

**(12) STANDARD PATENT
(19) AUSTRALIAN PATENT OFFICE**

(11) Application No. AU 2010276271 B2

(54) Title
Particulate tissue graft with components of differing density and methods of making and using the same

(51) International Patent Classification(s)
A61L 27/14 (2006.01)

(21) Application No: **2010276271** (22) Date of Filing: **2010.07.21**

(87) WIPO No: **WO11/011484**

(30) Priority Data

(31) Number **12/507,311** (32) Date **2009.07.22** (33) Country **US**

(43) Publication Date: **2011.01.27**
(44) Accepted Journal Date: **2014.05.22**

(71) Applicant(s)
ACell, Inc.

(72) Inventor(s)
Bosley Jr., Rodney W.;Fette, Clay;Tullius, Robert S.

(74) Agent / Attorney
Davies Collison Cave, Level 15 1 Nicholson Street, MELBOURNE, VIC, 3000

(56) Related Art
D2: EP 0747067 A2 (COLLAGEN CORPORATION) 11 December 1996
D1: WO 2008/012828 A2 (COLBAR LIFESCIENCE LTD et al.) 31 January 2008

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
27 January 2011 (27.01.2011)

(10) International Publication Number
WO 2011/011484 A4

(51) International Patent Classification:
A61L 27/14 (2006.01) *A61L 27/58* (2006.01)
A61L 27/36 (2006.01)

AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(21) International Application Number:
PCT/US2010/042701

(22) International Filing Date:
21 July 2010 (21.07.2010)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
12/507,311 22 July 2009 (22.07.2009) US

(71) Applicant (for all designated States except US): **ACELL, INC.** [US/US]; 8671 Robert Fulton Drive, Suite B, Columbia, Maryland 21046 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **BOSLEY, JR., Rodney, W.** [US/US]; c/o Acell, Inc., 8671 Robert Fulton Drive, Suite B, Columbia, Maryland 21046 (US). **FETTE, Clay** [US/US]; c/o Acell, Inc., 8671 Robert Fulton Drive, Suite B, Columbia, Maryland 21046 (US). **TULLIUS, Robert, S.** [US/US]; c/o Acell, Inc., 8671 Robert Fulton Drive, Suite B, Columbia, Maryland 21046 (US).

(74) Agents: **MOORE, Ronda, P.** et al.; Burns & Levinson, LLP, 125 Summer Street, Boston, Massachusetts 02110 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM,

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))

Published:

— with international search report (Art. 21(3))
— with amended claims (Art. 19(1))

(88) Date of publication of the international search report: 21 April 2011

Date of publication of the amended claims: 16 June 2011

(54) Title: PARTICULATE TISSUE GRAFT WITH COMPONENTS OF DIFFERING DENSITY AND METHODS OF MAKING AND USING THE SAME

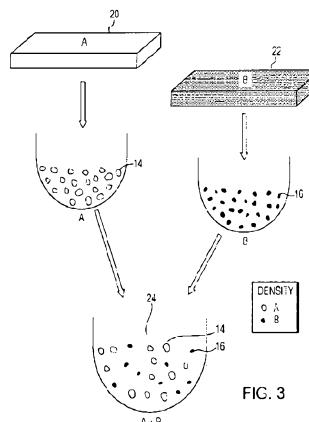


FIG. 3

(57) Abstract: Disclosed are tissue graft compositions made of particles having different densities, methods of making these compositions, and methods of using these compositions for promoting tissue restoration in a patient.

PARTICULATE TISSUE GRAFT WITH COMPONENTS OF DIFFERING DENSITY AND
METHODS OF MAKING AND USING THE SAME

Technical Field of the Invention

5 The present invention is related to particulate tissue graft compositions, methods of making them, and methods of using them to repair or restore tissues in a patient.

Background

Tissue graft compositions made of devitalized native tissues have been widely used to
10 repair or restore damaged, deformed, or missing tissues in patients. Typically these compositions are made from the extracellular matrix of tissues, for example, the submucosa of the intestine (SIS), or urinary bladder (UBS), and the epithelial basement membrane of, for example, the urinary bladder (UBM). Collectively these compositions are termed extracellular matrices or ECMs. SIS, UBS, and UBM are further described in U.S. Patent Nos. 6,576,265,
15 6,579,538, 5,573,784, 5,554,389, 4,956,178, and 4,902,508, each of which are incorporated by reference herein.

Briefly, SIS and UBS are made from gastrointestinal tissue and urinary bladder, respectively, by delaminating the submucosa from all other layers of the tissue source and retaining the submucosa to form a tissue graft composition. UBM is made by delaminating the epithelial basement membrane and, optionally, delaminating one or more of the deeper layers from the epithelial cells of the urinary bladder and retaining at least the epithelial basement membrane to form a tissue graft composition. Other tissues including, but not limited to skin and tissues of the gastrointestinal tract, e.g., esophagus, stomach, and intestine, may also be used to make an ECM including epithelial basement membrane by delaminating the epithelial basement membrane from the epithelial cells and, optionally, one or more of the deeper layers of the tissue source. Still other native ECMs may be made from connective tissues such as aponeurosis, tendon, ligament, smooth and skeletal muscle, and treatment-site specific ECMs.

ECMs are used to restore, for example, epithelial tissues. Restoration of epithelial tissues requires restoration of the epithelium and the connective tissue elements underlying the epithelial tissue. Epithelial restoration occurs rapidly when epithelial basement membrane

is present. However, restoration of the connective tissue elements is slower than epithelial tissue restoration, prolonging the complete restoration of damaged epithelial tissues.

Each of the ECMs, such as UBS, UBM, and SIS, known in the prior art, are resorbed at the site of implantation in a patient. For example, a single sheet of urinary bladder matrix (UBM) may resorb, for example, within a week when implanted in a topical wound. FIGS. 5 1A-C depict the rapid resorption profile of a prior art ECM material 12 implanted at a tissue site 10 in a patient. FIG. 1 A shows the sheet of ECM 12 on day 0 of implantation. As shown, the ECM 12 fills the entire tissue site 10. At day X after implantation, FIG. 1 B shows that the ECM 12 has been partially resorbed at the tissue site 10 as shown by the reduced surface area 10 of the ECM 12 as compared to the surface area of the ECM 12 in FIG. 1A. At day Y after implantation, FIG. 1 C shows that the ECM 12 has been completely resorbed by the tissue site 10 as no ECM 12 remains.

Summary of the Invention

15 The invention disclosed herein has identified and solved the problem of prior art ECM implants that are rapidly resorbed and are subsequently no longer available to influence restoration and repair of damaged or missing epithelial tissues and the underlying connective tissue elements. While the rapid initial resorption of prior art ECMs is beneficial in providing an initial restoration response to the tissue site in need of restoration or repair, the invention 20 described herein is advantageous because it provides, at the site in need of restoration or repair, an ECM composition that takes longer to resorb than the prior art ECMs which prolongs the ECM's influence on the repair process at the site of tissue injury. Accordingly, the invention disclosed herein is directed to an ECM composition having at least one component that resorbs quickly to provide a rapid initial repair response, but also having at 25 least one additional component that resorbs more slowly in order to prolong the influence of the ECM composition combination on the restoration process at the site of tissue injury.

According to one aspect, the invention relates to compositions of bioresorbable particles useful for tissue repair or restoration and methods of using these compositions. The compositions include bioresorbable particles of differing density which resorb at different

rates, thereby providing the ability to adjust the length of time provided by the particles to effect repair as compared to compositions having materials of a single density.

According to one aspect, the invention includes a composition of bioresorbable particles comprising:

a first plurality of bioresorbable particles comprising a native extracellular matrix and having a first density and a first rate of resorption upon implantation at a site in a patient, and,

a second plurality of bioresorbable particles comprising another extracellular matrix different than the extracellular matrix of the first plurality of bioresorbable particles, or comprising a non-extracellular matrix material and having a second density and a second rate of resorption upon implantation at the site in a patient, wherein the second density of the second plurality of bioresorbable particles is at least 150% of the first density of the first plurality of bioresorbable particles, and the second rate of resorption is in the range of about 1 to about 10 times slower than the first rate of resorption.

According to one aspect, the invention includes tissue graft compositions of bioresorbable particles. In one embodiment of the invention, a mixture of bioresorbable particulate matter comprises a first plurality of bioresorbable particles having a first density and a first rate of resorption on implantation at a tissue site in the patient and a second plurality of particles having a second density and a second rate of resorption upon implantation at the tissue site in the patient. The second density is at least 150% of the first density. The second rate of absorption is in the range of about 1 to about 10 times slower than the first rate of absorption. The first plurality and second plurality of bioresorbable particles promote tissue restoration and repair when implanted at a tissue site in a mammalian patient.

In one embodiment, the first plurality of particles comprises a first bioresorbable material and the second plurality of particles comprises a second bioresorbable material, whereas in another embodiment the first plurality of particles and the second plurality of particles comprise the same bioresorbable material. The first or second bioresorbable material may be selected from, for example, extracellular matrix, collagen, a bioresorbable polymer, or hyaluronic acid. The particles may be in powder form, dispersed in a liquid or gel medium, or compressed into a sheet form. The size of the particles may range from 1 μm to 1000 μm . In a further embodiment, the particles may include one or more bioactive

factors, pharmaceutical agents, or DNA vectors. The first plurality of particles and the second plurality of particles may be present in a ratio of 1:1. The second density may be in the range from 150% to 500% of the first density.

In yet another embodiment, the tissue graft composition includes a third plurality of particles having a third density that is more dense than the first and second densities and a third rate of resorption that is slower than the first and second rates of resorption on implantation at the tissue site in a patient.

According to another aspect, the invention includes methods for preparing mixtures of bioresorbable particles having different densities. For example, in one embodiment, a 10 method for preparing a mixture of bioresorbable particulate matter having differing densities comprises providing a first sheet of bioresorbable material having a first density and a first rate of bioresorption and milling the sheet into a first plurality of particles. A second sheet of a bioresorbable material having a second density and a second rate of bioresorption is milled into a second plurality of particles. A predetermined quantity of the 15 first plurality of particles is mixed with a predetermined quantity of the second plurality of particles, wherein the density of the second plurality of particles is at least 150% of the density of the first plurality of particles and wherein the first rate of resorption is at least 1.5 times that of the second rate of resorption. The first plurality of particles and the second plurality of particles are capable of promoting tissue restoration and repair when 20 implanted at a tissue site in a mammalian patient.

In one aspect, the invention includes a method for preparing a mixture of bioresorbable particulate matter, comprising:

- (a) providing a first sheet of bioresorbable material comprising a native extracellular matrix comprising a plurality of naturally associated native extracellular matrix components and having a first density and a first rate of resorption, and milling said sheet into a first plurality of particles;
- (b) providing a second sheet of a bioresorbable material comprising another extracellular matrix different than the extracellular matrix of the first sheet, or a non-extracellular matrix material and having a second density and a second rate of resorption, 30 and milling said sheet into a second plurality of particles;
- (c) providing a predetermined quantity of said first plurality of particles and a predetermined quantity of said second plurality of particles, and mixing said predetermined

quantity of said first plurality of bioresorbable particles with a predetermined quantity of said second plurality of bioresorbable particles.

In one aspect, the invention includes a method for preparing a mixture of bioresorbable particulate matter having differing densities comprising:

5 providing a sheet of bioresorbable material comprising a native extracellular matrix and having a first density and compressing a portion of said sheet such that said compressed portion comprises a second density that is at least 150% of the first density of said sheet of bioresorbable material; and

10 providing a second sheet of bioresorbable material comprising a native extracellular matrix;

joining said first sheet to said second sheet to form a multilaminate sheet; and milling said multilaminate sheet into a plurality of particles, wherein said mixture of bioresorbable particles promotes tissue restoration and repair when implanted at a tissue site in a mammalian patient.

15 In one aspect, the invention includes a method of modulating the rate of tissue repair at an anatomical site in a patient in need of repair or restoration, the method comprising:

20 administering at the anatomical site of the patient a mixture comprising (i) a first plurality of bioresorbable particles comprising a native extracellular matrix comprising a plurality of naturally associated native extracellular matrix components and having a first density and (ii) a second plurality of bioresorbable particles comprising another extracellular matrix different than the first plurality of bioresorbable particles and having a second density at least 150% of the first density of the first plurality of bioresorbable particles,

25 wherein the second plurality of bioresorbable particles are resorbed by the patient's tissue at a slower rate than the absorption of the first plurality of bioresorbable particles, thereby prolonging the therapeutic effect of the second plurality of particles at the site and wherein said first plurality and second plurality of bioresorbable particles promote tissue restoration and repair when implanted at the anatomical site in the patient.

30 In one aspect, the invention includes a method of preparing a mixture of bioresorbable extracellular matrix particles, comprising:

preparing a plurality of bioresorbable particles of native extracellular matrix each having the same density;

dividing the plurality of particles into a first group and a second group;

coating the first group of said plurality of particles with a coating comprising a

5 material selected from the group consisting of an extracellular matrix, polyglycolic acid, hyaluronic acid, collagen, or combinations thereof, said coating having a first density;

coating the second group of said plurality of particles with a coating comprising a material selected from the group consisting of an extracellular matrix, polyglycolic acid, hyaluronic acid, collagen, or combinations thereof, said coating having a second density;

10 mixing said first group of particles with said second group of particles to form a mixture of bioresorbable particles, wherein said mixture of particles promotes tissue restoration and repair when implanted at a tissue site in a mammalian patient.

According to another embodiment of the invention, the first sheet may be compressed or laminated prior to milling to increase its density relative to the first sheet.

15 In an alternative embodiment, the first sheet may be lyophilized prior to milling to reduce its density relative to the second sheet.

According to another embodiment of the invention, the sheet may be milled into particles by comminuting, grinding, chopping, blending, pulverizing, or mincing. In a further embodiment, the first or second sheet is dried, hydrated or frozen prior to milling.

20 According to another embodiment of the invention, the bioresorbable material comprises extracellular matrix, hyaluronic acid or a bioresorbable polymer. The hyaluronic acid or bioresorbable polymer may be chemically synthesized or precipitated to form sheets of a first density and sheets of a second density prior to milling.

According to yet another embodiment, the first sheet and the second sheet may

25 comprise the same bioresorbable material. For example, in one embodiment the first and second sheet are extracellular matrix. In another embodiment, the first and second sheet are aponeurosis. In an alternative embodiment, the first sheet and the second sheet comprise different bioresorbable materials. For example, in one embodiment the first sheet comprises extracellular matrix and the second sheet comprises hyaluronic acid. In

30 another embodiment, the first or second sheet further comprises one or more pharmaceutical agents, DNA vectors, or bioactive factors such as a growth factor.

In yet another embodiment, the second density of the second sheet is 150%-500% of the first density.

In yet another embodiment, the particles of a first density and the particles of a second density prepared by the method are compressed into a sheet form.

5 In another embodiment of the invention, a method for preparing a mixture of bioresorbable particulate matter having differing densities comprises providing a sheet of bioresorbable material of a first density and compressing a portion of the sheet such that the portion comprises a second density that is at least 150% or as much as 150%-500% of the first density. The sheet is milled into a plurality of particles. In a further embodiment, the
10 bioresorbable material is ECM.

In another aspect, the invention provides methods of promoting tissue repair and restoration at an anatomical site in a patient. For example, according to one embodiment, a method of modulating the rate of tissue repair at an anatomical site in a patient in need of repair or restoration comprises administering to a patient at the site a mixture comprising a
15 first plurality of bioresorbable particles having a first density and a second plurality of bioresorbable particles having a second density at least 150% of the first density, wherein the second plurality of bioresorbable particles are resorbed by the patient's tissue at a slower rate than the absorption of the first plurality of bioresorbable particles, thereby prolonging the therapeutic effect of the second plurality of particles at the site. The first plurality and second
20 plurality of bioresorbable particles promote tissue restoration and repair when implanted at the anatomical site in a mammalian patient. In a further embodiment, the mixture comprises a third plurality of particles having a third density.

In a further embodiment, the method also includes the steps of preparing the mixture of bioresorbable particles which include the steps of (a) determining the optimal rate of
25 absorption of a resorbable composition for repair or restoration of the site in the patient, (b) selecting the first plurality of bioresorbable particles to have a first density and a first rate of absorption based on step (a) on implantation at a site in the patient, and (c) selecting the second plurality of bioresorbable particles to have a second density more dense than the density of the first plurality of particles and having a second rate of absorption based on step

(a) and step (b) slower than the rate of absorption of the first plurality of bioresorbable particles on implantation at the site in the patient.

According to one embodiment of the method, the bioresorbable particles are extracellular matrix, collagen, a bioresorbable polymer, or hyaluronic acid. The

5 bioresorbable particles may be delivered to the site in the patient in powder form or in a liquid or gel medium. The bioresorbable particles may also be injected at the site in the patient. The bioresorbable particles may contain one or more bioactive factors, DNA vectors, or pharmaceutical agents.

In another embodiment of the method, a mixture of bioresorbable particles is
10 administered to the patient via coating the mixture on a medical device prior to implantation of the medical device at the site in the patient, whereas in an alternative embodiment, the mixture is administered topically to the site. In a further embodiment, the mixture is administered to the patient as a component of a bone substitute material.

According to a further embodiment of the method, the site in need of repair or
15 restoration in the patient is a tendon, ligament, bone, kidney, liver, spleen, lymph node, urinary bladder, ureter, uterus, blood vessel, skin, breast, heart, or a body cavity. In one embodiment, the patient is a human.

According to another aspect, the invention includes an implant for implanting in a patient comprising particles of extracellular matrix of a first density and having a first rate
20 of resorption in a patient and particles of extracellular matrix of a second density and having a second rate of resorption in a patient, where said particles are compressed to form a three-dimensional object. The particles of the first density and the particles of the second density promote tissue restoration and repair when implanted at a tissue site in a human patient.

25 In another aspect, the invention includes an implant for implanting at the site of an anatomical tissue in a human patient, comprising:

first particles comprising a first native extracellular matrix and comprising a first density;

30 second particles of a second native extracellular matrix and comprising a second density;

said first particles and said second particles compressed together to form a three dimensional object corresponding to the site of said anatomical tissue of said patient.

According to one embodiment, the extracellular matrix is UBS, SIS, or UBM. In another embodiment, the particles of a first density are UBS and the particles of a second density are UBM. In another embodiment of the implant, the particles of the first density are more dense than the particles of the second density and the particles of the first density 5 form a core of the implant and the particles of the second density form the exterior surface of the implant.

In another aspect, the invention includes implanting the implant in a patient in need of tissue repair. In a further embodiment, the implant site may be a tendon, ligament, bone, kidney, liver, spleen, lymph node, urinary bladder, ureter, uterus, blood vessel, skin, breast, heart, or a body cavity.

5 In yet another aspect, the invention includes a method of preparing a mixture of particles of extracellular matrix which comprises preparing a plurality of particles of extracellular matrix having the same density, coating a first portion of the plurality of particles with a coating having a first density and a first rate of absorption in a patient, coating a portion of the plurality of particles with a coating having a second density and a second rate of
10 absorption in a patient, and mixing the first portion of the plurality of particles with the second portion of the plurality of particles. The plurality of particles promote tissue restoration and repair when implanted at a tissue site in a mammalian patient. In one embodiment, the
15 particles of extracellular matrix include urinary basement membrane (UBM), the coating of the first portion of the plurality of particles having a first density is UBM, and the coating of the second portion of the plurality of particles having a second density is small intestinal submucosa (SIS).

In yet another aspect, the invention is a composition of extracellular matrix. The composition comprises a first sheet of extracellular matrix, a second sheet of extracellular matrix, and a first plurality of extracellular matrix particles having a first density and a second plurality of extracellular matrix particles having a second density at least 150% of the first density, wherein the first plurality of particles and the second plurality of particles are contained between the first sheet and the second sheet of extracellular matrix. The first plurality and second plurality of extracellular matrix particles promote tissue restoration and repair when implanted at a tissue site in a mammalian patient.

25 In a further embodiment, the composition includes a further plurality of extracellular matrix particles having a density of either the first density, the second density, or a third density, wherein the further plurality of particles are positioned between the surface of either the first sheet or second sheet of extracellular matrix and a third sheet of extracellular matrix.

Brief Description of the Figures

FIGS. 1 A-C depict the bioresorption of a prior art ECM such as UBM, UBS, or SIS in a mammalian patient tissue. FIG. 1A depicts the ECM on Day 0 at implantation. FIG. 1B depicts the ECM on Day X after implantation where the ECM has been partially resorbed by the patient tissue. FIG. 1C depicts the ECM on Day Y after implantation where the ECM has been fully resorbed by the patient tissue.

FIGS. 2A-C depict the bioresorption of an exemplary particulate tissue graft made up of two types of particles of different density according to one embodiment of the invention. The open circles (o) represent a lower density particle and the solid circles (•) represent a higher density particle. FIG. 2A depicts the particles on Day 0 at implantation. FIG. 2B depicts the particles on Day X after implantation where the lower density particles have been partially resorbed by the patient tissue, as evidenced by their decrease in size. FIG. 2C depicts the particles on Day Y after implantation where the lower density particles have been fully resorbed by the patient tissue, and the higher density particles have not yet been fully resorbed by the patient tissue.

FIG. 3 depicts an exemplary composition of ECM particles according to one embodiment of the invention where the particles are of two different densities and the stages of an exemplary method of making the composition according to one embodiment of the invention. Two sheets of ECM are shown, one having density A and the other having density B. The sheets are comminuted and a portion of A particles and B particles are mixed together.

FIG. 4 depicts an exemplary composition of ECM particles according to one embodiment of the invention where the particles are of two different densities and the steps of an exemplary method of making the composition according to one embodiment of the invention. A sheet of ECM is treated so that a portion of the sheet has a density A and a second portion of the sheet has a density B. The sheet is then comminuted to make a composition of particles of two different densities.

FIG. 5 depicts an exemplary composition of ECM particles according to one embodiment of the invention where the particles have a density A, B, or C and the steps of an exemplary method of making the composition according to one embodiment of the invention. A first sheet A of ECM having density A is adhered to a second sheet B of ECM having

density B. The sheet is then comminuted to create a composition of particles having density A and particles having density B and composite particles of density C, the composite particles having components of both sheets A and B.

FIG. 6 depicts an exemplary composition of ECM particles according to one embodiment of the invention where the particles have a core particle of the same density, but are coated with different materials having different densities A, B, and C. Accordingly, the coated particles have differing densities A, B, and C.

FIG. 7 depicts an exemplary ECM composition according to one embodiment of the invention where ECM particles having densities A, B, and C are adhered to the surface of a sheet 25 of ECM.

FIG. 8A depicts an exemplary ECM composition according to one embodiment of the invention where ECM particles of a higher density B are compressed together to form a core of particles of density B coated with particles of lower density A or layered with a layer of particles of density A to form a construct in the shape of a mammalian body part, here exemplified as a kidney.

FIG. 8B depicts an exemplary ECM composition according to one embodiment of the invention where ECM particles of a higher density B are compressed together to form a core of particles of density B coated with particles of lower density A or layered with a layer of particles of density A to form a construct in the shape of a mammalian body part, here exemplified as a human nose.

FIG. 9 depicts an exemplary ECM composition according to one embodiment of the invention where a mixture of ECM particles of a higher density B and of a lower density A are compressed together to form a three-dimensional construct in the shape of a mammalian body part, here exemplified as a human nose.

FIG. 10 depicts an exemplary ECM composition according to one embodiment of the invention where a mixture of ECM particles of a higher density B and of a lower density A are compressed together to form a sheet.

FIG. 11 depicts an exemplary multi-laminate ECM composition according to one embodiment of the invention where a mixture of ECM particles of a higher density B and a lower density A are sandwiched between sheets of ECM.

Detailed Description of the Invention

Extracellular matrix materials (ECMs) have been used to promote repair and restoration of damaged, deformed, injured, and even missing body tissues in mammals, such as humans. For example, ECMs such as SIS or UBM are implanted at a treatment site to 5 promote repair and restoration of epithelial tissues and connective tissues, including connective tissue layers associated with epithelial tissues. Tissue sites in need of repair or restoration include, but are not limited to tendons, ligaments, breast, bone, kidney, liver, spleen, lymph nodes, urinary bladder, ureter, uterus, blood vessels, intestine, stomach, skin, heart, or a body cavity. "Restore," "restored," "restoring," or "restoration" means that tissue 10 function and structure is substantially returned to its original condition by the patient's endogenous tissue repair mechanisms in combination with the tissue graft composition.

ECMs such as SIS, UBS, and UBM are rapidly resorbed by host tissues in which the ECM has been implanted. Once the material has been resorbed by the host, the ECM no longer has an influence over tissue restoration and repair at the site of implantation. As ECMs 15 are resorbed, they release bioactive components such as growth factors and other bioactive components naturally present in the native ECM that influence tissue restoration and repair at the site of implantation of the ECM. Once the ECM has been fully resorbed, these growth factors and bioactive components are no longer released by the ECM at the implantation site to influence tissue repair and restoration. In order to moderate the appearance of these factors 20 and components contributed by ECMs during repair of tissues, disclosed herein are compositions of ECM particles that have resorption times that vary from the rapid resorption times of known native ECMs.

In one aspect, the invention relates to tissue graft compositions of ECM particles where the particles vary in density. "Density" as used herein refers to mass per unit volume of 25 the particle. According to one embodiment of the invention, ECMs are manipulated to alter their densities and are then particularized. The particles, when implanted in a patient, resorb at rates related to their densities, with lower density particles being resorbed by the patient at a faster rate than more dense particles. Lower density particles resorb more quickly than higher density particles, releasing growth factors and other bioactive components quickly, thereby 30 modulating an initial repair response at the tissue site. In contrast, higher density particles

resorb slowly, releasing growth factors and other bioactive components more slowly, thereby maintaining the repair response at the site of implantation beyond the initial repair response influenced by the growth factors and bioactive components released by the lower density particles.

5 According to the invention, having both lower and higher density particles as opposed to just higher density particles alone permits the released growth factors and other bioactive components provided by the lower density particles to modulate an initial rapid repair response at the tissue site due to the rapid resorption of the low density particles. Ideally, such a rapid repair response cannot be provided by the higher density particles alone because their
10 resorption is slower and therefore, the release of bioactive components from the material that encourages tissue repair and restoration is also slower.

Traditional ECMs have a rapid resorption time such that the ECMs may be fully resorbed at the tissue implantation site before the tissue site is completely repaired. Increased density ECM particles or aggregates of ECM particles of varying densities prepared according
15 to the invention have prolonged resorption times and can therefore have an extended influence over tissue repair and restoration at the site of the injury as compared to using traditional ECMs. In other words, tissue graft compositions according to the invention allow the tissue site to repair completely or close to completely before the tissue graft composition is bioresorbed at the tissue site. According to the invention, compositions of ECM particles
20 provide a specific bioresorption profile tailored to the restoration needs of a specific tissue site in a patient.

The invention as described herein has the advantage of reducing the amount of space required to accommodate an ECM tissue graft construct at an implantation site in a patient. For example, by increasing the density of a tissue graft construct according to the invention,
25 more ECM material can be included in a smaller volume. In addition, by increasing the density, the ECM material according to the invention takes longer to be resorbed at the implantation site in comparison to thicker, less dense ECM materials in the patient, thereby exerting a prolonged influence on tissue repair and restoration at the implantation site in the patient.

According to the invention, the rate of resorption of compositions can be measured based on the number of days required for a certain volume of ECM to be resorbed by host tissue at the site of implantation. For example, a composition according to the invention, in one embodiment, includes a lower density particle having a volume of $100 \mu\text{M}^2$ and a density 5 of X that takes 15 days to be resorbed and a higher density particle having a volume of $100 \mu\text{M}^2$ and having a density of 2X that takes 45 days to be resorbed. According to the invention, there is not necessarily a direct linear relationship between density and resorption time. As shown by this example, a particle that is twice the density of a first less dense particle may take longer than twice the resorption time of the first less dense particle to be resorbed.

10 As previously mentioned, ECMs known in the art have a rapid bioresorption rate when implanted at a tissue site in a patient. In contrast to the rapid bioresorption profile demonstrated by known ECMs, compositions according to the invention have varied bioresorption times based on the density of particles in the tissue graft composition. For example, FIGS. 2A-C demonstrate the varied bioresorption profile of a tissue graft 15 composition of particles of differing density according to the invention. FIG. 2A shows ECM particles 16 (of a higher density than the ECM shown in FIGS. 1A-C) and ECM particles 14. (of comparable density to the ECM shown in FIGS. 1A-C) on day 0, the day of implantation at the tissue site. At day X after implantation, as shown in FIG. 2B, the low density particles 14 have been partially resorbed as indicated by their reduced size. The more dense particles 16 20 have also not yet been fully resorbed. By day Y after implantation, as shown in FIG. 2C, the lower density particles 14 have been fully resorbed, while the more dense particles 16 have not, permitting the more dense particles to continue to promote tissue repair and restoration at the tissue site 10.

25 In one embodiment of the invention, a tissue graft composition of particles includes particles of a first density and particles of a second, different density. In a further embodiment, the composition also includes particles of a third density; particles of a third and fourth density; particles of a third, fourth, and fifth density; or particles of a third, fourth, fifth, and sixth density, and so on. For example, in one embodiment, the particle composition has particles of two densities; in another, three densities; in yet another, four densities; in still 30 another, five densities; and in yet another, six densities. According to one embodiment of the

invention, the composition has particles of more than six different densities. In yet another embodiment, a particle may be a composite of more than one material, for example, a combination of different ECM types, each ECM type having a different density.

In one embodiment of the invention, the composition has particles of a first density
5 and particles of a second different density where the particles of the second density are more dense than the particles of the first density. In a further embodiment, the particles of the second density are at least about 150% of the density of the particles of the first density (i.e., 1.5 times more dense). This magnitude of difference in density is necessary such that the difference between the rate of bioresorption of the particles of the first density and the
10 rate of bioresorption of the particles of the second density is large enough to allow the tissue being treated to be repaired before the composition is fully resorbed. Particles of a second density having a density less than about 150% of the first density do not have a bioresorption profile significantly different from the bioresorption profile of particles of the first density to allow the tissue site to be repaired before the composition is completely
15 resorbed.

In a further embodiment, the composition further includes particles of a third density. Particles of the third density are more dense than particles of the second density; the particles of the second density are more dense than particles of the first density. In a particular embodiment, the particles of the third density are at least about 150% of the
20 density of the particles of the second density and the particles of the second density are at least about 150% of the density of the particles of the first density (i.e., 1.5 times more dense). Again, this magnitude of difference in density is necessary such that the difference between the rates of bioresorption between the particles of the first density, the second density, and the third density is large enough to allow the tissue being treated to be
25 repaired before the composition is fully resorbed.

In a further embodiment, the particles of a first density that are less dense than particles of a second density have a resorption rate of about 1 time, about 2 times, about 3 times, about 4 times, about 5 times, about 6 times, about 7 times, about 8 times, about 9 times, or about 10 times, for example, the rate of resorption of the particles of a second
30 density.

In a further embodiment, the particles of a second density are at least about 2 times, 2.5 times, 3 times, 3.5 times, 4 times, 4.5 times, or 5 times more dense than the density of the particles of a first density. In another embodiment, the particles of a third density are at least about 2 times, 2.5 times, 3 times, 3.5 times, 4 times, 4.5 times, or 5 times more dense than the 5 particles of a second density.

Preferably, the second density particles are at least about 150-500% of the density of the lower first density particles, more preferably about 200-400% of the density of the lower first density particles, and most preferably about 200-300% of the density of the lower first density particles.

10 The particles according to one embodiment of the invention are extracellular matrix particles (ECM). According to the invention, ECM from which the particles of differing density are made may be derived from native tissues including, but not limited to submucosa, dermis, epithelial basement membrane, aponeurosis, tendon, ligament, smooth and skeletal muscle, and treatment site-specific ECM. The native tissue source may be porcine, bovine, or 15 ovine, for example. The native tissue source may be allogenic, autogenic, or xenogenic to the patient. For example, if the patient is a human, the native tissue source is from another human (allogenic) or from the patient (autogenic). Alternatively, if the patient is a human, the native tissue is from a non-human species (xenogenic).

ECM particles according to the invention have densities ranging from about 150 20 mg/cm³ to about 1800 mg/cm³, more preferably from about 150 mg/cm³ to about 1200 mg/cm³, and most preferably from about 150 mg/cm³ to about 600 mg/cm³.

In another embodiment of the invention, particles of the invention are composed of, for example, collagen, i.e., type I, II, III and/or IV collagen; a bioresorbable polymer such as 30 poly (L) lactic acid, poly (D) lactic acid, polyglycolic acid, polydioxanone, or 25 tyrosinepolycarbonate, and/or hyaluronic acid. Collagen particles according to the invention have densities ranging from about 150 mg/cm³ to about 1800 mg/cm³, more preferably from about 150 mg/cm³ to about 1200 mg/cm³, and most preferably from about 150 mg/cm³ to about 600 mg/cm³. Bioresorbable polymer particles according to the invention have densities ranging from about 150 mg/cm³ to about 1800 mg/cm³, more preferably from about 150

mg/cm³ to about 1200 mg/cm³, and most preferably from about 150 mg/cm³ to about 600 mg/cm³.

According to one embodiment of the invention, bioactive materials such as growth factors, DNA, or other proteins are added to particles of bioresorbable polymers for release as the polymers are resorbed in the patient. In another embodiment, bioactive materials such as growth factors, DNA, or other proteins are added to ECM compositions of the invention for release as the ECM is resorbed in the patient.

In one embodiment according to the invention, all particles of the first density are made from a first starting material and all particles of the second density are made from the same first starting material. For example, in one embodiment, all particles of the first density are made of an ECM material having a first density and all particles of the second density are particles of the same ECM material, but manipulated to have a second, different density. For example, the particles of a first density and the particles of the second density are each UBM particles. In another embodiment, all particles of the first density are a bioresorbable polymer and all particles of the second density are the same bioresorbable polymer. For example, the particles of the first density and the particles of the second density are poly (L) lactic acid.

In another embodiment, all particles of the first density are made from a first material and all particles of the second density are made from a second material. For example, all particles of the first density are made from a first ECM material and all particles of the second density are made from a second ECM material. For example, in one embodiment, the particles of the first density are made of UBM while particles of the second density are made from SIS. In another embodiment, all particles of the first density are made from an ECM material while all particles of the second density are made from a bioresorbable polymer. In another embodiment, the first material is UBM, while the second material is aponeurosis. In another embodiment, the first material is UBM, while the second material is liver basement membrane (LBM). In yet another embodiment, the first material is UBM, while the second material is hyaluronic acid. In an even further embodiment, the first material is UBM, while the second material is poly(lacticco-glycolicacid) (PLGA).

Particles according to one embodiment of the invention range from about 0.1 μ M to about 2000 μ M. More preferably, the particles range in size from about 1 μ M - about 2000

μM , even more preferably from about 1 μM - about 1000 μM , and most preferably from about 50 μM - about 750 μM in width, for example, diameter.

According to one embodiment of the invention, the mixture of differing density particles is maintained in a powder form, while in another embodiment, the mixture of 5 particles is maintained in a gel or liquid carrier for injection at a tissue site in a patient.

Examples of suitable carriers include but are not limited to hyaluronic acid, gelatin, lecithin, collagen gel, and saline.

In a further embodiment, particles according to the invention contain growth factors and/or other biologically active molecules. For example, particles such as ECM may naturally 10 contain biologically active molecules that are released at the site of implantation as the ECM is bioresorbed by the patient. Alternatively, biologically active molecules may be added to the composition of particles to be delivered to the site of implantation with the particles. In yet another embodiment, particles according to the invention are coated with biologically active molecules. In yet another embodiment, particles of bioresorbable polymers are synthesized 15 and formed into particles in the presence of biologically active molecules so that the biologically active molecules are contained within the bioresorbable polymer particle.

Examples of biologically active molecules include, but are not limited to epidermal growth factor, TGF-alpha, TGF-beta, fibroblast growth factor, platelet derived growth factor, vascular endothelial growth factor, insulin-like growth factor, keratinocyte growth factor, and 20 bone morphogenic protein to name a few, as well as pharmaceutical agents, and DNA vectors.

According to the invention, particles release biologically active molecules in a time dependent fashion. For example, given that lower density particles are bioresorbed by patient tissue at a more rapid rate than higher density particles, biologically active molecules, such as growth factors, are released from lower density particles relatively quickly after implantation 25 as compared to their release from higher density particles. Higher density particles bioresorb more slowly and therefore release biologically active molecules at the tissue site over a longer period of time than the lower density particles, thereby exposing the tissue site to particular biologically active molecules for a longer period of time than if only lower density particles are present.

According to another aspect, the invention relates to methods of making mixtures of particles where a first type of particle has a first density and a second type of particle has a second density.

According to one embodiment of the method of making mixtures of particles of the invention, sheets of ECM are comminuted to create a mixture of particles of differing density. For example, as shown in FIG. 3, a mixture 24 of A particles 14 and B particles 16 where A particles 14 are less dense than B particles 16 is made from two ECM sheets 20, 22 of differing densities. A Sheet 20 is comminuted to form A particles by chopping, cutting, pulverizing, milling, or grinding A Sheet 20 with a suitable device that forms particles such as a blender, a hammer mill, a knife mill, a centrifugal mill, a die and press system, or a roller crusher, for example. B Sheet 22 is comminuted to form B particles 16 by chopping, cutting, crushing, pulverizing, milling, mincing, or grinding B Sheet 22 with a suitable device to form particles 16. A particles 14 and B particles 16 have size ranges and density ranges as described above.

To form the mixture, a predetermined number of A particles 14 is then measured and 15 mixed with a predetermined number of B particles 16 to form a composite mixture 24 of A particles 14 and B particles 16, where the concentration of A particles 14 to B particles 16 is predetermined based on a particular desired resorption profile of the mixture at a tissue site in a patient. For example, for a tissue site that takes a long time to repair, such as a joint, fewer 20 less dense A particles 14 are selected compared to the number of higher density B particles 16 in order to provide a prolonged resorption of ECM at the tissue site. A particles 14 may be selected at a ratio of (A:B) 1:1, 1:2, 1:3, 1:4: 2:3, 3:4, 2:1, 3:1, 3:2, 4:3, or 4:1 B particles 16, for example, depending on the particular tissue site to be treated and the desired resorption profile of the particles for treating that tissue site.

According to a further embodiment of the invention, a mixture of particles includes 25 three types of particles, each type of a differing density. For example, the mixture includes low density particles, intermediate density particles, and higher density particles. For example, according to the invention, a sheet of a third density (C) is comminuted to form particles of a third density C, higher than the density of particles formed from A sheet 20 and B sheet 22. A 30 predetermined number of particles of the third density are measured out and added to the

mixture of A particles 14 and B particles 16. Particles of a third density maybe be selected at a ratio of (A:B:C) 1:1:1, 1:1:2, 1:1:3, 1:1:4, 1:2:1, 1:2:2, 1:2:3, 1:2:4, 1:3:1, 1:3:2, 1:3:3, 1:3:4, 1:4:1, 1:4:2, 1:4:3, 1:4:4, 2:1:1, 2:1:2, 2:1:3, 2:1:4, 2:2:1, 2:2:3, 2:3:1, 2:3:2, 2:3:3, 2:3:4, 2:4:1, 2:4:3, 3:1:1, 3:1:2, 3:1:3, 3:1:4, 3:2:1, 3:2:2, 3:2:3, 3:2:4, 3:3:1, 3:3:2, 3:3:4, 3:4:1, 3:4:2, 3:4:3, or 3:4:4, for example, depending on the particular tissue site to be treated and the predetermined resorption profile of the particles for treating the tissue site.

For example, in one embodiment, a composition of the invention has two parts lower density particles to 1 part higher density particles (2:1) and is used for wound treatment, i.e., of the skin and skin adnexa. The higher concentration of lower density particles, when applied to a wound, provides a rapid influx of bioactive components to activate the restoration and repair process as the lower density particles are bioresorbed, followed by a longer term release of bioactive components over time at the implantation site as the higher density particles resorb slowly over time. Additionally, specific bioactive components may be added to the lower or higher density particles to tailor the release of bioactive components to the stage of restoration and repair at the tissue site.

In another embodiment, a composition of the invention has 1 part lower density particles to four parts higher density particles (1:4) or 1 part lowest density particles, 2 parts lower density particles, and 2 parts highest density particles, (1:2:2) and is used for tendon or ligament repair. Restoration and repair of tendons and ligaments takes longer than at body sites that are typically more vascular and more cellular. Accordingly, the 1:4 or 1:2:2 density profile composition provides a longer resorption profile of the composition for restoring or repairing tendons and ligaments as compared to traditional ECMs.

In yet another embodiment, a composition of the invention has 2 parts lower density particles to 3 parts higher density particles to (2:3) and is used for scar revision or plastic surgery applications to promote a rapid early tissue restoration response while also having prolonged bioactive component release at the site to help prevent hypertrophic scar formation over a longer time.

In yet another embodiment, a composition of the invention has one part lower density particles to 2 parts higher density particles (1:2) and is used for applications involving soft tissue augmentation. In a further embodiment, a composition of the invention has one part

lower density particles to 3 parts higher density particles (1:3) and is used for ophthalmological applications.

According to the invention, sheets of ECM 20, 22 can be treated to alter their densities prior to being comminuted. The density of a sheet of ECM can be increased by mechanically compressing the material, dehydrating the material, vacuuming the material, crosslinking the material, or lyophilizing the material, for example. The density of a sheet of ECM can be decreased by hydration, expanding the pore structure under vacuum, mechanically expanding the material, or freezing the material in water, for example.

In another embodiment of the method of making a mixture of particles having a first density A and particles having a second density B, particulate matter from a sheet of ECM having areas of density A and density B are removed from the sheet of ECM and mixed together. For example, as shown in FIG. 4, sheet 30 is partially compressed to form a sheet 30 having an area A 31 of a first lower density and area B 32 of a second higher density. The sheet 30 is then milled to produce particles 33 from area A 31 having a lower density A and particles 34 from area B 32 having a higher density B.

According to one embodiment of the invention, the portion of sheet 30 that is compressed relative to the portion that is not compressed or less compressed is determined based on the predetermined bioresorption profile for the particles at a given tissue site in a patient. Accordingly, for example, if a 1:1 ratio of A:B (low density:high density) particles is sought to achieve the desired bioresorption profile, one half of the sheet is compressed to the desired density prior to comminuting the sheet into a mixture of particles of A and B densities. In one embodiment, the size of sheet 30 is selected prior to compression of a portion of the sheet 30 and the whole sheet 30 is comminuted after compression. Alternatively, in another embodiment, a portion of the sheet 30 is compressed after which a portion of sheet 30 is cut away from the whole of sheet 30 with the cut-away portion being comminuted.

In a further embodiment, a portion of the sheet 30 is compressed to have a third density C. The sheet 30 is then milled to produce a mixture of particles having a density A, a density B, and the third density C. The third density may be higher than A or higher than A and B.

Another embodiment of the method of making a mixture of particles having differing densities is shown in FIG. 5. According to one embodiment, a sheet 40 of ECM of a first density A is joined with a sheet 41 of ECM of a second density B to form a composite sheet 45. The composite sheet 45 is then comminuted to create a mixture of particles 46 having 5 different densities. For example, when composite sheet 45 is comminuted, the mixture includes A particles 42 of low density derived from sheet A, B particles 43 of a higher density derived from sheet B, and C particles 44 of an intermediate density formed from the interfacing area 47 of the A sheet 40 and the B sheet 41.

A further embodiment of the method of making a mixture of particles having 10 different densities is shown in FIG. 6. According to this embodiment, particles 65 have a core 61 and a coating 62. The core 61 has a density X and may be coated with any of a variety of coatings 63, 64, 66 having a variety of densities. For example, some particles 65 have cores 61 coated with a first material 66 having a first density to produce particles having a density A, while other particles 65 have cores 61 that are coated with a second material having a 15 second density 63 to produce particles having a density B, while yet other particles 65 have cores 61 coated with a third material having a third density 64 to produce particles having a third density C. Coating the core 61 can be performed, for example, by precipitating the coating onto a particle from a solution, by a roll coater, or by a granulator. Materials for coatings may include, for example, an ECM, a gel made from an ECM such as UBM gel or 20 SIS gel, PLGA, hyaluronic acid, or collagen, for example. The core 61 of particles 65 may include, for example, ECMS as described herein.

In another aspect of the invention, mixtures of comminuted particles of differing density are coated on devices such as synthetic or natural wound repair matrices for implantation into a patient. For example, as shown in FIG. 7, a mixture of A lower density 25 particles 52 and B higher density particles 53 is applied to a sheet 50 of ECM. The particles 52, 53 are adhered to the sheet by compression or use of an adhesive, for example. Accordingly, when the sheet 50 is implanted at a tissue site in a patient, the particles 52, 53 provide more surface area to contact with the tissue site in the patient. The A particles 52 and B particles 53 are more dense in one embodiment than the ECM sheet 50 so that after the 30 sheet 50 has been fully resorbed, the tissue continues to repair in the presence of particles 52

and 53. The remaining particles 52, 53 allow the tissue to repair before the particles 52, 53 of the composition are completely resorbed.

In a further embodiment, mixtures of comminuted particles of differing density coated on a device such as a synthetic or natural wound repair matrix is implanted in a patient 5 for use as an adhesion barrier. For example, as shown in FIG. 7, a matrix in sheet form 50, for example, is coated on one side with particles 52, 53 of differing density according to the invention and is implanted in a patient. Any adhesions forming at the implant site adhere to the particulate side of the matrix and as the particles 52, 53 are resorbed, the adhesions no longer remain attached to the matrix, but to the particles and the adhesions are subsequently 10 resorbed. In an alternative embodiment, particles of differing density according to the invention are sandwiched between a first matrix sheet of a first heavier density and a second matrix sheet of a lighter density to form an implant. The implant is implanted at a site in the patient with the more dense matrix sheet implanted against the tissue requiring repair and the less dense sheet facing outward into the body of the patient. As adhesions form on the 15 outward facing less dense sheet, the less dense sheet is resorbed allowing any adhesions to become unattached from the more dense portion of the implant, the adhesions then being subsequently resorbed by the patient along with the less dense sheet of matrix.

In one embodiment, the mixture of particles 52, 53 is adhered to one surface of the sheet 50, while in another embodiment the mixture of particles 52, 53 is adhered to all 20 surfaces of the sheet 50. For example, the sheet 50 bioresorbs in the patient within 60 days, the lower density A particles bioresorb in 7 days, and the higher density B particles bioresorb in the patient within 30 days.

In a further embodiment, a mixture of comminuted particles according to the invention is coated on a surgical medical device prior to the devices' implantation at a tissue site in a patient. For example, a mixture of lower density A particles and higher density B particles 25 are coated on a medical implant such as, but not limited to, a stent, a cardiac occluder, suture material, or a mesh. The particles are adhered to the device by, for example, a chemical bond, a peptide linker, a bioresorbable adhesive, such as cyanoacrylate or fibrin glue, or by mechanical means such as lyophilization of the particles on the device or compressing the 30 particles onto the device. Once the device is implanted at the tissue site in the patient, the

lower density A particles bioresorb quickly providing an initial restoration response at the tissue site in the patient, while the higher density B particles are not fully bioresorbed until the tissue site is completely repaired. For example, the lower density A particles are bioresorbed by the patient within, for example, 7 days, while the higher density B particles are bioresorbed by the patient within, for example, 30 days.

In another aspect, the invention includes aggregates of particles of differing density which form an implant with portions of differing densities for implantation at a tissue site in a patient. According to the invention, as shown in FIG. 8A and 8B, high density particles B are compressed to form a shape replicating a body part or portion of a body part in a mammal, for example, a human, and form the core 71 a, b of an implant 70, 72. Lower density particles A are then compressed or coated around the 71 a, b core of B particles to form the peripheral layer of the implant 70, 72. As shown in FIG. 8A, the A and B particles form an ovoid shape implant 70 to be used in replacement or repair, for example, of a kidney or liver. As shown in FIG. 8B, the peripheral A and core B particles form roughly a triangular shape implant 72 to be used in replacement or repair, for example, of a nose.

The implant 70, 72 is implanted into a patient at a site requiring restoration or repair, for example. Upon implantation, the peripheral A particles having a lower density provide an immediate repair and restoration response at the tissue site in the patient. After the peripheral A particles rapidly bioresorb, the higher density core 71 a, b B particles continue to provide a repair and restoration response at the tissue site in the patient over a longer period of time than the peripheral A particles alone. The core 71 a, b B particles are typically not fully resorbed by the patient until the tissue site is repaired. For example, the peripheral A particles of lower density are bioresorbed by the patient within, for example, 14 days, while the core B particles of higher density are bioresorbed by the patient within, for example, 60 days.

In a further embodiment, as shown in FIG. 9, an implant 74 can be formed by compressing a mixture of lower density A particles 76 with higher density B particles 78. The lower density particles 76 resorb at a faster rate which create channels in the implant and provide space for body tissue to grow into the implant, while the higher density particles resorb more slowly, providing structural support for the construct as tissue restoration and repair occurs and releasing bioactive components over time as the higher density particles

resorb. The implant 74 can be compressed into any number of three dimensional shapes such as a kidney, spleen, nose, ear, liver, bone, or other body organ.

In another embodiment of the invention, a three dimensional construct of compressed particles of varying density is made to mimic natural biologic tissue densities. For example, in 5 one embodiment, a construct is made with denser particulate matter on one side, of a sheet for example, and more dense particulate matter is placed on the other side. Such a construct can be used at a bone-cartilage interface where the more dense portion is inserted into the bone defect while the less dense portion is positioned in the cartilage defect. The slower resorbing, 10 higher density particulate remodels according to the slower rate of bone generation resulting in more dense tissue restoration consistent with bone, while the less dense portion remodels more quickly and creates a less dense tissue restoration consistent with cartilage. The invention also contemplates three dimensional constructs of compressed particulate having 15 different density regions that are designed, for example, to mimic the native density variations of a spinal disc or the muscle-tendon interface.

15 In a further embodiment, as shown in FIG. 10, a sheet 80 is formed by compressing a mixture of lower density A particles 81 with higher density B particles 82. The sheet 80 can be formed, for example, by hydrating the particles and compressing the particles together by application of a mechanical force or vacuum to form a cake or wafer-like construct. The sheet 80 can be used to treat topical wounds, for example, of the skin, but can also be used to treat 20 other areas of the body such as a hernia, intestine, bowel, blood vessels, the heart, or any other organ, by cutting the sheet to size and/or bending or wrapping the sheet to fit the implant location. The sheet 80, with its differing density particles, will break down on implantation as a result of bioresorption of the lower density particles, providing greater surface area for tissue 25 restoration and repair at the site of implantation. The sheet form 82 has the benefit of being easy to apply to the implant site, but also has the ability to provide a prolonged restoration response at the implant site as the particles 81, 82 are resorbed at different rates, in contrast to sheets made of only one density of material.

30 In a further embodiment, as shown in FIG. 11, mixtures of particles 91 of differing densities, i.e., A particles of a first density 92 and B particles of a second density 93, are sandwiched between sheets 94 of an ECM. For example, a powder form mixture of particles

of a first density A 92 and a second density B 93 is sandwiched between sheets 94 of UBM or SIS to form a multi-laminate construct 95 of loose particles 91 and sheets 94. Any number of alternating layers of sheets 94 and the mixture 91 of particles 92, 93 can be constructed according to the invention. For example, in one embodiment, a first sheet 94A is covered with 5 the particle mixture 91; the particle mixture 91 is covered with a second sheet 94B; the second sheet 94B is covered with the particle mixture 91; and a third sheet 94C applied to form a sheet-particle-sheet-particle-sheet multi-laminate construct 95. The sheets of multi-laminate construct 95, when implanted at a site in a patient, provide a microarchitecture for tissue restoration at the site while the particles 92, 93 breaking down at varying rates provide a 10 continuous supply of bioactive factors to assist in restoration at the implant site.

In a further embodiment, particles of a lower density A and particles of a higher density B can be combined with a bone substitute material such as beta-tricalcium phosphate (β TCP), hydroxyapatite, sintered bovine bone, sintered porcine bone, demineralized allograft bone, or mineralized allograft bone, for example. In an alternative embodiment, the bone 15 particles and the differing density ECM particles of the invention can be mixed together without an adhesive, binder, or any other additive. The particles are coated on and/or injected into the bone substitute material. When the bone substitute material is implanted at the tissue site in the patient, the bone substitute material provides a rigid form to stabilize the defect while the lower density particles promote rapid tissue repair at the site. Over time, the higher 20 density particles sustain tissue repair at the site of implantation as they are bioresorbed at a slower rate than the lower density particles. The higher density particles are typically not fully bioresorbed until the tissue site is repaired.

According to another embodiment of the invention, a mixture of particles having particles of a lower density A and particles of a higher density B are provided in powder form. 25 A powder has particles of 1-1000 μ m. The powder can be sprinkled topically on a tissue site in a patient, for example, a cut, scrape, incision, or puncture of the skin, or while a tissue site is exposed during surgery, the powder form can be sprinkled at the tissue site. In another embodiment, the differing density particles are administered via inhalation, for example, through use of an inhaler such as those used to administered asthma treatments.

In an alternative embodiment, a mixture of particles of a lower density A and particles of a higher density B are maintained in a liquid, gel or paste medium. The liquid or gel is injected via a syringe at a tissue site in a patient. Alternatively, the liquid or gel is maintained within a tube and is squeezed out of the tube and applied to the tissue site. In a further 5 embodiment, the powder, liquid, gel, or paste composition includes particles of a third density.

In another aspect, the invention encompasses methods for modulating the rate of tissue restoration at a tissue site in a patient in need of repair or restoration. The method requires administering to a site in a patient a mixture that includes at least two types of particles, each 10 type having a different density, one type of particle being more dense than the other type of particle. The differing density particles are selected to form the mixture based on their respective biore sorption profiles, i.e., the higher density particles are selected because they take longer to be resorbed at the tissue site than the particles of a lower density and the lower density particles are selected because they will be resorbed quickly at the tissue site. 15 According to the invention, a physician particularly selects the mixture to have particles with particular rates of bioresorption tailored for the particular tissue site to be repaired or restored. The mixture is administered to the patient topically, via injection to the tissue site, or it may be delivered to the tissue site surgically, for example.

Tissue sites treatable with compositions according to the invention include, but are not 20 limited to head and neck structures, joints including synovium, joint capsule, labrum, intraarticular ligaments and cartilage, intervertebral discs, pelvic floor, tendon, bone, ligament, kidney, spleen, liver, muscle tissue, urinary bladder, ureter, uterus, intestine, pancreas, blood vessel, skin and skin adnexa, heart including intracardiac structures, nose, ear, breast, or a body cavity.

25

Examples

1. Tissue restoration of a chronic skin ulcer

A mixture of ECM particles is made by taking 60 mg of particles of UBM of size 50-1000 μm and having a density of approximately 0.05 g/cm^3 and 20 mg of particles of UBM of

size 50-500 μm and having a density of approximately 0.25 g/cm^3 , for a 3:1 ratio of particles based on weight. The mixture is applied topically to a chronic skin ulcer. Within 10 days, the 0.05 g/cm^3 particles are resorbed and tissue restoration is observed at the wound site. Within 30 days, the 0.25 g/cm^3 particles are resorbed and tissue at the wound site is fully restored.

2. Tissue restoration of a kidney

A mixture of ECM particles is prepared for implantation in a kidney at a site from which a tumor has been excised. A mixture of ECM particles is made by taking 200 mg of particles of UBM of size 500-1000 μm and having a density of approximately 0.10 g/cm^3 and 1000 mg of particles of UBM of size 50-500 μm and having a density of approximately 0.35 g/cm^3 , for a 1:5 ratio of particles based on weight. The mixture is surgically implanted in the patient via a syringe. Within 20 days, the 0.10 g/cm^3 particles have resorbed and tissue restoration is observed at the wound site via magnetic resonance imaging. Within 60 days, the 0.35 g/cm^3 particles are resorbed and tissue at the wound site is fully restored as viewed via magnetic resonance imaging.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A composition of bioresorbable particles comprising:
 - a first plurality of bioresorbable particles comprising a native extracellular matrix derived from a sheet of epithelial tissues comprising at least one epithelial layer consisting of epithelial basement membrane or submucosa and having a first density and a first rate of resorption upon implantation at a site in a patient, and,
 - a second plurality of bioresorbable particles comprising another extracellular matrix different than the extracellular matrix of the first plurality of bioresorbable particles, and having a second density and a second rate of resorption upon implantation at the site in the patient, wherein the second density of the second plurality of bioresorbable particles is at least 150% of the first density of the first plurality of bioresorbable particles, and the second rate of resorption is in the range of about 1 to about 10 times slower than the first rate of resorption.
- 15 2. A composition according to claim 1 or claim 2, wherein the second density is 150%-500% of the first density.
3. A method for preparing a mixture of bioresorbable particulate matter, comprising:
 - (a) providing a first sheet of bioresorbable material comprising a native extracellular matrix derived from a sheet of epithelial tissues comprising at least one epithelial layer consisting of epithelial basement membrane or submucosa and having a first density and a first rate of resorption, and milling said sheet into a first plurality of particles;
 - (b) providing a second sheet of a bioresorbable material comprising another extracellular matrix different than the extracellular matrix of the first sheet, or a non-extracellular matrix-material and having a second density and a second rate of resorption, and milling said sheet into a second plurality of particles;
 - (c) providing a predetermined quantity of said first plurality of particles and mixing said predetermined quantity with a predetermined quantity of said second plurality of particles, and mixing said predetermined quantity of said first plurality of bioresorbable particles with a predetermined quantity of said second plurality of bioresorbable particles.

4. A method according to claim 3, further comprising compressing or laminating the second sheet prior to milling to increase its density relative to the first sheet.

5. A method according to claim 3 or claim 4, further comprising lyophilizing the first sheet prior to milling to reduce its density relative to the second sheet.

5 6. A method according to any one of claims 3 to 5, wherein the second density of the second sheet of bioresorbable materials is 150%-500% of the first density of the first sheet of bioresorbable materials.

7. A method for preparing a mixture of bioresorbable particulate matter having differing densities comprising:

10 providing a sheet of bioresorbable material comprising a native extracellular matrix derived from a sheet of epithelial tissues comprising at least one epithelial layer consisting of epithelial basement membrane or submucosa and having a first density and compressing a portion of said sheet such that said compressed portion comprises a second density that is at least 150% of the first density of said sheet of bioresorbable material;

15 providing a second sheet of bioresorbable material comprising a native extracellular matrix;

joining said first sheet to said second sheet to form a multilaminate sheet; and

20 milling said multilaminae sheet into a plurality of particles, wherein said mixture of bioresorbable particles promotes tissue restoration and repair when implanted at a tissue site in a mammalian patient.

8. A method of modulating the rate of tissue repair at an anatomical site in a patient in need of repair or restoration, the method comprising:

25 administering at the anatomical site of the patient a mixture comprising (i) a first plurality of bioresorbable particles comprising a native extracellular matrix derived from a sheet of epithelial tissues comprising at least one epithelial layer consisting of epithelial basement membrane or submucosa and having a first density and (ii) a second plurality of bioresorbable particles comprising another extracellular matrix different than the first plurality of bioresorbable particles and having a second density at least 150% of the first density of the first plurality of bioresorbable particles,

wherein the second plurality of bioresorbable particles are resorbed by the patient's tissue at a slower rate than the absorption of the first plurality of bioresorbable particles, thereby prolonging the therapeutic effect of the second plurality of particles at the site and wherein said first plurality and second plurality of bioresorbable particles promote tissue 5 restoration and repair when implanted at the anatomical site in the patient.

9. A method according to claim 8, further comprising the steps of preparing the mixture of bioresorbable particles, comprising:

10 (a) determining the optimal rate of absorption of a resorbable composition for repair or restoration of the site in the patient;

(b) selecting the first plurality of bioresorbable particles to have a first density and said optimal rate of absorption based on step (a) on implantation at a site in the patient; and

(c) selecting the second plurality of bioresorbable particles to have a second density more dense than the density of the first plurality of particles and having a slower rate of absorption based on step (a) and step (b) than the rate of absorption of the first plurality of 15 bioresorbable particles on implantation at the site in the patient.

10. A method according to claim 8 or claim 9, wherein the bioresorbable particles are delivered to the site in the patient in a liquid or a gel medium.

11. A method according to any one of claims 8 to 10, wherein the bioresorbable particles are injected at the site in the patient, or wherein the mixture is administered 20 topically to the site.

12. A method of preparing a mixture of bioresorbable extracellular matrix particles, comprising:

25 preparing a plurality of bioresorbable particles of native extracellular matrix derived from a sheet of epithelial tissues comprising at least one epithelial layer consisting of epithelial basement membrane or submucosa each having the same density;

dividing the plurality of particles into a first group and a second group;

coating the second group of said plurality of particles with a coating comprising a material selected from the group consisting of an extracellular matrix, polyglycolic acid, hyaluronic acid, collagen, or combinations thereof, said coating having a second density;

mixing said first group of particles with said second group of particles to form a
5 mixture of bioresorbable particles, wherein said mixture of particles promotes tissue restoration and repair when implanted at a tissue site in a mammalian patient.

13. A method according to claim 7, wherein said sheet of bioresorbable material comprises non-cross-linked extracellular matrix, preferably said extracellular matrix is non-cross-linked, or preferably said extracellular matrix comprises UBM.

10 14. A method according to claim 3, wherein said first sheet of said second sheet comprises non-cross-linked extracellular matrix, and preferably wherein at least one of said extracellular matrix of said first sheet and extracellular matrix of said second sheet comprises UBM.

15. An implant for implanting at the site of an anatomical tissue in a human patient,
15 comprising:

first particles comprising a first native extracellular matrix derived from a sheet of epithelial tissues comprising at least one epithelial layer consisting of epithelial basement membrane or submucosa and comprising a first density;

20 second particles of a second native extracellular matrix and comprising a second density;

said first particles and said second particles compressed together to form a three dimensional object corresponding to the site of said anatomical tissue of said patient.

16. An implant according to claim 15, wherein the extracellular matrix of one of the first and the second particles is selected from the group consisting of UBS, SIS, and UBM

25 17. An implant according to claim 15 or claim 16, wherein the particles of the first density are more dense than the particles of the second density and wherein the particles of the first density form a core of the implant and the particles of the second density form the exterior surface of the implant.

18. An implant according to any one of claims 15 to 17, wherein the first particles are coated with a coating having a first density and the second particles are coated with a coating having a second density.
19. An implant according to any one of claims 15 to 18, wherein said first particles 5 comprise extracellular matrix component derived from a native tissue source that differs from the native tissue source of said second particles.
20. An implant according to claim 19, wherein said native tissue source of said first particles comprises urinary bladder, said first particles comprising the urinary bladder epithelial basement membrane.
- 10 21. An implant according to any one of claims 15 to 20, wherein said extracellular matrix of said second plurality of particles differs from said extracellular matrix of said first plurality of particles.
22. An implant according to any one of claims 15 to 21, wherein said first particles and 15 said second particles comprise non-cross-linked extracellular matrix, or wherein one of said first and second extracellular matrix comprises UBM.
23. A composition of extracellular matrix comprising:
 - a first sheet of a native extracellular matrix derived from a sheet of epithelial tissues comprising at least one epithelial layer consisting of epithelial basement membrane or submucosa;
 - 20 a second sheet of a native extracellular matrix; and
 - a first plurality of extracellular matrix particles having a first density and a second plurality of extracellular matrix particles having a second density at least 150% of the first density, wherein the first plurality of particles and the second plurality of particles are contained between the first sheet and the second sheet of extracellular matrix.
- 25 24. Use of (i) a first plurality of bioresorbable particles comprising a native extracellular matrix derived from a sheet of epithelial tissues comprising at least one epithelial layer consisting of epithelial basement membrane or submucosa and having a first density and (ii) a second plurality of bioresorbable particles comprising another extracellular matrix different than the first plurality of bioresorbable particles and having a 30 second density at least 150% of the first density of the first plurality of bioresorbable

particles in the manufacture of a medicament for modulating the rate of tissue repair at an anatomical site in a patient in need of repair or restoration, wherein the second plurality of bioresorbable particles are resorbed by the patient's tissue at a slower rate than the absorption of the first plurality of bioresorbable particles, thereby prolonging the

5 therapeutic effect of the second plurality of particles at the site and wherein said first plurality and second plurality of bioresorbable particles promote tissue restoration and repair when implanted at the anatomical site in the patient.

25. A composition of matter comprising:

10 a first plurality of bioresorbable particles comprising a native extracellular matrix and having a first density and a first rate of resorption upon implantation at a site in a patient, and,

15 a second plurality of particles having a second density and a second rate of resorption upon implantation at the site in the patient, wherein the second density of the second plurality of particles is at least 150% of the first density of the first plurality of bioresorbable particles, and the first rate of resorption is at least 1.5 times faster than that of the second rate of resorption.

26. A composition according to claim 25 wherein the second plurality of particles 20 comprises a bioresorbable material that differs from the first plurality of bioresorbable particles, and preferably wherein the second plurality of particles is selected from the group consisting of extracellular matrix, collagen, a bioresorbable polymer, and hyaluronic acid.

25 27. A composition according to claim 25, wherein the second density is 150%-500% of the first density.

28. A method for preparing a mixture of bioresorbable particulate matter, comprising:

30 (a) providing a first sheet of bioresorbable material comprising a devitalized native extracellular matrix having a first density and a first rate of resorption, and milling said sheet into a first plurality of particles;

(b) providing a second sheet of a bioresorbable material having a second density and a second rate of resorption, and milling said sheet into a second plurality of particles;

(c) providing a predetermined quantity of said first plurality of particles and a predetermined quantity of said second plurality of particles, and mixing said predetermined quantity of said first plurality of bioresorbable particles with a predetermined quantity of said second plurality of bioresorbable particles.

5

29. A method according to claim 28, further comprising compressing or laminating the second sheet prior to milling to increase its density relative to the first sheet.

10 30. A method according to claim 28 further comprising lyophilizing the first sheet prior to milling to reduce its density relative to the second sheet.

31. A method according to claim 28, wherein the first sheet and the second sheet comprise extracellular matrix.

15 32. A method according to claim 28, wherein the second density of the second sheet of bioresorbable materials is 150%-500% of the first density of the first sheet of bioresorbable materials.

20 33. A method for preparing a mixture of bioresorbable particulate matter having differing densities comprising:

providing a sheet of bioresorbable material comprising a devitalized native extracellular matrix and having a first density and compressing a portion of said sheet such that said compressed portion comprises a second density that is at least 150% of the first density of said sheet of bioresorbable material; and

25 milling said sheet into a plurality of particles, wherein said plurality of bioresorbable particles promote tissue restoration and repair when implanted at a tissue site in a mammalian patient.

30 34. A method of modulating the rate of tissue repair at an anatomical site in a patient in need of repair or restoration, the method comprising:

35 (i) administering at the anatomical site of the patient a mixture comprising (i) a first plurality of bioresorbable particles comprising a native extracellular matrix having a first density and a (ii) second plurality of bioresorbable particles having a second density at least 150% of the first density of the first plurality of bioresorbable particles, wherein the anatomical site is

selected from the group consisting of tendon, ligament, bone, kidney, liver, spleen, lymph node, urinary bladder, uterus, blood vessel, skin, skin adnexa, breast, heart, intracardiac anatomical structures, nose, ear, body cavity, intervertebral discs, synovium, joint capsule, labrum, intraarticular ligaments and cartilage, pelvic floor, muscle tissue, ureter, intestine, pancreas, and eye,

5 wherein the second plurality of bioresorbable particles are resorbed by the patient's tissue at a slower rate than the absorption of the first plurality of bioresorbable particles, thereby prolonging the therapeutic effect of the second plurality of particles at the site and
10 wherein said first plurality and second plurality of bioresorbable particles promote tissue restoration and repair when implanted at the anatomical site in a mammalian patient.

35. A method according to claim 34 further comprising the steps of preparing the mixture of bioresorbable particles, comprising:

15 (a) determining the optimal rate of absorption of a resorbable composition for repair or restoration of the site in the patient;
(b) selecting the first plurality of bioresorbable particles to have a first density and said optimal rate of absorption based on step (a) on implantation at a site in the patient; and
20 (c) selecting the second plurality of bioresorbable particles to have a second density more dense than the density of the first plurality of particles and having a slower rate of absorption based on step (a) and step (b) than the rate of absorption of the first plurality of bioresorbable particles on implantation at the site in the patient.

36. A method according to claim 34, wherein the bioresorbable particles are delivered
25 to the site in the patient in a liquid or a gel medium.

37. A method according to claim 34, wherein the bioresorbable particles are injected at the site in the patient, or wherein the mixture is administered topically to the site.

30 38. A composition according to claim 25 wherein said particles are compressed together to form a three dimensional object.

39. A composition according to claim 25, wherein the extracellular matrix of one of the first and the second particles is selected from the group consisting of UBS, SIS, and UBM.

40. A composition according to claim 25, wherein the particles of the second density are more dense than the particles of the first density and wherein the particles of the first density form a core of the composition and the particles of the second density form the exterior surface of the composition.

41. A method of preparing a mixture of bioresorbable extracellular matrix particles, comprising:

preparing a plurality of bioresorbable particles comprising devitalized native extracellular matrix each having the same density;

dividing the plurality of particles into a first group and a second group;

coating the first group of said plurality of particles with a coating comprising a material selected from the group consisting of an extracellular matrix, polyglycolic acid, hyaluronic acid, or combinations thereof, said coating having a first density;

coating the second group of said plurality of particles with a coating comprising a material selected from the group consisting of an extracellular matrix, polyglycolic acid, hyaluronic acid, or combinations thereof, said coating having a second density;

mixing said first group of particles with said second group of particles to form said mixture of bioresorbable particles, wherein said mixture of bioresorbable particles promotes tissue restoration and repair when implanted at a tissue site in a mammalian patient.

42. A composition according to claim 25 further comprising:

a first sheet of an extracellular matrix; and

a second sheet of an extracellular matrix,

wherein the first plurality of particles and the second plurality of particles are contained between the first sheet and the second sheet of extracellular matrix.

43. A method according to claim 34 further comprises coating a medical device with said particle mixture, and, said administering said particle mixture in step (i) comprises introducing said mixture-coated medical device to said anatomical site in the patient.

44. A method according to claim 43 wherein said medical device is selected from the group consisting of a stent, a cardiac occlude, suture material and a mesh; or wherein said device comprises a synthetic device or a natural material device; wherein said coating is selected from the group consisting of a liquid medium, a gel medium, and a paste medium; or wherein said mixture of particles further comprises a bone-substitute material selected from the group consisting of beta-tricalcium phosphate, hydroxyapatite, sintered bone, demineralized allograft bone, and mineralized allograft bone.

45. A method according to claim 43 further comprising adhering said mixture to said medical device by the introduction of a chemical bonding agent, a peptide linker, or an adhesive, or by lyophilizing or compressing said mixture onto said medical device.

46. A method according to claim 43 wherein the second plurality of particles is selected from the group consisting of extracellular matrix, collagen, a bioresorbable polymer, and hyaluronic acid.

47. A method according to claim 25 wherein the second density is 150%-500% of the first density.

48. A method according to claim 34 wherein the bioresorbable particles are administered to said site in a powder form; or wherein the bioresorbable particles are compressed together and administered to said site as a three-dimensional object; or wherein said bioresorbable particles further comprise a bioactive factor; or wherein said bioresorbable particles further comprise an agent selected from the group consisting of nucleic acids and a pharmaceutical.

49. A method according to claim 34 wherein said mixture of bioresorbable particles comprise said first plurality of bioresorbable particles coated with said second plurality of bioresorbable particles.

50. A composition, method, implant or use according to any one of claims 1 to 49 substantially as hereinbefore described.

1/9

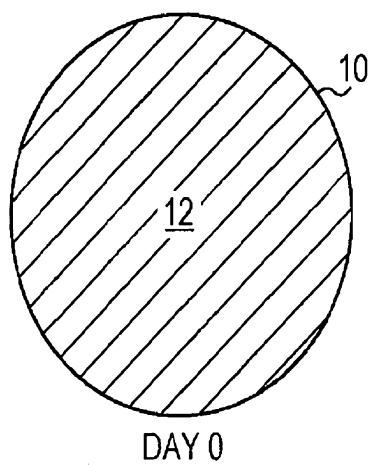


FIG. 1A

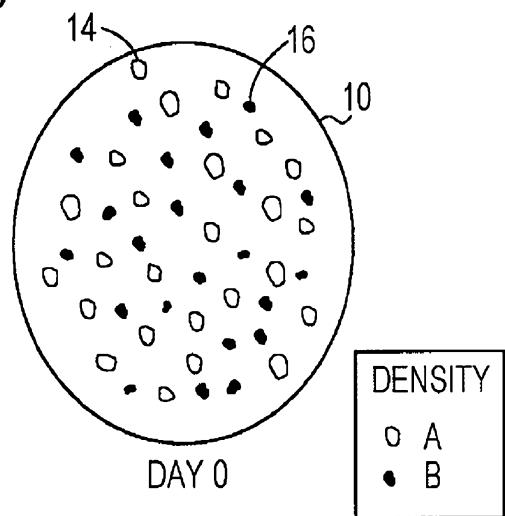


FIG. 2A

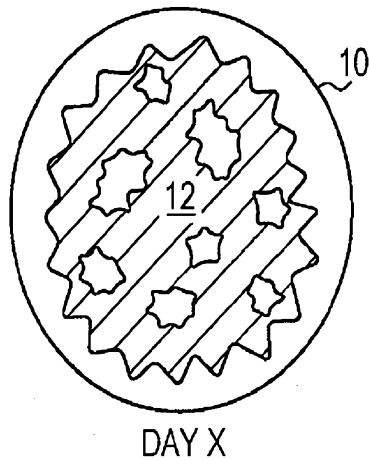


FIG. 1B

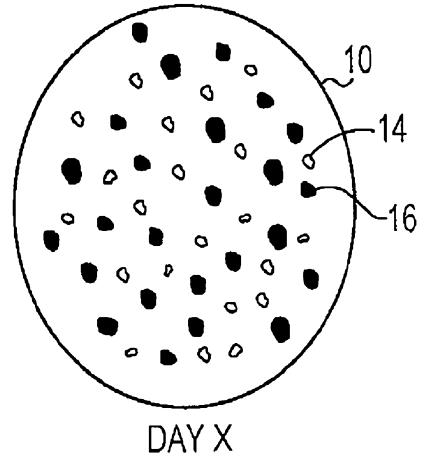


FIG. 2B

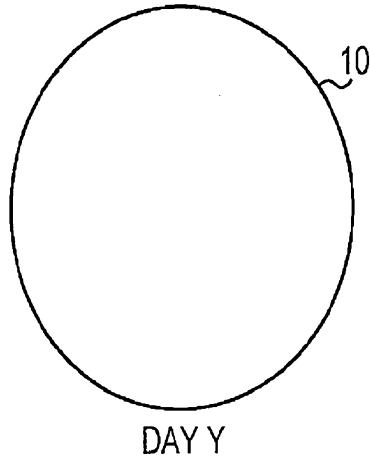


FIG. 1C

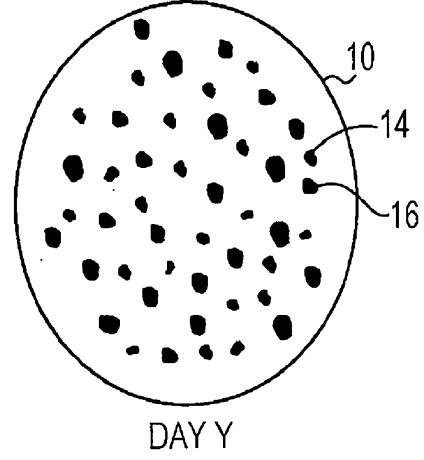


FIG. 2C

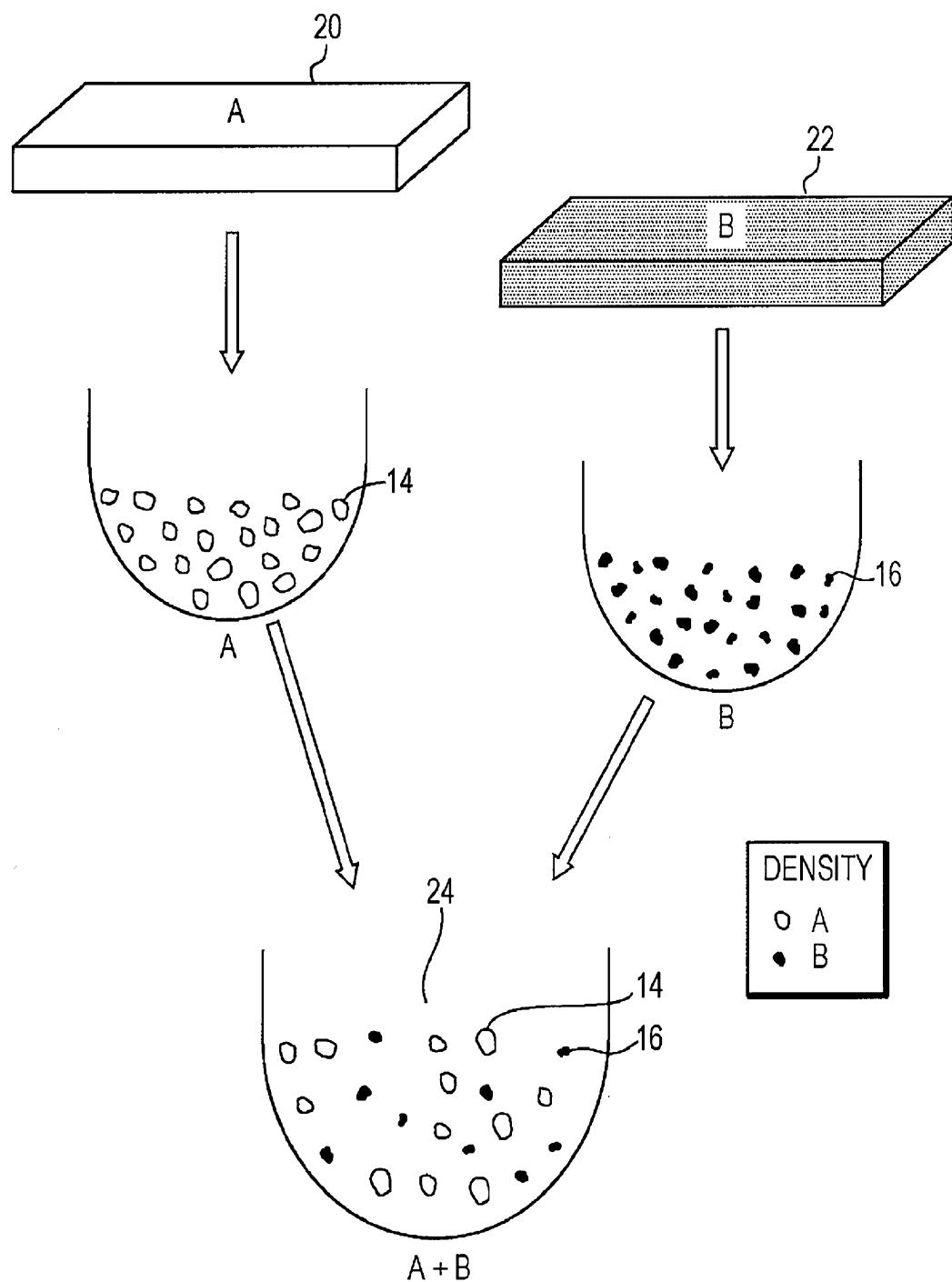


FIG. 3

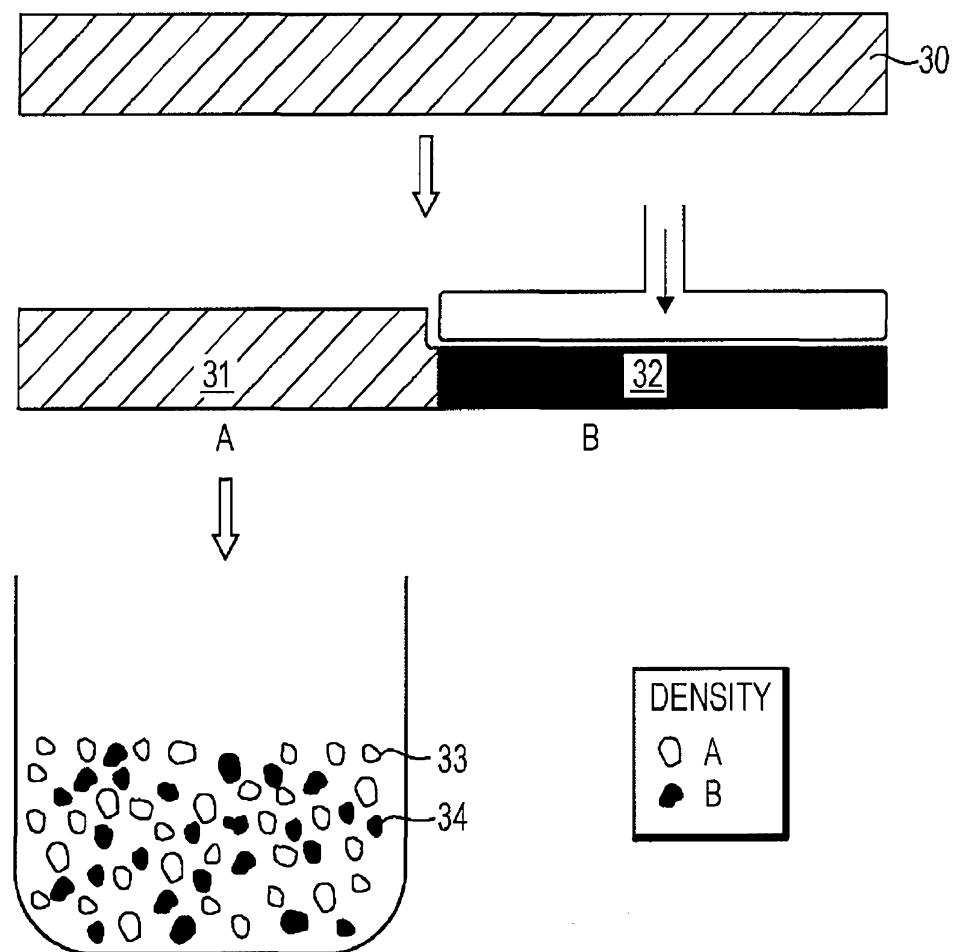


FIG. 4

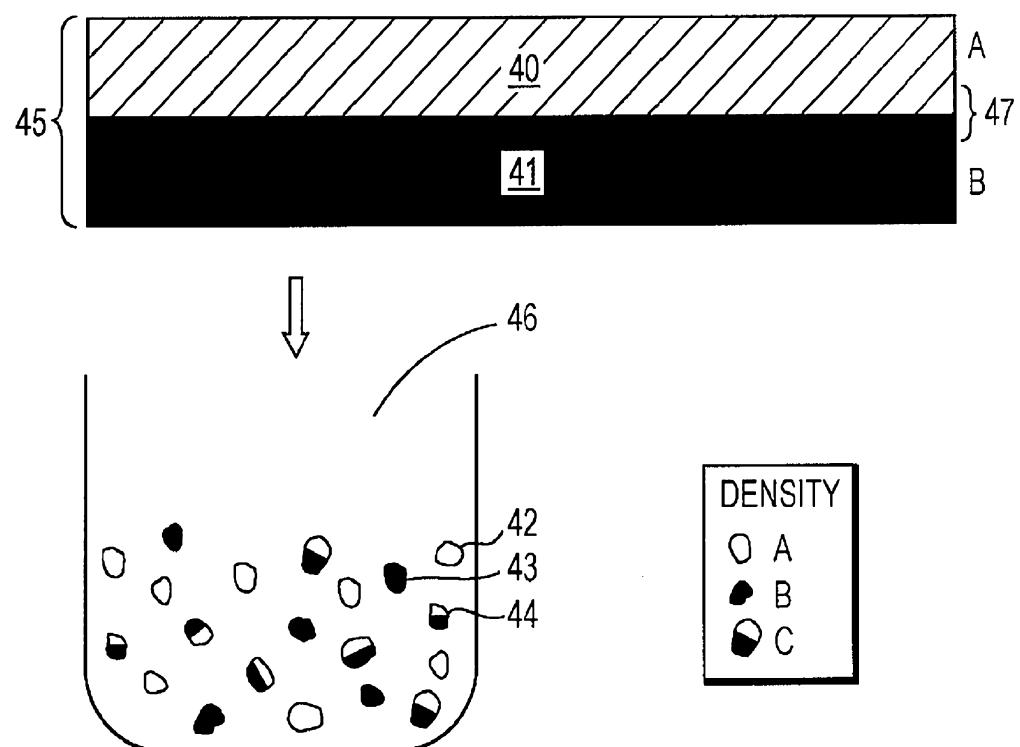


FIG. 5

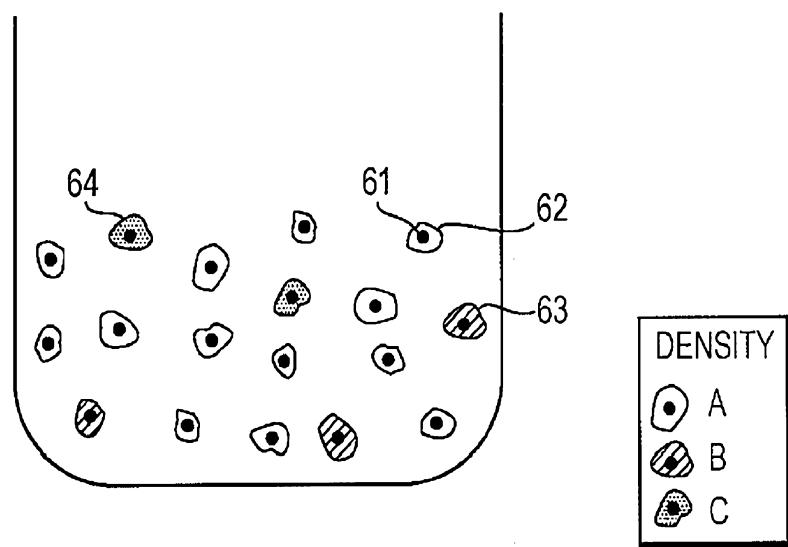


FIG. 6

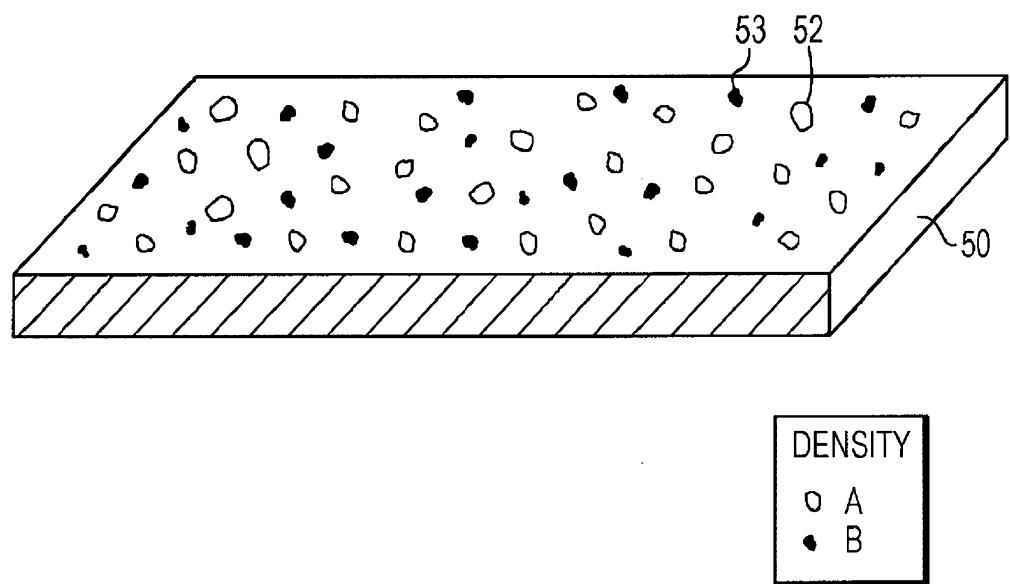


FIG. 7

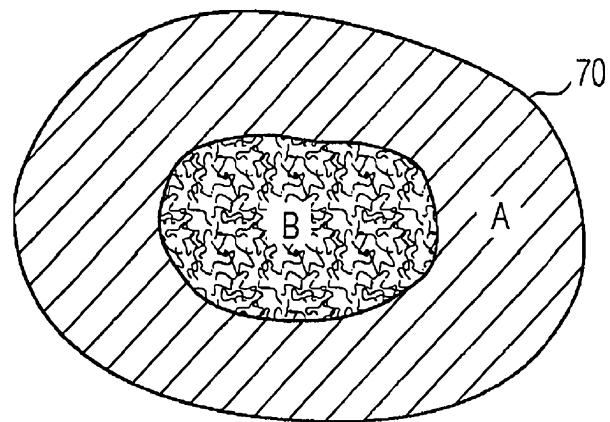


FIG. 8A

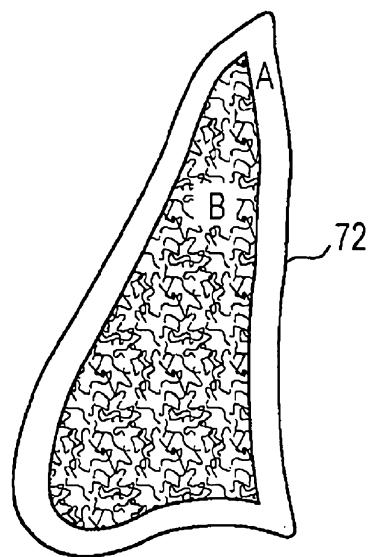


FIG. 8B

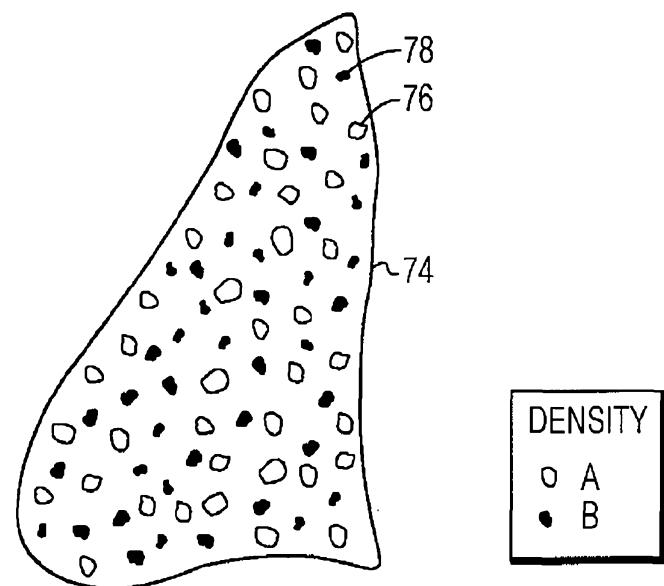


FIG. 9

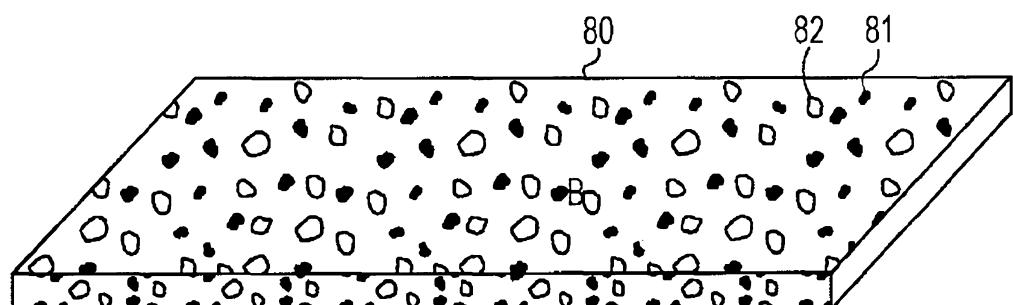
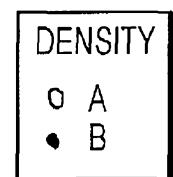


FIG. 10



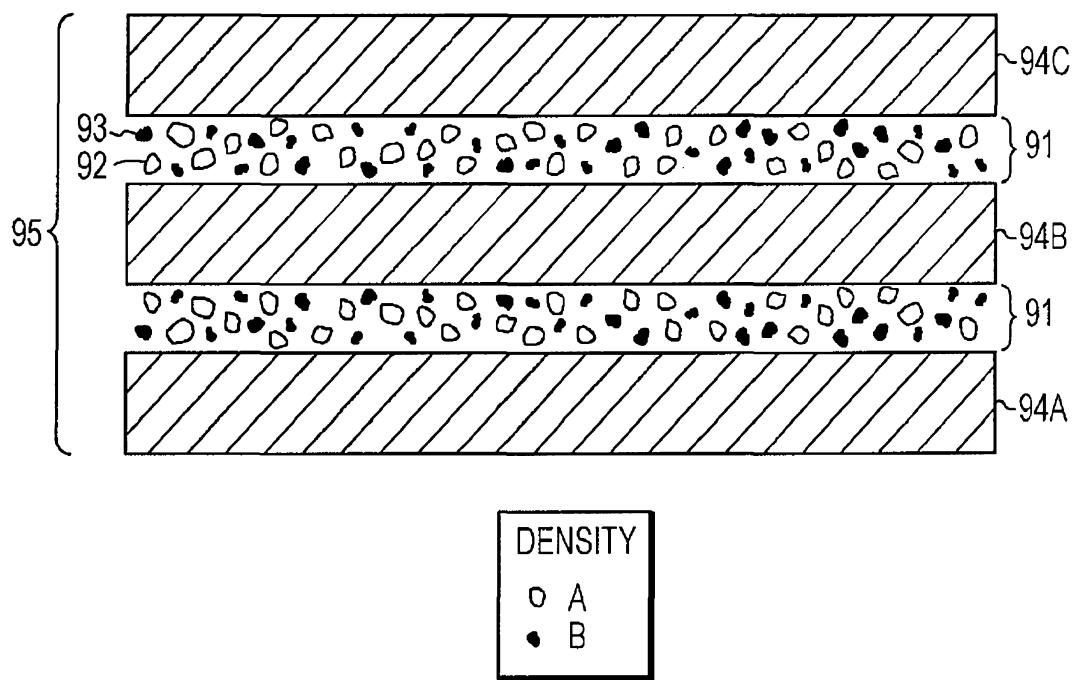


FIG. 11