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(71) Applicant: **SCRIPPS HEALTH** [US/US]; 4275 Campus Point Court, San Diego, CA 92121 (US).

(72) Inventors: **D'LIMA, Darryl, D.**; 4275 Campus Point Court, San Diego, CA 92121 (US). **OLEE, Tsaiwei**; 4275 Campus Point Court, San Diego, CA 92121 (US). **COLWELL, Clifford, W.**; 4275 Campus Point Court, San Diego, CA 92121 (US).

(74) Agent: **POULOS, Sabrina, D.**; Wilson Sonsini Goodrich & Rosati, 650 Page Mill Road, Plao Alto, CA 94304 (US).

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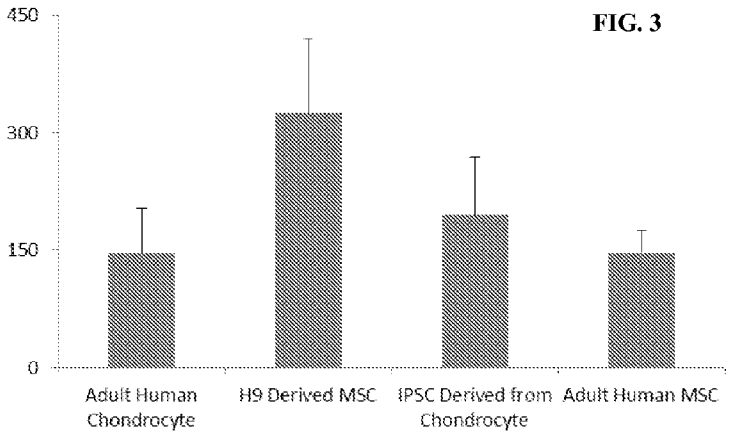

FIG. 3

(57) Abstract: Provided are tri-component matrices having collagen, hyaluronan, and chondroitin sulfate. Also provided are processes for producing a tri-component matrix. Additionally, provided are processes for providing cells capable of producing cartilage to a bone or cartilage defects.

METHODS OF TRANSPLANTING CHONDROCYTES

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Application Serial No. 61/719,902, filed October 29, 2012 which is hereby incorporated by reference in its entirety.

SUMMARY OF THE INVENTION

[0002] Disclosed herein, in some embodiments, is a tri-component matrix comprising: a) isolated and purified non-denatured type II collagen; b) hyaluronan; and c) chondroitin sulfate. In some embodiments, the tri-component matrix further comprises water. In some embodiments, the tri-component matrix further comprises a basal media. In some embodiments, the basal media is Medium 199. In some embodiments, the type II collagen is bovine-derived type II collagen. In some embodiments, the concentration of type II collagen is from about 0.5 mg/ml to about 5 mg/ml. In some embodiments, the concentration of type II collagen is about 3 mg/ml. In some embodiments, the concentration of hyaluronan is from about 0.25 mg/ml to about 3 mg/ml. In some embodiments, the concentration of hyaluronan is about 1 mg/ml. In some embodiments, the concentration of chondroitin sulfate is from about 0.25 mg/ml to about 3 mg/ml. In some embodiments, the concentration of chondroitin sulfate is about 1 mg/ml. In some embodiments, the tri-component matrix further comprises chondrocytes, chondroprogenitor cells, mesenchymal stem cells, induced pluripotent stem cells (iPS cells), iPS cells derived from chondrocytes, H9-derived chondroprogenitor cells, Sox-9 transduced chondrocytes, osteoblasts, osteoprogenitors, or any combinations thereof. In some embodiments, the tri-component matrix further comprises chondrocytes. In some embodiments, the tri-component matrix further comprises chondroprogenitor cells. In some embodiments, the tri-component matrix further comprises iPS cells.

[0003] Disclosed herein, in some embodiments, is a process for producing a transplantation composition for cartilage engineering comprising: a) dissolving isolated and purified non-denatured type II collagen in a solution comprising an acid, wherein the final concentration of type II collagen is from about 0.5 mg/ml to about 5 mg/ml; b) neutralizing the acid; c) adding from about 0.25 mg/ml to about 3 mg/ml of hyaluronan; and d) adding from about 0.25 mg/ml to about 3 mg/ml of chondroitin sulfate, wherein a tri-component matrix

is produced. In some embodiments, the acid is acetic acid. In some embodiments, the acetic acid is at a concentration of about 0.01 M. In some embodiments, the acetic acid is neutralized with $NaHCO_3$/HEPES. In some embodiments, the tri-component matrix comprises Medium 199. In some embodiments, the hyaluronan is added at a concentration of about 1 mg/ml. In some embodiments, the chondroitin sulfate is added at a concentration of about 1 mg/ml. In some embodiments, the final concentration of type II collagen is about 3 mg/ml. In some embodiments, the process further comprises adding chondrocytes, chondroprogenitor cells, mesenchymal stem cells, iPS cells, iPS cells derived from chondrocytes, H9-derived chondroprogenitor cells, Sox-9 transduced chondrocytes, or any combinations thereof, to the tri-component matrix. In some embodiments, the process further comprises adding chondrocytes to the tri-component matrix. In some embodiments, the process further comprises adding chondroprogenitor cells to the tri-component matrix. In some embodiments, the process further comprises adding iPS cells to the tri-component matrix.

[0004] Disclosed herein, in some embodiments, is a method for inducing endogenous cartilage growth in a subject in need thereof, including the step of implanting in the subject a tri-component matrix as disclosed herein.

[0005] Disclosed herein, in some embodiments, is a method of treating a bone or cartilage defect in a subject in need thereof, comprising administering a transplantation composition comprising: i) a tri-component matrix, as disclosed herein, and ii) a population of cells, at the site of the bone or cartilage defect. In some embodiments, the population of cells comprises chondrocytes, chondroprogenitor cells, iPS cells, mesenchymal stem cells, osteoblasts, osteoprogenitors, or combinations thereof. In some embodiments, new tissue is produced. In some embodiments, the new tissue restores the surface of the cartilage or bone. In some embodiments, the new tissue comprises collagen type II. In some embodiments, the new tissue comprises superficial, intermediate, and deep zones characteristic of normal articular cartilage. In some embodiments, the superficial zone of the new tissue comprises lubricin. In some embodiments, the new tissue does not comprise teratomas, neoplastic cells, evidence of deformation, abnormal architectural features, or other inappropriate cell types. In some embodiments, the population of cells comprises chondrocytes. In some embodiments, the population of cells comprises chondroprogenitor cells. In some embodiments, the population of cells comprises iPS cells. In some embodiments, the iPS cells are derived from chondrocytes. In some embodiments, the

chondroprogenitor cells are H9-derived chondroprogenitor cells. In some embodiments, the chondrocytes are Sox-9 transduced chondrocytes.

[0006] Disclosed herein, in some embodiments, is a method of treating a cartilage-related disorder in a subject in need thereof, comprising administering a mixture of a tri-component matrix, as claimed in claim 1, and chondrocytes, chondroprogenitor cells, mesenchymal stem cells, iPS cells, iPS cells derived from chondrocytes, H9-derived chondroprogenitor cells, Sox-9 transduced chondrocytes, osteoblasts, osteoprogenitors, or any combinations thereof, to a site of cartilage injury or defect. In some embodiments, the cartilage-related disorder is articular cartilage trauma, meniscus injury, a chonodrogenesis disorder, arthritis, chondropathy, chondrosarcoma, chondromalacia, polychondritis, relapsing polychondritis, slipped epiphysis, osteochondritis dissecans, chondrodysplasia, costochondritis, osteochondroma, spondylosis, osteochondroses, Tietze syndrome, dermochondrocorneal dystrophy of Francois, epiphyseal dysplasia, carpotarsal osteochondromatosis, achondropasia, chondrocalcinosis, genochondromatosis, chondroma, achondrogenesis, echondromata, hyprochondroplasia, or Keutel syndrome. In some embodiments, the cartilage-related disorder is arthritis. In some embodiments, the arthritis is osteoarthritis. In some embodiments, the osteoarthritis occurs in the knee, finger, wrist, hip, spine, shoulder, elbow, toe, ankle, or neck of a subject.

[0007] Disclosed herein, in some embodiments, is a cartilage repair implant comprising: a) a tri-component matrix, as disclosed herein; and b) cells selected from chondrocytes, chondroprogenitor cells, mesenchymal stem cells, iPS cells, iPS cells derived from chondrocytes, H9-derived chondroprogenitor cells, Sox-9 transduced chondrocytes, osteoblasts, osteoprogenitors, or any combinations thereof. In some embodiments, the cartilage repair implant further comprises a biomaterial substrate, wherein the biomaterial substrate is selected from: polyglycolic acid (PGA), polylactic acid, alginates, polyethylene oxide, fibrin adhesive, polylactic acid-polyglycolic acid copolymer, human dermis, a membrane such as a sheet, a porous body such as sponges, a mesh such as a knit, a textile, a non-woven fabric, cotton, porous material, or combinations thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative

embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0009] **Figure 1** exemplifies tissue repair by H9-derived chondroprogenitors and chondroprogenitors derived from adult human chondrocytes (CHON-1 and CHON-2). Alcian Blue and Safranin O staining was comparable between the H9-derived chondroprogenitors and the chondroprogenitors derived from adult human chondrocytes. Poor tissue repair was seen in empty osteochondral defects or osteochondral defects filled with only Matrigel.

[0010] **Figure 2** exemplifies tissue repair by H9-derived chondroprogenitors (H9-D MSC), adult human chondrocytes (chondrocyte), pluripotent cell-derived chondroprogenitors (IPSC), and adult human mesenchymal stem cells (Adult MSC). Cells were embedded in a tri-component matrix of type II collagen, chondroitin sulfate, and hyaluronic acid prior to surgical implantation.

[0011] **Figure 3** exemplifies mechanical stiffness of tissue generated by H9-derived chondroprogenitors (H9 Derived MSC), adult human chondrocytes, pluripotent cell-derived chondroprogenitors (IPSC derived from chondrocyte), and adult human mesenchymal stem cells (Adult Human MSC) which had been embedded in a tri-component matrix of type II collagen, chondroitin sulfate, and hyaluronic acid prior to surgical implantation . The exemplified units are Newtons/mm.

[0012] **Figure 4** exemplifies tissue repair by H9-derived chondroprogenitors and adult human chondrocyte derived chondroprogenitors. Cells were embedded in alginate prior to surgical implantation.

DETAILED DESCRIPTION OF THE INVENTION

[0013] Disclosed herein, in some embodiments, is a tri-component matrix comprising: a) isolated and purified non-denatured type II collagen; b) hyaluronan; and c) chondroitin sulfate. In some embodiments, the tri-component matrix further comprises water. In some embodiments, the tri-component matrix further comprises a basal media. In some embodiments, the basal media is Medium 199. In some embodiments, the type II collagen is bovine-derived type II collagen. In some embodiments, the concentration of type II collagen is from about 0.5 mg/ml to about 5 mg/ml. In some embodiments, the concentration of type II collagen is about 3 mg/ml. In some embodiments, the concentration of hyaluronan is from about 0.25 mg/ml to about 3 mg/ml. In some embodiments, the concentration of hyaluronan is about 1 mg/ml. In some embodiments, the concentration of

chondroitin sulfate is from about 0.25 mg/ml to about 3 mg/ml. In some embodiments, the concentration of chondroitin sulfate is about 1 mg/ml. In some embodiments, the tri-component matrix further comprises chondrocytes, chondroprogenitor cells, mesenchymal stem cells, induced pluripotent stem cells (iPS cells), iPS cells derived from chondrocytes, H9-derived chondroprogenitor cells, Sox-9 transduced chondrocytes, osteoblasts, osteoprogenitors, or any combinations thereof. In some embodiments, the tri-component matrix further comprises chondrocytes. In some embodiments, the tri-component matrix further comprises chondroprogenitor cells. In some embodiments, the tri-component matrix further comprises iPS cells.

[0014] Disclosed herein, in some embodiments, is a process for producing a transplantation composition for cartilage engineering comprising: a) dissolving isolated and purified non-denatured type II collagen in a solution comprising an acid, wherein the final concentration of type II collagen is from about 0.5 mg/ml to about 5 mg/ml; b) neutralizing the acid; c) adding from about 0.25 mg/ml to about 3 mg/ml of hyaluronan; and d) adding from about 0.25 mg/ml to about 3 mg/ml of chondroitin sulfate, wherein a tri-component matrix is produced. In some embodiments, the acid is acetic acid. In some embodiments, the acetic acid is at a concentration of about 0.01 M. In some embodiments, the acetic acid is neutralized with $NaHCO_3$/HEPES. In some embodiments, the tri-component matrix comprises Medium 199. In some embodiments, the hyaluronan is added at a concentration of about 1 mg/ml. In some embodiments, the chondroitin sulfate is added at a concentration of about 1 mg/ml. In some embodiments, the final concentration of type II collagen is about 3 mg/ml. In some embodiments, the process further comprises adding chondrocytes, chondroprogenitor cells, mesenchymal stem cells, iPS cells, iPS cells derived from chondrocytes, H9-derived chondroprogenitor cells, Sox-9 transduced chondrocytes, or any combinations thereof, to the tri-component matrix. In some embodiments, the process further comprises adding chondrocytes to the tri-component matrix. In some embodiments, the process further comprises adding chondroprogenitor cells to the tri-component matrix. In some embodiments, the process further comprises adding iPS cells to the tri-component matrix.

[0015] Disclosed herein, in some embodiments, is a method for inducing endogenous cartilage growth in a subject in need thereof, including the step of implanting in the subject a tri-component matrix as disclosed herein.

[0016] Disclosed herein, in some embodiments, is a method of treating a bone or cartilage defect in a subject in need thereof, comprising administering a transplantation composition

comprising: i) a tri-component matrix, as disclosed herein, and ii) a population of cells, at the site of the bone or cartilage defect. In some embodiments, the population of cells comprises chondrocytes, chondroprogenitor cells, iPS cells, mesenchymal stem cells, osteoblasts, osteoprogenitors, or combinations thereof. In some embodiments, new tissue is produced. In some embodiments, the new tissue restores the surface of the cartilage or bone. In some embodiments, the new tissue comprises collagen type II. In some embodiments, the new tissue comprises superficial, intermediate, and deep zones characteristic of normal articular cartilage. In some embodiments, the superficial zone of the new tissue comprises lubricin. In some embodiments, the new tissue does not comprise teratomas, neoplastic cells, evidence of deformation, abnormal architectural features, or other inappropriate cell types. In some embodiments, the population of cells comprises chondrocytes. In some embodiments, the population of cells comprises chondroprogenitor cells. In some embodiments, the population of cells comprises iPS cells. In some embodiments, the iPS cells are derived from chondrocytes. In some embodiments, the chondroprogenitor cells are H9-derived chondroprogenitor cells. In some embodiments, the chondrocytes are Sox-9 transduced chondrocytes.

[0017] Disclosed herein, in some embodiments, is a method of treating a cartilage-related disorder in a subject in need thereof, comprising administering a mixture of a tri-component matrix, as claimed in claim 1, and chondrocytes, chondroprogenitor cells, mesenchymal stem cells, iPS cells, iPS cells derived from chondrocytes, H9-derived chondroprogenitor cells, Sox-9 transduced chondrocytes, osteoblasts, osteoprogenitors, or any combinations thereof, to a site of cartilage injury or defect. In some embodiments, the cartilage-related disorder is articular cartilage trauma, meniscus injury, a chonodrogenesis disorder, arthritis, chondropathy, chondrosarcoma, chondromalacia, polychondritis, relapsing polychondritis, slipped epiphysis, osteochondritis dissecans, chondrodysplasia, costochondritis, osteochondroma, spondylosis, osteochondroses, Tietze syndrome, dermochondrocorneal dystrophy of Francois, epiphyseal dysplasia, carpotarsal osteochondromatosis, achondropasia, chondrocalcinosis, genochondromatosis, chondroma, achondrogenesis, echondromata, hyprochondroplasia, or Keutel syndrome. In some embodiments, the cartilage-related disorder is arthritis. In some embodiments, the arthritis is osteoarthritis. In some embodiments, the osteoarthritis occurs in the knee, finger, wrist, hip, spine, shoulder, elbow, toe, ankle, or neck of a subject.

[0018] Disclosed herein, in some embodiments, is a cartilage repair implant comprising: a) a tri-component matrix, as disclosed herein; and b) cells selected from chondrocytes,

chondroprogenitor cells, mesenchymal stem cells, iPS cells, iPS cells derived from chondrocytes, H9-derived chondroprogenitor cells, Sox-9 transduced chondrocytes, osteoblasts, osteoprogenitors, or any combinations thereof. In some embodiments, the cartilage repair implant further comprises a biomaterial substrate, wherein the biomaterial substrate is selected from: polyglycolic acid (PGA), polylactic acid, alginates, polyethylene oxide, fibrin adhesive, polylactic acid-polyglycolic acid copolymer, human dermis, a membrane such as a sheet, a porous body such as sponges, a mesh such as a knit, a textile, a non-woven fabric, cotton, porous material, or combinations thereof.

Cartilage

[0019] Articular cartilage is a type of hyaline cartilage that lines the surfaces of the opposing bones in a diarthrodial joint (*e.g.* knee, hip, shoulder, *etc.*). Articular cartilage provides a near-frictionless articulation between the bones, while also functioning to absorb and transmit the compressive and shear forces encountered in the joint. Further, since the tissue associated with articular cartilage is aneural, these load absorbing and transmitting functions occur in a painless fashion in a healthy joint.

[0020] Fibrocartilage is found in diarthrodial joints, symphyseal joints, intervertebral discs, articular discs, as inclusions in certain tendons that wrap around a pulley, and at insertion sites of ligaments and tendons into bone. Made of a mixture of collagen type I and type II fibers, fibrocartilage may be damaged, causing pain in the affected joint.

[0021] It is understood for purposes of this application that the term "cartilage" includes articular cartilage and fibrocartilage.

Tri-Component Implant Matrix

[0022] Disclosed herein are novel materials useful for medical grafting and cell culture, and methods for making and using them. Additional embodiments as well as features and advantages of the invention will be apparent from the descriptions herein.

[0023] Disclosed herein, in some embodiments, is a tri-component matrix. In some embodiments, the tri-component matrix comprises collagen; hyaluronan; and chondroitin sulfate. In some embodiments, the collagen is type II collagen. In some embodiments, the collagen is non-denatured. In some embodiments, the tri-component matrix further comprises water.

[0024] In some embodiments, the tri-component matrix further comprises chondrocytes or chondroprogenitor cells. In some embodiments, the tri-component matrix further comprises mesenchymal stem cells. In some embodiments, the tri-component matrix further comprises induced pluripotent stem (iPS) cells. In some embodiments, the tri-component matrix further comprises iPS cells derived from chondrocytes. In some embodiments, the

tri-component matrix further comprises H9-derived chondroprogenitor cells. In some embodiments, the tri-component matrix further comprises Sox-9 transduced chondrocytes.

[0025] In some embodiments, the tri-component matrix is a gel. In some embodiments, the tri-component matrix is a molded solid. In some embodiments, the tri-component matrix is a liquid.

[0026] In some embodiments, the tri-component matrix further comprises a basal media. In some embodiments, the basal media is Medium 199, DMEM (for example, DMEM supplemented with fetal bovine serum), RPMI-1640, Ham's F-12, or any combinations thereof. In some embodiments, the basal media is a chondrocyte basal medium. In some embodiments, the basal media comprises the following components: Glycine; L-Alanine; L-Arginine hydrochloride; L-Aspartic acid; L-Cysteine hydrochloride-H_2O; L-Cystine 2HCl; L-Glutamic Acid; L-Glutamine; L-Histidine hydrochloride-H_2O; L-Hydroxyproline; L-Isoleucine; L-Leucine; L-Lysine hydrochloride; L-Methionine; L-Phenylalanine; L-Proline; L-Serine; L-Threonine; L-Tryptophan; L-Tyrosine disodium salt dehydrate; L-Valine; Alpha-tocopherol Phosphate; Ascorbic Acid; Biotin; Choline chloride; D-Calcium pantothenate; Folic Acid; Menadione (Vitamin K3); Niacinamide; Nicotinic acid (Niacin); Para-Aminobenzoic Acid; Pyridoxal hydrochloride; Pyridoxine hydrochloride; Riboflavin; Thiamine hydrochloride; Vitamin A (acetate); Vitamin D2 (Calciferol); i-Inositol; Calcium Chloride ($CaCl_2$) (anhyd.); Ferric nitrate ($Fe(NO_3)$-$9H_2O$); Magnesium Sulfate ($MgSO_4$) (anhyd.); Potassium Chloride (KCl); Sodium Bicarbonate ($NaHCO_3$); Sodium Chloride (NaCl); Sodium Phosphate monobasic (NaH_2PO_4-H_2O); 2-deoxy-D-ribose; Adenine sulfate; Adenosine 5'-phosphate; Adenosine 5'-triphosphate; Cholesterol; D-Glucose (Dextrose); Glutathione (reduced); Guanine hydrochloride; Hypoxanthine Na; Phenol Red; Ribose; Sodium Acetate; Thymine; Tween 80®; Uracil; and Xanthine-Na. In some embodiments, the basal media is Medium 199.

[0027] In some embodiments, the basal media comprises platelet-derive growth factor (PDGF) (*e.g.*, PDGF-BB, PDGF-AB, and PDGF-AA), a lipid (*e.g.*, stearic acid, myristic acid, oleic acid, linoleic acid, palmitic acid, palmitoleic acid, arachidonic acid, linolenic acid, cholesterol, alpha-tocopherol acetate), bone morphogenic protein (BMP) (*e.g.*, BMP-4 or BMP-6), TGF-β (*e.g.*, TGF-β1, TGF-β2, TGF-β3), insulin-like growth factor (IGF), hydrocortisone, fibronectin, bFGF, albumin, insulin, FBS, or any combinations thereof.

[0028] In some embodiments, the tri-component matrix comprises type II collagen (*e.g.*, isolated and purified non-denatured type II collagen). In some embodiments, the type II collagen is

non-denatured. In some embodiments, the tri-component matrix further comprises type VI, type IX, type X, type XI and/or type XIII collagen.

[0029] In some embodiments, the type II collagen is derived from any suitable source. In some embodiments, the type II collagen is derived from a non-mammalian vertebrate (e.g., a shark). In some embodiments, the type II collagen is derived from a mammal. In some embodiments, the type II collagen is derived from a human. In some embodiments, the type II collagen is derived from a non-human mammal. In some embodiments, the type II collagen is bovine-derive, equine-derive, or porcine-derived. In some embodiments, the type II collagen is bovine-derived type II collagen.

[0030] In some embodiments, the concentration of type II collagen is from about 0.1 mg/ml to about 50 mg/ml. In some embodiments, the concentration of type II collagen is from about 0.5 mg/ml to about 25 mg/ml. In some embodiments, the concentration of type II collagen is from about 1 mg/ml to about 10 mg/ml. In some embodiments, the concentration of type II collagen is from about 1 mg/ml to about 5 mg/ml. In some embodiments, the concentration of type II collagen is about 3 mg/ml.

[0031] Hyaluronan (also referred to as hyaluronic acid or HA) is an anionic, nonsulfated glycosaminoglycan. It is composed on disaccharides, composed of D-glucuronic acid and D-N-acetylglucosamine, linked via alternating β-1,4 and β-1,3 glycosidic bonds. The size of hyaluronan varies. Hyaluronans have been found with 25,000 disaccharide repeats. They range in size from 5,000 to 20,000,000 Da *in vivo*. The average molecular weight in human synovial fluid is 3-4 million Da, and hyaluronan purified from human umbilical cord is 3,140,000 Da.

[0032] In some embodiments, the tri-component matrix comprises high molecular weight hyaluronan (i.e., greater than about 1000 Da). In some embodiments, the tri-component matrix comprises low molecular weight hyaluronan (i.e., less than about 1000 Da). In some embodiments, the tri-component matrix comprises hyaluronan having a size from about 500 Da to about 20,000,000 Da. In some embodiments, the tri-component matrix comprises hyaluronan having a size from about 500 to about 5000 Da. In some embodiments, the tri-component matrix comprises hyaluronan having a size from about 80,000 to about 800,000 Da. In some embodiments, the tri-component matrix comprises hyaluronan having a size of about 1000 to about 15,000,000 Da. In some embodiments, the tri-component matrix comprises hyaluronan having a size of about 2000 to about 10,000,000 Da. In some embodiments, the tri-component matrix comprises hyaluronan having a size of about 3000 to about 7,500,000 Da. In some embodiments, the tri-component matrix comprises

hyaluronan having a size of about 4000 to about 5,000,000 Da. In some embodiments, the tri-component matrix comprises hyaluronan having a size of about 5000 to about 2,500,000 Da. In some embodiments, the tri-component matrix comprises hyaluronan having a size of about 6000 to about 1,000,000 Da. In some embodiments, the tri-component matrix comprises hyaluronan having a size of about 7000 to about 900,000 Da. In some embodiments, the tri-component matrix comprises hyaluronan having a size of about 8000 to about 800,000 Da. In some embodiments, the tri-component matrix comprises hyaluronan having a size of about 9000 to about 700,000 Da. In some embodiments, the tri-component matrix comprises hyaluronan having a size of about 10,000 to about 600,000 Da. In some embodiments, the tri-component matrix comprises hyaluronan having a size of about 15,000 to about 500,000 Da. In some embodiments, the tri-component matrix comprises hyaluronan having a size of about 20,000 to about 400,000 Da. In some embodiments, the tri-component matrix comprises hyaluronan having a size of about 25,000 to about 300,000 Da. In some embodiments, the tri-component matrix comprises hyaluronan having a size of about 30,000 to about 200,000 Da. In some embodiments, the tri-component matrix comprises hyaluronan having a size of about 35,000 to about 200,000 Da. In some embodiments, the tri-component matrix comprises hyaluronan having a size of about 40,000 to about 100,000 Da. In some embodiments, the tri-component matrix comprises hyaluronan having a size of about 50,000 to about 100,000 Da.

[0033] In some embodiments, the concentration of hyaluronan is from about 0.1 mg/ml to about 25 mg/ml. In some embodiments, the concentration of hyaluronan is from about 0.25 mg/ml to about 10 mg/ml. In some embodiments, the concentration of hyaluronan is from about 0.25 mg/ml to about 5 mg/ml. In some embodiments, the concentration of hyaluronan is from about 0.25 mg/ml to about 3 mg/ml. In some embodiments, the concentration of hyaluronic acid is about 1 mg/ml.

[0034] Chondroitin sulfate is a sulfated glycosaminoglycan (GAG) composed of a chain of alternating sugars: N-acetyl-D-galactosamine (GalNAc) and D-glucuronic acid (GlcA). In certain instances, chondroitin chain have over 100 individual sugars, each of which can be sulfated in variable positions and quantities. Chondroitin sulfate is a structural component of cartilage. The tightly packed and highly charged sulfate groups of chondroitin sulfate generate electrostatic repulsion that provides much of the resistance of cartilage to compression. The site of sulfation varies amongst chondroitin chains. Chondroitin sulfate A is sulfated on carbon 4 of the N-acetylgalactosamine (GalNAc) sugar. Chondroitin sulfate C is sulfated on carbon 6 of the GalNAc sugar. Chondroitin sulfate D is sulfated on

carbon 2 of the glucuronic acid and 6 of the GalNAc sugar. Chondroitin sulfate E is sulfated on carbons 4 and 6 of the GalNAc sugar.

[0035] In some embodiments, the tri-component matrix comprises any suitable chondroitin sulfate. In some embodiments, the tri-component matrix comprises Chondroitin sulfate A, Chondroitin sulfate C, Chondroitin sulfate D, and/or Chondroitin sulfate E.

[0036] In some embodiments, the concentration of chondroitin sulfate is from about 0.1 mg/ml to about 25 mg/ml. In some embodiments, the concentration of chondroitin sulfate is from about 0.25 mg/ml to about 10 mg/ml. In some embodiments, the concentration of chondroitin sulfate is from about 0.25 mg/ml to about 5 mg/ml. In some embodiments, the concentration of chondroitin sulfate is from about 0.25 mg/ml to about 3 mg/ml.

[0037] In some embodiments, the tri-component matrix is a medium for the ingrowth of native chondrocytes. In some embodiments, the matrix further comprises chondrocytes either prior to or following implantation *in vivo*. In some embodiments, the matrix is impregnated with chondrocytes immediately prior to implantation in a subject, *e.g.* by injection.

[0038] In some embodiments, the tri-component matrix further comprises additional additives. In some embodiments, additional additives included in the tri-component matrix include, for example, chondronectin, laminin, fibronectin, calcium alginate, anchorin II, biglycan, decorin, versican, fibromodulin, lumican, bone, cartilage cell growth-promoting hormones, and growth factors such as cartilage inducing factor (CIP), insulin-like growth factor (IGF), IGF-1, transforming growth factor [beta] (TGFß), TGF-ß1, osteogenic protein-1 (OP-1) and bone morphogenetic factors (BMPs) such as native or recombinant human BMP-2, BMP-3 (osteogenin), BMP-4, BMP-7, BMP-8, bFGF, CDMP or other skeletal matrix molecules, as well as signaling peptides such as vascular endothelial growth factor (EGF/VEGF), parathyroid hormone related protein (PTHrP) and platelet derived growth factor (PDGF). In some embodiments, nucleic acid sequences (e.g., mammalian expression vectors) encoding any of the above listed additional additives, or which are capable of inducing or promoting *in vivo* production of the above listed additional additives, is incorporated into the tri-component matrix material.

[0039] In some embodiments, the tri-component matrix further comprises an active agent. In some embodiments, the tri-component matrix further comprises an antibacterial agent, *e.g.*, taurolidine, taurultam, or antibiotics such as tetracyclines and gentamycins. In some embodiments, the tri-component matrix further comprises an anti-inflammatory agent. In some embodiments, the tri-component matrix further comprises an NSAID. In some embodiments, the tri-component matrix further comprises celecoxib, oxaprozin, salsalate,

diflunisal, indomethacin, meloxicam, ketorolac, diclofenac, naproxen, sulindac, tenoxicam, etodolac, nabumetone, piroxicam, carprofen, flubiprofen, ibuprofen, aspirin, paracetamol, or any combinations thereof. In some embodiments, the tri-component matrix further comprises a steroid, *e.g.*, cortisone, hydrocortisone, prednisone, prednisolone, solumedrol, triamcinolone, kenalog, celestone, and depomedrol. In some embodiments, the tri-component matrix further comprises a disease-modifying anti-rheumatic drug (DMARD). In some embodiments, the tri-component matrix further comprises abatacept, adalimumab, azathioprine, chloroquine, hydroxychloroquine, Cyclosporin A, D-penicillamine, etanercept, golimumab, infliximab, leflunomide, methotrexate, minocycline, rituximab, sulfasalazine, or any combinations thereof.

[0040] In some embodiments, the tri-component matrix further comprises an agent that prevents or reduces vascularization. Examples of agents that prevent or reduce vascularization include, but are not limited to, tyrosine kinase inhibitors, such as inhibitors of the tyrosine kinase receptors Flt-1 (VEGFR1) and Flk-1/KDR (VEGFR2), inhibitors of epidermal-derived, fibroblast-derived, or platelet derived growth factors, MMP (matrix metalloprotease) inhibitors, integrin blockers, interferon-α, interleukin-12, pentosan polysulfate, cyclooxygenase inhibitors, including nonsteroidal anti-inflammatories (NSAIDs) like aspirin and ibuprofen as well as selective cyclooxy-genase-2 inhibitors like celecoxib and rofecoxib steroidal anti-inflammatories (such as corticosteroids, mineralocorticoids, dexamethasone, prednisone, prednisolone, methylpred, betamethasone), carboxyamidotriazole, combretastatin A-4, squalamine, 6-O-chloroacetyl-carbonyl)-fumagillol, thalidomide, angiostatin, troponin-1, angiotensin II antagonists, and antibodies to VEGF .

[0041] In some embodiments, the tri-component matrix disclosed herein elicits less of an inflammatory response than an alginate matrix.

Methods of Culturing Cells

[0042] The present invention also provides a method for culturing cells, comprising culturing cells in a tri-component matrix, as disclosed herein, comprising a) isolated and purified non-denatured type II collagen; b) hyaluronan; and c) chondroitin sulfate. In some embodiments, the cells within the tri-component matrix are cultured in a tissue culture vessel. In some embodiments, the tissue culture vessel is tissue culture-grade plastic. In some embodiments, the plastic is polystyrene. In some embodiments, the tissue culture vessel is glass.

[0043] In some embodiments, the tri-component matrix further comprises a cell-culture media. In some embodiments, the cell-culture media is a basal media. . In some embodiments, the basal media is Medium 199, DMEM (for example, DMEM supplemented with fetal bovine serum), RPMI-1640, Ham's F-12, or any combinations thereof. In some embodiments, the basal media is a chondrocyte basal medium. In some embodiments, the basal media comprises the following components: Glycine; L-Alanine; L-Arginine hydrochloride; L-Aspartic acid; L-Cysteine hydrochloride-H_2O; L-Cystine 2HCl; L-Glutamic Acid; L-Glutamine; L-Histidine hydrochloride-H_2O; L-Hydroxyproline; L-Isoleucine; L-Leucine; L-Lysine hydrochloride; L-Methionine; L-Phenylalanine; L-Proline; L-Serine; L-Threonine; L-Tryptophan; L-Tyrosine disodium salt dehydrate; L-Valine; Alpha-tocopherol Phosphate; Ascorbic Acid; Biotin; Choline chloride; D-Calcium pantothenate; Folic Acid; Menadione (Vitamin K3); Niacinamide; Nicotinic acid (Niacin); Para-Aminobenzoic Acid; Pyridoxal hydrochloride; Pyridoxine hydrochloride; Riboflavin; Thiamine hydrochloride; Vitamin A (acetate); Vitamin D2 (Calciferol); i-Inositol; Calcium Chloride ($CaCl_2$) (anhyd.); Ferric nitrate ($Fe(NO_3)$-$9H_2O$); Magnesium Sulfate ($MgSO_4$) (anhyd.); Potassium Chloride (KCl); Sodium Bicarbonate ($NaHCO_3$); Sodium Chloride (NaCl); Sodium Phosphate monobasic (NaH_2PO_4-H_2O); 2-deoxy-D-ribose; Adenine sulfate; Adenosine 5'-phosphate; Adenosine 5'-triphosphate; Cholesterol; D-Glucose (Dextrose); Glutathione (reduced); Guanine hydrochloride; Hypoxanthine Na; Phenol Red; Ribose; Sodium Acetate; Thymine; Tween 80®; Uracil; and Xanthine-Na. In some embodiments, the basal media is Medium 199.

[0044] In some embodiments, the basal media comprises platelet-derive growth factor (PDGF) (*e.g.*, PDGF-BB, PDGF-AB, and PDGF-AA), a lipid (*e.g.*, stearic acid, myristic acid, oleic acid, linoleic acid, palmitic acid, palmitoleic acid, arachidonic acid, linolenic acid, cholesterol, alpha-tocopherol acetate), bone morphogenic protein (BMP) (*e.g.*, BMP-4 or BMP-6), TGF-β (*e.g.*, TGF-β1, TGF-β2, TGF-β3), insulin-like growth factor (IGF), hydrocortisone, fibronectin, bFGF, albumin, insulin, FBS, or any combinations thereof.

Methods of Use

[0045] Disclosed herein, in certain embodiments, are methods for inducing endogenous cartilage growth in a subject in need thereof, comprising implanting in the subject a tri-component matrix as disclosed herein, comprising a) isolated and purified non-denatured type II collagen; b) hyaluronan; and c) chondroitin sulfate. In some embodiments, the tri-component matrix further comprises chondrocytes and/or chondroprogenitor cells. In some embodiments, the method comprises injecting the tri-component into a cartilageonous area

with a needle. In some embodiments, the method comprises injecting the tri-component matrix into a joint, *e.g.*, a synovial joint. In some embodiments, the subject has a defect in cartilage (*e.g.*, degeneration or absence of cartilage). In some embodiments, the subject has a cartilage related disorder. In some embodiments, the subject has osteoarthritis, rheumatoid arthritis, costochondritis, relapsing polychondritis, or any combinations thereof. In some embodiments, the subject has osteoarthritis. In some embodiments, the subject has rheumatoid arthritis. In some embodiments, the subject has damage to cartilage caused by a physical trauma.

[0046] In some embodiments, a tri-component matrix disclosed herein is used for methods of regenerating cartilaginous tissue. In some embodiments, the tri-component matrix further comprises chondrocytes, chondroprogenitor cells, mesenchymal stem cells, iPS cells, iPS cells derived from chondrocytes, H9-derived chondroprogenitor cells, Sox-9 transduced chondrocytes, osteoblasts, osteoprogenitors or any combinations thereof. In some embodiments, tri-component matrix is placed directly into an area of bone or cartilage defect. In some embodiments, the tri-component matrix is placed into an area of bone or cartilage defect with additional supportive biomaterial. In some embodiments, new cartilaginous tissue is formed. In some embodiments, the new cartilaginous tissue integrates with the tissue of the bone or cartilage defect. In some embodiments, the new cartilaginous tissue restores the surface of the cartilage or bone. In some embodiments, the new cartilaginous tissue comprises collagen type II. In some embodiments, the new cartilaginous tissue comprises superficial, intermediate, and deep zones characteristic of normal articular cartilage. In some embodiments, the superficial zone of the new cartilaginous tissue comprises lubricin. In some embodiments, the new cartilaginous tissue does not comprise teratomas, neoplastic cells, evidence of deformation, abnormal architectural features, or other inappropriate cell types.

[0047] Disclosed herein, in some embodiments, is a method of treating a cartilage-related disorder, comprising administration of a tri-component matrix, as described herein, to a site of cartilage injury or defect in a subject in need thereof. In some embodiments, the tri-component matrix further comprises chondrocytes, chondroprogenitor cells, mesenchymal stem cells, iPS cells, iPS cells derived from chondrocytes, H9-derived chondroprogenitor cells, Sox-9 transduced chondrocytes, osteoblasts, osteoprogenitors or any combinations thereof. In some embodiments, the cartilage-related disorder is articular cartilage trauma, meniscus injury, a chonodrogenesis disorder, arthritis, chondropathy, chondrosarcoma, chondromalacia, polychondritis, relapsing polychondritis, slipped epiphysis, osteochondritis

dissecans, chondrodysplasia, costochondritis, osteochondroma, spondylosis, osteochondroses, Tietze syndrome, dermochondrocorneal dystrophy of Francois, epiphyseal dysplasia, carpotarsal osteochondromatosis, achondropasia, chondrocalcinosis, genochondromatosis, chondroma, achondrogenesis, echondromata, hyprochondroplasia, or Keutel syndrome. In some embodiments, the cartilage-related disorder is arthritis. In some embodiments, the arthritis is osteoarthritis. In some embodiments, the osteoarthritis occurs in the knee, finger, wrist, hip, spine, shoulder, elbow, toe, ankle, or neck of a subject.

[0048] Disclosed herein, in some embodiments, is a cartilage repair implant comprising a tri-component matrix as disclosed herein, further comprising chondrocytes, chondroprogenitor cells, mesenchymal stem cells, iPS cells, iPS cells derived from chondrocytes, H9-derived chondroprogenitor cells, Sox-9 transduced chondrocytes, osteoblasts, osteoprogenitors or any combinations thereof. In some embodiments, the cartilage repair implant further comprises an additional biomaterial substrate. Examples of additional biomaterials include: polyglycolic acid (PGA), polylactic acid, alginates (for example, the calcium salt), polyethylene oxide, fibrin adhesive, polylactic acid-polyglycolic acid copolymer, proteoglycans, glycosaminoglycans, human dermis, or a combination thereof. In some embodiments, proteoglycans and glycosaminoglycans are sulfated. In some embodiments, the additional biomaterial substrate is a membrane such as sheet, a porous body such as sponges, a mesh such as a knit, a textile, a non-woven fabric, cotton and the like. In some embodiments, the additional biomaterial is a porous material.

[0049] In some embodiments, the cartilage repair implant forms cartilage tissue and induces cartilaginous ossification. In some embodiments, ossification is also promoted by a growth factor which promotes bone formation, such as bone morphogenetic protein (BMP).

[0050] Prior to transplantation of the described cartilage therapy materials, it is preferable to prepare the area that is to be treated. For this purpose, the defect must be prepared to ensure that the implant will take more effectively (particularly as regards its attachment), to reduce the risks of vascularization, etc. Generally, the defect is treated in advance (in order to eliminate all defective cartilage from the area), then it is cleaned. Next, various implantation techniques can be implemented, according to the material being implanted (suspension, matrix, cartilage reconstituted in vitro).

[0051] Generally, implantation of the described cartilage therapy materials is performed by applying various surgical techniques known to the skilled person, such as affixing the implant during surgery, through biodegradable sutures or by the application of bioadhesives. Examples of bioadhesives include, notably, biological glues made of fibrin and/or thrombin,

or other biocompatible materials. More particularly, the resorbable biocompatible film is affixed onto the area to be treated, by means of a biological or biocompatible glue. In a preferred variant, the film is positioned on the cartilaginous defect, then the pocket which is thus constituted is filled with cartilage therapy material.

[0052] In some embodiments, the method for inducing endogenous cartilage growth further comprises athroscopic lavage and/or debridement of the area where the implant will be administered. In some embodiments, part or all of the damaged cartilage is removed and a tri-component matrix (for example, the tri-component matrix further comprising chondrocytes or chondroprogenitor cells) is implanted (*e.g.*, injected) into the area.

[0053] In some embodiments, the method for inducing endogenous cartilage growth further comprises marrow stimulating techniques (*e.g.*, microfracture surgery). In some embodiments, (a) part or all of the damaged cartilage is removed from an area in need and underlying bone is partially or fully exposed, (b) microfractures are created in the subchondral bone (*e.g.*, with the use of an awl), and (c) the tri-component matrix (for example, the tri-component matrix further comprising chondrocytes or chondroprogenitor cells) is implanted (*e.g.*, injected) into the area.

[0054] In some embodiments, the tri-component matrix (for example, the tri-component matrix further comprising chondrocytes or chondroprogenitor cells) is injected with any suitable needle, *e.g.*, a 16 gauge needle, 17 gauge needle, 18 gauge needle, 19 gauge needle, 20 gauge needle, 21 gauge needle, 22 gauge needle, 23 gauge needle, 24 gauge needle, 25 gauge needle, 26 gauge needle, 27 gauge needle, 28 gauge needle, 29 gauge needle, or a 30 gauge needle. In some embodiments, the tri-component matrix is injected with a 22 gauge needle. In some embodiments, the tri-component matrix is injected with a 25 gauge needle.

EXAMPLES

Example 1: Production of Tri-Component Matrix Comprising Chondrocytes

[0055] An exemplary method of manufacturing the tri-component matrix is as follows.

[0056] First, Bovine type II collagen was dissolved in 0.01 M acetic acid. Bovine Type II collagen (10 mg) was dissolved in in 2.4 ml 10 mM acetic acid O/N. 300 µl of 10 X Medium 199 and 300 µl $NaHCO_3$/HEPES were added to the collagen/acetic acid mixture to neutralize.

[0057] 330 µl of hyaluronic acid (HA at 10 mg/ml) (final concentration of 1 mg/ml) was added to the mixture.

[0058] 60 µl of chondrotin sulfate (50 mg/ml, Bovine) was added to the mixture to a final concentration of 1 mg/ml to form the tri-component matrix.

[0059] Chondrocytes were then added to the tri-component matrix.

Example 2: Treatment of Osteochondral Defects with Chondroprogenitors Embedded in Alginate

[0060] Using a surgical drill, 3-mm osteochondral defects were created in rabbit femoral trochlea grooves and condyles. The defects were repaired using two different treatments. As a control, nothing was implanted into one defect site. In the first treatment adult human chondrocyte derived chondroprogenitor cell pellets were embedded in alginate and then surgically implanted into the defect site. In the second treatment H9-derived chondroprogenitor cell pellets were embedded in alginate and then surgically implanted into the defect site.

[0061] Alginate was cross linked in the defect site using surgical gauze soaked with calcium chloride.

[0062] Histologic examination (Figure 4) indicated that tissue repair generated by chondroprogenitors embedded in alginate was inferior to that generated by adult human chondrocyte derived chondroprogenitors embedded in alginate. However, both were superior to defects implanted with nothing.

Example 3: Treatment of Osteochondral Defects with Chondroprogenitors Embedded in Matrigel

[0063] Using a surgical drill, 3-mm osteochondral defects were created in rabbit femoral trochlea grooves and condyles. The defects were repaired using four different treatments. As a control, nothing was implanted into one defect site. In the first treatment, as a control Matrigel alone was surgically implanted into the defect site. In the second treatment adult

human chondrocyte derived chondroprogenitor cell pellets from donor #1 were embedded in Matrigel and then surgically implanted into the defect site. In the third treatment adult human chondrocyte derived chondroprogenitor cell pellets from donor #2 were embedded in Matrigel and then surgically implanted into the defect site. In the fourth treatment H9-derived chondroprogenitor cell pellets were embedded in Matrigel and then surgically implanted into the defect site.

[0064] Matrigel (BD Biosciences) was maintained as a liquid at 4 degrees Celsius and after implantation became gelatinous at 37 degrees Celsius.

[0065] Histologic examination (Figure 1) indicated that tissue repair generated by H9-derived chondroprogenitors embedded in Matrigel was comparable to that generated by adult human chondrocyte derived chondroprogenitors embedded in Matrigel. However, both treatments were superior to defects implanted with nothing or with Matrigel alone.

Example 4: Treatment of Osteochondral Defects with Chondroprogenitors Embedded in Tri-Component Matrix

[0066] Using a surgical drill, 3-mm osteochondral defects were created in rabbit femoral trochlea grooves and condyles. The defects were repaired using four different treatments. In all four treatments (adult human chondrocytes, H9 derives MSCs, iPSC derived from chondrocytes, and adult human MSCs) a tri-component matrix composed of collagen II, chondroitin sulfate, and hyaluronic acid was used.

[0067] The following protocol was used to assemble the tri-component matrix:

1) Bovine type II collagen (Innovative Research) was dissolved in 0.01 M acetic acid (add 10mg of bovine type II collagen to 2.4 ml of 10 mM acetic acid, 300 µl of 10X Medium 199, and 300 µl of $NaHCO_3$/HEPES to neutralize the acetic acid);
2) 300 µg of hyaluronic acid was added at a concentration of 1 mg/ml;
3) 60 µg of chondrotin sulfate (bovine) was added at a concentration of 1 mg/ml;
4) The final concentration of the type II collagen is approximately 3 mg/ml.

[0068] Histologic examination (Figure 2) indicated that tissue repair generated by H9-derived chondroprogenitors embedded in the tri-component matrix was better at tissue regeneration and had better mechanical properties (Figure 3) than adult human chondrocyte derived chondroprogenitors, pluripotent cell-derived chondroprogenitors, and adult human mesenchymal stem cells.

Example 5: Clinical Study of Using Tri-Component Matrix in Treatment of Osteoarthritis

[0069] Patients are given a single 5 mL injection of the tri-component matrix with chondrocytes (or a phosphate buffered saline control) into the synovial, with a possible repeat treatment after the week 16 visit.

[0070] Study Type: Interventional

[0071] Allocation: Randomized

[0072] Endpoint Classification: Safety/Efficacy Study

[0073] Intervention Model: Parallel Assignment

[0074] Masking: Double Blind (Subject, Outcomes Assessor)

[0075] Primary Purpose: Treatment

[0076] Outcome Measures:

[0077] Participants' assessment of pain. Mean scores were used for baseline (day 0) and for all visits up to week 16 (weeks 2, 4, 8, 12 and 16). The WOMAC Pain Subscale has a score range of 0-4, where 0=no pain and 4=extreme pain.

[0078] Participants categorized the pain they felt while walking using the WOMAC LK 3.1 A1 (Walking Pain) Subscale. The scale rates pain as none, mild, moderate, severe and extreme.

[0079] The change from baseline over the course of the 16-week initial treatment period using participants' assessment of physical function. Mean scores were used for baseline (day 0) and for all visits up to week 26 (weeks 4, 8, 12, 18 and 26). The WOMAC Function Subscale has a score range of 0-4 to assess the degree of difficulty completing tasks within the past 48 hours, where 0=no difficulty and 4=extreme difficulty.

[0080] Ages Eligible for Study: 40 Years and older

[0081] Genders Eligible for Study: Both

[0082] Accepts Healthy Volunteers: No

[0083] *Inclusion Criteria:*

[0084] Patient with documented diagnosis of primary osteoarthritis (OA) of the target knee made at least 3 months prior to trial.

[0085] Has radiographic evidence of OA in the tibio-femoral compartment of the target, knee with at least 1 definite osteophyte and a measureable joint space, as diagnosed by standard X-rays taken not longer than 3 months prior to Screening, and before any baseline assessment.

[0086] Has continued target knee pain despite conservative treatment (*e.g.* weight reduction, physical therapy, analgesics).

[0087] Has pain in the target knee as demonstrated by a score of 2 or 3 on the Western Ontario and McMaster Universities Osteoarthritis Index Likert Scale Version 3.1 (WOMAC LK 3.1) A1 (Walking Pain) Subscale.

[0088] Has a mean score of 1.5 to 3.5 on the Western Ontario and McMaster Universities Osteoarthritis Index Likert Scale Version 3.1 (WOMAC LK 3.1) A (Pain) Subscale.

[0089] Inclusion Criteria for Repeat Phase: Must have no major safety concerns during the first course of treatment as assessed by the Investigator; Must have a WOMAC LK 3.1 A score of at least 1.

[0090] *Exclusion criteria:*

[0091] Has modified Kellgren-Lawrence Numerical Grading System of grade IV in the patello-femoral compartment of the target knee confirmed by standard X-rays taken not longer than 3 months prior to Screening, and before any baseline assessment.

[0092] Has clinically apparent tense effusion of the target knee.

[0093] Has had viscosupplementation in any joint including the target knee within 9 months prior to Screening.

[0094] Has concomitant inflammatory disease or other condition that affects the joints (*e.g.* rheumatoid arthritis, metabolic bone disease, psoriasis, gout, symptomatic chondrocalcinosis and active infection, *etc.*).

[0095] Symptomatic OA of the contralateral knee or of either hip that is not responsive to paracetamol and requires other therapy.

[0096] Has related hypersensitivities to avian proteins and/or any components of hyaluronan-based injection devices.

Example 6: Treatment of Osteoarthritis with Tri-Component Matrix comprising Chondrocytes

[0097] A patient presents with osteoarthritis in his knees. The calcified cartilage is removed from both knees. The surgeon creates fractures in the subchondral bone. The tri-component matrix with chondrocytes is injected into the knee. The patient returns to the doctor 4 weeks, 8 weeks, and 16 weeks later for an injection of the tri-component matrix with chondrocytes into both knees. The cartilage in the knees regenerates.

[0098] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that

various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. A tri-component matrix comprising:
    a) isolated and purified non-denatured type II collagen;
    b) hyaluronan; and
    c) chondroitin sulfate.

2. The tri-component matrix of claim 1, further comprising water.

3. The tri-component matrix of claim 1, further comprising a basal media.

4. The tri-component matrix of claim 3, wherein the basal media is Medium 199.

5. The tri-component matrix of claim 1, wherein the type II collagen is bovine-derived type II collagen.

6. The tri-component matrix of claim 1, wherein the concentration of type II collagen is from about 0.5 mg/ml to about 5 mg/ml.

7. The tri-component matrix of claim 6, wherein the concentration of type II collagen is about 3 mg/ml.

8. The tri-component matrix of claim 1, wherein the concentration of hyaluronan is from about 0.25 mg/ml to about 3 mg/ml.

9. The tri-component matrix of claim 8, wherein the concentration of hyaluronan is about 1 mg/ml.

10. The tri-component matrix of claim 1, wherein the concentration of chondroitin sulfate is from about 0.25 mg/ml to about 3 mg/ml.

11. The tri-component matrix of claim 10, wherein the concentration of chondroitin sulfate is about 1 mg/ml.

12. The tri-component matrix of claim 1, further comprising chondrocytes, chondroprogenitor cells, mesenchymal stem cells, induced pluripotent stem cells (iPS cells), iPS cells derived from chondrocytes, H9-derived chondroprogenitor cells, Sox-9 transduced chondrocytes, osteoblasts, osteoprogenitors, or any combinations thereof.

13. The tri-component matrix of claim 12, further comprising chondrocytes.

14. The tri-component matrix of claim 12, further comprising chondroprogenitor cells.

15. The tri-component matrix of claim 12, further comprising iPS cells.

16. A process for producing a transplantation composition for cartilage engineering comprising:
    a) dissolving isolated and purified non-denatured type II collagen in a solution comprising an acid, wherein the final concentration of type II collagen is from about 0.5 mg/ml to about 5 mg/ml;
    b) neutralizing the acid;
    c) adding from about 0.25 mg/ml to about 3 mg/ml of hyaluronan; and
    d) adding from about 0.25 mg/ml to about 3 mg/ml of chondroitin sulfate,

    wherein a tri-component matrix is produced.

17. The process of claim 16, wherein the acid is acetic acid.

18. The process of claim 16, wherein the acetic acid is at a concentration of about 0.01 M.

19. The process of claim 16, wherein the acetic acid is neutralized with $NaHCO_3$/HEPES.

20. The process of claim 16, wherein the tri-component matrix comprises Medium 199.

21. The process of claim 16, wherein hyaluronan is added at a concentration of about 1 mg/ml.

22. The process of claim 16, wherein chondroitin sulfate is added at a concentration of about 1 mg/ml.

23. The process of claim 16, wherein the final concentration of type II collagen is about 3 mg/ml.

24. The process of claim 16, further comprising adding chondrocytes, chondroprogenitor cells, mesenchymal stem cells, iPS cells, iPS cells derived from chondrocytes, H9-derived chondroprogenitor cells, Sox-9 transduced chondrocytes, or any combinations thereof, to the tri-component matrix.

25. The process of claim 24 further comprising adding chondrocytes to the tri-component matrix.

26. The process of claim 24, further comprising adding chondroprogenitor cells to the tri-component matrix.

27. The process of claim 24, further comprising adding iPS cells to the tri-component matrix.

28. A method for inducing endogenous cartilage growth in a subject in need thereof, including the step of implanting in the subject the tri-component matrix as recited in claim 1.

29. A method of treating a bone or cartilage defect in a subject in need thereof, comprising administering a transplantation composition comprising: i) a tri-component matrix, as claimed in claim 1, and ii) a population of cells, at the site of the bone or cartilage defect.

30. The method of claim 29, wherein the population of cells comprises chondrocytes, chondroprogenitor cells, iPS cells, mesenchymal stem cells, osteoblasts, osteoprogenitors, or combinations thereof.

31. The method of claim 29, wherein new tissue is produced.

32. The method of claim 31, wherein the new tissue restores the surface of the cartilage or bone.

33. The method of claim 31, wherein the new tissue comprises collagen type II.

34. The method of claim 31, wherein the new tissue comprises superficial, intermediate, and deep zones characteristic of normal articular cartilage.

35. The method of claim 34, wherein the superficial zone of the new tissue comprises lubricin.

36. The method of claim 31, wherein the new tissue does not comprise teratomas, neoplastic cells, evidence of deformation, abnormal architectural features, or other inappropriate cell types.

37. The method of claim 29, the population of cells comprises chondrocytes.

38. The method of claim 29, the population of cells comprises chondroprogenitor cells.

39. The method of claim 29, the population of cells comprises iPS cells.

40. The method of claim 39, wherein the iPS cells are derived from chondrocytes.

41. The method of claim 38, wherein the chondroprogenitor cells are H9-derived chondroprogenitor cells.

42. The method of claim 37, wherein the chondrocytes are Sox-9 transduced chondrocytes.

43. A method of treating a cartilage-related disorder in a subject in need thereof, comprising administering a mixture of a tri-component matrix, as claimed in claim 1, and chondrocytes, chondroprogenitor cells, mesenchymal stem cells, iPS cells, iPS cells derived from chondrocytes, H9-derived chondroprogenitor cells, Sox-9 transduced chondrocytes, osteoblasts, osteoprogenitors, or any combinations thereof, to a site of cartilage injury or defect.
44. A method according to claim 43, wherein the the cartilage-related disorder is articular cartilage trauma, meniscus injury, a chonodrogenesis disorder, arthritis, chondropathy, chondrosarcoma, chondromalacia, polychondritis, relapsing polychondritis, slipped epiphysis, osteochondritis dissecans, chondrodysplasia, costochondritis, osteochondroma, spondylosis, osteochondroses, Tietze syndrome, dermochondrocorneal dystrophy of Francois, epiphyseal dysplasia, carpotarsal osteochondromatosis, achondropasia, chondrocalcinosis, genochondromatosis, chondroma, achondrogenesis, echondromata, hyprochondroplasia, or Keutel syndrome.
45. The method of claim 44, wherein the cartilage-related disorder is arthritis.
46. The method of claim 45, wherein the arthritis is osteoarthritis.
47. The method of claim 46, wherein the osteoarthritis occurs in the knee, finger, wrist, hip, spine, shoulder, elbow, toe, ankle, or neck of a subject.
48. A cartilage repair implant comprising:
    a) a tri-component matrix according to claim 1; and
    b) cells selected from chondrocytes, chondroprogenitor cells, mesenchymal stem cells, iPS cells, iPS cells derived from chondrocytes, H9-derived chondroprogenitor cells, Sox-9 transduced chondrocytes, osteoblasts, osteoprogenitors, or any combinations thereof.
49. The cartilage repair implant of claim 48, further comprising a biomaterial substrate, wherein the biomaterial substrate is selected from: polyglycolic acid (PGA), polylactic acid, alginates, polyethylene oxide, fibrin adhesive, polylactic acid-polyglycolic acid copolymer, human dermis, a membrane such as a sheet, a porous body such as sponges, a mesh such as a knit, a textile, a non-woven fabric, cotton, porous material, or combinations thereof.

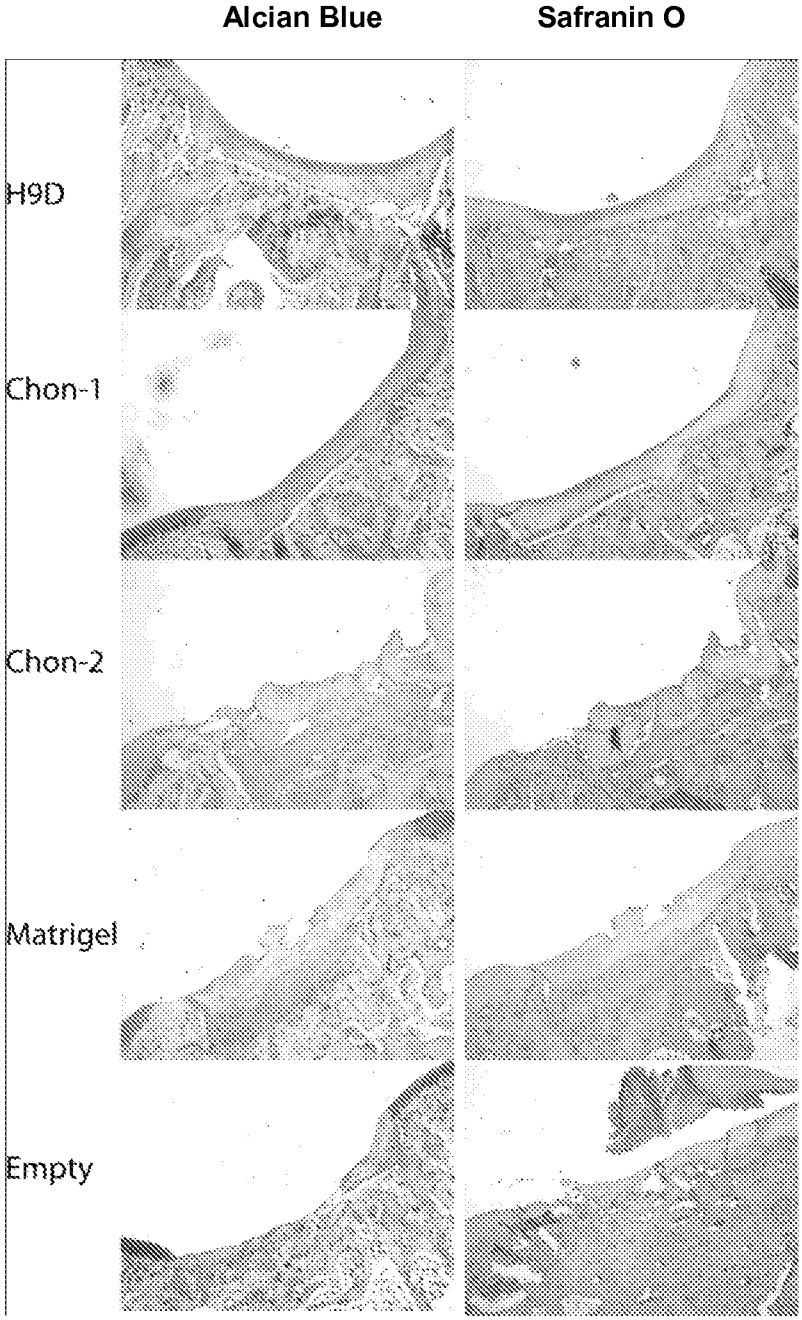

FIG. 1

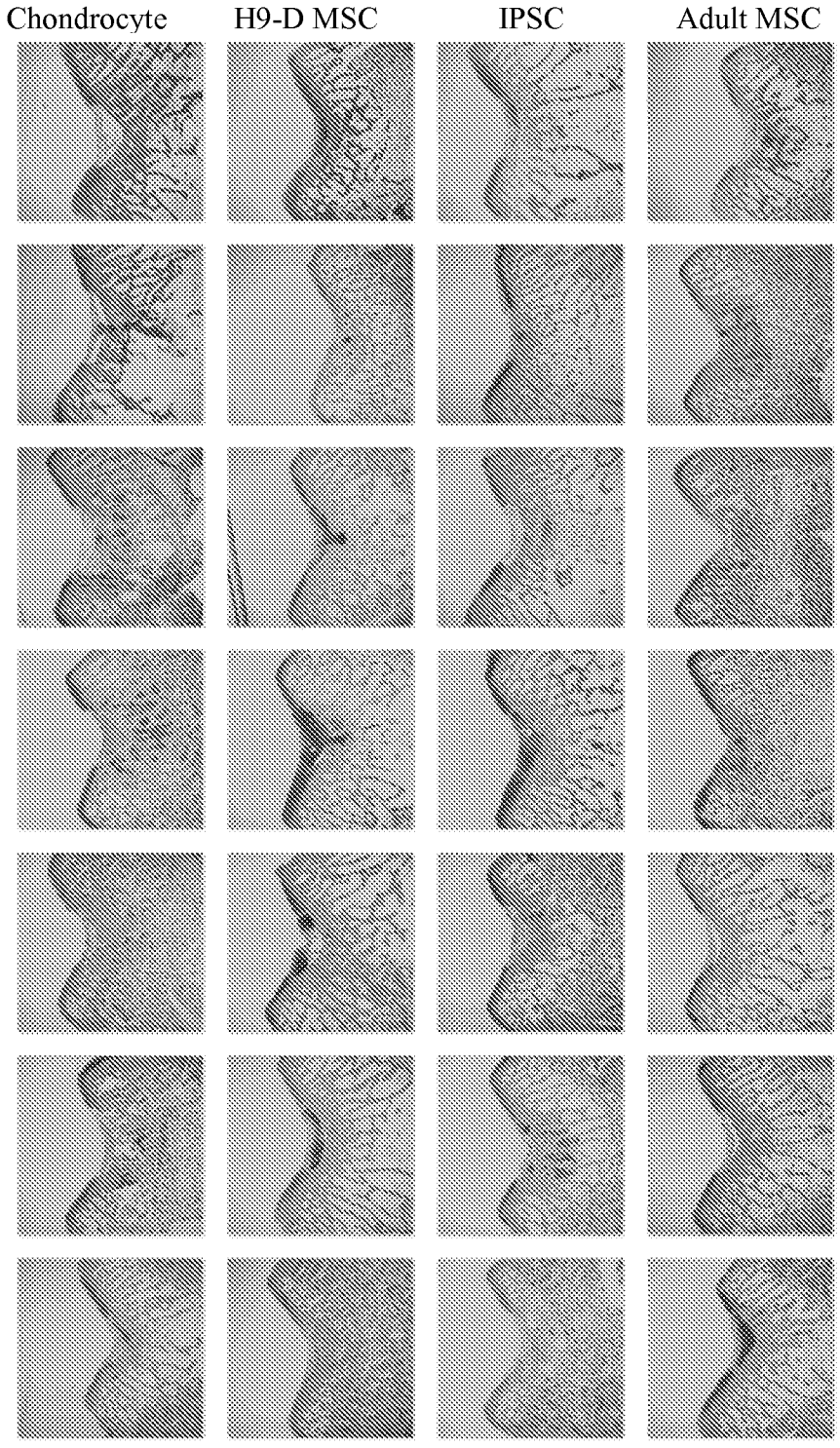

FIG. 2

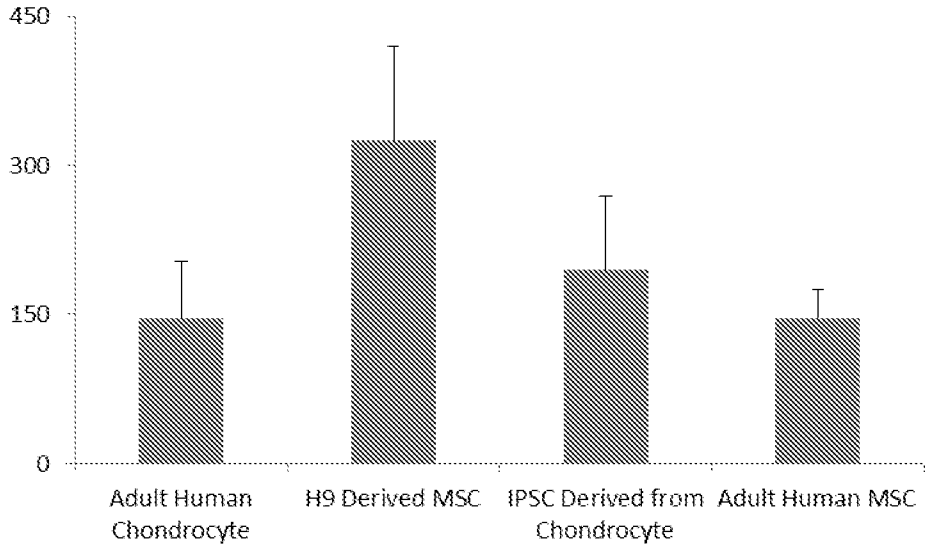
450
300
150
0
Adult Human Chondrocyte
H9 Derived MSC
IPSC Derived from Chondrocyte
Adult Human MSC

FIG. 3

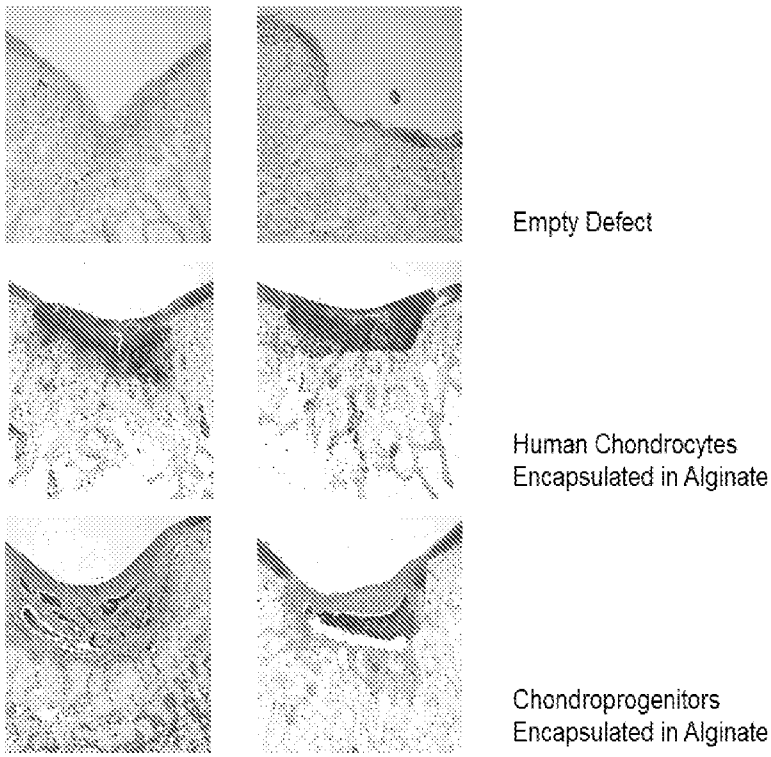
Empty Defect
Human Chondrocytes
Encapsulated in Alginate
Chondroprogenitors
Encapsulated in Alginate

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/067349

A. CLASSIFICATION OF SUBJECT MATTER

A61L 27/24(2006.01)i, A61L 27/20(2006.01)i, A61L 27/56(2006.01)i, A61L 27/38(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61L 27/24; A61K 9/14; A61K 9/10; A61K 9/20; A61K 31/728; A61K 31/737; A61K 38/39; A61L 27/20; A61L 27/56; A61L 27/38

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & Keywords: chondrocyte, transplant, non-denatured type II collagen, hyaluronan, chondroitin sulfate

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2002-0090391 A1 (GEISTLICH et al.) 11 July 2002 see claims 1, 7, 10 and 12; paragraphs [0015]-[0017] and [0042]-[0061].	1-27,48-49
Y	CROWLEY et al., `Safety and efficacy of undenatured type II collagen in the treatment of osteoarthritis of the knee: a clinical trial`, International Journal of Medical Sciences, 09 October 2009, Vol. 6, No. 6, pp. 312-321 see abstract; page 313.	1-27,48-49
A	US 2004-0213852 A1 (VAN KUPPEVELT et al.) 28 October 2004 see abstract; claims 1-35.	1-27,48-49
A	US 2007-0293427 A1 (VOULAND et al.) 20 December 2007 see abstract; claims 8-14.	1-27,48-49
A	US 5645851 A (MOORE) 08 July 1997 see abstract; claim 1.	1-27,48-49

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 December 2013 (19.12.2013)	**20 December 2013 (20.12.2013)**

Name and mailing address of the ISA/KR
Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon Metropolitan City, 302-701, Republic of Korea
Facsimile No. +82-42-472-7140

Authorized officer
KIM, Seung Beom
Telephone No. +82-42-481-3371

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/067349

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 28-47
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 28-47 pertain to methods for treatment of the human body by therapy, and thus relate to a subject matter which this International Searching Authority is not required, under Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/US2013/067349

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/US2013/067349

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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