

BONE GROWTH COMPOSITION

FIELD OF THE INVENTION

[0001] This invention disclosure relates generally to the field of bone growth compositions, methods of making of such compositions, and methods for treating bone defects in a subject using such compositions. Disclosed herein are heretofore unknown bone growth compositions for use as a bone graft, bone implant or bone filler having applications in treating a subject having a bone defect in a subject.

BACKGROUND

[0002] Bone is a composite material comprised of collagen, cells, a form of calcium hydroxyapatite crystals and small quantities of other proteins and organic molecules. The chemistry and physical nature of this composite affords it unique properties of high strength, rigidity, and an ability to adapt to changing loads in the body. However, when injuries to bone occur it is necessary to find a way to fill voids or gaps, and to encourage the repair and regeneration of the bone tissue.

[0003] Bone injury due to trauma or diseases requires surgical intervention to replace the diseased area of bone or temporarily fill voids in the bone structure that are to be replaced by the ingrowth of a new bone tissue.

[0004] There are several examples of surgical procedures related to orthopedic surgery, in which the insertion of bone filler material into the damaged bone extremities, pelvis or spine has generally functioned by providing a bioresorbable substitute for bone tissue to grow onto/into during the healing process. In maxillofacial surgery, implants are utilized to reconstruct damaged tissue of the face. In dentistry, the implants are mainly utilized to create a stable bone within the maxillary or mandibular bone for metal element implantation to which prostheses will then be affixed. In aesthetic surgery, implants are mostly utilized for reconstructing defects, including congenital defects.

[0005] Products which consist of or contain hydroxyapatite (HA) ($\text{Ca}_5(\text{PO}_4)_3\text{OH}$) and/or the crystalline form β of tricalcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$) have been historically known and used for the preparation of scaffolds useful for the bone tissue regeneration.

[0006] For the production of scaffolds for bone regrowth, HA is therefore used in a mixture with a second mineral component, the crystalline form β of tricalcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$), commonly known as β -tricalcium phosphate or by its abbreviation β -TCP. β -

TCP has a faster resorption than HA, and therefore promotes the regrowth of the bone tissue.

[0007] A variety of biocompatible and biodegradable materials consisting of HA/ β -TCP for use in orthopedic surgery, are known.

[0008]

[0009] The product OPTEMX by Exactech (Gainesville, Fla., USA) consists of porous HA/ β -TCP granular composition only and has limited osteoconductive properties when admixed with autologous bone marrow aspirate or autologous blood.

[0010] The product MASTERGRAFT by Medtronic (Minneapolis, Minn., USA), available in granular form of the HA/ β -TCP composition only, has osteoconduction properties.

[0011] However, these materials are granular in their form with limited moldability and flexibility. Thus, they are poorly suitable for use in bone lumens or surfaces which are irregular in shape.

[0012] Despite the beneficial performance features of HA/ β -TCP and other osteoconductive materials, a granular form factor presents challenges from a clinical use perspective. If not combined with a handling agent to retain the material in a defined location, ceramic granules are prone to migration from the surgical site (known as "spitting" from the defect). Historically, polymeric materials or animal-derived collagen biomaterials have been used in combination with osteoconductive granules to address this problem. Polymeric/animal derived collagen and ceramic constructs create a three-dimensional framework to retain granules and create additional porosity between granules. Numerous devices possessing similar design and performance characteristics have been developed. Several products are known on the market which consist of HA and/or β -TCP in a bovine collagen matrix.

[0013] The product MASTERGRAFT Putty by Medtronic, having osteoconductive properties, is formed by the same granules as the product MASTERGRAFT mentioned above, evenly distributed in a bovine collagen matrix.

[0014] The product Integra MOZAIK by Integra Life Sciences Corporation (Plainsboro, N.J., USA), with osteoconductive properties, consists of a mixture containing approximately 80% β -TCP and 20% crosslinked bovine collagen.

[0015] The product VITOSS Scaffold Foam Bone Graft Material is composed of approximately 80% β -TCP and 20% bovine crosslinked collagen.

[0016] The product COLLAGRAFT Bone Graft Matrix by Zimmer (Warsaw, Ind., USA) is commercialized in the form of strips made of HA/ β -TCP in a crosslinked bovine collagen matrix.

- [0017]** The product HEALOS by DePuy (Warsaw, IN., USA) consists of a matrix of bovine cross-linked collagen fibers coated with HA. This product is combined with autologous bone marrow aspirate or autologous blood.
- [0018]** The U.S. Pat. No. 6,764,517 and U.S. Pat. No. 6,902,584 describe a 3-dimensional mineralized collagen composite produced by creating a collagen slurry, freezing and lyophilizing the mixture and then subjecting it to calcium and phosphate solutions to form a porous mineralized matrix.
- [0019]** These patents describe adding a soluble collagen in an additional step and lyophilizing that mixture to form the porous composite. The inventions further describe the ability to use various cross-linking agents to enhance physical stability and increase implant resorption time and shape retention. While this technology can produce an improvement over the previous technologies, the manufacturing process consists of many different steps which become costly and very time consuming.
- [0020]** Further refinements of these general methods for producing collagen-calcium phosphate composite materials can be found in U.S. Pat. No. 7,156,880 and U.S. Pat. No. 7,166,133. These inventions describe the manufacture of implants that consist of an osteoconductive matrix that comprises a blend of both insoluble and soluble collagen where at least a portion of the implant is porous. In addition, these structures may contain osteoinductive molecules as well as biodegradable synthetic polymers. The inventions also describe the incorporation of ceramic materials such as calcium phosphate, calcium sulfate or hydroxyapatite in the form of discrete particles, rather than forming the compounds through precipitation of salts.
- [0021]** More recent technologies such as those found in U.S. Pat. No. 7,531,004 and U.S. Pat. No. 7,534,451 describe a bone restorative composite material that consists of a resorbable polymer that can be collagen, a range of meso, micro and macro porosity to allow for the inclusion of fluid and to assist in bone ingrowth, as well as the inclusion of calcium phosphate particles. The inventions further utilize a specific oxidation-reduction reaction of calcium and phosphorous containing salts to precipitate calcium phosphate within the collagen structure. These devices typically require very precise control of the chemistry to obtain the desired results of the precipitation of the calcium phosphate materials and appear to be limited to calcium based osteoconductive materials.
- [0022]** Patent application WO 2011/064724 A1 describes mono-, bi- or multi-layer biomimetic materials for use in orthopedic surgery, and the process for their production. The basic material described in this application is a bicomponent material made of the natural polymer bovine collagen added with chitosan (a polymer obtained by basic

deacetylation of chitin, the natural component of exoskeleton of crustaceans). The mono-layer biomimetic materials of this application are made of collagen-chitosan only. This bicomponent material contains between 30 and 90% (preferably 50-80%) by weight of collagen, the remainder being chitosan. The role of chitosan is said to be for favoring the fibrillation of the bicomponent material.

[0023] While bovine collagen-based materials have a long clinical history in a number of medical devices hypersensitivity reactions and immunological responses remains a recognized limitation. The processing steps involved in producing any collagen based medical device can have a significant influence on the in vivo response in terms of tissue infiltration and local inflammatory response and degradation of the collagen material (Chvapil M 1977; Weadock et al., 1983; Bailey AJ 2000).

[0024] What is needed is a bone growth composition having applications as a bone graft, bone filler, or bone implant that does not suffer from the problems and limitations of heretofore materials.

[0025] The citation of any reference herein should not be deemed as an admission that such reference is available as prior art to the instant disclosure.

SUMMARY OF THE DISCLOSURE

[0001] The present disclosure is based upon the discovery that, surprisingly and unexpectedly, a novel bone growth composition of the instant disclosure readily has applications for treating a bone defect in subject that exhibits an efficacious result when used to treat a bone defect in a subject, does not suffer from the shortcomings of heretofore known compositions for use in such treatments. Broadly, the present disclosure extends to a bone growth composition that comprises decellularized placenta derived extracellular matrix substantially free of residual material that comprises a natural biocompatible substrate, and source of Ca^{2+} and PO_4^{3-} ions, such as a mineral source. Examples of such bone defects that can be treated with a bone growth composition of the instant disclosure include but certainly are not limited to an injury from trauma, a congenital or acquired bone defect or anomaly, resection of bone due to surgery, a sequelae of bone infection; as a result of a fracture, e.g. a comminuted fracture, accompanied by bone mass loss, to name only a few. Moreover, the form of a bone growth composition of the instant disclosure can vary depending upon the bone defect it is being used to treat. Thus, a bone growth composition of the instant invention can be a bone implant, a bone graft, or a bone filler. Moreover, a bone growth composition as disclosed herein can be porous, osteoconductive, or porous and osteoconductive

[0002] Numerous natural biocompatible substrates have applications in a bone growth composition of the instant disclosure, including but not limited to a collagen, elastin, tropoelastin, an elastin-like protein, or any combination these biocompatible substrates. In a particular embodiment of the instant disclosure, the natural biocompatible substrate comprises a combination of a collagen and elastin, such the amount of collagen of the decellularized placenta derived extracellular matrix substantially free of residual material comprises at least about 60% by weight collagen, and no greater than about 20% by weight elastin. The type of collagen having applications in a bone growth composition of the instant disclosure can vary. In particular, the collagen can be Type I collagen, Type III collagen, Type V collagen, Type VI collagen, Type VII collagen, or any combination of these collagens. Type I Collagen is the most abundant collagen type in bone tissue, constituting about 90% of the total collagen content in bone. Type I collagen provides structural support and strength to bones; it also serves as a scaffold for cell attachment, proliferation, and differentiation during the bone healing process. Type III Collagen, although less abundant in bone compared to type I collagen, type III collagen plays a role in bone regeneration. It is often found in association with type I collagen, contributing to the formation of the collagen matrix. Type III collagen is involved in the early stages of tissue repair and remodeling. Collagen types I and III are the most suitable for bone regeneration due to their abundance in bone tissue and their roles in providing structural integrity and supporting cellular processes essential for bone healing and regeneration. There are also minor types of collagen: Type V collagen is a minor component of extracellular matrix but is still present in bone tissue. It is often found in association with type I collagen fibrils and is believed to play a regulatory role in collagen fibrillogenesis and organization. While type V collagen may not be as predominant as types I and III in bone tissue, it could potentially influence the mechanical properties and organization of the extracellular matrix, thereby indirectly impacting bone regeneration processes. Type VI Collagen is found in bone; it forms distinct microfibrillar structures within the ECM and contributes to tissue organization and integrity. Type VII collagen is also a minor collagen that may play a role in the attachment of bone cells to the underlying matrix or basement membrane during bone development and repair processes.

[0026] Synthetic biocompatible substrates also have applications in a bone growth composition of the instant disclosure. Examples of such synthetic biocompatible substrates includes a polylactic acid (PLA), a poly(lactic-co-glycolic acid) PLGA, a biodegradable polyester amide polymer, a hydrogel, a polyether ketone, or any combination of these synthetic biocompatible substrates, to name only a few.

- [0003]** Moreover, in a bone growth composition of the instant disclosure, the source of Ca^{2+} and PO_4^{3-} ions can vary. For example, the mineral source for these ions can be minerals such $\text{Ca}(\text{H}_2\text{PO}_4)_2$, CaHPO_4 , $\text{Ca}_3(\text{PO}_4)_2$ (β -TCP), $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$, or any combination of these minerals. In a particular embodiment, the mineral source is in particle form, e.g., a particulate mineral. Moreover, the size of the particles of the mineral source, and the range of the size of the particles having applications in a bone growth composition of the instant disclosure can vary. For example, the range of the particle size of mineral source particles in a bone growth composition of the instant disclosure can range from about 250 μm to about 3000 μm , about 250 μm to about 2000 μm , about 250 μm to about 1000 μm ; or about 1000 μm to about 2000 μm .
- [0004]** The mass ratio by weight of the mineral source to the decellularized placenta derived extracellular matrix substantially free of residual material in a bone growth composition of the instant disclosure can vary. For example, this mass ratio can be about 5% to about 95%, about 10% to about 90%, about 15% to about 85%, about 20% to about 80%, about 15% to about 85%, or about 30% to about 70%, (ECM:mineral source).
- [0005]** Moreover, a bone growth composition of the instant disclosure can further comprise a biological material, such as, for example, cells, conditioned cell medium, cell secretome, plasma, genetic material, e.g. nucleic acid molecules (e.g., siRNA and mRNA) and nucleotides, exosomes, bone marrow aspirate, platelet plasma, growth factors or any combination of such biological material. Examples of cells having applications herein include but certainly are not limited to mesenchymal stem cells, adipose tissue derived stem cells, osteoblasts, placental derived cells. In particular embodiments, conditioned medium and exosomes are produced by the cells discussed above.
- [0006]** The instant disclosure further extends to a method for treating a bone defect in a subject, which comprises administering to the subject at the point of the bone defect an effective amount of a bone growth composition of the instant disclosure.
- [0007]** Also provided herein is a kit that comprises a bone growth composition of the instant disclosure and packaging container specifically designed to allow for different product sizes and the addition of rehydration fluids.
- [0008]** Optionally, a kit of the instant disclosure can further comprise biological material, such as, for example cells, conditioned cell medium, cell secretome, plasma, genetic material, e.g. nucleic acid molecules (e.g., siRNA and mRNA) and nucleotides, exosomes, bone marrow aspirate, platelet plasma, growth factors or any combination of such biological material. Examples of cells having applications herein include but certainly are not limited to mesenchymal stem cells, adipose tissue derived stem cells, osteoblasts,

placental derived cells. In particular embodiments, conditioned medium and exosomes are produced by the cells discussed above. The biological material can be housed in a container that is included in the kit, or alternatively, the packaging container is designed to provide a sealed chamber that houses the biological material.

[0009] These and other aspects of the present disclosure will be better appreciated by reference to the following drawings and Detailed Description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] FIG. 1 is an SDA-PAGE analysis decellularized placenta derived extracellular matrix of a bone growth composition of the instant disclosure, wherein the biocompatible substrate comprises collagen, wherein MW refers to molecular weight, kDa refers to kilodaltons, COL I refers to Type I collagen, COL III refers to Type III collagen, COL IV refers to Type IV collagen, COL V refers to Type V collagen, and COL VI refers to Type VI collagen.

[0011] FIG. 2 illustrates several forms of a lyophilized bone growth composition of the instant disclosure in cuboidal configurations with the mineral source in particulate form, wherein the size of the particles of the mineral source range from about 250 μm to about 2000 μm distributed throughout the bone growth composition and the ratios of decellularized placenta derived extracellular matrix substantially free of residual material and β -TCP includes 10/90%, 15/85% and 20/80%.

[0012] FIG. 3 illustrates a form of a bone growth composition of the instant disclosure in cylindrical form having particulate mineral source with a particle size range of about 250-about 500 μm distributed throughout the bone growth composition of the instant disclosure.

[0013] FIG. 4 are top and size views of a form of a bone growth composition of the instant disclosure in cylindrical form having particulate mineral source with particle size range of about 1000-about 220 μm distributed throughout the bone growth composition of the instant disclosure.

[0014] FIG. 5 is a bone growth composition of the instant disclosure in a strip configuration containing about 95% by weight mineral source in particulate form with the range of the size of the particles of about 2050- about 1000 μm and about 5% by weight a decellularized placenta derived extracellular matrix substantially free of residual material.

[0015] FIG. 6 illustrates crosslinking of decellularized placenta derived extracellular matrix substantially free of residual material with thermal dehydration treatment of a bone growth composition of the instant disclosure.

[0016] FIG. 7A, B, and C illustrate the moldability and plasticity of a bone growth composition of the instant disclosure in which the mass ratio of mineral source to

decellularized placenta derived extracellular matrix substantially free of residual materials about 20%: about 80%. Sterile water was added to a bone growth composition as disclosed herein with a mass ratio of decellularized placenta derived extracellular matrix substantially free of residual material to β -TCP is about 20:80% (FIG. 7A). The rehydrated implant was molded into a desired ball shape as depicted in FIGs. 7B and 7C

[0017] FIGs. 8A, 8B and 8C illustrate a bone growth composition of the instant disclosure in which the mass ratio of decellularized placenta derived extracellular matrix substantially free of residual material to β -TCP is about 20: about 80%, rehydrated with blood.

[0018] FIGs. 9A, 9B, 9C, and 9D illustrate rehydration and handling of a bone growth composition of the instant disclosure having a mass ratio of decellularized placenta derived extracellular matrix substantially free of residual material to β -TCP is about 20:80% as compared to β -TCP alone. FIG. 8A shows a bone growth composition as described herein rehydrating in saline for 15 minutes and remained intact with beta-TCP particles retained therein. and FIG. 8B shows the rehydrated of FIG. 8A bone growth composition molded into a desired shape. FIGs 9C and 9D shows beta-TCP alone soaked in saline for 15 minutes. The well after transfer of the beta-TCP shows residual beta-TCP and FIG. 9D shows an inability to mold rehydrated beta-TCP alone.

[0019] FIGs. 10A and 10B illustrate the morphological analysis of a bone growth composition of the instant disclosure having a mass ratio of decellularized placenta derived extracellular matrix substantially free of residual material to β -TCP is about 20: about 80% as compared to β -TCP alone, wherein the range of the particle size of the β -TCP is (left) about 250 to about 2000 μ m and (right) about 1000- about 2000 μ m. FIG. 10 shows radio-opaque beta-TCP particles (white) and porous ECM (black surrounding the beta-TCP particles). Micro Computed Tomography (Micro-CT) of the beta-TCP granules show they were uniformly surrounded by porous placental structure ECM as described herein.

[0020] FIG. 11 illustrates XRD spectrum and overlay analysis of a bone growth composition of the instant disclosure in which the mass ratio of decellularized placenta derived extracellular matrix substantially free of residual material to β -TCP is about 20:about 80%. The correct spectra were observed to be indicative of β -TCP, and the β -TCP components are fully characterized, and no additional phases or impurities were detected.

[0021] FIG. 12 illustrates FTIR spectrum and stability analysis for a placenta derived ECM substantially free of residual material that is the natural biocompatible material of a bone growth composition of the instant disclosure. FIG. 12(A) illustrates collagen standards and decellularized ECM. FIG. 12(B) illustrates the stability profile of a bone growth composition of the instant disclosure over 115 days at ambient temperature. FIG. 12(C) is

the FTIR profile for a bone growth composition of the instant disclosure. FIG. 12D is the FTIR spectra showing stability of a bone growth composition of the instant disclosure at 40° C for 10 days.

- [0022]** FIG. 13 shows a bone growth composition of the instant disclosure in the form of bone implants in packaging trays accommodating 10 cc, 4 cc and 2.5 cc and providing a system for adding an appropriate rehydration agent in a clinical setting (kits). FIG. 13A is a double tray prototype with retainer; FIG. 13B shows a double tray prototype with a 10 cc bone growth composition of the instant disclosure; FIG. 13C shows a double tray prototype with 5 cc bone growth composition of the instant disclosure and retainer; and FIG. 13D shows a double tray prototype with a 2.5 cc bone growth composition of the instant disclosure with retainer.
- [0023]** FIG. 14 illustrates a bone growth composition of the instant disclosure post admixed with β -TCP dispersed therein.
- [0024]** FIG. 15 illustrates a bone growth composition of the instant disclosure alone at 12 weeks.
- [0025]** FIG. 16 illustrates the autograft at 12 weeks.
- [0026]** FIG. 17 illustrates Test Group 1 (FUSE 1) at 12 weeks.
- [0027]** FIG. 18 illustrates Test Group 2 (FUSE 2) at 12 weeks.
- [0028]** FIG. 19 illustrates bone marrow aspirate generation and application of bone marrow aspirate and a bone growth composition of the instant disclosure in the form of a bone implant.
- [0029]** FIG. 20 illustrates rehydration and placement of a bone growth composition of the instant disclosure in the form of a bone implant in a critical size bone defect.
- [0030]** FIG. 21 illustrates a micro-CT image of a bone growth composition of the instant disclosure in the form of a bone implant in a critical size at 3 and 6 weeks with β -TCP having a particle size range of about 250 to about 500 μm .
- [0031]** FIG. 22 is a micro-CT image of a bone growth composition of the instant disclosure in the form of a bone implant in a critical size bone defect at 3 and 6 weeks with β -TCP having a particle size range of about 1000 to about 2000 μm .
- [0032]** FIG. 23 illustrates histology of a bone growth composition of the instant disclosure in the form of a bone implant in a critical bone size defect at 3 and 6 weeks with β -TCP having a particle size range of about 1000 to about 2000 μm .
- [0033]** FIG. 24 illustrates histology of a bone growth composition of the instant disclosure in the form of a bone implant in a critical size bone defect with β -TCP having a particle size range of about 1000 to about 2000 μm .

- [0034]** FIG. 25 illustrates a bone growth composition of the instant disclosure in the form of a bone implant with a mass ratio of decellularized placenta derived extracellular matrix substantially free of residual material to β -TCP of 20:80%, rehydrated with bone marrow aspirate and loaded into a syringe for implantation/placement in a rabbit bone defect in a posterolateral fusion model.
- [0035]** FIG. 26 illustrates 8-week histology results of a bone growth composition of the instant disclosure with a mass ratio of decellularized placenta derived extracellular matrix substantially free of residual material to β -TCP is 20:80% rehydrated with bone marrow aspirate in a rabbit bone defect in the posterolateral fusion rabbit model. New bone formation as well as neo vasculature is denoted with arrows. "TP" in the figure denotes tricalcium phosphate and "ECM" in the figure denotes decellularized placenta derived extracellular matrix substantially free of residual material.
- [0036]** FIG. 27 illustrates 8-week histology results of a bone growth composition of the instant disclosure having a mass ratio of decellularized placenta derived extracellular matrix substantially free of residual material to β -TCP is 20:80% rehydrated with bone marrow. "TP" in the figure denotes tricalcium phosphate and "ECM" in the figure denotes decellularized placenta derived extracellular matrix substantially free of residual material. New bone formation as well as neo vasculature is denoted with arrows.
- [0037]** FIG. 28 is an illustration of the use of a bone growth composition of the instant disclosure as a dental graft.
- [0038]** FIG. 29 is an illustration of the use of a 3D printed mold with rods for creating channels in a bone growth composition of the instant disclosure with controlled channels therein to guide new blood vessel ingrowth.
- [0039]** FIG. 30 illustrates human Mesenchymal Stem cells grown on bone growth composition as described herein in the form of an implant as compared to a commercial product (Control Product) containing 20% human bovine collagen and 80% beta TCP.

DETAILED DESCRIPTION

- [0040]** The present disclosure is based upon the discovery that surprisingly and unexpectedly, a bone growth composition comprising a decellularized placenta derived extracellular matrix substantially free of residual materials, such as DNA, cells, cell debris, growth factors, exosomes and cytokines comprising a natural biocompatible material, and a mineral source of Ca^{3+} and PO_4 ions readily has applications in treating a bone defect in a subject.
- [0041]** Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this disclosure

belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure.

[0042] All numerical designations, e.g. volume, mass, number of particles, etc. are approximations which are varied by (+) or (-) by increments of 1.0 or 0.1, as appropriate. It is to be understood, although not always explicitly stated, that all numerical designations are preceded by the term "about".

[0043] Numerous terms and phrases are used throughout the instant specification and claims and are defined below.

[0044] "About" and "approximately" are interchangeable and mean plus or minus a percent (e.g. $\pm 5\%$) of the number, parameter, or characteristic so qualified, which would be understood as appropriate by a skilled artisan to the scientific context in which the term is utilized.

[0045] As used here, the singular form "a", "an" and "the" include plural reference unless the context clearly dictates otherwise.

[0046] As used herein, " β -TCP" is a mineral source for Ca^{2+} and PO_4^{3-} ions and is Beta-tricalcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$), a biocompatible ceramic material having applications in orthopedic and dental applications, including bone void filling and bone regeneration..

[0047] As used herein, the terms "comprising", "comprises" and "comprise" are intended to mean that the compositions, preparations and methods disclosed herein include recited elements, but do not exclude others.

[0048] As used herein, the phrase "critical size bone defect" refers to a bone defect created in an animal that is unable to heal spontaneously within a reasonable timeframe without intervention. The critical size varies depending on factors such as the location of the defect, and the specific characteristics of the bone tissue involved. Critical size bone defects typically exceed the body's natural regenerative capacity and require surgical intervention or the use of tissue-engineered constructs or biomaterials to facilitate healing. In clinical practice, defining the critical size of a bone defect is essential for determining the appropriate treatment strategy. Surgeons often assess factors such as the size, location, and stability of the defect, as well as the surrounding soft tissue condition, to determine whether surgical intervention is necessary and what type of intervention would be most effective. Various techniques and materials may be employed to address critical size bone defects, including bone grafts (autografts, allografts, or xenografts), synthetic bone substitutes, growth factors, and tissue engineering approaches

[0049] The terms "delivery" and "administration" are used interchangeably herein and mean and include providing a "pharmaceutical composition" or "biologically active agent" or "active

agent formulation" or a biologically acceptable material to a treatment site, e.g., damaged tissue, through any method appropriate to deliver the functional agent or formulation or pharmaceutical composition to the treatment site. Non-limiting examples of delivery methods include direct injection, percutaneous delivery and topical application at the treatment site.

[0050] As used herein, the terms "extracellular matrix" and "ECM" are used interchangeably herein and mean and include a collagen-rich substance that is found in between cells in mammalian tissue, and any material processed therefrom, e.g. decellularized ECM. In a particular embodiment, ECM material is derived from human placenta.

[0051] As used herein, the terms "lyophilized" and "dehydrated" are used interchangeably, and refer to the state, but not the method of having water removed as a means of preservation.

[0052] As used herein, the term "placenta" refers to a disc of tissue that connects a mother's uterus to the umbilical cord and is ultimately responsible for delivering nutrients and oxygen to a fetus. Three layers of membranes make up the placenta: (1) the amnion, which is a single layer of ectodermal epithelium completely enclosing the embryo; (2) the chorion which surrounds the amniotic sac and includes the villi and trophoblast; and (3) the decidua of the maternal endometrium. The umbilical cord is the conduit between the placenta and the mother's circulatory system, and consequently, is not a part of the placenta.

[0053] As used herein, the term "hydrate" refers to the addition of a liquid, e.g., a diluent, to a dehydrated material in order suspend the material in a solution and to allow for injection through an adequately sized needle.

[0054] As used herein, the phrase "substantially free of residual material" refers to an extracellular matrix that does not contain a quantifiable amount of residual material, e.g., (1) cells, (2) cell debris, (3) nucleic acid molecules, (4) nucleotides, (5) growth factors, (6) cytokines, (7) chemokines, (8) hormones, or (9) any combination of (1)-(8).

[0055] As used herein The term "effective amount", as used herein, means that the amount of a "pharmaceutical composition" and/or "biologically active agent" and/or "active agent formulation" such as a bone growth composition as disclosed herein, that is administered at a sufficient quantity to ameliorate one or more causes, symptoms, or sequelae of a disease or condition, such as, for example, a bone defect. Such amelioration only requires a reduction or alteration, not necessarily elimination, of the cause, symptom, or sequelae of a disease or disorder. An effective amount will depend on several factors including severity and course of the disease or condition, previous therapy, the patient's health status, age, weight, and response to the drugs. It is considered well within the skill of the art for one to determine

therapeutically effective amounts by routine experimentation (including, but not limited to, a dose escalation clinical trial).

[0056] As used herein, the terms "exosome" or "exosomes" refer to naturally existing nanoparticles that are secreted endogenously by many types of in vitro cell cultures and in vivo cells, and are commonly found in vivo in body fluids, such as blood, urine and malignant ascites. Exosomes are cup-like multivesicular bodies (MVBs) varying in size between 30-100 nm. MVBs are specialized endosomes in the endocytosis pathway of cells and are formed by inward budding and scission of vesicles from the limiting membranes into the endosomal lumen. During the formation of MVBs, transmembrane and peripheral membrane proteins are absorbed into the vesicle membrane, and at the same time, cytosolic components are also embedded in the vesicles. As this process progresses, the MVBs ultimately fuse with the cellular membrane, triggering the release of the exosomes from the cells.

[0057] As used herein, the terms "extracellular matrix" and "ECM" are used interchangeably and mean and include a collagen-rich substance that is found in between cells in mammalian tissue, and any material processed therefrom, e.g., decellularized ECM. In a particular embodiment, ECM material is derived from human placenta. Naturally, placenta derived extracellular matrix is derived from the placenta.

[0058] As used herein, the term "hydrogel" refers to a water-insoluble, three-dimensional network of polymer chains capable of holding large amounts of water.

[0059] As used herein, the terms "lyophilized" and "dehydrated" are used interchangeably, and refer to the state, but not the method of having water removed as a means of preservation.

[0060] As used herein, the term "osteoconductive" refers to a material or substance that supports and promotes bone growth. In the context of medical devices or implants, osteoconductivity describes the ability of a material to serve as a scaffold or framework for new bone formation. Osteoconductive materials encourage the attachment, proliferation, and differentiation of bone cells, facilitating the integration of the implant with surrounding bone tissue. This property is crucial for the success of bone grafts, implants, and other orthopedic or dental devices designed to repair or replace bone tissue. Materials such as certain ceramics, calcium phosphates, and bioactive glasses are often considered osteoconductive due to their ability to stimulate bone regeneration.

[0061] As used herein, the term "pharmaceutical composition" refers to a composition that comprises at least one ophthalmic therapeutic agent, excipient, a carrier, etc. Pharmaceutical compositions are administered to a patient rather than the ophthalmic therapeutic agent alone.

[0062] As used herein, the terms "particle" and "granule" are used interchangeably and refer to a minute portion, piece, fragment, or amount.

[0063] As used herein, the term "porous" refers to a material or structure characterized by the presence of interconnected voids or pores within its substance. These voids allow for the passage or diffusion of substances, such as fluids or gases, through the material. The porous nature of a material provides properties such as increased surface area, permeability, or absorbency, which may be advantageous for various applications including filtration, insulation, catalysis, or controlled release of substances. The extent of porosity can vary, ranging from fine porosity with very small interconnected voids to coarse porosity with larger voids. "Osteoconductive" refers to a material or substance that supports and promotes bone growth.

[0064] As used herein, the phrase "porous and osteoconductive" refers to a material or structure that possesses both porous characteristics and osteoconductive properties. Such a material is not only permeable, allowing for the passage or diffusion of substances, but also promotes bone growth and integration with surrounding bone tissue. This combination of traits is particularly advantageous for medical devices or implants intended for orthopedic or dental applications. The porous nature of the material provides a framework for cellular ingrowth and vascularization, while its osteoconductive properties stimulate bone formation and facilitate the osseointegration of the implant with the surrounding tissue. This dual functionality enhances the effectiveness and long-term success of the implant in supporting bone regeneration and structural integrity.

[0065] As used herein, the term "placenta" refers to a disc of tissue that connects a mother's uterus to the umbilical cord and is ultimately responsible for delivering nutrients and oxygen to a fetus. Three layers of membranes make up the placenta: (1) the amnion, which is a single layer of ectodermal epithelium completely enclosing the embryo; (2) the chorion which surrounds the amniotic sac and includes the villi and trophoblast; and (3) the decidua of the maternal endometrium. For purposes of this disclosure, umbilical cord is not considered part of a placenta.

[0066] As used herein the term "placenta derived" with respect to a biomaterial indicates that the source of the biomaterial is the placenta, which is typically obtained from animals (such as pigs or sheep) or from human placental tissue. Examples of placenta derived biomaterials having applications herein are a decellularized placenta derived extracellular matrix and placenta derived exosomes. Placental derived biomaterials been proposed to be immune-privileged such that, when presented physically and biochemically to a "native" administration site on a subject, they are proposed to rapidly re-establish healing cues and

limit foreign body reactions. A particular example of a decellularized placenta derived extracellular matrix having applications in a pharmaceutical composition of the instant disclosure is a decellularized, dehydrated human amniotic membrane (DDHAM).

[0067] As used herein, "therapeutic agent" refers to a compound or substance in a pharmaceutical composition that is biologically active and produces the effects of the pharmaceutical composition

[0068] A bone growth composition of the present disclosure may be hydrated for administration at the point of care or may be stored at a cold temperature, such as from about 2° C to about 8° C. An example of a hydration fluid for a bone growth composition of the instant disclosure can vary. Examples of such fluids include sterile sodium chloride solution, for example about 0.9% sodium chloride (hereinafter referred to as "saline"), blood, plasma, bone marrow, etc. An effective amount of a bone growth composition can vary depending upon magnitude of the bone defect, whether the composition is being used as a bone graft, a bone implant, or a bone filler

[0069] In a particular embodiment, a bone growth composition of the instant disclosure is administered directly to the target site (e.g., the bone defect to be treated.).

[0070] The mass ratio of the mineral source to biocompatible material, e.g., decellularized placenta derived extracellular matrix substantially free of residual material in a bone growth composition as described herein can vary. Examples of such ratios having applications herein include 5:95%, 10:90%, 15:85%, 20:80%, 25:75%, or 30:80%. In a particular embodiment, the biocompatible material comprises a collagen and an elastin, wherein the amount collagen is more than about 60% by weight of a bone growth composition as described herein, and elastin is less than about 20% by weight of a bone growth composition.

[0071] Optionally, a bone growth composition of the instant disclosure can comprise pharmaceutically acceptable diluent(s), excipient(s), or carrier(s). Furthermore, a bone growth composition of the instant disclosure can further comprise a medicinal or pharmaceutical agent, carrier, or adjuvants to address possible infection, pain, prevent or limit development of blood clots (an anti-coagulant), etc.

[0072] The present disclosure may be better understood by reference to the following non-limiting examples, which are provided as exemplary of the disclosure. The following examples are presented in order to more fully illustrate the preferred embodiments of the disclosure. They should in no way be construed, however, as limiting the broad scope of the disclosure.

EXAMPLES

Example 1.

Placental Sourcing for Creating a Bone Growth Composition as described Herein

[0073] The placental starting material is sourced by established methods designed to reduce the risk of additional contamination and prevent mislabeling and/or tracking errors. The tissue originates from accredited hospitals, with collections performed by licensed health care professionals. The tissue collection kit utilized includes sufficient containers and barcoded labels for biomaterials along with appropriate documentation and instructions for collecting the umbilical cord. Furthermore, the placental tissue is screened for the presence of transfusion transmissible viruses in accordance with the FDA Guidance for Industry: Eligibility Determination for Donors of Human Cells, Tissues and Cellular and Tissue-Based Products (HCT/Ps) - August 2007.

Example 2.**Generation a Bone Growth Composition of the Instant Disclosure**

[0074] Making a bone growth composition as disclosed herein begins with the procurement of placental tissue, obtained from a normal, full-term delivery and thus is sourced from whole human placentas. The frozen placental tissue is released from quarantine for processing and is thawed at 4 - 8°C for up to 96 hours. The placental tissue is cut into small segments and homogenized using an Omni mixer homogenizer. The homogenization step serves to generate small tissue particulates, with increased surface area, allowing for more effective separation and removal of cells and cellular debris from the placental tissue. The homogenized tissue from a single placenta is transferred into a sterile processing container with sodium chloride (NaCl) solution. The tissue is washed repeatedly with sterile NaCl by shaking on an orbital shaker whereby the NaCl solution is exchanged by centrifugation to settle tissue particulate, followed by removal of the supernatant, and replacement with fresh sterile NaCl solution. The placental tissue is incubated at controlled room temperature (20 to 25°C) for up to 24 hours while shaking in sterile NaCl solution, followed by centrifugation to remove supernatant and washed with sterile water. The exposure of the placental tissue to a high concentration of NaCl, followed by hypotonic water constitutes an "osmotic shock" to the tissue. The osmotic shock serves to remove blood, blood components, cells, and cell debris from the tissue. The placental tissue is subjected to a second osmotic shock before a nucleic acid removal step.

[0075] The supernatant is removed from the sterile processing container after the completion of the water wash and replaced with a solution containing BENZONASE and is incubated up to 24 hours on an orbital shaker at room temperature to aid in degradation of all forms of nucleic acids (deoxyribonucleic acid (DNA) and ribonucleic acid (RNA)).

[0076] Following removal of BENZONASE with extensive sterile water rinses, the tissue is subjected to detergent washes with sodium deoxycholate (DOC) solution for up to 24 hours followed by freshly exchanged DOC solution for up to an additional 72 hours. DOC is a water soluble, bile-acid derived, mild ionic detergent used to remove residuals such as cells, cell membranes, and other cell debris. Upon completion of the detergent washes, the tissue is washed with sterile water to remove debris and residual DOC.

[0077] To enrich for the placental structural protein, collagen, the placental ECM is treated with sodium hydroxide (NaOH) solution to denature and remove any soluble extracellular matrix elements, non-crosslinked proteins, i.e., cytokines/chemokines, growth factors, and hormones. Thereafter, the ECM is subjected to hydrochloric acid (HCl) treatment to neutralize the NaOH and to remove the above-mentioned impurities. The incubation of NaOH followed by HCl is utilized as an "enrichment step". Titration of this step allows for careful selection of the matrix composition containing enriched collagen content and titrated desirable levels of elastin content of less than 20%. This enrichment process, followed by extensive water washes further facilitates the removal of solubilized product residuals such as cells, cellular debris, nucleic acids, growth factors, chemokines/cytokines, hormones, and process reagents, such as salt, DOC, and BENZONASE.

[0078] The resultant placental structural protein enriched composition is tested for collagen and elastin content. The methods used are well established in the art for biochemical testing and are based on hydroxyproline- based colorimetric assay for collagen and a dye-5,10,15,20-tetraphenyl- 21H,23H-porphine tetra-sulfonate based colorimetric assay for elastin.

[0079] Upon confirmation of the ratios of collagen and elastin, the placental decellularized structural protein enriched composition is transferred into a sterile mold, frozen at -80° C, and lyophilized. The lyophilized material is sealed and stored at room temperature until it is used in formulation with β -TCP granules at different ratios, including examples discussed below.

Example 3.

Determination of the Composition of a Bone Growth Composition of the Instant Disclosure

[0080] The resultant placental structural protein enriched composition, when tested for the level of collagen and elastin, showed that within its 100% composition there is enrichment for collagen greater than 60%, elastin is titrated to optimally less than 20% and residual water content to less than 20%.

Table 1. A Bone Growth Composition of the Instant Disclosure

Component	Rounded Range
Collagen	Greater than 60%
Elastin	Less than 20%
Moisture Content	Less than 20%
Sulfated Glycosaminoglycans (sGAGs)/ Proteoglycans	Less than 1%
Laminin	Non detectable
Fibronectin	Non detectable

[0081] To investigate the collagen types in placental structural protein enriched composition, an analysis using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was conducted for collagen Types I, III, IV, V and VI by molecular weight and comparison to respective human placenta collagen standards as per ASTM 2122-192. The results confirmed that the predominant bands in placental structural protein enriched composition correspond to collagen Type I and Type III as the alpha 1 Type I collagen and alpha 1 Type III collagen chains move at the same rate on SDS-PAGE gel with overlapping gel band patterns. Collagen Type IV was below the assay limit of detection (LOD). Collagen Type V and VI were not detectable in placental structural protein enriched composition due to lack of their signature bands. Only the protein bands representing collagen type I and collagen type III were observed. These collagen types represent the majority of the collagen content in the placental structural protein enriched composition. FIG.1 shows results of SDS-PAGE analysis of growth composition of the instant disclosure denoted as ECM in comparison to collagen standards.

Example 4.

Determination of the Ratio by mass of Mineral source to decellularized placenta derived extracellular matrix substantially free of Residual Material

[0082] A bone growth composition of the instant disclosure developed with desired ratio of collagen to elastin, as confirmed by biochemical assays, is then transferred into a sterile mold, frozen at -80° C, lyophilized and stored at room temperature. The lyophilized matrix is formulated with β -TCP granules of sizes including, but not limited to about 250 micrometers to about 3000 micrometers at different mass ratios, including but not limited

to 5/95%, 10/90%; 15/85%, 20/80%, 25/75%, and 30/70% β -TCP to the biocompatible material, e.g., decellularized placenta derived extracellular matrix substantially free of residual material.

[0083] The dry weight of the decellularized placenta derived extracellular matrix substantially free of residual material was calculated for the required ratio of the mineral component, β -TCP, to form a ratio of 5/95%, 10/90%, 15/85%, 20/80%, 25/75%, and 30/70%, to name only a few

[0084] Sterile water was added to the decellularized placental matrix, transferred into a plastic sterile container and homogenized using a probe. The placental ECM mixture was combined with β -TCP granules at the desired mass ratio and distributed into sterile molds of a desired shape, cylindrical, cuboidal etc. The molds were frozen and dehydrated via lyophilization.

[0085] FIG. 2 illustrates several forms of the lyophilized biocompatible graft material in cuboidal configurations with granules or particles of β -TCP having a size ranging from about 250- about 2000 micrometers distributed throughout the bone growth composition of the instant disclosure, and matrix/ β -TCP ratios including 10/90%, 15/85%, and 20/80%. FIG. 3 illustrates one form of the lyophilized biocompatible graft material in a cylindrical form having small size mineral granules having a size ranging from about 250- about 1000 micrometers distributed throughout a bone growth composition of the instant disclosure. FIG. 4 illustrates another form of a bone growth composition as described herein in a cylindrical form having particle size ranging from about 1000-2000 micrometers, distributed throughout the bone growth composition, top and side view.

[0086] FIG. 5 illustrates another form of a bone growth composition described herein in a strip configuration containing 95% of mineral source in particle form with the size of the particles ranging from about 250-1000 micrometers and 5% of biocompatible material, e.g., placenta derived extracellular matrix substantially free of residual material.

Example 5.

Crosslinking Decellularized Placenta Derived Extracellular Matrix

[0087] Placental structural protein enriched extracellular matrix that is developed with desired ratio of collagen to elastin as discussed above and confirmed by biochemical assays, is then transferred into a sterile mold, frozen at -80° C, lyophilized and stored at room temperature. The lyophilized matrix is formulated with β -TCP granules of sizes ranging from about 250 micrometers to about 3000 micrometers at different mass ratios, including but

not limited to 5/95%, 10/90%; 15/85%, 20/80%, 25/75%, and 30/70% placental Matrix / β -TCP.

[0088] The dry weight of the placental structural protein enriched extracellular matrix was calculated for the required ratio of the mineral component, β -TCP, to form a ratio of 5/95%, 10/90%, 15/85%, 20/80%, 25/75%, and 30/70%.

[0089] Sterile water was added to the decellularized placental matrix, transferred into a plastic sterile container and homogenized using a probe. The placental ECM mixture was combined with β -TCP granules at the desired mass ratio and distributed into sterile molds of a desired shape, cylindrical, cuboidal etc. The molds were frozen and dehydrated via lyophilization.

Example 5.

Crosslinking of a Bone Growth Composition of the Instant Disclosure in the Form of a Bone Implant

[0090] Crosslinking studies were performed testing different methods for stabilizing the structure and handling properties of a bone growth composition of the instant disclosure. The crosslinking methods tested included a) chemical crosslinking, using carbodiimides and glutaraldehyde chemistry, which require lengthy rinsing procedures to remove chemicals, such as crosslinkers, catalysts, etc., b) a physical crosslinking method, using thermal treatment, which is one of the earliest published physical techniques utilized for Collagen cross-linking (Yannas I V, Tobolsky A V. Nature. 1967). As a result of thermal dehydration, collagen cross-links were formed in the absence of any catalysts, excipients or crosslinkers. During the thermal dehydration crosslinking process, there were no additional chemicals, excipient, or crosslinkers present. FIG. 6 illustrates one embodiment of superior crosslinking a bone growth composition as disclosed herein with thermal dehydration treatment.

Example 7.

Blood Rehydration of a Bone growth Composition of the Instant Disclosure with Mass Ratio of 20:80% Decellularized Placenta Derived ECM substantially free of Residual Material to Mineral Resource

[0091] Blood was added to a molded bone growth composition as described herein at 20:80% ratio of β -TCP to biocompatible material, e.g., decellularized placenta derived ECM substantially free of residual materials in the form of an implant as described above. The

rehydrated implant was molded into a desired ball shape as depicted in FIG. 7, allowing for superior positioning and implantation within a defect. When the implant was cut in half post rehydration, it was demonstrated that the blood had penetrated and hydrated the implant entirely to the core. FIG. 8 illustrates the bone growth composition of the instant disclosure in the form of an implant at 20:80% β -TCP rehydrated with blood.

Example 8. Bone Growth Composition of the Instant Disclosure Rehydration and Handling

[0092] When placental structural protein enriched bone implant at 20/80 ratio with β -TCP, e.g. a bone growth composition as disclosed herein, was rehydrated with saline for 15 minutes, it remained intact with β - TCP granules retained within implant. The rehydrated implant was removed from the well and molded into a ball shape mimicking the clinical procedure. The implant unexpectedly demonstrated superior integrity, moldability and handling, without apparent separation of β -TCP. Such superior integrity, moldability and handling however were not observed when β -TCP alone was rehydrated and molded, as evidenced by the multiple granules left in the well. FIG. 9 illustrates rehydration and handling of the bone implant at 20:80% β -TCP as compared to β -TCP alone.

Example 9.

Morphological Analysis of a Bone Growth Composition of the instant Disclosure in the form of a Bone Implant

[0093] FIG. 10 illustrates the morphological analysis of a bone growth composition of the instant disclosure in the form of a bone implant, at 20:80% β -TCP with β -TCP particle size ranges of about 250 to about 2000 micrometers (Left) and about 1000 to about 2000 micrometers (Right). SEM analysis was used to perform morphological analysis of the bone implant at 20:80% β -TCP.

Example 10.

Ratio Analysis of a Bone Growth Composition of the Instant Disclosure in the form of a Bone Implant

[0094] The organic to inorganic ratio and inorganic phase composition of a bone growth composition as described herein in the form of a bone implant was tested. Acquiring the organic and inorganic weight composition involves heating/sintering the samples to a temperature that will decompose (ash) the organic components, leaving behind the inorganic granules. The heat treatment/sintering will simultaneously prepare the inorganic

granules, which are left over after sintering, to be analyzed for phase composition using x-ray diffraction (XRD). Ash testing was performed to establish a specification for the weight percentage of organic material to inorganic components. Prior to the samples undergoing XRD analysis to acquire the phase purity and phase weight composition, the samples were sintered at $1100^{\circ}\text{C} \pm 25^{\circ}\text{C}$ in order to increase and normalize the crystallite size relative to the XRD standards used to calibrate the XRD. Increasing the crystallite size is necessary to better define the peaks and provide a flat baseline during the XRD scan. The required sintering of the samples takes place during the heat treatment for ash testing.

[0095] The sintered granules resultant to ash testing were pulverized in a mortar and pestle composed of synthetic sapphire and analyzed according to established testing procedure. The pulverized sample(s) were scanned at a rate of 1.20 degrees per minute from 20° – 60° using a Rigaku Miniflex 11 X-ray diffractometer following the established procedure. Calcium phosphate patterns from the International Center for Diffraction Data/ Joint Committee on Powder Diffraction Standards (ICDD/JCPDS) library that are stored in the JADE 9 analysis software were compared to the sample pattern. All matching calcium phosphate patterns will be reported as crystalline phase impurities. The sample diffraction patterns were provided with superimposed patterns of each phase from the pattern library.

[0096] Phase Purity Quantification: Calibration curves for various calcium phosphate phases (Hydroxyapatite/P-Tricalcium Phosphate/ pyrophosphate) have been prepared. These calibration curves were used to quantify the impurity phases detected in the test samples. The detection limit for hydroxyapatite and P-tricalcium phosphate is 1%, while pyrophosphate has a detection limit of 4% but can be visible as low as 0.5%. FIG. 11 illustrates XRD spectrum and overlay analysis of bone growth composition as described herein in the form of a bone implant at 20:80% β -TCP.

Example 11.

FTIR Analysis of a Bone Growth Composition of the Instant Disclosure in the Form of a Bone Implant

[0097] Fourier-transform infrared spectroscopy (FTIR) in Attenuated Total Reflectance (ATR) mode was used to perform characterization of placental structural protein enriched biocomposite bone implant, e.g., a bone growth composition as disclosed herein in the form of a bone implant, and control samples. Minimum test sample dimensions of 10 mm x 10 mm were used for analysis. Five (5) batches were included as part of this feasibility study. A random 5 FTIR spectra were taken from each test sample. A minimum of 32 scans were collected per spectrum at 4 cm^{-1} spectral resolution. Data processing was performed using

OMNIC software at two levels: 1) Identifying collagen characteristic peaks; Comparing and contrasting characteristic peaks from product test groups and control test groups for possible change due to additive or degradation; Performing additional spectral processing to establish range and standard deviation. Level 2 analysis included: Performing curve fitting for selected regions to access comparative percentage structural proteins in the product. Establishing range and standard deviations for selected peaks or indices (ratio of peaks). FIG. 12 illustrates FTIR spectrum and stability analysis of Placental decellularized ECM and PSPE-BI.

Example 12.

Placement of a Bone Growth Composition of the Instant Disclosure in the Form of a Bone Implant into Packaging Designed for Shipment and Clinical Rehydration.

[0098] The lyophilized bone implants are placed into an inner plastic tray sealed by an aluminum cover using medical grade sealing equipment. The sealed inner tray is then placed inside an outer tray which is sealed with aluminum foil as seen in FIG. 13. The double sealed trays will be labeled and prepared for sterilization. FIG. 13 shows bone growth compositions of the instant disclosure in the form of bone implants in packaging trays accommodating 10cc, 5ccc and 2.5 cc and providing a system for adding an appropriate rehydration agent in a clinical setting.

Example 13.

A Bone Growth Compositions formed with Varying Mass Ratios of ECM to Mineral Resource

[0099] Placental structural protein enriched matrix, e.g., decellularized placenta derived extracellular matrix substantially free of residual material and comprising collagen and elastin, as discussed above, was mixed with β -TCP at various ratios, 5/95%, 10/90%, 15/85%, 20/80%, 25/75%, 30/70%, 40/60% and various β -TCP particle sizes including about 250 to about 3000 micrometers and homogenized to fully disperse the β -TCP within the ECM to form a bone growth composition as described herein with dispersed β -TCP granules. FIG. 14 illustrates the bone growth composition post homogenization with β -TCP dispersed therein. The method of dispersing the mineral resource within a bone growth composition of the instant disclosure provides an unexpected way of generating micronized small size of the mineral source in particle form.

Example 14.

In Vivo Testing of a Bone Growth Composition of the instant Disclosure at Ratios 15/85 and 30/70% ECM to β -TCP.

[0100] Pilot Evaluation Study of a bone growth composition as disclosed herein in the form of a bone filler was implanted into Critical Sized Defects in the Long Bones of New Zealand White Rabbits: a 6 millimeter (mm) femoral condyle defect was drilled to a depth of 10-12 mm, with the implant placed within the defect. Implant#1 = 15% ECM: 85% β -TCP and Implant#2 = 30% ECM: 70% β -TCP rehydrated with saline or BMA (Bone marrow aspirate). Implant#1 and Implant#2 were compared to Autograft and Tri-Calcium Phosphate (β -TCP) alone at 0, 42 and 84 Days. BMA was obtained from the iliac crest and combined with test articles. New bone growth was evaluated by micro-computed tomography (micro-CT) analysis at 0-, 6- and 12-weeks post-implantation and histologic evaluation at 6- and 12-weeks post-implantation. Evaluation of potential local tissue response, inflammation, and bone ingrowth following implantation of the test article as compared to β -TCP and autograft were conducted. FIG. 15 illustrates the bone growth composition alone at 12 weeks. FIG. 16 illustrates the autograft at 12 weeks. FIG. 17 illustrates Test group 1 (FUSE 1) at 12 weeks. FIG. 18 illustrates group 2 (FUSE2) at 12 weeks.

Example 15.

In Vivo Testing a Bone Growth Composition of the Instant Disclosure in Critical Sized Bone Defects in the Long Bones of New Zealand White Rabbits – β -TCP of Different particle size.

[0101] A 6 mm femoral condyle defect was drilled to a depth of 10-12 mm. Bone growth composition in the form of two implants with one having β -TCP particles ranging in size from about 250 to about 2000 micrometers and the other having β -TCP particles ranging in size from about 1000 to about 2000 micrometers were placed within the bone defect. The bone growth composition in the form of a bone implant was further combined with a biological material, e.g., Bone Marrow Aspirate (BMA) and was tested at 3 and 6 weeks. As part of the procedure, BMA was obtained from each tibia and combined with test articles. Evaluation by micro-CT and histology analysis at 3- and 6-weeks post-implantation was conducted for local tissue response, inflammation, and bone ingrowth following implantation. FIG. 19 shows BMA generation and application of bone marrow aspirate the bone growth composition implant FIG. 20 Rehydration and placement of the bone growth composition implant in a critical size bone defect. FIG. 21 micro-CT imaging of the bone growth composition implant in a critical size defect at 3 and 6 weeks with β -TCP particles ranging

in size from 250 to 2000 micrometers. FIG. 22 micro-CT imaging of the bone growth composition implant in a critical size defect at 3 and 6 weeks – β -TCP particle size range of about 1000 to about 2000 micrometers. FIG. 23 Histology of a bone growth composition of the instant disclosure in the form of a bone implant in a critical size defect at 3 and 6 weeks – β -TCP particle size range is 1000-2000 micrometers. FIG. 24 Histology of a bone growth composition of the instant disclosure in the form of a bone implant in a critical size defect at 3 and 6 weeks – β -TCP particle size range is 1000-2000 micrometers.

Example 16.***In Vivo* Testing of a Bone Growth Composition of the Instant Disclosure in the form of a Bone Implant in Critical Sized Bone Defects in the Long Bones of New Zealand White Rabbits.**

[0102] Bilateral critical distal femur (cancellous region) defects (6mm x 10mm) will be created in skeletally mature New Zealand White rabbits. Treatment of both defects in a single animal will consist of no treatment (empty defect), a bone growth composition as disclosed herein in the form of a bone implant, or Comparator Scaffold Foam Material. New bone growth will be evaluated at both 6- and 12-weeks post implantation into the defect. Bone growth will be measured by micro-CT and polymethylmethacrylate (PMMA) histology and histomorphometry. The Day 3 timepoint will provide a baseline of micro-CT and histomorphometry. The 6-week time allows for an assessment of the initial biologic responses to the device. The final time point (12-weeks) should be of a sufficient duration to demonstrate bone healing and the effects of any residual device material. Paraffin histology sections will also be used for ISO 10993-6 evaluation from the timepoints referenced above.

Example 17.***In Vivo* Testing of a Bone Growth Composition of the instant Disclosure in the form of a Bone Implant in a Rabbitt Posterolateral Fusion Model**

[0103] An 8 Week Posterolateral Fusion Study was conducted in skeletally mature New Zealand White Rabbits (n=15). Each rabbit underwent aseptic bilateral surgery to decorticate the transverse processes (TPs) of the fourth and fifth lumbar vertebrae (L4-5). Bone marrow aspirate was obtained from the iliac crest and combined with a bone growth composition as disclosed herein in the form of bone implants. Approximately 2.5-3 cc of test

article mixture was deployed onto the paraspinal beds bridging the decorticated L4-L5 TPs. Radiography was performed immediately prior to surgery to identify the L4-5 operative sites, after surgery to verify implant placement, and at the study term (explanted specimens) to assess fusion. On Day 0, surgery on L4-L5 and bone marrow aspirate from the iliac crest was combined with a bone growth composition as described herein in the form of a bone implant and implanted in L4-L5. At 8 weeks, at the study term (explanted specimens) was evaluated by radiography, micro-CT, and histology to assess fusion for test article as compared to historical autograft and negative control, β -TCP fusion rates. FIG. 25 illustrates the bone growth composition in the form of a bone implant at 20:80% β -TCP rehydrated with bone marrow aspirate and loaded into a syringe for implantation/placement in a rabbit defect in a posterolateral fusion model. FIG. 27 illustrates 8-week histology results of the bone growth composition implant at 20:80% β -TCP rehydrated with bone marrow aspirate in a rabbit defect in the posterolateral fusion model. A line of osteoblast cells migrating into the defect and neovascularization are denoted by arrows. FIG. 27 illustrates 8-week histology results of the bone growth composition implant at 20:80% β -TCP rehydrated with bone marrow aspirate in a rabbit defect in the posterolateral fusion model. A line of osteoblast cells migrating into the defect and neovascularization are denoted by arrows.

Example 18.***In Vivo* Testing of a Bone Growth Composition as Disclosed Herein in the Form of a Bone Implant in a Canine Model**

- [0104]** A bilateral canine humeral defect implant study is undertaken to evaluate bone growth composition as described herein in the form of a bone graft (the bone graft material) that is in direct contact with bone tissue. Bone remodeling, new bone formation, and implant resorption is evaluated at periodic time intervals. The bone graft material is supplied in a form that results in a flexible graft upon wetting. The bovine collagen containing bone void filler, such as VITROSS Foam Bone Implant, will be used as a control.
- [0105]** The test or control materials will be implanted into bilateral drill defects surgically created in the cancellous bone of the proximal humerus of 18 canines. Bone graft material graft will be implanted into the drill defect of one humerus, and the control article will be implanted into the drill defect in the opposite humerus of each animal in accordance with a randomization schedule generated prior to study start. The drill defects will be approximately 10 mm in diameter and approximately 25 mm in depth. A lateral and dorso-palmar view will be obtained immediately post-operatively and all animals will be

subsequently radiographed at each sacrifice time point. After the predetermined exposure period (3, 6, 12, 24, or 52 weeks), the animals will be sacrificed, and the implantation sites exposed. The implant sites will be grossly observed, harvested, wrapped in a saline soaked gauze sponge and frozen at approximately -20°C . for further analysis.

[0106] Analysis of the harvested implant sites will include mechanical testing to assess the bony ingrowth and remodeling of the defect site. The tissue implant sites will also be examined using standard histology techniques well-known in the art. The extent of healing and nature of tissue contained within the defect will be characterized by the histopathological and histomorphometry evaluation. Defects treated with bone growth composition as described herein will show bone healing with the rate or extent of healing in defects treated with graft material equal to or better than that observed in defects treated with the control graft material.

Example 19.

Clinical Application of a Bone Growth Composition of the Instant Disclosure in the Form of a Bone Implant in a Critical Size Defect

[0107] Several surgical techniques have been described for the operative treatment of late-stage osteochondritis dissecans (OCD) in the knee and ankle that have failed nonoperative management. A bone growth composition as described herein in the form of a bone implant may be an optimal surgical option. All surgeries will be performed using retrograde drilling procedures for OCD in the talar dome and the femoral condyles. Approximately 10 patients will be treated by retrograde drilling or trephine drilling under arthroscopic and fluoroscopic control preventing injury to the cartilage surface. The subchondral cavity will be filled with a mixture of 20–40 mg bone growth composition bone implant either rehydrated with saline, blood, or morselized bone graft. It will be tamped through the drill guide and into position with a Steinmann pin. Thus, only the subchondral defect will be filled but the peripheral area of the drill hole will remain empty. Evaluation will be achieved by clinical assessment, radiographic, and magnetic resonance imaging examination. The follow-up will average 24 months up to 48 months. In all cases, osseous density is expected to increase in the bone growth composition bone implant filled subchondral area and mechanical impression of the joint surface could be prevented. There is expected good clinical (range of motion, pain) and radiographic results (bony healing) after this new treatment modality.

Example 20.

A bone Growth Composition as Disclosed herein with Fine Particle Size β TCP for Dental Bone Growth

[0108] A bone growth composition as disclosed herein prepared from finer particle size Calcium phosphate can serve as dental implants (FIG. 28) and will be evaluated in rat calvarial critical size- defect dental implant model to demonstrate osteoconductivity, bone formation and to study remodeling. The bone growth composition will be used to fill a 5.0 mm- diameter calvarial defect in rats. The defects will be evaluated by histological and histomorphological analyses of decalcified histological sections with hematoxylin and eosin staining at 6 and 10 weeks, respectively, after surgery. FIG. 28 illustrates use a Bone Growth Composition of the instant disclosure in the form of a dental graft.

Example 21.**A Bone Growth Composition of the Instant Disclosure in the form of a Bone Implant as a Scaffold for Delivery of Exosomes**

[0109] To fill in the large critical size bone defect sites and achieve desired therapeutic outcomes, a bone growth composition of the instant disclosure in the form of a bone implant will be integrated with exosomes. Exosomes isolated from placental cells or placental tissue will be loaded into the Bone Implant by physical absorption at the operating theater and the exosome-containing implant will be positioned within the critical size bone defect in rat tibia critical defects using a Bone Implant as the carrier. Bone regeneration will be quantified using micro-computed tomography and histology. Along with inducing early migration and mineral deposition by pre- osteoblasts, exosome treatment will demonstrate a positive effect on bone mineralization by providing a local delivery of exosomes loaded onto a slowly resorbing Bone Implant can provide a potential alternate to autografts for bone reconstruction.

Example 22.**A Bone Growth Composition as Disclosed in the Form of a Bone Implant as a Scaffold for Delivery of Biological Material**

[0110] To fill in the large critical size bone defect sites and achieve desired therapeutic outcomes, a bone growth composition as described herein in the form of a bone implant will be seeded with cells known to stimulate bone tissue formation including but not limited to Mesenchymal Stem Cells, Adipose Tissue Derived Stem Cells, Osteoblasts, Placental derived Cells. Cells will be seeded onto the Bone Implant and allowed to colonize it generating cell byproducts within the Implant. The cell and cell byproducts-containing implant will be

positioned within the critical size bone defect in a rat tibia model. Bone regeneration will be quantified using micro-computed tomography and histology. Along with inducing early migration and mineral deposition by pre-osteoblasts, cell- and cell-byproducts - based treatment will demonstrate a positive effect on bone mineralization by providing local delivery of cells or cell byproduct - loaded onto a slowly resorbing Bone Implant. Cell byproducts include but are not limited to cell conditioned media, cell secretome content. Such application can provide a potential alternative to autografts for bone reconstruction.

Example 23.

A Bone Growth Composition as Disclosed Herein in the form a Bone Implant as a Scaffold for Delivery of Biological Material

[0111] To fill in the large critical size bone defect sites and achieve desired therapeutic outcomes, a bone growth composition as described herein in the form of a bone implant will be combined with biological material, including but not limited to growth factors known to stimulate bone growth, such as Platelet Derived Growth Factor BB (PDGF-BB), Bone Morphogenetic Protein 2 (BMP-2). The biological material will be loaded onto the Bone Implant and the resulting bioactive - containing implant will be positioned within the critical size bone defect in a rat tibia model. Bone regeneration will be quantified using micro-computed tomography and histology. Along with inducing early migration and mineral deposition by pre-osteoblasts, bioactive- treatment will demonstrate a positive effect on bone mineralization by providing local delivery of cells loaded onto a slowly resorbing Bone Implant, which can provide a potential alternate to autografts for bone reconstruction.

Example 24.

A Bone Growth Composition as Disclosed herein in the Form of a Bone Implant with Controlled Channels Guiding New Blood Vessel Ingrowth

[0112] Scaffolds with controlled pore structures were produced using 3 dimensionally printed molds by computer-aided design (CAD) models having uniformly spaced rods within a mold. These rods following casting created horizontal channels in the implant. These channels are to guide new blood vessel ingrowth into the bone tissue. FIG. 29 illustrates use of 3D printed mold with rods for creating channels a bone growth composition as described herein in the form of a bone implant with controlled channels to guide new blood vessel ingrowth into the bone tissue. Rods of different spacing and configuration are utilized allowing for more effective blood vessel ingrowth. FIG. 29 illustrates use of 3D printed mold with rods for creating channels in bone growth composition as described herein

in the form of a bone implant with controlled channels to guide new blood vessel ingrowth. The dimensions of these horizontal projections are optimized for implant integrity and handling. Pores ranging in size from 20–1,500 μm have been used. Initial studies demonstrated that the minimum pore size for significant bone growth is 75–100 μm with an optimal range of 100–270 μm .

[0113] In addition to using molds to modify the geography of a bone growth composition of the instant disclosure, and because of the beneficial properties of a bone growth composition described herein, e.g., its plasticity and moldability properties it can readily be used as material available to modifying the shape of a bone growth composition of the instant disclosure readily have applications as a three dimensional printing material. Thus, for example, to create an extremely precise bone implant or graft for a bone defect, optionally the bone defect can be mapped using lasers, and that information fed into a three dimension printer, which can print an exact fitting bone graft or implant from a bone growth composition as described herein.

Example 25.

A Bone Growth Composition as Disclosed Herein in the Form of a Bone Implant that Supports Mesenchymal Stem Cell (MSC) Attachment and Growth.

[0114] Human Mesenchymal Stem cells were seeded on a bone growth composition of the instant disclosure in the form of a bone implant as compared to a commercial product containing 20% human bovine collagen and 80% β -TCP. FIG. 30 illustrates human Mesenchymal Stem cells grown on bone growth composition of the instant disclosure in the form of a bone implant as compared to a commercial product (Control Product) containing 20% bovine collagen and 80% β -TCP, e.g. VITOSS Bone implant.

[0115] The present disclosure is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the disclosure in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A bone growth composition comprising:

- (a) decellularized placenta derived extracellular matrix substantially free of residual material and comprising a natural biocompatible substrate; and
- (b) a mineral source of Ca^{2+} and PO_4^{3-} ions.

2. The bone growth composition of Claim 1, wherein the natural biocompatible substrate comprises: (a) a collagen; (b) elastin; (c) tropoelastin; (d) an elastin-like protein; or (e) any combination of (a) - (d).

3. The bone growth composition of either of Claims 1 or 2, wherein the natural biocompatible substrate comprises a collagen and elastin.

4. The bone growth composition of any of Claims 2-3, wherein the collagen of the decellularized placenta derived extracellular matrix substantially free of residual material comprises at least about 60% by weight collagen, and no greater than about 20% by weight elastin.

5. The bone growth composition of any of Claims 1-4, further comprising a synthetic biocompatible substrate.

6. The bone growth composition of Claim 5, wherein the synthetic biocompatible substrate comprises: (a) a PLA; (b) a PLGA; (c) a biodegradable polyester amide polymer; (d) a hydrogel; (e) a polyether ketone; or (f) any combination of (a)-(e).

7. The bone growth composition of any of Claims 1-6, wherein the mass ratio in the bone growth composition of the mineral source to the decellularized placenta derived extracellular matrix substantially free of residual material comprises: (a) about 5% to about 95%; (b) about 10% to about 90%; (c) about 15% to about 85%; (d) about 20% to about 80%; (e) about 15% to about 85%; or (f) about 30% to about 70%.

8. The bone growth composition of any of Claims 1-7, wherein the mineral source is in the form of particles ranging in size of: (a) about 250 μm to about 3000 μm ; (b) about 250 μm

to about 2000 μm ; (c) from about 250 μm to about 1000 μm ; or (d) about 1000 μm to about 2000 μm .

9. The bone growth composition of any of Claims 1-8 wherein the mineral source of Ca^{2+} and PO_4^{3-} ions comprises:

(a) $\text{Ca}(\text{H}_2\text{PO}_4)_2$; (b) CaHPO_4 ; (c) $\text{Ca}_3(\text{PO}_4)_2$; (d) $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$; or (e) any combination of (a)-(d).

10. The bone growth composition of any of Claims 1-9, further comprising biological material.

11. The bone growth composition of Claim 10, wherein the biological material comprises: cells, conditioned cell medium, cell secretome, plasma, genetic material, exosomes, bone marrow aspirate, platelet plasma, growth factors, or any combination thereof.

12. The bone growth composition of Claim 11, wherein:

- (a) the genetic material comprises a nucleic acid, a nucleotide, or a combination thereof;
- (b) the cells comprise mesenchymal stem cells, adipose tissue derived stem cells, osteoblasts, placental derived cells or any combination thereof;
- (c) the exosomes are produced by the cells of (b); and
- (d) the condition medium is produced by the cells of (b).

13. The bone growth composition of any of Claims 1-12, wherein the bone growth composition is: (a) porous; (b) osteoconductive; or (c) porous and osteoconductive.

14. The bone growth composition of any Claims 1-13, wherein the bone composition is a bone graft, a bone implant, or a bone filler.

15. A method for treating a bone defect in a subject, comprising administering to the subject at the point of the bone defect an effective amount of the bone growth composition of any of Claims 1-14.

16. The method of Claim 15, where the bone defect comprises: (a) an injury from trauma; (b) a congenital or acquired bone defect or anomaly; (c) resection of bone due to surgery;

(d) a sequelae of bone infection; (e) a comminuted fracture accompanied by bone mass loss.

17. A kit comprising:

- (a) the bone growth composition of any of 1-14; and
- (b) a packaging container specifically designed to allow for different product sizes and the addition of rehydration fluids.

18. The kit of Claim 17, further comprising a container that houses a biological material.

19. The kit of Claim 17, wherein the packaging container is designed to provide a sealed chamber that houses a biological material.

20. The kit of either of Claims 18 or 19, wherein the biological material comprises cells, conditioned cell medium, cell secretome, plasma, genetic material, exosomes, bone marrow aspirate, platelet plasma, a growth factor, or any combination thereof.

21. The kit of Claim 20, wherein:

- (a) the genetic material comprises a nucleic acid, a nucleotide, or a combination thereof;
- (b) the cells comprise mesenchymal stem cells, adipose tissue derived stem cells, osteoblasts, placental derived cells or any combination thereof;
- (c) the exosomes are produced by the cells of (b); and
- (d) the condition medium is produced by the cells of (b).