Inc., and, being authorized to do so, executed the foregoing instrument for the purposes therein contained by signing the name of the said company by herself/himself as such Jamie Grooms.

IN WITNESS WHEREOF, I have hereunto set my hand and official seal.

Jamie Grooms
G 652-453-59-383-0
Exp 10/23/27

Stormy Posey
Notary Public
GG 908716
Exp 9/28/23

(SEAL)



SCHEDULE A

Country	Application Number	Filing Date
Canada	2,897,662	January 9, 2014
China	201780013359.0	January 9, 2014
Europe	14737892.1	January 9, 2014
Hong Kong	16108111.9	January 9, 2014
Israel	239828	January 9, 2014
Japan	2015-552775	January 9, 2014
	2018-224475 (Divisional)	November 30, 2018
Korea	10-2015-7021367	January 9, 2014
United States	61/750,555	January 9, 2013
	14/759,895	July 8, 2018
	16/801956 (Continuation)	April 30, 2019
WIPO	PCT/US2014/010890	January 9, 2014

Form PTO-1449 US DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE LIST OF PUBLICATIONS CITED BY APPLICANT <i>(Use several sheets if necessary)</i>					ATTY. DOCKET NO. SYG-19-1252US-CON2		SERIAL NO. 16/801,956	
					APPLICANT ISE Professional Testing & Consulting Services, Inc.			
					FILING DATE February 26, 2020		GROUP TBD	
US PATENT DOCUMENTS								
EXAMINER INITIAL*		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE	
	AA							
	AB							
	AC							
	AD							
	AE							
	AF							
	AG							
	AH							
	AI							
	AJ							
	AK							
FOREIGN PATENT DOCUMENTS								
		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION	
							YES	NO
	AL							
	AM							
	AN							
	AO							
	AP							
OTHER PUBLICATIONS (Including Author, Title, Date, Pertinent Pages, Etc.)								
	AR	Notice of Reasons of Refusal from counterpart Japanese Patent Appln. No 2018-224475 dated October 13, 2020, and its English translation.						
	AS							
	AT							
EXAMINER					DATE CONSIDERED			
*EXAMINER: Initial if publication considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.								

Notice of Reasons for Refusal

Application number: Japanese Patent Application No. 2018-224475

Date of Drafting: Reiwa 2(2020) October 9

Patent examiner: DATE, Rina 3960 Four B00

Representative/Applicant: KUZUWA, Kiyoshi

Applied Provisions: Article 36

This application should be refused for the reason mentioned below. If the applicant has any opinion(s) against the reason, a written opinion should be submitted within 3 months from the date on which this notification was dispatched.

Reason

1. (Clarity) This patent application does not fulfill the requirement of the provision of Patent Act Article 36(6)(ii) in terms of the item(s) listed below regarding the statement of the scope of claims.

2. (Support Requirement) This patent application does not fulfill the requirement of the provision of Patent Act Article 36(6)(i) in terms of the item(s) listed below regarding the statement of the scope of claims.

Note

- Reason 1

- Claim 1-54

Claim 1 "Maintaining the structural and functional integrity of the tissue sample" and "removing or eliminating the cellular components of the sample sufficient to reduce or eliminate the antigenicity of the biomaterial as a heterologous implant in the mammal in a mammal in a mammal" It is unclear what kind of operation means the retention of integrity and the removal of cellular components. Not defined in the description in the description, and what kind of operation is specifically included even in consideration of common general technical knowledge is unclear. Therefore, the invention according to Claim 1 and the invention according to Claim 2-54 which is dependent on the invention are not clear.

- Claim 1-54

"A Urodela extracellular matrix (ECM) for use in a separated, decellularized mammal" described in Claim 1 "obtains a tissue sample comprising extracellular matrix from a (A) (i) a structural and functional integrity of the tissue sample, and (B) retains the structural and functional integrity of the tissue sample ; and (B) Urodela (ii) is produced by a method involving decellularizing the sample by a method involving removing the cellular component of the sample sufficient to reduce or eliminate the antigenicity of the biomaterial as a xenograft in the mammal, and is characterized by the method for the preparation of the product. ". However, what kind of operation the (A) and (B) refers to is unclear. In the description, [0024] exemplifies various tissues, such as the dermis and the basement membrane, the body wall mesothelium, the cartilage, the tendons, the bones, the dura, and the fascia, as the starting material for the decellularization. [0026] As a technology for decellularizing tissues, freezing, direct pressing, and direct application of pressure Examples illustrate physical treatment such as ultrasonic treatment, stirring, chemical methods such as alkali or acidic treatment, surfactant, organic solvents, hypotonic or hypertonic solutions, chelating agents, and enzymatic approaches including protease digestion and nuclease treatment. It cannot be said that any of the ECM produced by any of the techniques described in [0026] or a combination thereof is of the same physical property, using these various tissues as the starting material. Consequently, even if it is difficult to specify the ECM of the present application by a structure or characteristics not by a manufacturing method, the manufacturing method described in Claim 1 is ambiguous, and thus it is unclear what kind of product manufactured by the manufacturing method is unclear. Therefore, the invention according to Claim 1 and the invention according to Claim 2-54 which is dependent on the invention are not clear.

- Claim 2-54

Claim 2, "Isolated extracellular matrix (EC)" described in Claim 1 M)" is described. However, the invention according to Claim 1 is "a Urodela extracellular matrix (ECM) for use in a separated and decellularized mammal," and the tail ends of Claim 2 and Claim 1 are different from each other. Therefore, it is unclear whether Claim 2 refers to the entirety of Claim 1. The same applies to Claims 3-10 and 15-29. Similarly, Claim 11 describes the "extracellular matrix components derived from Urodela" described in Claim 1, and thus the same deficiency as Claim 2 is present. Claim 12-14 which is dependent on Claim 11 is similar to Claim 1. Claim 30 describes "a sterilized Urodela ECM isolated from sterilization or a sterilized Urodela fraction derived from isolated decellularized Urodela ECM" described in Claim 1, and thus the same deficiency as Claim 2 is present. Claim 31-38 which is dependent on Claim 30 is similar to Claim 1. Claim 39 describes the "isolated decellularized Urodela ECM" described in Claim 1, and thus the same deficiency as Claim 2 is present. Claim 40 which is dependent on Claim 39 is similar to Claim 1. Claim 41 describes the "isolated decellularized Urodela extracellular matrix (ECM)" described in Claim 1, and thus the same deficiency as Claim 2 is present. Claim 42 describes the "isolated decellularized Urodela extracellular matrix (ECM)" described in the claim, and thus the same deficiency as Claim 2 is present. Claim 43-54 which is dependent on Claim 42 is similar to Claim 1. Therefore, the invention according to Claim 2-54 is not clear.

- Claim 5

Claim 5 describes a "human agent" while referring to Claim 4, but Claim 4 does not describe a "human agent." Therefore, the identification in Claim 5 is unclear.

- Claim 11-14

Claim 11 describes "extracellular matrix components derived from Urodela" according to Claim 1, but it is unclear whether the substances mean those obtained by separating only specific components from the ECM according to Claim 1. Claim 12-14 which is dependent on Claim 11 is similar to Claim 1. Therefore, the invention according to Claim 11-14 is not clear.

- Claim 16-18

Claim 16 describes the "tissue implant according to Claim 15," but Claim 15 relates to the invention relating to the "isolated ECM," so that the cited Document 16 is inappropriate in terms of the other Claims. Claims 17 and 18 which are dependent on Claim 16 are similar to Claim 1. Therefore, the invention according to Claim 16-18 is not clear.

- Claim 27

In Claim 27, since expression using "(here, a sheet of the ECM is ... or a combination thereof)" is used, whether or not the matters described in parentheses are optional matters to identify the invention is unclear. Therefore, the invention according to Claim 27 is not clear.

- Claims 28 and 29

In Claim 28, there is described "the isolated ECM according to Claim 1, which includes at least 2 separate cellular Urodela ECM layered with each other." However, since Claim 1 does not describe the ECM "including at least 2 separate cellular Urodela ECM layered with each other," the citation of the other claims in Claim 28 is inappropriate. Claim 29 which is dependent on Claim 28 is similar to Claim 1. Therefore, the inventions according to Claims 28 and 29 are not clear.

- Claims 28 and 29

It is understood that the isolated ECM described in Claim 28 is one in which "at least 2 separate cells laminated with each other" are separately added to the ECM stated in Claim 1. Urodela. In that case, it is unclear how the "at least 2 separate cellular Urodela ECM layered with each other" differs from the ECM described in Claim 1. Claim 29 which is dependent on Claim 28 is similar to Claim 1. Therefore, the inventions according to Claims 28 and 29 are not clear.

- Claims 28 and 29

In Claim 28, "the extracellular matrix component" is described ; however, since there is no description of the "extracellular matrix component," the statement that the

description refers to is unclear. Further, in Claim 28, "the tissue" is described ; however, since there is no statement of the "tissue," it is unclear what the description does not indicate. Claim 29 which is dependent on Claim 28 is similar to Claim 1. Therefore, the inventions according to Claims 28 and 29 are not clear.

- Claim 30-38

In Claim 30, "a sterilized Urodela fraction derived from isolated decellularized Urodela ECM" is described. Since the fraction does not refer to Claim 1, it is unclear whether or not the ECM is an ECM other than the ECM described in Claim 1. When the "sterile Urodela fraction derived from the isolated decellularized Urodela ECM" is an ECM different from the ECM described in Claim 1, it is not specified whether or not the fraction is a fraction obtained by an operation to separate a fraction having any characteristics. Therefore, the invention claimed in Claim 30 is unclear. Claim 31-38 which is dependent on Claim 30 is similar to Claim 1. Therefore, the invention according to Claim 30-38 is not clear.

- Claim 32

In Claim 32, it is described that "the Urodela ECM includes a base membrane.". However, the base membrane is included in the starting material for preparing the ECM, and the expression that the basement membrane itself is included in the ECM is technically inappropriate. Therefore, the invention according to Claim 32 is not clear.

- Claim 37

It is recognized that the term "significance" described in Claim 37 includes an error.

- Claim 41

Claim 41 describes, while referring to Claim 1, an expression specifying "Urodela extracellular skin matrix (ECM)". However, since "Urodela extracellular skin matrix (ECM)" is not described in Claim 1, what specifies what is specified in Claim 41 is unclear. Therefore, the invention according to Claim 41 is not clear.

- Claim 42-54

Claim 42 describes "... (ECM) according to Claim 1 included in the composition.". It is unclear whether the Description means only the ECM parts in the composition, not the composition as a whole. If the above description means only the ECM part in the composition, it is unclear whether the ECM is identical to the ECM described in Claim 1, and how different it is. Claim 43-54 which is dependent on Claim 42 is similar to Claim 1. Therefore, the invention according to Claim 42-54 is not clear.

(Regarding the entirety of Claim 1-54, it should be noted that the terms are coordinated and the citation relationship of the claims is properly organized.)

- Reason 2 (Support Requirement)

- Claim 1-54

In Claim 1, the ECM produced by the method including steps A and B is described, but the specific operation of step A and step B is not specified. In the description, what is disclosed as an ECM suitable as an implant to a mammal is : As step A, it is an ECM produced by a method involving preparation of the dermis as a starting material, and subsequent dehydration with ethanol followed by dehydration with ethanol and drying, as step B, after a plurality of low osmotic pressure and high osmotic pressure dipping using NaCl solution and water. At the time of the filing of the present application, it cannot be said that common general technical knowledge exists in transplanting Urodela derived material into a mammal and preparing an implantable material to be applied to a mammal from the Urodela. Consequently, even if the ECM is produced by arbitrarily selecting various kinds of Urodela tissues and various processing methods exemplified in [0024] and [0026] of the description, it is unclear whether the ECM suitable for transplantation into a mammal can be obtained. Therefore, the matters disclosed in the description cannot be expanded or generalized to the scope of the invention claimed in Claim 1. Claim 2-54 which is dependent on Claim 1 is similar to Claim 1. Hence, the invention according to Claim 1-54 is not described in the detailed description of the invention.

Record of the result of prior art search etc.

- Fields searched :

IPC

C12N 5/00

DB name PubMed

- Prior art documents :

International Publication No. WO 2008/129997

JP 2004-521935A

National Publication of International Patent Application No. 2009 - 513269, The Journal of Experimental Zoology, 1982, Vol.220, pp.243 - 250

JP 2009-510091A

National Publication of International Patent Application No. 2007 - 532153, ZOOLOGICAL SCIENCE, 2012, Vol.29, pp.191 - 197

National Publication of International Patent Application No. 2006 - 507851 The recording of the results of the prior art document does not constitute a reason for refusal.

<Suggestions in amending>

(1) When making amendment to the description or the scope of claims, the amended parts should be underlined so as to be clearly identified (please refer to Note 6 and 7 of Form 13, Regulations under the Patent Act).

(2)The amendment shall be made within the scope of the matters stated in the

translation of the description or drawings (limited to descriptions in the drawings) of the international patent application at the international filing date, the translation of the scope of claims of the international patent application at the international filing date (in the case where a translation of the amended scope of claims under the provisions of Article 19 (1) of the Patent Cooperation Treaty is submitted, the relevant translation), or the drawing of the international patent application at the international filing date (except the descriptions in the drawings) (hereinafter referred to as a translation, etc.) (in the case of having amended the description, scope of claims or the drawings by submitting a mistranslation correction document, the translation, etc. or the amended description, amended claims or amended drawings). In addition, for the evidence of the legality of each amendment, allege the legality of each amendment in a written opinion with clear indication of the corresponding parts in the translation, etc. (in the case of amending the description or drawings by submitting a Correction of Mistranslation, the translation, etc., or the amended description, or the scope of claims, or the drawings).

(3) One should make sure not to perform any corrections that invoke a violation against Patent Act Article 17-2(4) when amending the scope of claims.

If there is an inquiry concerning the content of the notice of reasons for refusal, please refer to the next contact. If it is desired to send an amendment draft or the like by e-mail or FAX, the notice shall be notified by a telephone.

Examination No. 3, BIOENGINEERING Date, Susumu
TEL. 03 to 3581 - 1101 Extensions 3448

A Director General(p.p.), a Director(p.p.), an examiner, an assistant examiner, SAKU, Takashi, DATE, Rina, and 3037 3960

拒絶理由通知書

特許出願の番号	特願 2 0 1 8 - 2 2 4 4 7 5
起案日	令和 2 年 1 0 月 9 日
特許庁審査官	伊達 利奈 3 9 6 0 4 B 0 0
特許出願人代理人	葛和 清司 様
適用条文	第 3 6 条

この出願は、次の理由によって拒絶をすべきものです。これについて意見がありましたら、この通知書の発送の日から 3 か月以内に意見書を提出してください。

理由

1. (明確性) この出願は、特許請求の範囲の記載が下記の点で、特許法第 3 6 条第 6 項第 2 号に規定する要件を満たしていない。
2. (サポート要件) この出願は、特許請求の範囲の記載が下記の点で、特許法第 3 6 条第 6 項第 1 号に規定する要件を満たしていない。

記

●理由 1 について

・請求項 1 - 5 4

請求項 1 に記載されている、「該組織試料の構造的および機能的完全性を保持すること」及び「哺乳動物における異種移植片としての生体材料の抗原性を低減または排除するために十分なように試料の細胞成分を除去すること」とは、どのような操作による完全性の保持及び細胞成分の除去を意味しているのかが不明確である。明細書において上記記載について定義されていないし、技術常識を考慮しても具体的にどのような操作が含まれるのかが不明確である。

したがって、請求項 1 に係る発明及びこれを引用する請求項 2 - 5 4 に係る発明は明確でない。

・請求項 1 - 5 4

請求項 1 に記載されている「分離され、脱細胞化された哺乳動物への使用のための有尾類細胞外マトリックス (ECM)」は、「(A) 有尾類からの、細胞外

マトリックスを含む組織試料を得ること、および（B）（i）該組織試料の構造および機能的完全性を保持すること、および（i i）哺乳動物における異種移植片としての生体材料の抗原性を低減または排除するために十分なように試料の細胞成分を除去すること、を含む方法により前記試料を脱細胞化すること、を含む方法により生産される」という、その物の製造方法によって特定されている。

しかし、上記（A）及び（B）が、どのような操作を意味しているのかが不明確である。

明細書において、〔0024〕には、脱細胞化の出発物質として、真皮及び基底膜、体壁中皮組織、軟骨、腱、骨、硬膜、筋膜等の種々の組織が例示されている。〔0026〕には、組織を脱細胞化するための技術として、凍結、直接加圧、超音波処理、攪拌等の物理的処理、アルカリ又は酸処理、界面活性剤、有機溶剤、低張又は高張溶液、キレート化剤等の化学的方法、及び、プロテアーゼ消化、ヌクレアーゼ処理を含む酵素的アプローチが例示されている。これら種々の組織を出発物質として、〔0026〕に記載されている技術のいずれか又はそれらの組み合わせによって製造されるECMのいずれもが同じ物性のものであるとはいえない。

そうすると、本願のECMを製造方法によらず構造又は特性によって特定することは困難であるとしても、請求項1に記載されている製造方法はあいまいであるため、当該製造方法によって製造される物についてもどのようなものであるのかが不明確となっている。

したがって、請求項1に係る発明及びこれを引用する請求項2－54に係る発明は明確でない。

・請求項2－54

請求項2において、請求項1に記載の「分離された細胞外マトリックス（ECM）」が記載されている。しかし、請求項1に係る発明は「分離され、脱細胞化された哺乳動物への使用のための有尾類細胞外マトリックス（ECM）」であり、請求項2と請求項1の末尾が異なっているため、請求項2は請求項1の全体を引用しているのか否かが不明確となっている。請求項3－10、15－29についても同様である。

同様に、請求項11には、請求項1に記載の「有尾類由来の細胞外マトリックス成分」が記載されているため、請求項2と同じ不備が存在する。請求項11を引用する請求項12－14についても同様である。

請求項30には、請求項1に記載の「滅菌の分離された脱細胞化有尾類ECM、または分離された脱細胞化有尾類ECMに由来する滅菌有尾類画分」が記載されているため、請求項2と同じ不備が存在する。請求項30を引用する請求項31－38についても同様である。

請求項39には、請求項1に記載の「分離された脱細胞化有尾類ECM」が記載されているため、請求項2と同じ不備が存在する。請求項39を引用する請求

項 4 0 についても同様である。

請求項 4 1 には、請求項 1 に記載の、「分離された、脱細胞化された有尾類細胞外マトリックス（E C M）」が記載されているため、請求項 2 と同じ不備が存在する。

請求項 4 2 において、請求項に記載の「分離され、脱細胞化された有尾類細胞外マトリックス（E C M）」が記載されているため、請求項 2 と同じ不備が存在する。請求項 4 2 を引用する請求項 4 3 － 5 4 についても同様である。

したがって、請求項 2 － 5 4 に係る発明は明確でない。

・請求項 5

請求項 5 において、請求項 4 を引用しつつ「ヒト薬剤」が記載されているが、請求項 4 には「ヒト薬剤」が記載されていないため、請求項 5 における特定が何に対するものなのかが不明確となっている。

・請求項 1 1 － 1 4

請求項 1 1 において、請求項 1 に記載の「有尾類由来の細胞外マトリックス成分」が記載されているが、当該物質は、請求項 1 に記載の E C M から特定の成分だけを分離したものを意味しているのか否かが不明である。

請求項 1 1 を引用する請求項 1 2 － 1 4 についても同様である。

したがって、請求項 1 1 － 1 4 に係る発明は明確でない。

・請求項 1 6 － 1 8

請求項 1 6 において、「請求項 1 5 に記載の組織移植片」が記載されているが、請求項 1 5 は「分離された E C M」に係る発明であるから、請求項 1 6 における他の請求項の引用が不適切である。

請求項 1 6 を引用する請求項 1 7、1 8 についても同様である。

したがって、請求項 1 6 － 1 8 に係る発明は明確でない。

・請求項 2 7

請求項 2 7 において、「（ここで該 E C M のシートが、・・・、または結合されている）」と括弧を用いた表現がされているため、括弧内に記載されている事項が任意付加的事項であるのか必須の発明特定事項であるのかが不明確である。

したがって、請求項 2 7 に係る発明は明確でない。

・請求項 2 8、2 9

請求項 2 8 において、「互いに積層された少なくとも 2 枚の分離脱細胞化有尾類 E C M を含む請求項 1 に記載の分離された E C M」が記載されている。しかし、請求項 1 には、「互いに積層された少なくとも 2 枚の分離脱細胞化有尾類 E C M を含む」E C M は記載されていないため、請求項 2 8 における他の請求項の引

用が不適切である。

請求項 28 を引用する請求項 29 についても同様である。

したがって、請求項 28、29 に係る発明は明確でない。

・請求項 28、29

請求項 28 に記載されている分離された E CM は、請求項 1 に記載の E CM に対して、それとは別の物質である「互いに積層された少なくとも 2 枚の分離脱細胞化有尾類 E CM」が別途添加されたものとも解される。

その場合、「互いに積層された少なくとも 2 枚の分離脱細胞化有尾類 E CM」は、請求項 1 に記載の E CM とはどのように異なるのかが不明である。

請求項 28 を引用する請求項 29 についても同様である。

したがって、請求項 28、29 に係る発明は明確でない。

・請求項 28、29

請求項 28 において、「該細胞外マトリックス成分」が記載されているが、それより前に「細胞外マトリックス成分」が記載されていないため、当該記載が何を指しているのかが不明確となっている。

また、請求項 28 において、「該組織」が記載されているが、それより前に「組織」が記載されていないため、当該記載が何を指しているのかが不明確となっている。

請求項 28 を引用する請求項 29 についても同様である。

したがって、請求項 28、29 に係る発明は明確でない。

・請求項 30－38

請求項 30 において、「分離された脱細胞化有尾類 E CM に由来する滅菌有尾類画分」が記載されている。当該画分は請求項 1 を引用していないため、請求項 1 に記載されている E CM とは別の E CM であるのか否かが不明である。

上記「分離された脱細胞化有尾類 E CM に由来する滅菌有尾類画分」が、請求項 1 に記載されている E CM とは別の E CM である場合、どのような特徴を有する画分を分離する操作によって得られた画分であるのかが特定されていないため、請求項 30 に係る発明が不明確となっている。

請求項 30 を引用する請求項 31－38 についても同様である。

したがって、請求項 30－38 に係る発明は明確でない。

・請求項 32

請求項 32 において、「有尾類 E CM が、基底膜を含む」ことが記載されている。しかし、基底膜は E CM を調製するための出発物質に含まれるものであって、基底膜そのものが E CM に含まれるという表現は、技術的に不適切である。

したがって、請求項 32 に係る発明は明確でない。

・請求項 3 7

請求項 3 7に記載されている「有意類」は、誤記を含むものと認められる。

・請求項 4 1

請求項 4 1において、請求項 1 を引用しつつ、「有尾類細胞外皮膚マトリックス (E C M)」を特定する記載がされている。しかし、請求項 1 において「有尾類細胞外皮膚マトリックス (E C M)」は記載されていないため、請求項 4 1 において何を特定しているのかが不明確となっている。

したがって、請求項 4 1 に係る発明は明確でない。

・請求項 4 2 - 5 4

請求項 4 2 において「組成物に含まれる請求項 1 に記載の・・・ (E C M)」が記載されている。当該記載は、組成物全体ではなく、組成物中の E C M 部分のみを意味しているのか否かが不明確である。

上記記載が、組成物中の E C M 部分だけを意味している場合、請求項 1 に記載されている E C M と同じであるのか否か、どのように異なるのかが不明である。

請求項 4 2 を引用する請求項 4 3 - 5 4 についても同様である。

したがって、請求項 4 2 - 5 4 に係る発明は明確でない。

(請求項 1 - 5 4 の全体について、用語を統一し、請求項の引用関係を適切に整理されたい。)

●理由 2 (サポート要件) について

・請求項 1 - 5 4

請求項 1 において、工程 A 及び工程 B を含む方法によって生産される E C M が記載されているが、工程 A 及び工程 B について具体的な操作は特定されていない。

明細書において、哺乳動物への移植片として適する E C M として開示されているものは、工程 A として、真皮を出発物質として用意すること、工程 B として、N a C l 溶液と水を使用した複数回の低浸透圧及び高浸透圧浸漬の後、エタノールによって脱水し、乾燥させること、を含む方法によって生産された E C M である。

本願出願時に、有尾類由来物質を哺乳動物へ移植することや、哺乳動物に適用される移植可能物質を有尾類から調製することについての技術常識が存在したとはいえない。そうすると、明細書の [0 0 2 4] 及び [0 0 2 6] に例示されている、種々の有尾類組織及び種々の処理方法を、任意に選択して E C M を生産し

ても、哺乳動物への移植に適するECMが得られるかは不明である。

そのため、明細書に開示された事項を、請求項1に係る発明の範囲まで拡張ないし一般化することはできない。請求項1を引用する請求項2-54についても同様である。

したがって、請求項1-54に係る発明は、発明の詳細な説明に記載したものではない。

先行技術文献調査結果の記録等

・調査した分野

I P C C 1 2 N 5 / 0 0

D B 名 PubMed

・先行技術文献

国際公開第2008/129997号

特表2004-521935号公報

特表2009-513269号公報

The Journal of Experimental Zoology, 1982, Vol.220, pp.243-250

特表2009-510091号公報

特表2007-532153号公報

ZOOLOGICAL SCIENCE, 2012, Vol.29, pp.191-197

特表2006-507851号公報

この先行技術文献調査結果の記録は、拒絶理由を構成するものではありません。

<補正をする際の注意>

(1) 明細書、特許請求の範囲について補正をする場合は、補正により記載を変更した個所に下線を引いてください(特許法施行規則様式第13備考6、7)。

(2) 補正は、この出願の国際出願日における国際特許出願の明細書若しくは図面(図面の中の説明に限る。)の翻訳文、国際出願日における国際特許出願の請求の範囲の翻訳文(特許協力条約第19条(1)の規定に基づく補正後の請求の範囲の翻訳文が提出された場合にあっては、当該翻訳文)又は国際出願日における国際特許出願の図面(図面の中の説明を除く。)(以下、翻訳文等という。)

(誤訳訂正書を提出して明細書、特許請求の範囲又は図面について補正をした場合にあっては、翻訳文等又は当該補正後の明細書、特許請求の範囲若しくは図面)に記載した事項の範囲内で行わなければなりません。また、意見書で、各補正事項について補正が適法なものである理由を、根拠となる翻訳文等(誤訳訂正書を提出して明細書、特許請求の範囲又は図面について補正をした場合にあっては、翻訳文等又は当該補正後の明細書、特許請求の範囲若しくは図面)の記載箇所

を明確に示したうえで主張してください。

(3) 特許請求の範囲について補正をする際には、特許法第17条の2第4項に違反する補正とならないよう、注意してください。

この拒絶理由通知の内容に関するお問合せがありましたら次の連絡先までご連絡ください。電子メール又はFAXで補正案等の送付を希望される場合は、その旨を電話でお知らせください。

審査第三部生命工学 伊達利奈

TEL. 03-3581-1101 内線3448

Electronic Acknowledgement Receipt

EFS ID:	41107163
Application Number:	16801956
International Application Number:	
Confirmation Number:	9621
Title of Invention:	DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE
First Named Inventor/Applicant Name:	Ryanne Early
Customer Number:	35811
Filer:	Sima Singadia Kulkarni/Stephanie De marco
Filer Authorized By:	Sima Singadia Kulkarni
Attorney Docket Number:	SYG-19-1252US-CON2
Receipt Date:	12-NOV-2020
Filing Date:	26-FEB-2020
Time Stamp:	14:45:50
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	SYG_19_1252US_CON2_SIDS. pdf	97512	no	1
			2cc89034c08bd9394f02da41b3c20cd6163 c6969		

Warnings:

Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	SYG_19_1252US_CON2_1449. pdf	134419 91b8e6e331a5decb3e756d17aa796c82415 aca1b	no	1
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
3	Non Patent Literature	20201013_JPOA.pdf	223881 072f909cec3109c58f19c7fcd798d20ad0442 130	no	13
Warnings:					
Information:					
Total Files Size (in bytes):			455812		
This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503. <u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application. <u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course. <u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.					

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Art Unit	: TBD	Customer No.	035811
Examiner	: TBD		
Appl. No.	: 16/801,956	Docket No.:	SYG-19-1252US-CON2
Appl. Filed	: February 26, 2020		
First Named Inventor	: Ryanne Early	Confirmation No.:	9621
Title	: DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE	Dated:	November 12, 2020

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

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

Sir:

The Applicant encloses Form PTO-1449 together with a copy of the Notice of Reasons for Refusal from corresponding Japanese Patent Application No. 2018-224475 dated October 13, 2020, and its English translation. The document is submitted under 37 CFR §1.56.

Applicant certifies that each item of information contained in the Information Disclosure Statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months ago.

The Applicant respectfully requests that this Information Disclosure Statement be officially entered into the file and that appropriate notification be made that all the publications were considered by the Examiner.

Respectfully submitted,

/sima singadia kulkarni/

Sima Singadia Kulkarni
Reg. No. 43,732

SSK/sh
(215) 656-2478

Form PTO-1449 US DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE LIST OF PUBLICATIONS CITED BY APPLICANT <i>(Use several sheets if necessary)</i>	ATTY. DOCKET NO. SYG-19-1252US-CON2	SERIAL NO. 16/801,956
	APPLICANT ISE Professional Testing & Consulting Services, Inc.	
	FILING DATE February 26, 2020	GROUP TBD

US PATENT DOCUMENTS							
EXAMINER INITIAL*		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE
	AA						
	AB						
	AC						
	AD						
	AE						
	AF						
	AG						
	AH						
	AI						
	AJ						
	AK						

FOREIGN PATENT DOCUMENTS								
		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION	
							YES	NO
	AL							
	AM							
	AN							
	AO							
	AP							

OTHER PUBLICATIONS (Including Author, Title, Date, Pertinent Pages, Etc.)		
	AR	Notice of Decision for Rejection received in corresponding Korean Appln. No. 10-2015-7021367 dated February 19, 2021, and its English translation.
	AS	
	AT	

EXAMINER	DATE CONSIDERED
*EXAMINER: Initial if publication considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.	

**PATENT TERM ADJUSTMENT
STATEMENT UNDER 37 CFR 1.704(d)**

Application Number	16/801,956
Filing Date	February 26, 2020
First Named Inventor	Ryanne Early
Art Unit	TBD
Examiner Name	TBD
Practitioner Docket No.	SYG-19-1252US-CON2

APPLICANT HEREBY STATES THE FOLLOWING (please review 37 CFR 1.704(d) before filing this form):

☐ Each item of information contained in the information disclosure statement was first cited in any communication from a patent office in a counterpart foreign or international application or from the Office, and this communication was not received by any individual designated in 37 CFR 1.56(c) more than thirty days prior to the filing of the information disclosure statement.

AND/OR

☒ Each item of information contained in the information disclosure statement is a communication that was issued by a patent office in a counterpart foreign or international application or by the Office, and this communication was not received by any individual designated in 37 CFR 1.56(c) more than thirty days prior to the filing of the information disclosure statement.

INSTRUCTIONS:

- **This form will not satisfy the requirement of 37 CFR 1.97(e).** The present statement is filed under 37 CFR 1.704(d) and will not substitute for compliance with any of the requirements of 37 CFR 1.97 and 1.98. For an information disclosure statement to comply with 37 CFR 1.97(c) or (d), the information disclosure statement must be accompanied by a statement under 37 CFR 1.97(e) notwithstanding any statement filed under 37 CFR 1.704(d).
- The present form (PTO/SB/133) should be filed concurrently with the information disclosure statement to derive benefit under 37 CFR 1.704(d).

Signature	/sima singadia kulkarni/	Date	March 12, 2021
Typed or Printed Name	Sima Singadia Kulkarni	Practitioner Registration Number	43,732
Note: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required in accordance with 37 CFR 1.33 and 11.18. Please see 37 CFR 1.4(d) for the form of the signature. If necessary, submit multiple forms for more than one signature, see below*.			
☐	*Total of _____ forms are submitted.		

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

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1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
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5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

[Translation]

Issuing No.: 9-5-2021-014520001
Issuing Date: February 19, 2021

Recipient: Eun-sub JUNG
AJU Kim Chang & Lee

Korean Intellectual Property Office Notice of Decision for Rejection

Applicant:

Name: Synogen Management Group, Inc. (Applicant Code: 520210053998)
Address: 101 SE 2nd Place, Suite 201B, Gainesville, FL 32601, U.S.A.

Attorney:

Name: AJU Kim Chang & Lee
Address: 13th-14th Fl. Donghee Building 302, Gangnam-daero Gangnam-gu, Seoul 06253, Republic of Korea

Application No.: 10-2015-7021367

Title of Invention: DECELLULARIZED BIOMATERIAL FORM NON-MAMMALIAN TISSUE

As a result of re-examination of the subject application in light of the Response / Amendment filed on September 29, 2020, we hereby decide to reject this application according to Article 62 of the Korean Patent Act because the reason for rejection issued on June 29, 2020 has not been overcome.

[Re-examination Result]

- ☐ Claims to be examined: Claims 1-33
- ☐ Rejected Claims and Relevant Law supporting the rejections

No.	Rejected Claims	Relevant Law
1	Claims 16-27	Article 29(2) of the Patent Act

- ☐ Patentable Claims: Claims 1-15, 28-33

※ The above-mentioned Patentable Claims are allowed based on the examination result at the time of this notice of decision for rejection report's being issued, and accordingly, they can be changed later. In order to be issued a Notice of Allowance, all of the reasons for rejection pointed out to the subject application should be overcome.

[Reasons of Decision for Rejection]

The invention disclosed in Claims 16 to 27 of the subject application could have been easily conceived of by one of ordinary skill in the art prior to the filing of the subject application for the reasons set forth in the "Details" section below. Thus, pursuant to Article 29(2) of the Patent Act, the subject application cannot be allowed.

- Details -

Cited Reference 1: Akira Satoh et al., ZOOLOGICAL SCIENCE (2012), Vol. 29, pp. 191-197
Cited Reference 2: U.S. Patent Publication Laid-Open No. 2009-0142836 (June 4, 2009)
Cited Reference 3: U.S. Patent Publication Laid-Open No. 2007-0248638 (October 25, 2007)

1. Progress of the subject application

In relation to the subject application, a Notification of Preliminary Rejection was issued on June 29, 2020, on the ground of lack of novelty and inventive step, and unclarity. In response to the Notification of Preliminary Rejection, the applicant filed an amendment and an argument on September 29, 2020.

2. Amendments and the applicant's argument

In the amendment submitted by the applicant on September 29, 2020, in response to the rejection for deficiencies in description, the subject matter of Claims 7 to 15 was amended to be consistent with the subject matter of Claim 6, unclear descriptions in Claims 9 and 10 were clarified, and the dependencies of Claims 17, 21, and 24 were amended to clarify the technical scope of each of the above claims. In addition, in response to the rejection for lack of novelty and inventive step, Claims 5 and 6 were amended to refer to Claim 1 such that the technical scope of each of Claims 5 and 6 was restricted.

Further, in the argument of the same date, the applicant asserted that **one of ordinary skill in the art would not have been motivated to combine the technical constitutions of Cited References 2 and 3 with the technical constitution of Cited Reference 1** because, according to Cited Reference 1, **disrupted components can be retained in the cross-linked ECM as a result of glutaraldehyde treatment of the ECM in the presence of cellular debris.**

3. Review of the applicant's argument

The independent Claims 16, 20, 22, 23, and 25 to 27 have the same technological feature in that they all relate to a decellularized urodele ECM. However, Cited Reference 1 discloses the constitution of taking a tissue sample from the skin and the constitution of removing a nucleus from each of incubated cells, thereby obtaining a cell-free collagen sheet from an axolotl (refer to lines 1 to 5 of the right hand column of page 192 and lines 39 to 42 of the right hand column of page 194). In the argument, **the applicant asserted that, according to Cited Reference 1, an ECM has been fixed with glutaraldehyde, and thus cell debris may remain in the ECM.** However, Cited Reference 1 discloses the constitution of removing remaining nucleus by DNase, and also discloses that no nuclei remain in a sheet dyed with hematoxylinh (refer to lines 36 to 89 of page 194), and thus it is not acknowledged that the ECM disclosed in Claims 16, 20, 22, 23, and 25 to 27 is distinctive from the cell-free ECM disclosed in Cited Reference 1 in terms of technical constitution. In addition, **Cited References 2 and 3 both disclose methods for obtaining an ECM by decellularizing tissues or incubated cells for use in the field of tissue engineering**, and one of ordinary skill in the art could have easily applied the ECM of Cited Reference 1, which belongs to the same technical field as Cited References 2 and 3, to Cited References 2 and 3 as necessary. Moreover, it is not acknowledged that the decellularized urodele ECM leads to a remarkable functional effect of the present invention. Thus, the applicant's argument above cannot be accepted.

4. Conclusion

The subject application has been reexamined on the basis of the argument and the amendment, but the rejections of Claims 16 to 27 under Ground for Rejection 2 in the Notification of Preliminary Rejection have still not been overcome. Accordingly, the present invention has been rejected as a final action.

Dated this 19th of February, 2021

**Korean Intellectual Property Office
Chemistry & Biotechnology Examination Bureau
Medical Technology Examination Division**

<<Information on Appellate Trial against a Decision to Refuse a Patent >>

* Where a person has received a decision to refuse a patent, he/she may file a request of a re-examination with claim amendment or an appeal to the Intellectual Property Trial and Appeal Board without claim amendment within the thirty-day period after the date on which this decision was received. A divisional application can be filed during the same period. Where the description or drawing(s) is amended in a request of re-examination, the amendment must be limited in scope provided under Article 47(2) and (3).

* The period for demanding an appeal against the decision of rejection may extend according to Article 15(1) of Korean Patent Law. If a person wishes to extend the period, please file the Request for Extension of Period before the deadline with IPTAB.

발송번호: 9-5-2021-014520001
발송일자: 2021.02.19.

수신 서울특별시 강남구 강남대로 302, 13, 14층
(역삼동, 동회빌딩)(특허법인아주)
특허법인아주[정은섭] 귀하(귀중)

06253

YOUR INVENTION PARTNER

특 허 청
특허거절결정서

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지정된변리사 정은섭

출 원 번 호 10-2015-7021367
발 명 의 명 칭 비-포유류 조직으로부터의 세포제거된 생체재료

2020.09.29.자 접수된 [명세서등 보정]보정서 및 [거절이유 등 통지에 따른 의견]의견서·
답변서·소명서에 의하여 다시 심사한바 2020.06.29.자 거절이유를 해소하지 못하였으므로
이 출원에 대하여 특허법 제62조에 따라 거절결정합니다.

[다시 심사한 결과]

- ☐ 심사 대상 청구항 : 제1-33항
☐ 이 출원의 거절이유가 해소되지 않은 부분과 관련 법조항

순번	거절이유가 해소되지 않은 부분	관련 법조항
1	청구항 제16항 내지 제27항	특허법 제29조제2항

- ☐ 특허 가능한 청구항 : 제1-15, 28-33항

※ 위 특허 가능한 청구항은 거절결정시점에서의 심사의견이며 추후 변경될 수
있습니다. 이 출원이 특허결정을 받기 위해서는 이 출원에 대해 지적된 거절결정의
이유가 모두 해소되어야 합니다.

[거절결정의 이유]

이 출원의 특허청구범위의 청구항 제16항 내지 제27항에 기재된 발명은 아래와 같이 그 출
원 전에 이 발명이 속하는 기술분야에서 통상의 지식을 가진 자가 용이하게 발명할 수 있는
것이므로 특허법 제29조제2항에 따라 특허를 받을 수 없습니다.

- 아 래 -

인용발명1: Akira Satoh et al., *ZOOLOGICAL SCIENCE*(2012), Vol. 29, pp.191-197

인용발명2: 미국공개특허공보 제2009-0142836호 (2009.06.04.)

1. 심사경과

출원발명에 대해 2020.06.29.자 의견제출통지서를 통해 신규성, 진보성 및 기재불비 거절이유를 통지한 바 있고, 이에 대해 출원인은 2020.09.29자로 보정서와 의견서를 제출하였습니다.

2. 보정서 및 의견서 내용

출원인은 의견제출통지서의 기재불비 거절 이유에 대응하여 제7항 내지 제15항의 종결어를 제6항과 동일하게 보정하고, 제9항 및 제10항의 불명확한 표현과 제17항, 제21항 및 제24항의 인용되는 항을 보정하여 청구범위를 명확하게 보정 하였으며, 신규성 및 진보성 거절 이유에 대응하여 제5항 및 제6항을 제1항을 인용하는 항으로 보정하여 청구범위를 감축하는 보정을 하였습니다.

또한 보정서와 동일자 접수된 의견서에서 ‘인용발명1’의 재료는 글루타르 알데히드 고정으로 세포 파편 성분이 가교된 ECM에 유지될 수 있으며, 이로 인해 ‘인용발명2 및 3’의 구성을 ‘인용발명1’과 조합할 동기가 없다고 주장하고 있습니다.

3. 출원인의 주장 검토

상기 출원인의 주장을 살펴보면, 독립항인 16, 20, 22, 23 및 25 내지 27 발명은 세포제거된 유미목 양서류 ECM을 공통된 특징으로 하고 있으나, ‘인용발명1’에도 피부를 분리한 후 핵을 제거하여 무세포 아홀로틀 콜라겐 시트를 얻는 점(p. 192의 우측컬럼 1-5째 줄, p. 194의 우측컬럼 39-42 참조)이 개시되어 있고, 다만 출원인은 ‘인용발명1’의 ECM은 글루타르 알데히드 고정으로 세포 파편 성분이 ECM에 남아 있다고 주장하고 있으나, ‘인용발명1’에도 남아 있는 핵은 DNase로 제거되며 시트를 헤마톡실린으로 염색했을 때 남아 있는 핵이 없음이 개시되어 있는바(p. 194의 36-89 참조), 상기 청구항에 기재된 구성만으로는 ‘인용발명1’의 무세포 ECM과 출원발명의 ECM이 차이가 있다고 볼만한 점이 없습니다. 또한 ‘인용발명2 및 3’에는 조직 공학 분야에 이용하기 위한 목적으로 조직 또는 배양된 세포를 탈세포화하여 ECM을 얻는 점이 개시되어 있고, 통상의 기술자라면 동일 기술분야의 ‘인용발명1’의 ECM을 ‘인용발명2 및 3’의 구성에 쉽게 적용해 볼 수 있으며, 이로 인해 각별한 작용 효과가 발생한다고 볼 수 없으므로, 상기 출원인의 주장은 받아들일 수 없습니다.

4. 결론

이에 따라, 상기 의견서를 참조하여 다시 심사한 바, 청구항 16 내지 27 발명은

의견제출통지서의 ‘거절이유2’를 여전히 해소하지 못하였으므로, 이 발명을 거절 결정합니다. 끝.

2021.02.19.

특허청

화학생명기술심사국

의료기술심사과

심사관 이수희

이수희

<< 재심사 청구, 거절결정 불복심판 청구 및 분할출원 안내 >>

1. 출원인은 이 거절결정서를 송달받은 날부터 30일 이내(심판청구기간)에 보정을 하여 특허청(출원과)에 재심사를 청구하거나 특허심판원에 거절결정불복심판 청구서를 제출하여 보정 없이 심판을 청구할 수 있습니다. 또한, 심판 청구기간(재심사 청구 이후는 제외)에는 분할출원도 할 수 있습니다. 재심사 청구시에는 특허법 제47조제2항 및 제3항에 따른 범위 내에서 보정하실 수 있습니다.
 2. 심판 청구기간(재심사 청구기간)은 특허법 제15조제1항에 따라 연장할 수 있습니다. 심판 청구기간을 연장하려는 경우에는 심판청구기간 만료일 전까지 법정기간 연장신청서(특허법 시행규칙 별지 제10호 서식)를 특허청장에게 제출해 주시기 바랍니다.
 3. 명세서 등의 보정은 반드시 특허법 시행규칙 별지 제9호서식을 이용해야 합니다.
(재심사 청구 시 반드시 ‘재심사청구 란’에 체크하시기 바랍니다.)
- ※ 위에서 안내한 후속 절차 또는 신청 서식 등에 관한 사항은 특허고객상담센터 ☎1544-8080로, 이 통지서 결정 내용에 대한 사항은 특허청 ☎042-481-5128(담당심사관 이수희)로 문의하시기 바랍니다.
- ※ 특허청 및 특허청 직원은 법령에서 정한 법정 수수료 외에 출원인 또는 대리인으로부터 일체의 금품·향응 및 편의제공을 요구하거나 받지 않습니다. 이러한 요구나 사례를 인지 또는 목격하였을 경우, 특허청 홈페이지(<https://www.kipo.go.kr>) 공직비리신고센터(특허청-민원/참여-국민신고-공직비리신고센터)로 신고하여 주시기 바랍니다.
- ※ (특허청) 우 35208 대전광역시 서구 청사로 189, 4동 (둔산동, 정부대전청사)

Electronic Acknowledgement Receipt

EFS ID:	42172504
Application Number:	16801956
International Application Number:	
Confirmation Number:	9621
Title of Invention:	DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE
First Named Inventor/Applicant Name:	Ryanne Early
Customer Number:	35811
Filer:	Sima Singadia Kulkarni/Stephanie De marco
Filer Authorized By:	Sima Singadia Kulkarni
Attorney Docket Number:	SYG-19-1252US-CON2
Receipt Date:	12-MAR-2021
Filing Date:	26-FEB-2020
Time Stamp:	19:44:11
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	SYG_19_1252US_CON2_SIDS. pdf	97899	no	1
			cad79812c27e496bac7987dbccc5eec5c1879e5e		

Warnings:

Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	SYG_19_1252US_CON2_1449. pdf	134698 8942d6b770f4ce9eae08e40337db00b55056b89	no	1
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
3	Patent Term Adjustment Stmt Under 37CFR1.704(d)	SYG_19_1252US_CON2_IDS_C ert.pdf	258775 fc7d4145d4687f5a0ab02ad2e2b5eb228927d475	no	2
Warnings:					
Information:					
4	Non Patent Literature	20210219_KR_OA.pdf	1638769 7accd7209fb17ca2c8541b62a5191c39dd5dff0b	no	6
Warnings:					
Information:					
Total Files Size (in bytes):			2130141		
This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503. <u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application. <u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course. <u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.					

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Art Unit	: TBD	Customer No.	035811
Examiner	: TBD		
Appl. No.	: 16/801,956	Docket No.:	SYG-19-1252US-CON2
Appl. Filed	: February 26, 2020		
First Named Inventor	: Ryanne Early	Confirmation No.:	9621
Title	: DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE	Dated:	March 12, 2021

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

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

Sir:

The Applicant encloses Form PTO-1449 together with a copy of the Notice of Decision for Rejection received in corresponding Korean Appln. No. 10-2015-7021367 dated February 19, 2021, and its English translation. The document is submitted under 37 CFR §1.56.

Applicant certifies that each item of information contained in the Information Disclosure Statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months ago.

A Patent Term Adjustment Statement under 37 CFR 1.704(d) is attached herewith.

The Applicant respectfully requests that this Information Disclosure Statement be officially entered into the file and that appropriate notification be made that all the publications were considered by the Examiner.

Respectfully submitted,

/sima singadia kulkarni/

Sima Singadia Kulkarni
Reg. No. 43,732

SSK/sh
(215) 656-2478

Form PTO-1449 US DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE				ATTY. DOCKET NO. SYG-19-1252US-CON2		SERIAL NO. 16/801,956	
LIST OF PUBLICATIONS CITED BY APPLICANT (Use several sheets if necessary)				APPLICANT ISE Professional Testing & Consulting Services, Inc.			
				FILING DATE February 26, 2020		GROUP TBD	
US PATENT DOCUMENTS							
EXAMINER INITIAL*		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE
	AA	2005/0013872 A1	01/20/05	Freyman			
	AB	2004/048796 A1 (corr. to KR '247)	03/11/04	Hariri et al.			
	AC						
	AD						
	AE						
	AF						
	AG						
	AH						
	AI						
	AJ						
	AK						
FOREIGN PATENT DOCUMENTS							
		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION
							YES NO
	AL	100999247 B1 (corr. to US '796)	12/07/10	KR			
	AM						
	AN						
	AO						
	AP						
OTHER PUBLICATIONS (Including Author, Title, Date, Pertinent Pages, Etc.)							
	AR	Notice of Allowance received in corresponding Korean Appln. No. 10-2015-7021367 dated May 18, 2021, and its English translation.					
	AS						
	AT						
EXAMINER				DATE CONSIDERED			
*EXAMINER: Initial if publication considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.							

**PATENT TERM ADJUSTMENT
STATEMENT UNDER 37 CFR 1.704(d)**

Application Number	16/801,956
Filing Date	February 26, 2020
First Named Inventor	Ryanne Early
Art Unit	TBD
Examiner Name	TBD
Practitioner Docket No.	SYG-19-1252US-CON2

APPLICANT HEREBY STATES THE FOLLOWING (please review 37 CFR 1.704(d) before filing this form):

☒ Each item of information contained in the information disclosure statement was first cited in any communication from a patent office in a counterpart foreign or international application or from the Office, and this communication was not received by any individual designated in 37 CFR 1.56(c) more than thirty days prior to the filing of the information disclosure statement.

AND/OR

☒ Each item of information contained in the information disclosure statement is a communication that was issued by a patent office in a counterpart foreign or international application or by the Office, and this communication was not received by any individual designated in 37 CFR 1.56(c) more than thirty days prior to the filing of the information disclosure statement.

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Signature	/sima singadia kulkarni/	Date	June 16, 2021
Typed or Printed Name	Sima Singadia Kulkarni	Practitioner Registration Number	43,732
Note: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required in accordance with 37 CFR 1.33 and 11.18. Please see 37 CFR 1.4(d) for the form of the signature. If necessary, submit multiple forms for more than one signature, see below*.			
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전체 청구항 수 : 총 78 항

심사관 : 김정아

(54) 콜라겐 생구조물 및 그 제조방법 및 용도

(57) 요약

본 발명은 양막으로부터 생산된 본 명세서에서는 콜라겐 생구조물(biofabric)로 정의되는 콜라겐 막에 관한 것이다. 본 발명의 콜라겐 생구조물은 천연 비-처리된 양막의 구조적 형태, 즉 천연 3차 또는 4차 구조를 갖는다. 본 발명은 양막을 탈세포화(decellularize)하여 용도막 및 양막을 갖는 태반 막 바람직하게는 인간 태반 막으로부터 콜라겐 생구조물을 제조하는 방법을 제공한다. 바람직한 실시예에서 상기 양막은 완전히 탈세포화된다. 본 발명의 콜라겐 생구조물은 예를 들어 혈관 재생, 혈관의 제조 및 대체, 건 및 인대 대체, 상처-드레싱, 외과적 이식, 안과적 사용, 봉합 및 다른 것들을 포함하는 의학 및 수술 분야에 다양한 용도를 갖는다. 본 생구조물의 잇점은 생역학적 강도, 유연성, 융합성 및 특히 인간 태반으로부터 유래한 경우에 낮은 항원성에 부분적으로 기인한다.

도 4

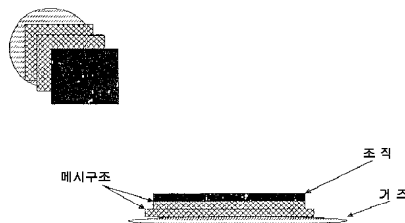


FIG. 4

특허청구의 범위

청구항 1

(a) 융모막으로부터 양막을 분리하는 단계;
 (b) 상기 양막이 효소와 접촉하지 않기 위하여 양막을 탈세포화하는 단계; 및
 (c) 상기 탈세포화된 양막을 탈수하는 단계를
 포함하는 양막과 융모막을 갖는 태반으로부터 콜라겐 생구조물을 제조하는 방법.

청구항 2

제1항에 있어서, 상기 방법은 상기 탈세포화된 양막을 세척하고 건조하는 것을 더욱 포함하는 방법.

청구항 3

제1항에 있어서, 상기 태반은 인간 태반인 것을 특징으로 하는 방법.

청구항 4

제1항에 있어서, 상기 태반은 제왕 절개 분만 또는 자연 분만을 겪은 인간 여성으로부터 온 것을 특징으로 하는 방법.

청구항 5

제1항에 있어서, 상기 방법은 상기 태반 막이 적어도 하나의 전염병에 대한 테스트를 한 공여자로부터 온 것인지 결정하는 단계를 부가적으로 포함하는 것을 특징으로 하는 방법.

청구항 6

제1항에 있어서, 상기 태반은 인간 여성의 태반인 것을 특징으로 하는 방법.

청구항 7

제1항에 있어서, 상기 태반은 출생 후 48시간 내에 공급된 것을 특징으로 하는 방법.

청구항 8

제1항에 있어서, 상기 방법은 (a)단계 후, (b)단계 전에 72시간까지 상기 양막을 저장하는 단계를 부가적으로 포함하는 것을 특징으로 하는 방법.

청구항 9

제8항에 있어서, 상기 저장은 (a)단계에서 얻은 상기 양막을 5일까지 냉장하는 것을 포함하는 방법.

청구항 10

제1항에 있어서, 상기 (b)단계에서 상기 양막의 탈세포화는 상기 양막으로부터 모든 가시적인 세포 물질 및 세포 찌꺼기를 제거하는 것을 포함하는 것을 특징으로 하는 방법.

청구항 11

제1항에 있어서, 상기 (b)단계에서 상기 양막의 탈세포화는 상기 양막의 태아 측과 상기 양막의 산모 쪽 면과 태아 쪽 면에서 모든 가시적인 세포 물질 및 세포 찌꺼기를 제거하는 것을 포함하는 것을 특징으로 하는 방법.

청구항 12

제1항에 있어서, 상기 (b)단계에서 상기 양막의 탈세포화는 상기 막을 물리적으로 벗겨내는 것을 포함하는 것을 특징으로 하는 방법.

청구항 13

제1항에 있어서, 상기 (b)단계에서 상기 양막의 탈세포화는 용액을 갖는 세제로 상기 양막을 탈세포화하는 것을 포함하는 것을 특징으로 하는 방법.

청구항 14

제13항에 있어서, 상기 용액을 갖는 세제는 0.01-1.0% 테옥시콜린산(deoxycholic acid) 나트륨 염 1수화물을 포함하는 용액인 것을 특징으로 하는 방법.

청구항 15

제13항에 있어서, 상기 용액을 갖는 세제는 비이온성 세제, 트리톤 X-100, 음이온성 세제 및 황산도데실나트륨(SDS) 또는 그것의 혼합물로 구성된 군으로 부터 선택된 것을 특징으로 하는 방법.

청구항 16

제12항에 있어서, 상기 물리적 벗겨냄은 세포 스크레이퍼(scraper)를 가지고 벗겨내는 것을 포함하는 방법.

청구항 17

제1항에 있어서, 상기 (b)단계에서 상기 양막의 탈세포화는 살균 용액에서 수행되는 것을 특징으로 하는 방법.

청구항 18

제2항에 있어서, 상기 양막의 세척은 살균 용액에서 수행되는 것을 특징으로 하는 방법.

청구항 19

제2항에 있어서, 상기 탈세포화된 양막의 건조는 35℃에서 50℃에서 수행되는 것을 특징으로 하는 방법.

청구항 20

제1항에 있어서, 상기 방법은 (d) 양막 래미네이트(laminate)를 형성하기 위하여 적어도 두 개의 상기 탈세포화 및 탈수화 양막을 서로 접촉시켜 적층하는 단계를 추가로 포함하는 것을 특징으로 하는 방법.

청구항 21

삭제

청구항 22

삭제

청구항 23

제20항에 있어서, 상기 방법은 적어도 두 개의 상기 양막 래미네이트를 복잡한 3차원 스캐폴드(complex three-dimensional scaffold)로 조립하는 것을 더욱 포함하는 것을 특징으로 하는 방법.

청구항 24

제1항의 방법에 의해 제조된, 양막 내의 콜라겐이 천연 3차 및 4차 구조를 가지며, 양막이 무기질(無基質 substrate-free)인 것을 특징으로 하는 콜라겐 생구조물.

청구항 25

제1항의 방법에 의해 제조된, 양막이 무기질(無基質 substrate-free)이며, 콜라겐, 엘라스틴 및 피브로넥틴을 포함하는 콜라겐 생구조물.

청구항 26

제1항의 방법에 의하여 제조된 콜라겐 생구조물.

청구항 27

제24항에 있어서, 상기 양막은 인간 양막인 것을 특징으로 하는 콜라겐 생구조물.

청구항 28

제24항에서 제26항 중 어느 한 항에 있어서, 상기 생구조물은 10에서 40 마이크론의 두께를 갖는 것을 특징으로 하는 콜라겐 생구조물.

청구항 29

제24항에서 제26항 중 어느 한 항에 있어서, 상기 생구조물은 항생물질, 호르몬, 성장 인자, 항-종양제, 항진균제, 항바이러스제, 통증 치료제, 항히스타민제, 항염증제, 항감염제, 상처 치유제, 상처 봉합제, 세포 유인제 및 스캐폴딩제로 구성된 군으로부터 선택된 하나 이상의 생분자들을 더욱 포함하는 것을 특징으로 하는 콜라겐 생구조물.

청구항 30

삭제

청구항 31

제29항에 있어서, 상기 콜라겐 생구조물은 몰 당 10,000그램 보다 작은 분자량을 가진 펩타이드, 펩타이드 유사체(peptidomimetic), 아미노산, 아미노산 유사체(analog), 폴리뉴클레오티드, 폴리뉴클레오티드 유사체, 뉴클레오티드, 뉴클레오티드 유사체, 유기 또는 무기 화합물 및 이들의 염, 에스터, 및 다른 약학적으로 허용 가능한 형태로 구성된 군으로부터 선택된 하나 이상의 소분자들을 더욱 포함하는 콜라겐 생구조물.

청구항 32

제24항에서 제26항 중 어느 한 항에 있어서, 상기 생구조물은 균일하고 합류 세포(confluent cell) 상태를 이루도록 군집된 세포를 더 포함하는 것을 특징으로 하는 콜라겐 생구조물.

청구항 33

제32항에 있어서, 상기 세포들은 인간 줄기세포 또는 인간 분화된 성인 세포들인 것을 특징으로 하는 콜라겐 생구조물.

청구항 34

제24항에서 제26항 중 어느 한 항에 있어서, 상기 생구조물은 하나 이상의 치료제를 더욱 포함하는 것을 특징으로 하는 콜라겐 생구조물.

청구항 35

제34항에 있어서, 상기 치료제는 호르몬, 펩타이드, 항생제, 항진균제 및 효소로 구성된 군으로부터 선택된 치료제인 것을 특징으로 하는 콜라겐 생구조물.

청구항 36

제24항에서 제26항 중 어느 한 항에 있어서, 상기 생구조물은 하나 이상의 하이드로겔 조성물을 더욱 포함하는 것을 특징으로 하는 콜라겐 생구조물.

청구항 37

제36항에 있어서, 상기 하이드로겔 조성물은 폴리비닐 알콜, 폴리에틸렌 글리콜, 히알루론산(hyaluronic acid), 텍스트란 및 이들의 유도체 또는 유사물질(analog)로 구성된 군으로부터 선택된 고분자를 포함하는 것을 특징으로 하는 콜라겐 생구조물.

청구항 38

제24항에서 제26항 중 어느 한 항의 콜라겐 생구조물을 포함하는 3차원 스캐폴드.

청구항 39

제38항에 있어서, 상기 스캐폴드는 튜브인 것을 특징으로 하는 3차원 스캐폴드.

청구항 40

제38항에 있어서, 상기 스캐폴드는 하나 이상의 하이드로겔 조성물을 더욱 포함하는 것을 특징으로 하는 3차원 스캐폴드.

청구항 41

제40항에 있어서, 상기 하이드로겔 조성물은 폴리비닐 알콜, 폴리에틸렌 글리콜, 히알루론산(hyaluronic acid), 텍스트란 및 이들의 유도체 또는 유사물질(analog)로 구성된 군으로부터 선택된 고분자를 포함하는 것을 특징으로 하는 3차원 스캐폴드.

청구항 42

제20항의 방법에 의하여 제조된 양막 래미네이트.

청구항 43

제24항에서 제26항 중 어느 한 항의 콜라겐 생구조물을 두 층 이상 포함하는 양막 래미네이트.

청구항 44

제24항에서 제26항 중 어느 한 항의 콜라겐 생구조물을 포함하는 양막 래미네이트.

청구항 45

제42항에 있어서, 상기 래미네이트는 하나 이상의 하이드로겔 조성물을 더욱 포함하는 양막 래미네이트.

청구항 46

제45항에 있어서, 상기 하이드로겔 조성물은 폴리비닐 알콜, 폴리에틸렌 글리콜, 히알루론산, 텍스트란 및 이들의 유도체 또는 유사물질(analog)로 구성된 군으로부터 선택된 고분자를 포함하는 것을 특징으로 하는 양막 래미네이트.

청구항 47

제24항에서 제26항 중 어느 한 항의 콜라겐 생구조물을 포함하는 수술 이식물(surgical graft).

청구항 48

수술 대상의 수술 자리에 직접 적용되는 것을 특징으로 하는, 수술 절차(surgical procedure)에서의 이용을 위한 제47항에 따른 수술 이식물.

청구항 49

제48항에 있어서, 상기 수술(surgical) 자리는 눈, 피부, 복부의 장막 표면, 흉강의 장막 표면, 장 심장막, 구강의 점액질 표면, 비강의 점액질 표면, 호흡기관 표면, 위장관 표면 및 비뇨생식기관 표면으로 구성된 군으로부터 선택된 것을 특징으로 하는 수술 이식물.

청구항 50

제48항에 있어서, 상기 수술 이식물은 수술 대상의 몸의 내부 자리에 적용하는 것을 특징으로 하는 수술 이식물.

청구항 51

제48항에 있어서, 상기 수술 이식물은 대상의 몸의 외부 자리에 적용하는 것을 특징으로 하는 수술 이식물.

청구항 52

제48항에 있어서, 상기 대상은 인간인 것을 특징으로 하는 수술 이식물.

청구항 53

개체(subject)의 질환 있는 눈 표면에 위치시켜지는 것을 특징으로 하는, 개체(subject)의 안과 질환 치료 또는 예방용 제24항에서 제26항 중 어느 한 항에 따른 콜라겐 생구조물.

청구항 54

제53항에 있어서, 상기 안과질환은 궤양(ulceration)/천공(perforation), 수포각막병증, 안 유피종(ocular dermoid)/종양, 1차 익상편(primary pterygium), 지속적 각막 상피 결합, 급성 및 만성 알칼리 화상들, 열 화상들, 홍채결여증(aniridia), 아토피성 각막염, 특발성 윤부 줄기 세포 결핍(idipathic limbal stem cell deficiency), 각막 판누스(pannus), 신혈관 신생, 류머티스성 각막 용해, 안 반흔성유착포창(cicatricial pemphigoid), 누수되는 여과 수포(leaking filtering bleb), 노출된 아메드 밸브관(exposed Ahmed valve tube), 차후 검구유착을 갖는 세라시아 봉와직염(Serratia cellulitis with subsequent symblepharon), 급성 및 만성 스티븐슨 존슨(Stevenson Johnson) 증후군으로 구성된 군으로부터 선택된 질환인 것을 특징으로 하는 콜라겐 생구조물.

청구항 55

안과 수술; 심혈관 수술; 치주 수술; 신경학적 수술, 치과수술 및 정형외과 수술로 구성된 군으로부터 선택된 수술 절차에서의 사용을 위한 제24항에서 제26항 중 어느 한 항에 따른 콜라겐 생구조물.

청구항 56

개체(subject)의 비노기적 실금의 치료용 제24항에서 제26항 중 어느 한 항에 따른 콜라겐 생구조물.

청구항 57

개체와 접촉하여 치료제를 전달하는, 치료제 전달용 제24항에서 제26항 중 어느 한 항에 따른 콜라겐 생구조물.

청구항 58

제56항에 있어서, 상기 개체는 인간인 것을 특징으로 하는 콜라겐 생구조물.

청구항 59

제57항에 있어서, 상기 치료제는 항생물질, 항암제, 항세균제, 항바이러스제, 백신, 마취제, 진통제, 항천식제, 항염증제, 항우울제, 항당뇨제, 항정신제, 중추신경계 자극제, 호르몬, 면역 억제제, 근육 이완제 및 프로스타그란딘으로 구성된 군으로부터 선택된 치료제인 것을 특징으로 하는 콜라겐 생구조물.

청구항 60

콜라겐 생구조물의 제조 시간에 콜라겐 생구조물의 수화를 포함하는 제24항에서 제26항 중 어느 한 항의 콜라겐 생구조물을 이용하는 방법.

청구항 61

제60항에 있어서, 상기 생구조물의 수화는 살균된 식염수로 수화하는 것을 포함하는 방법.

청구항 62

제60항에 있어서, 상기 생구조물은 사용 전 적어도 2분간 수화하는 것을 특징으로 하는 방법.

청구항 63

살아 있는 세포를 양막 래미네이트 표면에 증식시키는 단계를 더욱 포함하는 것을 특징으로 하는 양막 래미네이트의 사용 방법.

청구항 64

제63항에 있어서, 상기 살아 있는 세포는 성인 조직 세포 및 줄기 세포로 구성된 군으로부터 선택된 방법.

청구항 65

제64항에 있어서, 상기 줄기 세포는 분화전능한(totipotent) 것을 특징으로 하는 방법.

청구항 66

제64항에 있어서, 상기 줄기 세포는 만능(pluripotent)인 것을 특징으로 하는 방법.

청구항 67

제64항에 있어서, 상기 줄기 세포는 조직 특이적인 것을 특징으로 하는 방법.

청구항 68

피부 질환과 접촉하는 것을 특징으로 하는, 개체 내 피부 질환의 치료 또는 예방용 제24항에서 제26항 중 어느 한 항에 따른 콜라겐 생구조물.

청구항 69

제68항에 있어서, 상기 피부 질환은 피부 손상, 주름, 세선(fine line), 얇은 피부, 감소된 피부 신축성, 거친 피부, 여드름 흉터, 미간 후로스(glabellar furros), 절개 흉터, 연 조직 결함, 선천적 피부 질환, 퇴행성 피부 질환, 콜라겐 VII 결함 및 태양 손상된 피부로 구성된 군으로부터 선택된 질환인 것을 특징으로 하는 콜라겐 생구조물.

청구항 70

제68항에 있어서, 상기 콜라겐 생구조물은 개체에 하나 이상의 치료제를 투여하기에 적합한 것임을 특징으로 하는 콜라겐 생구조물.

청구항 71

제70항에 있어서, 상기 하나 이상의 치료제는 비타민, 미네랄, 카테킨 기반 조제물 및 글루코사민으로 구성된 군으로부터 선택된 것을 특징으로 하는 콜라겐 생구조물.

청구항 72

상처와 접촉하는 것을 특징으로 하는, 개체 내 상처의 치료용 제24항에서 제26항 중 어느 한 항에 따른 콜라겐 생구조물.

청구항 73

제72항에 있어서, 상기 상처는 표피 상처, 피부 상처, 압력(pressure) 궤양, 만성 상처, 급성상처, 외부 상처, 내부 상처, 선천적 상처, 화상 상처, 외과 상처 및 상처 감염으로 이루어진 군으로부터 선택된 상처인 것을 특징으로 하는 콜라겐 생구조물.

청구항 74

화상 부위와 접촉하는 것을 특징으로 하는, 개체 내 화상의 치료용 제24항에서 제26항 중 어느 한 항에 따른 콜라겐 생구조물.

청구항 75

제74항에 있어서, 상기 화상은 1도 화상, 2도 화상, 3도 화상, 감염된 화상 상처 및 화상 상처 농가진으로 이루어진 군으로부터 선택된 것을 특징으로 하는 콜라겐 생구조물.

청구항 76

제68항에 있어서, 상기 개체는 인간인 것을 특징으로 하는 콜라겐 생구조물.

청구항 77

제43항에 있어서, 상기 래미네이트는 하나 이상의 하이드로겔 조성물을 더욱 포함하는 것을 특징으로 하는 양막 래미네이트.

청구항 78

제44항에 있어서, 상기 래미네이트는 하나 이상의 하이드로겔 조성물을 더욱 포함하는 것을 특징으로 하는 양막 래미네이트.

청구항 79

제57항에 있어서, 상기 개체는 인간인 것을 특징으로 하는 콜라겐 생구조물.

청구항 80

제72항에 있어서, 상기 개체는 인간인 것을 특징으로 하는 콜라겐 생구조물.

청구항 81

제74항에 있어서, 상기 개체는 인간인 것을 특징으로 하는 콜라겐 생구조물.

명세서

기술분야

[0001] 본 발명은 양막으로부터 생산된 본 명세서에서는 콜라겐 생구조물(biofabric)로 정의되는 콜라겐 막에 관한 것이다. 본 발명의 콜라겐 생구조물은 천연 비-처리된 양막의 구조적 형태, 즉 천연 3차 또는 4차 구조를 갖는다. 본 발명은 양막을 탈세포화(decellularize)하여 용모막 및 양막을 갖는 태반 막 바람직하게는 인간 태반 막으로부터 콜라겐 생구조물을 제조하는 방법을 제공한다. 바람직한 실시예에서 상기 양막은 완전히 탈세포화된다. 본 발명의 콜라겐 생구조물은 예를 들어 혈관 재생, 혈관의 제조 및 대체, 건 및 인대 대체, 상처-드레싱, 외과적 이식, 안과적 사용, 봉합 및 다른 것들을 포함하는 의학 및 수술 분야에 다양한 용도를 갖는다. 본 생구조물의 잇점은 생역학적 강도, 유연성, 봉합성 및 특히 인간 태반으로부터 유래한 경우에 낮은 항원성에 부분적으로 기인한다.

배경기술

[0002] 2.1 양막: 해부 및 조직학

[0003] 태반 낭(sac)은 느슨한 결합조직에 의해 밀접하게 연결된 두 층으로 구성되어 있다. 그들은 양막 및 용모막 층들로 알려졌다(도1 참조). 양막 층은 가장 안쪽의 두층이고, 태아를 둘러싼 양수와 접촉하고 그들은 함께 양막 낭을 형성한다. 양막 층은 무혈성(avascular)이고 기저막 위에 있는 단층 원주상피세포에 의해 정렬(line)되어 있고 30에서 60 마이크론의 두께를 갖는다. 용모막은 낭의 가장 외부 층이고 심하게 세포화(cellularize)되어 있다. 혈관계(vascular tree)는 태반에서 기원하고 용모막 층을 통하여 태반막으로 확장한다. 용모막 층은 느슨한 결합조직에 의해 양막 층으로부터 분리되고 결합되며, 그 두 층은 120에서 180 마이크론이다. 태반 막들은 무코다당류들(mucopolysaccharides)과 심하게 적재된 콜라겐 매트릭스를 가지고 그들은 태아가 자라기 위한 보호 낭으로 주로 작용하는 것으로 알려졌다. 그 막은 어미 순환에 존재하는 감염원 및 면역원에 대한 장벽을 유지하기도 한다. 태반들은 능동 및 수동 수송 모두를 갖는다. 가장 작은 분자와 단백질들은 그들을 통하여 자유롭게 통과할 수 있으나 IgM과 같은 큰 단백질들은 기저막을 통과할 수 없다.

[0004] 95 % 에틸알콜 또는 조직 배양 배지와 50대 50 % 비율에 섞인 글리세롤에 태반 막들(동물 또는 인간으로부터 온)의 보존은 냉동 전에 양막의 보존을 위하여 활용되었다. 이 보존제들은 태반 조직의 생명을 앗아가기 때문에 핵의 응축(pyknotic) 일으키지만 콜라겐 매트릭스 및 기저막들은 보존된다. 흥미롭게, 보존의 이러한 두 형태들은 이식된 막들의 항원성을 제거하고 또한 잠재적인 독성원을 제거한다. 보존은 종종 양막이 용모로부터 조심스레 분리된 후에 보존은 대체로 달성된다. 기저 막(basal lamina) 및 상피를 갖는 양막 쪽은 광택이 있고(shiny), 용모 반대편은 무디다(dull).

- [0005] 2.2 양막의 전 진료 적용(PREVIOUS CLINICAL APPLICATIONS OF AMNIOTIC MEMBRANES)
- [0006] 동물원성 감염증 병을 운반하거나 교차-종(cross-species) 거부를 일으키는 이종 조직들(다른 종으로부터 온 조직들)을 갖는 가능한 잠재적 문제들은 이들 조직들을 덜 바람직하게 만들었다. 동종 또는 같은 종의 다른 개인들로부터 이식들은 인간 이식 물질에 대한 바람직한 소스로 계속되었다. 이식을 위한 인간의 기증자 조직들의 부족은 조직 이식을 위한 새로운 물질의 개발을 촉진해 온 커가는 문제이다. 아주 종종 생물학적 생(raw) 물질의 공급원은 부족하고 얻기 어렵고 매우 비싸다. 그러나, 인간의 양막으로부터 콜라겐 시트는 조직 이식 적용에 유용한 바람직한 생역학적 특성을 갖는다. 따라서 양막들은 동종 이식 물질에 대한 좋은 공급원이다.
- [0007] 양막 및 용모막을 포함하는 태반 막은 1910년 만큼 이른 시기에 문서화된 수술에 사용되었고 1979년에 Trelford와 Trelford-Sauder에 의하여 재검토 되었다(Trelford와 Trelford-Sauder, 1979, Am. J. Obstet. Gynecol. 833 참조). 1910년에 Davis는 예를 들어 화상 및 궤양 피부에 대한 피부 이식 수술에 수술 재료로서 태아 막들의 사용을 처음 보고하였다(Davis 외, 1910, John Hopkins Med. J; 15 : 307 참조). 이 연구는 주로 동물들에게 있었고 인체 실험들은 기대에 어긋나게 판명 되었다. 그 후 외과수술에 양막들의 사용은 확장되었다(Stern 외, 1913, JAMA, 13: 973-4; Sabella 외, 1913 Med. Records NY, 83: 478-80; de Rotth 외, 1940 Arch. Ophthalmol, 23: 522-5; Sorsby 외, 1946, Br. J.Ophthalmol. 30: 337-45 참조). 그것은 현재 화상 피부, 피부 상처들 및 다리의 만성 궤양에 대한 생물학적 드레싱으로, 인공 질의 외과적 재건에서 부수적 조직으로 및 배꼽 탈장을 보수하는데 사용된다(예를 들어 Trelford 및 Trelford-Sauder, 1979, 134 Am.J. Obstet. Gynecol. 833; Colacho 외, 1974 Arch. Surg. 109: 370-3; Faulk 외, 1980, Lancet, 1156; Prasad 외, 1986, J. Trauma, 26: 945-6; Subrahmanyam 외, 1995, J. Plastic Surg. 48: 477-8; Gruss 외, 1978, J. Can. Med. Assoc. 118: 1237-46; Ward 외, 1984, Br.J. Plastic Surg. 37: 191-3; Dhall, 1984, J. Obstet. Gynaecol. 91: 279-82 참조). 그것은 또한 복부 머리 및 골반의 수술과정에서 조직 결합을 막는데 사용되어 왔다(Gharib 외, 1996, Pediatr. Surg. Int. 11: 96-9; Rennekampff 외, 1994, Invest. Surg. 7: 187-93). 1940 년대에, 몇몇 저자들은 여러가지 는 표면 이상을 치료하는데 상기 양막의 유일한 역할을 발표하였다(예를 들어 de Rotth 외, 1940 Arch. Ophthalmol, 23: 522-5; Sorsby 외, 1946, Br. J.Ophthalmol. 30: 337-45; Lavery 외, 1946, Trans.Ophthalmol. Soc. UK, 66: 668 참조).
- [0008] 이식 수술에서 용도를 위하여 양막의 조제 및 보존을 최적화하려는 상기 분야의 많은 시도들이 이전에 기재되었다(리뷰를 위해선 Dua 외, 1999, Br. J. Ophthalmol. 83: 748-52 ("Dua") 참조). 양막들의 여러가지 조제는 용모막 층에서 양막층의 분리 또는 분리없는 식염수 및 항생물질 혼합물, 알콜 탈수에 의한 보존을 포함하였다. 그러나 Dua 및 상기에서 언급한 참고문헌들에 설명된 모든 방법들은 여전히 양막들의 조제 및 보존에서 개량에 의하여 발표될 필요가 있는 단점을 갖는다. 더 최근에는 각각 상피 결합을 보정하기 위한 조직 이식 수술과정에 적용하기 위하여 양막의 보존에 대한 해동에 의존하는 방법들이 공지되었다. 예를 들어 미국 특허 번호 6,152,142 및 6,326,019 B1("Tseng")을 참조. 트셡(Tseng)은 기질에 올려지고 Dulbecco 변형 이글 배지 및 글리세롤의 혼합물에 보존하고 -80℃에 냉동된 양막이 공지되었다. 그것의 제조동안에 특정 시간에서 조직의 냉동 과정은 트셡 양막을 부서지게하고 심지어는 해동 및 활성화의 단계 후에 더 부서지게한다. 이 외에도 해동 및 활성화 단계는 양막의 취급을 위해 필요한 시간을 증가한다. 게다가 보존 및 제조에서 냉동 단계에 의한 트셡 양막의 부서짐때문에 구조적 지지대 또는 뒷받침(backing)이 저장 동안 트셡 양막의 구조적 보전을 위해 필요하다. 이것은 뒷받침으로부터 보존된 양막을 분리하는 어려움 즉 그것의 부서짐에 기인하여 원형대로 취급하고 분리하기 어려울 수 있는 증가된 어려움을 제공한다. 따라서 뒷받침으로부터 양막의 분리는 막의 파열의 가능성을 증진시키고, 수술 진행에 조제 시간의 증가를 이끄는 수술 과정을 수행하기 전에 냉동된 양막으로 활성화 용액을 흡수되게 하는 양막 활성화에 필요한 시간의 길이를 증가시킨다. 저장 및 선적(shipping)도 -80℃ 냉동에 의하여 복잡화된다. 결국 트셡의 막들은 일반적으로 탈세포화되지 않는다; 결과적으로 그렇게 제조된 양막들은 전형적으로 불투명하고 단일한 구조적 조성물을 가지고 있지 않다.
- [0009] 더 최근에 Yui 등의 미국 특허 번호 5,618,312("312 특허")는 치밀층으로부터 콜라겐 시트의 제조를 설명하였다. 비록 설명된 재료가 주로 콜라겐이지만 그것은 매우 약해서 의학적 사용, 예컨대 봉합(suturable)을 위하여 그것을 충분히 강하게 만들기 위하여 가교(crosslinking)의 분리 단계를 필요로 하는 가공이 필요하다. '312 특허'에서 설명된 가교의 한 방법은 콜라겐의 자연 구조에 역 효과를 주는 것으로 알려진 높은 열처리 바람직하게는 100에서 110℃를 채택한다. Yui의 미국 특허 번호 5,876,451호는 태반으로부터 유도된 유사한 콜라겐 재료를 설명한다. 그러나, 이 재료는 제조과정에서 매트릭스 컴포넌트의 천연 구조의 파괴 및/또는 분열을 야기할 것 같은 탈세포화 단계의 일부로 프로테아제로 처리된다. 따라서 개량된 구조적 특성을 가지고 의학적, 진단적 및 화장품에 이용되는 개량된 양막에 대한 것이 당업계에 필요하다. 본 발명은 천연 콜라겐 4차 구조를 유지하

며 기존의 콜라겐 기초된 생구조물과는 다른 콜라겐을 포함하는 그러한 생구조물을 제공한다. 결과적으로, 본 발명의 생구조물은 용이하게 제조되고, 용이하게 사용되며 의학적 및 수술적 목적에 충분한 강도를 가지며 상처 치료에 대한 뛰어난 기질을 제공한다.

발명의 상세한 설명

3. 발명의 요약

본 발명은 부분적으로 본 발명자들이 태반, 바람직하게는 사람 태반으로부터 콜라겐 생구조물의 제조하는 새로운 방법을 발견한 것에 관련되는데, 이 제조법은 개량된 물리적 및 생물리적 특성들을 갖는 새로운 콜라겐 생구조물을 낳는다. 바람직하게는 이 제조 방법은 양막의 최소한도로 조작한다. 본 발명의 콜라겐 생구조물은, 선행 기술과 달리, 생구조물의 처리 방식 때문에, 본래의 천연 3차 및 4차 구조를 갖는다. 본 발명은 또한 증가된 인장 강도, 불합성 및 감소된 숙주 이식 거부를 야기하는 감소된 면역성의 우수한 특성을 갖는 태반 유래 양막 또는 생구조물을 제공한다. 본 발명은 또한 냉동 또는 저온보존없이 탈수된 시트로 보존할 수 있는 태반 유래 양막 또는 생구조물을 제공한다. 바람직하게는 태반 유래 양막은 인간 환자에게 사용하기 위한 인간태반으로부터 유래한다. 그러나, 동일한 방법은 동물 환자들에 수의학적 사용을 위하여 여러가지 동물 종으로부터 태반들을 사용하여 채택될 수 있다.

본 발명은 콜라겐 생구조물을 제조하는 방법을 제공하는데, 여기서는 태반, 바람직하게는 인간 태반을 제공하는 단계, 용모막 층과 양막 층을 서로서로 분리하는 단계와 양막을 탈세포화되 그 밑에 있는 세포의 매트릭스의 구조를 보존하는 단계를 포함하는 콜라겐 생구조물을 제조하는 방법을 제공한다. 본 발명은 상기 탈세포화된 양막을 세척하고 건조하는 것을 더욱 포함한다. 이 방법은 상온에서 살균 저장 조건하에서 안정할 수 있는 탈수되고 탈세포화된 생구조물을 만들고, 그것은 차후에 재수화되고 대상에 이식된다.

특정 실시예에서 상기 태반은 제왕 절개 분만 또는 자연 분만을 겪은 인간 여성으로부터 온 것이고, 바람직한 실시예에서 상기 태반이 적어도 하나의 전염병에 대한 테스트를 한 공여자로부터 온 것인지를 결정하기 위하여 당업계에 알려진 혈청학적 및 세균학적 분석이 사용된다. 또 다른 실시예에서 상기 태반의 공급원은 공여자의 의학적 역사, 혈액형, 면역 데이터 및 유전자형 특성을 포함하여 알려졌다. 비록 태반은 어떤 포유류에서 올수 있지만 바람직하게는 상기 공여자는 인간 여성이다. 일 실시예에서 상기 태반은 용모막으로부터 양막을 분리하기 전에 당업계에 공지된 일반적인 방법을 사용하여 방혈된다.

본 발명에 따른 양막의 탈세포화는 상기 양막 예를 들어 상기 양막의 태아 측과 산모 측 면에서 모든 가지적인 세포 물질 및 세포저거기를 제거하는 것을 포함한다. 양막의 탈세포화는 양막의 구조적 일체성을 붕괴시키거나 생화학적 조성을 변화시켜서는 안된다. 따라서 본 발명에 따른 양막의 탈세포화는 양막의 제조의 어느 지점에서 냉동 또는 프로테이즈로 막을 접촉하는 것으로 이루어지지 않는다. 바람직하게는 상기 양막은 용액을 갖는 약한 세제 예를 들어 0.01-1.0% 테옥시콜린산(deoxycholic acid) 나트륨 염 1수화물, 비이온성 세제, 트리톤 X-100, 음이온성 세제 및 황산도데실나트륨(sodium dodecyl sulfate) 또는 이들의 혼합물을 포함하는 용액을 사용하여 탈세포화한다.

일단 본 발명의 방법에 따라 양막이 탈세포화되면 상기 막은 세척되고 바람직하게는 진공하에서 낮은 열로 건조될 수 있다.

일 실시예에서 본 발명은 천연 3차 또는 4차 구조를 갖는 막이고, 탈수되고, 탈세포화되고 기질 없는 양막을 포함하는 콜라겐 생구조물을 제공한다. 다른 실시예에서 본 발명은 콜라겐, 에라스틴 및 피브로넥틴을 포함하는 탈세포화되고 기질 없는 양막을 포함하는 콜라겐 생구조물을 제공한다. 또 다른 실시예에서 상기 양막이 결코 프로테이즈와 접촉되지 않는 조건에서 탈수되고, 탈세포화되고 단일하고, 반투명하고 기질 없는 양막을 포함하는 콜라겐 생구조물을 제공한다.

일 실시예에서 콜라겐 생구조물은 하나 이상의 생분자들 예를 들어 항생물질, 호르몬, 성장 인자, 항-종양제, 항진균제, 항바이러스제, 통증 치료제, 항히스타민제, 항염증제, 항감염제, 상처 치유제, 상처 봉합제, 세포 유인제 및 스케폴딩제 및 그 유사체를 포함하나 이에 한정되지 않는 치료제를 더욱 포함한다. 특정 실시예에서 상기 콜라겐 생구조물은 예를 들어 피브로브라스트 성장 인자, 상피 성장 인자 등의 하나 이상의 성장 인자를 포함할 수 있다. 상기 생구조물은 또 특정 생화학적 과정의 특정 저해제 예를 들어 막 수용체 저해제, 카이네이즈 저해제, 성장 저해제, 항암제, 항생제 등과 같은 작은 유기 분자를 포함하나 이에 한정되지 아니하는 하나 이상의 작은 분자들을 포함할 수 있다. 일 실시예에서 콜라겐 생구조물은 그것의 의도된 사용에 따라 수술에 대한 생

산 또는 제조 동안에 한 생 분자를 포함한다.

- [0018] 일 실시예에서 본 발명은 본 발명의 생구조물의 적어도 두층들을 포함하는 래미네이트(laminate) 및 그 제조방법을 포함한다. 다른 실시예에서 본 발명은 시트, 화이버, 구, 튜브를 포함하나 이에 한정되지 아니하는 의도된 사용에 따른 복잡한 3차 스캐폴딩으로 그 래미네이트를 형성하는 것을 포함한다.
- [0019] 일 실시예에서 본 발명은 양막 및 음모막을 포함하는 태반을 제공하고; 음모막으로부터 양막을 분리하고; 양막을 탈세포화하는 것을 포함하는 양막 래미네이트 제조방법을 포함한다. 다른 실시예에서 본 발명은 적어도 한번 상기 탈세포화된 양막을 세척하고 양막 래미네이트를 형성하기 위하여 적어도 두 탈세포화된 양막을 서로 접촉하여 적층하고 탈세포화된 양막 래미네이트를 건조하는 단계를 더욱 포함한다. 대안적으로 다른 실시예에서 양막 래미네이트 제조방법은 본 발명의 방법에 따라 제조된 적어도 두 양막을 건조하고 양막 래미네이트를 형성하기 위하여 적어도 두 탈세포화된 양막을 서로 접촉하여 적층하는 것을 포함한다.
- [0020] 몇몇 실시예에서 본 발명의 방법에 따라 제조된 상기 양막층들은 양막 래미네이트를 형성하기 위하여 접착제 존 재하에서 서로 접촉하여 위치할 수 있다. 본 발명의 조성물 및 방법에 따라 사용된 접착제는 당업자에 공지된 생물학적 접착제 바람직하게는 생체들과 잘 교합하는 접착제 예를 들어 피브로넥틴, 피브린과 같은 천연 접착제, 합성 접착제를 포함하나 이에 한정되지 아니한다. 타 실시예에서 본 발명의 방법에 따라 제조된 상기 양막층들은 양막 래미네이트를 형성하기 위하여 서로 가교(cross-linking)된다. 당업자에게 공지된 가교제 및 방법은 본 발명의 범위에 포함되고 화학 가교, 펩타이드 가교, UV 가교, 방사선 가교, 피브로넥틴 가교, 피브리노겐 가교, 하이드로겔 가교를 포함하나 이에 한정되지 아니한다. 타 실시예에서 본 발명의 방법에 따라 제조된 양막 래미네이트는 접착제를 포함하지 않는다.
- [0021] 일 실시예에서 본 발명은 수술 이식물(surgical graft)로서 콜라겐 생구조물을 포함한다. 특정 실시예에서 본 발명은 수술 자리에 직접 이식체를 위치하는 것을 포함하는 대상 바람직하게는 인간에서 수술 과정에서 수술 이식의 사용을 포함한다.
- [0022] 본 발명은 콜라겐 생구조물, 양막 래미네이트 또는 하나 이상의 하이드로 겔 조성물을 더욱 포함하는 3차원 스캐폴드를 제공하며, 이들을 제조하는 방법을 제공한다. 상기 하이드로 겔 조성물은 폴리비닐 알콜, 폴리에틸렌 글리콜, 히알루론산(hyaluronic acid), 및 그것의 유도체(derivative) 또는 유사체(analog)를 포함하나 이에 한정되지 아니하는 고분자를 포함할 수 있다.
- [0023] 일 실시예에서 본 발명의 콜라겐 생구조물, 양막 래미네이트, 3차원 스캐폴드, 또는 그 하이드로 겔 조성물 상에 세포들이 균일하고 합류 세포(confluent cell) 상태에 있도록 세포를 증식시킬 수 있는데, 이들 세포의 예로는 줄기 세포, 분화된 성인세포, 전구세포 등, 바람직하게는 사람 세포를 들 수 있다.
- [0024] 본 발명은 특히 상업적인 규모의 생산을 위한, 하지만 그에 한정되지 아니하는 콜라겐 생구조물, 상기 생구조물의 래미네이트, 그것의 3차 스캐폴드 및 그 하이드로 겔 조성물의 대규모 생산방법을 제공한다. 본 발명은 임상 시험과 상업적 판매 용도를 위하여 양막을 대규모로 생산하는데 있어서의 어려움을 해결하였다.
- [0025] 본 발명의 콜라겐 생구조물은 약물 운반; 조직 공학; 예를 들어 요실금의 보정과 같은 비뇨기 관련 용도; 눈 표면 이상의 치료와 같은 안과적 사용; 눈 수술 이식; 혈관 재생, 혈관의 신설 및 대체와 같은 혈관적 사용; 감소된 밸브를 세우는 의치(prosthetic) 장치와 같은 심장학적 사용; 예를 들어 손상된 신경 특히 두랄루민 대체 및 신경 교통 주위의 의치로서 잘려진 말초 신경의 재생과 같은 신경 관련 사용; 골 대체물로서 정형학적 치료와 같은 골 관련 사용; 상처(외부 및 내부), 급성 및 만성 상처, 천천적 상처 및 화상의 치료와 같은 피부과적 사용; 피부 결함, 노화된 피부, 주름들, 세선들, 피부 가늘어짐, 감소된 피부 탄력성, 거친 피부, 및 태양 손상 피부와 같은 피부 이상 치료; 상처 드레싱; 및 상처 감염의 치료에 적합하다.
- [0026] 특정한 실시예에서 본 발명은 예를 들어 대상의 질병화된 각막 표면 상에 수술 이식으로 생구조물을 위치하여 본 발명의 콜라겐 생구조물을 사용하는 것을 포함하는, 대상에서 예를 들어 눈 표면 질병과 같은 눈 관련 질환 또는 이상을 치료 및/또는 예방하는 방법을 포함한다. 또 다른 실시예에서 본 발명은 본 발명의 생구조물을 사용하는 것을 포함하는 예를 들어 생구조물로 피부에 접촉하여 대상에서 피부 이상을 치료 및/또는 예방하는 방법을 포함한다. 또 다른 실시예에서 본 발명은 본 발명의 생구조물로 상처 및/또는 화상에 접촉하는 것을 포함하는 대상에서 상처 및/또는 화상을 치료 및/또는 예방하는 방법을 포함한다. 다른 실시예에서 본 발명은 임플란트로 본 발명의 콜라겐 생구조물을 사용하는 것을 포함하는 대상 바람직하게는 인간에서 요실금을 치료하는 방법을 포함한다.
- [0027] 다른 실시예에서 본 발명은 본 발명의 콜라겐 생구조물로 대상에 접촉하는 것을 포함하는 대상에 치료제를 운반

하는 방법을 포함한다. 본 발명은 예를 들어 조직 공학용으로 살아 있는 세포들을 본 발명의 콜라겐 생구조물 또는 래미네이트에서 증식시키는 단계를 포함하는, 대상에 세포들을 운반하는 방법을 또한 망라한다.

[0028] 본 발명은 수술 과정에 사용을 위하여 본 발명의 생구조물을 포함하는 수술 이식을 제공한다. 수술 이식은 대 상 바람직하게는 인간의 내부 또는 외부에 적용할 수 있다.

[0029] 발명의 상세한 설명

[0030] 본 발명은 포유류 바람직하게는 인간의 태반에서 유래한 콜라겐 막 또는 생구조물을 제공한다. 상기 콜라겐 생 구조물은 최종 산물에 천연 콜라겐 구조 즉 천연 3차 및 4차 구조를 유지하도록 제조되었다. 콜라겐 생구조물 외에 본 발명은 또한 콜라겐 생구조물을 제조하는 방법과, 의학적 환경에서 상기 생구조물을 사용하는 방법을 제공한다.

[0031] 본 발명은 천연 3차 및 4차 구조를 갖는 탈수되고 탈세포화된 무기질(無基質 substrate-free) 양막을 포함하는 콜라겐 생구조물을 제공한다. 일 실시예에서 콜라겐 에라스틴 및 피브로넥틴을 포함하는 탈세포화된 무기질 콜라겐 생구조물을 제공한다.

[0032] 몇몇 실시예에서 본 발명은 본 발명의 콜라겐 생구조물을 포함하는 양막 래미네이트를 제공한다.본 발명의 방 법에 따라 제조된 양막 래미네이트는 양막 래미네이트 형성하기 위하여 서로 접촉하여 위치하는 적어도 두 층 의 콜라겐 생구조물을 포함한다. 다른 실시예들에서 본 발명은 본 발명의 콜라겐 생구조물을 포함하는 튜브 같 은 3차 구조 스캐폴드를 제공한다.다른 실시예에서 본 발명은 본 발명의 콜라겐 생구조물, 그것의 래미네이트 또는 하이드로겔 조성물을 더욱 포함하는 그것의 3차 스캐폴드를 제공한다.

[0033] 따라서 본 발명은 래미네이트, 3차 스캐폴드 및 하이드로겔 조성물을 포함하나 이에 한정되지 아니하는 콜라겐 생구조물의 여러 형태 및 형상을 제공한다. 본 발명에서는 본 발명에 따른 조성물의 의학적으로 유용한 모든 형태를 제공한다. 특정한 형태 또는 형상에 관계없이 본 발명의 조성물은 하나 이상의 생분자들 바람직하게는 치료제를 더욱 포함할 수 있다.본 발명의 조성물은 명세서에서 자세하게 기재된 것과 같은 의학 분야에서 여러 유용성을 갖는 생분자를 포함한다. 몇몇 실시예에서 본 발명은 세포들이 균일하고 합류 세포 상태를 유지하도 록 살아있는 세포들을 본 발명의 조성물에 증식시키는 것을 포함한다. 세포들로 군집화된 본 발명의 조성물은 예를 들어 조직 공학 목적을 위한 것과 같이 의료 및 치과 분야에 다양한 유용성을 갖는다.

[0034] 본 발명은 또한 콜라겐 생구조물, 그것의 래미네이트 그것의 3차 스캐폴드 또는 하이드로겔 조성물을 제조하는 방법들에 관한 것이다. 특정한 실시예에서 본 발명은 용모막으로부터 양막을 분리하는 단계; 및 상기 양막이 효 소 예를 들어 프로테이즈와 접촉하지 않도록 양막을 탈세포화하는 단계를 포함하는, 양막과 용모막을 갖는 태반 으로부터 콜라겐 생구조물을 제조하는 방법을 제공한다. 다른 실시예에서 본 방법은 상기 탈세포화된 양막을 세 척하고 건조하는 것을 더욱 포함한다. 또 다른 특정한 실시예에서, 본 발명은 용모막으로부터 양막을 분리하는 단계; 상기 양막을 탈세포화하는 단계; 및 양막 래미네이트(laminate)를 형성하기 위하여 적어도 두 개의 상기 탈세포화된 양막을 서로 접촉하여 적층하는 단계를 포함하는 양막과 용모막을 갖는 태반으로부터 양막 래미네 이트를 제조하는 방법을 제공한다.특정한 실시예에서 상기 탈세포화된 양막은 적층 전에 건조된다.또 다른 특정 한 실시예에서 상기 탈세포화된 양막은 적어도 두 상기 탈세포화된 양막을 서로 접촉하여 적층된 후에 건조된다.

[0035] 본 발명은 의료, 치과 및 수술 세팅에 본 발명의 콜라겐 생구조물, 그것의 래미네이트, 그것의 3차 스캐폴드 또는 그것의 하이드로겔 조성물을 사용하는 방법을 제공한다. 사실상, 본 발명의 조성물들은 당업계에 공지된 다른 생물질에 비하여 증가된 치료 및 진료적 유용성을 갖는다고 기대된다. 몇몇 실시예에서 본 발명은 본 발 명의 조성물을 사용하여 대상에서 눈 관련 질병 또는 이상을 치료 및/또는 예방하는 방법을 제공한다. 특정한 실시예에서 본 발명은 대상의 질환있는 눈 표면에 상기 생구조물을 위치하는 것을 포함하는 대상에서 눈 관련 질병 또는 이상을 치료 및/또는 예방하는 방법을 제공한다.

[0036] 다른 실시예들에서 본 발명은 본 발명의 조성물을 사용하여 대상 바람직하게는 인간의 피부 질환을 치료료 및 /또는 예방하는 방법을 제공한다. 특정한 실시예에서 본 방법은 조성물을 대상의 피부에 접촉하는 것을 포함한 다. 또 다른 특정한 실시예에서, 본 조성물은 피부 질환의 자리인 대상의 피부 표면에 직접 위치된다.본 발명 은 또한 상처 또는 화상의 위치에 본 발명의 조성물을 접촉하여 대상 바람직하게는 인간에서 상처 또는 화상을 치료하는 방법을 제공한다.

[0037] 다른 실시예에서 본 발명은 눈 수술; 심혈관 수술; 치주 수술; 신경학상 수술; 치과 수술 및 정형외과 수술과

같은 수술 과정에 본 발명의 조성물(예를 들어 콜라겐 생구조물, 양막 래미네이트)을 사용하는 것을 제공한다.

[0038] 본 발명은 대상 바람직하게는 인간에 생분자 바람직하게는 치료제를 전달하기 위하여 본 발명의 조성물을 사용하는 방법을 제공한다. 본 발명은 또한 대상 바람직하게는 인간에 본 발명의 조성물을 사용하여 세포들을 전달하는 방법을 제공하는데 여기서 본 발명의 조성물에서는 세포들이 증식하도록 세포를 더 모아놓았다.

[0039] **5.1 콜라겐 생구조물**

[0040] 본 발명은 본 명세서에서 콜라겐 생구조물로 정의되는 콜라겐 양막을 제공한다. 본 발명의 콜라겐 생구조물은 천연, 비처리된 양막의 구조적 형태 유지하는데, 즉 콜라겐, 에라스틴 및 가능하게는 피브로넥틴과 같이 그 조성물 내에 구조 단백질의 3차 및 4차 구조를 유지한다. 이처럼, 본 발명의 콜라겐 생구조물은 천연 또는 비처리된 양막과 같은 구조 단백질로 구성된다. 양막을 생산하는 종래의 방법은 프로테아제나 고열처리의 사용을 요구하는데, 그 결과로서 이러한 막들은 조성물 내에서 구조 단백질의 3차 및 4차구조를 유지하지 않는다.

[0041] 특정 실시예에서, 본 발명은 탈수되고, 탈세포화되며, 기질이 없는(즉, 필터 뒷받침없는) 양막을 제공하는데, 그러한 양막은 천연 3차 및 4차 구조를 갖는다(도 2A 및 B 참조). 본 발명의 탈수되고 탈세포화된 양막은 균일한, 즉 최소한 세포 물질이 없는 반투명한 생구조물로서, 탈세포화된 매트릭스의 좌선성 삼중 나선 알파 나선형 시트를 포함하고 도 3에 나타난 바와 같은 외관을 갖는다 (예를들면, Molecular Biology of the Cell, 1989, Alberts et al., ed., Garland Publishing Inc., New York, NY 참조; 본 명세서에서 본 인용문헌은 참고로서 그대로 포함된다.).

[0042] 본 발명의 콜라겐 생구조물은 예를 들면 말, 소, 돼지나 험비류 원숭이와 같은 어떠한 포유류의 양막으로부터 얻어질 수 있는데, 가장 바람직한 것은 인간의 태반으로부터 얻어지는 것이다. 바람직한 실시예에서, 본 발명의 생구조물은 그 조성물내에 콜라겐 물질의 천연 3차 및 4차구조를 포함한다.

[0043] 본 발명의 콜라겐 생구조물은 종래 기술분야에서 기술된 것과는 대조적으로 최소한으로 가공되는데, 즉, 본 발명의 콜라겐 생구조물은 예를 들면 약한 세제에서 탈세포화되는 것처럼 기껏해야 하나의 화학적 또는 생물학적 처리 또는 가공을 겪게되는 것이다. 본 명세서에서 사용되는 바와 같이, "최소한으로 가공된"이라는 것은 콜라겐 생구조물의 제조 중에 어떠한 단계에서라도 본 발명의 양막이 효소 처리, 예를 들면 프로테아제 처리, 고열 처리, 거친 화학 처리, 강한 세제나 산에 노출이 없는 것을 의미한다. 양막의 프로테아제 처리는 탈세포화 단계의 일부분으로서, 생구조물의 구조적 일체성을 손상하는데, 예를 들면 콜라겐 물질의 3차 및/또는 4차 구조에 영향을 준다. 반면에, 본 발명의 생구조물의 제조에 있어서 양막의 최소 가공은 종래 기술에 비하여 향상된 기계적 강도와 반투명성을 지니는 제품을 낳는다.

[0044] 탈세포화되고 기질이 없는 본 발명의 콜라겐 생구조물은 피브로넥틴 및 에라스틴 뿐만 아니라 콜라겐(콜라겐 타입 I, IV 및 II를 포함하되 이에 한정되지는 않는다)을 포함한다. 이러한 조합은 본 명세서에서 기술하는 방법에 따라 제조된 본 발명의 생구조물이 향상된 기계적 강도를 가지는데 부분적으로 기여한다. 최소 처리 및 가공의 결과로서, 본 발명의 콜라겐 생구조물은 천연 막의 조성물을 유지한다. 콜라겐은 척추동물의 주된 구조 물질이고, 주된 기계적 기능을 하는 조직에 존재한다. 콜라겐 분자는 정상 좌선성 슈퍼나선(superhelix)을 형성하는 세 가닥 로프에서처럼 서로 주위에 짝을 이룬 알파 사슬(각각 약 1000개의 아미노산의 길이)로 둘러싸이는 3개의 폴리펩타이드 사슬로 구성된다. 적어도 19개 타입의 콜라겐이 밝혀졌으며, 그들 모두 동일한 3차 구조를 포함한다. (예를 들면, Lee et al., 2001, International J. of Pharmaceutics, 221: 1-22 참조)

[0045] 본 발명의 양막은 형태, 생역학적 및 구조적 특성이 종래 기술에 보고되고 발명자들에게 알려진 것들보다도 우수한데, 본 명세서에 기재된 바와 같이, 부분적으로 양막과 인간 태반의 조절되고 풍부한 근원의 신규한 제조방법의 발견에 근거된다(5.2절 참조).

[0046] 특히, 본 발명의 콜라겐 생구조물은 종래의 하나 또는 그 이상의 양막과 비교하여 다음과 같이 하나 또는 그 이상의 특징을 갖는다: 향상된 인장강도; 우수한 융합성; 숙주-이식(host-graft) 거부반응의 감소를 초래하는 감소된 항원성; 냉동이나 저온보존 필요 없는 저장과 운송의 용이성; 사용 전에 취급과 재수화와 같은 활성화 공정을 위한 최소한의 후조제(post-preparation) 요구; 및 구조적, 기능적 형태를 유지하면서 장기간 상온에서 저장될 수 있는 능력이다. 표 1은 종래 기술에 비하여 본 발명의 생구조물의 잇점을 정리한 것이다.

[0047] 대안적인 실시예에서, 본 발명은 탈수되고, 탈세포화되고, 기질이 없는 용모막, 바람직하게는 인간 용모막을 포함하는 콜라겐 생구조물을 제공한다. 용모막을 포함하는 콜라겐 생구조물은 양막을 포함하는 본 발명의 콜라겐 생구조물과 유사한 특성을 갖을 것으로 기대된다. 본 발명은 모두 래미네이트, 3차 스캐폴드 및 하이드로겔 조성물을 포함하되 이에 한정되지는 않는 용모막을 포함하는 콜라겐 생구조물의 모든 의학적으로 유용한 형태를

제공한다.

표 1

[0048] 본 발명의 생구조물 대 종래의 양막 제제

특징	본 발명의 콜라겐 생구조물	종래의 양막
혈청학적 측정	완전함: 항체전별(ATY); 알라닌 아미노 전이 효소 선별(ALT); 간염 핵심항체(핵산 및 ELISA); B형간염 표면항원(핵산 및 ELISA); C형간염 바이러스항체(핵산 및 ELISA); HIV-1 및 HIV-2; HTLV-1 및 HTLV-2; 매독 테스트(RPR); CMV항체 테스트; C형간염 및 HIV 테스트(핵산테스트)	불완전함: CMV 테스트 없음.
저장	건조: 저열 진공으로 탈수; 냉동이나 냉장 필요 없음; 선반 보관	냉동: 배양 배지에 보관; 드라이 아이스 선적 및 냉동기/냉장기 필요
수술적 제조	수 분: 수분내 제조; 외과적 자리에서 직접 수화; 예를 들면 식염수 방울로 눈에서; 해동, 적시기 또는 린스 필요 없음.	길다: 장황한 제조/해동/적심시간이 요구됨; 반드시 필터 뒷받침(backing)을 제거해야 함
수술적 취급	간단: 건조 중 흔들어 줄 수 있고; 외과적 자리에 도포되고 난 다음 그 자리에서 수화됨.	장황함: 필터 페이퍼로부터 반드시 제거되어야 함; 외과수술 동안 둥글게 말리는 경향 있음(ball up); 외과적으로 가공하기 어려움
세포충실성	탈세포화: 최소한으로 경피세포가 없음; 최소한으로 세포 찢겨기가 없음; 더 빨라진 재형성	사세포(dead cell)와 함께: 사세포가 존재; 임상적 증거가 긴 치료시간/높은 거부율을 나타낸다(일화적 증거)
기질	없음: 기질이 없음(예를 들면 필터 뒷받침)	있음: 니트로셀룰로오스/필터 페이퍼에 지지됨.
살균(선택적)	있음: 전자선 조사; 적어도 18kGy; 조직 안전성의 증가된 보증	없음: 항생제 및 글리세롤(유독성)을 포함하는 배지에 저장; 공식적인 최종적인 살균 없음
조직 투명도	반투명: 광학적으로 맑음	불투명: 우유빛 또는 탁한 외관

[0049] 바람직한 실시예에서, 본 발명의 콜라겐 생구조물은 반투명, 즉 광학적으로 맑다. 다른 바람직한 실시예에서, 본 발명의 콜라겐 생구조물은 옅고 경량이다. 특정 실시예에서, 본 발명의 탈수된 콜라겐 생구조물은 $0.3\text{--}0.6\text{mg/cm}^2$ 이다. 특정 실시예에서, 본 발명의 콜라겐 생구조물은 적어도 두께가 30 마이크론이다. 또 다른 실시예에서, 본 발명의 콜라겐 생구조물은 두께가 약 10 - 40 마이크론이다.

[0050] 본 발명은 다양한 형태, 예를 들면 삽입, 차폐에서 본 발명의 콜라겐 생구조물의 용도를 포함한다. 일반적으로, 생구조물은 어떠한 의학적으로 유용한 형태로도 형성될 수 있다. 예를 들면, 콜라겐 생구조물은 그대로 사용을 위하여, 즉, 전체 대반으로부터 콜라겐 생구조물의 사용을 위하여 형성될 수 있다. 대안적으로, 콜라겐 생구조물은 띠, 패치 또는 롤로 절단되거나 또는 실로 짜여질 수 있다. 다른 실시예에서, 단일-두께 시트 또는 래미네이트로서 콜라겐 생구조물은 일정한 간격으로 잘릴 수 있고, 예를 들면 메쉬를 형성하기 위하여 확장될 수도 있다.

[0051] 일부 실시예에서, 콜라겐 생구조물은 평평한데, 예를 들면 기울기나 굴곡이 없는 표면을 갖는다. 다른 실시예에서, 본 발명의 콜라겐 생구조물은 완전히 평평하지는 않고, 보조개가 생긴 표면을 갖는데, 예를 들면 표면이 톱하 또는 함입된 것이다.

[0052] 특정의 바람직한 실시예에서, 본 발명은 적어도 본 발명의 콜라겐 생구조물의 두 층을 포함하는 래미네이트를 포함한다. 층들은 물리적으로 함께 결합될 수 있는데, 예를 들면 접착제, 패스너의 사용으로, 또는 상기 층들을 융합시키기 위해서 생구조물의 일부분, 예를 들면 적어도 상기 층들 중 어느 하나의 주변을 열-스탬핑함으로써 될 수 있다. 생구조물은 "그레인"을 가지고 있기 때문에, 상기 생구조물은 다양한 방법으로 래미네이트될 수 있다. 하나의 실시예에서, 래미네이트의 모든 층들은 동일한 그레인 배치를 갖는다. 다른 실시예에서, 층들 중 적어도 하나는 적어도 다른 하나의 층의 그레인 배치에 대하여 90도 회전된(즉, 거의 수직인) 그레인 배치이다. 더욱 특정된 실시예에서, 상기 수직 층은 서로 인접한다. 또 다른 실시예에서, 상기 래미네이트는 적어도 3층의 콜라겐 생구조물을 포함하는데, 전술한 적어도 3층의 각각의 그레인 구조는 두개의 나머지 층의 그레인 구조에

대해 약 60도 정도 회전되어 있다. 다른 실시예에서, 상기 래미네이트는 적어도 첫 번째 물질과 두 번째 물질을 포함하는데, 여기에서 전술한 첫 번째 물질은 본 발명의 콜라겐 생구조물이고, 전술한 두 번째 물질은 콜라겐 생구조물과 함께 래미네이트를 형성할 수 있는 어떠한 다른 성분이다. 특정 실시예에서, 전술한 두 번째 물질은 양막 이외의 막으로부터 형성된 시트 또는 막이다. 다른 특정 실시예에서, 전술한 두 번째 물질은 비천연 물질인데, 예를 들면 폴리락톤, 폴리아세테이크, 플라스틱 필름 등이다. 일부 실시예에서, 양막 래미네이트는 다층인데, 적어도 6, 적어도 8, 적어도 10, 적어도 20, 적어도 80, 적어도 100, 적어도 1000개의 본 발명의 방법에 따라서 제조된 양막을 포함한다. 본 발명의 양막 래미네이트는 제한없는 층 수를 포함할 수 있다.

[0053] 래미네이트는 래미네이트를 복잡한 3차 구조로 형성시키게 하는 증가된 구조적 경직성을 갖는다. 그러한 3차 구조는 시트, 튜브, 중심체를 포함할 수 있다.

[0054] 콜라겐 생구조물은 또한 단일 시트 또는 래미네이트로서 다른 물질과 결합될 수 있다. 예를 들면, 생구조물은 연성 플라스틱 필름, 거즈, 플라스틱 시팅, 부목(stent), 밸브, 정형외과 장치, 봉대, 패치 등에 결합, 즉 묶일 수 있다.

[0055] 콜라겐 생구조물은 생구조물의 콜라겐 매트릭스의 일부분이 아닌 하나 또는 그 이상의 화합물이나 성분들을 포함할 수 있다. 예를 들면, 콜라겐 생구조물은 제조 과정 중 또는 외과 수술 준비 중에 생분자를 도입할 수 있다. 그러한 생분자는 항생제(클린다마이신, 미노사이클린, 독시사이클린, 겐타마이신과 같은), 호르몬, 성장인자, 항종양제, 항진균제, 항바이러스제, 통증치료제, 항히스타민제, 항염증제, 은(질산은과 실파디아진 은을 포함하되 이에 한정되지는 않는 은 염과 같은)을 포함하되 이에 한정되지는 않는 항감염제, 원소성 은(elemental silver), 항생제, 살균효소(bactericidal enzymes) (리소좀과 같은), 상처 치유제 (PDGF, TGF를 포함하되 이에 한정되지는 않는 시토킨; 티모신과 같은), 상처 치유제로서 히알루론산, 상처 봉합제(트롬빈을 포함 또는 불포함하는 피브리노겐과 같은), 세포 유인제 및 스캐폴딩제(피브로넥틴과 같은) 등을 포함하는데, 이에 한정되는 것은 아니다. 특정 예에서, 콜라겐 생구조물은 적어도 하나의 성장 인자를 포함하는데, 예를 들면 섬유모세포 성장인자, 상피 성장인자 등이 있다. 생구조물은 또한 특별한 생화학적 과정의 특정 억제제, 예를 들면 막 수용체 억제제, 키나아제 억제제, 성장 억제제, 항암제, 항생제 등과 같은 작은 유기 분자를 포함할 수 있다.

[0056] 또 다른 실시예에서, 본 발명의 콜라겐 생구조물은 하이드로겔과 결합될 수 있다. 본 발명이 속하는 기술분야에서 당업자에게 알려진 어떠한 하이드로겔 조성물이라도 본 발명 내에서 포함될 수 있는데, 예를 들면 다음 문헌에 개시된 하이드로겔 조성물 중 하나이다: Graham, 1998, Med. Device Technol. 9 (1) : 18-22; Peppas et al., 2000, Eur. J. Pharm. Biopharm. 50 (1) : 27-46; Nguyen et al., 2002, Biomaterials, 23 (22): 4307-14; Henin et al., 2002, Adv. Drug Deliv. Rev. 54 (1) : 13-36; Skelhorne et al., 2002, Med. Device Technol. 13 (9): 19-23; Schmedlen et al., 2002, Biomaterials 23: 4325-32; 상기 모든 문헌은 그대로 본 명세서에 참고문헌으로서 포함된다. 특정 실시예에서, 하이드로겔 조성물은 콜라겐 생구조물에 적용되는데, 다시 말하면 콜라겐 생구조물의 표면에 내려진다. 예를 들면, 하이드로겔 조성물은 콜라겐 생구조물 위에 뿌려지고, 생구조물의 표면에 흡착되고, 콜라겐 생구조물에 적셔지고, 콜라겐 생구조물과 담겨지거나 콜라겐 생구조물의 표면에 코팅되어질 수 있다.

[0057] 본 발명의 방법과 조성물에 유용한 하이드로겔은 당업계에 알려진 어떠한 물과 상호작용하는 또는 수용성 고분자로부터 만들어질 수 있는데, 폴리비닐알콜(PVA), 폴리히드록시에틸 메타크릴레이트, 폴리에틸렌 글리콜, 폴리비닐 피롤리돈, 히아루론산, 텍스트린 또는 이들의 유도체와 유사물들을 포함하되 이에 한정되는 것은 아니다.

[0058] 일부 실시예에서, 본 발명의 콜라겐 생구조물은 하이드로겔과의 결합 이전에 하나 또는 그 이상의 생분자를 더 포함한다. 다른 실시예에서, 하이드로겔 조성물은 본 발명의 콜라겐 생구조물과 결합하기 이전에 하나 또는 그 이상의 생분자를 더 포함한다. 그러한 생분자들은 항생제(클린다마이신, 미노사이클린, 독시사이클린, 겐타마이신과 같은), 호르몬, 성장인자, 항종양제, 항진균제, 항바이러스제, 통증치료제, 항히스타민제, 항염증제, 은(질산은과 실파디아진 은을 포함하되 이에 한정되지는 않는 은 염과 같은)을 포함하되 이에 한정되지는 않는 항감염제, 원소성 은(elemental silver), 항생제, 살균효소(bactericidal enzymes) (리소좀과 같은), 상처 치유제 (PDGF, TGF를 포함하되 이에 한정되지는 않는 시토킨; 티모신과 같은), 상처 치유제로서 히알루론산, 상처 봉합제 (트롬빈을 포함 또는 불포함하는 피브리노겐과 같은), 세포 유인제 및 스캐폴딩제 (피브로넥틴과 같은) 등을 포함하는데, 이에 한정되는 것은 아니다. 특정 예에서, 콜라겐 생구조물 또는 하이드로겔 조성물은 적어도 하나의 성장 인자를 포함할 수 있는데, 예를 들면 섬유모세포 성장인자, 상피 성장인자 등이 있다. 바람직하게, 생

분자는 치료제이다.

- [0059] 일부 실시예에서, 하이드로겔 조성물은 본 발명의 생구조물을 포함하는 래미네이트와 결합된다.
- [0060] 하이드로겔/콜라겐 생구조물 조성물은 의학분야에서 이용되는데, 상처, 화상, 피부 컨디션(예를 들면 흉터의 치료)의 치료, 성형용(예를 들면 성형수술) 및 이식물로서의 어떠한 용도라도 포함되되 이에 한정되지는 않는다. 일부 실시예에서, 하이드로겔/콜라겐 생구조물 조성물은 대상물에 국소적으로, 즉 피부의 표면에 도포되는데, 예를 들면 상처의 치료를 위함이다. 다른 실시예에서, 하이드로겔/콜라겐 생구조물 조성물은 대상물의 내부에서 사용될 수 있는데, 예를 들면 이식물로서 신체 내에서 영구적 또는 반영구적 구조가 된다. 일부 실시예에서, 하이드로겔 조성물은 생분해되지 않도록 조제된다. 또 다른 실시예에서, 하이드로겔 조성물은 생분해되도록 조제된다. 특정 실시예에서, 하이드로겔 조성물은 수일 내에 분해되도록 조제된다. 다른 특정 실시예에서, 하이드로겔 조성물은 수 개월내에 분해되도록 조제된다.
- [0061] 일부 실시예에서, 본 발명의 콜라겐 생구조물에는 단일하고 합류 세포 상태가 되도록 세포가 증식된다(populated). 본 발명의 생구조물에서 세포를 증식시키는데 사용될 수 있는 세포들은 줄기세포, 바람직하게는 인간 줄기세포, 인간 분화된 성인세포, 분화 전능성의 줄기세포(totipotent stem cells), 만능성 줄기세포(pluripotent stem cells), 다기능성(multipotent) 줄기세포, 조직 특이 줄기세포(tissue specific stem cells), 태아성(embryonic like) 줄기세포, 수임 전구세포(committed progenitor cells), 피브로블라스토이드(fibroblastoid) 세포를 포함하되 이에 한정되는 것은 아니다. 다른 실시예에서, 본 발명은 본 발명의 생구조물을 콘드로사이트, 간세포, 조혈세포, 췌장 선세포 조직세포(pancreatic parenchymal cells), 신경모세포 및 근육 전구 세포를 포함하되 이에 제한되지는 않는 전구 세포(progenitor cell)의 특정 계층으로 군집시키는 것을 포함한다.
- [0062] **5.2 콜라겐 생구조물의 제조과정**
- [0063] 본 발명은 본 발명의 콜라겐 생구조물을 제조하는 방법을 제공한다.
- [0064] 특히, 본 발명은 양막과 음모막을 포함하는 태반을 제공하고; 음모막으로부터 양막을 분리하고; 양막을 탈세포화하는 것을 포함하는 콜라겐 생구조물을 제조하는 방법을 포함한다. 특정 실시예에서, 본 방법은 탈세포화된 양막을 세척하고 건조하는 것을 더 수반한다.
- [0065] 바람직하게, 상기 태반은 인간 대상물에서 사용하기 위하여 인간 태반으로부터 기인한 것이다. 일부 실시예에서, 본 발명은 인간 대상물에서 사용하기 위하여 동물 종으로부터 기인한 태반을 포함한다. 다른 실시예에서, 본 발명은 동물 대상물에서 수의학적 사용을 위하여 동물 종으로부터 기인한 태반을 포함한다.
- [0066] 바람직한 실시예에서, 본 발명의 방법에서 사용을 위한 태반은 신생아 분만 후에 가능한한 빨리 취해진 것이다. 또 다른 바람직한 실시예에서, 상기 태반은 정상적인 건강한 유아의 제왕절개 분만 후에 즉시 취해진 것이다. 제공된 태반은 무균 조건 하에서 수집된다. 일부 실시예에서, 태반은 어떤 추가적인 처리에 앞서 분만 시간으로부터 48시간동안 저장된다. 다른 실시예에서, 태반은 어떤 추가적인 처리에 앞서 분만 시간으로부터 5일까지 저장된다.
- [0067] 바람직하게, 태반, 탯줄 및 탯줄 혈액은 추가적인 처리를 위해서 분만 또는 출산실로부터 다른 장소, 예를 들면 실험실로 이동된다. 바람직하게, 태반은 살균 가방 또는 컨테이너와 같은 살균된 운송 장치로 수송되는데, 선택적으로는 단열된 것이다. 일부 실시예에서, 태반은 다음 처리가 있을 때까지 상온에 보관된다. 다른 실시예에서, 태반은 다음 처리가 있을 때까지 냉장되는데, 즉, 2 내지 8℃에서 보관된다. 또 다른 실시예에서, 태반은 다음 처리가 있을 때까지 살균 조건하에서 5일까지 보관된다. 가장 바람직한 실시예에서, 태반은 당업자에게 알려진 바와 같이 무균 조건 하에서 다루어지고 처리된다. 바람직하게는 상기 실험실은 HEPA 여과 시스템(청정실 분류법에 의한 정의에 따라, 클래스 1000을 갖추거나 또는 그보다 나은)을 갖춘 것이 바람직하다. 바람직한 실시예에서, HEPA 여과 시스템은 본 발명의 방법을 실시하기 위하여 실험실을 사용하기 전 적어도 1시간 작동시킨다.
- [0068] 특정 실시예에서, 태반은 방혈되는데, 즉, 출생 후 남아있는 탯줄 혈액을 완전히 빼낸다. 일부 실시예에서, 상기 태반은 70% 방혈, 80% 방혈, 90% 방혈, 95% 방혈, 99% 방혈된다.
- [0069] 본 발명은 HIV, HBV, HCV, HTLV, 매독, CMV 및 태반 조직을 오염시킨다고 알려진 다른 바이러스성 병원균들을 포함하되 이에 한정되지는 않는 전염병에 대해서 당업자에게 알려진 표준 기법을 이용하여 출산 시간 전에 임신 중인 모체를 선별하는 것을 포함한다. 바람직하게, 전염병에 대해서 선별하는데 사용되는 방법은 연방 의약국

(Federal Drug Administration)에 의해 설명된 바와 같은 규정을 따른다. 임신중인 모체는 출산 한달 이내에, 바람직하게는 출산 두주 이내에, 보다 바람직하게는 출산 한주 이내에, 가장 바람직하게는 출산시에 선별될 수 있다(예를 들면, 진단 목적으로 혈액 샘플을 채취한다). 진술한 병원체들에 대해 음성 또는 비반응성인 모체의 공여자들로부터 수집된 조직만이 본 발명의 생구조물을 생산하는데 사용된다. 바람직하게, 태반 막의 공여자의 완전한 부계와 의학적 및 사회적 역사가 얻어지는데, 예를 들면 상세한 가족 역사를 포함한다.

[0070] 공여자는 본 발명이 속하는 기술분야에서 당업자에게 알려진 표준 혈청학적 및 세균학적 테스트를 이용하여 선별된다. 병원체를 확인하는 어떠한 측정 또는 진단 테스트도 본 발명의 방법의 범위 내에 있으나, 바람직한 측정은 높은 처리량을 위한 용량과 높은 정확성이 결합된 것이다. 특정 실시예에서, 본 발명은 당업자에게 알려진 표준 기술을 이용하여 항원 및/또는 항체에 대해서 공여자를 선별하는 것을 포함한다. 항원과 항체의 제한없는 예는 항체 선별(ATY); 알라닌 아미노 전이 효소 선별(ALT); 간염 핵심 항체(핵산과 ELISA); B형 간염 표면 항원; C형 간염 바이러스 항체; HIV-1과 HIV-2; HTLV-1과 HTLV-2; 매독 테스트(RPR); CMV 항체 테스트; 및 C형 간염과 HIV 테스트를 포함한다. 사용된 측정은 당업자에게 알려진 바와 같이 핵산에 근거한 측정 또는 ELISA에 근거한 측정일 수 있다.

[0071] 본 발명은 당업자에게 알려진 표준 기술을 이용하여 신생아의 탯줄로부터 혈액을 더 테스트하는 것을 포함한다(예를 들면, Cotorruelo et al., 2002, Clin Lab. 48 (5-6): 271-81; Maine et al., 2001, Expert Rev. Mol.Diagn.,1(1): 19-29; Nielsen et al., 1987, J. Clin. Microbiol. 25 (8):1406-10 참조; 상기 모든 문헌은 본 명세서에서 참고문헌으로서 그대로 포함된다.) 하나의 실시예에서, 신생아의 탯줄로부터 얻어진 혈액은 당업자에게 알려진 표준 기술을 사용하여 세균 병원체(그람 양성 및 그람 음성균을 포함하되 이에 한정되지는 않는)와 곰팡이에 대하여 테스트된다. 특정 실시예에서, 신생아의 탯줄 혈액의 혈액형과 혈액의 Rh 인자는 당업자에게 알려진 기술을 이용하여 결정된다. 다른 실시예에서, 특이한 CBC는 신생아의 탯줄에 기인하는 혈액으로부터 당업자에게 알려진 표준 방법을 이용하여 얻어진다. 또 다른 실시예에서, 호기성 세균 배양은 본 발명이 속하는 기술분야의 당업자에게 알려진 표준 방법을 사용하여 신생아의 탯줄로부터 얻어지는 혈액으로부터 취해진다. 정상범위내의(예를 들면, 정상 레벨로부터 큰 이상이나 벗어남이 없는) CBC를 갖고, 혈청학 및 세균학에 대해서 음성으로 측정되고, 전염성 질병 및 오염에 대해 음성 또는 비반응성으로 측정되는 공여자들로부터 수집된 조직만이 본 발명의 생구조물을 생산하는데 사용된다.

[0072] 본 발명의 콜라겐 생구조물을 제조하는 하나의 전형적인 방법은 다음의 단계들을 포함한다.

[0073] I 단계. 본 발명은 탯줄을 태반 원반으로부터 분리하기 위해서(선택적으로) 태반 막을 처리하고, 융모막으로부터 양막을 분리하는 것을 포함한다. 바람직한 실시예에서, 양막은 태반 막을 절단하기 전에 융모막으로부터 분리된다. 융모막으로부터 양막의 분리는 태반막의 가장자리로부터 출발하여 이루어지는 것이 바람직하다. 다른 바람직한 실시예에서, 양막은 비절개 박리를 이용하여, 예를 들면 장갑 낀 손가락으로 융모막으로부터 분리된다. 융모막과 태반 원반으로부터 양막을 분리한 다음, 탯줄 밀봉은 예를 들면 가위로 자르고 태반 원반으로부터 분리한다. 특정 실시예에서, 양막과 융모막의 분리가 조직을 찢지 않고는 할 수 없는 경우, 본 발명은 양막과 융모막을 태반 원반으로부터 하나의 조각으로 잘라낸 다음 그것을 벗겨내어 분리하는 것을 포함한다.

[0074] 그 다음, 바람직하게 상기 양막은 살균 염수 용액에 보관된다. 일부 실시예에서, 살균 염수 용액은 완충되어 있다. 특정 바람직한 실시예에서, 양막을 보관하기 위한 상기 살균 염수 용액은 0.9% 살균 NaCl 용액이다. 바람직하게, 양막은 적어도 4℃에서 냉장 보관된다. 특정 실시예에서, 양막은 적어도 2℃, 적어도 6℃, 또는 8℃까지의 온도에서 냉장된다. 여기서, 양막은 냉장되고 살균 염수로 보호되어 유지되면 5일까지 저장될 수 있다. 바람직하게, 분리된 양막은 처리과정에서 다음 단계 이전에 분만시로부터 최대 72시간동안 냉장된다.

[0075] II 단계. 일단 양막이 융모막으로부터 분리되면, 본 발명은 양막을 탈세포화하는 것을 포함한다. 본 발명의 양막을 탈세포화하는 공정은 양막의 어떠한 냉동도 포함하지 않는다는 조건하에, 본 발명이 속하는 기술분야에서 당업자에게 알려진 어떠한 탈세포화 공정도 본 발명의 방법에 포함된다. 본 명세서에서 사용되는 바와 같이, 탈세포화는 본 발명의 양막으로부터 모든 세포물질과 세포 찌꺼기(예를 들면, 모든 가시적인 세포물질과 세포 찌꺼기)를 제거하는 것을 의미한다. 양막의 탈세포화는 실질적으로 양막의 콜라겐 매트릭스와 정상적으로 결합된 모든 세포가 제거되는 것을 확실하게 한다. 콜라겐 매트릭스와 결합된 "실질적으로 모든" 세포를 제거하는 본 발명의 양막의 탈세포화는 바람직하게는 적어도 90%의 세포를 제거하고, 보다 바람직하게는 적어도 95%의 세포를 제거하며, 가장 바람직하게는 적어도 99%의 세포를 제거한다. 본 발명의 방법에 따라 탈세포화된 양막은 균일하게 얇고, 즉, 10-40 마이크론의 두께이고, 매끄럽고(접촉에 의해 결정된 바와 같이), 외관이 깨끗하다.

[0076] 바람직한 실시예에서, 본 발명의 양막의 탈세포화는 양막의 모체 측면으로부터 실질적으로 모든 세포 물질과 세

포 찌꺼기를 제거한 다음, 양막의 태아 측면으로부터 모든 세포 물질과 세포 찌꺼기를 제거하는 것을 포함한다. 특정 실시예에서, 본 발명의 양막의 탈세포화는 살균 용액 세척과 결합한 물리적인 벗겨냄(physical scraping)을 포함한다. 다른 특정 실시예에서, 양막의 물리적인 벗겨냄은 살균 세포 스크레이퍼(scraper)로 벗겨내는 것을 포함한다. 또 다른 특정 실시예에서, 탈세포화 중 양막을 세척하기 위한 살균 용액은 수용액, 생리학적 완충액을 포함하는 용액 또는 예를 들면 0.9% NaCl 용액과 같은 염수 용액이다.

[0077] 양막의 탈세포화는 양막으로부터 천연 세포와 다른 항원체 및 세포 찌꺼기를 제거하는 것을 포함하며, 선택적으로, 새로운 면역학적 자리의 생성을 억제하기 위한 처리를 포함한다. 양막의 탈세포화 단계에서, 유해한 면역반응을 유도할 수 있는 다른 세포 및 비세포 구조 또는 성분 뿐만 아니라 천연 생세포는 제거된다. 본 발명에 따라 행해지는 탈세포화 기술은 양막의 해부학적 구조를 크게 파괴하거나 양막의 구조적 조성이 가지는 특성을 바꾸어서도 안 되는데, 즉 콜라겐, 엘라스틴 여기에 피브로넥틴을 포함할 수도 있는 조성의 구조적·생물학적 일체성이 탈세포화에 의하여 영향을 받아서는 안된다. 특히, 양막의 거친 화학적 처리와 프로테아제 처리는 본 발명의 탈세포화 기술의 범위내가 아니다.

[0078] 바람직하게, 양막의 탈세포화는 세제를 포함한 용액의 사용을 포함한다. 본 발명의 방법에 따라 사용될 수 있는 세제는 비이온성 세제, 트리톤 X-100, 음이온성 세제, 황산도데실나트륨을 포함하는데, 이에 한정되지는 않는다. 본 발명의 방법에서 세제는 단독으로 또는 조합하여 사용될 수 있다. pH 6 ~ 8이고 거품이 적은 모든 순한 음이온성 세제, 즉, 비자극성 세제는 본 발명의 방법에 따라서 사용될 수 있다. 특정 실시예에서, 0.01 ~ 1% 테옥시콜린산 나트륨 염 1수화물이 양막의 탈세포화에 사용된다. 특별한 작용 방식에 따른 설명에 열매이고자 하는 것은 아니나, 본 발명의 방법에 따른 양막의 탈세포화는 세포막을 붕괴할 수 있고, 양막으로부터 세포 찌꺼기를 제거하는데 도움이 될 수 있다. 그러나, 양막 내에 어떠한 수준의 잔류 세제도 남지 않도록 하기 위해서는 조치가 취해져야 하는데, 이러한 조치는 예를 들면 살아 있는 세포를 양막에서 재증식시킬 때 간섭을 피할 수 있기 위함이다.

[0079] 본 발명의 생구조물의 제조에 있어서 프로테아제의 활성을 제한하는 것은 필수적이다. 금속 이온 킬레이트제, 예를 들면 1,10-페난트롤린(phenanthroline) 및 에틸렌디아민테트라아세트산(EDTA)와 같은 첨가물은 많은 단백질 가수분해 효수들에게 바람직하지 않은 환경을 만든다. 콜라게나제와 같은 단백질 분해 효소의 반응 조건을 최적에 못 미치도록 하면 용해(lysis) 단계 동안 콜라겐과 같은 양막 조성물을 분해로부터 보호하는데 도움이 될 수 있다. 단백질 분해 효소에 대한 최적 미만의 조건은 용액 내 칼슘 및 아연 이온의 양을 제거하거나 제한하는 저장 용해액(hypotonic lysis solution)을 조제함으로써 이루어질 수 있다. 많은 단백질 분해 효소는 칼슘 및 아연 이온의 존재하에 활성을 가지며, 칼슘 및 아연 이온이 없는 환경에서는 그들의 활성을 많이 잃는다. 저장 용해액을 제조할 때 pH를 조절하고 칼슘 및 아연 이온의 이용 가능성을 낮추며, 금속 이온 킬레이트제를 포함시키고 콜라게나제에 대한 특정 단백질분해 억제제를 사용—하는 조건을 선택하는 것이 바람직한데, 그리하게 되면 저장 용해액이 천연 세포들을 최적으로 용해하는 동안, 밑에 있는 양막은 유해한 단백질 가수 분해로부터 보호받을 것이다. 예를 들면, 저장 용해액은 칼슘 및 아연 이온을 포함하지 않고 EDTA와 같은 금속 이온 킬레이트제를 포함하는 pH 5.5 ~ 8, 바람직하게는 pH 7 ~ 8인 물의 완충 용액을 포함할 수 있다. 추가적으로, 저장 용해액으로 양막을 처리하는 동안 온도와 시간 파라미터의 조절도 또한 프로테아제의 활성을 제한하기 위하여 채용될 수 있다.

[0080] 또한, 양막의 탈세포화 처리는 새로운 면역학적 자리의 발생을 제한하는 것이 바람직하다. 콜라겐의 효소 분해는 증가된 면역원성을 초래한다고 여겨지므로, 본 발명은 세포 대사, 단백질 생성 및 세포분열을 억제하는데 효과적이고, 양막의 조성물의 단백질 분해를 최소화하여 양막의 기초 구조를 유지하는 효소, 예를 들면 뉴클레아제로 양막의 처리를 포함한다. 본 발명의 방법에 따라 사용될 수 있는 뉴클레아제의 예는 엑소뉴클레아제와 엔도뉴클레아제 모두를 포함하여 천연 세포 DNA와 RNA의 소화에서 효력이 있는 것들이다. 본 발명의 방법에 따라 사용될 수 있는 뉴클레아제의 제한없는 예는 예를 들면 DNAase I (SIGMA Chemical Company, St. Louis, Mo.) 및 RNAase A (SIGMA Chemical Company, St. Louis, Mo.)와 같이 세포 활성을 억제하는 엑소뉴클레아제와, 예를 들면 EcoR I (SIGMA Chemical Company, St. Louis, Mo.) 및 Hind III (SIGMA Chemical Company, St. Louis, Mo.)와 같이 세포 활성을 억제하는 엔도뉴클레아제를 포함한다. 선택된 뉴클레아제는, 예를 들어 마그네슘, 칼슘과 같은 이온을 포함하는 생리학적 완충용액에 적용되는 것이 바람직한데, 이것은 뉴클레아제의 활성에 최적이다. 바람직하게, 완충 용액의 이온 농도, 처리 온도 및 처리 길이는 원하는 레벨의 뉴클레아제 활성을 확실하게 하기 위한 전형적인 실험에 의해 본 발명이 속하는 기술분야의 당업자에 의해 선택된다. 바람직하게 완충액은 세포 내부로 뉴클레아제의 접근을 촉진하는 저장(hypotonic)인 것이다.

[0081] 특정 실시예에서, 본 발명의 양막을 탈세포화하는 것은 다음의 단계들을 포함한다. 먼저, 양막을 깨끗한 살균

컨테이너로 옮기고, 선택적으로 살균수로 세척하고, 살균 거즈로 건조시킨다. 그런 다음, 양막은 모체 측면이 위로 향하게 하여 살균 트레이에 놓여진다. 살균 세포 스크레이퍼(예를 들면, 32 cm, PE 블레이드, PS 핸들, NalgeNunc International)을 사용하여, 양막의 모체 측면으로부터 모든 가시적인 세포물질을 물리적으로 제거함으로써 양막을 부분적으로 탈세포화 시킨다. 필요하다면, 살균수는 세포 및 세포 찌꺼기를 제거하는 것을 도와주는데 사용된다. 양막의 모체 측면에서 부분적인 탈세포화가 완료된 다음, 양막은 태아 측면이 위로 올라오도록 돌려진다. 찢김을 방지하기 위해 양막에 최소의 압력을 가하여 세포 스크레이퍼로 태아 측면 상의 모든 가시적인 세포 찌꺼기를 부드럽게 제거한다. 살균수는 세포 및 세포 찌꺼기를 제거하는 것을 도와주는데 사용될 수 있다.

- [0082] 하나의 실시예에서, 탈세포화된 양막은 다음 공정에 앞서 살균 생리액, 예를 들면 살균 0.9% NaCl 용액으로 채워진 살균 컨테이너에 놓여진다. 본 발명의 방법에 따라서, 양막의 다음 처리단계는 양막이 살균 생리액에 놓여진 후 2-3 시간 내에 시작되어야 한다. 특정 실시예에서, III단계는 II단계를 즉시 뒤따르는데, 양막은 살균 용액으로 채워진 컨테이너에 놓여질 필요는 없다.
- [0083] 양막은 보다 쉬운 세척을 위해 모양을 만들거나 세척되지 않는 부위를 제거하기 위하여, 예를 들면 살균 작은 칼을 이용하여 세척 과정 동안 절단될 수 있다.
- [0084] III 단계. 탈세포화 후에, 양막은 세포의 가용성 단백질, 지질 및 프로테오글리칸(proteoglycan)과 같은 어떠한 세포의 찌꺼기 뿐만 아니라 세포 단백질, 세포 지질 및 세포 핵산을 포함할 수 있는 세포 찌꺼기를 확실하게 제거하기 위하여 세척된다. 비록 어떤 작용 방식을 특정하여 설명하고자 하는 것은 아니지만, 이러한 세포 및 세포의 찌꺼기를 제거하는 것은 양막의 면역원성을 감소시킨다.
- [0085] 일단 본 발명의 양막이 탈세포화되면, 막은 양막의 양 면으로부터 모든 가시적인 세포 물질과 세포 찌꺼기를 완벽하게 효율적으로 제거하기 위하여 더 세척된다. 바람직하게 용액은 천연 조직 세포를 효과적으로 용해하기 위하여 조제된 수용성 저장 또는 낮은 이온 강도 용액이다. 그러한 수용성 저장 용액은 탈이온수 또는 수용성 저장 완충액이 될 수 있다. 바람직하게는 상기 수용성 저장 완충액이 추가적으로 프로테아제, 예를 들어 콜라게나제의 활성을 최적에 못 미치는 조건으로 만드는 첨가제를 포함할 수 있는데, 이들은 세포 용해의 결과로서 방출될 수 있다.
- [0086] 바람직하게, 탈세포화를 도와주기 위해 양막은 세제에서, 예를 들면 흔들리는 판(rocking platform) 위에서 부드럽게 흔들어진다. 특정 실시예에서, 양막은 적어도 15분간, 적어도 20분간, 적어도 30분간 또는 120분까지 흔들어진다. 양막은, 세제 탈세포화 후에, 앞에서 설명한 바와 같이 다시 물리적으로 탈세포화될 수 있다; 물리적 및 세제 탈세포화 단계는 양막의 형태가 유지되는 한, 가시적인 세포 물질 및 세포 찌꺼기가 남아있지 않을 때까지 필요시 반복될 수 있다.
- [0087] 특정 실시예에서, 양막의 세척은 다음 단계를 포함한다: 탈세포화된 양막은 살균 컨테이너에 놓여지고, 그 다음 양막을 충분히 덮을 수 있는 양으로 탈세포화 용액을 채운다; 그런 다음, 양막과 탈세포화 용액이 담긴 컨테이너는 흔들리는 판 위에 놓여진다(예를 들면, Model 100, VWR Scientific Products Corp., P. O. Box 640169, Pittsburgh, PA 15264-0169). 그 다음, 탈세포화 용액 내의 양막은 흔들리는 판 위에서 15 내지 120분동안 흔들어진다. 흔들림 단계 후, 양막은 컨테이너로부터 제거되고, 살균 용액, 예를 들면 0.9% NaCl 용액으로 채워진 깨끗한 살균 트레이에 놓여진다. 새로운 살균 세포 스크레이퍼를 이용하여, 잔류 탈세포화 용액을 제거하고, 양막의 양 면으로부터 남아있는 어떠한 세포 물질을 제거한다. 이 단계는 모든 가시적인 잔류 세포 물질을 제거하기 위하여 필요한 횟수만큼 반복될 수 있다.
- [0088] 특정 실시예에서, 양막은 탈세포화 단계 이후에 즉시(즉, 30분 이내) 건조된다. 대안적으로, 다음 처리가 즉시 이루어지지 않는 경우, 양막은 냉장, 예를 들면 2-8℃의 온도에서, 건조 전에 28일까지 저장될 수 있다. 탈세포화된 양막이 3일 이상 28일 이하동안 저장되는 경우, 양막을 덮는 살균 용액은 주기적으로, 예를 들면 매 1-3일마다 교체해 주는 것이 바람직하다.
- [0089] 특정 실시예에서, 양막이 세척 후에 냉장되지 않는 경우, 양막은 제조의 IV단계를 진행하기 전에 적어도 3회 세척된다. 다른 실시예에서, 양막이 냉장되고 살균 용액이 한번 교체된 경우, 양막은 제조의 IV단계를 진행하기 전에 적어도 2회 세척된다. 또 다른 실시예에서, 양막은 냉장되고 살균 용액은 2회 이상 교체된 경우, 양막은 제조의 IV단계를 진행하기 전에 적어도 한번 세척된다.
- [0090] 특정 실시예에서, 탈세포화된 양막은 살균 조건하에 저장되고, 더 이상의 추가적인 공정, 예를 들면 건조와 같은 공정을 행하지 않는다. IV단계를 진행하기 전에, 모든 세균학적 및 혈청학적 시험에서 모든 테스트가 음성을

나타내는 것이 확실하도록 평가되어야 한다.

- [0091] IV 단계. 본 발명의 방법의 마지막 단계는 콜라겐 생구조물을 생산하기 위하여 본 발명의 탈세포화된 양막을 건조하는 것을 포함한다. 콜라겐의 평평하고 건조한 시트를 만들기 위해서는 양막을 건조하는 어떠한 방법도 사용될 수 있다. 그러나, 양막은 진공하에서 건조되는 것이 바람직하다.
- [0092] 특정 실시예에서, 본 발명의 탈세포화된 양막을 건조하기 위한 전형적인 방법은 다음과 같은 단계를 포함한다:
- [0093] 건조를 위한 탈세포화된 양막의 조립. 탈세포화된 양막을 살균 용액에서 꺼낸 다음, 과잉의 유동액은 부드럽게 짜낸다. 그런 다음, 탈세포화된 양막은 태아 측면이 아래쪽, 예를 들면 트레이를 향하게 하여 평평하게 될 때까지 부드럽게 눌러 펼쳐진다. 그런 다음, 탈세포화된 양막은 태아 측면이 위쪽을 향하도록 뒤집고, 건조 프레임, 바람직하게는 플라스틱 메쉬 건조 프레임(예를 들면, Quick Count[®] Plastic Canvas, Uniek, Inc., Waunakee, WI) 위에 놓는다. 다른 실시예에서, 건조 프레임은 약 0.5센티미터의 멸균 가능(autoclavable) 스테인레스강 메쉬일 수 있다. 가장 바람직한 실시예에서, 양막은 약 0.5 센티미터 분량이 건조 프레임의 가장자리와 겹쳐진다. 특정 실시예에서, 건조 프레임 밖으로 불거져 나오는 겹쳐진 양막으로, 예를 들면 클램프나 지혈기를 이용하여 건조 프레임의 상단 상부를 감싸게 된다. 일단 양막이 건조 프레임에 위치하게 되면, 살균 거르는 열 건조기(또는 젤 건조기)(예를 들면, Model 583, Bio-Rad Laboratories, 200 Alfred Nobel Drive, Hercules, CA 94547)의 건조 판 상에 위치되어, 플라스틱 메쉬 건조 프레임 위에 놓인 양막보다 조금 더 큰 면적을 덮게끔 한다. 바람직하게는, 거즈 층의 전체 두께는 한번 접힌 4X4 거즈의 두께를 넘지 않는다. 시트상 물질의 건조에 적합한 모든 열 건조 기기가 사용될 수 있다. 건조 프레임은 건조 판 상에서 거즈의 상부에 위치되는데, 플라스틱 프레임의 경계가 거즈의 경계 이상으로 확장될 수 있게 바람직하게는 0.1 - 10cm, 더욱 바람직하게는 0.5-1.0cm가 되도록 한다. 가장 바람직한 실시예에서, 양막을 갖는 건조 프레임은 양막의 태아 측면이 위쪽을 향하게 하여 살균 거즈의 상부에 놓여진다. 일부 실시예에서, 다른 플라스틱 프레임 메쉬는 양막의 상부에 놓여진다. 메쉬 프레임과 거기에서 건조된 막의 관찰 결과는 도 4에 나타났다. 다른 실시예에서는 얇은 플라스틱(예를 들면, SW182, 투명 PVC, AEP Industries Inc., South Hackensack, NJ 07606) 또는 생체 상용적인 실리콘의 시트가 모든 모서리를 넘어 펼쳐지도록 메쉬를 덮은 막의 위에 놓인다. 이 실시예에서 두 번째 메쉬 프레임은 필요하지 않다.
- [0094] 대안적인 실시예에서, 상기 양막은 티벡(Tyvek) 재질의 살균 시트(예를 들면, 의료용 포장을 위한 티벡 한 시트 장, DupontTyvek 등록상표, P. O. Box 80705, Wilmington, DE 19880-0705) 한 장 또는 여러 장 위에 놓이게 되며, 선택적으로는 상기 양막 위에 티벡 시트 한 장이 (상기 플라스틱 필름을 설치하기 전에) 놓이게 된다. 이러한 대안적 과정은 더 부드러운 재질의 생구조물(즉, 재료의 축을 따른 방향과 수직 방향에 따라 섬유 압착 패턴의 차이가 없는)을 생산할 것인데, 이것은 예를 들면 본 명세서에 기술된 바와 같이 세포의 팽창을 위한 매트릭스로서 사용하는 경우 등의 특정 적용예에 유리할 것이다.
- [0095] 양막 건조. 바람직한 실시예에서, 본 발명은 진공 하에서 본 발명의 양막을 열 건조하는 것을 포함한다. 진공 하에서의 건조는 약 0도 내지 약 60℃의 어떠한 온도에서라도 이루어질 수 있는 반면, 양막은 약 35℃에서 약 50℃ 사이에서 건조되는 것이 바람직하고, 약 50℃에서가 가장 바람직하다. 콜라겐의 일부 분해는 50℃를 넘는 온도에서 예상된다는 것을 인식해야 한다. 바람직하게는, 건조 온도는 연장된 탐침을 사용하여 교정된 디지털 온도계를 사용하여 맞춰지고 입증된다. 바람직하게는, 진공압력은 약 -22 인치Hg로 맞춰진다. 건조 단계는 양막의 콜라겐 매트릭스가, 예를 들면 수분 분석기에 의해 결정된 바와 같이 3-12% 미만의 수분을 함유할 때까지 계속된다. 이것을 달성하기 위해서, 양막은 , 예를 들면 약 60분동안, 탈수된 양막을 얻기 위해서 열-진공 건조될 수 있다. 일부 실시예에서, 양막은 약 30분에서 2시간동안, 바람직하게는 약 60분동안 건조된다. 비록 특정한 작용 방식에 구애받고자 하는 것은 아니지만, 진공 압력과 결합된 낮은 온도 조건은 콜라겐을 변성시키지 않으면서 양막을 탈수 상태에 이르게 할 수 있다고 여겨지고 있다.
- [0096] 본 발명에 따라서 건조 공정이 완결된 후, 양막은 진공 펌프를 가동하면서 약 2분간 냉장된다.
- [0097] 양막의 포장 및 저장. 진술한 바와 같이 본 발명의 방법에 따라서 양막이 일단건조되면, 막은 부드럽게 건조 프레임으로부터 들어올려진다. 막을 "들어올려짐"은 다음과 같은 단계를 포함할 수 있다: 펌프를 계속 작동하면서, 플라스틱 필름은 양막을 아래로 잡아당기면서 코너부터 시작하여 부드럽게 양막으로부터 제거된다; 양막을 구비한 프레임은 건조 판으로부터 들어 올려져서 양막 면이 위로 가는 절단 보드에 놓여진다; 프레임의 가장자리로부터 1-2mm 떨어진 가장자리를 따라서 잘라내어 절개가 이루어진다; 그런 다음, 양막은 프레임으로부터 벗겨진다; 그리고 다음에 사용될 바에 의해 결정된 적절한 크기대로 절단된다. 바람직하게, 이 단계에서의 양막의 취

굽은 살균 장갑을 가지고 이루어진다.

[0098] 양막은 살균 컨테이너, 예를 들면 필 파우치에 놓여지고 밀봉된다. 본 발명의 방법에 따라 만들어진 생구조물은 진술한 바와 같이 장기간 동안 상온에서 저장될 수 있다.

[0099] 대안적인 실시예에서, 본 발명은 용모막을 포함하는 콜라겐 생구조물을 제조하는 방법을 제공한다. 진술한 방법은 용모막을 포함하는 생구조물을 제조하는 방법에 적용할 수 있을 것으로 기대된다. 특정 실시예에서, 본 발명은 양막과 용모막을 포함하는 태반을 제공하고; 용모막으로부터 양막을 분리하고; 용모막을 탈세포화 하는 것을 포함하는 콜라겐 생구조물을 제조하는 방법을 포함한다. 특정 실시예에서, 본 방법은 탈세포화된 용모막을 세척하고 건조하는 것을 더 수반한다.

[0100] 5.2.1 3차 스캐폴드와 래미네이트를 제조하는 방법

[0101] 본 발명은 본 발명의 콜라겐 생구조물을 포함하는 3차원 스캐폴드, 3차원 배열(configuration) 및 래미네이트를 제조하는 방법을 제공한다.

[0102] 일부 실시예에서, 본 발명은 본 명세서에 개시된 방법을 이용하여, 양막과 용모막을 포함하는 태반, 바람직하게는 인간 태반을 제공하고, 용모막으로부터 양막을 분리하고; 본 명세서에 개시된 방법을 이용하여 양막을 탈세포화하고; 본 명세서에 개시된 방법을 이용하여 적어도 한번 탈세포화된 양막을 세척하고; 양막 래미네이트가 형성되도록 서로 접촉하여 탈세포화된 양막의 적어도 두개를 적층하고; 본 명세서에 개시된 방법을 이용하여 양막 래미네이트를 건조하는 것을 포함하는 양막 래미네이트를 제조하는 방법을 제공한다.

[0103] 대안적으로, 다른 실시예에서, 양막 래미네이트를 제조하는 방법은 본 발명의 방법에 따라 제조된 양막 적어도 두개를 건조하고, 양막 래미네이트가 형성되도록 적어도 두개의 양막을 서로 접촉하여 적층하는 것을 포함한다.

[0104] 일부 실시예에서, 본 발명의 방법에 따라 제조된 양막 층은 양막 래미네이트를 형성하기 위하여 접착제 존재하에 서로 접촉하게 놓여질 수 있다. 본 발명의 방법과 조성물에 따라 사용된 접착제는 본 발명이 속하는 기술분야의 당업자에게 알려진 어떠한 생물학적 접착제일 수 있으며, 바람직하게는, 예를 들면 피브로넥틴, 피브린과 같은 천연 접착제, 합성 접착제를 포함하되 이에 한정되지는 않는 생물학적 상용성이 있는 접착제일 수 있다. 다른 실시예에서, 본 발명의 방법에 따라 제조된 양막 층은 양막 래미네이트를 형성하기 위하여 서로 가교결합된다. 본 발명이 속하는 기술분야의 당업자에게 알려진 어떠한 가교제나 방법도 본 발명의 범위 내에서 사용될 수 있는데, 화학적 가교, 펩타이드 가교, UV 가교, 방사선 가교, 피브로넥틴 가교, 피브리노겐 가교, 하이드로겔 가교를 포함하되 이에 한정되지는 않는다. 다른 실시예에서, 본 발명의 방법에 따라 형성된 양막 래미네이트는 접착제를 포함하지 않는다.

[0105] 5.3 콜라겐 생구조물의 저장 및 취급

[0106] 본 발명은 상온(예를 들면 25℃)에서 탈수된 시트로서 본 발명의 콜라겐 생구조물을 저장하는 것을 포함한다. 특정 실시예에서, 본 발명의 콜라겐 생구조물은 적어도 10℃, 적어도 15℃, 적어도 20℃, 적어도 25℃, 또는 적어도 29℃의 온도에서 저장될 수 있다. 바람직하게는, 본 발명의 콜라겐 생구조물은 냉장되지 않는다. 일부 실시예에서, 본 발명의 콜라겐 생구조물은 약 2 내지 8℃의 온도에서 냉장될 수 있다. 다른 실시예에서, 본 발명의 콜라겐 생구조물은 장기간동안 상기 확인된 어떠한 온도에서라도 저장될 수 있다. 가장 바람직한 실시예에서, 본 발명의 생구조물은 살균 및 비산화 조건하에서 저장된다. 본 발명의 방법에 따라 생성된 생구조물은 콜라겐 생구조물의 생화학적 또는 생물리적 성질의 어떠한 변화 없이, 생화학적 또는 구조적 형태의 변화 없이(예를 들면, 분해없이) 어떠한 특정 온도에서 12개월 또는 그 이상 저장될 수 있다. 본 발명의 방법에 따라 생성된 생구조물은 콜라겐 생구조물의 생화학적 또는 생물리적 성질의 어떠한 변화 없이, 생화학적 또는 구조적 형태의 변화없이(예를 들면, 분해없이) 수년동안 저장될 수 있다. 본 발명의 방법에 따라 제조된 본 발명의 생구조물은 무기한적으로 지속될 것으로 기대된다. 생구조물은 장기간 저장에 적합한 어떤 컨테이너에서 저장될 수 있다. 바람직하게, 본 발명의 콜라겐 생구조물은 살균 이중 필-파우치 포장으로 저장된다.

[0107] 본 발명은 그것의 건조 상태에서 본 발명의 콜라겐 생구조물의 취급을 포함한다. 특정 실시예에서, 콜라겐 생구조물은 사용전에, 예를 들면 외과적 이식 전에 정돈된다. 본 발명은 본 발명이 속하는 기술분야의 당업자에 의해 정해진 바와 같이, 그 사용에 양립할 수 있는 본 발명의 생구조물의 어떠한 차원이라도 포함한다. 일부 실시예에서, 본 발명은 1x2cm; 2x3 cm; 4x4 cm, 5x5, 또는 6x8인 콜라겐 생구조물을 포함한다. 본 발명의 생구조물

은 양막의 크기의 범위 내에서 필요한 어떠한 크기로도 절단될 수 있다.

[0108] 본 발명의 콜라겐 생구조물의 표면 배치는 시각적으로 확인될 수 있다. 본 발명의 콜라겐 생구조물은 "격자(grid)" 패턴을 갖는데, 본 발명이 속하는 기술분야의 당업자에 의해 모체 및 태아 표면의 시각적 확인이 가능하다. 특정 실시예에서, 콜라겐 생구조물의 표면 배치는 확대 하에서 확인된다. 콜라겐 생구조물의 태아 측면은 그것의 오목면, 즉 오목해진 격자 패턴에 의해 구별될 수 있다는 것이 본 발명이 속하는 기술분야의 당업자에 의해 평가될 것이다. 역으로, 모체 측면은 볼록면, 즉 높아진 격자 패턴에 의하여 확인될 수 있다.

[0109] 본 발명의 콜라겐 생구조물은 사용 전에 최소 제조 시간이 요구된다. 바람직한 실시예에서, 본 발명의 콜라겐 생구조물은 5분 또는 그 이하 이내, 10분 또는 그 이하 이내에, 15분 또는 그 이하 이내에 사용할 준비가 된다. 사용 전, 예를 들면 외과적 이식 전에 본 발명에 따른 콜라겐 조성물의 제조 시간은 탈수된 콜라겐 생구조물의 재수화에 의한 활성화를 포함한다. 일부 실시예에서, 본 발명의 콜라겐 생구조물은 외과수술 자리에 있는 동안 수화된다. 다른 실시예에서, 본 발명의 콜라겐 생구조물은 집시에서 살균 조건 하에서 수화된다. 본 발명은 살균 생리학적 완충액을 이용하여 본 발명의 콜라겐 생구조물의 수화를 포함한다. 특정 실시예에서, 본 발명은 살균 염수액, 예를 들면 살균 0.9% NaCl 용액으로 본 발명의 콜라겐 생구조물을 수화하는 것을 포함한다. 일부 실시예에서, 살균 염수액은 완충되었다. 특정 실시예에서, 본 발명의 콜라겐 생구조물의 수화는 적어도 2분, 적어도 5분, 적어도 10분, 적어도 15분 또는 적어도 20분을 요구한다. 바람직한 실시예에서, 본 발명의 콜라겐 생구조물의 수화는 5분 이내에 완결된다. 또 다른 바람직한 실시예에서, 본 발명의 콜라겐 생구조물의 수화는 단지 10분 걸린다.

[0110] 예를 들면, 외과적 이식물로서의 사용을 위하여 일단 수화된 본 발명의 콜라겐 생구조물은 본 발명이 속하는 기술분야의 당업자들에 의해 평가된 바와 같이 종래 기술의 양막보다 향상된 봉합성(suturability)을 갖는다. 본 발명의 콜라겐 생구조물은 종래 기술의 양막처럼 쉽게 찢어지거나 쉽게 부서지지 않는다. 본 발명은 효과적으로 봉합될 수 있는 콜라겐 생구조물을 포함한다.

[0111] **5.3.1 살균**

[0112] 본 발명의 생구조물의 살균은 본 발명이 속하는 기술분야의 당업자에게 알려진 방법, 예를 들면 Gorham, D. Byrom(ed.), 1991, Biomaterials, Stockton Press, New York, 55-122을 이용하여 전자선 조사에 의해 이루어지는 것이 바람직하다. 적어도 99.9%의 세균이나 또는 다른 잠재적으로 오염을 일으키는 유기체들을 죽이는데 충분한 어떠한 방사선량이라도 본 발명의 범위내이다. 바람직한 실시예에서, 본 발명의 생구조물의 최종적인 살균을 얻기 위하여 적어도 18-25 kGy의 선량이 사용되었다. 그러나, 본 발명의 생구조물의 살균은 항생제나 글리세롤에서 저장을 포함하지 않는다.

[0113] **5.4 본 발명의 콜라겐 생구조물을 사용하는 방법**

[0114] 본 발명의 콜라겐 생구조물은 부분적으로 본 발명이 속한 분야에서 사용되어온 전통적 막과 비교하여 생체역학적(biomechanical) 강도, 유연성, 봉합능력(suturability), 낮은 면역원성과 같은 물리적 성질을 때문에 의학 및 수술 영역에서 많은 유용성을 지닌다. 예를 들어, 본 발명의 콜라겐 생구조물은 예를 들어 GoretexTM와 같은 합성 비-재흡수 PTFE 막; 글리코라이드 및 락타이드 코폴리머로 제조된 합성 재흡수 막; PCT특허 제W0-88/08305호, 독일특허 제DE-2631909호, 미국특허 제5,837,278호에 개시된 막과 같은 본 발명이 속한 분야의 기존 막들에 비하여 가이드된 조직 재생성에 있어 촉진된 치료적 유용성을 가질 것으로 기대된다.

[0115] 본 발명의 콜라겐 생구조물을 제조하는 방법은 생구조물의 3차 및 4차 구조의 보존을 보장하고, 따라서 의학 및 수술 영역에서 그것의 의도된 사용에 있어 생구조물을 이상적으로 만든다. 아래에서 상세히 설명되는 것처럼, 본 발명은 혈관 치유, 자궁 치유, 힘줄 대체, 각막 대체, 인공 피부, 치주질환의 치료, 및 상처 치유를 포함하는, 하지만 이에 한정되지 않는, 다양한 의학 및 치의학 적용에 있어 사용하기에 적당한 물리적 성질을 가진 콜라겐 생구조물을 제공한다. 의도한 사용 분야에 따라, 본 발명은 요세관(tubular) 혈관을 형성하도록 모양 지어질 수 있는 막과 같은 2차원 막; 임플란트와 같은 3차원 스케폴드, 또는 1차원 섬유(fiber)와 같은 콜라겐 생구조물의 사용을 포함한다.

[0116] **5.4.1 본 발명의 콜라겐 생구조물을 사용하는 치료방법**

[0117] **5.4.1.1 피부상태의 치료방법**

- [0118] 인간피부는 표피와 진피의 혼성 물질이다. 표피의 윗부분은 각질층이고, 이것은 피부의 가장 뽀뽀한 층이며 외부 환경에 의하여 가장 영향을 많이 받는 층이다. 각질층 아래가 표피의 내부부분이다. 표피 아래는 유두진피인데, 이것은 피부의 마이크로-렐리프(micro-relief)로 정의되는 다소 느슨한 연결조직으로 만들어져 있다. 유두진피 아래에 위치한 망상진피는 공간적으로 조직된 단단한 결합 조직이다. 망상진피는 또한 거친 주름과 관련된다. 진피 아래에 피하층이 있다.
- [0119] 피부의 주 기능은 보호, 배설, 분비, 흡수, 체온조절, 색소생성(pigmentogenesis), 축적, 체온조절, 시각 인식, 및 면역 과정의 조절을 포함한다. 이러한 기능은 노화 및 태양 과다 노출에 의한 피부 내 구조적 변화에 유해하게 영향을 받는다. 피부 노화와 관련된 생리적 변화는 예를 들어 Cerimele, D. et al., 1990, Br. J. Dermatol., 122 Suppl. 35, p. 13-20에 개시된 것과 같은 막 기능의 손상과 표피 세포의 감소된 회전율(turnover)을 포함한다. 그러나 본 발명이 속한 분야의 방법 및 조성물은 피부 탄력과 부드러움의 개선, 또는 주름의 제거와 같은 피부 상태를 개선하는데 있어 제한된 성공을 하였을 뿐이다.
- [0120] 본 발명의 콜라겐 생구조물은 의학적 용도뿐만 아니라 화장 용도로 사용될 수도 있다. 본 발명의 콜라겐 생구조물은 피부손상, 노화된 피부, 주름, 세션, 얇아짐(thinning), 감소된 피부탄력, 거친 피부, 선천적인 퇴행성 피부 상태, 콜라겐 VII 결핍, 및 태양 손상 피부를 포함하는, 하지만 이에 한정되지 않는, 피부 상태를 치료하는데 임상적이고 치료적인 유용성을 가진다. 몇몇 실시예에서, 본 발명의 콜라겐 생구조물은 여드름 흉터, 미간고랑(glabellar furros), 절개 흉터, 또는 본 발명이 속한 분야에 알려진 어떠한 다른 부드러운 조직 결함과 같은 피부 상태를 위한 피하 이식물로써 사용될 수 있다. 본 발명의 콜라겐 생구조물은 피부 노화와 관련된 변화를 치료하는데 임상적이고 치료적인 유용성을 가진다. 몇몇 실시예에서, 본 발명의 콜라겐 생구조물은 피부주름, 및/또는 탄력성 및 부드러움과 같은 피부 상태를 개선하는데 유용성을 가진다. 본 발명의 콜라겐 생구조물은 이식물로써, 라미네이트(laminate)로써, 또는 피부 상태의 치료를 위한 3차원의 둘둘 말린 형태로서 사용될 수도 있다. 본 발명의 콜라겐 생구조물은 부분적으로 긴 지속 효과를 제공할 수 있는 그것의 안정성 때문에 예를 들어 미국특허 제5,972,999호; 제5,418,875호; 제5,332,579호, 제5,198,465호와 같은 피부 상태를 치료하기 위한, 본 발명이 속한 분야에 알려진 방법과 비교하여, 향상된 임상성 유용성을 가질 것으로 기대된다.
- [0121] 바람직한 실시예로, 본 발명의 콜라겐 생구조물은 피부 상태를 치료하기 위하여 본 발명이 속한 분야에 알려진 하나 이상의 치료제로 더 보충된다. 그러한 치료제의 예는 비타민, 미네랄, 카테킨-기초 제제, N-아세틸글루코사민, 및 글루코사민을 포함하나 이에 한정되는 것은 아니다 (Neldner, 1993, Amer. Acad. Derm. Annl. Mtg. Wash. DC.; Lubell 1996, Cosmetic Dermatol, 9 (7): 58-60; Swaine et al., 1995, J. Am. Board of Family Practice, 8 (3): 206-16; Shan et al., 1994, Kidney International, 46 (2): 388-95 참조; 상기 모든 문헌은 인용에 의하여 그 전체가 본 명세서에 포함된다).
- [0122] 본 발명의 콜라겐 생구조물은 피부 상태의 치료에 있어 유용한 (클린다마이신, 미노사이클린, 독시사이클린, 겐타마이신과 같은) 항생제, 호르몬, 성장 인자, 항-종양 치료제, 항-진균 치료제, 항-바이러스성 치료제, 통증 치료제, 항-히스타민제, 항-염증치료제, (질산은 및 은 실파디아진을 포함하는, 하지만 이에 한정되지 않는, 은염(silver salt)과 같은) 은을 포함하는, 하지만 이에 한정되지 않는, 항-감염제, 은 원소(elemental silver), 항생제, (라이소좀과 같은) 박테리아살균성 효소, (PDGF, TGF를 포함하는, 하지만 이에 한정되지 않는, 사이토카인; 티모신과 같은) 상처 치료제, 상처 치유제로서 히아루로닉 산, (트롬빈이 있는 또는 없는 피브리노겐과 같은) 상처 실란트, (피브로넥틴과 같은) 세포 부착성 및 스케폴드성 시약 등을 포함하는, 하지만 이에 한정되지 않는, 일정 생체분자(biomolecule)를 함유할 수도 있다. 특정 실시예에서, 콜라겐 생구조물은 예를 들어, 섬유모세포 성장 인자, 표피 성장 인자 등과 같은 적어도 하나의 성장 인자를 함유할 수도 있다. 생구조물은 예를 들어 막 수용체 억제제, 키나제 억제제, 성장 억제제, 항암제, 항생제 등과 같은 작은 유기 분자 또한 함유할 수 있다.
- [0123] **5.4.1.2 상처 및 화상**
- [0124] 본 발명의 콜라겐 생구조물은 경질 및/또는 연질 조직 회복을 보장하기 위한 상처 드레싱으로서 종래 기술에서 알려진 생구조물, 예를 들어 미국특허 제3,157,524호; 제4,320,201호; 제3,800,792호; 제4,837,285호; 제5,116,620호에 개시된 것보다 향상된 임상적 유용성을 가지리라고 예상되는데, 이는 부분적으로는 상기 생구조물의 물리적 특성 덕택이다. 본 발명의 콜라겐 생구조물은 콜라겐의 천연 4차 구조를 유지하기 때문에 콜라겐 매트릭스의 간극(interstices) 내로 세포 이동을 통하여 향상된 조직 내성장(tissue in-growth)을 제공한다. 본

발명의 콜라겐 생구조물은 세포가 콜라겐 매트릭스 내에 부착하고 성장하도록 하고, 그들의 자연적 거대분자(macromolecules)를 합성하도록 한다. 세포는 그로써 새로운 조직의 성장을 위한 새로운 매트릭스를 생성한다. 그러한 세포 발달은 섬유, 플리스(fleece) 및 가용성 콜라겐과 같은 다른 알려진 콜라겐의 형태에서는 관찰되지 않는다.

[0125] 몇몇 실시예에서, 본 발명은 개체의 피부 위에 직접 즉, 상처부위 위 각질층 위에 직접 콜라겐 생구조물을 놓아 상처를 치유하는 것을 포함하고, 상처는 예를 들어 부작성 테이프를 사용하여 덮어진다. 다른 실시예로, 본 발명은 예를 들어 피하 이식물과 같은 이식물로서 본 발명의 콜라겐 생구조물을 사용하여 상처를 치료하는 것을 포함한다.

[0126] 본 발명은 본 발명의 콜라겐 생구조물에 조직 내 성장을 촉진할 수 있는 거대분자를 첨가하여 상처 치료 속도를 높이는 것을 포함한다. 그러한 거대분자는 히알루론산, 피브로넥틴(fibronectin), 라미닌, 및 프로테오글리칸을 포함하나 이에 한정되는 것은 아니다 (예를 들어, Doillon et al. (1987) Biomaterials 8: 195-200; 및 Doillon and Silver (1986) Biomaterials 7: 3-8 참조).

[0127] 본 발명은 또한 혈소판-유래 성장 인자, 인슐린-유사 성장 인자, 표피 성장 인자, 전환 성장 인자 베타, 혈관신생 인자, 항생제, 항진균제, 살정제(spermicidal agents), 호르몬, 효소, 효소 억제제를 포함하는, 하지만 이에 한정되지 않는, 약리학적으로 활성인 성분 및 위에서 설명된 어떠한 생체분자를 5.4.2.7장에서 설명된 것처럼 피부에 전달하기 위하여 본 발명의 콜라겐 생구조물 내에 함유하는 것을 포함한다. 바람직하게 약리학적으로 활성인 성분은 생리학적으로 효과적인 양만큼 제공된다.

[0128] 몇몇 실시예에서, 콜라겐 생구조물은 상처 부위에 적용하기 전에 동종이형(allogenic) 줄기세포, 줄기세포, 및 자가 성인 세포를 포함하는, 하지만 이에 한정되지 않는, 살아있는 세포를 더 함유할 수 있다.

[0129] 본 발명의 콜라겐 생구조물은 특히 외과적 또는 외상의(traumatic) 상처 파손에 의한 상처 감염과 같은 상처를 치료하는데 유용하다. 특정 실시예에서, 콜라겐 생구조물은 항생제, 항-미생물제, 및 항-박테리아제를 포함하는, 하지만 이에 한정되지 않는, 상처 감염의 치료에 유용한 성분을 치료학적으로 유효한 양만큼 함유한다. 본 발명의 콜라겐 생구조물은 예를 들어 인체 내 또는 외부 환경 기원의, 상처를 감염시킬 수 있는, 병원성 세균에 대한 알려진 레저보어인 미생물과 같은 본 발명이 속한 분야에 잘 알려진 어떠한 미생물로부터의 상처감염의 치료에 있어서 임상적 및 치료학적 유용성을 가진다. 상처 내 미생물의 성장이 본 발명의 방법 및 조성물에 의해 감소 또는 억제될 수 있는 미생물의 비-한정적 예는 *S. aureus*, *St. epidermis*, 베타 해모라이틱(haemolytic) *Streptococci*, *E. coli*, *Klebsiella* 및 *Pseudomonas* 종, 및 혐기성 박테리아로 깊은 외상성 상처의, 가스 괴저의 원인인 *Clostridium welchii* 또는 *tartium*이 있다.

[0130] 다른 실시예로, 본 발명의 콜라겐 생구조물은 표피 상처, 피부 상처, 만성 상처, 급성 상처, 외부 상처, 내부 상처(예를 들어 콜라겐 생구조물은 봉합선으로부터 혈액의 누출을 막기 위해, 및 신체가 봉합 물질에 부착하는 것을 막기 위해 수술 동안 아나스토모시스(anastomosis) 부위 둘레로 감싸질 수 있다), 선천적 상처(예를 들어 위축물집표피박리증(dystrophic epidermolysis bullosa))를 포함하는, 하지만 이에 한정되지 않는, 상처 치료를 위해 사용된다. 특히 콜라겐 생구조물은 압박 궤양(예를 들어 욕창 궤양)의 치료에 있어 향상된 유용성을 가진다. 압박 궤양은 주로 오랫동안 침대에 있어야 하는, 예를 들어 피부 손실로 고통 받는 사지마비환자 및 하반신마비환자와 같은 환자에게 국소적 압력의 효과 때문에 발생한다. 결과적인 압박 상처는 진피 마손 및 표피와 피부 부속물의 손실을 나타낸다.

[0131] 본 발명의 콜라겐 생구조물은 또한 1도 화상, 2도 화상(부분적 두께 화상), 3도 화상(총 두께 화상), 화상 상처의 감염, 절제 및 비절제(unexcised) 화상 상처의 감염, 이식 상처의 감염, 공여 부위의 감염, 전에 이식된 또는 치유된 화상 상처 또는 피부 이식 공여 부위로부터 표피의 손실, 및 화상 상처 능가진을 포함하는, 하지만 이에 한정되지 않는, 화상의 치료에 사용될 수 있다.

[0132] 5.4.1.3 조직 공학(engineering)

[0133] 본 발명은 예를 들어 표피 성장 및 분화를 위한 보충으로, 배양된 피부 세포의 운송을 위한 운반체로 본 발명의 콜라겐 생구조물을 사용하는 것을 포함한다. 일정 실시예에서, 조직 및/또는 오르가노이드(즉, 표면 모습 또는 구조에 있어 신체의 일정 기관 또는 선(gland)과 유사체) 형성용으로 생구조물에 세포를 증식시키기 위하여, 생구조물은 그러한 새로운 세포를 생구조물에 재증식시키는(repopulate) 과정 동안 생구조물에 세포의 부착을 촉진하는 세포 부착 인자로 처리될 수 있다. 일정 실시예에서, 세포 부착의 정도는 예를 들어 인간 또는 소태아

혈청과 같은 혈청으로 양막을 처리함으로써 증가한다. 다른 실시예에서, 세포 부착의 정도는 피브로넥틴으로 양막을 처리함으로써 증가한다.

[0134] 본 발명의 콜라겐 생구조물은 예를 들어 질환이 있는 또는 손상된 조직을 재생성하거나 대체하기 위하여 유도된 조직 재생성에서 사용될 수 있다. 본 발명은 치료부위에 생구조물을 직접 이식하여 또는 인공기구(prosthetic device)의 형성으로 본 발명의 생구조물을 사용하는 것을 포함한다. 본 발명의 콜라겐 생구조물은 예를 들어 특히 경구 또는 치과 수술 등의 수술 후와 같은 일정 환경에서 특히 유용하다 (5.4.2.2장에서 좀더 자세히 설명함). 이러한 경우에 촉진된 상처 치유 및/또는 진피 대체는 바람직하다. 유도된 조직 재생성에 있어 본 발명의 콜라겐 생구조물의 유용성은 부분적으로, 재생성이 요구되어지는 영역에 다른 조직의 내 성장을 막는 환경을 제공하는 능력 때문이다.

[0135] 예를 들어, 붕괴 또는 질환 때문에 치근의 실질 부분이 제거된 경우에 제거된 뼈 조직을 대체하기 위하여 건강한 뼈 재생성이 일어나는 것이 바람직하다. 그러나 뼈의 제거로 남겨진 공간은 결합 조직으로 빠르게 채워지고, 결합 조직의 이러한 내 성장은 결과적으로 뼈 재생성을 막는다. 그러한 내 성장을 막기 위하여, 본 발명의 콜라겐 생구조물은 수술적으로 상처 공간의 주위에 삽입될 수 있다. 생구조물은 바람직하지 않은 세포 타입이 상처 공간으로 침입하는 것을 막거나 방해하고, 이것은 바람직한 세포가 공간 내에서 성장하도록 한다. 이것에 의하여 상처를 치유한다.

[0136] 몇몇 실시예에서, 콜라겐 생구조물은 망막 표면 재건을 위한 이식물로써 예를 들어 스티븐 존슨 신드롬(Stevens Johnson syndrome)을 가진 환자, 2차적 화학적 화상이 있는 림볼(limbal) 줄기세포 결핍을 가진 환자, 또는 화학적 및/또는 열적 화상을 가진 환자에 있어 유용하다. 본 발명의 콜라겐 생구조물은 예를 들어 상피화(epithelialization)를 촉진함으로써 임상적 유용성을 가지리라 기대된다. 본 발명의 콜라겐 생구조물은 조직 공학에 있어 본 발명이 속한 분야에서 사용되어온 양막과 비교하여 뛰어난 임상적 유용성을 가질 것으로 기대된다. Gomes et al., 2003, Ophthalmology, 119: 166-73; Ti et al., 2001, Ophthalmology, 108: 1209-1217; Meller et al., 2000, Ophthalmology, 107: 980-9; Gris et al., 2002 Ophthalmology, 109: 508-12; Koizumi et al., 2000, Invest. Ophthal. and Visual Science, 41: 2506-13; Pires et al., 1999, Arch. Ophthalmol. 117: 1291-7; Tseng et al., 1998, Arch. Ophthalmol. 116: 431-441; Heiligenhaus et al., 2001 Invest. Ophthal. and Visual Science 42: 1969-74; Anderson et al. 2001, Br. J. Ophthalmol. 85: 567-75 참조.

[0137] 본 발명은 성인 조직 세포, 자가 세포, 및 줄기세포를 포함하는, 하지만 이에 한정되지 않는, 살아있는 세포를 콜라겐 생구조물에 증식시키는 것을 포함한다. 본 발명의 방법에서 사용하기 위한 줄기세포는 분화전능성(totipotent) 또는 분화만능성(pluripotent)이거나, 또는 조직 특이 세포로 분화될 수 있다. 본 발명의 방법에서 사용하기 위한 줄기세포는 본 발명이 속한 분야에서 통상의 지식을 가진 자에게 알려진 표준 방법에 의하여 얻어질 수 있다. 바람직하게는, 줄기세포는 미국특허출원 제10/74,976호(2002년 2월 13일 출원)에 개시된 방법에 따라 모아진다. 전술한 인용문헌은 인용에 의하여 본 명세서에 포함된다.

[0138] 본 발명은 혈관, 심장 밸브, 간, 궤장, 및 인대를 포함하는, 하지만 이에 한정되지 않는, 생체공학적 조직 및 오르가노이드의 발달을 위하여 본 발명의 콜라겐 생구조물(예를 들어 3차원 스캐폴드)을 사용하는 것을 포함한다. 비록 일정 작용기전에 본 발명이 제한되도록 의도되는 것은 아니지만, 생체공학적 조직으로써 본 발명의 콜라겐 생구조물의 유용성은 부분적으로 혈액 응고를 촉진하는 지혈 성질에서 말미암은 것이다. 본 발명의 콜라겐 생구조물은 특히 혈관 수술에서 혈관 인공삽입물 및 이식물로 유용하다. 본 발명의 콜라겐 생구조물은 예를 들어 봉합 라인으로부터 혈액의 누출을 막고 봉합 물질에 신체가 부착하는 것을 막기 위하여 혈관 수술 시술 동안에 혈관(또는 이식 혈관)의 연결 부위 위에 주위(circumferential) 커버링으로 사용할 수 있다.

[0139] 5.4.2 수술 시술에서 콜라겐 생구조물을 사용하는 방법

[0140] 본 발명은 외과적 이식물로 본 발명의 콜라겐 생구조물을 사용하는 것을 포함한다. 본 발명은 본 발명의 콜라겐 생구조물 또는 그것의 라미네이트를 포함하는 외과적 이식물을 포함한다. 본 발명은 또한 외과적 이식물을 준비하고 사용하는 방법을 포함한다.

[0141] 몇몇 실시예에서, 본 발명은 외과적 시술에서 외과적인 이식물을 사용하는 방법을 포함하고, 외과적 이식물은 직접적으로 예를 들어 내면 부위, 또는 외면 부위인 개체의 외과수술 부위에 적용된다. 몇몇 실시예에서, 콜라겐 생구조물은 아래에서 자세히 설명되는 것처럼, 예를 들어 봉합 라인으로부터 혈액의 누출을 막고 봉합물질에 신체가 부착하는 것을 막기 위하여 외과 시술 동안 외과적 이식물로 사용된다. 다른 실시예에서, 외과적 이식물은 예를 들어 봉합 라인으로부터 장관액 및 담즙의 누출을 막고 봉합물질에 신체가 부착하는 것을 막기 위하여

위장관 수술 동안 위장관의 연결 부위 위에 커버링으로 사용된다.

[0142] 본 발명은 화상 또는 외과기술 피부 상처를 커버하기 위하여; 복부, 흉부 강(cavity) 및 심낭을 덮고 있는 장막 표면 위의 모든 내부 복막 수술 또는 다른 재건술 내 부착을 막기 위하여; 구강 및 비강, 호흡관, 위장관, 및 비뇨생식기관을 따라 있는 점막 표면을 재구성하기 위하여 이식물 또는 드레싱으로; 뇌 수술에 있어 뇌경질막 복원을 보충하기 위한 대체물로; 중추 및 말초 신경계 내 신경 재생성을 촉진하기 위한 대체물로; 및 관절 또는 힘줄 회복에서 부착을 방지하는 부드러운 조직 재건을 위하여 생구조물을 사용하는 것을 포함한다.

[0143] 본 발명은 특정 의도 외과기술 용도에 따라 본 발명의 외과적 이식물에 하나 이상의 생체분자, 바람직하게는 치료 성분을 함유시키는 것을 포함한다. 그러한 생체분자는 (클린다마이신, 미노사이클린, 독시사이클린, 젠타마이신과 같은) 항생제, 호르몬, 성장 인자, 항-종양 치료제, 항-진균 치료제, 항-바이러스성 치료제, 통증 치료제, 항-히스타민제, 항-염증치료제, (질산은 및 은 설파디아진을 포함하는, 하지만 이에 한정되지 않는, 은염(silver salt)과 같은) 은을 포함하는, 하지만 이에 한정되지 않는, 항-감염제, 은 원소(elemental silver), 항생제, (라이소좀과 같은) 박테리아살균성 효소, (PDGF, TGF를 포함하는, 하지만 이에 한정되지 않는, 사이토카인; 티모신과 같은) 상처 치료제, 상처 치유제로서 히아루로닉 산, (트롬빈이 있는 또는 없는 피브린과 같은) 상처 실란트, (피브로넥틴과 같은) 세포 부착성 및 스케폴드성 시약 등을 포함하지만 이에 한정되는 것은 아니다. 특정 실시예에서, 콜라겐 생구조물은 적어도 하나 이상의 성장 인자, 예를 들어 섬유모세포 성장 인자, 표피 성장 인자 등과 같은 성장 인자를 함유할 수 있다. 생구조물은, 예를 들어 막 수용체 억제제, 키나제 억제제, 성장 억제제, 항암제, 항생제 등의, 특정 생화학 프로세스의 특이적 억제제와 같은 작은 유기 분자를 함유할 수 있다.

[0144] 본 발명은 또한 본 발명의 외과적 이식물에 줄기세포, 분화전능성 줄기세포, 분화만능성 줄기세포, 분화능성 줄기세포, 조직 특이 줄기세포, 배아 유사 줄기세포, 수임 전구세포, 피브로블라스토이드(fibroblastoid) 세포를 포함하는, 하지만 이에 한정되지 않는, 살아있는 세포를 함유시키는 것을 포함한다. 다른 실시예에서, 본 발명은 본 발명의 외과적 이식물에 연골세포, 간세포, 조혈세포, 췌장실질세포, 신경세포, 및 근 전구세포를 포함하는, 하지만 이에 한정되지 않는, 특정 클래스의 전구세포를 함유시키는 것을 포함한다.

[0145] 5.4.2.1 안과학(ophthalmology)

[0146] 본 발명의 콜라겐 생구조물은 눈과 관련된 질환 또는 장애의 치료에 임상적이고 치료학적인 유용성을 가진다. 본 발명의 콜라겐 생구조물은 특히 각막 궤양/천공, 수포성 각막병증, 망막 유피낭/종양, 주요 익상편(pterygium), 저항성 표피 장애, 급성 및 만성 알칼리 화상, 열화상, 무홍채증, 아토피성 각막염, 원인불명의 림발(limbal) 줄기세포 결핍, 각막 판누스, 신생혈관증식, 류마티스성 각막 용해, 안구홍터 유사물집증, 누설 여과 기포, 노출된 Ahmed 밸브 튜브, 계속적 검구유착이 있는 세라시아 봉와직염(Serratia cellulitis), 급성 및 만성 스티벤슨 존슨 신드롬(Stevenson Johnson syndrome)을 포함하는, 하지만 이에 한정되지 않는, 안구 표면 질환의 치료 및/또는 예방에 유용하다. 본 발명의 콜라겐 생구조물은 특히 궤양이 있는 저항성 표피 장애의 치료를 촉진; 상피화를 촉진; 상피 및 줄기세포의 성장을 촉진; 염증 및 통증의 감소; 혈관신생 및 홍터화의 억제; 상피 표현형의 회복에 있어 유용하고, 익상편(ptyerygia)의 "노출 공막(bare sclera)" 제거 동안 결막 자가이식물의 대안적 대체물로 유용하다.

[0147] 본 발명은 증상성(症狀性) 물질 각막병증(symptomatic bullous keratopathy)의 치료를 위해, 바람직하게는 인간에 있어, 더욱 바람직하게는 시력이 나쁜 인간에 있어 본 발명의 콜라겐 생구조물을 사용하는 이식을 포함한다. 본 발명의 콜라겐 생구조물은 증상성 물질 각막병증의 치료, 특히 결막 플랩 구축(conjunctival flap construction)을 위해 본 발명이 속한 분야에서 사용되는 다른 표준 절차와 비교하여 향상된 치료적 및 임상적 유용성을 가질 것으로 기대된다. 증상성 물질 각막병증의 치료, 특히 결막 플랩 구축을 위해 본 발명이 속한 분야에서 사용되는 표준 절차에 비해 본 발명의 콜라겐 생구조물을 사용하는 이식의 이점은 예를 들어 통증의 감소, 수술의 편리함, 미용학적으로 좋은 외형, 및 눈꺼풀 처짐 및 림발(limbal) 줄기세포 결핍과 같은 합병증의 감소를 포함한다. 본 발명의 콜라겐 생구조물은 궤양이 있는 각막 표피 상해의 치료를 촉진하기 위한 결막 플랩의 향상된 대안물; 검구유착(symbelpharon) 용해에 대한 결막 표면 재건을 위한 향상된 방법; 결막 또는 각막 표면의 종양, 병변, 또는 상흔 조직의 외과적 제거를 위한 향상된 방법; 결합 수포 누출에 의한 녹내장 수술을 위한 향상된 방법; 익상편(ptyerygia)의 "노출 공막(bare sclera)" 제거 동안 결막 자가이식물의 대안적으로 향상된 대체물; 및 때 각막병증의 재발 방지를 위한 향상된 방법을 제공한다.

[0148] 본 발명은 또한 안과영역의 외과적 이식물로 생구조물을 사용하는 것을 제공한다. 본 발명은 적용 대상에 부착될 때 그 대상에게 전달될 수 있는 하나 이상의 치료 성분을 더 함유하는 본 발명의 생구조물을 포함하는 이식

조제물을 포함한다. 본 발명의 생구조물을 사용하여 전달될 수 있는 치료적 성분의 예는 펠로카핀, 에리스로마 이신, 겐타미신, 반코마이신, 토브라마이신, 네틸마이신, 폴리믹신 B 설페이트, 트리메토프림, 암포테리신 B, 항-암제, 항생제, 사이클로스포린 등을 포함하지만 이에 한정되는 것은 아니다.

[0149] 본 발명의 콜라겐 생구조물은 또한 엑시머 레이저 사진굴절의/치료의(photorefractive/therapeutic) 각막절제술에 의해 야기된 각막혼탁을 줄이는데 유용하다.

[0150] 안과 수술에서 사용하기 위한 본 발명의 콜라겐 생구조물은 인서트, 쉴드(shield), 입자(particle), 겔, 수성 주사, 스폰지, 필름을 포함하는, 하지만 이에 한정되지 않는, 다양한 구성으로 제공될 수 있다.

[0151] 본 발명이 속한 분야에서 통상의 지식을 가진 자에게 알려진 안과 이식을 위한 통상의 외과적 기술은 본 발명 내에 포함된다.

[0152] 안과영역 외과적 이식물로 본 발명의 콜라겐 생구조물을 사용하기 위한 예시적 프로토콜은 다음의 단계를 포함한다: 질병이 있는 각막 및/또는 결막 조직이 제거된다; 본 발명의 방법에 따라 제조된 외과적 이식물이 멸균 상태에서 제공되고, 각막 표피 또는 노출 공막을 덮기 위하여 free hand 테크닉에 의하여 손질된다. 봉합을 사용하여, 이식물은 노출 공막 및 결막의 접합부위에 연결된다; 기본 봉합이 행하여진다; 그 후 끊긴 봉합이 이식 표면 위에 긴장을 부여하기 위하여 사용된다; 그래서 이식물과 적용 대상의 공막 또는 각막 사이의 일정 영역은 피하여 진다. 이식물과 호스트의 연결부위에서, 공여 조직과 연결된 지점의 기초 라미나(lamina) 위로 호스트 표피가 슬라이딩하도록 하기 위하여 적용 대상 결막 아래로 이식물의 자유 모서리(free edge)가 있어야 한다. 그렇지 않다면 이식물은 적용 대상에 의해 배출된다.

[0153] 5.4.2.2 치과(Dental)

[0154] 본 발명의 콜라겐 생구조물은 예를 들어 치주 수술, 치주 조직의 재생성을 위한 조직의 유도 재생성, 유도된 뼈 재생성 및 치근 덮음과 같은 치과 분야에서 특히 유용하다. 본 발명은 양쪽 매치 치주 결함, 치아 사이 내 뼈 결함(interdental intrabony defect), 깊은 3-벽(wall) 내 뼈 결함, 2-벽 내 뼈 결함, 및 내 뼈 결함 2 및 3을 포함하는, 하지만 이에 한정되지 않는, 치주 내 뼈 결함(periodontal intrabony defect)의 재생성을 촉진하기 위하여 본 발명의 콜라겐 생구조물을 사용하는 것을 포함한다. 본 발명의 콜라겐 생구조물은 예를 들어 Quteish et al., 1992, J. Clin. Periodontol. 19 (7): 476-84; Chung et al., 1990, J. Periodontol. 61 (12): 732-6; Mattson et al., 1995, J. Periodontol. 66 (7): 635-45; Benque et al., 1997, J. Clin. Periodontol. 24 (8): 544-9; Mattson et al., 1999, J. Periodontol. 70 (5):510-7에 개시된 것과 같은 가교된 콜라겐 막을 사용하는 등의 본 발명이 속한 분야에 알려진 다른 기술과 비교하여 치주 내 뼈 결함의 치료에 있어 향상된 치료적 유용성 및 향상된 임상적 파라미터를 가질 것으로 기대된다. 본 발명의 콜라겐 생구조물을 사용하여 향상된 임상적 파라미터의 예는 플라크 및 잇몸 인덱스 스코어링, 포켓 깊이(pocket depth)의 검사, 부착 깊이(attachment depth)의 검사 및 분기 관련성(furcation involvement)과 뼈 결함의 분류를 포함하지만 이에 한정되는 것은 아니며, 이들은 당업자에게 잘 알려져 있다.

[0155] 본 발명은 또한 양쪽 결함(bilateral defect), 양쪽 볼 클래스 II 턱뼈 어금니 분기 결함(paired buccal Class II mandibular molar furcation defects) 및 양쪽 턱뼈 분기 결함(bilateral mandibular furcation defect)을 포함하는, 하지만 이에 한정되지 않는, 클래스 II 분기 결함을 치료하기 위하여 본 발명의 콜라겐 생구조물을 사용하는 것을 포함한다. 클래스 II 분기 결함을 치료하는데 있어서 본 발명의 콜라겐 생구조물의 유용성은 부분적으로 분기 결함 내 없어진 치주를 재생성하는 그것의 능력으로 설명될 수 있다. 본 발명의 콜라겐 생구조물은 Paul et al., 1992, Int. J. Periodontics Restorative Dent. 12: 123-31; Wang et al., 1994, J. Periodontol. 65: 1029-36; Blumenthal, 1993, J. Periodontol. 64: 925-33; Black et al., 1994, J. Periodontol. 54: 598-604; Yukna et al., 1995, J. Periodontol. 67: 650-7에 개시된 것과 같은 클래스 II 분기 결함을 치료하는데 있어 본 발명이 속한 분야에서 사용된 콜라겐 막과 비교하여 향상된 치료적 및 임상적 유용성을 가지리라 예상된다.

[0156] 본 발명은 또한 치근(root) 덮음 시술에 있어 본 발명의 콜라겐 생구조물을 사용하는 것을 포함한다. 치근 덮음(root coverage)에서 본 발명의 생구조물의 유용성은 부분적으로 가이드된 조직 재생성의 원리에 기초한 없어진, 손상된 또는 질병 있는 잇몸 조직을 대체하는 그것의 능력으로 설명될 수 있다. 본 발명의 생구조물은 여러 이유로 위에서 인용되었던 Shieh et al., 1997 J. Periodontol., 68: 770-8; Zahedi et al., 1998 J. Periodontol. 69: 975-81; Ozcan et al., 1997 J. Marmara Univ. Dent. Fa. 2: 588-98; Wang et al., 1997 J.

Dent. Res. 78 (Spec Issue): 119 (Abstr. 106)에 개시된 것과 같은 치근 덮음에 전통적으로 사용되어온, 본 발명이 속한 분야의 콜라겐 막과 비교하여 향상된 임상적 유용성을 가질 것으로 기대된다.

[0157] 본 발명은 또한 치주염 및 치은염(gingivitis)을 포함하는, 하지만 이에 한정되지 않는, 치주 질환을 가진 개체에 콜라겐 생구조물을 사용하는 것을 포함한다. 본 발명의 생구조물은 또한 스케일링 및 치근 계획 기술(root planning procedures)에 있어 부속물(adjunct)로써 임상적 유용성을 가진다. 본 발명은 본 발명의 콜라겐 생구조물을 이용하여 치주 질환이 있는 개체를 치료하는 것을 포함한다. 본 발명의 콜라겐 생구조물을 사용하여 개체의 치주 질환을 치료하는 예시적 방법은 예를 들어 5mm 이상의 크기를 가진 하나 이상의 치주 포켓 내로 콜라겐 생구조물을 삽입하는 것을 포함하며, 이 경우 바람직하게 콜라겐 생구조물은 클로헥시딘 글루코네이트와 같은 항생제를 함유한다. 바람직하게 콜라겐 생구조물은 생분해된다.

[0158] 치과학에서의 사용을 위한 본 발명의 콜라겐 생구조물은 치료되는 치과 장애의 타입에 따라 하나 이상의 생체분자를 함유할 수 있다. 치과 장애를 치료하는데 본 발명이 속한 분야에서 알려진 어떠한 생체분자도 본 발명의 방법 및 조성물에 포함된다. 특정 실시예에서, 감염과 관련된 치과 장애를 치료하기 위해 사용된 콜라겐 생구조물은 독사이클린, 테트라사이클린, 클로헥시딘 글루코네이트, 및 미노사이클린을 포함하는, 하지만 이에 한정되지 않는, 하나 이상의 항생제를 함유할 수 있다.

[0159] 5.4.2.3 신경병(neurology)

[0160] 본 발명의 콜라겐 생구조물은 또한 손상된 신경을 회복하는데, 특히 심각한 말초 신경을 회복하는데, 및 신경외과 수술에 있어 유용하다. 본 발명은 예를 들어 경질(dural) 대체 및 말초 신경 회복에 있어 콜라겐 생구조물을 사용하는 것을 포함한다. 본 발명의 콜라겐 생구조물은 여러 이유로 위에서 언급된 예를 들어 Berger et al., 1970, Acta. Neurochir. 23: 141; Ducker et al., 1968, Mil. Med. 133: 298; 미국특허 제4,778,467호; 제4,883,618호; 제3,961,805호; 제5,354,305호와 같은 본 발명이 속한 분야에서 사용된 다른 방법들과 비교하여 경질 대체물로써 또는 신경 회복에 있어 향상된 임상적 유용성을 가진다.

[0161] 몇몇 실시예에서, 본 발명의 콜라겐 생구조물은 신경 연결 주위에 인공삽입물로써 사용될 수 있다. 예를 들어 그것은 말초 신경 연결을 둘러싸기 위해 사용될 수 있다. 본 발명의 콜라겐 생구조물은 예를 들어 회복 영역을 가로질러 세로적(longitudinal) 결합 조직 뼈대의 형성을 촉진하고, 따라서 집(sheath) 세포 및 축삭(axonal) 재생의 세로적 패턴(longitudinal pattern)을 향상함으로써 특히 신경 회복에 유용하다.

[0162] 말초 신경 회복은 일반적으로 네로라피(nerorrhapy, Jennings et al., 1955, Surgery: 206)로 알려진 수술에서 봉합을 이용하여 이루어진다. 그러나 이러한 접근법은 심각하게 손상된 신경을 봉합하는 방법이 어렵기 때문에 제한적 성공을 거두었다. 본 발명의 콜라겐 생구조물은 따라서 신경 회복에 있어 봉합 없는 방법의 대안을 제공하고, 그것에 의하여 예를 들어 신경 말단은 본 발명의 생구조물로 이루어진 관(tubular) 보철 장치 내로 예워싸인다. 따라서 심각한 말단을 재생하기 위하여 서로 근접시킨다.

[0163] 5.4.2.4 비뇨기학(urology)

[0164] 본 발명의 콜라겐 생구조물은 특히 요실금의 교정에 특히 유용하다. 요실금은 저장 동안 요도가 막혀지지 않아 발생한다. 그 원인은 외인적일 수 있는데, 예를 들어 요도 및 방광 목(neck)의 부실한 해부학적 뒷받침이 요실금(incontinence)과 골반저부의 중량감(resuspension)에 대한 반응성을 가져온다. 요도 기능 상실은 한편으로 내인성일 수 있다. 즉 나쁜 요도 기능(urethral function) 때문일 수 있다. 전통적으로, 요실금은 콜라겐, 지방, 실리콘과 같은 팽화 성분(bulking agent)를 사용하여 교정된다(review Lightner, 2002, Current Opinion in Urology, 12 (4): 333-8 참조). 본 발명의 콜라겐 생구조물은 본 발명이 속한 분야에 알려진 팽화 성분과 비교하여 향상된 유용성 즉 자체력을 향상시키는 유용성을 가진다. 더욱이 본 발명의 콜라겐 생구조물은 예를 들어 감소된 국소 합병증, 감소된 요도관 감염, 피이식체의 반응 부재(no host reaction), 믿을만한 내구성, 및 높은 안전성과 같은 향상된 치료적 유용성을 가진다.

[0165] 요실금을 교정하는데 있어 본 발명의 콜라겐 생구조물의 유용성은 부분적으로 본 명세서에서 설명된 콜라겐 생구조물의 독특한 물리적 형태, 특히 그것의 비-면역원성 때문이다. 몇몇 실시예에서, 콜라겐 생구조물은 예를 들어 요도 임플란트와 같이 이식물로 사용된다. 비록 어떠한 특정 이론에 의해 본 발명이 한정되도록 의도된 것은 아니지만, 본 발명의 콜라겐 생구조물은 예를 들어 요도 폐쇄 압력 및 소변의 수동적 누출에 대한 저항성을 증가시킴으로써 향상된 치료적 유용성을 가질 수 있다.

5.4.2.5 정형외과학(orthopedics)

본 발명의 콜라겐 생구조물은 정형외과 수술 시술에 사용될 수 있다. 일정 실시예에서, 콜라겐 생구조물은 예를 들어 후천적 또는 선천적 결합과 같은 정형외과적 결합에 대하여 사용될 수 있다. 외상에서 오는 국소 결합의 재건과 뼈 증양의 외과적 복구(resurrection)는 정형외과 또는 턱얼골 수술에 있어 중요한 문제이다. 전형적으로, 합성 뼈 대체물 또는 콜라겐 막이 부분적으로 그들의 뼈 유도(osteoinductive) 활성 때문에 사용되어 왔다 (예를 들어 Rao et al., 1995, J. Biomater. Sci. Polymer Edn. 7 (7): 623-45 참조). 그러나 일반적 문제는 이식된 물질의 결과인 전신적 감염이다. 본 발명의 콜라겐 생구조물은, 그러나, 특히 정형외과 결합에서 사용된 뼈 대체물로써의 낮은 면역원성 때문에 본 발명이 속한 분야에서 사용되는 물질에 비하여 유리하다. 몇몇 실시예에서, 콜라겐 생구조물은 힘줄, 인대 및 연골을 재건하기 위한 인공삽입물(prosthesis)로 사용될 수 있다. 다른 실시예에서, 본 발명의 콜라겐 생구조물은 뼈 대체물로써의 인공삽입물로 사용될 수 있다.

5.4.2.6 심장혈관 수술

본 발명의 콜라겐 생구조물은 예를 들어 큰 및 작은 혈관을 구성하기 위한 인공삽입물로써; 혈관의 선천적 기형 및 질병이 있는 밸브를 회복하기 위하여 심장혈관 외과 시술에서 사용될 수 있다.

본 발명의 콜라겐 생구조물은 특히 예를 들어 정맥 또는 동맥 이식과 같은 혈관 수술에서 이식물로써 유용성을 가진다. 혈관 수술에서 생구조물의 유용성은 부분적으로 그것의 비-독성, 비-면역원성; 안전성; 취급용이성; 항-트롬보제닉 특성; 혈관 벽의 최소 이식 구멍(minimal implantation porosity); 다양한 크기의 가능성; 물질의 재현성 때문이다. 몇몇 실시예에서, 본 발명의 생구조물은 밸브 대체물로 사용될 수 있다.

5.4.2.7 약물 전달

본 발명의 콜라겐 생구조물은 예를 들어 치료 성분과 같은 약물의 조절 전달을 위한 약물 전달 운반체로 사용될 수 있다. 몇몇 실시예에서, 콜라겐 생구조물은 하나 이상의 치료성분을 개체, 바람직하게는 인간에게 전달한다. 본 발명의 범주 내에 포함된 치료 성분은 단백질, 펩타이드, 다당류, 다당류 접합체(conjugate), 유전자 기반 백신, 생 약화 백신, 전체의 세포들이다. 본 발명의 방법에서 사용되는 약물의 비-한정적 예는 항생제, 항-암제, 항-박테리아제, 항-바이러스제; 백신; 마취제; 진통제; 항-천식제; 항-염증 치료제; 항-우울제; 항-관절염 치료제; 항-당뇨 치료제; 항-정신병제; 중추 신경계 자극제; 호르몬; 면역-억제제; 근 이완제; 프로스타글란딘이다.

콜라겐 생구조물은 개체, 특히 인간에게 하나 이상의 작은 분자의 조절 전달을 위한 전달 운반체로 사용될 수 있다. 몇몇 실시예에서, 콜라겐 생구조물은 개체, 특히 인간에게 하나 이상의 작은 분자를 전달한다. 여기에서 사용된 것처럼, 용어 "작은 분자" 및 유사한 용어는 몰 당 약 10,000그램 보다 작은 분자량을 가진 펩타이드, 펩타이드 유사체(peptidomimetic), 아미노산, 아미노산 유사체(analog), 폴리뉴클레오티드, 폴리뉴클레오티드 유사체, 뉴클레오티드, 뉴클레오티드 유사체, 유기 또는 무기 화합물(즉 헤테로유기화합물 및 유기금속 화합물을 포함함), 몰 당 약 5,000그램보다 작은 분자량을 가진 유기 또는 무기 화합물, 몰 당 약 1,000그램보다 작은 분자량을 가진 유기 또는 무기 화합물, 몰 당 약 500그램 보다 작은 분자량을 가진 유기 또는 무기 화합물, 몰 당 약 100그램보다 작은 분자량을 가진 유기 또는 무기 화합물 및 그러한 화합물의 염, 에스터, 및 다른 약학적으로 허용 가능한 형태를 포함하나 이에 한정되는 것은 아니다. 그러한 화합물의 염, 에스터, 및 다른 약학적으로 허용 가능한 형태 또한 포함된다.

일정 실시예에서, 약물 전달을 위한 운반체로서의 본 발명의 콜라겐 생구조물은 본 발명이 속한 분야에서 알려진 다른 약물 전달 시스템에 비하여 약물의 향상된 흡수; 개선된 약동학적 프로파일, 및 약물의 전신적 분포를 가져온다. 개선된 약동학에 있어, 약동력학적 프로파일의 개선은 예를 들어 최고 혈장 농도에 도달하는 시간(T_{max}); 최고 혈장 농도의 크기(C_{max}); 검출 가능한 혈액 또는 혈장 농도를 나타내는 시간($T_{1/3}$)과 같은 표준 약동력학적 파라미터로 측정된다. 향상된 흡수에 있어, 약물의 흡수는 그러한 파라미터로 측정되는 것처럼 향상된다. 약동력학적 파라미터의 평가는 본 발명이 속한 분야에서 통상적으로 수행된다.

실시예

6.1 콜라겐 생구조물을 만드는 방법

- [0180] **물질(Materials)**
- [0181] 다음의 물질들이 콜라겐 생구조물의 제조에 사용된다.
- [0182] 물질/장치
- [0183] * 전달 기록의 사본(Copy of Delivery Record)
- [0184] * 물질/가족 병력/동의서의 사본(Copy of Material/Family Health History/Informed Consent)
- [0185] * 소스 바코드 라벨(Source Bar Code Label) (공여자 ID 번호)
- [0186] * 콜렉션 번호 (투입 물질에 지정되는 연속 숫자)
- [0187] * 조직 프로세싱 기록(서류 ID #ANT-19F); 각 롯트 숫자의 프로세싱의 자세한 기록이 유지됨.
- [0188] * 인간 태반 (프로세싱의 시작 전 48 시간이 넘지 않은)
- [0189] * 멸균 외과수술용 클램프/지혈제
- [0190] * 멸균 가위
- [0191] * 멸균 외과용 메스(Scalpel)
- [0192] * 멸균 세포 스크래퍼(Nalgene NUNC Int. R0896)
- [0193] * 멸균 거즈(비-멸균 PSS 4416, 멸균된)
- [0194] * 멸균 린싱(rinsing) 스테인리스 스틸 트레이
- [0195] * 소독된 프로세싱 스테인리스 스틸 트레이
- [0196] * 소독된 플라스틱 주머니
- [0197] * 멸균 0.9% NaCl 용액 (Baxter 2F7124)
- [0198] * 멸균수 (Milli Q plus 09195 또는 Baxter 2F7113)
- [0199] * 멸균 표본 저장용기 (VWR15704-014)
- [0200] * 개인 보호 장비 (멸균 및 비-멸균 장갑을 포함)
- [0201] * 인증된 클린 룸
- [0202] * 미리 제조된 탈세포 용액 (D-cell); 0.01-1% 테옥시콜릭 산 소디움 일수화물
- [0203] * 소독된 주머니
- [0204] * 흔들리는 플랫폼 (VWR Model 100)
- [0205] * 타이머 (VWR 21376890)
- [0206] * 소독된 플라스틱 플레임 메쉬
- [0207] * PVC 랩 필름
- [0208] * 진공 펌프 (Schuco-Vac 5711-130)
- [0209] * 겔 건조기 (즉 열 건조기; BioRad Model 583)
- [0210] * 소독된 스테인리스 스틸 절단 보드
- [0211] * 포장을 위한 파우치
- [0212] * 멸균 스테인리스 스틸 자 (General Tools MFG. Co 1201)
- [0213] * 추적 가능한 디지털 열측정기 (Model 61161-364, Control Company)
- [0214] * Accu-Seal 자동 밀봉기 (Accu-Seal, Model630-1B6)
- [0215] 산모는 분만 시에 HIV, HBV, HCV, HTLV, 매독, CMV 및 모아지는 태반 조직을 오염시킬 수 있는 다른 바이러스,

및 다른 병원체와 같은 전염 가능한 질환이 조사되었다. 시험된 산모가 위에서 언급된 병원체에 대하여 음성 또는 비-반응성인 경우 공여자로부터 모아진 다른 조직들이 콜라겐 생구조물을 제조하기 위해 사용되었다.

[0216] 정상적 출산 후에, 태반, 탯줄 및 탯줄 혈액은 자발적으로 수축하는 자궁으로부터 방출된다. 태반, 탯줄, 및 탯줄 혈액은 출생 후에 모아졌다. 그 물질들은 실험실로 운반되고, 실험실에서 그것들은 프로세싱의 적어도 1시간 전부터 가동된 HEPA 여과 시스템을 사용한 클린 룸 내 무균 상태에서 처리되었다. 그 물질을 다루는 모든 경우에 있어 장갑(멸균 또는 비-멸균 장갑을 적절히 사용)을 착용하였다. 조직 프로세싱 동안 생성된 양막/용모막의 모든 사용되지 않은 (폐기) 부분 및 오염된 용액은 가능한 빨리 처리되었다.

[0217] 단계 I

[0218] 멸균 필드는 멸균 Steri-Wrap 시트로 설치되었고, 프로세싱을 위한 다음의 기구와 액세서리가 그 위에 놓아졌다.

[0219] * 멸균 트레이 팩

[0220] * 멸균 세포 스크래퍼

[0221] * 멸균 외과용 메스

[0222] * 소독된 프로세싱 트레이

[0223] 멸균 팩 ID #는 프로세싱 레코드에 기록되었다.

[0224] 태반이 운반 용기로부터 분리되고 소독된 스테인리스 스틸 트레이에 놓여졌다. 외과용 클램프와 가위를 사용하여 탯줄이 태반 디스크로부터 약 2 인치가 되도록 잘랐다. 탯줄은 나중의 프로세싱을 위하여 분리된 멸균 용기에 놓여졌다. 용기는 조직 ID 바코드로 표지되었고; 존재하는 물질 및 보관 용액 (즉 매체의 타입)이 확인되었다. 몇몇 케이스에서, 탯줄은 다른 프로젝트에서 요구되지 않는 경우 버려졌다.

[0225] 태반 막의 모서리로부터 시작하여, 손가락으로 비절개박리하여 용모막으로부터 양막을 분리하였다. 이것은 막을 절단하기에 앞서 수행되었다.

[0226] 양막을 용모막의 전체 표면 및 태반 디스크로부터 분리한 후에, 양막은 가위를 사용하여 탯줄 스텝프(stump) 주위로 잘려졌고 태반 디스크로부터 분리되었다. 몇몇 예에서, 양막과 용모막의 분리가 조직을 손상시키지 않고 가능하지 않다면 양막과 용모막은 태반 디스크로부터 하나의 조각으로 잘려지고, 그 후 따로 분리하며 벗겨졌다.

[0227] 용모막은 분리된 표본 용기에 다른 프로젝트에서 사용하기 위하여 놓여졌다. 용기는 조직 ID 바코드로 표지되고, 존재하는 물질 및 보관 용액 (즉 매체의 타입)이 확인되고, 서명되고, 날짜가 기입되었다.

[0228] 양막의 일정 조각이 태반 디스크에 아직 부착되어 있는 경우, 그것은 디스크로부터 벗겨지고 가위를 사용하여 탯줄 주위에서 잘려졌다. 태반은 다른 프로젝트에서 사용하기 위하여 운송 용기에 다시 놓여졌다.

[0229] 적절한 데이터가 조직 프로세싱 레코더에 기록되었다.

[0230] 양막은 멸균 0.9% NaCl 용액의 트레이에 보관되었다. 바람직하게는, 양막은 프로세스의 다음 단계에 앞서 전달 시간으로부터 최대 72시간 동안 동결되어 저장된다.

[0231] 단계 II

[0232] 양막은 한번에 하나씩 표본 용기로부터 분리되고, 소독된 스테인리스 스틸 트레이에 놓여진다. 다른 조각들은 그들이 세척을 위해 준비될 때까지 멸균수로 채워진 분리된 멸균 스테인리스 스틸 트레이에 놓여진다. 프로세싱 트레이의 양막의 여분 조각은 제거되고 멸균수로 채워진 분리된 린싱 스테인리스 스틸 트레이에 놓여진다.

[0233] 양막이 어머니 혈액 또는 태아 액/물질로 많이 오염된 경우 양막은 필요한 만큼 멸균수를 교환하여 멸균수로 행구어졌다.

[0234] 양막은 어머니쪽 부위가 위로 향하게 프로세싱 트레이에 놓여졌다. 멸균 세포 스크레이퍼를 사용하여, 양막의 어머니쪽 면에서 세포성 물질과 육안으로 관찰되는 오염물질을 가능한 많이 주의깊게 제거하였다. (참조: 막이

찢어지는 것을 막기 위하여 이번 단계에서는 최소 압력이 적용되어야 한다) 멸균수가 세포 및 세포 파편의 제거를 돕기 위해 사용되었다. 양막은 분리된 멸균 스테인리스 스틸 린싱 트레이에서 멸균수를 사용하여 더 행구어졌다.

- [0235] 양막을 뒤집어 태아쪽이 위로 향하게 하고, 프로세싱 트레이에 다시 놓고 멸균수로 행구었다. 육안으로 보이는 세포성 물질 및 파편들을 세포 스크래퍼를 사용하여 부드럽게 제거하였다. (참조: 막이 찢어지는 것을 막기 위하여 이번 단계에서는 최소 압력이 적용되어야 한다) 멸균수가 세포 및 세포 파편의 제거를 돕기 위해 사용되었다.
- [0236] 양막은 분리된 멸균 린싱 트레이에서 세척 라운드(rounds) 사이에 멸균수로 행구어졌다. 모든 육안으로 보이는 세포 물질 및 파편들이 막의 양쪽부위에서 제거되지 않은 경우 대부분을 제거하기 위하여 필요한 만큼 많이 조직은 세척되었다. 멸균수는 행굼 사이에 린싱 트레이에서 교환되었다.
- [0237] 프로세싱 트레이는 각각의 세척 라운드 후에 멸균수로 행구어졌다.
- [0238] 양막의 모든 다른 조각들이 동일한 방법으로 조각되었고, 동일한 용기 내에 놓여졌다. 조직 ID 바코드가 첨부되고, 존재하는 물질 및 보관 용액 (즉 매체의 타입)이 확인되고, 서명 데이터가 첨부되었다.
- [0239] 적절한 정보 및 데이터가 조직 프로세싱 레코더에 기록되었다.
- [0240] **단계 III**
- [0241] 양막은 린싱 트레이(또는 보관 용기)로부터 분리되었고, 여분의 용액은 손가락으로 부드럽게 짜서 제거하였고, 막은 멸균 표본 용기에 놓여졌다. 용기는 양막의 모든 부분이 덮여지도록 150ml 마크까지 D-세포 용액으로 채워졌고, 밀폐하였다.
- [0242] 용기는 흔들리는 플랫폼 위 주머니(bin) 내에 놓여졌다. 흔들리는 플랫폼은 켜지고, 막은 최소 15분 및 최대 120분 동안 #6 세팅으로 D-세포 용액 내에서 교반되었다.
- [0243] 새로운 멸균 필드가 단계 I에서와 동일한 방법으로 새로운 멸균 장치와 소독된 트레이를 이용하여 설치되었다. 멸균 팩 ID 숫자는 프로세싱 레코더에 기록되었다.
- [0244] 교반이 완료된 후에, 흔들리는 플랫폼은 꺼졌고, 막은 용기에서 꺼내었다. 막은 새로운 멸균 스테인리스 스틸 프로세싱 트레이 내에 놓여졌다. 멸균 0.9% NaCl 용액이 트레이의 바닥을 덮기 위하여 첨가되었다.
- [0245] 새로운 멸균 세포 스크래퍼를 사용하여, 잔여 D-세포 및 세포성 물질(만약 존재한다면)이 조직의 양면으로부터 제거되었다. 이러한 단계는 양쪽 면의 전체 표면으로부터 육안으로 보이는 잔여 세포성 물질을 가능한 많이 제거하기 위하여 필요한 만큼 수회 반복되었다. 막은 세척 라운드 사이에 멸균 린싱 트레이 내에서 멸균 0.9% NaCl 용액으로 행구어졌다. 멸균 0.9% NaCl 용액은 행굼 사이에 린싱 트레이 내에서 교환되었다.
- [0246] 마지막 세척 라운드가 완결된 후에, 막은 멸균 0.9% NaCl 용액으로 행구어졌고, 멸균 0.9% NaCl 용액으로 채워진 새로운 멸균 표본 용기 내에 놓여졌다.
- [0247] 양막의 모든 남은 조각이 완전히 동일한 방법으로 처리되었다.
- [0248] 모든 양막 조각이 처리되고 멸균 0.9% NaCl 용액의 용기 내에 놓여졌을 때, 용기는 #6 세팅으로 최소 5분 동안 교반되기 위하여 흔들리는 플랫폼 위 주머니 내에 놓여졌다. 교반이 완결된 후에, 막은 표본 용기에서 꺼내지고, 멸균 0.9% NaCl 용액이 용기 내에서 교환되고, 막은 다시 표본 용기 내에 놓여졌다.
- [0249] 표본 용기는 조직 ID 바코드와 격리 라벨로 표시되었다. 존재하는 물질 및 보존 용액 (즉 용액의 타입)은 확인되고, 서명되고 및 날짜가 기입되었다. 표본 용기는 깨끗한 zip-밀폐 용기 내에 놓여지고, 냉장고(2-8℃) 내에 놓았다.
- [0250] 모든 적절한 데이터는 조직 프로세싱 레코드 내에 기록되었다.
- [0251] 혈청검사 결과가 나왔을 때, 적절한 표지(혈청 음성 또는 단지 연구용도용)가 격리 라벨 위에 놓여졌고, 그러한 용기는 격리된 것들로부터 분리되었다.

- [0252] **단계 IV**

- [0253] 단계 IV를 진행하기 전에, 조직 상태 검토(Tissue Status Review)가 모든 이용 가능한 시험 결과가 음성인지 확인하기 위하여 이루어졌다.
- [0254] 멸균 필드가 멸균 Steri-Wrap 시트를 사용하여 세팅되었고, 모든 멸균 및 소독된 장치와 액세서리가 단계 II 및 III와 동일한 방식으로 세팅되었다.
- [0255] 막은 냉장고에서 꺼내지고, 멸균 스테인리스 스틸 프로세싱 트레이 내에 놓여졌다. 멸균 0.9% NaCl 용액이 트레이의 바닥을 덮기 위하여 첨가되었다.
- [0256] 모든 육안으로 보이는 세포성 물질 및 파편(만약 있다면)이 새로운 멸균 세포 스크레이퍼를 이용하여 부드럽게 제거되었다. (참조: 막이 찢어지는 것을 막기 위해 이 단계에서는 최소 압력이 적용되어야 한다) 멸균 0.9% NaCl 용액이 세포 및 파편의 제거를 돕기 위해 사용되었다.
- [0257] 막은 멸균 0.9% NaCl 용액으로 채워진 분리된 멸균 스테인리스 스틸 린싱 트레이 내에서 행구어졌다. 멸균 0.9% NaCl 용액은 세척 라운드 사이에 교환되었다. 막은 새로운 멸균 표본 용기에 놓아졌고, 용기는 새로운 멸균 0.9% NaCl 용액으로 채워졌고, #6 세팅으로 최소 5분 동안 진탕하기 위하여 흔들리는 플랫폼 위에 놓아졌다.
- [0258] 앞 단계가 3회 반복되었고, 멸균 0.9% NaCl 용액이 각 진탕 사이에 교환되었다. 적절한 데이터가 조직 프로세싱 레코드에 기록되었다.
- [0259] 막은 표본 용기에서 한번에 한 조각씩 꺼내어졌고, 과량의 액은 손가락을 이용하여 부드럽게 짜서 제거하였고, 막은 멸균 프로세싱 트레이에 놓아졌다. 막은 평평해질 때까지 부드럽게 눌러졌다; 태아쪽 면이 아래로 향하도록 확실히 하였다.
- [0260] 프레임은 멸균 가위를 이용하여 소독된 플라스틱 시트를 잘라 준비되었다. 프레임의 크기는 막 세그먼트 보다 각 방향에 있어 약 0.5cm는 작아야 한다. 프레임은 멸균 0.9% NaCl 용액으로 채워진 린싱 트레이 내에서 행구어졌다.
- [0261] 프레임은 가볍게 늘려진 막 표면 위에 놓여졌고, 그 위에 부드럽게 압력을 가하였다. 플라스틱 프레임의 부드러운 면이 조직을 향하도록 하는 것은 중요하다.
- [0262] 외과용 메스를 사용하여, 막은 프레임 모서리부터 약 0.5cm가 남도록 프레임 주위로 잘려졌다. 과량의 막은 표본 용기 내에 다시 놓여졌다.
- [0263] 프레임을 벗어내는 막 모서리는 클램프 또는 족집게를 사용하여 프레임의 모서리 위로 감싸졌고, 동일한 트레이 위에 놓아졌다.
- [0264] 막의 다른 조각은 동일한 방법으로 처리되었다. 건조되는 총 면적이 열 건조기 당 300cm²을 초과하지 않는 것이 중요하다. 막의 조각을 '프레임 아웃(framing out)'하는 동안, 비-프레임 조각은 용기 내 멸균 0.9% NaCl 용액 내에 있어야 한다.
- [0265] 드라이어의 건조 온도는 확장 프로브를 가진 캘리브레이트된 디지털 온도계를 사용하여 세팅되고, 확인되었다. 건조 온도는 50℃로 세팅되었다. 데이터는 조직 프로세싱 레코드에 기록되었다.
- [0266] 진공 펌프는 켜졌다.
- [0267] 멸균거즈는 열 건조기의 건조 플랫폼 위에 놓여졌고, 프레임된 막의 영역 보다 약간 넓은 영역을 덮었다. 거즈 층의 총 두께가 한번 접혀진 4x4 거즈의 두께를 초과하지 않는 것이 중요하다.
- [0268] 플라스틱 프레임(framing) 메쉬 한 시트가 거즈 위에 놓여졌다. 플라스틱 메쉬 모서리는 거즈 모서리보다 약 0.5-1.0cm 초과하여야 한다.
- [0269] 프레임된 막은 부드럽게 들어올려져 막 쪽이 위로 향하도록 열 건조기 플랫폼 위 플라스틱 메쉬의 위에 놓여졌다. 이것은 막의 최대 양(300cm²을 초과하지 않음)이 열 건조기 플랫폼 위에 놓여질 때까지 반복되었다. (참조: 양막의 태아쪽 면이 위로 향함)
- [0270] PVC 랩 필름이 열 건조기의 전체 건조 플랫폼과 그의 다리를 덮을 정도로 크게 잘려졌다.
- [0271] 진공 펌프를 작동시키고, 열 건조기의 전체 건조 플랫폼을 부드럽게 플라스틱 필름으로 덮어, 양쪽 면 건조 플랫폼 모서리를 넘어 다리의 반만 남도록 하였다. 주의 깊게 필름을 막 및 프레임 시트에 대하여 단단히 끌고

(즉, 그것은 진공에 의하여 "빨아들어졌다(sucked in)") 조직 영역에 공기 누출 및 주름이 없었다. 리드(lid)는 그 후 밀폐되었다.

[0272] 진공 펌프는 약-22 인치 Hg 진공으로 세팅되었다. 펌프 게이지는 건조 사이클의 2-3분 후에 기록되었다. 막은 약 60분 동안 열 진공 건조되었다. 건조 프로세스 내 약 15-30분, 멸균 거즈 층은 새로운 것으로 열 건조기 내에 놓여졌다. 거즈 층의 총 두께는 한번 접힌 4x4 거즈의 두께를 넘지 말아야 한다.

[0273] 교환 후, 주의 깊게 플라스틱 필름이 막 및 프레임 시트에 대하여 단단히 잡아당겨졌고, 막 영역에는 공기 누출 및 주름이 없었다.

[0274] 진공 밀봉의 완전성은 주기적으로 펌프 압력 모노미터를 체크하여 확인되었다. 건조 프로세스의 완결 후에, 열 건조기는 열려졌고 막은 펌프가 켜진 상태에서 약 2분 동안 식혀졌다.

[0275] 새로운 멸균 필드가 멸균 Steri-wrap을 이용하여 세팅되었고, 소독된 스테인리스 스틸 커팅 보드(board)가 그 아래 놓여졌다. 이 시점에서 멸균 장갑이 사용되었다. 펌프를 계속 작동시킨 상태에서, 플라스틱 필름이 코너에서 시작하여 막 시트로부터 부드럽게 제거되었고 막 시트를 장갑 낀 손아래에 유지하였다. 프레임이 막과 함께 건조 플랫폼에서 들어올려졌고 막쪽이 위로 향하도록 소독된 스테인리스 스틸 커팅 보드의 위 멸균 필드 위에 놓여졌다. 외과용 메스를 사용하여, 막 시트를 프레임의 모서리부터 1-2mm 떨어지도록 모서리를 따라 칼 새김(incision)을 내며 잘랐다. 막은 장갑 (멸균 장갑) 낀 손으로 위치 지어졌다. 막 시트를 그것을 부드럽게 벗겨내어 프레임에서 분리하였고, 커팅 보드 위 멸균 필드 위에 놓았다.

[0276] 외과용 메스 또는 날카로운 가위를 사용하여, 막 시트는 특정 크기의 세그먼트로 잘려졌다. 모든 조각은 잘려졌고, 포장 전에 멸균 필드 위에 안전하게 놓았다. 하나의 막 조각은 다른 한 손(비-멸균)으로 파우치를 잡고 있는 동안 다른 한 손(멸균)으로 이너(inner) 필-파우치(peel-pouch) 포장 내에 넣어졌다. '멸균' 손으로 파우치를 건드리지 않도록 주의하였다. 모든 조각이 이너 파우치 내로 넣어진 후, 그것들은 봉해졌다. 적절한 정보(예를 들어 파트 #, 롯트 # 등)가 있는 표지가 파우치의 바깥 지정된 위치에 첨부되었다. 모든 조각의 막이 동일한 방식으로 프로세스 되었다. 표지되고 밀봉된 필-파우치 포장은 그들이 멸균 시설 또는 분배자에게 운송되기 위해 준비될 때 까지 보관을 위하여 방수 집(zip)-락(lock) 가방에 넣어졌다. 모든 적절한 데이터가 조직 프로세싱 레코드에 기록되었다.

[0277] 6.2 수화/융합능력(suturability)에 대한 콜라겐 생구조물의 평가

[0278] 본 발명의 생구조물의 외과 수술적 취급성, 수화 기간 및 융합능력에 관하여 정성적 및 정량적 피드백을 얻기 위하여, 본 발명의 방법에 따라 제조된 양막 샘플이 4명의 경험 있고, 존경받는 눈표면 외과 의사들에게 평가를 위해 제공되어졌다. 샘플은 본 발명의 생구조물의 외과 수술적 취급 성질 및 융합능력을 결정하기 위하여 돼지 눈 표본 위에 조직 이식을 수행함으로써 외과 의사들에 의해 평가되었다.

[0279] 다음의 방법들이 각 외과 의사에 의해 사용될 수 있다.

[0280] (1) 돼지 눈의 사분역(quadrant) 하나에 알맞게 생구조물을 자른 후 건조한다.

[0281] (2) 잘려진 생구조물은 돼지 눈의 표면 위에 놓여진다.

[0282] (3) 생구조물은 멸균 생리식염수 용액으로 수화되고, 이식이 활성화되도록 된다. 즉 2, 5, 10 및 20분의 수화 기간동안 돼지 눈 위에서 재-수화된다.

[0283] (4) 생구조물은 여러 개의 9-0 비크릴(vicryl) 봉합 바이트(bite)로 돼지 눈의 표피에 봉합된다. 및

[0284] (5) 외과 의사는 수화된 양막의 조직 질(quality), 일치정도(consistency), 및 융합능력에 대하여 정성적 평가를 한다.

[0285] 균등물(Equivalents):

[0286] 본 발명은 본 명세서에서 설명된 특정 실시예에 의하여 그 범위가 한정되는 것은 아니다. 실로, 설명된 것들에 더하여 본 발명의 다양한 변형은 전술한 설명 및 도면들로부터 본 발명이 속한 분야에서 통상의 지식을 가진 자에게 자명하다. 그러한 변형은 다음의 청구항의 범위 내에 있도록 의도된 것이다.

[0287] 다양한 문헌, 특허 및 특허출원이 본 명세서에서 인용되었고, 그것의 개시들은 인용에 의하여 전체적으로 본 명세서에 포함된다.

도면의 간단한 설명

[0175] 도1은 인간 태반의 융모막 및 양막이다.

[0176] 도2의 A는 처리 전, B는 처리 후의 콜라겐 생구조물의 사진이다.

[0177] 도3은 콜라겐 생구조물이다. 본 발명의 콜라겐 생구조물은 양각(emboss)형태를 갖는 단일한 반투명 표면을 갖는 것으로 예시된다.

[0178] 도4는 메시 구조 및 그것의 건조된 생구조물 그림이다.

도면

도면1



FIG. 1

도면2

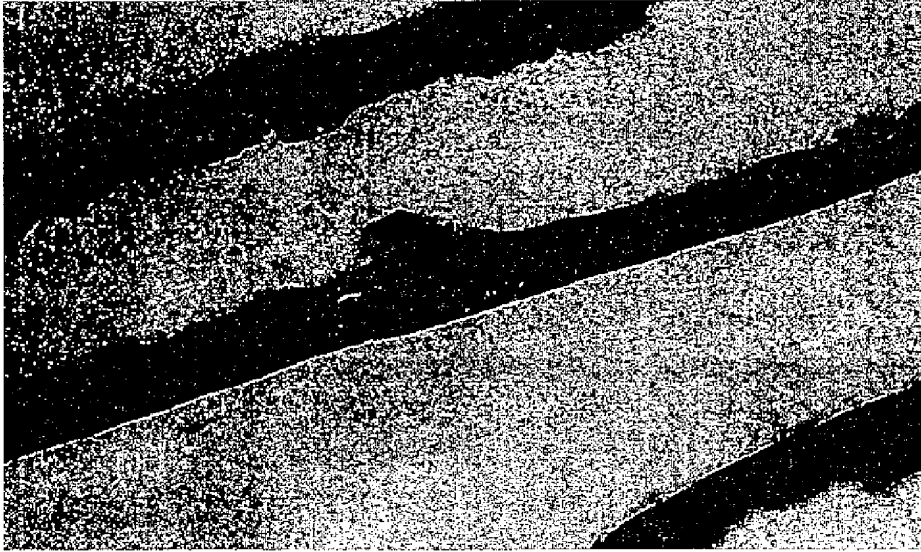


FIG. 2A



FIG. 2B

도면3

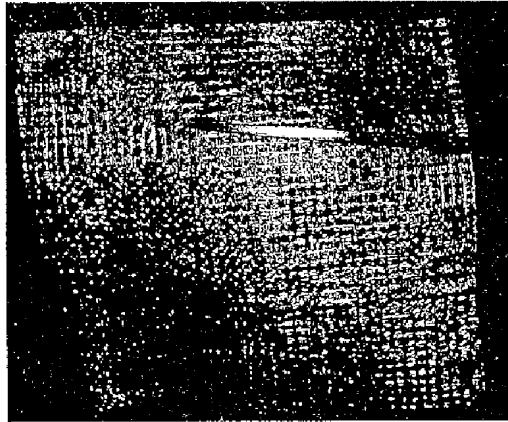


FIG. 3

도면4

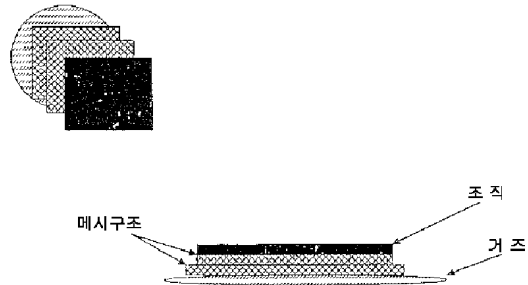


FIG. 4

[Translation]

Issuing No.: 9-5-2021-039550809
Issuing Date: May 18, 2021

To: AJU Kim Chang & Lee
13th-14th Floor, Donghee
Building, 302, Gangnam-daero,
Gangnam-gu, Seoul 06253

Korean Intellectual Property Office
Notice of Allowance

Applicant:

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Application No.: 10-2015-7021367

Title of Invention: DECELLULARIZED BIOMATERIAL FORM NON-MAMMALIAN
TISSUE

Number of Claims: 21

It is hereby decided that a patent is granted to this application, under Article 66 of the Korean Patent Law. (The patent right will come into effect upon the payment of the issue fee under Article 87 of the Korean Patent Law. Meanwhile, according to Article 67(2)-2, formal decision of refusal (February 19, 2021) for the subject application is considered void resulted from request for re-examination.)

[Priorities Claimed]

Country: US Priority No.: 61/750,555 (Date: January 9, 2013)

[References]

1. Akira Satoh et al., ZOOLOGICAL SCIENCE(2012), Vol. 29, pp.191-197
2. US20090142836 A1
3. US20070248638 A1
4. US20050013872 A1
5. KR100999247 B1
6. WO2008129997 A1.

Dated this 18th day of May, 2021

Korean Intellectual Property Office
Chemistry & Biotechnology Examination Bureau
Medical Technology Examination Division

발송번호: 9-5-2021-039550809
발송일자: 2021.05.18.

수신 서울특별시 강남구 강남대로 302, 13, 14층
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특허법인아주[정은섭] 귀하(귀중)

06253

특 허 청 특허결정서

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520210236663)
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불러바드 13709
대 리 인 명 칭 특허법인아주
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동희빌딩)(특허법인아주)
지정된변리사 정은섭
발 명 자 성 명 얼리, 라이앤
주 소 미국 플로리다주 32003 플레밍 아일랜드 히커리 트레이스
드라이브 1909
출 원 번 호 10-2015-7021367
발 명 의 명 칭 비-포유류 조직으로부터의 세포제거된 생체재료
청 구 항 수 21

이 출원에 대하여 특허법 제66조에 따라 특허결정합니다.
(특허권은 특허료를 납부하여 특허법 제87조에 따라 설정등록을 받음으로써 발생하게 됩니
다. 한편, 특허법 제67조의2제2항에 따라 이 출원에 대한 종전 거절결정(2021.02.19)은 재
심사 청구로 인하여 취소된 것으로 간주되었습니다.)

[우선권주장]

우선권주장국가: US, 우선권주장번호: 61/750,555 (우선권주장일자: 2013.01.09)

[참고문헌]

1. Akira Satoh et al., ZOOLOGICAL SCIENCE(2012), Vol. 29, pp.191-197
2. US20090142836 A1
3. US20070248638 A1
4. US20050013872 A1
5. KR100999247 B1
6. W02008129997 A1. 끝.

2021.05.18.

특허청

화학생명기술심사국

의료기술심사과

심사관 이수희

이수희 (인)

<< 안내 >>

【 분할 출원 및 전자등록증 발급 】

1. 출원인은 이 특허결정서를 송달받은 날로부터 3개월 이내의 기간(다만, 특허법 제79조에 따른 설정등록을 받으려는 날이 3개월보다 짧은 경우에는 그 날까지의 기간)에 분할출원을 할 수 있습니다.
2. 2018. 7. 1. 설정 등록부터는 특허로(<http://www.patent.go.kr>)에서 「전자등록증 신청」을 사전에 하거나, 납부서(특허권 등의 등록령 시행규칙 별지 제25호 서식)에 등록증 수령 방법을 전자문서 수령으로 선택하면 「전자등록증 수신함」을 통해 전자등록증 파일을 내려받을 수 있습니다. 만약, 전자등록증을 사전에 신청하지 않고 동봉된 납입고지서로 설정등록료를 납부하면 기존과 동일하게 서면 등록증으로 발급됩니다.

※ 위에서 안내한 후속 절차 또는 신청 서식 등에 관한 사항은 특허고객상담센터 ☎1544-8080로, 이 통지서 내용에 대한 사항은 특허청 ☎042-481-5128(담당심사관 이수희)로 문의하시기 바랍니다.

※ 특허로(www.patent.go.kr) 또는 특허청(www.kipo.go.kr) 홈페이지를 방문하시면 심사 진행 상황, 서류 제출 및 절차에 관한 보다 많은 정보를 제공받을 수 있습니다.
(특허청) 우 35208 대전광역시 서구 청사로 189, 4동 (둔산동, 정부대전청사)

Electronic Acknowledgement Receipt

EFS ID:	43000248
Application Number:	16801956
International Application Number:	
Confirmation Number:	9621
Title of Invention:	DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE
First Named Inventor/Applicant Name:	Ryanne Early
Customer Number:	35811
Filer:	Sima Singadia Kulkarni/Stephanie De marco
Filer Authorized By:	Sima Singadia Kulkarni
Attorney Docket Number:	SYG-19-1252US-CON2
Receipt Date:	16-JUN-2021
Filing Date:	26-FEB-2020
Time Stamp:	11:24:25
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	SYG_19_1252US_CON2_SIDS. pdf	98513	no	2
			abd76bae1f0f93c2f214ef5dd0ccb62ad0a 7927		

Warnings:

Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	SYG_19_1252US_CON2_1449.pdf	135166 3a47ec176695a91fdac42acc5aaed274d312981	no	1
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
3	Patent Term Adjustment Stmt Under 37CFR1.704(d)	SYG_19_1252US_CON2_IDS_Cert.pdf	258776 ae93792ce3979de6eb6a21eb6b4051463bff104b	no	2
Warnings:					
Information:					
4	Foreign Reference	KR100999247B1.pdf	3723167 e13c8c87cb43a8c7ff44d23e749d7d9c523ff3a8	no	37
Warnings:					
Information:					
5	Non Patent Literature	20210518_KRNOA.pdf	702080 275f18949a4b59fc7763008f16656184a6f51df5	no	3
Warnings:					
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Total Files Size (in bytes):			4917702		
This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503. <u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application. <u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course. <u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.					

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Art Unit	: TBD	Customer No.	035811
Examiner	: TBD		
Appl. No.	: 16/801,956	Docket No.:	SYG-19-1252US-CON2
Appl. Filed	: February 26, 2020		
First Named Inventor	: Ryanne Early	Confirmation No.:	9621
Title	: DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE	Dated:	June 16, 2021

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

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

Sir:

The Applicant encloses Form PTO-1449 together with a copy of one (1) non-US publication. Also enclosed is a copy of the Notice of Allowance received in corresponding Korean Appln. No. 10-2015-7021367 dated May 18, 2021, and its English translation. The documents are submitted under 37 CFR §1.56. The enclosed publication is believed related in view of its mention in the Search Report for the reasons stated.

Applicant certifies that each item of information contained in the Information Disclosure Statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months ago.

A Patent Term Adjustment Statement under 37 CFR 1.704(d) is attached herewith.

The Applicant respectfully requests that this Information Disclosure Statement be officially entered into the file and that appropriate notification be made that all the publications were considered by the Examiner.

Respectfully submitted,

/sima singadia kulkarni/

Sima Singadia Kulkarni
Reg. No. 43,732

SSK/sh
(215) 656-2478

Form PTO-1449 US DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE LIST OF PUBLICATIONS CITED BY APPLICANT <i>(Use several sheets if necessary)</i>				ATTY. DOCKET NO. SYG-19-1252US-CON2		SERIAL NO. 16/801,956	
				APPLICANT ISE Professional Testing & Consulting Services, Inc.			
				FILING DATE February 26, 2020		GROUP TBD	
US PATENT DOCUMENTS							
EXAMINER INITIAL*		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE
	AA						
	AB						
	AC						
	AD						
	AE						
	AF						
	AG						
	AH						
	AI						
	AJ						
	AK						
FOREIGN PATENT DOCUMENTS							
		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION
							YES NO
	AL						
	AM						
	AN						
	AO						
	AP						
OTHER PUBLICATIONS (Including Author, Title, Date, Pertinent Pages, Etc.)							
	AR	Final Rejection from corresponding Japanese Patent Appln. No. 2018-224475 dated August 26, 2021, and an English summary of the action.					
	AS						
	AT						
EXAMINER				DATE CONSIDERED			
*EXAMINER: Initial if publication considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.							

**PATENT TERM ADJUSTMENT
STATEMENT UNDER 37 CFR 1.704(d)**

Application Number	16/801,956
Filing Date	February 26, 2020
First Named Inventor	Ryanne Early
Art Unit	TBD
Examiner Name	TBD
Practitioner Docket No.	SYG-19-1252US-CON2

APPLICANT HEREBY STATES THE FOLLOWING (please review 37 CFR 1.704(d) before filing this form):

☐ Each item of information contained in the information disclosure statement was first cited in any communication from a patent office in a counterpart foreign or international application or from the Office, and this communication was not received by any individual designated in 37 CFR 1.56(c) more than thirty days prior to the filing of the information disclosure statement.

AND/OR

☒ Each item of information contained in the information disclosure statement is a communication that was issued by a patent office in a counterpart foreign or international application or by the Office, and this communication was not received by any individual designated in 37 CFR 1.56(c) more than thirty days prior to the filing of the information disclosure statement.

INSTRUCTIONS:

- **This form will not satisfy the requirement of 37 CFR 1.97(e).** The present statement is filed under 37 CFR 1.704(d) and will not substitute for compliance with any of the requirements of 37 CFR 1.97 and 1.98. For an information disclosure statement to comply with 37 CFR 1.97(c) or (d), the information disclosure statement must be accompanied by a statement under 37 CFR 1.97(e) notwithstanding any statement filed under 37 CFR 1.704(d).
- The present form (PTO/SB/133) should be filed concurrently with the information disclosure statement to derive benefit under 37 CFR 1.704(d).

Signature	/sima singadia kulkarni/	Date	September 23, 2021
Typed or Printed Name	Sima Singadia Kulkarni	Practitioner Registration Number	43,732
Note: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required in accordance with 37 CFR 1.33 and 11.18. Please see 37 CFR 1.4(d) for the form of the signature. If necessary, submit multiple forms for more than one signature, see below*.			
☐	*Total of _____ forms are submitted.		

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

To: Ms. Stephanie Henke

Final Rejection

Appln. No.	2018-224475
Examiner	Ms. Rina Date
Drafting Date	August 26, 2021
Dispatching Date	August 30, 2021
Period for Appeal	4 months from Dispatching Date (inextensible)
Due Date for Appeal	December 30, 2021

Summary of reasons for final rejection

Reasons (JPL)	Claims	References
Support requirements (§ 36 VI (i))	1-54	
Description requirements (§ 36 VI (ii))	1-54	

JPL (Japanese Patent Law)

36 VI The statement of the patent claim(s) under Subsection II shall comply with each of the following paragraphs:

- (i) statements setting forth the invention(s) for which patent is sought and which is described in the detailed explanation of the invention;
- (ii) statements setting forth the invention(s) for which a patent is sought and which is clear;
- (iii) statements setting forth the claim(s) which is concise;
- (iv) statements which are as provided for in an ordinance of the Ministry of Economy, Trade and Industry.

Examiner's remarks

Reason 1 (§ 36 VI (ii))

Claims 1-54

The amendment in claim 1 is the change from the phrase "(B) decellularizing the sample, by a process comprising: (i) retaining the structural and functional integrity of said tissue sample; and (ii) removing cellular components of the sample sufficient to reduce or eliminate antigenicity of the biomaterial as a xenograft in a human mammal" to "(B) decellularizing the sample, by a process comprising: removing cellular components of the sample sufficient to retain the structural and functional integrity of said tissue sample; and to reduce or eliminate antigenicity of the biomaterial as a xenograft in a mammal", however, it is substantially not a change of the subject matter.

The applicant asserted in the written opinion dated March 15, 2021 as follows:

"We believe the meaning of "removing cellular components" is clear, since the Examiner points out "[P]aragraph 0026 shows physical methods, such as freezing, direct pressure application, sonication and agitation; chemical methods, such as alkaline and acid treatments, detergents, organic solvents, hypotonic or hypertonic

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solutions and chelating agents; and enzymatic approaches including protease digestion and treatment with nucleases, as techniques for decellularizing tissues. Accordingly, we believe that the above Reason was overcome."

The specification, paragraph 0024, exemplifies various kinds of tissues, such as dermis and basement membrane, parietal mesothelial tissues, cartilage, tendon, bone, dura mater, and fascia, as a starting material of decellularization. Furthermore, the specification, paragraph 0026 exemplifies enzymatic approaches, including freezing, direct pressure application, sonication and agitation; chemical methods, such as alkaline and acid treatments, detergents, organic solvents, hypotonic or hypertonic solutions and chelating agents; and enzymatic approaches including protease digestion and treatment with nucleases, as techniques for decellularizing tissues.

The probability that each of ECM prepared by one of the techniques stated in paragraph [0026] or the combinations thereof has the different physical properties, using the above various kinds of tissues as a starting materials is high.

Then, even though it is difficult to specify ECM of the present inventions with the structure or property not depending on the process, since the process recited in claim 1 is unclear, it is unclear what products are prepared by the aforesaid process.

Accordingly, the inventions of claim 1 and claims 2-54 citing claim 1 are unclear.

Therefore, this application cannot be patented since the descriptions of claims 1-54 do not meet the requirements of Japanese Patent Law § 36 VI (ii).

Reason 2 (§ 36 VI (i))
Claims 1-54

The applicant asserts in the written opinion above as follows:

"The features of the present inventions are that the origin is an urodele, and it is possible to obtain ECM suitable for implanting into a mammal even if preparing ECM by optionally selecting the various kinds of urodele tissues and the treating methods exemplified in the specification, paragraphs 0024 and 0026, as long as preparing it to be sufficient to retain the structural and functional integrity of said tissue sample; and to reduce or eliminate antigenicity of the biomaterial as a xenograft in a mammal. Accordingly, we believe that the indication of the Examiner that the matter disclosed in the specification cannot be expanded and generalized to the scope of the invention of claim 1, and the inventions of claims 1-54 are not regarded to be described in the specification is improper is improper."

However, the specification does not indicate that ECM suitable for implanting into a mammal could be obtained even if preparing ECM by optionally selecting the

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various kinds of urodele tissues and the treating methods exemplified in the specification, paragraphs 0024 and 0026. Moreover, there was no common technical knowledge at the time of filing the present application regarding the method for preparing ECM suitable for implanting into a mammal from any urodele tissues.

Then, it is unclear whether or not the ECM suitable for implanting into mammal can be obtained even though preparing ECM by optionally selecting various kinds of urodele tissues and various processes exemplified in the specification, paragraphs [0024] and [0026].

Therefore, the matter disclosed in the specification cannot be expanded and generalized to the scope of the invention of claim 1.

Accordingly, the inventions of claim 1 and claims 2-54 citing claim 1 are not regarded to be described in the specification.

Therefore, this application cannot be patented since the claims thereof do not meet the requirements set forth in JPL § 36 VI (i).

Note that there are defects in this application as mentioned below.

Claim 29

§ 36 VI (ii) (Description requirements)

The invention of claim 29 is unclear since the aforesaid claim includes the phrase "[T]he device of claim 32" which number is larger than 29. (Our comments: This is caused by our typing error. We apologize for this.)

Claims 55-68

§ 37 (Unity)

1. Determination for subjects of the examination from the view point of special technical feature

The special technical feature "[A]n isolated, decellularized urodele extracellular matrix (ECM) for use in mammals, produced by the process that comprises: (A) obtaining a tissue sample from a urodele, which tissue sample comprises extracellular matrix, and (B) decellularizing the sample, by a process comprising: removing cellular components of the sample sufficient to retain the structural and functional integrity of said tissue sample; and to reduce or eliminate antigenicity of the biomaterial as a xenograft in a mammal." was found in the invention of claim 1.

Accordingly, the invention of claim 1 and those of claims 2-54, which have the identical or corresponding special technical features to that of claim 1, are to be examined.

To: Ms. Stephanie Henke

2. Determination for subjects of the examination from the viewpoint of efficiency

The inventions of claims 55-68 do not include all features of claim 1 and do not fall into the same category as claim 1. Furthermore, the inventions of claims 55-68 cannot be examined without an substantially additional prior art search or decision, as a result of the examination of the aforesaid subjects, and there is no reason why it is efficient to examine those with the subjects.

Therefore, the inventions of claims 55-68 are not to be examined other than requirements stipulated in the Japanese Patent Law Article 37.

Our comments

Our comments will be reported shortly as mentioned above.

拒絶査定

特許出願の番号	特願 2018-224475
起案日	令和 3年 8月26日
特許庁審査官	伊達 利奈 3960 4N00
発明の名称	非哺乳動物組織からの脱細胞化生体材料
特許出願人	アイエスイー プロフェッショナル テスティング アンド コンサルティング サービスズ , インコーポレーテッド
代理人	葛和 清司

この出願については、令和 2年10月 9日付け拒絶理由通知書に記載した理由1及び理由2によって、拒絶をすべきものです。

なお、意見書及び手続補正書の内容を検討しましたが、拒絶理由を覆すに足りる根拠が見いだせません。

備考

●理由1（特許法第36条第6項第2号）について

・請求項1-54

請求項1における補正は、「(B) (i) 該組織試料の構造的および機能的完全性を保持すること、および (i i) 哺乳動物における異種移植片としての生体材料の抗原性を低減または排除するために十分なように試料の細胞成分を除去すること」なる記載を「(B) 該組織試料の構造的および機能的完全性を保持し哺乳動物における異種移植片としての生体材料の抗原性を低減または排除するために十分なように試料の細胞成分を除去すること」と変更するものであり、実質的に発明特定事項が変更されていない。

出願人は令和3年3月15日付け意見書において、「細胞成分の除去は、審査官殿が「[0026]には、組織を脱細胞化するための技術として、凍結、直接加圧、超音波処理、攪拌等の物理的处理、アルカリ又は酸処理、界面活性剤、有機溶剤、低張又は高張溶液、キレート化剤等の化学的方法、及び、プロテアーゼ消化、ヌクレアーゼ処理を含む酵素的アプローチが例示されている。」と指摘されているように、明確であると思料します。したがって、本拒絶理由は解消したと思料します。」と主張している。

明細書の段落[0024]には、脱細胞化の出発物質として、真皮及び基底膜

、体壁中皮組織、軟骨、腱、骨、硬膜、筋膜等の種々の組織が例示されている。そして、明細書の段落〔0026〕には、組織を脱細胞化するための技術として、凍結、直接加圧、超音波処理、攪拌等の物理的処理、アルカリ又は酸処理、界面活性剤、有機溶剤、低張又は高張溶液、キレート化剤等の化学的方法、及び、プロテアーゼ消化、ヌクレアーゼ処理を含む酵素的アプローチが例示されている。

上記種々の組織を出発物質として、段落〔0026〕に記載されている種々の技術のいずれか又はそれらの組み合わせによって製造されるECMの各々は異なる物性を有するものである蓋然性が高い。

そうすると、本願のECMを製造方法によらず構造又は特性によって特定することは困難であるとしても、請求項1に記載されている製造方法はあいまいであるため、当該製造方法によって製造される物についてもどのようなものであるのかが不明確となっている。

したがって、請求項1に係る発明及びこれを引用する請求項2～54に係る発明は明確でない。

よって、この出願は、請求項1～54の記載が特許法第36条第6項第2号に規定する要件を満たしていないため特許を受けることができない。

●理由2（特許法第36条第6項第1号）について

・請求項1～54

出願人は上記意見書において、「本願発明の特徴は、有尾類が由来であることであり、組織試料の構造的および機能的完全性を保持し哺乳動物における異種移植片としての生体材料の抗原性を低減または排除するために十分のように調製できれば、明細書の〔0024〕及び〔0026〕に例示されている、種々の有尾類組織及び種々の処理方法を、任意に選択してECMを生産しても、哺乳動物への移植に適するECMを得ることが可能であります。したがって、審査官殿の明細書に開示された事項を、請求項1に係る発明の範囲まで拡張ないし一般化することはできず、現請求項1～54に係る発明が、発明の詳細な説明に記載したものでないとの指摘は、当たらないと思料します。」と主張している。

明細書において、段落〔0024〕及び段落〔0026〕に例示されている種々の有尾類組織及び種々の処理方法を任意に選択する幾通りかの方法によってECMを生産しても、哺乳動物への移植に適するECMを取得することができたことは示されていない。

本願出願時に、任意の有尾類組織から哺乳動物への移植に適するECMを製造する手法に関する技術常識は存在していない。

そうすると、〔0024〕及び〔0026〕に例示されている、種々の有尾類組織及び種々の処理方法を任意に選択してECMを生産しても、哺乳動物への移植に適するECMを得ることが可能であるかは不明であり、明細書に開示された

事項を請求項 1 に係る発明の範囲まで拡張ないし一般化することはできない。

したがって、請求項 1 及びこれを引用する請求項 2 - 5 4 に係る発明は、発明の詳細な説明に記載されたものでない。

よって、この出願は、請求項 1 - 5 4 の記載が、特許法第 3 6 条第 6 項第 1 号に規定する要件を満たしていないため特許を受けることができない。

なお、この出願には以下に示す不備が存在する。

- ・請求項 2 9

- ・特許法第 3 6 条第 6 項第 2 号

請求項 2 9 において、「請求項 3 2 に記載のデバイス」という自身より大きい項番の請求項を引用する記載が含まれているため、請求項 2 9 に係る発明は明確でない。

- ・請求項 5 5 - 6 8

- ・特許法第 3 7 条

1. 特別な技術的特徴に基づく審査対象の決定

請求項 1 に係る発明に、「(A) 有尾類からの、細胞外マトリックスを含む組織試料を得ること、および (B) 該組織試料の構造的および機能的完全性を保持し哺乳動物における異種移植片としての生体材料の抗原性を低減または排除するために十分なように試料の細胞成分を除去すること、を含む方法により前記試料を脱細胞化すること、を含む方法により生産される、分離され、脱細胞化された哺乳動物への使用のための有尾類細胞外マトリックス (ECM) 。」という特別な技術的特徴が発見された。

したがって、請求項 1 に係る発明と、当該特別な技術的特徴と同一の又は対応する特別な技術的特徴を有する、請求項 2 - 5 4 に係る発明とを、審査対象とする。

2. 審査の効率性に基づく審査対象の決定

請求項 5 5 - 6 8 に係る発明は、請求項 1 に係る発明の発明特定事項を全て含む同一カテゴリーの発明ではない。そして、請求項 5 5 - 6 8 に係る発明は、審査対象とされた発明を審査した結果、実質的に追加的な先行技術調査や判断を必要とすることなく審査を行うことが可能である発明ではなく、当該発明とまとめて審査を行うことが効率的であるといえる他の事情も無い。

したがって、請求項 5 5 - 6 8 に係る発明は、特許法第 3 7 条以外の要件についての審査対象としない。

この査定に不服があるときは、この査定の謄本の送達があった日から3月以内（在外者にあつては、4月以内）に、特許庁長官に対して、拒絶査定不服審判を請求することができます（参照条文：特許法第121条第1項）。

＜行政事件訴訟法第46条第2項に基づく教示＞

この査定に対しては取消訴訟を提起することはできません。この査定についての審判請求に対する審決に対してのみ取消訴訟を提起することができます（参照条文：特許法第178条第6項）。

上記はファイルに記録されている事項と相違ないことを認証する。

認証日 令和 3年 8月27日 経済産業事務官 持村 和則

Electronic Acknowledgement Receipt

EFS ID:	43844771
Application Number:	16801956
International Application Number:	
Confirmation Number:	9621
Title of Invention:	DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE
First Named Inventor/Applicant Name:	Ryanne Early
Customer Number:	35811
Filer:	Sima Singadia Kulkarni/Stephanie De marco
Filer Authorized By:	Sima Singadia Kulkarni
Attorney Docket Number:	SYG-19-1252US-CON2
Receipt Date:	23-SEP-2021
Filing Date:	26-FEB-2020
Time Stamp:	13:02:56
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	SYG_19_1252US_CON2_SIDS. pdf	98185 1aa0e465df2956d0bb354f626d2c79d39227 ba52e	no	1

Warnings:

Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	SYG_19_1252US_CON2_1449. pdf	134767 1da516321d8086a6e1f3f227b8b04ff41f1f9 051	no	1
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
3	Patent Term Adjustment Stmt Under 37CFR1.704(d)	SYG_19_1252US_CON2_IDS_C ert.pdf	258802 2eb2728974e458f342a78f48c0b770f1419b a806	no	2
Warnings:					
Information:					
4	Non Patent Literature	20210826_JPOA.pdf	219636 ba086ca2fe08b53abc7959eae9c65e263ea 2fd33	no	8
Warnings:					
Information:					
Total Files Size (in bytes):			711390		
This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503. <u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application. <u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course. <u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.					

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Art Unit	: TBD	Customer No.	035811
Examiner	: TBD		
Appl. No.	: 16/801,956	Docket No.:	SYG-19-1252US-CON2
Appl. Filed	: February 26, 2020		
First Named Inventor	: Ryanne Early	Confirmation No.:	9621
Title	: DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE	Dated:	September 23, 2021

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

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

Sir:

The Applicant encloses Form PTO-1449 together with a copy of the Final Rejection received in corresponding Japanese Appln. No. 2018-224475 dated August 26, 2021, and an English summary of the Action. The document is submitted under 37 CFR §1.56.

Applicant certifies that each item of information contained in the Information Disclosure Statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months ago.

A Patent Term Adjustment Statement under 37 CFR 1.704(d) is attached herewith.

The Applicant respectfully requests that this Information Disclosure Statement be officially entered into the file and that appropriate notification be made that all the publications were considered by the Examiner.

Respectfully submitted,

/sima singadia kulkarni/

Sima Singadia Kulkarni
Reg. No. 43,732

SSK/sh
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/801,956	02/26/2020	Ryanne Early	SYG-19-1252US-CON2	9621
35811 7590 04/28/2022 IP GROUP OF DLA PIPER LLP (US) ONE LIBERTY PLACE 1650 MARKET ST, SUITE 5000 PHILADELPHIA, PA 19103			EXAMINER DAVIS, RUTH A	
			ART UNIT	PAPER NUMBER
			1699	
			NOTIFICATION DATE	DELIVERY MODE
			04/28/2022	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

pto.phil@us.dlapiper.com

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

DETAILED ACTION

Election/Restrictions

Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claims 1 - 10, 18 - 27, 34, drawn to decellularized ECM, classified in C12N 5/0625, for example.

II. Claims 11 - 12, drawn to a tissue culture system, classified in C12N 5/00, for example.

III. Claims 13 - 17, drawn to a method for making a decellularized ECM, classified in A61K 35/12, for example.

IV. Claims 28 - 32, 35, drawn to a method for making a decellularized ECM, classified in A61K 35/65, for example.

V. Claim 33, drawn to a method for making a material, classified in A61K 38/1703, for example.

The inventions are independent or distinct, each from the other because:

Inventions I-III, IV, V are related as process of making and product made. The inventions are distinct if either or both of the following can be shown: (1) that the process as claimed can be used to make another and materially different product or (2) that the product as claimed can be made by another and materially different process (MPEP § 806.05(f)). In the

Art Unit: 1699

instant case decellularized ECM can be obtained by decellularizing other materials such as a human pancreas.

The inventions of the remaining groups are directed to different inventions which are not connected in design, operation, and/or effect. These groups are independent since they are not disclosed as capable of use together, they have different modes of operation, they have different functions, and/or they have different effects. One would not have to practice the various inventions at the same time to practice just one alone.

The several inventions above are independent and distinct, each from the other. They have acquired a separate status in the art as a separate subject for inventive effect and require independent searches (as indicated by the different classification). The search for each of the above inventions is not co-extensive particularly with regard to the literature search. Further, a reference which would anticipate the invention of one group would not necessarily anticipate or even make obvious another group.

These inventions are distinct for the reasons given above and because the search required for one group is not required for the other groups, resulting in a serious burden for searching and examination of the claims. Thus, restriction for examination purposes as indicated is proper.

The examiner has required restriction between product or apparatus claims and process claims. Where applicant elects claims directed to the product/apparatus, and all product/apparatus claims are subsequently found allowable, withdrawn process claims that include all the limitations of the allowable product/apparatus claims should be considered for rejoinder. All claims directed to a nonelected process invention must include all the limitations of an allowable product/apparatus claim for that process invention to be rejoined.

In the event of rejoinder, the requirement for restriction between the product/apparatus claims and the rejoined process claims will be withdrawn, and the rejoined process claims will be fully examined for patentability in accordance with 37 CFR 1.104. Thus, to be allowable, the rejoined claims must meet all criteria for patentability including the requirements of 35 U.S.C. 101, 102, 103 and 112. Until all claims to the elected product/apparatus are found allowable, an otherwise proper restriction requirement between product/apparatus claims and process claims may be maintained. Withdrawn process claims that are not commensurate in scope with an allowable product/apparatus claim will not be rejoined. See MPEP § 821.04. Additionally, in order for rejoinder to occur, applicant is advised that the process claims should be amended during prosecution to require the limitations of the product/apparatus claims. **Failure to do so may result in no rejoinder.** Further, note that the prohibition against double patenting rejections of 35 U.S.C. 121 does not apply where the restriction requirement is withdrawn by the examiner before the patent issues. See MPEP § 804.01.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to RUTH A DAVIS whose telephone number is (571)272-0915. The examiner can normally be reached Monday - Friday (8am - 4pm).

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at <http://www.uspto.gov/interviewpractice>.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Fereydoun Sajjadi can be reached on 571-272-3311. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of published or unpublished applications may be obtained from Patent Center. Unpublished application information in Patent Center is available

Art Unit: 1699

to registered users. To file and manage patent submissions in Patent Center, visit:

<https://patentcenter.uspto.gov>. Visit <https://www.uspto.gov/patents/apply/patent-center> for more information about Patent Center and <https://www.uspto.gov/patents/docx> for information about filing in DOCX format. For additional questions, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/RUTH A DAVIS/

Primary Examiner, Art Unit 1699

Bibliographic Data

Application No: 16/801,956

Foreign Priority claimed: ☐ Yes ☒ No

35 USC 119 (a-d) conditions met: ☐ Yes ☐ No ☐ Met After Allowance

Verified and Acknowledged: /RUTH A DAVIS/

Examiner's Signature

Initials

Title:

DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN
TISSUE

FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
02/26/2020	424	1699	SYG-19-1252US-CON2
RULE			

APPLICANTS

ISE Professional Testing & Consulting Services, Inc., Fleming Island, FL,

INVENTORS

Ryanne Early, Fleming Island, FL, UNITED STATES

CONTINUING DATA

This application is a CON of 14759895 07/08/2015 PAT 10617790

14759895 is a 371 of PCT/US2014/010890 01/09/2014

PCT/US2014/010890 has PRO of 61750555 01/09/2013

FOREIGN APPLICATIONS

IF REQUIRED, FOREIGN LICENSE GRANTED**

03/11/2020

** SMALL ENTITY **

STATE OR COUNTRY

UNITED STATES

ADDRESS

IP GROUP OF DLA PIPER LLP (US)

ONE LIBERTY PLACE

1650 MARKET ST, SUITE 5000

PHILADELPHIA, PA 19103

UNITED STATES

FILING FEE RECEIVED

\$1,535

To: pto.phil@us.dlapiper.com,,
From: PAIR_eOfficeAction@uspto.gov
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Apr 28, 2022 04:57:54 AM

Dear PAIR Customer:

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Application	Document	Mailroom Date	Attorney Docket No.
16801956	CTRS	04/28/2022	SYG-19-1252US-CON2

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If you have any questions, please email the Electronic Business Center (EBC) at EBC@uspto.gov with 'e-Office Action' on the subject line or call 1-866-217-9197 during the following hours:

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PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Art Unit	: 1699	Customer No.	035811
Examiner	: Ruth A. Davis		
Appl. No.	: 16/801,956	Docket No.:	SYG-19- 1252US-CON2
Appl. Filed	: February 26, 2020		
First Named Inventor:	Ryanne Early	Confirmation No.:	9621
Title	: DECELLULARIZED BIOMATERIAL		
	: FROM NON-MAMMALIAN TISSUE		
		Dated:	June 21, 2022

RESPONSE

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

Sir:

This is in response to the Official Action dated April 28, 2022.

The Official Action requires election of one of the following five groups:

- 1) Group I: Claims 1 - 10, 18 – 27 and 34, drawn to decellularized ECM;
- 2) Group II: Claims 11 and 12, drawn to a tissue culture system;
- 3) Group III: Claims 13 - 17, drawn to a method for making a decellularized ECM;
- 4) Group IV: Claims 28 – 32 and 35, drawn to a method for making a decellularized ECM; and
- 5) Group V: Claim 33, drawn to a method for making a material.

The Applicant elects Group I, including Claims 1-10, 18-27 and 34, drawn to decellularized ECM. This election is without traverse.

In light of the foregoing, the Applicant respectfully requests examination of the application on the merits.

Respectfully submitted,

/Christopher I. Halliday/

Christopher I. Halliday
Reg. No. 42,621

CIH/vbm
(215) 656-2452



ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #
16/801,956

RECEIPT DATE / TIME
06/21/2022 03:35:55 PM ET

ATTORNEY DOCKET #
SYG-19-1252US-CON2

Title of Invention

DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE

Application Information

APPLICATION TYPE Utility - Nonprovisional Application
under 35 USC 111(a)

PATENT # -

CONFIRMATION # 9621

FILED BY Victorine Meise

PATENT CENTER # 60730884

FILING DATE 02/26/2020

CUSTOMER # 35811

FIRST NAMED INVENTOR
Ryanne Early

CORRESPONDENCE ADDRESS -

AUTHORIZED BY Thomas Christenbury

Documents

TOTAL DOCUMENTS: 1

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
Response.pdf	1	Response to Election / Restriction Filed	93 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
Response.pdf	C1D2F589B5D029CCC506F5AC660A658A0395930A00D458EE3 FF66CD852D721F4E3B8E9F0AEE4CAC34CE9299553F9C7BFB 25E5D6E7B1AA0D0EC87DB7D7AE7A36F

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized

by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.



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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/801,956	02/26/2020	Ryanne Early	SYG-19-1252US-CON2	9621
35811	7590	08/18/2022	EXAMINER	
IP GROUP OF DLA PIPER LLP (US)			DAVIS, RUTH A	
ONE LIBERTY PLACE			ART UNIT	PAPER NUMBER
1650 MARKET ST, SUITE 5000			1699	
PHILADELPHIA, PA 19103			NOTIFICATION DATE	DELIVERY MODE
			08/18/2022	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

pto.phil@us.dlapiper.com

DETAILED ACTION

Notice of Pre-AIA or AIA Status

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

Election/Restrictions

Applicant's election without traverse of Group I, claims 1 – 10, 18 – 27 and 34 in the reply filed on June 21, 2022 is acknowledged.

Claims 1 – 35 are pending; claims 11 – 17, 28 – 33 and 35 are withdrawn; claims 1 – 10, 18 – 27 and 34 have been considered on the merits.

Applicant is reminded that non-elected claims may be considered for rejoinder when all claims directed to the elected invention are in condition for allowance. In order to be eligible for rejoinder, a claim to a nonelected invention must depend from or otherwise require all the limitations of an allowable claim. In order to retain the right to rejoinder, applicant is advised that the claims to the nonelected invention(s) should be amended during prosecution to require the limitations of the elected invention. Failure to do so may result in a loss of the right to rejoinder. MPEP 821.04

Information Disclosure Statement

The information disclosure statements (IDS) submitted on February 26, July 27, and November 12, 2020; March 12, June 16 and September 23 2021 are in compliance with the

provisions of 37 CFR 1.97. Accordingly, the information disclosure statements are being considered by the examiner.

Claim Objections

Claims 8 and 9 are objected to because of the following informalities:

Claims 8 fails to end with a “.”

Claim 9 recites “a sheet” in duplicate.

Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of 35 U.S.C. 112(b):

(b) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 7 – 8 are rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor (or for applications subject to pre-AIA 35 U.S.C. 112, the applicant), regards as the invention.

Claim 7 is drawn to a decellularized extracellular matrix (ECM), however is rendered vague and indefinite for reciting “human agent” as the phrase is not adequately defined by the claim language or specification.

Claim 8 is rendered indefinite because the claim does not appear to further define the claim on which it depends. Claim 7 requires the decellularized ECM to be infused with one of the listed agents. However, a cell or human cell does not further characterize any of the recited listed agents, rendering the scope of the claim unclear.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the “right to exclude” granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP §

2146 *et seq.* for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/process/file/efs/guidance/eTD-info-L.jsp.

Claims 1 – 10, 18 – 27 and 34 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1 – 17 of U.S. Patent No.10,617,790. Although the claims at issue are not identical, they are not patentably distinct from each other because the only distinction is that the issued claims are recited as a tissue graft that implies a specific use of the decellularized ECM as currently claimed. Both the issued claims and those pending are otherwise drawn to the same decellularized ECM, obtained from the same source, having the same treatments, additives and characteristics. Regarding the intended use of the ECM in the issued claims, the intended use of the claimed composition does not patentably distinguish the composition, per se, since such undisclosed use is inherent in both compositions. In order to be limiting, the intended use must create a structural difference between the claimed composition and the composition of the prior art. In the instant case, the intended use fails to create a structural difference, thus, the intended use is not limiting.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to RUTH A DAVIS whose telephone number is (571)272-0915. The examiner can normally be reached Monday - Friday (8am - 4pm).

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at <http://www.uspto.gov/interviewpractice>.

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Information regarding the status of published or unpublished applications may be obtained from Patent Center. Unpublished application information in Patent Center is available to registered users. To file and manage patent submissions in Patent Center, visit: <https://patentcenter.uspto.gov>. Visit <https://www.uspto.gov/patents/apply/patent-center> for more information about Patent Center and <https://www.uspto.gov/patents/docx> for information about filing in DOCX format. For additional questions, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/RUTH A DAVIS/
Primary Examiner, Art Unit 1699

Notice of References Cited	Application/Control No. 16/801,956		Applicant(s)/Patent Under Reexamination Early, Ryanne	
	Examiner RUTH A DAVIS		Art Unit 1699	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
*	A	US-10617790-B2	04-2020	Early; Ryanne	A61L27/3604	1/1
	B					
	C					
	D					
	E					
	F					
	G					
	H					
	I					
	J					
	K					
	L					
	M					

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

<i>Search Notes</i> 	Application/Control No. 16/801,956	Applicant(s)/Patent Under Reexamination Early, RYANNE
	Examiner RUTH A DAVIS	Art Unit 1699

CPC - Searched*		
Symbol	Date	Examiner
A61K 35/12	08/13/2022	rad
A61L 27/3604, 54	08/13/2022	rad
A61L 2300/412	08/13/2022	rad

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
SEARCH, PALM, inventor search. See notes.	08/13/2022	rad

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

	/RUTH A DAVIS/ Primary Examiner, Art Unit 1699
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Form PTO-1449 US DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE				ATTY. DOCKET NO. SYG-19-1252US-CON2		SERIAL NO. 16/801,956	
LIST OF PUBLICATIONS CITED BY APPLICANT (Use several sheets if necessary)				APPLICANT ISE Professional Testing & Consulting Services, Inc.			
				FILING DATE February 26, 2020		GROUP TBD	
US PATENT DOCUMENTS							
EXAMINER INITIAL*		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE
	AA	2005/0013872 A1	01/20/05	Freyman			
	AB	2004/048796 A1 (corr. to KR '247)	03/11/04	Hariri et al.			
	AC						
	AD						
	AE						
	AF						
	AG						
	AH						
	AI						
	AJ						
	AK						
FOREIGN PATENT DOCUMENTS							
		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION
							YES NO
	AL	100999247 B1 (corr. to US '796)	12/07/10	KR			
	AM						
	AN						
	AO						
	AP						
OTHER PUBLICATIONS (Including Author, Title, Date, Pertinent Pages, Etc.)							
	AR	Notice of Allowance received in corresponding Korean Appln. No. 10-2015-7021367 dated May 18, 2021, and its English translation.					
	AS						
	AT						
EXAMINER /RUTH A DAVIS/				DATE CONSIDERED 08/13/2022			
*EXAMINER: Initial if publication considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.							

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US PATENT DOCUMENTS							
EXAMINER INITIAL*		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE
	AA						
	AB						
	AC						
	AD						
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	AI						
	AJ						
	AK						
FOREIGN PATENT DOCUMENTS							
		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION
							YES NO
	AL						
	AM						
	AN						
	AO						
	AP						
OTHER PUBLICATIONS (Including Author, Title, Date, Pertinent Pages, Etc.)							
	AR	Final Rejection from corresponding Japanese Patent Appln. No. 2018-224475 dated August 26, 2021, and an English summary of the action.					
	AS						
	AT						
EXAMINER				DATE CONSIDERED			
/RUTH A DAVIS/				08/13/2022			
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LIST OF PUBLICATIONS CITED BY APPLICANT (Use several sheets if necessary)				APPLICANT ISE Professional Testing & Consulting Services, Inc.			
				FILING DATE February 26, 2020		GROUP TBD	
US PATENT DOCUMENTS							
EXAMINER INITIAL*		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE
	AA						
	AB						
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	AF						
	AG						
	AH						
	AI						
	AJ						
	AK						
FOREIGN PATENT DOCUMENTS							
		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION
							YES NO
	AL						
	AM						
	AN						
	AO						
	AP						
OTHER PUBLICATIONS (Including Author, Title, Date, Pertinent Pages, Etc.)							
	AR	Notice of Reasons of Refusal from counterpart Japanese Patent Appln. No 2018-224475 dated October 13, 2020, and its English translation.					
	AS						
	AT						
EXAMINER /RUTH A DAVIS/				DATE CONSIDERED 08/13/2022			
*EXAMINER: Initial if publication considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.							

PE2E SEARCH - Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
L1	6	((("EARLY") near3 ("Ryane"))).INV.	(US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT)	OR	ON	ON	2022/08/13 02:49 PM
L2	124474	(A61K35/12 OR A61L27/3604 OR A61L27/54).cpc.	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/08/13 02:50 PM
L3	27	L1 AND L2	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/08/13 02:50 PM

PE2E SEARCH - Search History (Interference)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
N1	5	((("EARLY") near3 ("Ryane"))).INV.	(US-PGPUB; USPAT)	OR	ON	ON	2022/08/13 02:49 PM

Form PTO-1449 US DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE LIST OF PUBLICATIONS CITED BY APPLICANT <i>(Use several sheets if necessary)</i>				ATTY. DOCKET NO. SYG-19-1252US-CON2		SERIAL NO. 16/801 956 TBD	
				APPLICANT ISE Professional Testing & Consulting Services, Inc.			
				FILING DATE February 26, 2020		GROUP TBD	
US PATENT DOCUMENTS							
EXAMINER INITIAL*		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE
	AA	2006/153797 A1 (corr. to CN '039)	07/13/06	Bortolotto et al.			
	AB	6,485,969 B1	11/26/02	Asem et al.			
	AC	4,298,002	11/03/81	Ronel et al.			
	AD	4,361,552 A	11/30/82	Baur			
	AE	2004/0181240 A1	09/16/04	Tseng et al.			
	AF	2008/0046095 A1	02/21/08	Daniel			
	AG	2010/0104539 A1	04/29/10	Daniel et al.			
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FOREIGN PATENT DOCUMENTS							
		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION
							YES NO
	AL	2009-510091 A (corr. to WO '686)	03/12/09	JP			X
	AM	2007/038686 A2 (corr. to JP '091)	04/05/07	WO			
	AN	2004-521935 A (corr to. WO '720)	04/22/04	JP			X
	AO	02/078720 A2 (corr. to JP '935)	10/10/02	WO			
	AP	2007-532153 A (corr. to WO '219)	11/15/07	JP			X
OTHER PUBLICATIONS (Including Author, Title, Date, Pertinent Pages, Etc.)							
	AR	Notice for Reasons of Refusal from counterpart Japanese Patent Appln. No. 2018-224475 dated February 3, 2020, and its English translation.					
	AS	Second Office Action from counterpart Chinese Patent Appln No. 201480013359.0 dated May 22, 2019, along with its English translation.					
	AT	Duan et al., "Preliminary study on porous scaffold prepared with decellularized artery" Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi, 2010 Sep., 24(9), pages 1052-1057, Abstract only.					
EXAMINER				DATE CONSIDERED			
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							YES NO
	AL	2005/097219 A1 (corr. to JP '153)	10/20/05	WO			
	AM	2009-513269A	04/02/09	JP			X
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	AO	101337089 A	01/07/09	CN			Abstract
	AP	1791331 A	06/21/06	CN			X
OTHER PUBLICATIONS (Including Author, Title, Date, Pertinent Pages, Etc.)							
	AR	Meng et al., "An experimental study of extracellular matrix of conjunctiva", <u>Journal of Injuries and Occupational Diseases of the Eye with Ophthalmic Surgeries</u> , 2007 May; Vol.1, pages 26-28.					
	AS	Umashankar et al., "Glutaraldehyde Treatment Elicits Toxic Response Compared to Decellularization in Bovine Pericardium", <u>Toxicol Int.</u> , 2012, 19(1), pages 51-58.					
	AT	Sato et al., "Collagen reconstitution is inversely correlated with induction of limb regeneration in Ambystoma mexicanum", <u>Zoological Science</u> , 2012 Mar., Vol. 3, pages 191-197; doi: 10.2018/zsj.29.191.					
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	AL	102227225 A	10/26/11	CN			X
	AM	2008/129997 A1	10/30/10	WO			X
	AN	03/082201 A2	10/09/03	WO			
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OTHER PUBLICATIONS (Including Author, Title, Date, Pertinent Pages, Etc.)							
	AR	Smith et al., "Collagens from the Skin and Cartilage of the Larval Salamander Ambystoma tigrinum", <u>J. of Exp. Zoology</u> , Vol. 220, pages 243-250, 1982.					
	AS	Seifert et al., "Skin Regeneration in Adult Axolotls: A Blueprint for Scar-Free Healing in Vetebrates", <u>PLoS ONE</u> , www.plosone.org , April 2012, Vol. 7, Issue 4, e32875.					
	AT	Extended European Search Report from counterpart European Appln. No. 14797892.1 mailed September 28, 2016					
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	AL	2012/131104	10/04/12	WO			
	AM	2012/131106	10/04/12	WO			
	AN						
	AO						
	AP						
OTHER PUBLICATIONS (Including Author, Title, Date, Pertinent Pages, Etc.)							
	AR	International Search Report and Written Opinion from counterpart International Appln. No. PCT/US2014/010890 mailed May 15, 2014.					
	AS	International Preliminary Report on Patentability from counterpart International Appln. No. PCT/US2014/010890 mailed July 23, 2015.					
	AT	Cima et al., "Hepatocyte culture on biodegradable polymeric substrates" <i>Biotechnol Bioeng.</i> , 1991 Jun 20, 38(2): 145-158.					
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OTHER PUBLICATIONS (Including Author, Title, Date, Pertinent Pages, Etc.)							
	AR	Meller et al., "Amniotic Membrane Transplantation in the Human Eye", <u>Deutsches Arzteblatt International</u> , 2011 April, 108(14): 243-248.					
	AS	Totter et al., "The effect of source animal age upon extracellular matrix scaffold properties", <u>Biomaterials</u> , 2011 January, 32(1): 128-136.					
	AT	Vacanti et al., "Selective cell transplantation using bioabsorbable artificial polymers as matrices", <u>J. Pediatr. Surg.</u> , 1988 Jan, 12 (1 Pt 2), 3-9.					
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OTHER PUBLICATIONS (Including Author, Title, Date, Pertinent Pages, Etc.)							
	AR	Wiens et al., "Ontogeny discombobulates phylogeny: paedomorphosis and higher-level salamander relationships", <u>Systematic Biology</u> , 2005 Feb., 54(1): 91-110.					
	AS	Zaiou, "Multifunctional antimicrobial peptides: therapeutic targets in several human diseases", <u>Journal of Molecular Medicine</u> , 2007 Apr., 85(4): 317-329, Epub 2007 Jan 10.					
	AT	Vancanti et al., "Synthetic polymers seeded with chondrocytes provide a template for new cartilage formation", <u>Plast. Reconstr. Surg.</u> , 1991 Nov., 88(5): 753-759.					
EXAMINER /RUTH A DAVIS/				DATE CONSIDERED 08/13/2022			
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US PATENT DOCUMENTS							
EXAMINER INITIAL*		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE
	AA	2009/0142836 A1	06/04/09	Wang et al.			
	AB	2007/0248638 A1	10/25/07	Van Dyke et al.			
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OTHER PUBLICATIONS (Including Author, Title, Date, Pertinent Pages, Etc.)							
	AR	Notice of Reasons for Refusal from corresponding Korean Patent Appln. No. 10-2015-7021367 dated June 29, 2020, and its English translation.					
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EXAMINER /RUTH A DAVIS/				DATE CONSIDERED 08/13/2022			
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OTHER PUBLICATIONS (Including Author, Title, Date, Pertinent Pages, Etc.)		
	AR	Notice of Decision for Rejection received in corresponding Korean Appln. No. 10-2015-7021367 dated February 19, 2021, and its English translation.
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Application	Document	Mailroom Date	Attorney Docket No.
16801956	CTNF	08/18/2022	SYG-19-1252US-CON2
	892	08/18/2022	SYG-19-1252US-CON2
	1449	08/18/2022	SYG-19-1252US-CON2
	1449	08/18/2022	SYG-19-1252US-CON2
	1449	08/18/2022	SYG-19-1252US-CON2
	1449	08/18/2022	SYG-19-1252US-CON2
	1449	08/18/2022	SYG-19-1252US-CON2
	1449	08/18/2022	SYG-19-1252US-CON2

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PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Art Unit	: 1699	Customer No. 035811
Examiner	: Ruth A. Davis	
Appl. No.	: 16/801,956	Confirmation No.: 9621
Appl. Filed	: February 26, 2020	
First Named Inventor	: Ryanne Early	Docket No.: SYG-19- 1252US-CON2
Title	: DECELLULARIZED	
	: BIOMATERIAL	
	: FROM NON-MAMMALIAN	
	: TISSUE	Dated: November 11, 2022

RESPONSE

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

Sir:

In response to the Official Action dated August 18, 2022, the Applicant amends the application as follows:

In the Specification

Kindly amend para. [0071] as follows:

[0071] Although numerous methods exist, two exemplary methods may be used to produce a particulate form of the scaffold. The first method involved lyophilizing the disinfected material and then immersing the sample in liquid nitrogen. The snap frozen material is then reduced to small pieces with a blender so that the particles are small enough to be placed in a rotary knife mill, such as a Wiley mill. A #60 screen can be used to restrict the collected powder size to a desired size, for example less than ~~250 mm~~ 250 microns.

Kindly amend Example 4, bridging pages 25 and 26, as follows:

Example 4. Preparation of sterilized particulated or powder form of mineralized and non-mineralized decellularized and dehydrated urodele connective tissue matrix

Following decellularization of sections of mineralized collagen urodele connective tissue, one can perform a demineralization process, similar to that employed by Urist, and solvent or lyophilization dehydration, cryomilling of sectioned acellular demineralized, mineralized, or non-mineralized urodele connective tissue extracellular matrix, thereby to obtain particulated or powder form of the ECM with preserved histoarchitecture and function. “The final particle size distribution can be varied depending on duration and sieving, ~~post-cryomilling~~ cryomilling, between 125 and 850 microns. Low-dose cold gamma irradiation or e-beam irradiation (<25 Kgy) can be employed to sterilize acellular ECM sheets, particulate or powder and custom engineered constructs.

In the Claims

1. (Currently Amended) Decellularized extracellular matrix (ECM) 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 ~~of the biomaterial~~, making it suitable for use as a xenograft.

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

3. (Original) The decellularized ECM of claim 1, wherein said decellularized ECM is subjected to sterilization.

4. (Original) The decellularized ECM of claim 3 further comprising devitalized cells.

5. (Original) The decellularized ECM of claim 1 wherein the ECM is derived from Axolotl tissue.

6. (Original) The decellularized ECM of claim 1, wherein the ECM includes basement membrane.

7. (Currently Amended) 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 ~~human agent~~.

8. (Currently Amended) The decellularized ECM of claim 7, wherein the agent is a ~~cell, optionally a~~ human cell.

9. (Currently Amended) 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, ~~a sheet~~, or a mesh, and wherein the ECM is parietal ECM, mesothelial ECM, thoracic cavity ECM, abdominal cavity ECM, pericardium ECM or dermal tissue ECM.

10. (Original) The decellularized ECM according to claim 1 contained within a package.

11-12. (Cancelled)

13. (Withdrawn-Currently Amended) 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 ~~of the biomaterial~~, making it suitable for use as a xenograft.

14. (Withdrawn) The method of claim 13, 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.

15. (Withdrawn) The method of claim 13, further comprising homogenizing the decellularized ECM to form a powder, and, optionally reconstituting the powder as a gel.

16. (Withdrawn) The method of claim 13, further comprising sterilizing the decellularized ECM.

17. (Withdrawn) The method of claim 13, further comprising attaching the decellularized ECM to an agent xenogenic to a Urodele.

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

19. (Currently Amended) The ECM according to claim ~~9~~ 18, wherein the powder has a particle size of less than 250 microns.

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

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

22. (Original) The ECM of claim 21, wherein the gel comprises a synthetic polymer.

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

24. (Currently Amended) The ECM according to claim 1 comprising sterile, isolated, decellularized Urodele extra cellular matrix (ECM), wherein the ECM is ~~[[a]]~~ 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.

25. (Original) The ECM of claim 24, wherein the agent is an anti-microbial agent.

26. (Original) The ECM of claim 24, wherein the agent is a growth factor.
27. (Original) The ECM of claim 1, wherein the sheet is at least two sheets of isolated Urodele decellularized ECM laminated to one another.
28. (Withdrawn-Currently Amended) 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 ~~of the biomaterial~~, making it suitable for use as a xenograft.
29. (Withdrawn) The method according to claim 28, 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.
30. (Withdrawn-Currently Amended) A method for making the ECM of claim 29, 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 ~~of the biomaterial~~, making it suitable for use as a xenograft; and sterilizing said tissue.
31. (Withdrawn) The method according to claim 30, 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.
32. (Withdrawn) The method according to claim 30, 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.
33. (Cancelled)
34. (Original) A biomaterial comprising decellularized extracellular matrix (ECM)

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

35. (Withdrawn) A method of making a biomaterial comprising decellularized extracellular matrix (ECM) derived from a Urodele said decellularized ECM said method comprising:

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

Electronic Acknowledgement Receipt

EFS ID:	47005873
Application Number:	16801956
International Application Number:	
Confirmation Number:	9621
Title of Invention:	DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE
First Named Inventor/Applicant Name:	Ryanne Early
Customer Number:	35811
Filer:	Thomas Daniel Christenbury/Christine Aguado
Filer Authorized By:	Thomas Daniel Christenbury
Attorney Docket Number:	SYG-19-1252US-CON2
Receipt Date:	11-NOV-2022
Filing Date:	26-FEB-2020
Time Stamp:	09:41:29
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	Amendment-Transmittal-Letter.pdf	116821	no	1
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Warnings:

Information:					
2		Response-with-PDF-Enclosure. pdf	90013	yes	12
			7c0075660a74756bfbdd3e888cad7a9ec6bfb8fc12		
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	Document Description		Start	End	
	Amendment/Request for Reconsideration-After Non-Final Rejection		1	1	
	Specification		2	2	
	Claims		3	6	
	Applicant Arguments/Remarks Made in an Amendment		7	12	
Warnings:					
Information:					
Total Files Size (in bytes):			206834		
This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503. <u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application. <u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course. <u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.					

In re Application of RYANNE EARLY

Serial No.: 16/801,956

Filed: February 26, 2020

For: DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE

Commissioner for Patents

P.O. Box 1450

Alexandria, VA 22313-1450

Sir:

A Response to the outstanding Official Action in the above-identified application is enclosed, along with a copy of the AZO Materials PDF for the Examiner's convenience and a Terminal Disclaimer.

☒ Small entity status of this application under 37 CFR §1.9 and §1.27 has been established.

☐ This is a Petition for an Extension of Time for the period noted below, as well as for any additional period necessary to render this submission timely.

☒ No additional fee is required.

						SMALL ENTITY		OTHER THAN SMALL ENTITY	
TIME EXTENSION PETITION FEE				No. of month(s): 0		\$0.00			
Subtract time extension fee previously paid				No. of month(s): 0		(\$0.00)			
TOTAL EXTENSION FEE DUE						\$0.00			
CLAIM FEE	CLAIM(S) REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	EXTRA CLAIM(S) PRESENT	RATE	ADD'L CLAIM FEE	RATE	ADD'L CLAIM FEE	
TOTAL	32	MINUS	35	= 0	x 50=	\$0.00	x 100=		
INDEPENDENT	3	MINUS	3	= 0	x 240=	\$0.00	x 480=		
☐	FIRST PRESENTATION OF MULTIPLE CLAIM(S)				+ 430=	\$0.00	+ 860=		
TOTAL ADDITIONAL CLAIM FEE DUE						\$0.00			
TOTAL FEE DUE: \$0.00									

☒ Please charge Deposit Account No. 50-2719 in the amount of **\$85.00**. (Terminal Disclaimer Fee).

☒ The Commissioner is authorized to charge payment of the following fees associated with this communication or credit any overpayment to Deposit Account No. 50-2719.

☒ Any filing fees under 37 CFR §1.16 for the presentation of extra claims.

☒ Any patent application processing fees under 37 CFR §1.17.

Respectfully submitted,

/Chris Halliday/

Date: November 11, 2022

Christopher I. Halliday
Reg. No. 42,621

CIH/LML/cma
(215) 656-2452

Doc Code: DIST.E.FILE Document Description: Electronic Terminal Disclaimer - Filed		PTO/SB/26 U.S. Patent and Trademark Office Department of Commerce
Electronic Petition Request	TERMINAL DISCLAIMER TO OBIATE A DOUBLE PATENTING REJECTION OVER A "PRIOR" PATENT	
Application Number	16801956	
Filing Date	26-Feb-2020	
First Named Inventor	Ryenne Early	
Attorney Docket Number	SYG-19-1252US-CON2	
Title of Invention	DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE	
☒ Filing of terminal disclaimer does not obviate requirement for response under 37 CFR 1.111 to outstanding Office Action ☒ This electronic Terminal Disclaimer is not being used for a Joint Research Agreement.		
Owner	Percent Interest	
ISE Professional Testing & Consulting Services, Inc.	100%	
The owner(s) with percent interest listed above in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of prior patent number(s) 10617790 as the term of said prior patent is presently shortened by any terminal disclaimer. The owner hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and the prior patent are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns. In making the above disclaimer, the owner does not disclaim the terminal part of the term of any patent granted on the instant application that would extend to the expiration date of the full statutory term of the prior patent, "as the term of said prior patent is presently shortened by any terminal disclaimer," in the event that said prior patent later: - expires for failure to pay a maintenance fee; - is held unenforceable; - is found invalid by a court of competent jurisdiction; - is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321; - has all claims canceled by a reexamination certificate; - is reissued; or - is in any manner terminated prior to the expiration of its full statutory term as presently shortened by any terminal disclaimer. ☒ Terminal disclaimer fee under 37 CFR 1.20(d) is included with Electronic Terminal Disclaimer request.		

☐ I certify, in accordance with 37 CFR 1.4(d)(4), that the terminal disclaimer fee under 37 CFR 1.20(d) required for this terminal disclaimer has already been paid in the above-identified application.	
Applicant claims the following fee status: ☒ Small Entity ☐ Micro Entity ☐ Regular Undiscounted	
I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or 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 application or any patent issued thereon.	
THIS PORTION MUST BE COMPLETED BY THE SIGNATORY OR SIGNATORIES I certify, in accordance with 37 CFR 1.4(d)(4) that I am: ☒ An attorney or agent registered to practice before the Patent and Trademark Office who is of record in this application Registration Number <u>31750</u> ☐ A sole inventor ☐ A joint inventor; I certify that I am authorized to sign this submission on behalf of all of the inventors as evidenced by the power of attorney in the application ☐ A joint inventor; all of whom are signing this request	
Signature	/T. Daniel Christenbury/
Name	T. Daniel Christenbury

*Statement under 37 CFR 3.73(b) is required if terminal disclaimer is signed by the assignee (owner).
 Form PTO/SB/96 may be used for making this certification. See MPEP § 324.

Electronic Patent Application Fee Transmittal

Application Number:	16801956			
Filing Date:	26-Feb-2020			
Title of Invention:	DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE			
First Named Inventor/Applicant Name:	Ryanne Early			
Filer:	Thomas Daniel Christenbury/Christine Aguado			
Attorney Docket Number:	SYG-19-1252US-CON2			
Filed as Small Entity				
Filing Fees for Utility under 35 USC 111(a)				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
STATUTORY OR TERMINAL DISCLAIMER	2814	1	170	170
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				170

Doc Code: DISQ.E.FILE

Document Description: Electronic Terminal Disclaimer – Approved

Application No.: 16801956

Filing Date: 26-Feb-2020

Applicant/Patent under Reexamination: Early

Electronic Terminal Disclaimer filed on November 11, 2022

☒ APPROVED

This patent is subject to a terminal disclaimer

☐ DISAPPROVED

Approved/Disapproved by: Electronic Terminal Disclaimer automatically approved by EFS-Web

U.S. Patent and Trademark Office

Electronic Acknowledgement Receipt

EFS ID:	47006076
Application Number:	16801956
International Application Number:	
Confirmation Number:	9621
Title of Invention:	DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE
First Named Inventor/Applicant Name:	Ryanne Early
Customer Number:	35811
Filer:	Thomas Daniel Christenbury/Christine Aguado
Filer Authorized By:	Thomas Daniel Christenbury
Attorney Docket Number:	SYG-19-1252US-CON2
Receipt Date:	11-NOV-2022
Filing Date:	26-FEB-2020
Time Stamp:	10:11:50
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	\$ 170
RAM confirmation Number	E2022AAA11482935
Deposit Account	502719
Authorized User	Christine Aguado

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

37 CFR 1.16 (National application filing, search, and examination fees)

37 CFR 1.17 (Patent application and reexamination processing fees)

37 CFR 1.19 (Document supply fees)
 37 CFR 1.20 (Post Issuance fees)
 37 CFR 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Terminal Disclaimer-Filed (Electronic)	eTerminal-Disclaimer.pdf	41395	no	2
			20348393e8ef9f8fb92cf43a298b692ceab11266		

Warnings:

Information:

2	Fee Worksheet (SB06)	fee-info.pdf	37834	no	2
			dfbcf816e41233efcf08153c4229becdd356a608		

Warnings:

Information:

Total Files Size (in bytes):			79229
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This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Remarks

The Specification is amended to correct obvious typographic errors.

The “250 mm” is clearly a typographic error, as supported by the following comments and evidence.

Para. [0071] states:

A #60 screen can be used to restrict the collected powder size to a desired size, for example less than 250 mm....

A #60 screen permits particles smaller than 250 microns to pass through, as follows:

US Sieve Size	Tyler Equivalent	Opening	
		mm	in
No. 60	60 Mesh	0.250	0.0098

See the attached pdf and the listing of standard US Sieve Sizes Tyler Equivalents, mm and inch opening sizes, <https://www.azom.com/article.aspx?ArticleID=1417#>.

See also, Example 4, pages 25 and 26, and, in particular, page 26, lines 1-3, which provides:

The final particle size distribution can be varied depending on duration and sieving, post cryomilling, between 125 and 850 microns....

This disclosure, therefore, defines a range of 125-850 microns, thereby bracketing 250 microns.

250 mm, by contrast, would be a 25 cm sized opening which would permit similarly sized “particles” to pass through. This, of course, is clearly not what is intended, as such a size does not only not require sieving, but it is difficult to obtain intact tissue samples of such a size.

See also that size range claimed in original Claim 18, thereby making the 250 micron size a selection within that size range defined by the 125-850 micron range.

Note that Claim 19 correctly states the selected size 250-125 microns, upon being amended to depend from Claim 18.

The term “cyromilling” is likewise an obvious error. There is no such English term, and the correct term, “cryomilling” appears on page 25, second to last line, within Example 4.

The claims are amended, starting on page 3 herein above, to clarify the claims, ensure antecedent basis, and put the claims in condition for allowance.

Rejoinder of withdrawn method claims upon allowance of the product claims is earnestly solicited.

Claims 1, 7-9, 19, 24, 28, and 30 have been amended to clarify the claimed subject matter and to remove superfluous terms (“of the biomaterial”), from Claims 1, 13, 28, and 30 for which there is no antecedent basis in the claim. Note that the “biomaterial” limitation is maintained in Claims 34 and 35, for which there is antecedent basis.

Claims 11, 12 and 33 have been cancelled without prejudice or disclaimer.

Claims 13-17, 28-32, 34 and 35 stand withdrawn.

The Applicant respectfully requests rejoinder of method Claims 13-17, 28-32, 34 and 35 upon allowance of the elected product claims.

Claims 8 and 9 are objected to over informalities. Claims 8 and 9 have been amended as helpfully suggested by the Examiner. Withdrawal of the objection is respectfully requested.

Claims 7 and 8 are rejected under 35 USC §112(b) as being indefinite. Specifically, the rejection states that the term “human agent” in Claim 7 is unclear and that Claim 8 does not appear to further limit Claim 7.

Claim 7 has been amended to replace the term “human agent” with the term “cell.” Thus, Claim 8 further limits Claim 7 inasmuch as Claim 8 requires that the cell is a human cell.

Accordingly, the Applicant respectfully submits that Claims 7 and 8 are clear and definite. Withdrawal of the rejection is respectfully requested.

The dependency of Claim 19 to Claim 18 has been corrected to ensure a bounded lower limit to the range specified in Claim 19 is provided.

The language of Claim 24 has been amended to provide proper antecedent basis for Claim 27, which depends from Claim 24.

Claims 1-10, 18-27 and 34 are rejected on the ground of non-statutory double patenting over Claims 1-17 of US Pat. No. 10, 617,790. In light of the Terminal Disclaimer filed herewith, the Applicant respectfully submits that the rejection has been rendered moot.

In light of the foregoing, the Applicant respectfully requests examination of the application on the merits and expeditious issuance to patent.

Respectfully submitted,

/Chris Halliday/

Christopher I. Halliday
Reg. No. 42,621

CIH/LML/cma
(215) 656-2452

Enclosure
AZO Materials PDF

Particle Size - US Sieve Series and Tyler Mesh Size Equivalents

May 15 2002

Background

Sieving or screening is a method of separating a mixture of grains or particles into 2 or more size fractions, the over sized materials are trapped above the screen, while undersized materials can pass through the screen.

Sieves can be used in stacks, to divide samples up into various size fractions and hence determine particle size distributions.

Sieves and screen are usually used for larger particle sized materials i.e., greater than approximately 50µm (0.050mm).

Size Equivalents

Two scales that are used to classify particle sizes are the US Sieve Series and Tyler Equivalent, sometimes called Tyler Mesh Size or Tyler Standard Sieve Series. The most common mesh opening sizes for these scales are given in the table below and provide an indication of particle sizes.

US Sieve Size	Tyler Equivalent	Opening	
		mm	in
-	2½ Mesh	8.00	0.312
-	3 Mesh	6.73	0.265
No. 3½	3½ Mesh	5.66	0.233
No. 4	4 Mesh	4.76	0.187
No. 5	5 Mesh	4.00	0.157

No. 6	6 Mesh	3.36	0.132
No. 7	7 Mesh	2.83	0.111
No. 8	8 Mesh	2.38	0.0937
No.10	9 Mesh	2.00	0.0787
No. 12	10 Mesh	1.68	0.0661
No. 14	12 Mesh	1.41	0.0555
No. 16	14 Mesh	1.19	0.0469
No. 18	16 Mesh	1.00	0.0394
No. 20	20 Mesh	0.841	0.0331
No. 25	24 Mesh	0.707	0.0278
No. 30	28 Mesh	0.595	0.0234
No. 35	32 Mesh	0.500	0.0197
No. 40	35 Mesh	0.420	0.0165
No. 45	42 Mesh	0.354	0.0139
No. 50	48 Mesh	0.297	0.0117
No. 60	60 Mesh	0.250	0.0098
No. 70	65 Mesh	0.210	0.0083
No. 80	80 Mesh	0.177	0.0070
No.100	100 Mesh	0.149	0.0059

No. 120	115 Mesh	0.125	0.0049
No. 140	150 Mesh	0.105	0.0041
No. 170	170 Mesh	0.088	0.0035
No. 200	200 Mesh	0.074	0.0029
No. 230	250 Mesh	0.063	0.0025
No. 270	270 Mesh	0.053	0.0021
No. 325	325 Mesh	0.044	0.0017
No. 400	400 Mesh	0.037	0.0015

The mesh number system is a measure of how many openings there are per linear inch in a screen. Sizes vary by a factor of $\sqrt{2}$. This can easily be determined as screens are made from wires of standard diameters, however, opening sizes can vary slightly due to wear and distortion.

US sieve sizes differ from Tyler Screen sizes in that they are arbitrary numbers.

Primary author: AZoM.com



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

35811 7590 01/24/2023
IP GROUP OF DLA PIPER LLP (US)
ONE LIBERTY PLACE
1650 MARKET ST, SUITE 5000
PHILADELPHIA, PA 19103

EXAMINER

DAVIS, RUTH A

ART UNIT

PAPER NUMBER

1699

DATE MAILED: 01/24/2023

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/801,956	02/26/2020	Ryanne Early	SYG-19-1252US-CON2	9621

TITLE OF INVENTION: DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	\$480	\$0.00	\$0.00	\$480	04/24/2023

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 40% the amount of undiscounted fees, and micro entity fees are 20% the amount of undiscounted fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Maintenance fees are due in utility patents issuing on applications filed on or after Dec. 12, 1980. It is patentee's responsibility to ensure timely payment of maintenance fees when due. More information is available at www.uspto.gov/PatentMaintenanceFees.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via EFS-Web.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

By fax, send to: (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via EFS-Web or by facsimile to (571) 273-2885, on the date below.

_____ (Typed or printed name)
_____ (Signature)
_____ (Date)

35811 7590 01/24/2023
IP GROUP OF DLA PIPER LLP (US)
ONE LIBERTY PLACE
1650 MARKET ST, SUITE 5000
PHILADELPHIA, PA 19103

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/801,956	02/26/2020	Ryanne Early	SYG-19-1252US-CON2	9621

TITLE OF INVENTION: DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	\$480	\$0.00	\$0.00	\$480	04/24/2023

EXAMINER	ART UNIT	CLASS-SUBCLASS
DAVIS, RUTH A	1699	424-093100

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

☐ Change of correspondence address (or Change of Correspondence Address form PTO/AIA/122 or PTO/SB/122) attached.

☐ "Fee Address" indication (or "Fee Address" Indication form PTO/AIA/47 or PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,

1 _____

(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

2 _____

3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

(B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☐ Corporation or other private group entity ☐ Government4a. Fees submitted: ☐ Issue Fee ☐ Publication Fee (if required) ☐ Advance Order - # of Copies _____

4b. Method of Payment: (Please first reapply any previously paid fee shown above)

☐ Electronic Payment via EFS-Web ☐ Enclosed check ☐ Non-electronic payment by credit card (Attach form PTO-2038)☐ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. _____

5. Change in Entity Status (from status indicated above)

☐ Applicant certifying micro entity status. See 37 CFR 1.29☐ Applicant asserting small entity status. See 37 CFR 1.27☐ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____

Date _____

Typed or printed name _____

Registration No. _____



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www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/801,956	02/26/2020	Ryanne Early	SYG-19-1252US-CON2	9621
35811 7590 01/24/2023 IP GROUP OF DLA PIPER LLP (US) ONE LIBERTY PLACE 1650 MARKET ST, SUITE 5000 PHILADELPHIA, PA 19103			EXAMINER DAVIS, RUTH A	
			ART UNIT 1699	PAPER NUMBER
DATE MAILED: 01/24/2023				

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b) (Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 16/801,956	Applicant(s) Early, Ryanne	
	Examiner RUTH A DAVIS	Art Unit 1699	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to reply filed 11.11.2022.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

3. ☒ The allowed claim(s) is/are 1-10,13-32 and 34-35. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov**.

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a) ☐ All b) ☐ Some* c) ☐ None of the:

1. ☐ Certified copies of the priority documents have been received.

2. ☐ Certified copies of the priority documents have been received in Application No. _____.

3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

* Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892) 2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____. 3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____. 4. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____.	5. ☒ Examiner's Amendment/Comment 6. ☐ Examiner's Statement of Reasons for Allowance 7. ☐ Other _____.
---	--

	/RUTH A DAVIS/ Primary Examiner, Art Unit 1699
--	---

DETAILED ACTION

Notice of Pre-AIA or AIA Status

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

Applicant's amendment, reply and terminal disclaimer filed November 11, 2022 have been received and entered into the case. Claims 11 – 12 and 33 are canceled; claims 1 – 10, 13 – 32 and 34 – 35 are pending and have been considered on the merits. All arguments have been fully considered.

Claim Objections

Previous objections are withdrawn due to applicant's amendments.

Claim Rejections - 35 USC § 112

Previous rejections under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, are withdrawn due to applicant's amendment removing unclear language.

Double Patenting

The previous nonstatutory double patenting rejection is withdrawn due to applicant's filing of a proper terminal disclaimer on November 11, 2022.

Election/Restrictions

Claims 1 – 10, 18 – 27 and 34 are allowable. Claims 13 – 17, 28 – 32 and 35, previously withdrawn from consideration as a result of a restriction requirement, require all the limitations of an allowable claim. Pursuant to the procedures set forth in MPEP § 821.04(a), **the restriction requirement between inventions I - V, as set forth in the Office action mailed on April 28, 2022, is hereby withdrawn** and claims 13 – 17, 28 – 32 and 35 are hereby rejoined and fully examined for patentability under 37 CFR 1.104. In view of the withdrawal of the restriction requirement, applicant(s) are advised that if any claim presented in a continuation or divisional application is anticipated by, or includes all the limitations of, a claim that is allowable in the present application, such claim may be subject to provisional statutory and/or nonstatutory double patenting rejections over the claims of the instant application. Once the restriction requirement is withdrawn, the provisions of 35 U.S.C. 121 are no longer applicable. See *In re Ziegler*, 443 F.2d 1211, 1215, 170 USPQ 129, 131-32 (CCPA 1971). See also MPEP § 804.01.

Allowable Subject Matter

Claims 1 – 10, 13 – 32 and 34 – 35 are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to RUTH A DAVIS whose telephone number is (571)272-0915. The examiner can normally be reached Monday - Friday (8am - 4pm).

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is

Art Unit: 1699

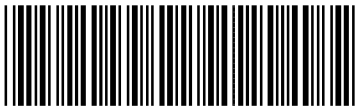
encouraged to use the USPTO Automated Interview Request (AIR) at <http://www.uspto.gov/interviewpractice>.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Fereydoun Sajjadi can be reached on 571-272-3311. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of published or unpublished applications may be obtained from Patent Center. Unpublished application information in Patent Center is available to registered users. To file and manage patent submissions in Patent Center, visit: <https://patentcenter.uspto.gov>. Visit <https://www.uspto.gov/patents/apply/patent-center> for more information about Patent Center and <https://www.uspto.gov/patents/docx> for information about filing in DOCX format. For additional questions, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/RUTH A DAVIS/

Primary Examiner, Art Unit 1699

Issue Classification 	Application/Control No. 16/801,956	Applicant(s)/Patent Under Reexamination Early, RYANNE	
	Examiner RUTH A DAVIS	Art Unit 1699	

CPC						
Symbol					Type	Version
A61L	/	27	/	3687	F	2013-01-01
A61K	/	9	/	70	I	2013-01-01
A61L	/	27	/	58	I	2013-01-01
A61L	/	27	/	60	I	2013-01-01
A61L	/	27	/	54	I	2013-01-01
A61L	/	27	/	362	I	2013-01-01
A61L	/	27	/	3691	I	2013-01-01
A61K	/	38	/	1703	I	2013-01-01
A61K	/	35	/	12	I	2013-01-01
A61L	/	27	/	3625	I	2013-01-01
A61L	/	27	/	3604	I	2013-01-01
A61L	/	27	/	3633	I	2013-01-01
A61L	/	27	/	38	I	2013-01-01
A61K	/	35	/	65	I	2013-01-01
A61L	/	27	/	52	I	2013-01-01
C12N	/	5	/	0625	I	2013-01-01
A61L	/	2430	/	40	A	2013-01-01
A61L	/	2430	/	34	A	2013-01-01
A61L	/	2300	/	64	A	2013-01-01
A61L	/	2300	/	606	A	2013-01-01
A61L	/	2300	/	604	A	2013-01-01
A61L	/	2300	/	412	A	2013-01-01
A61L	/	2300	/	252	A	2013-01-01
C12N	/	2533	/	90	A	2013-01-01
C12N	/	2533	/	92	A	2013-01-01

CPC Combination Sets					
Symbol				Type	Set

NONE (Assistant Examiner) _____ (Date) _____		Total Claims Allowed: 32	
/RUTH A DAVIS/ Primary Examiner, Art Unit 1699 (Primary Examiner) _____ (Date) _____		14 January 2023 (Date)	O.G. Print Claim(s) 1
			O.G. Print Figure NONE

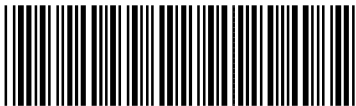
Issue Classification 	Application/Control No. 16/801,956	Applicant(s)/Patent Under Reexamination Early, RYANNE
	Examiner RUTH A DAVIS	Art Unit 1699

INTERNATIONAL CLASSIFICATION			
CLAIMED			
A61L27/36	/	27	/ 36
A61L27/54	/	27	/ 54
A61K35/12	/	35	/ 12
A61L27/52	/	27	/ 52
NON-CLAIMED			
	/		/

US ORIGINAL CLASSIFICATION	
CLASS	SUBCLASS

CROSS REFERENCES(S)						
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)					

NONE		Total Claims Allowed:	
(Assistant Examiner)	(Date)	32	
/RUTH A DAVIS/ Primary Examiner, Art Unit 1699 (Primary Examiner)	14 January 2023 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure NONE

Issue Classification 	Application/Control No. 16/801,956	Applicant(s)/Patent Under Reexamination Early, RYANNE
	Examiner RUTH A DAVIS	Art Unit 1699

☐ Claims renumbered in the same order as presented by applicant
 ☐ CPA
 ☒ T.D.
 ☐ R.1.47

CLAIMS															
Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
1	1	12	10	11	19	26	28								
2	2		11	13	20	27	29								
3	3		12	14	21	28	30								
4	4	21	13	15	22	29	31								
5	5	22	14	16	23	30	32								
6	6	23	15	17	24		33								
7	7	24	16	18	25	31	34								
8	8	25	17	19	26	32	35								
9	9	10	18	20	27										

NONE (Assistant Examiner) _____ (Date) _____		Total Claims Allowed: 32	
/RUTH A DAVIS/ Primary Examiner, Art Unit 1699 (Primary Examiner) _____ (Date) 14 January 2023		O.G. Print Claim(s) 1	O.G. Print Figure NONE

<i>Search Notes</i> 	Application/Control No. 16/801,956	Applicant(s)/Patent Under Reexamination Early, Ryanne
	Examiner RUTH A DAVIS	Art Unit 1699

CPC - Searched*		
Symbol	Date	Examiner
A61K 35/12	01/13/2023	rad
A61L 27/3604, 54	01/13/2023	rad
A61L 2300/412	01/13/2023	rad

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
SEARCH, PALM, inventor search updates. See notes.	01/13/2023	rad

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner
A61K	35/12	01/13/2023	rad
A61L	27/3604, 3687, 54	01/13/2023	rad
A61L	2300/412	01/13/2023	rad

	/RUTH A DAVIS/ Primary Examiner, Art Unit 1699
--	---

PE2E SEARCH - Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
L1	6	((("EARLY") near3 ("Ryanne"))).INV.	(US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT)	OR	ON	ON	2023/01/13 09:55 PM
L2	154055	(A61L27/3687 OR A61K35/12 OR A61L27/3604 OR A61L27/52 OR A61L27/54).cpc.	(US-PGPUB; USPAT; USOCR; FIT (AU, AP, AT, BE, BG, BR, BY, CA, CH, CN, CS, CU, CZ, DD, DE, DK, EA, EE, EP, ES, FI, FR, GB, HR, HU, ID, IE, IL, IS, IT, JP, KR, LT, LU, LV, MA, OA, RU, SU, WO, MC, MD, MY, NL, NO, NZ, PH, PL, PT, RO, RS, SE, SG, SI, SK, TH, TN, TR, TW, UA, VN); FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/01/13 09:56 PM

PE2E SEARCH - Search History (Interference)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
N1	5	((("EARLY") near3 ("Ryanne"))).INV.	(US-PGPUB; USPAT)	OR	ON	ON	2023/01/13 09:55 PM
N2	22981	(A61L27/3687 OR A61K35/12 OR A61L27/3604 OR A61L27/52 OR A61L27/54).cpc.	(US-PGPUB; USPAT)	OR	ON	ON	2023/01/13 09:56 PM

PE2E SEARCH - Search History (Similarity Search)

No Similarity Results available

To: pto.phil@us.dlapiper.com,,
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 35811

Jan 24, 2023 03:27:14 AM

Dear PAIR Customer:

IP GROUP OF DLA PIPER LLP (US)
ONE LIBERTY PLACE
1650 MARKET ST, SUITE 5000
PHILADELPHIA, PA 19103
UNITED STATES

The following USPTO patent application(s) associated with your Customer Number, 35811 , have new outgoing correspondence. This correspondence is now available for viewing in Private PAIR.

The official date of notification of the outgoing correspondence will be indicated on the form PTOL-90 accompanying the correspondence.

Disclaimer:

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Application	Document	Mailroom Date	Attorney Docket No.
16801956	NOA	01/24/2023	SYG-19-1252US-CON2

To view your correspondence online or update your email addresses, please visit us anytime at <https://portal.uspto.gov/secure/myportal/privatepair>.

If you have any questions, please email the Electronic Business Center (EBC) at EBC@uspto.gov with 'e-Office Action' on the subject line or call 1-866-217-9197 during the following hours:

Monday - Friday 6:00 a.m. to 12:00 a.m.

Thank you for prompt attention to this notice,

UNITED STATES PATENT AND TRADEMARK OFFICE
PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

PATENT ASSIGNMENT COVER SHEET

Electronic Version v1.1
 Stylesheet Version v1.2

EPAS ID: PAT7785334

SUBMISSION TYPE:	CORRECTIVE ASSIGNMENT
NATURE OF CONVEYANCE:	Corrective Assignment to correct the ASSIGNEE'S NAME previously recorded on Reel 053947 Frame 0941. Assignor(s) hereby confirms the ASSIGNEE'S NAME IS SYNOGEN MANAGEMENT GROUP, INC..
CONVEYING PARTY DATA	
Name	Execution Date
ISE PROFESSIONAL TESTING & CONSULTING SERVICES, INC.	07/24/2020
RECEIVING PARTY DATA	
Name:	SYNOGEN MANAGEMENT GROUP, INC.
Street Address:	101 SE 2ND PLACE
Internal Address:	SUITE 201 B
City:	GAINESVILLE
State/Country:	FLORIDA
Postal Code:	32601
PROPERTY NUMBERS Total: 3	
Property Type	Number
Application Number:	61750555
Application Number:	14759895
Application Number:	16801956
CORRESPONDENCE DATA	
Fax Number:	(215)656-3301
<i>Correspondence will be sent to the e-mail address first; if that is unsuccessful, it will be sent using a fax number, if provided; if that is unsuccessful, it will be sent via US Mail.</i>	
Phone:	2156562452
Email:	pto.phil@us.dlapiper.com
Correspondent Name:	IP GROUP OF DLA PIPER LLP (US)
Address Line 1:	ONE LIBERTY PLACE
Address Line 2:	1650 MARKET ST, SUITE 5000
Address Line 4:	PHILADELPHIA, PENNSYLVANIA 19103
ATTORNEY DOCKET NUMBER:	SYN-19-1252US-CON2
NAME OF SUBMITTER:	CHRIS HALLIDAY
SIGNATURE:	/chris halliday/
DATE SIGNED:	02/08/2023
This document serves as an Oath/Declaration (37 CFR 1.63).	

Total Attachments: 5

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PATENT ASSIGNMENT COVER SHEET

Electronic Version v1.1
 Stylesheet Version v1.2

EPAS ID: PAT6330614

SUBMISSION TYPE:	NEW ASSIGNMENT
NATURE OF CONVEYANCE:	ASSIGNMENT
CONVEYING PARTY DATA	
Name	Execution Date
ISE PROFESSIONAL TESTING & CONSULTING SERVICES, INC.	07/24/2020
RECEIVING PARTY DATA	
Name:	~~SYNOGEN MANAGEMENT, INC.~~ <u>SYNOGEN MANAGEMENT GROUP, INC.</u>
Street Address:	101 SE 2ND PLACE
Internal Address:	SUITE 201 B
City:	GAINESVILLE
State/Country:	FLORIDA
Postal Code:	32601
PROPERTY NUMBERS Total: 3	
Property Type	Number
Application Number:	61750555
Application Number:	14759895
Application Number:	16801956
CORRESPONDENCE DATA	
Fax Number:	(215)656-3301
<i>Correspondence will be sent to the e-mail address first; if that is unsuccessful, it will be sent using a fax number, if provided; if that is unsuccessful, it will be sent via US Mail.</i>	
Phone:	215-656-3300
Email:	pto.phil@us.dlapiper.com
Correspondent Name:	IP GROUP OF DLA PIPER LLP (US)
Address Line 1:	ONE LIBERTY PLACE
Address Line 2:	1650 MARKET STREET, SUITE 5000
Address Line 4:	PHILADELPHIA, PENNSYLVANIA 19103
ATTORNEY DOCKET NUMBER:	SYG-19-1252/US-CON
NAME OF SUBMITTER:	SIMA SINGADIA KULKARNI
SIGNATURE:	/sima singadia kulkarni/
DATE SIGNED:	10/01/2020
Total Attachments: 3	
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source=ISE to Synogen#page2.tif	

ASSIGNMENT

WHEREAS, ISE Professional Testing & Consulting Services, Inc., a corporation of Florida, with its principal place of business located at 2785 S. Bay Street G, Eustis, FL 32726 ("Assignor") is the owner of the entire right, title and interest in and to U.S. Provisional Patent Application No. 61/750,555, PCT International Patent Application No. PCT/US2014/010890, together with the patents and patent applications including but not limited to those listed in Schedule A, and of the invention(s) therein described; and

WHEREAS, Synogen Management Group, Inc., a corporation of Florida, with its principal place of business located at 101 SE 2nd Place, Suite 201 B, Gainesville, FL 32601 ("Assignee"), is desirous of acquiring the entire right, title and interest in and U.S. Provisional Patent Application No. 61/750,555, PCT International Patent Application No. PCT/US2014/010890, together with the patents and patent applications listed in Schedule A, and of the invention(s) therein described.

NOW, THEREFORE, in consideration of One Dollar (\$1.00) and of other good and valuable consideration, receipt of which is hereby acknowledged, the undersigned, intending to be legally bound, does hereby sell, assign and transfer to the assignee the entire right, title and interest, for the United States of America, its territories and possessions, and for all foreign countries, in said U.S. Provisional Patent Application No. 61/750,555, PCT International Patent Application No. PCT/US2014/010890, all divisions and continuations thereof, all rights to claim priority based thereon, all rights to file foreign applications on said inventions, and all letters patents and reissues thereof, issuing for said inventions and any improvements thereon in the United States of America and in any and all foreign countries including the patents and patent applications listed in Schedule A.

It is agreed that the undersigned shall be legally bound, upon request of the assignee, or its successors or assigns or a legal representative thereof, to execute all instruments proper to patent the inventions in the United States of America and foreign countries in the name of the assignee, and to execute all instruments proper to carry out the intent of this instrument.

The rights and property herein conveyed by the undersigned are free and clear of any encumbrance.

[Signature Page Follows]

EXECUTED under seal on July 24, 2020.

ISE Professional Testing & Consulting Services, Inc.

By: [Signature]

Name: Ryanne Early

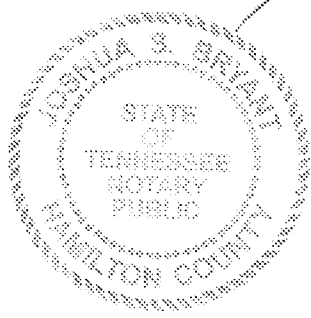
Title: President

STATE OF Tennessee :
COUNTY OF Hamilton : ss
:

On July 24, 2020, before me, the undersigned, personally appeared, Ryan Early, known to me or satisfactorily proven to be the person(s) who executed the within instrument, and who acknowledged herself/himself to be the President of ISE Professional Testing & Consulting Services, Inc., and, being authorized to do so, executed the foregoing instrument for the purposes therein contained by signing the name of the said company by herself/himself as such Ryan Early.

IN WITNESS WHEREOF, I have hereunto set my hand and official seal.

[Signature] (SEAL)
Notary Public
ex 11-07-2021



SCHEDULE A

Country	Application Number	Filing Date
Canada	2,897,662	January 9, 2014
China	201780013359.0	January 9, 2014
Europe	14737892.1	January 9, 2014
Hong Kong	16108111.9	January 9, 2014
Israel	239828	January 9, 2014
Japan	2015-552775 2018-224475 (Divisional)	January 9, 2014 November 30, 2018
Korea	10-2015-7021367	January 9, 2014
United States	61/750,555 14/759,895 16/801956 (Continuation)	January 9, 2013 July 8, 2018 April 30, 2019
WIPO	PCT/US2014/010890	January 9, 2014

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Art Unit	: 1699	Customer No.	035811
Examiner	: Ruth A. Davis		
Serial No.	: 16/801,956	Docket No.:	SYG-19-1252US-CON2
Filed	: February 26, 2020		
Inventor	: RYANNE EARLY	Confirmation No.:	9621
Title	: DECELLULARIZED		
	: BIOMATERIAL FROM	Not. of Allow.:	01/24/23
	: NON-MAMMALIAN TISSUE	Dated:	April 24, 2023

TRANSMITTAL LETTER

Mail Stop ISSUE FEE

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

Sir:

Applicant submits herewith Form PTOL-85B.

The Commissioner is authorized to charge the \$480.00 fee to Deposit Account No. 50-2719 to cover the fee for payment of the issue fee.

The Commissioner is also authorized to charge any additional fees to Deposit Account No. 50-2719.

Respectfully submitted,

/Christopher I. Halliday/

Christopher I. Halliday
Reg. No. 42,621

CH/rk
(215) 656-2452

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via EFS-Web.

By mail, send to: Mail Stop ISSUE FEE
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Alexandria, Virginia 22313-1450

By fax, send to: (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

35811 7590 01/24/2023
IP GROUP OF DLA PIPER LLP (US)
ONE LIBERTY PLACE
1650 MARKET ST, SUITE 5000
PHILADELPHIA, PA 19103

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I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via EFS-Web or by facsimile to (571) 273-2885, on the date below.

(Typed or printed name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/801,956	02/26/2020	Ryanne Early	SYG-19-1252US-CON2	9621

TITLE OF INVENTION: DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	\$480	\$0.00	\$0.00	\$480	04/24/2023

EXAMINER	ART UNIT	CLASS-SUBCLASS
DAVIS, RUTH A	1699	424-093100

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

☐ Change of correspondence address (or Change of Correspondence Address form PTO/AIA/122 or PTO/SB/122) attached.

☐ "Fee Address" indication (or "Fee Address" Indication form PTO/AIA/47 or PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,

(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 DLA Piper LLP (US)

2

3

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

(B) RESIDENCE: (CITY and STATE OR COUNTRY)

NeXtGen Biologics, Inc.

Alachua, FL

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☒ Corporation or other private group entity ☐ Government

4a. Fees submitted: ☒ Issue Fee ☐ Publication Fee (if required) ☐ Advance Order - # of Copies _____

4b. Method of Payment: (Please first reapply any previously paid fee shown above)

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☒ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. 50-2719

5. Change in Entity Status (from status indicated above)

☐ Applicant certifying micro entity status. See 37 CFR 1.29

☐ Applicant asserting small entity status. See 37 CFR 1.27

☐ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature /Christopher I. Halliday/

Date April 24, 2023

Typed or printed name Christopher I. Halliday

Registration No. 42,621



ELECTRONIC PAYMENT RECEIPT

APPLICATION #
16/801,956

RECEIPT DATE / TIME
04/24/2023 04:17:42 PM ET

ATTORNEY DOCKET #
SYG-19-1252US-CON2

Title of Invention

DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE

Application Information

APPLICATION TYPE Utility - Nonprovisional Application
under 35 USC 111(a)

PATENT # -

CONFIRMATION # 9621

FILED BY Rachel Kirk

PATENT CENTER # 61973613

AUTHORIZED BY Thomas Christenbury

CUSTOMER # 35811

FILING DATE 02/26/2020

CORRESPONDENCE
ADDRESS -

FIRST NAMED
INVENTOR Ryanne Early

Payment Information

PAYMENT METHOD
DA / 502719

PAYMENT TRANSACTION ID
E20234NG19335658

PAYMENT AUTHORIZED BY
Rachel Kirk

PRE-AUTHORIZED ACCOUNT
502719

PRE-AUTHORIZED CATEGORY

37 CFR 1.16 (National application filing, search, and examination fees); 37 CFR 1.17 (Patent application and reexamination processing fees); 37 CFR 1.19 (Document supply fees); 37 CFR 1.20 (Post issuance fees); 37 CFR 1.21 (Miscellaneous fees and charges)

FEE CODE	DESCRIPTION	ITEM PRICE(\$)	QUANTITY	ITEM TOTAL(\$)
2501	UTILITY ISSUE FEE	480.00	1	480.00
TOTAL AMOUNT:				\$480.00

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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #
16/801,956

RECEIPT DATE / TIME
04/24/2023 04:17:42 PM ET

ATTORNEY DOCKET #
SYG-19-1252US-CON2

Title of Invention

DECELLULARIZED BIOMATERIAL FROM NON-MAMMALIAN TISSUE

Application Information

APPLICATION TYPE Utility - Nonprovisional Application
under 35 USC 111(a)

PATENT # -

CONFIRMATION # 9621

FILED BY Rachel Kirk

PATENT CENTER # 61973613

FILING DATE 02/26/2020

CUSTOMER # 35811

FIRST NAMED INVENTOR
Ryanne Early

CORRESPONDENCE ADDRESS -

AUTHORIZED BY Thomas Christenbury

Documents

TOTAL DOCUMENTS: 2

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
SYG-19-1252US- CON2_Transmittal.pdf	1	Transmittal Letter	95 KB
SYG-19-1252US- CON2_PTOL85B.pdf	1	Issue Fee Payment (PTO-85B)	121 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
SYG-19-1252US- CON2_Transmittal.pdf	79460B5DEAE4C11FD97B6743F350541960E40FEEC81DDA159 B6307BD4504FA9798440BCF24DFE19AD77619242830F62BDA

EDF0A786B70ABA37ED6F60B0A6C9CD

SYG-19-1252US-
CON2_PTOL85B.pdfB9A1D5D0D8758AF99223396C6FBCDAB264204186508CEE0B5
1C9A679839D13BCEE5C37D5812E83BF13B18592331DE52E71
3BF191A0870ECB795ABFD93CEB1A56

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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/801,956	05/30/2023	11660376	SYG-19-1252US-CON2	9621

35811 7590 05/10/2023
IP GROUP OF DLA PIPER LLP (US)
ONE LIBERTY PLACE
1650 MARKET ST, SUITE 5000
PHILADELPHIA, PA 19103

ISSUE NOTIFICATION

The projected patent number and issue date are specified above. The patent will issue electronically. The electronically issued patent is the official patent grant pursuant to 35 U.S.C. § 153. The patent may be accessed on or after the issue date through Patent Center at <https://patentcenter.uspto.gov/>. The patent will be available in both the public and the private sides of Patent Center. Further assistance in electronically accessing the patent, or about Patent Center, is available by calling the Patent Electronic Business Center at 1-888-217-9197.

The USPTO is implementing electronic patent issuance with a transition period, during which period the USPTO will mail a ceremonial paper copy of the electronic patent grant to the correspondence address of record. Additional copies of the patent (i.e., certified and presentation copies) may be ordered for a fee from the USPTO's Certified Copy Center at <https://certifiedcopycenter.uspto.gov/index.html>. The Certified Copy Center may be reached at (800)972-6382.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b) (application filed on or after May 29, 2000)

The Patent Term Adjustment is 460 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (<http://pair.uspto.gov>).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Patents Stakeholder Experience (OPSE), Stakeholder Support Division (SSD) at (571)-272-4200.

INVENTOR(s) (Please see PAIR WEB site <http://pair.uspto.gov> for additional inventors):

Ryanne Early, Fleming Island, FL;

APPLICANT(s) (Please see PAIR WEB site <http://pair.uspto.gov> for additional applicants):

ISE Professional Testing & Consulting Services, Inc., Fleming Island, FL;

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TO: pto.phil@us.dlapiper.com
FROM: noreply@uspto.gov
CC: patentcenter_eofficeaction@uspto.gov
SUBJECT: USPTO: Patent Electronic System - Correspondence Notification for Customer Number 35811

Thu May 11 08:38:36 EDT 2023

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Correspondence Address:

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Application	Document	Mailroom Date	Attorney Docket No.
16801956	ISSUE.NTF	05/10/2023	SYG-19-1252US-CON2

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APPLICATION NO.	ISSUE DATE	PATENT NO.
16/801,956	30-MAY-23	11660376

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PHILADELPHIA, PA 19103

EGRANT NOTIFICATION

Your electronic patent grant (eGrant) is now available, which can be accessed via Patent Center at <https://patentcenter.uspto.gov>

The electronic patent grant is the official patent grant under 35 U.S.C. 153. For more information, please visit <https://www.uspto.gov/electronicgrants>

TO: pto.phil@us.dlapiper.com
FROM: noreply@uspto.gov
CC: patentcenter_eofficeaction@uspto.gov
SUBJECT: USPTO: Patent Electronic System - Correspondence Notification for Customer Number 35811

Tue May 30 05:22:04 EDT 2023

Dear Patent Center Customer:

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Application	Document	Mailroom Date	Attorney Docket No.
16801956	EGRANT.NTF	05/30/2023	SYG-19-1252US-CON2

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