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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
13 November 2008 (13.11.2008)

PCT

(10) International Publication Number
WO 2008/134814 A1

(51) International Patent Classification:
A61P 29/00 (2006.01) *A61L 27/50* (2006.01)
A61K 35/32 (2006.01) *A61L 31/14* (2006.01)

(21) International Application Number:
PCT/AU2008/000627

(22) International Filing Date: 5 May 2008 (05.05.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
2007902394 4 May 2007 (04.05.2007) AU

(71) Applicant *(for all designated States except US)*: **PERTH BONE & TISSUE BANK** [AU/AU]; Gate 3, Verdun Street, Nedlands, Western Australia 6008 (AU).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **FAN, Ying** [AU/AU]; 2 Kingsland Avenue, City Beach, Western Australia 6015 (AU). **ZHENG, Ming-Hao** [AU/AU]; 2 Kingsland Avenue, City Beach, Western Australia 6015 (AU).

(74) Agent: **GRIFFITH HACK**; 109 St George's Terrace, Perth, Western Australia 6000 (AU).

(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, 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*

WO 2008/134814 A1

(54) Title: A METHOD FOR TREATING INFLAMMATION AND CONTROLLED-RELEASE MATERIAL CAPABLE OF PROVIDING SAME

(57) Abstract: The present invention relates to methods for treating inflammation associated with bone, joint or connective tissue and an implantable controlled-release material capable of providing these anti-inflammatory activities. In particular, the present invention relates to a method for reducing inflammation in a subject's tissue comprising the step of implanting a material comprising allogenic bone gel into or adjacent to said tissue, wherein said allogenic bone gel reduces the inflammation.

A METHOD FOR TREATING INFLAMMATION AND CONTROLLED-RELEASE MATERIAL CAPABLE OF PROVIDING SAME

FIELD

The present invention relates to methods for treating inflammation associated with bone, joint or connective tissue and an implantable controlled-release material capable of providing these anti-inflammatory activities. In particular the present invention relates to a method for reducing inflammation in a subject's tissue by implanting therein a material comprising allogenic bone gelatin (ABG) as described herein.

BACKGROUND

Inflammation is normally a localized, protective response to trauma or microbial invasion that destroys, dilutes, or walls-off the injurious agent and the injured tissue. It is most often characterized by dilation of the microvasculature, leakages of the elements of blood into the interstitial spaces, and migration of polymorphonuclear leukocytes into the inflamed tissue. On a macroscopic level, this is usually accompanied by the familiar clinical signs of erythema (redness), oedema (fluid build up), hyperalgesia (tenderness), heat, and pain. During this complex response, chemical mediators such as histamine, 5-hydroxytryptamine, various chemotactic factors, bradykinin, leukotrienes, and prostaglandins are liberated locally. Phagocytic cells migrate into the area, and cellular lysosomal membranes may be ruptured, releasing lytic enzymes. All of these events may contribute to the inflammatory response.

While inflammation commonly occurs as a defensive response to invasion of the host by foreign material, it is also triggered by a response to mechanical trauma, toxins, and

neoplasia. Excessive inflammation caused by abnormal recognition of host tissue as foreign, or prolongation of the inflammatory process, may lead to inflammatory diseases such as rheumatoid arthritis and osteolysis.

In recent years the use of implantable material has increased dramatically in the field of orthopaedics. There is a variety of apparatus and methods for reducing, fixing and generally assisting the healing of fractured or grafted bone using these implantable materials. However, recognition of implants as foreign bodies by the immune system can often trigger the recruitment of killer cells to their host tissue interface leading to tissue inflammation, unwanted cell growth, aseptic loosening of joint implants or rejection. Thus, one of the most significant factors in the success or failure of orthopaedic surgery is the effect of general and local inflammation.

Inflammation is traditionally treated with anti-inflammatory, analgesic, and/or anti-pyretic drugs, which form a heterogeneous group of compounds, often chemically unrelated, which nevertheless share certain therapeutic actions and side-effects. Corticosteroids represent the most widely used class of compounds for the treatment of the inflammatory response. Proteolytic enzymes represent another class of compounds which are thought to have anti-inflammatory effects. Hormones which directly or indirectly cause the adrenal cortex to produce and secrete steroids represent another class of anti-inflammatory compounds. A number of non-hormonal anti-inflammatory agents have been described. These agents are generally referred to as non-steroidal anti-inflammatory drugs (NSAIDS). Among these, the most widely used are the salicylates. Acetylsalicylic acid, or aspirin, is the most widely prescribed analgesic-antipyretic and anti-inflammatory agent. Examples of steroidal and non-

steroidal anti-inflammatory agents are listed in the Physicians Desk Reference, 54th Edition, 2000 (see pp. 202 and 217 for an index of such preparations).

To date, the majority of these anti-inflammatory agents are administered by traditional routes such as subcutaneous, intravenous or intramuscular injections. In recent times a number of authors have reported the delivery of anti-inflammatory or therapeutic agents directly to sites of orthopaedic surgery. However, the vast majority of these reports failed to report that many of these trials failed to provide adequate benefits to the patient as the agents used defused too rapidly from the wound site or in some cases exacerbated the inflammation.

Thus, there is a continuing need for methods and materials for treating inflammation, especially associated with orthopaedic conditions. Moreover, there is a need for a material that is capable of providing the controlled-release of biologically active agents *in situ*, especially when associated with the use of implantable material, devices and/or orthopaedic surgical techniques, such that inflammation is reduced or eliminated.

SUMMARY

The inventors have previously developed a method of producing insoluble bone gelatin (see US Pat. Applc. No. 20030065392 to Zheng *et al.*) useful in lumbar fusion surgery. However, they have surprisingly discovered that a modified form of the insoluble bone gelatin produced by the method of Urist and colleagues termed herein allogenic bone gel (ABG) is anti-inflammatory *per se*, which is capable of overcoming or at least alleviating the problems identified above. Moreover, the ABG is capable of providing an implantable material for the controlled-release of other agents *in situ*.

Accordingly, in a first aspect, the present invention provides a method for reducing inflammation in a subject's tissue comprising the step of implanting a material comprising allogenic bone gel into or adjacent to said tissue, wherein said allogenic bone gel reduces the inflammation.

It will be appreciated by those skilled in the art that the four classic symptoms of inflammation are redness, elevated temperature, swelling, and pain in the affected area. Therefore, the implantable material of the present invention is suitable for inhibiting one or more of these four symptoms of inflammation. The implantable material is also suitable for inhibiting the influx of polymorphonuclear leukocytes (PMNs) into a tissue involved in inflammation.

Thus, in a second aspect the present invention provides a method for reducing polymorphonuclear leukocytes in a subject's tissue comprising the step of implanting a material comprising allogenic bone gel into or adjacent to said tissue, wherein said allogenic bone gel reduces the number of polymorphonuclear leukocytes present by at least 3 fold.

The invention furthermore relates to the medical uses of allogenic bone gel (ABG) as an inhibitor of inflammation, wherein said material is used locally as a topical agent or as a coating for biological implants such as medical devices.

Accordingly, in a third aspect the present invention provides an implantable anti-inflammatory material comprising allogenic bone gel, which gel provides at least a 3 fold reduction in the number of polymorphonuclear

leukocytes in a subject's tissue when said gel is topically applied on to or adjacent to said tissue.

In a fourth aspect the present invention provides a medical device coated with allogenic bone gel, which device, when implanted, results in a reduction in inflammation compared to the level of inflammation produced by the implantation of the same device not coated with allogenic bone gel.

In some aspects, the inflammation is cytokine-induced inflammation. In other aspects, the inflammation is associated with bone disorders such as osteolysis.

It will be appreciated that the implantable anti-inflammatory material of the present invention substantially comprises allogenic bone gel *per se*. For example, the implantable material preferably includes at least 15% (w/w) allogenic bone gel (ABG). Desirably, the implantable material comprises at least 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or even 90% (w/w) ABG. However, the implantable anti-inflammatory material of the invention optionally includes a supplementary material selected from bioerodible materials (e.g., biodegradable and bioresorbable materials) and non-erodible materials. Bioerodible materials include polysaccharides, nucleic acids, carbohydrates, proteins, polypeptides, poly(.alpha.-hydroxy acids), poly(lactones), poly(amino acids), poly(anhydrides), poly(orthoesters), poly (anhydride-co-imides), poly(orthocarbonates), poly(.alpha.-hydroxy alkanoates), poly(dioxanones), poly(phosphoesters), or copolymers thereof. Desirably, the bioerodible material includes collagen, glycogen, chitin, starch, keratins, silk, hyaluronic acid, poly(L-lactide) (PLLA), poly(D,L-lactide) (PDLLA), polyglycolide (PGA), poly(lactide-co-glycolide (PLGA), poly(L-lactide-co-D, L-lactide),

poly(D,L-lactide-co-trimethylene carbonate), polyhydroxybutyrate (PHB), poly(.epsilon.-caprolactone), poly(.delta.-valerolactone), poly(.gamma.-butyrolactone), poly(caprolactone), or copolymers thereof. Non-erodible materials include dextrans, celluloses and cellulose derivatives (e.g., methylcellulose, carboxy methylcellulose, hydroxypropyl methylcellulose, and hydroxyethyl cellulose), polyethylene, polymethylmethacrylate, carbon fibers, poly(ethylene glycol), poly(ethylene oxide), poly(vinyl alcohol), poly(vinylpyrrolidone), poly(ethyloxazoline), poly(ethylene oxide)-co-poly(propylene oxide) block copolymers, poly(ethylene terephthalate)polyamide, or copolymers thereof. Bioerodible and non-erodible materials can be selected to introduce porosity or modify physical properties, such as strength and viscosity.

The anti-inflammatory implantable material of the invention optionally includes a biologically active agent. Biologically active agents that can be used in the compositions and methods described herein include, without limitation, osteogenic proteins, antibiotics, polynucleotides, anti-cancer agents, growth factors, and vaccines. Osteogenic proteins include, without limitation, BMP-2, BMP-3, BMP-3b, BMP-4, BMP-5, BMP-6, BMP-7, BMP-8, BMP-9, BMP-10, BMP-1 1, BMP-12, BMP-13, BMP-14, BMP-15, BMP-16, BMP-17, and BMP-18. Biologically active agents also include alkylating agents, platinum agents, antimetabolites, topoisomerase inhibitors, antitumor antibiotics, antimitotic agents, aromatase inhibitors, thymidylate synthase inhibitors, demineralized bone matrix, DNA antagonists, farnesyltransferase inhibitors, pump inhibitors, histone acetyltransferase inhibitors, metalloproteinase inhibitors, ribonucleoside reductase inhibitors, TNF alpha agonists, TNF alpha antagonists, endothelin A receptor antagonists, retinoic acid receptor agonists, immuno-modulators, hormonal agents, antihormonal

agents, photodynamic agents, and tyrosine kinase inhibitors.

Accordingly, in a fifth aspect, the present invention provides an implantable, anti-inflammatory controlled-release material comprising at least 15% w/w allogenic bone gel, at least one supplementary material and at least one biologically active agent, wherein said biologically active agent supplements the anti-inflammatory effect of the allogenic bone gel.

In some embodiments, the biologically active agents are BMP2 and/or OP1.

In a sixth aspect the present invention provides a controlled-release, implantable anti-inflammatory material consisting essentially of allogenic bone gel and bone-morphogenetic protein-7 (OP-1) and/or bone-morphogenetic proteins (BMP)-2.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the histo-pathological samples from sham operation + saline injection group.

Figure 2 shows the histo-pathological samples from sham operation + LPS injection group.

Figure 3 shows the histo-pathological samples from the allogenic bone gel + PLS injection group

Figure 4 shows the histo-pathological samples from the Lycoll™ + LPS group.

DETAILED DESCRIPTION

Before describing the present invention in detail, it is to be understood that this invention is not limited to particularly exemplified methods and may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting which will be limited only by the appended claims.

All publications, patents and patent applications cited herein, whether *supra* or *infra*, are hereby incorporated by reference in their entirety. However, publications mentioned herein are cited for the purpose of describing and disclosing the protocols and reagents which are reported in the publications and which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, and recombinant DNA, which are within the skill of the art. Such techniques are described in the literature. See, for example, Bailey & Ollis, 1986, "Biochemical Engineering Fundamentals", 2nd Ed., McGraw-Hill, Toronto; Coligan *et al.*, 1999, "Current protocols in Protein Science" Volume I and II (John Wiley & Sons Inc.); "DNA Cloning: A Practical Approach", Volumes I and II (Glover ed., 1985); Handbook of Experimental Immunology, Volumes I-IV (Weir & Blackwell, eds., 1986); Immunochemical Methods in Cell and Molecular Biology (Mayer and Walker, eds., Academic Press, London, 1987), Methods in Enzymology, Vols. 154 and 155 (Wu *et al.* eds. 1987); "Molecular Cloning: A Laboratory

Manual", 2nd Ed., (ed. by Sambrook, Fritsch and Maniatis) (Cold Spring Harbor Laboratory Press: 1989); "Nucleic Acid Hybridization", (Hames & Higgins eds. 1984); "Oligonucleotide Synthesis" (Gait ed., 1984); Remington's Pharmaceutical Sciences, 17th Edition, Mack Publishing Company, Easton, Pennsylvania, USA.; "The Merck Index", 12th Edition (1996), Therapeutic Category and Biological Activity Index; and "Transcription & Translation", (Hames & Higgins eds. 1984).

It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a protein" includes a plurality of such proteins, and a reference to "an agent" is a reference to one or more agents, and so forth. 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 invention belongs. Although any materials and methods similar or equivalent to those described herein can be used to practice or test the present invention, the preferred materials and methods are now described.

In its broadest aspect the present invention encompasses an allogenic bone gel, which, on implantation, reduces inflammation.

The term "allogenic bone gel" (ABG) as used herein refers to a modified form of "insoluble bone gelatin" (ISBG) as compared to the ISBG produced by Urist and others, which can be prepared by the methods disclosed herein to produce a material that has anti-inflammatory properties. The allogenic bone gel generally comprises bone morphogenic protein (BMP), fibroblast growth factors (FGF), transforming growth factor beta (TGF-β), and growth factor binding proteins eg insulin-like growth factor (IGF) and

BMP binding protein and any combination thereof. In particular, the ABG of the present invention has the features shown in the properties section of Table 1.

"Insoluble bone gelation" is a product produced from demineralised bone matrix (BDM). DBM has been readily available for over ten years and is essentially milled (powdered) bone that has been treated with acid and/or EDTA to demineralise the bone i.e. remove calcium and/or phosphate while retaining lipids, collagen and non-collagenous proteins, including growth factors. The term "DBM" is well understood in the art and is described, for example, in Nimni, "Polypeptide Growth Factors: Targeted Delivery Systems," *Biomaterials*, 10:1201-1225 (1997), incorporated herein by this reference, and articles referenced therein. In general, DBM is prepared from cortical bone of various animal sources. It is purified by a variety of procedures for the removal of non-collagenous proteins and other antigenic determinants. It typically consists of more than 99% Type I collagen. The DBM can be, for example, human DBM or rat DBM; DBM from other species can alternatively be used. For example, the DBM can be DBM from another animal such as a cow, a horse, a pig, a dog, a cat, a sheep, or another socially or economically important animal species.

DBM, which contains a mixture of bone morphogenic proteins (BMPs), consistently induces formation of new bone with a quantity of powdered matrices in the 10-25 mg range, while less than 10 mg fails to induce bone formation. Accordingly, in attempts to produce better DBM, different processes have been investigated including those disclosed in (Muthukumaran *et al.*, 1988, *Col. Rel. Res.*, 8:433-441; Hammonds, *et al.*, 1991, *Mol. Endocrinol.*, 5:149-155; Ripamonti *et al.*, 1992, *Matrix*, 12:202-212; Ripamonti *et al.*, 1992, *Plast. Reconstr. Surg.*, 89:731-739).

Generally, all of these methods produce DBM's which have the same inherent problems as the more traditional methods e.g. the products produced are comprised mainly of collagen, wherein the growth factors normally associated with bone are bound by binding proteins such that they are not readily available to patients' cells on administration. In recognition of this Urist and others have investigated the production and use of insoluble bone gelatin, which is a product produced by the further processing of DBM. Methods for isolating and purifying insoluble bone gelatin (ISBG) including, for example, in US Pat. No. 4,294,753, as well as Urist *et al*. 1973, *PNAS*, 70;12, pp 3511-3525 are well known. While Urist appreciated that DBM was not as capable of inducing bone formation as it should have been and that the lack of growth factors, especially BMP's was probably the cause, the methods disclosed by Urist had major flaws. For example, by treating DBM with chloroform and methanol many of the growth factors such as fibroblast growth factor (FGF) and transforming growth factor beta (TGF-β) were effectively removed. Moreover, the material produced by Urist was not sufficiently pliable to be used in most orthopaedic settings. Table 1 compares the preparation, physical and biochemical properties of DBM, ISBG and ABG.

Table 1

	ABG	ISBG Urist *et al.* 1973, *PNAS.*, 70;12, pp 3511-3525	DBM Chen *et al.* US 6,180,606	DBM Nimni, 1997, Biomaterials 10:1201-1225
Preparation	Washed in saline	Washed in saline	Washed in saline	Chloroform-methanol extraction of bone tissue. Dried overnight
	Mechanical grinding at 4°C to less than 1000 micron particle	Mechanical cutting at RT to ~3000 micron size	Mechanical grinding at 4°C to various sizes	Mechanical grinding at RT to 72-850 micron particles
	Decalcified in HCl for less than 24 h	Decalcified in HCl at least 24 h	Decalcified in HCl at least than 24 h	Decalcified in HCl for less than 24 h
	No chloroform-methanol extraction	Chloroform-methanol extraction of bone tissue	No Chloroform-methanol extraction used	N/A
	Treated with EDTA/$CaCl_2$; less than 24 h in order to enhance growth factor activity	Treated with either LiCl (24h)/EDTA (4h)/$CaCl_2$ (24h)	HCl only. Time dependent on the amount of calcium required to be removed	N/A
	Process at 4°C/below	Ambient to 4°C	Ambient to 4°C	Ambient to 4°C
	N/A	N/A	Treated with chaotropic agent (& protease)	N/A
Physical properties	Yellow-cream colour	White colour	White colour	White colour
	Gelatin-like	Rubber-like	Powder	Powder
	Very soft	soft	soft	soft
Biochemical properties	BMP's, FGF, TGF-β, IGF and growth factor binding proteins eg IGF and BMP binding protein and any combination	BMP only	Only Type I collagen	99% Type I collagen, BMP's; growth factors generally not active

In some embodiments of the present invention, ABG is prepared from milled bone powder up to about 1.0 millimetre particles (1000 microns). The powdered bone is pre-washed with saline at 35-55°C, preferably 40-45°C for 5 minutes. This washing procedure replaced the chloroform and methanol solution as described by Urist. The washing with warm saline removed lipids and bone marrow cells in the tissue. Using this procedure, 80% of lipids and bone marrow cells were removed at the end of washing. The bone powder rinsed with saline is clear, moist and not overly dry as compared to bone powder treated with a solution of chloroform and methanol.

The milled bone powder is then demineralized using an acid such as hydrochloric acid or acetic acid, then treated with a neutralizing salt such as calcium chloride or calcium phosphate, and then treated with a stabilizer such as ethylene diamine tetraacetic acid (EDTA) all at 4°C. The resulting ABG is then treated with sterilized water. The entire procedure takes approximately 48 hours as it is desirable to reduce the amount of processing time in order to maximize the amount of liable growth factors retained in the ABG. It should be noted that no chloroform or methanol extraction is used in the process.

The following two protocols are particular useful in the present invention; however, it will be appreciated by those skilled in the art that variations can be undertook without adversely affecting the ABG produced.

Protocol 1

Bone powders prepared by the method described above were treated as follows:

Step 1 0.6 N HCl up to 24 hours at 4°C;
Step 2 2.0 M $CaCl_2$ for 24 hours at 4°C;

Step 3 0.5 M EDTA for 24 hours at 4°C;
Step 4 8.0 M LiCl for 4 hours at 4°C; and
Step 5 sterilized H_2O for 4 hours at 55°C.

Protocol 2

Bone powders prepared by the method described above were treated as follows:

Step 1 0.6 N HCl up to 12 hours at 4°C;
Step 2 2.0 M $CaCl_2$ up to 12 hours at 4°C;
Step 3 0.5 M EDTA for 4 hours at 4°C; and
Step 4 sterilized H_2O for 4 hours at 55°C.

To eliminate non-crucial chemicals, a series of experiments was conducted to examine if the use of solutions of chloroform and methanol, and lithium chloride (LiCl) are necessary for isolating and purifying ABG. Based on the results of rat models, it was found that neither a solution of chloroform and methanol, nor a solution of LiCl is essential to produce ABG that is suitable for induction of bone formation. By eliminating one or both of these chemicals from the isolation and purification procedure, the duration of ABG extraction is reduced by up to approximately one-half according to the present invention.

Once the ABG is produced it can be used in the methods and materials of the present invention to reduce inflammation as described herein.

The term "inflammation," as used herein refers to an adverse immune response having a detrimental health effect in a subject. A "subject" is a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, humans, farm animals, sport animals, and pets.

It is well understood that inflammation is the first response of the immune system to infection or irritation and may be referred to as the innate cascade. Inflammation has two components: (i) cellular and (ii) exudative.

The exudative component involves the movement of fluid, usually containing many important proteins such as fibrin and immunoglobulins. Blood vessels are dilated upstream of an infection and constricted downstream while capillary permeability to the affected tissue is increased, resulting in a net loss of blood plasma into the tissue, giving rise to oedema or swelling.

The cellular component involves the movement of white blood cells from blood vessels into the inflamed tissue. The white blood cells, or leukocytes, take on a role in inflammation; they extravasate from the capillaries into tissue, and act as phagocytes, picking up bacteria and cellular debris. For instance, without being limited to any theory, lymphocytes and monocytes recruited to the inflamed tissue and also macrophages release chemokines that further recruit polymorphonuclear leukocytes. White blood cells may also aid by walling off an infection and preventing its spread.

Thus, in the present invention the allogenic bone gel is capable of modulating inflammatory cells including, but not limited to, monocytes, lymphocytes, eosinophils, neutrophils and basophils across the epithelial surface. Preferably, the inflammatory cells comprise neutrophils, such as polymorphonuclear leukocytes ("PMNs"). In particular, the allogenic bone gel of the present invention is suitable for inhibiting the influx of polymorphonuclear leukocytes (PMNs) into a tissue involved in inflammation.

As used herein, the term "modulating" means regulating or controlling as necessary, through eliminating, reducing, maintaining or increasing a desired effect. The desired effect can be an effect on inflammatory cell migration or transmigration or by reducing the symptoms of inflammation as described *supra*. In particular, the allogenic bone gel reduces inflammation by reducing the number of PMNs in a tissue by at least 3 fold.

The activity of the allogenic bone gel to reduce inflammation can alternatively referred to as "anti-inflammatory" activity, a term which is intended to include inflammatory response modifier, including all inflammatory responses such as production of stress proteins, white blood cell infiltration, fever, pain, swelling and so forth.

In some embodiments, the ABG of the present invention is used directly as described herein. In other embodiments, the ABG is further formulated or manufactured into a material suitable for implantation and/or controlled-release of biologically active agents. The implantable material of the invention may be prepared by combining the ABG with a selected supplementary material. The supplementary material is selected based upon its compatibility with the ABG and the other components and its ability to impart properties (biological, chemical, physical, or mechanical) to the implantable material, which are desirable for a particular prophylactic or therapeutic purpose. For example, the supplementary material may be selected to improve tensile strength and hardness, increase fracture toughness, and provide imaging capability of the material after implantation. The supplementary materials are desirably biocompatible. The supplementary material may also be selected as a cohesiveness agent.

The supplementary material may be added to the ABG in varying amounts and in a variety of physical forms, dependent upon the anticipated prophylactic or therapeutic use. For example, the supplementary material may be in the form of solid structures, such as sponges, meshes, films, fibres, gels, filaments or particles, including microparticles and nanoparticles. The supplementary material may be a composite, a particulate or liquid additive which is intimately mixed with the ABG. For example, the supplementary material may be dissolved in a non-aqueous liquid prior to mixing with the ABG.

In some embodiments, the supplementary material includes bone substitutes such as bone ceramics eg calcium phosphate ceramics including hydroxyapatites, tricalcium phosphate and biphasic calcium phosphate; calcium sulphate ceramics; and bioglass ie a group of artificial bone graft substitutes consisting of silico-phosphatic substitutes. In other embodiments, the supplementary material includes corals and porous coralline ceramics, including natural corals and synthetic porous coated hydroxyapatites.

In many instances, it is desirable that the supplementary material be bioresorbable. Bioresorbable material for use as supplementary material in the implantable material of the invention include, without limitation, polysaccharides, nucleic acids, carbohydrates, proteins, polypeptides, poly(.alpha.-hydroxy acids), poly(lactones), poly(amino acids), poly(anhydrides), poly(orthoesters), poly (anhydride-co-imides), poly(orthocarbonates), poly(.alpha.-hydroxy alkanoates), poly(dioxanones), and poly(phosphoesters). Preferably, the bioresorbable polymer is a naturally occurring polymer, such as collagen, glycogen, chitin, starch, keratins, silk, and hyaluronic acid; or a synthetic polymer, such as poly(L-lactide) (PLLA), poly(D,L-lactide) (PDLLA), polyglycolide (PGA), poly(lactide-co-glycolide (PLGA), poly(L-lactide-co-D, L-

lactide), poly(D,L-lactide-co-trimethylene carbonate), polyhydroxybutyrate (PHB), poly(.epsilon.-caprolactone), poly(.delta.-valerolactone), poly(.gamma.-butyrolactone), poly(caprolactone), or copolymers thereof. Such polymers are known to bioerode and are suitable for use in the implantable material of the invention. In addition, bioresorbable inorganic supplementary materials, such as compositions including SiO_2, Na_2O, CaO, P_2O_5, Al_2O_3 and/or CaF_2, may be used, as well as salts, e.g., NaCl, and sugars, e.g., mannitol, and combinations thereof.

Supplementary materials may also be selected from non-resorbable or poorly resorbable materials. Suitable non-resorbable or poorly resorbable materials for use in the implantable material of the invention include, without limitation, dextrans, cellulose and derivatives thereof (e.g., methylcellulose, carboxy methylcellulose, hydroxypropyl methylcellulose, and hydroxyethyl cellulose), polyethylene, polymethylmethacrylate (PMMA), carbon fibers, poly(ethylene glycol), poly(ethylene oxide), poly(vinyl alcohol), poly(vinylpyrrolidone), poly(ethyloxazoline), poly(ethylene oxide)-co-poly(propylene oxide) block copolymers, poly(ethylene terephthalate)polyamide, and lubricants, such as polymer waxes, lipids and fatty acids.

The implantable material of the invention is useful for the controlled-release of biologically active agents. In general, the only requirement is that the substance is encased within the material and remain active within the implantable material during fabrication or be capable of being subsequently activated or re-activated, or that the biologically active agent can be added at the time of implantation of the implantable material into a subject.

Biologically active agents that can be incorporated into the implantable material of the invention include, without

limitation, organic molecules, inorganic materials, proteins, peptides, nucleic acids (e.g., genes, gene fragments, gene regulatory sequences, and antisense molecules), nucleoproteins, polysaccharides, glycoproteins, and lipoproteins. Classes of biologically active compounds that can be loaded into a implantable material of the invention include, without limitation, anti-cancer agents, antibiotics, analgesics, anti-inflammatory agents, immunosuppressants, enzyme inhibitors, antihistamines, anti-convulsants, hormones, muscle relaxants, anti-spasmodics, prostaglandins, anti-depressants, anti-psychotic substances, trophic factors, osteoinductive proteins, growth factors, and vaccines.

Anti-cancer agents include alkylating agents, platinum agents, antimetabolites, topoisomerase inhibitors, antitumor antibiotics, antimitotic agents, aromatase inhibitors, thymidylate synthase inhibitors, DNA antagonists, farnesyltransferase inhibitors, pump inhibitors, histone acetyltransferase inhibitors, metalloproteinase inhibitors, ribonucleoside reductase inhibitors, TNF alpha agonists/antagonists, endothelin A receptor antagonists, retinoic acid receptor agonists, immuno-modulators, hormonal and antihormonal agents, photodynamic agents, and tyrosine kinase inhibitors.

Antibiotics include aminoglycosides (e.g., gentamicin, tobramycin, netilmicin, streptomycin, amikacin, neomycin), bacitracin, corbapenems (e.g., imipenem/cislastatin), cephalosporins, colistin, methenamine, monobactams (e.g., aztreonam), penicillins (e.g., penicillin G, penicillin V, methicillin, natcillin, oxacillin, cloxacillin, dicloxacillin, ampicillin, amoxicillin, carbenicillin, ticarcillin, piperacillin, mezlocillin, azlocillin), polymyxin B, quinolones, and vancomycin; and bacteriostatic agents such as chloramphenicol, clindanyan, macrolides (e.g., erythromycin, azithromycin,

clarithromycin), lincomyan, nitrofurantoin, sulfonamides, tetracyclines (e.g., tetracycline, doxycycline, minocycline, demeclocyline), and trimethoprim. Also included are metronidazole, fluoroquinolones, and ritampin.

Enzyme inhibitors are substances which inhibit an enzymatic reaction. Examples of enzyme inhibitors include edrophonium chloride, N-methylphysostigmine, neostigmine bromide, physostigmine sulfate, tacrine, tacrine, 1-hydroxy maleate, iodotubercidin, p-bromotetramisole, 10-(alpha-diethylaminopropionyl)-phenothiazine hydrochloride, calmidazolium chloride, hemicholinium-3,3,5-dinitrocatechol, diacylglycerol kinase inhibitor I, diacylglycerol kinase inhibitor II, 3-phenylpropargylamine, N_6-monomethyl-L-arginine acetate, carbidopa, 3-hydroxybenzylhydrazine, hydralazine, clorgyline, deprenyl, hydroxylamine, iproniazid phosphate, 6-MeO-tetrahydro-9H-pyrido-indole, nialamide, pargyline, quinacrine, semicarbazide, tranylcypromine, N,N-diethylaminoethyl-2,2-diphenylvalerate hydrochloride, 3-isobutyl-1-methylxanthne, papaverine, indomethacind, 2-cyclooctyl-2-hydroxyethylamine hydrochloride, 2,3-dichloro-a-methylbenzylamine (DCMB), 8,9-dichloro-2,3,4,5-tetrahydro-1H-2-benzazepine hydrochloride, p-aminoglutethimide, p-aminoglutethimide tartrate, 3-iodotyrosine, alpha-methyltyrosine, acetazolamide, dichlorphenamide, 6-hydroxy-2-benzothiazolesulfonamide, and allopurinol.

Antihistamines include pyrilamine, chlorpheniramine, and tetrahydrazoline, among others.

Anti-inflammatory agents include corticosteroids, non-steroidal anti-inflammatory drugs (e.g., aspirin, phenylbutazone, indomethacin, sulindac, tolmetin, ibuprofen, piroxicam, and fenamates), acetaminophen,

phenacetin, gold salts, chloroquine, D-Penicillamine, methotrexate colchicine, allopurinol, probenecid, and sulfinpyrazone.

Muscle relaxants include mephenesin, methocarbomal, cyclobenzaprine hydrochloride, trihexylphenidyl hydrochloride, levodopa/carbidopa, and biperiden.

Anti-spasmodics include atropine, scopolamine, oxyphenonium, and papaverine.

Analgesics include aspirin, phenybutazone, idomethacin, sulindac, tolmetic, ibuprofen, piroxicam, fenamates, acetaminophen, phenacetin, morphine sulfate, codeine sulfate, meperidine, nalorphine, opioids (e.g., codeine sulfate, fentanyl citrate, hydrocodone bitartrate, loperamide, morphine sulfate, noscapine, norcodeine, normorphine, thebaine, nor-binaltorphimine, buprenorphine, chlomaltrexamine, funaltrexamione, nalbuphine, nalorphine, naloxone, naloxonazine, naltrexone, and naltrindole), procaine, lidocain, tetracaine and dibucaine.

Prostaglandins are art recognized and are a class of naturally occurring chemically related, long-chain hydroxy fatty acids that have a variety of biological effects.

Anti-depressants are substances capable of preventing or relieving depression. Examples of anti-depressants include imipramine, amitriptyline, nortriptyline, protriptyline, desipramine, amoxapine, doxepin, maprotiline, tranylcypromine, phenelzine, and isocarboxazide.

Trophic factors are factors whose continued presence improves the viability or longevity of a cell. Trophic factors include, without limitation, platelet-derived growth factor (PDGP), neutrophil-activating protein, monocyte chemoattractant protein, macrophage-inflammatory

protein, platelet factor, platelet basic protein, and melanoma growth stimulating activity; epidermal growth factor, transforming growth factor (alpha), fibroblast growth factor, platelet-derived endothelial cell growth factor, insulin-like growth factor, glial derived growth neurotrophic factor, ciliary neurotrophic factor, nerve growth factor, bone growth/cartilage-inducing factor (alpha and beta), bone morphogenetic proteins, interleukins (e.g., interleukin inhibitors or interleukin receptors, including interleukin 1 through interleukin 10), interferons (e.g., interferon alpha, beta and gamma), hematopoietic factors, including erythropoietin, granulocyte colony stimulating factor, macrophage colony stimulating factor and granulocyte-macrophage colony stimulating factor; tumor necrosis factors, and transforming growth factors (beta), including beta-1, beta-2, beta-3, inhibin, and activin.

Hormones include estrogens (e.g., estradiol, estrone, estriol, diethylstibestrol, quinestrol, chlorotrianisene, ethinyl estradiol, mestranol), anti-estrogens (e.g., clomiphene, tamoxifen), progestins (e.g., medroxyprogesterone, norethindrone, hydroxyprogesterone, norgestrel), antiprogestin (mifepristone), androgens (e.g, testosterone cypionate, fluoxymesterone, danazol, testolactone), anti-androgens (e.g., cyproterone acetate, flutamide), thyroid hormones (e.g., triiodothyronne, thyroxine, propylthiouracil, methimazole, and iodixode), and pituitary hormones (e.g., corticotropin, sumutotropin, oxytocin, and vasopressin).

The biologically active agent is desirably selected from the family of proteins known as the transforming growth factors-beta (TGF-.beta.) superfamily of proteins, which includes the activins, inhibins and bone morphogenetic proteins (BMPs). Most preferably, the active agent includes at least one protein selected from the subclass

of proteins known generally as BMPs, which have been disclosed to have osteogenic activity, and other growth and differentiation type activities. These BMPs include BMP proteins BMP-2, BMP-3, BMP-4, BMP-5, BMP-6 and BMP-7 (OP-1), disclosed for instance 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 PCT application WO94/26893; BMP-11, disclosed in PCT application WO94/26892, or BMP-12 or BMP-13, disclosed in PCT application WO 95/16035; BMP-14; BMP-15, disclosed in U.S. Pat. No. 5,635,372; or BMP-16, disclosed in U.S. Pat. No. 5,965,403. Other TGF-.beta. proteins which may be useful as the active agent in the implantable material of the invention include Vgr-2, Jones et al., Mol. Endocrinol. 6:1961 (1992), and any of the growth and differentiation factors (GDFs), 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 invention may be BIP, disclosed in WO94/01557; HP00269, disclosed in JP Publication number: 7-250688; and BMP-14 (also known as MP52, CDMP1, and GDF5), disclosed in PCT application WO93/16099. The disclosures of all of the above applications are incorporated herein by reference. A subset of BMPs which are presently preferred for use in the invention include BMP-2, BMP-3, BMP-3b, BMP-4, BMP-5, BMP-6, BMP-7, BMP-8, BMP-9, BMP-10, BMP-11, BMP-12, BMP-13, BMP-14, BMP-15, BMP-16, BMP-17, and BMP-18. The active agent is most preferably BMP-2, the sequence of which is disclosed in U.S. Pat. No. 5,013,649, the disclosure of which is incorporated herein by reference. Other osteogenic agents known in the art can also be used, such as teriparatide (Forteo™), Chrysalin™, prostaglandin E2, or LIM protein, among others.

The biologically active agent may be recombinantly

produced, or purified from a protein composition. The active agent, if a TGF-.beta. such as a BMP, or other dimeric protein, 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 effective to stimulate increased osteogenic activity of present or infiltrating progenitor or other cells will depend upon the size and nature of the defect being treated. Generally, the amount of protein to be delivered is in a range of from about 0.1 to about 100 mg; preferably about 1 to about 100 mg; most preferably about 10 to about 80 mg.

Biologically active agents can be introduced into the implantable material of the invention during or after its formation. Agents may conveniently be mixed into the implantable material.

Standard protocols and regimens for delivery of the above-listed agents are known in the art. Typically, these protocols are based on oral or intravenous delivery. Biologically active agents are introduced into the implantable material in amounts that allow delivery of an appropriate dosage of the agent to the implant site. In most cases, dosages are determined using guidelines known to practitioners and applicable to the particular agent in question. The exemplary amount of biologically active agent to be included in the implantable material of the

invention is likely to depend on such variables as the type and extent of the condition, the overall health status of the particular patient, the formulation of the active agent, and the bioresorbability of the implantable material used. Standard clinical trials may be used to optimize the dose and dosing frequency for any particular biologically active agent.

The implantable material of the invention can be used to deliver biologically active agents to any of a variety of sites in a mammalian body, preferably in a human body. The implantable material can be implanted subcutaneously, intramuscularly, intraperitoneally and bony sites. Preferably, the implantable material is implanted into or adjacent to the tissue to be treated such that, by diffusion, the encased biologically active agent is capable of penetrating the tissue to be treated.

Such materials offer the advantage of controlled, localized delivery. As a result, less biologically active agent is required to achieve a therapeutic result in comparison to systemic administration, reducing the potential for side effects maximizing the agent's activity at the site of implantation.

The implantable material can be implanted into any acceptable tissue. The implantable material has particular advantages for delivery of biologically active agents to sites in bone. Implantation of the implantable material to a bony site includes either anchoring the vehicle to a bone or to a site adjacent to the bone.

The implantable material described herein can be implanted to support bone growth so that it is eventually replaced by the subject's own bone. It should be borne in mind, however, that bone ingrowth may well affect the resorbability rate of the drug delivery for implantable

material incorporating a biologically active agent. Accordingly, it may be desirable in certain circumstances (e.g., where the biologically active agent must be delivered according to a precise, predetermined administrative schedule) to reduce bone growth into the drug delivery vehicle, for example by blocking penetration of osteocytic or chondrocytic cells or precursors. In most circumstances, ossification can be avoided by placing the device at some distance away from bone. Generally, 1 mm will be sufficient, although greater distances are preferred.

To optimize ossification, the implantable material may be seeded with bone forming cells, such as progenitor cells, stem cells, and/or osteoblasts. This is most easily accomplished by placing the implantable material in contact with a source of the subject's own bone forming cells. Such cells may be found in bone-associated tissue, blood or fluids, including exogenous fluids which have been in contact with bone or bone materials or regions, including the periosteum, cancellous bone or marrow. When used in conjunction with devices such as screws and pins, the introduction of which into bone is accompanied by breach of the periosteum and/or bleeding, no further seeding is required. For plates, which oppose only cortical bone, induction of a periosteal lesion which will contact the device is recommended. In yet other embodiments, it will be useful to surgically prepare a seating within the bone by removing a portion of cortical bone at the implant site. Bone forming cells harvested from the subject may be introduced into the graft to augment ossification. Other steps may also be taken to augment ossification, including introduction bone forming cells harvested from the patient into the graft, or incorporation of trophic factors or bone growth inducing proteins into, or onto the device. Non-autologous bone cells can also be used to promote bone regeneration.

Immunosuppressants may be administered to the device recipient, either systemically or by incorporation into the device. Thus, cells or tissues obtained from primary sources, cell lines or cell banks may be used (See, U.S. Pat. No. 6,132,463 to Lee *et al.*, which is incorporated herein by reference).

Certain categories of biologically active agents are expected to be particularly suitable for delivery to bony sites. For example, where the implantable material is applied to a damaged bone site, it may be desirable to incorporate bone regenerative proteins (BRPs) into the implantable material. BRPs have been demonstrated to increase the rate of bone growth and to accelerate bone healing (see, for example, Appel et al., Exp. Opin. Ther. Patents 4:1461 (1994)). Exemplary BRPs include, but are in no way limited to, Transforming Growth Factor-Beta (TGF-.beta.), Cell-Attachment Factors (CAFs), Endothelial Growth Factors (EGFs), OP-1, and Bone Morphogenetic Proteins (BMPs). Such BRPs are currently being developed by Genetics Institute, Cambridge, Mass.; Genentech, Palo Alto, Calif.; and Creative Biomolecules, Hopkinton, Mass. Bone regenerative proteins and trophic factors can also be used to stimulate ectopic bone formation if desired. For example, an implantable material containing BMP-2 can be placed subcutaneously, and bone formation will occur within 2-4 weeks.

Antibiotics and antiseptics are also desirably delivered to bony sites using the implantable material of the invention. For example, as indicated *supra* one of the major clinical implications arising from bone-graft surgery is a need to control the post-operative inflammation or infection, particularly infection associated with osteomyelitis. A implantable material of the invention that includes an antibiotic can be used as, or in conjunction with, an improved bone graft to reduce

the chances of local infection at the surgery site, contributing to infection-free, thus faster, bone healing process. The efficacy of antibiotics is further enhanced by controlling the resorption of the poorly crystalline hydroxyapatite such that it dissolves at a rate that delivers antibiotic peptides or its active component at the most effective dosage to the tissue repair site. Antibiotics and bone regenerating proteins may be incorporated together into the implantable material of the invention, to locally deliver most or all of the components necessary to facilitate optimum conditions for bone tissue repair.

Other biologically active agents that are desirably delivered to bony sites include anti-cancer agents, for example for treatment of bone tumors (see, for example, Otsuka et al., J. Pharm. Sci. 84:733 (1995)). The delivery vehicles of the invention are useful, for example, where a patient has had a bone tumor surgically removed, because the implantable material can be implanted to improve the mechanical integrity of the bone site while also treating any remaining cancer cells to avoid metastasis

Additional biologically active agents can be incorporated into the implantable material of the invention for delivery to bony sites include agents that relieve osteoporosis. For example, amidated salmon calcitonin has been demonstrated to be effective against osteoporosis.

Vitamin D and Vitamin K are also desirably delivered to bony sites, as are angiogenic factors such as VEGF, which can be used when it is desirable to increase vascularization.

The implantable material of the invention can be useful for repairing a variety of orthopaedic conditions. The implantable material of the invention may be implanted

into the vertebral body for treatment of spinal fusion, spinal fractures, implanted into long bone or flat bone fractures to augment the fracture repair or to stabilize the fractured fragments, or implanted into intact osteoporotic bones to improve bone strength. It can be useful in the augmentation of a bone-screw or bone-implant interface. Additionally, it can be useful as bone filler in areas of the skeleton where bone may be deficient. Examples of situations where such deficiencies may exist include post-trauma with segmental bone loss, post-bone tumor surgery where bone has been excised, and after total joint arthroplasty. The implantable material can be used to hold and fix artificial joint components in subjects undergoing joint arthroplasty, as a strut to stabilize the anterior column of the spine after excision surgery, as a structural support for segmented bone (e.g., to assemble bone segments and support screws, external plates, and related internal fixation hardware), and as a bone graft substitute in spinal fusions.

The ABG *per se* or the implantable material can be used to coat medical devices such as prosthetic bone implants. For example, where the prosthetic bone implant has a porous surface, the ABG or implantable material may be applied to the surface to reduce inflammation and/or promote bone growth therein (i.e., bone ingrowth). The ABG or implantable material may also be applied to a prosthetic bone implant to enhance fixation within the bone.

The implantable material of the invention are easy to apply and can be readily modelled to accurately reconstruct bony cavities, missing bone, and to recreate contour defects in skeletal bone. The implantable material can be applied, for example, with a spatula, can be moulded and sculpted, and can hold its shape satisfactorily until set.

By "comprising" is meant including, but not limited to, whatever follows the word comprising". Thus, use of the term "comprising" indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present. By "consisting of" is meant including, and limited to, whatever follows the phrase "consisting of". Thus, the phrase "consisting of" indicates that the listed elements are required or mandatory, and that no other elements may be present. By "consisting essentially of" is meant including any elements listed after the phrase, and limited to other elements that do not interfere with or contribute to the activity or action specified in the disclosure for the listed elements. Thus, the phrase "consisting essentially of" indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present depending upon whether or not they affect the activity or action of the listed elements.

The invention will now be further described by way of reference only to the following non-limiting examples. It should be understood, however, that the examples following are illustrative only, and should not be taken in any way as a restriction on the generality of the invention described above.

EXAMPLE 1 PRODUCTION OF ALLOGENIC BONE GEL

Allogenic bone gel was produced from up to 10 grams of milled bone, which was immersed in 36% HCl solution at 4°C for 12 hours. The DBM was then immersed in 1000ml 2.0 M $CaCl_2$ at 4°C for 12 hours. After this, the material was immersed in 1000ml 0.5 M EDTA for 4 hours at 4°C, and at the same time NaOH was added to the solution until pH 8.0 was reached. The resulting material was immersed into H_2O at 55°C for 4 hours to produce the allogenic bone gel (ABG).

The ABG was then used directly as outline below or mixed with biologically active agents if required. For example, 0.5g ABG was mixed with 0.5g OP-1 (which contains 1.75mg of recombinant human osteogenic protein 1 in 0.5g of bovine collagen) to produce an implantable material that comprised at least 50% ABG. The recombinant human osteogenic protein 1 (OP-1) was provided by Stryker Biotech.

EXAMPLE 2 IMPLANTATION OF ABG IN ANIMALS

Thirty-two skeletally mature New Zealand White rabbits (age, 1 years old; weight, 3.5-4.5kg) were divided randomly into four groups, and in each rabbit one of the following materials was implanted:

1). 1g corticocancellous bone harvested from each side of the posterior iliac crest;
2). 1g of ABG as produced in Example 1 were placed into each side of fusion bed;
3). 3.5 mg of recombinant human OP-1 in 1.0g of bovine collagen for each side; and
4). ABG mixed with recombinant human OP-1 as described in Example 1.

Animals were housed in an established animal facility for a period of 1 week before surgery to allow acclimatization. Preoperative radiographs were obtained to rule out underlying disease.

Surgical anaesthesia was achieved with intramuscular injection of acepromazine (0.75mg/kg) followed by ketamine (35 mg/kg) and xylazine (5 mg/kg).(Lipman *et al.*, 1990) Enrofloxacin (5-10 mg/kg) was administered subcutaneously immediately before surgery.

The rabbits were shaved, positioned, draped, sterilised, and prepared in a standard surgical fashion. A dorsal midline skin incision is made in the lumbar region extending from L4-L7. Fascial incisions were made 2-3 cm on each side of the midline and a plane between the multifidus and longissimus muscles was made through blunt dissection until the transverse processes of L5-L6 and the intertransverse membrane exposed. Identification of vertebral levels was made by manual palpation of superficial landmarks using the sacrum as reference. The dorsal aspects of L5-L6 transverse processes were decorticated using a high-speed burr. Graft materials were then placed in the paraspinal muscle bed between the transverse processes. The wounds were closed using 3-0 absorbable sutures continuously to both the fascial and skin layers. Post-operative radiographs were taken to confirm the level of fusion.

All animals received 0.1mg/kg buprenorphine for post-operative analgesia and were individually housed. There were no post-operative restrictions on activity, and no supportive orthotic devices were used.

Follow-up was 6 weeks post-operatively as fusions have been shown to be distinguishable from non-unions by this time in previous research (Boden *et al.*, 1995, *Spine*, 20(4): 412-20; Minamide *et al.*, 1999, *Spine*, 24(18): 1863-70; Namikawa *et al.*, 2005, *Spine*, 30(15): 1717-22). Rabbits were killed with a sedating dose of intramuscular injection of xylazine (2.5mg/kg) followed by a lethal dose of intravenous pentobarbital.

Fusion masses were characterized and compared with manual, radiographic, biomechanical, and histologic evaluations.

At the time of harvest, the operated segments and the rest of the lumbar spine were manually palpated to assess

structural integrity by 2 blinded independent observers. Each segment was graded as solid or not solid. Only levels graded solid were considered fused.

All lumbar spines were examined by posteroanterior plain radiographs, mammography, and micro computed tomographic (microCT) scans(GE) 6 weeks post surgery. Each radiograph was assigned a numerical score using of the grading scale (see Table 2) by three observers in a blinded fashion (Yee *et al.*, 2003, *Spine*, 28(21): 2435-40). Table 2 shows the radiographic grading of spine fusions.

TABLE 2

Roentgenographic Score	Criteria
4	Intertransverse bone mass present bilaterally without lucency
3	Bone mass present bilaterally with lucency on one side only
2	Bone mass present bilaterally with lucency bilaterally
1	Bone mass present on one side only
0	No bone mass seen on either side

Biomechanical testing to evaluate the strength of the L5-L6 fusion site was performed by three-point flexion-bending test using a materials testing machine.

Harvested specimens were fixed in 4% formalin in a neutral buffer solution, decalcified in 10% formic acid solution, dehydrated in a gradient ethanol series, and embedded in paraffin. Sections of 4μm thickness at the intertransverse process region were cut in a sagittal plane, stained with hematoxylin and eosin, and observed under light microscopy to examine for the presence of bony fusion between the newly formed bone and transverse processes.

Average values were presented as mean ± standard deviation. Fusion rates determined by manual palpation and radiographic analysis were evaluated using Fisher's exact test. Comparisons of biomechanical testing of spines in each group were made using one-way analysis of variance (ANOVA). Significance for all tests was defined as $P < 0.05$.

Three rabbits were excluded (9%): one from autograft group died because of anaesthesia-related complications. Another two, one each from the OP-1 and ABG+OP-1 groups were sacrificed as they encountered deep wound infection. The remaining rabbits tolerated the surgical procedure without complications and started to gain weight after 1 week of post-operation.

Inspection by manual palpation of the fusion mass in the BMP group showed a bony mass in the intertransverse area that was more prominent than in the other groups. Solid spinal fusion was achieved in all seven rabbits in the ABG+OP-1 group (see Table 3).

TABLE 3

UNION RATE ON MANUAL PALPATION AFTER 6 WEEKS

Group Material	Results
Autograft group	3/7 *
ABG group	2/8 * *
OP-1 group	2/7 * *
ABG+OP-1 group	7/7 *, * *

Note: Significant difference (Fisher's exact test, * * P<0.01 * P<0.05).

PO = postoperative.

Six weeks after surgery, the degree of radiographic intertransverse processes fusion rate as assessed by the 5-point grading scale is presented in Table 4.

TABLE 4

RADIOGRAPHIC SCORE AFTER 6 WEEKS

Group Material	Mean Value	Significance *
Autograft group(A)	2.43±.98	A/IO: P = 0.031
ABG group(I)	2.12±.64	I/IO: P = 0.004
OP-1 group(O)	2.29±.18	O/IO: P = 0.014
ABG+OP-1 group(IO)	3.71±.18	

Note: The P values were derived using the One-way ANOVA, Bonferroni post hoc test.

A/IO = comparison between trial group A and IO; I/IO = comparison between trial group I and IO; O/IO = comparison between trial group O and IO

It can be seen that the ABG alone or combined with OP-1 produced equivalent or superior results as compared to the autogenous bone graft. The test was conducted in a critical bone defect model wherein the successful outcome was a solid posterolateral intertransverse process fusion. In the current study, autograft group did not result in a significant difference in fusion rate compared with results in the previous study in the same model (57% VS 66%) confirming the consistency of the model (Boden *et al.*, *J Bone Joint Surg Am.*, 77(9): 1404-17; Boden *et al.* (1995), *Spine,* 20(24): 2626-32).

Result of radiographic and histologic studies consistently showed fusion mass size of ABG /OP-1 composite group is larger than autograft, ABG alone and OP-1 alone. More mature fusion masses were also noted, ABG combined with OP-1 showed the greatest response in osteoid and new bone growth. However ABG alone and OP-1 alone showed osteoid formation, but no bony fusion after 6 weeks. Autograft showed more new bone growth than ABG alone and OP-1 alone. Quantitative Micro CT ray Tomography (MicroCT) results showed that bone volume in ABG/OP-1 group is significantly larger than the other three groups. We also found that the bone volume formed in outside zone is larger than central zone.

Pain relief and stability are the primary goals of spinal fusion. Although radiography and histology revealed fusion masses, these techniques can not be used to evaluate the stability of the fusion. Physiology biomechanical flexibility testing offers a precise method to characterize the changes in physiologic motion that result from spinal fusion. In the current study posterolateral fusion led to significant ROM decreases in lateral bending, flexion and extension between the ABG/OP-1 group and the autograft group.

	Autograft	ABG	OP-1	ABG+OP-1
Osteogenic cell	+	±	±	±
Osteoinductivity	+	+	++	+++
Osteoconductivity	+	+	±	+

Of most interest was the observation that the ABG and/or ABG plus OP-1 group had reduced inflammation relative to the other groups.

EXAMPLE 3 ANTI-INFLAMMATORY PROPERTIES OF ABG

Inflammatory reaction caused by failure of arthroplasty, bacterial infection or tumour metastasis is a major concern in patients exhibiting osteolysis. Pro-inflammatory cytokines, such as IL-1, IL-6, TNF and the cascade reaction of bone inductive growth factors including OP-1 and BMP-2, are considered to be major mediators of osteolysis and ultimately aseptic loosening. In addition, lipopolysaccharide (LPS)-induced pro-inflammatory cytokine released in bone cells is also linked to bacterial bone infection.

Based on the previous observation that ABG could significantly reduce the inflammation caused by OP-1 or its carrier in rabbit model of spinal fusion (Example 2), we proposed that ABG may inhibit the inflammatory reaction caused by failure of arthroplasty, bacterial infection or tumour metastasis.

The LPS-induced osteolysis in the mouse calvarium model was used to examine the anti-inflammatory effect of ABG *vivo*. LPS with or without ABG was introduced into mouse calvaria. The method used is described by Yip *et al*.

2004, *J Bone Miner Res.*, 19(11):1905-16 herein incorporated in its entirety by reference.

ABG was produced as described in Example 1. LPS (*Escherichia coli*, serotype 026-B6) (Sigma, Castle Hill, New South Wales, Australia) and Lycoll™ (Resorba, Nuernberg, Germany) were obtained through commercial outlets.

Twenty C57 Black mice were divided into four groups: sham operation + saline injection; sham operation + LPS injection; ABG implantation + LPS injection; and Lycoll™ implantation + LPS injection.

In the sham operation + saline injection group, a skin incision of 0.5cm long was made on top of calvaria and an injection of saline (50µl/mice) was given 3 days later. The sham operation + LPS group underwent the same operation procedure and was then given an injection of LPS (500µg/mice) 3 days later. In the ABG implantation + LPS group, the same operation procedure was employed and about 0.1g ABG was implanted into the space between the skin and the skull. Three days later, 500µg LPS was injected into the same area for each mouse. For Lycoll™ implantation + LPS group the same procedures as in the previous group were used except Lycoll™ was implanted. After 7 days of injections, histo-pathological assessment was performed and micrographs taken at 100x.

Four days after the operation procedure, it was noticed that the skin around the injection area of 2 mice in sham operation + LPS group was significantly inflamed and warm

to the touch. The eye on the same side of one of these 2 mice was swollen. This situation remained unchanged to the end of experimental period.

Histo-pathological samples from sham operation + saline injection group showed there were some acute inflammatory cells and fibroblasts in the injection area (Figure 1), while the samples from sham operation + LPS injection group showed prominent vasodilatation of precapillary arterioles and densely packed polymorphonuclear leukocytes in connective tissue in the injection area (Figure 2). In contrast, there were only a few polymorphonuclear leukocytes present in the operation area (Figure 3) of samples from the ABG + LPS injection group. There was no notable difference found between the sham operation + LPS and Lycoll™ + LPS (Figure 4) group, – in terms of inflammatory reaction.

This experiment demonstrated that ABG inhibits LPS-induced inflammation in mouse model. Combining these results with our observation that ABG could significantly reduce the inflammation caused by OP-1 in a rabbit model of spinal fusion (Example 2), we conclude that ABG can inhibit:

1. the inflammatory reaction caused by the failure of arthroplasty, bacterial infection or tumour metastasis;
2. the inflammatory reaction caused by other implant materials or carriers in the administration of OP-1, BMP-2 or other biologically active agents.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method for reducing inflammation in a subject's tissue comprising the step of implanting a material comprising allogenic bone gel into or adjacent to said tissue, wherein said allogenic bone gel reduces the inflammation.

2. A method for reducing polymorphonuclear leukocytes in a subject's tissue comprising the step of implanting a material comprising allogenic bone gel into or adjacent to said tissue, wherein said allogenic bone gel reduces the number of polymorphonuclear leukocytes present by at least 3 fold.

3. A method according to claim 1, wherein the inflammation comprises redness, elevated temperature, swelling or pain.

4. A method according to claim 1, wherein the inflammation is cytokine-induced inflammation associated with osteolysis.

5. A method according to any one of claims 1 to 4, wherein the material comprises at least 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or even 90% (w/w) allogenic bone gel.

6. A method according to claim 5, wherein the material further comprises a supplementary material selected from polysaccharides, carbohydrates, poly(.alpha.-hydroxy acids), poly(lactones), poly(amino acids), poly(anhydrides), poly(orthoesters), poly (anhydride-co-imides), poly(orthocarbonates), poly(.alpha.-hydroxy alkanoates), poly(dioxanones), poly(phosphoesters), or copolymers thereof.

7. A method according to claim 5 or 6, wherein the material further comprises a supplementary material selected from collagen, glycogen, chitin, starch, keratins, silk, hyaluronic acid, poly(L-lactide) (PLLA), poly(D,L-lactide) (PDLLA), polyglycolide (PGA), poly(lactide-co-glycolide (PLGA), poly(L-lactide-co-D, L-lactide), poly(D,L-lactide-co-trimethylene carbonate), polyhydroxybutyrate (PHB), poly(.epsilon.-caprolactone), poly(.delta.-valerolactone), poly(.gamma.-butyrolactone), poly(caprolactone), or copolymers thereof.

8. A method according to any one of claims 1 to 7, wherein the material further comprises a biologically active agent.

9. A method according to claim 8, wherein the biologically active agent is selected from the group consisting of osteogenic proteins, antibiotics, polynucleotides, anti-cancer agents, growth factors, and vaccines.

10. A method according to claim 9, wherein the osteogenic protein is selected from the group consisting of BMP-2, BMP-3, BMP-3b, BMP-4, BMP-5, BMP-6, BMP-7, BMP-8, BMP-9, BMP-10, BMP-1 1, BMP-12, BMP-13, BMP-14, BMP-15, BMP-16, BMP-17, and BMP-18.

11. An implantable anti-inflammatory material comprising allogenic bone gel, which gel provides at least a 3 fold reduction in the number of polymorphonuclear leukocytes in a subject's tissue when said gel is topically applied on to or adjacent to said tissue.

12. A medical device coated with allogenic bone gel, which device, when implanted, results in a reduction in inflammation compared to the level of inflammation produced by the implantation of the same device not coated

with allogenic bone gel.

13. An implantable, anti-inflammatory controlled-release material comprising at least 15% w/w allogenic bone gel, at least one supplementary material and at least one biologically active agent, wherein said biologically active agent supplements the anti-inflammatory effect of the allogenic bone gel

14. A controlled-release, implantable anti-inflammatory material consisting essentially of allogenic bone gel and bone morphogenetic protein-7 (OP-1) and/or bone-morphogenetic proteins (BMP)-2.

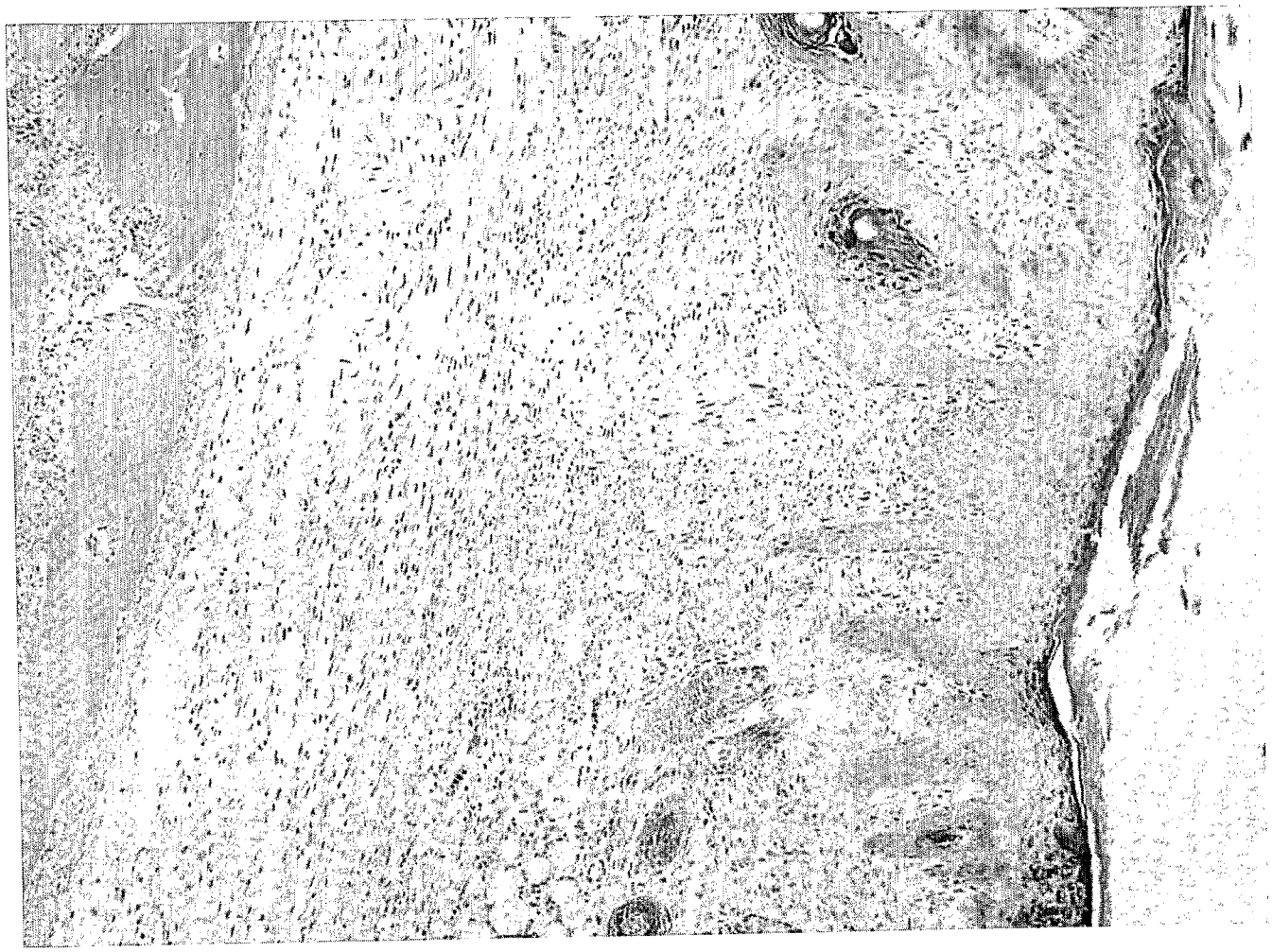

FIGURE 1

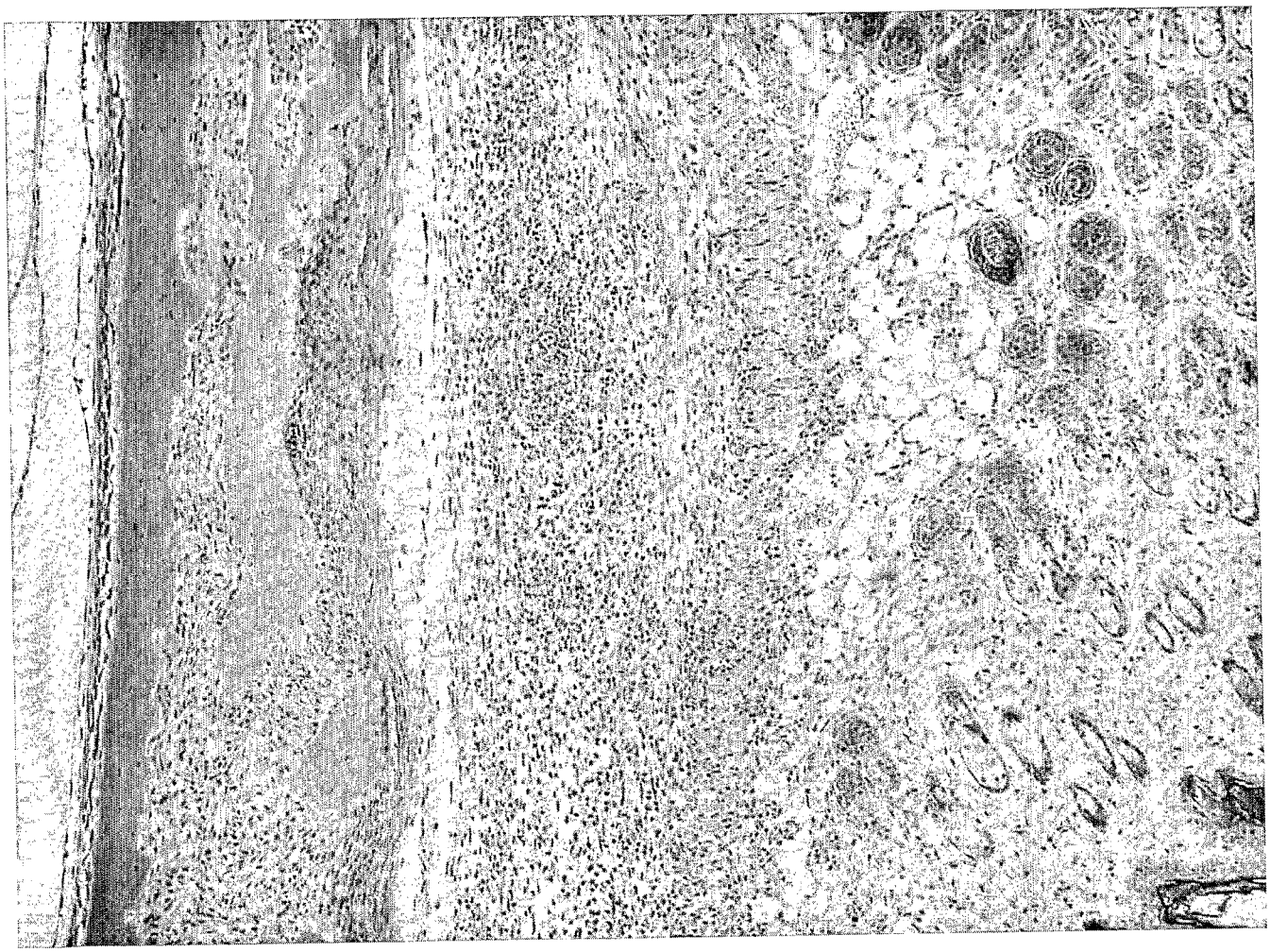

FIGURE 2

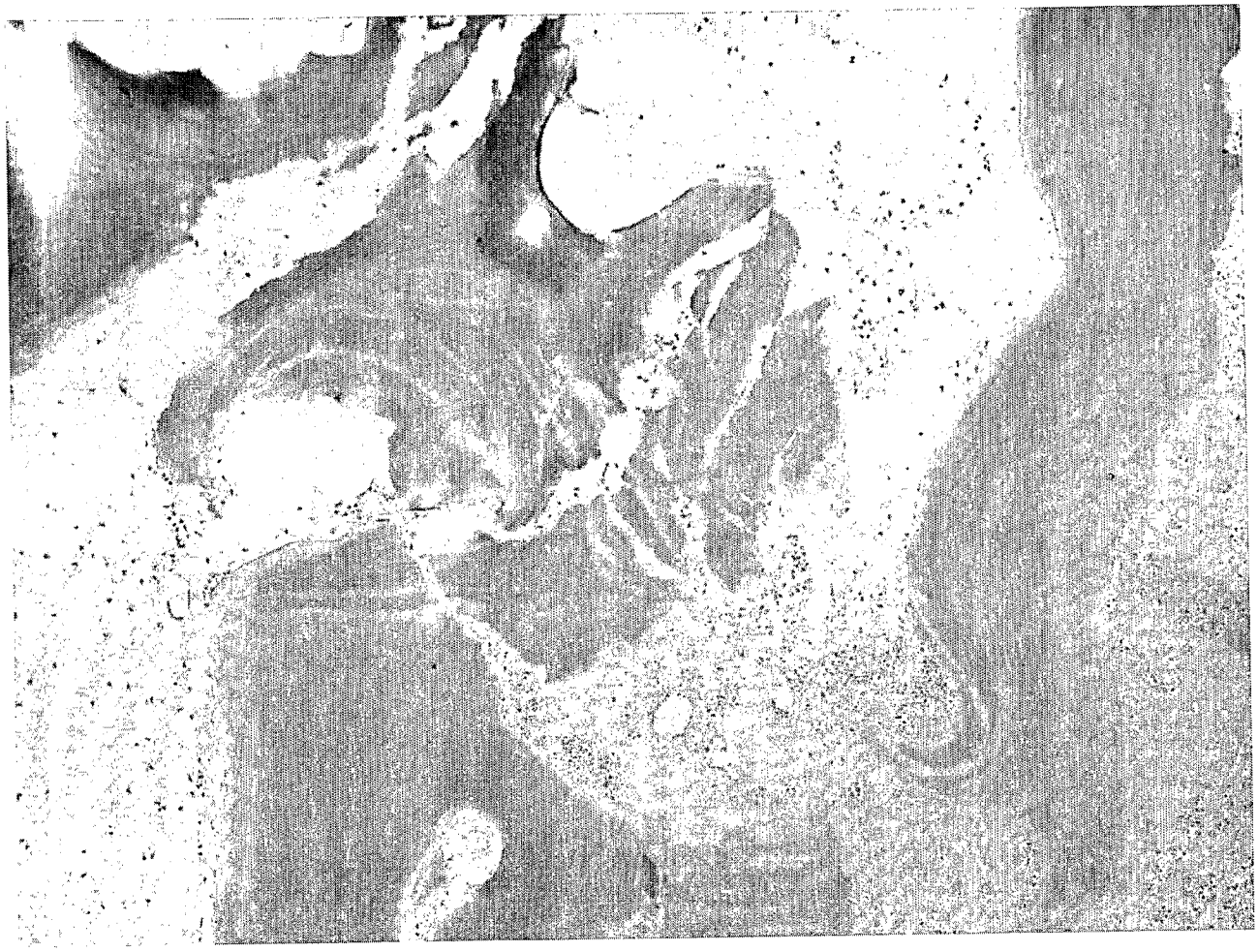

FIGURE 3

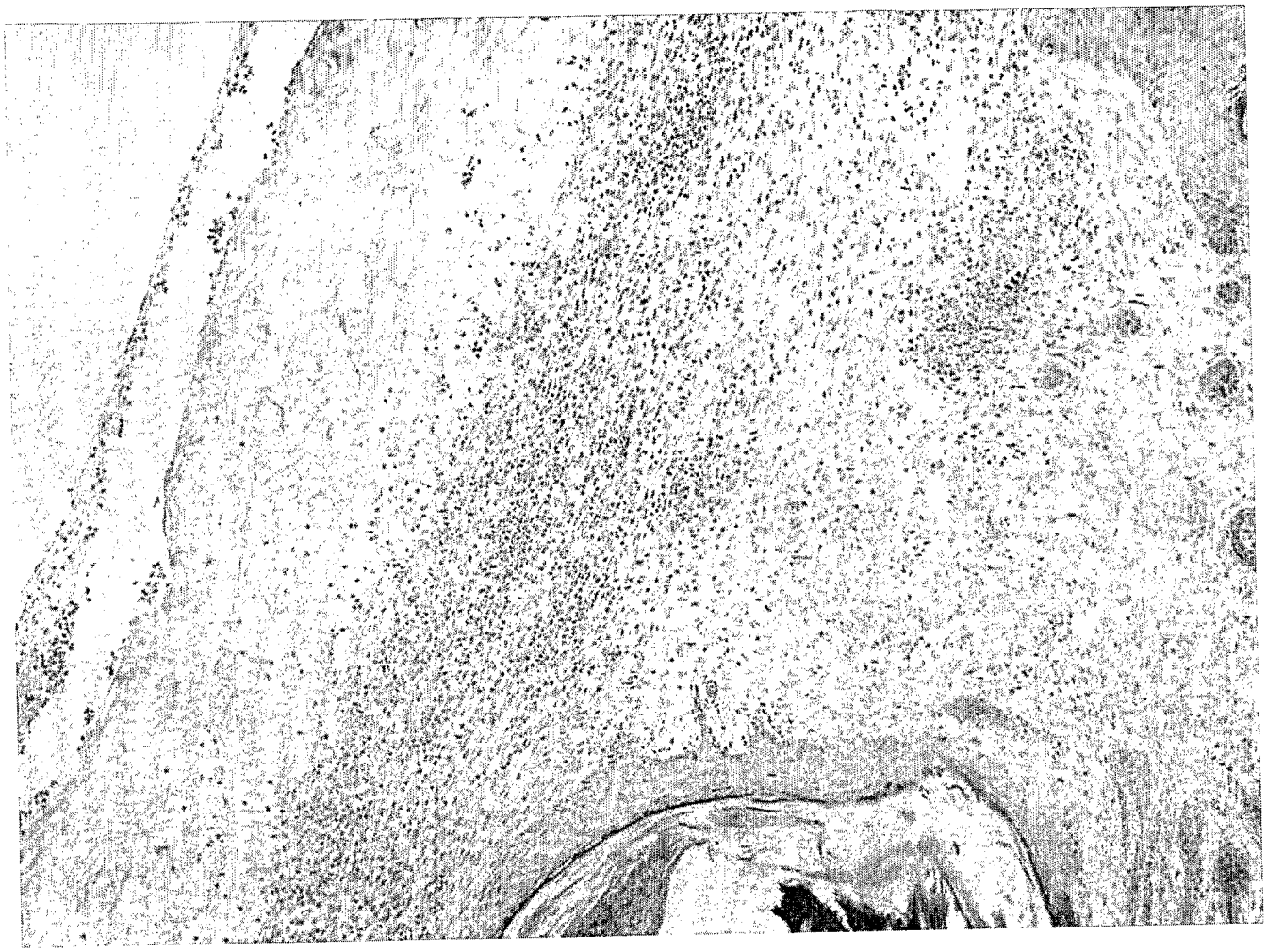

FIGURE 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2008/000627

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

A61P 29/00 (2006.01) ***A61L 27/50*** (2006.01)
A61K 35/32 (2006.01) ***A61L 31/14*** (2006.01)

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)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WIPDS, MEDLINE, CA & Keywords: insoluble bone gelatine, allogenic bone gel, bone morphogenetic protein, inflammation

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 2003/020840 A1 (PERTH BONE AND TISSUE BANK, INC.) 13 March 2003. Page 3 lines 5-13, page 4 lines 1-26, protocols 2 and 3, example and claims.	11 11-14
Y	US 6,214,049 B1 (GAYER *et al.*) 10 April 2001. Column 3-4, column 5 lines 35-55, column 10 lines 10-14, column 11 lines 19-47	11-14
Y	Maric I. *et al.* "Bone morphogenetic Protein-7 reduces the severity of colon tissue damage and accelerates the healing of inflammatory bowel disease in rats". *J Cell Physiol* 2003, Vol. 196, pages 258-264. Abstract	14

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

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

Date of the actual completion of the international search
25 June 2008

Date of mailing of the international search report 08 JUL 2008

Name and mailing address of the ISA/AU

AUSTRALIAN PATENT OFFICE
PO BOX 200, WODEN ACT 2606, AUSTRALIA
E-mail address: pct@ipaustralia.gov.au
Facsimile No. +61 2 6283 7999

Authorized officer
NATHALIE TOCHON-DANGUY
AUSTRALIAN PATENT OFFICE
(ISO 9001 Quality Certified Service)
Telephone No : (02) 6283 3107

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2008/000627

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Kaps C. *et al.* "Bone morphogenetic proteins promote cartilage differentiation and protect engineered artificial cartilage from fibroblast invasion and destruction". *Arthritis & Rheumatism*, 2002, Vol. 46, No. 1, pages 149-162 Abstract, pages 157-159.	11-14
A	Schneider U. "Autogenous bone cell transplantation". *Orthopäde* 1998, Vol. 27, pages 143-146. Summary	
A	Urist M.R. *et al.* "Bone morphogenesis in implants of insoluble bone gelatin". *Proc.Nat.Acad.Sci.USA*, 1973, Vol.70, No. 12, pages 3511-3515. Abstract, page 3511.	
A	Six N. *et al.* "Osteogenic proteins and dental pulp mineralization". *J Materials Sci: Materials Medicine* 2002, Vol. 13, pages 225-232. All document	
A	WO 2006/138690 A2 (ABBOTT LABORATORIES) 28 December 2006. Abstract, pages 2-4, pages 22, 31-32	
A	US 6,180,606 B1 (CHEN *et al.*) 30 January 2001 Examples 5 and claims	
A	WO 97/40137 A1 (OSIRIS THERAPEUTICS, INC.) 30 October 1997. Abstract, pages 3-5, 16, 17.	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2008/000627

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Kaps C. *et al.* "Bone morphogenetic proteins promote cartilage differentiation and protect engineered artificial cartilage from fibroblast invasion and destruction". *Arthritis & Rheumatism*, 2002, Vol. 46, No. 1, pages 149-162 Abstract, pages 157-159.	11-14
A	Schneider U. "Autogenous bone cell transplantation". *Orthopäde* 1998, Vol. 27, pages 143-146. Summary	
A	Urist M.R. *et al.* "Bone morphogenesis in implants of insoluble bone gelatin". *Proc.Nat.Acad.Sci.USA*, 1973, Vol.70, No. 12, pages 3511-3515. Abstract, page 3511.	
A	Six N. *et al.* "Osteogenic proteins and dental pulp mineralization". *J Materials Sci: Materials Medicine* 2002, Vol. 13, pages 225-232. All document	
A	WO 2006/138690 A2 (ABBOTT LABORATORIES) 28 December 2006. Abstract, pages 2-4, pages 22, 31-32	
A	US 6,180,606 B1 (CHEN *et al.*) 30 January 2001 Examples 5 and claims	
A	WO 97/40137 A1 (OSIRIS THERAPEUTICS, INC.) 30 October 1997. Abstract, pages 3-5, 16, 17.	