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

(19) **World Intellectual Property Organization**
International Bureau

(43) **International Publication Date**
6 May 2010 (06.05.2010)

PCT

(10) **International Publication Number**
WO 2010/050935 A1

(51) **International Patent Classification**:
A61L 27/40 (2006.01) ***A61L 31/00*** (2006.01)
A61L 24/00 (2006.01) ***A61L 27/00*** (2006.01)

(21) **International Application Number**:
PCT/US2008/081544

(22) **International Filing Date**:
29 October 2008 (29.10.2008)

(25) **Filing Language**: English

(26) **Publication Language**: English

(71) **Applicant** *(for all designated States except US)*: **WARSAW ORTHOPEDIC, INC.** [US/US]; 2500 Silveus Crossing, Warsaw, Indiana 46581 (US).

(72) **Inventors; and**
(75) **Inventors/Applicants** *(for US only)*: **DRAPEAU, Susan J.** [US/US]; 2009 Winly Lane, Cordova, Tennessee 38018 (US). **REMSEN, Kathy L.** [US/US]; 2635 Maple Grove Cove N., Germantown, Tennessee 38139 (US). **MCKAY, William F.** [US/US]; 3870 McElrie Cove, Memphis, Tennessee 38133 (US). **SHIMKO, Daniel** [US/US]; 8054 Bobwood Drive, Germantown, Tennessee 38138 (US).

(74) **Agents**: **PATEL, Samir R.** et al.; 710 Medtronic Parkway MS LC340, Minneapolis, Minnesota 55432 (US).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, 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, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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

Published:

— *with international search report (Art. 21(3))*

(54) **Title**: DEVICES CONTAINING DEMINERALIZED BONE MATRIX PARTICLES

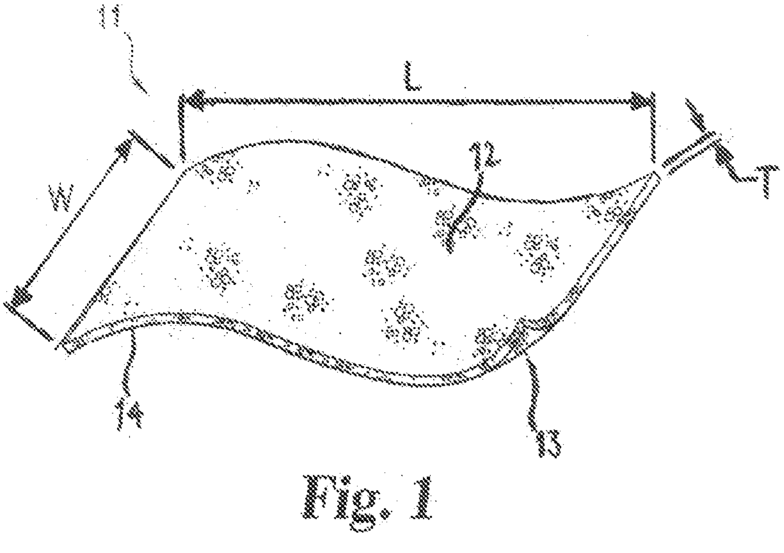

Fig. 1

(57) **Abstract**: Described are medical implant devices that include bone-derived particles. These and potentially other materials are held together in a three-dimensionally stable structure such as a porous, resilient sheet, by an ionically-crosslinked polysaccharide gel. Also described are methods for making and using such medical devices.

WO 2010/050935 A1

DEVICES CONTAINING DEMINERALIZED BONE MATRIX PARTICLES

BACKGROUND

The present invention relates generally to medical implants. In more particular aspects, the present invention relates to osteoinductive medical devices containing demineralized bone matrix.

Any publications or references discussed herein are presented to describe the background of the invention and to provide additional detail regarding its practice. Nothing herein is to be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention.

A wide variety of implant formulations have been suggested in the art for the treatment of bone defects. In addition to traditional bone grafting, a number of synthetic bone graft substitutes have been used or explored, including some materials that contain demineralized bone matrix. Demineralized bone matrix has been shown to exhibit the ability to induce and/or conduct the formation of bone. It is thus desirable to implant and maintain demineralized bone matrix at a site at which bone growth is desired.

However, the beneficial nature of demineralized bone matrix is susceptible to disruption by the incorporation of incompatible materials or techniques when formulating the medical implant material. At the same time, it is desirable to have implant devices exhibiting good physical integrity to retain the demineralized bone matrix at the implant site, and that handle well in the operating environment and during implantation. As well, it is of considerable commercial significance that the formulation be manufacturable without undue cost, equipment or material burdens.

In view of the background in the area of demineralized bone matrix devices, there exist needs for product configurations which exhibit the ability to induce and/or support bone growth through the desired region, which are readily manufacturable, and which demonstrate acceptable handling properties for surgeons.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides a demineralized bone matrix (DBM) device comprising a dried, flexible aggregate of bone-derived particles retained in a unitary body by an ionically-crosslinked polysaccharide gel, with the bone-derived particles including DBM particles alone or in combination with demineralized bone chips.

The bone-derived particles may constitute at least 90% by weight of the body on a dry weight basis, and the polysaccharide may constitute no more than 10% by weight of the body on a dry weight basis. Methods of using such DBM devices for implantation to promote bone growth also form aspects of the invention.

In another aspect, the present invention provides a method for preparing a DBM device. The method comprises providing wetted bone-derived particles, wherein the particles have pores occupied by a wetting liquid. The wetted bone-derived particles are blended into a gellable liquid medium comprising an ionically-crosslinkable polysaccharide under conditions wherein the gellable liquid medium does not displace the wetting liquid from the pores. The polysaccharide is ionically crosslinked to form a unitary body comprising an ionically-crosslinked polysaccharide gel and the bone-derived particles. The unitary body is dried to form a dried material in which bone-derived particles have pores free from the ionically-crosslinked polysaccharide gel.

In another aspect, the present invention provides a DBM device comprising a dried, flexible unitary body comprising an ionically-crosslinked polysaccharide gel and bone-derived particles having pores free from the ionically-crosslinked polysaccharide gel. Methods of using such DBM devices for implantation to promote bone growth also form aspects of the invention.

In another aspect, the present invention provides a sheet-form DBM device that may provide a persistent collagen scaffold in use. The device may comprise a resilient, porous sheet including particulate solids, wherein the particulate solids contain a particulate collagen material and a bone-derived particle, *e.g.*, DBM particles and demineralized bone chips. Particles of the particulate collagen material may be individually chemically or otherwise crosslinked, and the solids are retained in the resilient, porous sheet form by an ionically-crosslinked polysaccharide gel. The DBM material retained in the resilient, porous sheet may exhibit osteoinductivity.

In another aspect, the invention provides a DBM device that includes a three-dimensionally stable, porous implant structure which comprises particulate solids. The particulate solids may include a particulate collagen material and a particulate DBM material in a weight ratio of 1:12 to about 1:2, respectively. The particulate solids are retained in the porous implant structure form by an ionically-crosslinked polysaccharide gel. The bone-derived particles retained in the porous implant structure may exhibit osteoinductivity.

In another aspect, the invention provides a DBM device that includes a three-dimensionally stable, porous implant structure. The three-dimensionally stable, porous implant structure comprises particulate solids which include a fragmented bioresorbable sponge material and a particulate DBM material. The fragmented bioresorbable sponge material may comprise a sponge material that has been rendered to particulate form, such as a randomly fragmented (*e.g.*, milled) sponge material. The particulate solids are retained in the porous implant structure form by an ionically-crosslinked polysaccharide gel. The DBM material retained in the porous implant structure may exhibit osteoinductivity.

In another aspect, the invention provides a method for preparing a DBM implant material. The method includes preparing an admixture including DBM particles in a liquid medium containing a polysaccharide susceptible to ionic crosslinking, optionally the method may also include admixing individually chemically or otherwise crosslinked collagen particles. The admixture is contacted with a liquid medium containing a multivalent ionic agent effective to ionically crosslink the polysaccharide so as to form an ionically-crosslinked gel incorporating the DBM and, optionally, the collagen particles. In addition, the method may include the steps of freezing the ionically-crosslinked gel to form a frozen precursor material, and drying the frozen precursor material.

In another embodiment, the invention provides a method for preparing a DBM implant material. The method includes providing an admixture including collagen particles and DBM particles in a liquid medium containing a polysaccharide susceptible to ionic crosslinking, with the collagen particles and DBM particles present in a weight ratio of about 1:12 to about 1:2. The admixture is contacted with a liquid medium containing a multivalent ionic agent effective to ionically crosslink the polysaccharide, so as to form an ionically-crosslinked gel incorporating the DBM particles and collagen particles. The method may include the further steps of freezing the ionically-crosslinked gel to form a frozen precursor material, and drying the frozen precursor material.

In another embodiment, the invention provides a method for preparing a DBM implant material. The method includes providing an admixture including a particulated bioresorbable sponge material and osteoinductive DBM particles in a liquid medium containing a polysaccharide susceptible to ionic crosslinking. The admixture is contacted with a liquid medium containing a multivalent ionic species effective to ionically crosslink the polysaccharide, so as to form an ionically-crosslinked gel incorporating the particulated bioresorbable sponge material and DBM particles. The method may include the additional steps of freezing the ionically-crosslinked gel to form a frozen precursor material, and drying the frozen precursor material.

In another embodiment, the invention provides a bioactive demineralized bone matrix (DBM) implant device comprising a dried, porous implant body including osteoinductive DBM particles held in intimate contact with one another by an ionically-crosslinked polysaccharide matrix.

In still further embodiments, the invention provides methods of treating patients comprising implanting in the patients DBM devices as described herein, and the manufacture of a bone graft substitute and/or bone void filler for the treatment of voids or gaps in bone. Bone voids or gaps can be surgically created defects or bony defects resulting from traumatic injury and/or cancer.

All of the compositions of the invention provide excellent handling characteristics, osteoinductivity, validated viral inactivation and/or device-level sterility.

In another embodiment, the compositions of the invention are prepared and packaged in a sterile condition.

Additional aspects and embodiments, as well as features and advantages thereof, will be apparent to those skilled in the art from the descriptions herein.

BREIF DESCRIPTION OF THE FIGURES

FIG. 1 provides a perspective view of a DBM-containing medical device of the invention.

DETAILED DESCRIPTION OF THE INVENTION

For the purposes of promoting an understanding of the principles of the invention, reference will now be made to certain embodiments and specific language will be used to describe the same. It will nevertheless be understood that no limitation of the scope of the invention is thereby intended, such alterations and further modifications in the illustrated device, and such further applications of the principles of the invention as described herein being contemplated as would normally occur to one skilled in the art to which the invention relates.

The uses of the terms "a" and "an" and "the" and similar references in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context.

Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein.

All methods described herein may be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

As used herein, “comprising,” “including,” “containing,” “characterized by,” and grammatical equivalents thereof are inclusive or open-ended terms that do not exclude additional, unrecited elements or method steps, but will also be understood to include the more restrictive terms “consisting of” and “consisting essentially of.”

As disclosed herein, in certain aspects, the present invention relates to implantable medical devices, and to methods for making and using the devices. In particular embodiments, the present invention provides osteoinductive medical devices that include a three-dimensionally stable structure incorporating particulate solids. The solids include bone-derived particles that in particular embodiments are osteoinductive when implanted in a mammalian patient, including a human patient. The solids may optionally include a particulate collagen material, such as collagen fibers or a randomly fragmented bioresorbable material such as a collagen sponge. In certain embodiments, at least some of the collagen particles that are incorporated in the device are individually populated with non-native crosslinks, *e.g.*, by chemical, dehydrothermal, radiation or other crosslinking techniques, to increase their persistence as a scaffold at the implant site after implantation.

Devices of the invention include an ionically-crosslinked polysaccharide matrix which advantageously facilitates holding the solids in a defined form or shape. The inventive devices desirably retain the DBM in its bioactive, osteoinductive condition, while also providing beneficial properties during handling and implantation.

The incorporated solids or bone-derived particles of the disclosed devices include demineralized bone matrix particles (DBM particles) and demineralized bone chips. In this regard, as used herein, the term "DBM particles" refers to a matrix material prepared by demineralizing any bone source, including cortical and/or cancellous bone. More desirable demineralized bone matrix materials will contain less than about 5% by weight of residual calcium, less than about 4% by weight residual calcium, less than about 3% by weight residual calcium, less than about 2% by weight residual calcium, less than about 1% by weight residual calcium, less than about 0.5% by weight residual calcium, less than about 0.25% by weight residual calcium, less than about 0.1% by weight residual calcium, or less than about 0.05% by weight residual calcium. The source bone may be from any suitable source including autogenic, allogeneic, and/or xenogenic bone. When used in describing a demineralized bone matrix (DBM) or bone-derived paricles, the term "osteoinductive" refers to the ability of the material to induce bone growth. Alternatively, DBM or bone-derived particles may be provided lacking osteoinductive character, and nonetheless be used as osteoconductive materials that provide a scaffold capable of receiving bone growth induced by natural healing processes or other materials implanted in the patient.

DBM materials for use in the present invention may be obtained commercially or may be prepared by known techniques. In general, osteoinductive DBM materials may be prepared by decalcification of cortical and/or cancellous bone, which is often done by way of an acid extraction technique. This process may be conducted so as to retain collagen, noncollagenous proteins, and growth factors in the solid matrix. Methods for preparing such bioactive demineralized bone matrix are well known, for example, see U.S. Patent Nos. 5,073,373; 5,484,601; and 5,284,655. DBM products are also available commercially, including for instance, from sources such as Regeneration Technologies, Inc. (Alachua, FL), The American Red Cross (Arlington, VA), and others. In certain embodiments, the particulate DBM material may have an average particle size of less than about 1,000 μm. For instance, the DBM material may have particle sizes in the range of

50 to 850 μm. DBM materials that are solely osteoconductive may be prepared using similar techniques that have been modified or supplemented to remove or inactivate (e.g. by crosslinking or otherwise denaturing) components in the bone matrix responsible for osteoinductivity. Osteoinductive and/or osteoconductive DBM materials used in the present invention may desirably be derived from human donor tissue, especially in regard to implant devices intended for use in human subjects.

The sponge material of the invention may be made completely or in part from a bioresorbable material other than collagen. In embodiments herein, the device is produced without the addition of collagen. For example, the particulated sponge material may be made from any of the other natural or synthetic polymers disclosed herein. Likewise, in these particulated sponge device embodiments, another binder material or technique may be used instead of the ionically-crosslinked polysaccharide matrix, including for example other gel-forming substances which may, for example, be thermally reversible gel forming agents such as gelatin. As well, the particulated sponge material may be used in the same relative amounts disclosed herein for the optional collagen materials. Further, where a sponge starting material has been chemically crosslinked with an aldehyde crosslinker such as formaldehyde or glutaraldehyde, or another suitable chemical crosslinker such as a carbodiimide, or by other techniques such as dehydrothermal or radiation-induced crosslinking, the particulated collagen or other bioresorbable material retains the chemical crosslinking and provides an advantageous, lasting scaffold for bone ingrowth. These crosslinked particulate materials may be provided as starting materials for preparing devices as disclosed herein, and therefore as incorporated in the device these particles are individually crosslinked. Thus, as used herein, the term “individually crosslinked” means that the particles include non-native crosslinks other than those which might be introduced by any crosslinking technique used to hold the solids of the device together as a unitary structure, e.g. by introducing crosslinks between respective particles after they have been cast or otherwise formed into the shape of the device.

In certain embodiments of the invention, the bioresorbable solids include an optional particulate solid collagen material and the DBM material at a collagen material:DBM material weight ratio (dry basis) of about 1:12 to about 1:2, or about 1:12 to about 1:5, or about 1:10 to about 1:5, or about 1:8 to about 1:5.

In regard to the incorporated materials considered on a dry weight basis, the particulate DBM material may constitute about 20% to about 90% of a device in some embodiments, more preferably about 50% to about 90%, and most preferably about 70% to about 85% by weight. In certain embodiments, on a dry weight basis, there may be about 5% to about 30% by weight of particulate collagen, more preferably about 8% to about 20%, and most preferably about 10% to about 15%.

In certain preferred forms, inventive devices of the invention are comprised of dried, flexible aggregate bodies containing a major amount by weight of bone-derived particles that include DBM particles alone or in combination with demineralized bone particles, and a minor amount by weight of an ionically-crosslinked polysaccharide, especially alginate. Dried DBM-containing devices of the invention may comprise at least about 70%, by weight, particulate bone-derived material(s), and/or at least 90%, by weight, particulate bone-derived material(s). The bone-derived particles may include DBM only in certain embodiments, and a combination of DBM and larger demineralized bone particles in other embodiments. The device may comprise no more than about 30% by weight of a polysaccharide, and in other embodiments, no more than about 10% by weight of the polysaccharide. Devices of such embodiments are desirably free from any particulate collagen material as disclosed herein, or at least free from any particulate collagen material derived from a tissue source other than bone, and may, in certain embodiments, have a dried, flexible matrix that consists or consists essentially of DBM and polysaccharide components, or DBM, demineralized bone chips and polysaccharide components. When using osteoinductive, particulate DBM materials, such as demineralized bone chips, as disclosed herein, the invention provides devices that may have relatively high levels of DBM and very low levels of polysaccharide, where these devices are highly bioactive, posses good integrity during handling and implant, and provide ready cellular access to concentrated volumes of the bioactive DBM (and optionally the demineralized bone particles) upon implant. In their dried form, such devices may have a bulk density in the range of about 0.2 g/cc to about 0.6 g/cc, more preferably in the range of about 0.3 g/cc to about 0.6 g/cc. The dried devices of the invention may be rehydrated prior to use in a mammal. For example, the device may be rehydrated with bone marrow aspirate, other bone derived liquids, blood cells, blood product, a liquid preparation containing stem cells or any other desirable fluid.

When the bone-derived particles of the device include both DBM particles and demineralized bone (MB) particles, in certain embodiment, the DBM particles and MB particles may be used in a dry weight ratio of about 1:4 to 4:1, more preferably about 1:2 to 2:1. The MB particles may be obtained from human or animal donors, and may suitably be bone chips having a maximum particle dimension of about 1 to about 5 mm, or about 2 to about 4 mm. As well, relatively large demineralized bone particles such as demineralized bone chips having a maximum particle dimension of about 1 to about 5 mm, or about 2 to about 4 mm, may be used instead of or in addition to the demineralized bone chips or other particles.

When used together, the particulate collagen and DBM materials may at least largely make up the weight of the device on a dry weight basis, for example, these two materials may constitute at least about 50%, at least about 70%, or at least about 80% by weight of the overall device on a dry weight basis. In certain embodiments, the water-insoluble solids in the composition may consist or consist essentially of DBM particles. As well, in other embodiments of the invention, all of the water-insoluble solids in the composition will be bioresorbable. It will be understood, however, that these parameters are not necessary to all aspects of the invention.

In certain aspects of the invention, medical devices are provided which comprise one or more polysaccharides used to form an ionically-crosslinked gel or matrix which participates in providing three-dimensional stability to the devices. Preferred are ionic polysaccharides that are capable of forming thermally irreversible ionically-crosslinked gels upon combination with divalent or other polyvalent cationic materials. Suitable polysaccharides include, as examples, plant-derived polysaccharides such as alginates and pectins, and gel-forming derivatives thereof. Aqueous solutions of such ionic polysaccharides form ionically-crosslinked gels upon contact with aqueous solutions of counter-ions. For instance, useful agents for ionically crosslinking alginate and pectin polysaccharides include cationic gelling agents, preferably including divalent or trivalent cations. Useful divalent cations for these purposes include the alkaline earth metals, especially calcium and strontium. Aluminum is a useful crosslinking trivalent cation. These ionic crosslinking agents will usually be provided by salts. Useful anionic counter-ions for the calcium or other salts are desirably selected from pharmaceutically-acceptable anions such as chlorides, gluconates, fluorides, citrates, phosphates, tartrates, sulphates, acetates, borates, and the like. An especially preferred ionic crosslinking agent for use with an alginate or pectin compound is provided by calcium chloride. The ionic polysaccharide chitosan may also be used, and may be ionically crosslinked with multivalent, anionic gelling agents. Such agents include metal polyphosphates, such as an alkali metal or ammonium polyphosphate, pyrophosphates or metaphosphates. Citrates may also be used. These anionic crosslinking agents will also usually be provided by salts. The cationic counter-ion for the polyphosphate or other salt may be any suitable, biocompatible or pharmaceutically-acceptable cation including for instance sodium, potassium, or ammonium. Many other biocompatible polysaccharides, including plant-derived and animal-derived materials, as well as corresponding ionic crosslinking agents, are known and may also be used in aspects of the present invention.

The polysaccharide will typically be incorporated into devices of the invention at a relatively low level. Accordingly, in certain embodiments, when considered on a dry weight basis, the device may contain a polysaccharide at a level of about 1% to about 20%, about 5% to about 15%, and/or about 8% to about 12%. It will be understood, however, that other amounts of polysaccharide may be used within the broader aspects of the invention. Further, in additional embodiments, the polysaccharide, and especially alginate, will be included at a level of less than about 8% by dry weight, for example about 2% to about 8% by dry weight, or about 3% to about 7% by dry weight. The polysaccharide material, when ionically crosslinked, holds the incorporated particulate solids together and contributes by providing integrity to the overall three-dimensional structure of the device. In this regard, in certain advantageous embodiments, the particulate solids may be bound together to form the device without the use of any chemical crosslinker, such as glutaraldehyde, formaldehyde or a carbodiimide, to covalently link the solid particles to one another. In such devices, the solid particulates are thus held together in the substantial or complete absence of covalent chemical crosslinks between the particles. In other embodiments, covalent chemical crosslinks may be introduced by such chemical crosslinking agents; however, where osteoinductive DBM material is used and preservation of its osteoinductive character is desired, such chemical crosslinking is desirably used at a low, controlled level that does not abrogate such osteoinductive character.

Medical grade polysaccharides suitable for use in aspects of the invention may be prepared using known techniques or purchased from commercial sources. Illustratively, purification techniques for preparing medical grade polysaccharides may include conventional separation techniques such as chromatography, membrane filtration, precipitation, extraction, or other suitable techniques. Medical grade sodium alginate may be commercially obtained, for example, from Medipol SA (Lausanne, Switzerland), or from NovaMatrix FMC Biopolymer (Philadelphia, Pennsylvania, Ultrapure PRONOVA brand (endotoxin level < 100 endotoxin units per gram)).

In some embodiments, one or more alginate polymers may contain more than 50% alpha-L-guluronic acid, for example, in malleable compositions of the invention. In other embodiments, one or more alginate polymers may contain more than 60% alpha-L-guluronic acid. In yet other embodiments, one or more alginate polymers may contain 60% to 80% alpha-L-guluronic acid.

In certain embodiments, alginate polymers used in the invention may have average molecular weights ranging from 2 to 1000 kilodaltons (kD). The molecular weight of alginates may be utilized to affect the properties of the malleable composition. Generally, lower molecular weight alginates will be more biodegradable. In some embodiments, the alginate polymers have an average molecule weight of from 5 to 350 kD. In some embodiments, the alginate polymers have an average molecule weight of from 2 to 100 kD. In some embodiments, the alginate polymers have an average molecule weight of from 50 to 500 kD. In some embodiments, the alginate polymers have an average molecule weight of from 100 to 1000 kD. The molecular weights identified in this paragraph may similarly apply to other polysaccharides when used in the invention.

The alginate, when used, may possess a viscosity in a 1% solution measured at 20 degrees centigrade of from 25 to 1000 mPas and in some embodiments, 50 to 1000 mPas (1% solution, 20°C).

Devices of the present invention may be manufactured in a ready-to-use condition and packaged in medically acceptable packaging in a sterile condition, in either wet or dry formats. In some embodiments, as illustrated in FIG. 1, the ready-to-use medical product may be a porous, sheet-form product 11. The sheet-form material may be configured as a strip or other parallelepiped three dimensional structure.

Sheet-form product 11 includes a first face 12, a second face 13, and sidewalls 14 interconnecting the first and second faces 12 and 13. Sheet-form product may have any suitable length (L), width (W) and thickness (T). In certain embodiments, the length and width will range from about 1 mm to about 200 mm, and the thickness will range from about 0.1 mm to about 10 mm. More typically, the length and width will each range from about 1 mm to about 100 mm, and the thickness from about 0.2 mm to about 1 mm. From the standpoint of volume, devices may have a unitary body defining a volume of about 0.1 mm^3 to about 40 cm^3, in the range of about 500 mm^3 to about 10,000 mm^3, and/or in the range of about 600 mm^3 to about 10,000 mm^3. Exemplary dimensions include, but are not limited to, approximately 100mm x 20mm x 5mm; approximately 50 mm x 20 mm x 5 mm; approximately 10 mm x 12 mm x 5 mm and/or approximately 8 mm x 25 mm x 5 mm. It will be understood however that other linear and volumetric dimensions may also be employed within the scope of the present invention.

The bulk densities of the devices of the invention may vary, depending upon factors such as the extent of their porosity, the densities of the incorporated materials, and the state of the device (*e.g.*, hydrated or dehydrated condition). In certain embodiments, the device will be in a dried condition (containing less than about 5% by weight of water or other liquid), and will have a bulk density in the range of about 0.1 g/cc to about 0.4 g/cc, more typically in the range of about 0.1 g/cc to about 0.3 g/cc, and in certain embodiments about 0.15 g/cc to about 0.25 g/cc. Nonetheless, as noted above these densities may vary with many factors, and bulk densities within these ranges, or that are lower or higher, may be exhibited by devices within aspects of the invention.

Devices of the invention may be substantially porous. Devices of the invention may have a void volume of at least about 40%, about 40% to 90%, especially when in a dried condition. Moderate void volume levels of about 50% to less than 70%, or higher levels in the range of 70% to 90% or more, may be controllably achieved, for instance by varying the amount of water or other liquid included in a slurry used to prepare the devices and/or varying the lyophilization conditions.

Devices of the invention may be manufactured by preparing a mixture including the DBM material, the polysaccharide, and, when used, the collagen material (or the particulated bioresorbable sponge material, be it collagenous or otherwise), along with any other liquid or solid materials to be incorporated into the device upon original manufacture. This mixture may then be contacted with a liquid medium (*e.g.*, solution) containing the ionic crosslinking agent so as to form a wetted ionically-crosslinked gel material. This wet gel material may be sterilized and packaged in a wetted state; or, the wet gel material may be dried. Such drying advantageously utilizes lyophilization or other sublimation drying techniques that involve freezing the wet gel material and drying the material while frozen. In certain embodiments, the non-crosslinked admixture of materials is charged to a liquid-penetrable mold, such as a porous mold, in a desired shape. The filled mold is then immersed in a solution of the ionic crosslinking agent to form the ionically-crosslinked gel in the shape of the mold. The resultant wetted crosslinked gel may then be packaged and sterilized as the final product, or may be dried and potentially additionally processed as described herein to provide the final product.

In certain preparative methods of the invention, one or more of the solid particulates to be bound in a crosslinked polysaccharide matrix, such as the particulate DBM, may first be impregnated with a wetting liquid prior to admixture with the polysaccharide-containing medium. The impregnating liquid may for example be an aqueous medium, such as water or phosphate buffered saline, and may occupy pores located in the internal volume of the particles. The resulting admixture of the wetted particulate and the polysaccharide medium may then be processed under conditions wherein the impregnating liquid substantially remains within the pores of the particles, thus preventing or reducing the level of polysaccharide medium infiltrating into the pores. Thus, upon ionically-crosslinking the polysaccharide and drying the matrix, the internal pores of the DBM or other particulate will remain free or substantially free from the polysaccharide. In this manner, the pores will be more readily accessible to cells upon implant of the material in a patient.

The devices of the invention may be implanted in a patient in a dried or wetted condition. When wetted, any medically acceptable wetting agent may be used. These include, for example, aqueous substances such as sterile water, physiological saline, phosphate buffered saline, blood, bone marrow, bone marrow fractions or other liquid mediums, emulsions or suspensions that provide adequate wetting characteristics. Biocompatible organic liquids may also be used, alone or in combination with water. A wide variety of biocompatible organic liquids are known and suitable for these purposes, including for instance liquid polyols such as glycerol. In desired forms, molecules of the wetting agent (e.g. water) will be taken up into the dried, ionically-crosslinked polysaccharide matrix to form a wetted, firm gel device incorporating the particulate DBM material, and any other material of the device, *e.g.*, one or more of those additional (optional) substances described herein.

Devices of the invention may also contain other beneficial substances including for example preservatives, cosolvents, suspending agents, buffering agents (e.g. carrying active agents to be added to the device) viscosity enhancing agents, ionic strength and osmolality adjusters and/or other excipients.

Devices of the invention may also include other biocompatible and/or bioresorbable substances. These materials may include, for example, natural polymers such as proteins and polypeptides, glycosaminoglycans, proteoglycans, elastin, hyaluronic acid, dermatan sulfate, or mixtures or composites thereof. Synthetic polymers may also be incorporated into the devices of the invention. These include, for example biodegradable synthetic polymers such as polylactic acid, polyglycolide, polylactic polyglycolic acid copolymers ("PLGA"), polycaprolactone ("PCL"), poly(dioxanone), poly(trimethylene carbonate) copolymers, polyglyconate, poly(propylene fumarate), poly(ethylene terephthalate), poly(butylene terephthalate), polyethyleneglycol, polycaprolactone copolymers, polyhydroxybutyrate, polyhydroxyvalerate, tyrosine-derived polycarbonates and any random or (multi-)block copolymers, such as bipolymer, terpolymer, quaterpolymer, etc., that may be polymerized from the monomers related to previously-listed homo- and copolymers.

The devices of the invention may also include a mineral component. The mineral used may include a natural or synthetic mineral that is effective to provide a scaffold for bone ingrowth. Illustratively, the mineral matrix may be selected from one or more materials consisting of bone particles, Bioglass®, tricalcium phosphate, biphasic calcium phosphate, hydroxyapatite, corraline hydroxyapatite, and biocompatible ceramics. Biphasic calcium phosphate is a particularly desirable synthetic ceramic for use in the invention. Such biphasic calcium phosphate may have a tricalcium phosphate:hydroxyapatite weight ratio of about 50:50 to about 95:5, about 70:30 to about 95:5, about 80:20 to about 90:10, and/or about 85:15. The mineral material may be a granular particulate having an average particle diameter between about 0.2 and 5.0 mm, more typically between about 0.4 and 3.0 mm, and desirably between about 0.4 and 2.0 mm.

In another aspect of the invention, the demineralized bone particles may be obtained from cancellous and/or cortical bone that is ground to provide an average particle diameter among as described herein. Both human and non-human sources of bone are suitable for use in the instant invention, and the bone may be autograft, allograft or xenograft in nature relative to the mammal to receive the implant. Appropriate pre-treatments known in the art may be used to minimize the risks of disease transmission and/or immunogenic reaction when using bone particles as or in the mineral material.

In one embodiment, xenogenic bone that has been pretreated to reduce or remove its immunogenicity is used to provide a porous mineral matrix in the implant device. For example, the bone may be calcined or deproteinized to reduce the risks of immunogenic reactions to the implant material.

Bioactive agents may be delivered with devices of the invention. These bioactive agents may include, for example, antimicrobials, antibiotics, antimyobacterial, antifungals, antivirals, antineoplastic agents, antitumor agents, agents affecting the immune response, blood calcium regulators, agents useful in glucose regulation, anticoagulants, antithrombotics, antihyperlipidemic agents, cardiac drugs, thyromimetic and antithyroid drugs, adrenergics, antihypertensive agents, cholnergics, anticholinergics, antispasmodics, antiulcer agents, skeletal and smooth muscle relaxants, prostaglandins, general inhibitors of the allergic response, antihistamines, local anesthetics, analgesics, narcotic antagonists, antitussives, sedative-hypnotic agents, anticonvulsants, antipsychotics, anti-anxiety agents, antidepressant agents, anorexigenics, non-steroidal anti-inflammatory agents, steroidal anti-inflammatory agents, antioxidants, vaso-active agents, bone-active agents, osteogenic factors, antiarthritics, and diagnostic agents.

Bioactive agents may also be provided by tissue materials incorporated into the devices, including for instance autologous or allogenic tissue materials, which are incorporated into the material to be implanted in the patient. Such tissue materials may include blood or blood fractions, bone marrow or bone marrow fractions, and/or other sources of cells or other beneficial tissue components derived from the patient to be treated or another suitable animal source. These substances can, for example, be added to the device just prior to implantation into the patient.

Bioactive agents such as those described herein may be incorporated homogeneously or regionally into the implant devices by simple admixture or otherwise. Further, they may be incorporated alone or in conjunction with another carrier form or medium such as microspheres or another microparticulate formulation. Suitable techniques for forming microparticles are well known in the art, and may be used to entrain or encapsulate bioactive agents, whereafter the microparticles may be incorporated within or upon the inventive device during or after its preparation.

In certain embodiments, a device of the invention will include one or more substances, additional to the osteoinductive DBM material, that induces or generates the formation of bone. Suitable osteogenic materials may include a growth factor that is effective in inducing formation of bone. Desirably, the growth factor will be from a class of proteins known generally as bone morphogenic proteins (BMPs), and may in certain embodiments be recombinant human (rh) BMPs. These BMP proteins, which are known to have osteogenic, chondrogenic and other growth and differentiation activities, include rhBMP-2, rhBMP-3, rhBMP4 (also referred to as rhBMP-2B), rhBMP-5, rhBMP-6, rhBMP-7 (rhOP-1), rhBMP-8, rhBMP-9, rhBMP-12, rhBMP-13, rhBMP-15, rhBMP-16, rhBMP-17, rhBMP-18, rhGDF-1, rhGDF-3, rhGDF-5, rhGDF-6, rhGDF-7, rhGDF-8, rhGDF-9, rhGDF-10, rhGDF-11, rhGDF-12, rhGDF-14. For example, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6 and BMP-7, disclosed in U.S. Pat. Nos. 5,108,922; 5,013,649; 5,116,738; 5,106,748; 5,187,076; and 5,141,905; BMP-8, disclosed in PCT publication WO91/18098; and BMP-9, disclosed in PCT publication WO93/00432, BMP-10, disclosed in U.S. Pat. No. 5,637,480; BMP-11, disclosed in U.S. Pat. No. 5,639,638, or BMP-12 or BMP-13, disclosed in U.S. Pat. No. 5,658,882, BMP-15, disclosed U.S. Pat. No. 5,635,372 and BMP-16, disclosed in U.S. Patent Nos. 5,965,403 and 6,331,612. Other compositions which may also be useful include Vgr-2, and any of the growth and differentiation factors, including those described in PCT applications WO94/15965; WO94/15949; WO95/01801; WO95/01802; WO94/21681; WO94/15966; WO95/10539; WO96/01845; WO96/02559 and others. Also useful in the present invention may be BIP, disclosed in WO94/01557; HP00269, disclosed in JP Publication number: 7-250688; and MP52, disclosed in PCT application WO93/16099. The disclosures of all of these Patents and applications are hereby incorporated herein by reference. Also useful in the present invention are heterodimers of the above and modified proteins or partial deletion products thereof. These proteins may be used individually or in mixtures of two or more. rhBMP-2 and/or rhGDF-5 are preferred.

The BMP may be recombinantly produced, or purified from a protein composition. The BMP may be homodimeric, or may be heterodimeric with other BMPs (e.g., a heterodimer composed of one monomer each of BMP-2 and BMP-6) or with other members of the TGF-beta superfamily, such as activins, inhibins and TGF-beta 1 (e.g., a heterodimer composed of one monomer each of a BMP and a related member of the TGF-beta superfamily). Examples of such heterodimeric proteins are described for example in Published PCT Patent Application WO 93/09229, the specification of which is hereby incorporated herein by reference. The amount of osteogenic protein useful herein is that amount effective to stimulate increased osteogenic activity of infiltrating progenitor cells, and will depend upon several factors including the size and nature of the defect being treated, and the device and particular protein being employed.

Other therapeutic growth factors or substances may also be used in devices of the present invention, especially those that may be used to stimulate bone formation. Such proteins are known and include, for example, platelet-derived growth factors, insulin-like growth factors, cartilage-derived morphogenic proteins, growth differentiation factors such as growth differentiation factor 5 (GDF-5), and transforming growth factors, including TGF-α and TGF-β.

The osteogenic proteins or other biologically active agents, when used in the present invention, may be provided in liquid formulations, for example buffered aqueous formulations. In certain embodiments, such liquid formulations may be received upon and/or within, or otherwise combined with a dry-form device by a health care provider just prior to implantation. In other embodiments, such liquid formulations may be included within wet materials used to prepare a dry-form or wetted device during its manufacture. One suitable rhBMP-2 formulation is available from Medtronic Sofamor Danek, Memphis, TN, with its INFUSE® Bone Graft product.

Osteoinductive devices of the present invention may also comprise progenitor and/or stem cells derived from embryonic or adult tissue sources and/or taken from culture. Illustratively, compositions of the invention may incorporate cells derived from blood, bone marrow, or other tissue sources from the patient to be treated (autologous cells) or from a suitable allogenic or xenogenic donor source. In certain embodiments of the invention, the conformable compositions incorporate an enriched bone marrow fraction, prepared for example as described in US Patent Publication No. 2005/0130301 to McKay et al. published June 16, 2005, which is hereby incorporated herein by reference in its entirety. Thus, the devices of the invention may incorporate a bone marrow fraction enriched in connective tissue growth components, which may be prepared by centrifuging a biological sample (*e.g.*, from the patient to be treated) to separate the sample into fractions including a fraction rich in connective tissue growth components. The isolated fraction that is rich in connective tissue growth components may then be incorporated into or onto a device of the invention, e.g. by using the fraction in or as a wetting medium applied to the device by a health care provider prior to implantation.

In still further embodiments, the present invention provides methods for treating patients that involve implanting in the patients an osteoinductive DBM device as described herein. In such uses, an osteoinductive DBM device may be implanted at a site at which tissue growth is desired, *e.g.*, to treat a disease, defect or location of trauma, and/or in some instances to promote artificial arthrodesis. The medical devices of the invention may be used as surgical implants at, in, on, or near bone defect sites, cartilage repair sites, or other musculoskeletal sites. In certain embodiments, the device will exhibit a conformable or flexible character that enables its introduction and shaping within voids, defects or other areas in which new tissue growth is desired, and/or in certain embodiments in which the delivery of a bioactive agent is desired. Further in this regard, the device may have compression-resistant properties sufficient to resist substantial compression when impinged by adjacent soft tissues of the body at a bony implant site, for instance at a posterolateral spinal fusion implant site.

Illustrative bone repair sites that may be treated with medical devices of the invention include, for instance, those resulting from injury, defects brought about during the course of surgery, infection, malignancy or developmental malformation. The devices may be used in a wide variety of orthopedic, periodontal, neurosurgical and oral and maxillofacial surgical procedures including, but not limited to: the repair of simple and compound fractures and non-unions; external and internal fixations; joint reconstructions such as arthrodesis; general arthroplasty; cup arthroplasty of the hip; femoral and humeral head replacement; femoral head surface replacement and total joint replacement; repairs of the vertebral column including spinal fusion and internal fixation; tumor surgery, *e.g.*, deficit filing; discectomy; laminectomy; excision of spinal cord tumors; anterior cervical and thoracic operations; repairs of spinal injuries; scoliosis, lordosis and kyphosis treatments; intermaxillary fixation of fractures; mentoplasty; temporomandibular joint replacement; alveolar ridge augmentation and reconstruction; inlay osteoimplants; implant placement and revision; sinus lifts; cosmetic enhancement; etc. Specific bones which may be repaired or replaced with the device include, but are not limited to: the ethmoid; frontal; nasal; occipital; parietal; temporal; mandible; maxilla; zygomatic; cervical vertebra; thoracic vertebra; lumbar vertebra; sacrum; rib; sternum; clavicle; scapula; humerus; radius; ulna; carpal bones; metacarpal bones; phalanges; ilium; ischium; pubis; femur; tibia; fibula; patella; calcaneus; tarsal and metatarsal bones.

In accordance with certain aspects of the invention, the osteoinductive DBM device may be used as a bone void filler, or may be incorporated in, on or around a load bearing implants such as spinal implants, hip implants (e.g. in or around implant stems and/or behind acetabular cups), knee implants (e.g. in or around stems). Some embodiments provide an osteoinductive DBM device that may be incorporated in, on or around a load-bearing spinal implant device having a compressive strength of at least about 10000 N, such as a fusion cage, dowel, or other device potentially having a pocket, chamber or other cavity for containing the osteoinductive material of the invention, and used in a spinal fusion such as an interbody fusion. One illustrative such use is in conjunction with a load-bearing interbody spinal spacer to achieve interbody fusion. In these applications, the device may be placed in and/or around the spacer to facilitate the fusion.

Illustrative cartilage repair sites that may be treated with devices of the invention include, as examples, articular cartilage surfaces occurring in articular joints having at least two major bones. Examples include, but are not limited to the elbow, wrist, phalanx, knee, and ankle. Additionally, cartilage surfaces within shoulder and hip joints may be treated.

The present invention also provides medical kits or other products that include one or more osteoinductive DBM devices of the invention. Such products may include the device(s) of the invention in a dried (e.g. having less than about 5% residual water) or wet format, received in sterile condition in medical packaging. Such products may also include one or more additional surgical instruments or implants, for example a load-bearing implant (e.g. a spinal spacer), and/or a fluid transfer device such as a syringe, and/or a therapeutic substance, for example an osteogenic substance such as a BMP. In one specific form, such a medical kit may include a dried device of the invention, a BMP in lyophilized form (e.g. rhBMP-2), and an aqueous medium for reconstitution of the BMP to prepare an aqueous formulation that may then be added to the dried device.

The invention will now be more particularly described with reference to the following specific examples. It will be understood that these examples are illustrative and not limiting of the embodiments of the invention.

EXAMPLE 1

PREPARATION OF OSTEOINDUCTIVE DBM DEVICES

A DBM strip device comprising 8.3% by dry weight of sodium alginate and 91.7% by dry weight of DBM material was prepared as follows.

9g of sodium alginate (Ultrapure Pronova MVG grade, NovaMatrix) was combined with 144 cc of water for injection in a blender. The materials were mixed in the blender until all of the alginate was incorporated to produce a mixture termed WFI. Approximately 100g of lyophilized demineralized bone matrix (DBM) was added to a mixing bowl and was combined with 200cc of the WFI. The combined WFI/alginate solution was then transferred to a mixer bowl containing the hydrated DBM and mixing was initiated. Mixing was allowed to proceed until a uniform slurry was obtained. The uniform slurry was then decanted into pre-sized molds. Example mold dimensions may be sized to produce a product that is approximately 100mm x 20mm x 5mm; approximately 50 mm x 20 mm x 5 mm; approximately 10 mm x 12 mm x 5 mm or approximately 8 mm x 25 mm x 5 mm. Once filled with slurry, the molds were transferred to a freezer and the slurry was allowed to freeze. The frozen slurry was then unmolded and transferred into a continuously agitated pool of 1-10% by weight solution of calcium chloride dihydrate for a period of 1 to 24 hours to ionically crosslink the alginate. The resulting cross-linked blocks of alginate and DBM were then removed from the crosslinking solution, and placed into a pool of water of sufficient volume to extract any residual calcium chloride solution from the cross-linked blocks. The rinsed cross-linked blocks were then lyophilized under conditions that substantially preserved the original volume of the cross-linked blocks. The resulting lyophilized materials were, porous strip devices having a thickness of about 5 mm and exhibiting a bulk density in the range of about 0.3 g/cc to 0.6 g/cc. The strips were then packaged in dual barrier trays or equivalent and terminally sterilized with an E-beam to a level of 25 kiloGrays (kGy). Strip materials prepared as described above were evaluated for osteoinductivity in the widely-accepted athymic rat model.

Implants were placed singularly in the muscle pouch of each hind limb near the femur to allow for blood supply but not adjacent to the bone. At 28 days post-operative, the rats were sacrificed via carbon dioxide inhalation. The implants were then harvested. The explanted devices were then placed in labeled histology cassettes in 10% neutral buffered formalin. Samples were decalcified, embedded in paraffin, sectioned & stained. Three sections per implant were taken at 100 micrometer intervals and stained with H&E. Semi-quantitative scoring of the samples versus controls confirmed that the implant materials were osteoinductive.

EXAMPLE 2

PREPARATION OF OSTEOINDUCTIVE DBM/CHIP DEVICES

A DBM strip device comprising 7.7% by dry weight of sodium alginate, 42.2% by dry weight of small DBM particles, and 50.1% by dry weight of demineralized bone chips (2-4 mm maximum particle dimension) was prepared using the procedures described in Example 1, except that pre-hydrated human allograft demineralized bone chips were mixed into the water/alginate material along with the DBM particles prior to transfer to the molds.

EXAMPLE 3

PREPARATION OF LOW ALGINATE DBM DEVICES

A DBM strip device comprising 4.3% by dry weight of sodium alginate and 95.7% by dry weight of DBM particles was prepared as follows.

4.5g of sodium alginate (Ultrapure Pronova MVG grade, NovaMatrix) was combined with 144 cc of water in a blender. The materials were mixed in the blender until all of the alginate was incorporated, producing WFI. Approximately 100g of lyophilized demineralized bone matrix (DBM, 710 micron powder) was added to a mixing bowl and was combined with 200cc of WFI. Optionally, demineralized bone chips may be added to, or substituted for, the DBM powder. The DBM was mixed with the WFI until all DBM was fully hydrated. The combined WFI/alginate solution was then transferred to the mixer bowl containing the hydrated DBM and mixing was initiated. Mixing was allowed to proceed until a uniform slurry was obtained. The uniform slurry was then decanted into pre-sized molds. Example mold dimensions may be sized to produce a product that is approximately 100mm x 20mm x 5mm; approximately 50 mm x 20 mm x 5 mm; approximately 10 mm x 12 mm x 5 mm or approximately 8 mm x 25 mm x 5 mm. Once filled with slurry, the molds were transferred to a freezer and the slurry was allowed to freeze. The frozen slurry was then unmolded and transferred into a continuously agitated pool of 1-10% by weight calcium chloride dihydrate solution for a period of 1 to 24 hours to ionically crosslink the alginate. The resulting cross-linked blocks of alginate and DBM were then removed from the crosslinking solution, and placed into a sufficient volume of water to extract any residual calcium chloride solution from the cross-linked blocks. The rinsed cross-linked blocks were then lyophilized under conditions to substantially preserve the original volume of the cross-linked blocks. The resulting lyophilized materials were, porous strip materials having a thickness of about 5 mm and exhibiting a bulk density in the range of about 0.3 g/cc to 0.6 g/cc. The strips were then packaged in dual barrier trays or equivalent and terminally sterilized with E-beam to a level of 25 kiloGrays (kGy).

EXAMPLE 4

LOW ALGINATE OSTEOINDUCTIVE DBM/CHIP DEVICES

A DBM strip device constituted 4.0% by dry weight of sodium alginate, 44.0% by dry weight of small DBM particles, and 52.0% by dry weight of demineralized bone chips (2-4 mm maximum particle dimension) was prepared using the procedures described in Example 3, except that pre-hydrated human allograft demineralized bone chips were mixed into the water/alginate material along with the DBM particles prior to transfer to the molds.

EXAMPLE 5

LOW ALGINATE DBM DEVICES

A DBM strip device constituted 3.0% by dry weight of sodium alginate and 97.0% by dry weight of DBM particles is prepared as follows.

3.1g of sodium alginate (Ultrapure Pronova MVG grade, NovaMatrix) are combined with 144 cc of water for injection (WFI) in a blender. The materials are mixed in the blender until all of the alginate is incorporated. Approximately 100g of lyophilized demineralized bone matrix (DBM, 710 micron powder) is added to a mixing bowl and is combined with 200cc of WFI. The DBM is mixed with the WFI until all DBM is fully hydrated. The combined WFI/alginate solution is then transferred to the mixer bowl containing the hydrated DBM and mixing is initiated. Mixing is allowed to proceed until a uniform slurry is obtained. The uniform slurry was then decanted into pre-sized molds. Example mold dimensions may be sized to produce a product that is approximately 100mm x 20mm x 5mm; approximately 50 mm x 20 mm x 5 mm; approximately 10 mm x 12 mm x 5 mm or approximately 8 mm x 25 mm x 5 mm. Once filled with slurry, the molds are transferred to a freezer and the slurry is allowed to freeze. The frozen slurry is then unmolded and transferred into a continuously agitated pool of 1-10% by weight solution of calcium chloride dihydrate for a period of 1 to 24 hours to ionically crosslink the alginate. The resulting cross-linked blocks of alginate and DBM are then removed from the crosslinking solution, and placed into a pool of water for injection in sufficient volume to extract any residual calcium chloride solution from the cross-linked blocks. The rinsed cross-linked blocks are then lyophilized under conditions to substantially preserve the original volume of the cross-linked blocks. The resulting lyophilized materials are porous strip materials having a thickness of about 5 mm and exhibiting a bulk density in the range of about 0.3 g/cc to 0.6 g/cc. The strips are then packaged in dual barrier trays or equivalent and terminally sterilized with E-beam to a level of 25 kiloGrays (kGy).

EXAMPLE 6

LOW ALGINATE OSTEOINDUCTIVE DBM/CHIP DEVICES

A DBM strip device constituted 3.0% by dry weight of sodium alginate, 45.0% by dry weight of small DBM particles, and 52.0% by dry weight of demineralized bone chips (2-4 mm maximum particle dimension) is prepared using the procedures described in Example 5, except that pre-hydrated human allograft demineralized bone chips are mixed into the water/alginate material along with the DBM particles prior to transfer to the molds.

EXAMPLE 7

PREPARATION OF OSTEOINDUCTIVE DBM DEVICES

13.3 g of sodium alginate (USP grade, Spectrum Chemical) were combined with 1680 cc of water for injection (WFI) in a blender. The materials were mixed in the blender until all of the alginate was incorporated. The combined WFI/alginate material was then transferred to a mixer bowl and mixing was initiated. 18.2 g of milled, crosslinked collagen sponge were slowly added to the mixer bowl while mixing, and the resulting materials mixed to form a uniform mixture. The milled collagen was obtained by grinding a crosslinked collagen sponge material available from Collagen Matrix, Inc. (Franklin Lakes, NJ) and known as Collagen Matrix Sponge. A retch grinder operating at 8000 rpm with a 1 mm sieve was used for this purpose, resulting in the collection of the randomized sponge particulate as a wispy mass. 100 g of osteoinductive demineralized bone matrix having a particle size of 55-850 μm were then slowly added to the bowl while mixing, which was allowed to proceed until a uniform mixture was obtained. 260 cc aliquats of the resulting slurry were measured in a graduated cylinder and transferred to 260 cc capacity stainless steel screen molds (270 mesh screen size). The dimensions of the molds were 8 inches (~20.3 cm) by 10 inches (~25.4 cm), and the slurry was pooled to a depth of about 0.5 cm. The molds were capped with a screen top and completely submerged in a vessel containing a 1% by weight solution of calcium chloride dihydrate for a period of 6 to 24 hours in order the ionically crosslink the alginate. The molds were then removed from the cross-linking solution, placed into individual lyophilization pouches, and lyophilized under conditions to substantially preserve the original volume of the charged material. The resulting lyophilized materials were flexible, solid sheet materials having a thickness of about 5 mm and exhibiting a bulk density in the range of about 0.17 g/cc to 0.21 g/cc. The sheets were cut to desired strip sizes (some 8 mm X 25 mm; some 10 mm X 12 mm; some 50 mm X 20 mm and some 100 mm X 20 mm) and packaged in trays in a double-pouch format. The packaged products were terminally sterilized with E-beam to a level of 25 kiloGrays (kGy). The packaged product may contain any number of product strips, for example, a single strip, two strips, three strips, or four stips.

Strip materials prepared as described above were evaluated for osteoinductivity in the widely-accepted athymic rat model. Implants were placed singularly in the muscle pouch of each hind limb near the femur to allow for blood supply but not adjacent to the bone. At 28 days post-operative, the rats were sacrificed via carbon dioxide inhalation. The implants were then harvested. Explants were placed in labeled histology cassettes in 10% neutral buffered formalin. Samples were decalcified, embedded in paraffin, sectioned & stained. Three sections per implant were taken at 100 micrometer intervals and stained with H&E. Semi-quantitative scoring of the samples versus controls confirmed that the implant materials were osteoinductive.

While the invention has been illustrated and described in detail in the drawings and foregoing description, the same is to be considered as illustrative and not restrictive in character, it being understood that only the preferred embodiment has been shown and described and that all changes and modifications that come within the spirit of the invention are desired to be protected. In addition, all publications cited herein are hereby incorporated by reference in their entirety.

CLAIMS

WHAT IS CLAIMED IS:

1. A demineralized bone matrix device, comprising:

   a dried, flexible aggregate of bone-derived particles retained in a unitary body by an ionically-crosslinked polysaccharide gel, the bone-derived particles including demineralized bone matrix (DBM) particles;

   wherein the bone-derived particles constitute at least 90% by weight of the unitary body on a dry weight basis; and

   wherein the polysaccharide constitutes no more than 10% by weight of the unitary body on a dry weight basis.

2. The demineralized bone matrix device of claim 1, wherein the polysaccharide is alginate.

3. The demineralized bone matrix device of claim 1 or claim 2, wherein the alginate constitutes no more than about 8% by dry weight of the body.

4. The demineralized bone matrix device of claim 1, claim 2 or claim 3, wherein the DBM particles comprise demineralized bone chips having pores free from the polysaccharide gel.

5. The demineralized bone matrix device of any one of claims 1 to 4, wherein the bone-derived particles are osteoinductive.

6 The demineralized bone matrix device of any one of claims 1 to 5, wherein the unitary body is comprised of at least 70% by dry weight bone-derived particles.

7. The demineralized bone matrix device of any one of claims 1 to 6, wherein the unitary body has a volume of between 500 mm^3 and 20,000 mm^3.

8. The demineralized bone matrix device of any one of claims 1 to 6, wherein the unitary body has a volume of between 600 mm^3 and 10,000 mm^3.

9. The demineralized bone matrix device of any one of claims 1 to 8, wherein the unitary body has dimensions selected from the group consisting of: approximately 100 mm x 20mm x 5mm; approximately 50 mm x 20 mm x 5 mm; approximately 10 mm x 12 mm x 5 mm and approximately 8 mm x 25 mm x 5 mm.

10. A method for preparing a demineralized bone matrix (DBM) device, comprising:
    providing wetted bone-derived particles, wherein the particles have pores occupied by a wetting liquid;
    blending the wetted bone-derived particles into a gellable liquid medium comprising an ionically-crosslinkable polysaccharide under conditions wherein the gellable liquid medium does not displace the wetting liquid from the pores;
    ionically crosslinking the polysaccharide to form a unitary body comprising an ionically-crosslinked polysaccharide gel and the bone-derived particles; and
    drying the unitary body to form a dried material in which bone-derived particles have pores free from the ionically-crosslinked polysaccharide gel.

11. The method according to claim 10, wherein the wetting liquid is an aqueous medium.

12. The method according to claim 10 or claim 11, wherein the polysaccharide is alginate.

13. A demineralized bone matrix device produced by the method according to claim 10, claim 11 or claim 12.

14. Use of a demineralized bone matrix device according to any one of claims 1 to 8 for the treatment of a bone defect.

15. Use of a demineralized bone matrix device according to any one of claims 1 to 8 for the manufacture of a medicament for the treatment of a bone defect.

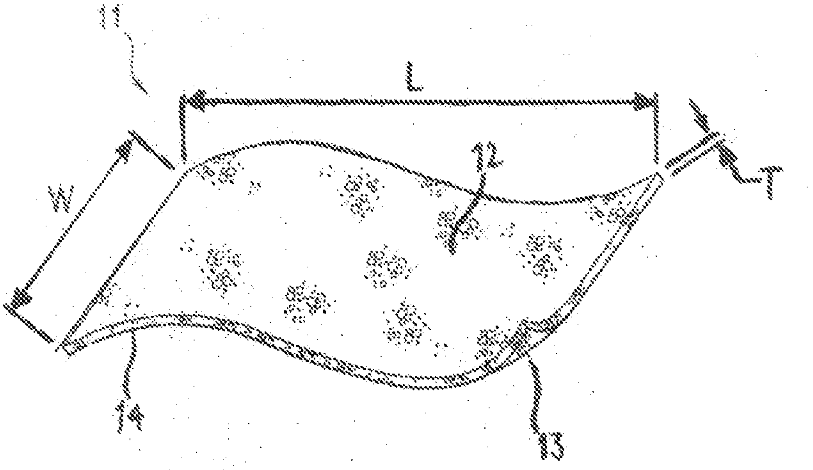

Fig. 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2008/081544

A. CLASSIFICATION OF SUBJECT MATTER

A61L 27/40(2006.01)i, A61L 24/00(2006.01)i, A61L 31/00(2006.01)i, A61L 27/00(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: A61L 27/40, A61L 24/00, A61L 27/00, A61L 31/00, A61K 9/14, A61K 35/37, A61K 38/18, A61F 2/00, A61F 2/28

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean Utility models and applications for Utility models since 1975
Japanese Utility models and applications for Utility models since 1975

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKIPASS(KIPO internal) & keywords: demineralized bone matrix, bone-derived, crosslink, polysaccharide, alginate, ionically

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2007/0254041 A1 (DRAPEAU, SUSAN J. et al.) 1 November 2007 See abstract, paragraphs 5-7, 11, 23, 46, claims 21, 23, 24, 30, 53-55	1-3, 10-12
A	US 6340477 B1 (ANDERSON, BILLY G.) 22 January 2002 See abstract, columns 4-5, claims 1, 3	1-3, 10-12
A	US 2001/0014667 A1 (CHEN, CHARLES C. and JEFFERIES, STEVEN R.) 16 August 2001 See abstract, paragraphs 13-20, claims 1-5	1-3, 10-12
A	US 2004/0097612 A1 (ROSENBERG, ARON D. and PELICHY, LAURENT GILLES DE) 20 May 2004 See abstract, paragraphs 11, 53, 63, claims 1, 8	1-3, 10-12
A	US 7163691 B2 (KNAACK, DAVID et al.) 16 January 2007 See abstract, column 2, claim 1	1-3, 10-12

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

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

Date of the actual completion of the international search 29 MAY 2009 (29.05.2009)	Date of mailing of the international search report **29 MAY 2009 (29.05.2009)**
Name and mailing address of the ISA/KR Korean Intellectual Property Office Government Complex-Daejeon, 139 Seonsa-ro, Seo-gu, Daejeon 302-701, Republic of Korea Facsimile No. 82-42-472-7140	Authorized officer Yang, Kyung Shik Telephone No. 82-42-481-8404

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2008/081544

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

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

3. ☒ Claims Nos.: 4-9, 13-15
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

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

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

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

Remark on Protest

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

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2008/081544

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2007-0254041 A1	01.11.2007	None	
US 6340477 B1	22.01.2002	None	
US 2001-0014667 A1	16.08.2001	US 6180605 B1	30.01.2001
		US 6180606 B1	30.01.2001
US 2004-0097612 A1	20.05.2004	AU 2003-290884 A1	15.06.2004
		CA 2505737 A1	03.06.2004
		EP 1567044 A2	31.08.2005
		JP 2006-508715 T	16.03.2006
		KR 10-2005-0075416	20.07.2005
		WO 2004-045372 A2	03.06.2004
US 7163691 B2	16.01.2007	US 2003-0143258 A1	31.07.2003