



(51) International Patent Classification:

A61L 27/20 (2006.01) A61L 27/52 (2006.01)
A61L 27/38 (2006.01) A61L 27/54 (2006.01)

(21) International Application Number:

PCT/GB2015/051988

(22) International Filing Date:

9 July 2015 (09.07.2015)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

1412236.0 9 July 2014 (09.07.2014) GB

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

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

Published:

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

(54) Title: METHOD OF PRODUCING AND USES OF ALGINATE HYDROGELS

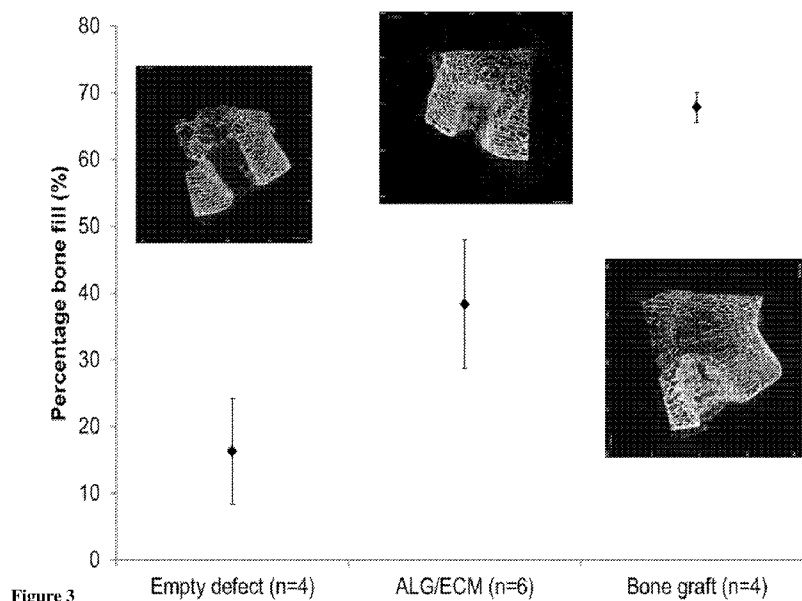


Figure 3

(57) Abstract: A method of forming an alginate hydrogel in a locus in the human or animal body, comprising i) mixing an alginate solution with a viscosity in the range of 200 – 3000 centipoise with a quantity of divalent ions to make an alginate containing composition; ii) administering the alginate containing composition to a locus in the human or animal body; and iii) forming an alginate hydrogel in the locus wherein at least 50% of the alginate containing composition is retained in the locus.

METHOD OF PRODUCING AND USES OF ALGINATE HYDROGELS

The present invention relates to a method of producing alginate hydrogels and to compositions for use in producing alginate hydrogels. In particular, such hydrogels may be used in tissue repair, and in particular in bone repair.

Degenerative disease (particularly osteoporosis), severe infection, trauma and the excision of tumours can result in large non-healing defects in bone and other tissues. Similarly, some surgical procedures, such as removal of diseased tissue, harvesting of tissue for a biopsy or autogenous transplant, and implant fixation, often require the formation of a cavity within either soft or hard tissue, including bone. Current treatment options for treating large non-healing defects have limited effectiveness, particularly in bone. Whilst autologous bone grafts are considered to be the gold standard with the best clinical outcome, significant limitations including restricted availability of donor tissue and morbidity at the harvest site mean such grafts are not always possible. Allografts also face issues of processing, sterilisation, disease transmission and potential immunogenic response, with high rates of fractures and complications attributed to their limited ability to revascularise and remodel.

In the US alone there are 13 million physician office visits and 3.7 million emergency room visits annually for back pain alone. There are over 3m spinal procedures performed globally each year. Current treatment strategies for spinal bone repair use non-biodegradable materials: typically metallic cages and fixation devices in spinal fusion, and stents and PMMA cements in vertebral compression fractures. PMMA cements are non-resorbable and do not promote bone healing. Furthermore, if cement leakage occurs the potential complications are devastating, and include neurological damage or even death. Although clinical outcomes are often excellent, the long term risk of severe complications from these materials remains a concern. Complications, including stress-shielding due to mechanical incompatibility with host bone, corrosive effects of metallic cages and implant migration, contribute to the incidence of revision surgery. There also remain concerns over cement leakage and the need for long term studies of the *in vivo* effects of PMMA (poly methyl methacrylates), thus the lifelong

implantation of non-degradable devices remains a subject of on-going debate. There is therefore a strong, and as yet unmet, need for alternative bone repair materials, in particular biocompatible and biodegradable materials. There is also a need to develop new products to non-invasively deliver osteoprogenitor cells and growth factors to harness the body's innate ability to form bone.

It is an aim of the present invention to provide a novel material for use in large non-healing defects in tissues, in particular for use in bone defects. In particular, it is an aim of the present invention to provide a product that can be used to enhance bone formation, in particular in spinal fusion and in the treatment of vertebral compression fractures.

According to a first aspect of the invention, there is provided a method of forming an alginate hydrogel in a locus in the human or animal body, comprising

i) mixing an alginate solution with a viscosity in the range of 200 – 3000 centipoise with a quantity of divalent ions to make an alginate containing composition;

ii) administering the alginate containing composition to a locus in the human or animal body; and

iii) forming an alginate hydrogel in the locus wherein at least 50% of the alginate containing composition is retained in the locus.

According to another aspect the invention provides an alginate solution with viscosity in the range of 200 – 3000 centipoise for use in the treatment of a human or animal subject, wherein the treatment comprises administering the alginate solution with a quantity of divalent ions to a locus in the subject wherein the alginate crosslinks to form a hydrogel, preferably at least 50% of the alginate solution remains at the locus.

According to another aspect the invention provides a quantity of divalent ions for use in the treatment of a human or animal subject, wherein the treatment comprises

administering the quantity of divalent ions with an alginate solution with viscosity in the range of 200 – 3000 centipoise to a locus in the subject wherein the alginate crosslinks to form a hydrogel, preferably at least 50% of the alginate solution remains at the locus.

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According to another aspect the invention provides an alginate containing composition for use in the treatment of a human or animal subject, wherein the treatment comprises administering the alginate containing composition which comprises a quantity of divalent ions and an alginate solution with viscosity in the range of 200 – 3000 centipoise to a locus in the subject, and wherein the alginate crosslinks to form a hydrogel, preferably at least 50% of the alginate solution remains at the locus.

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Preferably the locus is a void or cavity in a tissue, the tissue may be any tissue, in particular the tissue may be bone. The void or cavity may have arisen due to disease or damage or may be the result of a surgical procedure, such as removal of diseased tissue, harvesting of tissue for a biopsy or autogenous transplant, and implant fixation. The locus may also be a void created during the treatment of a disease or condition, for example, in vertebral compression fractures in the repair process a stent is placed into the fractured vertebra to open up the fracture thus creating a cavity.

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Alginate is commercially available and is typically extracted from brown algae by treatment with aqueous alkali solutions, such as NaOH. Alginate describes derivatives of alginic acid, including mono or divalent salts such as calcium, sodium or potassium salts or propylene glycol alginate. Preferably in the invention a monovalent metallic salt of alginate is used, more preferably sodium alginate. Sodium alginate is obtained from the brown seaweed *Phaeophyceae* and is the sodium salt of alginic acid. Its empirical formula is $(\text{NaC}_6\text{H}_7\text{O}_6)_n$.

25

Sodium alginate is a linear copolymer containing blocks of (1,4)-linked β -D-mannuronate (M) and α -L-guluronate (G) residues. The blocks are composed of consecutive G residues (GGGGGG), consecutive M residues (MMMMMM) and

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alternating M and G residues (GMGMGM). Examples of G-block, M-block and alternating G and M block structures of alginate are given in Figure 1 of Lee & Mooney, Progress in Polymer Science 37, 2012, 106-136. The amount, distribution and length of each block depends on the species, location and age of the seaweed from which the alginate is isolated.

Preferably the invention uses a between about 1% and about 5% w/v, preferably between about 2% and about 3% w/v, alginate solution to provide a solution with a viscosity in the range of 200 – 3000 centipoise. The skilled man will appreciate that if a lower or higher viscosity grade alginate is used then the percentage of alginate will have to be increased or decreased to give the desired solution viscosity.

The percentages recited refer to the percent w/v of a component, that is, the grams per 100ml of solution.

The alginate may be provided in a suitable carrier, preferably an aqueous carrier such as water, NaCl, phosphate buffered saline (PBS), saline, HBSS, HEPES, Ringer buffer, Krebs buffer or an aqueous calcium free media (particularly if cells are to be included).

Divalent cations used in the invention may be selected from Ca^{2+} , Ba^{2+} , Sr^{2+} , Mg^{2+} , Zn^{2+} and Fe^{2+} . Preferably the divalent cation used is Ca^{2+} . In a preferred embodiment this may be provided by calcium chloride (CaCl_2), calcium sulphate (CaSO_4) or calcium carbonate (CaCO_3), preferably the divalent cation is provided by CaCl_2 .

The divalent cation, preferably Ca^{2+} , may be provided at a concentration of between about 0.05% and about 1.5% w/v, for example between about 0.07% and about 1.4%. more preferably between about 0.05% and about 0.2%, or between about 0.05% and about 0.15%, more preferably between about 0.1% and about 0.15%, for example between about 0.09% and about 0.1%.

The divalent cation may be provided by adding CaCl_2 , preferably a concentration of between about 0.2% and about 4% w/v CaCl_2 is added, more preferably between about 0.25% and about 0.4%, more preferably between about 0.25% and about 0.3% CaCl_2 .

- 5 In a preferred embodiment the alginate solution comprising between about 1% and about 5% w/v, preferably between about 2% and about 3% w/v, alginate is mixed with a solution comprising between about 0.05% and about 0.2% w/v, more preferably between about 0.5% and about 0.15%, more preferably between about 0.1% and about 0.15% divalent cations, in a ratio of about 3:1 alginate solution to divalent cation solution.
- 10 The alginate solution and the cation containing solutions may be fixed in a ratio of about 5:1, 4:1, 3:1, 2:1, 1:1 of alginate solution to cation containing solution. Preferably if an about 3% alginate solution is used it is mixed in a 2:1 ratio, whereas if an about 2% alginate solution is used it is mixed in a 3:1 ratio.
- 15 In an embodiment the addition of a suitable cation concentration to an alginate solution will result in the production of an alginate hydrogel. The cation source may be added in an amount of 1 – 50 wt%, more preferably in an amount of 2 – 10% based on the dry weight of alginate when using low viscosity (eg 200 – 1000 cP) sodium alginate. For example 2 ml of a crosslinker solution of 0.3% (w/v) calcium chloride may be used per
- 20 6 ml solution of 2% w/v low viscosity sodium alginate.

- In an embodiment the divalent cations are believed to bind solely to the G-blocks of alginate chains as the structure of the G-blocks allows a high degree of coordination of the divalent cations. The G-blocks of one polymer may then form junctions with the G-
- 25 blocks on adjacent polymer chains in an egg box model of cross-linking to produce a gel structure. That is, the divalent cations may cooperatively interact with blocks of G monomers to form ionic bridges between different polymer chains.

- Preferably upon mixing the alginate solution with the divalent cations to form an
- 30 alginate containing composition it takes less than 30 minutes, less than 20 minutes, less than 15 minutes, less than 10 minutes, less than 5 minutes for the alginate in the

alginate containing composition to cross link and form a hydrogel. The time taken to form a hydrogel is a balance between having time to deliver the alginate containing composition to a locus in a subject and the alginate containing composition being retained as a hydrogel in the locus. In an embodiment the alginate solution and cation
5 containing solution may be mixed for up to 5 minutes, and the hydrogel will form about 5 minutes thereafter, this allows 5 minutes for administration of the alginate containing composition.

Preferably the alginate solution and divalent ions are mixed at temperature between
10 about room temperature and about body temperature, that is, between about 20°C and about 37°C.

In an embodiment the alginate solution and divalent ions are mixed about 15 minutes, about 10 minutes, about 5 minutes, preferably within about 1 minute or less, before
15 application to a locus, that is the clinical site of use, for example a bone.

Preferably the alginate containing composition forms a hydrogel at the locus where applied such that the majority of the composition is retained at the locus. Preferably at least 50%, 60%, 70%, 80%, 90% or more of the alginate containing composition applied
20 is retained at the locus.

The method may further comprise the step of adding a solution comprising further divalent cations, for example about 0.3% Ca^{2+} or about 1% CaCl_2 , to the hydrogel after application of the alginate containing composition to the locus to provide further
25 crosslinking in the hydrogel. The additional divalent ions may cause further crosslinking in the hydrogel which provides further rigidity and strength to the hydrogel and may further immobilise the gel at the site of administration.

By using a composition which solidifies to form a hydrogel after administration, a
30 hydrogel can be formed which conforms to the shape of where it is placed, for example,

the shape of the locus, tissue cavity, into which it is placed. This overcomes a problem with hydrogels having to be fabricated prior to administration, and allows administration to loci where it would otherwise require invasive surgery to administer a fabricated hydrogel.

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Preferably the alginate containing composition is intended to be administered by injection into the body of a human or non-human animal. If the composition is injected then the need for invasive surgery to position the hydrogel is removed. Preferably the alginate containing composition has a viscosity which allows it to be administered, using
10 normal pressure, from a syringe which has an orifice of about 4mm or less. The size of the orifice will depend on the medical application, for example, for many bone applications a syringe with an orifice of between about 2mm and about 4mm will be used, however, for other applications smaller or larger orifices may be preferred. Preferably “normal pressure” is that applied by a human administering the composition to
15 a patient using one hand.

Preferably the alginate containing composition is of sufficient viscosity when it reaches the delivery locus that it does not immediately dissipate, as water would, but instead takes the form of the site where it is administered and crosslinks to form a hydrogel.

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The alginate containing composition may be administered via a cannula to a subject, the cannula may be up to 50cm long and may have a diameter of up to 1cm. The alginate containing composition must retain sufficient viscosity to pass down the cannula before it forms a hydrogel, otherwise it will block the cannula. It is important for the balance to
25 be struck between being able to administer the alginate containing composition and the hydrogel forming in the locus where administered such that the majority of the alginate containing composition is retained at the locus as a hydrogel. The cannula may be up to 50cm, 45cm, 40cm , 35cm, 30cm, 25cm, 20cm, 15cm, or 10cm long. The cannula diameter may be about 10mm, about 8mm, about 6mm, about 5mm, about 4mm, about
30 3mm, about 2mm or about 1mm or less. The cannula may be a 10g, 11g or 13g cannula.

The alginate gel may further comprise one or more additional agents in addition to the alginate and divalent cations. The additional agents may be added before the hydrogel is formed in a subject or after the hydrogel has formed.

- 5 Generally, the agents include any substance that can be coated on, embedded into, absorbed into, adsorbed to, retained in or otherwise attached to or incorporated onto or into the alginate gel that would provide a benefit, preferably a therapeutic benefit to a patient.
- 10 The additional agents may be therapeutic agents. The additional agents may be used alone or in combination with other agents.

The agent may be a growth factor, such as a neurotrophic or angiogenic factor, which optionally may be prepared using recombinant techniques. Non-limiting examples of

- 15 growth factors include basic fibroblast growth factor (bFGF), acidic fibroblast growth factor (aFGF), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), insulin-like growth factors 1 and 2 (IGF-1 and IGF-2), platelet derived growth factor (PDGF), stromal derived factor 1 alpha (SDF-1 alpha), nerve growth factor (NGF), ciliary neurotrophic factor (CNTF), neurotrophin-3, neurotrophin-4,
- 20 neurotrophin-5, pleiotrophin protein (neurite growth-promoting factor 1), midkine protein (neurite growth-promoting factor 2), brain-derived neurotrophic factor (BDNF), tumor angiogenesis factor (TAF), corticotrophin releasing factor (CRF), transforming growth factors α and β (TGF- α and TGF- β), interleukin-8 (IL-8), granulocyte-macrophage colony stimulating factor (GM-CSF), interleukins, and interferons. In
- 25 certain embodiments, the growth factor is one or more useful growth factors useful in promoting bone regeneration/growth, such as platelet-derived growth factors (PDGFs), the transforming growth factor-beta (TGF- β) family, insulin-like growth factor (IGF-I) and the acidic and basic fibroblast growth factors (FGFs).

- 30 Alternatively or additionally the agent may be an antibiotic or antimicrobial agent, such as, without limitation: acyclovir, ofloxacin, ampicillin, amphotericin B, atovaquone,

azithromycin, ciprofloxacin, clarithromycin, clindamycin, clofazimine, dapsone, diclazuril, doxycycline, erythromycin, ethambutol, fluconazole, fluoroquinolones, foscarnet, ganciclovir, gentamicin, iatroconazole, isoniazid, ketoconazole, levofloxacin, lincomycin, miconazole, neomycin, norfloxacin, ofloxacin, paromomycin, penicillin, pentamidine, polymyxin B, pyrazinamide, pyrimethamine, rifabutin, rifampin, sparfloxacin, streptomycin, sulfadiazine, tetracycline, tobramycin, trifluorouridine, trimethoprim sulphate, Zn-pyrithione, and silver salts such as chloride, bromide, iodide and periodate.

Alternatively or additionally the agent may be an anti-inflammatory agent, such as, without limitation, an NSAID, such as salicylic acid, indomethacin, sodium indomethacin trihydrate, salicylamide, naproxen, colchicine, fenoprofen, sulindac, diflunisal, diclofenac, indoprofen, sodium salicylamide; an anti-inflammatory cytokine; an anti-inflammatory protein; a steroidal anti-inflammatory agent; or an anti-clotting agents, such as heparin, ebac, enoxaprin, aspirin, hirudin, plavix, bivalirudin, prasugrel, idraparinux, warfarin, coumadin, clopidogrel, PPACK, GGACK, tissue plasminogen activator, urokinase, and streptokinase. Other drugs that may promote wound healing and/or tissue regeneration may also be included.

Alternatively or additionally the agent may be an immunosuppressant/immunomodulatory agent, for example glucocorticoids such as hydrocortisone, betamethasone, dexamethasone, flumethasone, isoflupredone, methylpred-nisolone, prednisone, prednisolone, and triamcinolone acetonide; antibodies; drugs acting on immunophilins, such as cyclosporine, zotarolimus, everolimus, tacrolimus and sirolimus (rapamycin), interferons, and TNF binding proteins.

Alternatively or additionally the agent may be an antiangiogenic agent such as fluorouracil, paclitaxel, doxorubicin, cisplatin, methotrexate, cyclophosphamide, etoposide, pegaptanib, lucentis, tryptophanyl-tRNA synthetase, retene, CA4P, AdPEDF, VEGF-TRAP-EYE, AG-103958, Avastin, JSM6427, TG100801, ATG3, OT-551, endostatin, thalidomide, bevacizumab and neovastat.

Alternatively or additionally the agent may be an antiproliferative agent such as sirolimus, paclitaxel, perillyl alcohol, farnesyl transferase inhibitors, FPTIII, L744,

antiproliferative factor, Van 10/4, doxorubicin, 5-FU, Daunomycin, Mitomycin, dexamethasone, azathioprine, chlorambucil, cyclophosphamide, methotrexate, mofetil, vasoactive intestinal polypeptide, and PACAP.

- 5 Alternatively or additionally the agent may be a protein, including one or more that is useful for osteogenic purposes, such as bone morphogenetic proteins (BMPs) and osteogenic proteins (OPs).

10 The alginate gel may further include extracellular matrix (ECM). The ECM may comprise the secreted products of resident cells from specific tissues and organs. The ECM preferably comprises growth factors, cytokines, chemokines, and other signalling molecules. The ECM may be bone derived.

15 The alginate gel may further comprise microparticles, nanoparticles or particulate matter. The microparticles, nanoparticles or particulate matter may be polymeric, ceramic or glass. The ceramic may be a bioactive glass, bioglass, hydroxyapatite or calcium phosphate or a combination thereof.

20 The one or more additional agents may be administered in the microparticles, nanoparticles or particulate matter. The agents may be provided in polymeric microparticles. The discrete particles may be of one or more polymer, preferably one or more synthetic polymer. The particles may comprise one or more polymer selected from the group comprising poly (α -hydroxyacids) including poly (D,L-lactide-co-glycolide)(PLGA), poly D,L-lactic acid (PDLA), polyethyleneimine (PEI), polylactic
25 or polyglcolic acids, poly-lactide poly-glycolide copolymers, and poly-lactide poly-glycolide polyethylene glycol copolymers, polyethylene glycol (PEG), polyesters, poly (ϵ -caprolactone), poly (3-hydroxy-butyrate), poly (s-caproic acid), poly (p-dioxanone), poly (propylene fumarate), poly (ortho esters), polyol/diketene acetals addition polymers, polyanhydrides, poly (sebacic anhydride) (PSA), poly
30 (carboxybiscarboxyphenoxyphosphazene) (PCPP), poly [bis (p-carboxyphenoxy) methane] (PCPM), copolymers of SA, CPP and CPM (as described in Tamat and Langer

in Journal of Biomaterials Science Polymer Edition, 3, 315-353. 1992 and by Domb in Chapter 8 of The Handbook of Biodegradable Polymers, Editors Domb A J and Wiseman R M, Harwood Academic Publishers), poly (amino acids), poly (pseudo amino acids), polyphosphazenes, derivatives of poly [(dichloro) phosphazene], poly [(organo) phosphazenes], polyphosphates, polyethylene glycol polypropylene block co-polymers for example that sold under the trade mark Pluronics™, natural or synthetic polymers such as silk, elastin, chitin, chitosan, fibrin, fibrinogen, polysaccharides (including pectins), alginates, collagen, peptides, polypeptides or proteins, copolymers prepared from the monomers of any of these polymers, random blends of these polymers, any suitable polymer and mixtures or combinations thereof.

The microparticles may be designed to allow the release kinetics of the further therapeutic agents to be controlled. For example, the microparticles may have defined degradation rates. The microparticles may allow for agent release to be sustained for some time, preferably at least about 2 hours, at least about 4 hours, at least about 6 hours, at least about 10 hours, at least about 12 hours, at least about 24 hours, more preferably at least 48 hours, preferably at least a week, preferably more than one week, preferably more than 10 days.

The alginate hydrogel may further include cells, such as genetically modified cells, stem cells, progenitor cells, and the like. Cells may be mixed with the alginate solution or the cation containing solution prior to mixing, or to the alginate containing composition prior to gelation, or cells may be seeded on the hydrogel once gelation has occurred. In one non-limiting embodiment, genetically modified cells are capable of expressing a therapeutic or bioactive substance, such as a growth factor. Cells can be modified by any useful method in the art. For example and without limitation, the therapeutic agent is a growth factor that is released by cells transfected with cDNA encoding for the growth factor. Therapeutics agents that can be released from cells include, without limitation, a neurotrophic factor, such as nerve growth factor, brain-derived neurotrophic factor, neurotrophin-3, neurotrophin-4, neurotrophin-5, and ciliary neurotrophic factor; a growth factor useful in promoting bone regeneration/growth, such as platelet-derived growth factors (PDGFs), the transforming growth factor-beta (TGF- β) family, insulin-like growth factor (IGF-I) and the acidic and basic fibroblast growth

factors (FGFs); an anti-inflammatory cytokine; and an anti-inflammatory protein. The cells may be autologous, allogeneic, or xenogeneic.

5 The cells seeded on the hydrogel may be, for example, mesenchymal stem cells, adipose derived mesenchymal stem cells, bone marrow derived mesenchymal stem cells, induced pluripotent stem cells, osteoprogenitor cells, osteoblasts, chondrocytes, bone cells, bone marrow cells, soft connective tissue cells, fibrous tissue cells, cartilage cells, muscle tissue cells, mucous epithelium cells, ligament cells, tendon cells, liver cells, kidney cells, skin cells, endothelial cells, gut cells, intestinal cells, cardiovascular cells, 10 cardiomyocytes, pulmonary cells, placental cells, amnionic cells, chorionic cells, foetal cells or stem cells. Where stem cells are used, preferably non-embryonic stem cells are used. The cells may be included for delivery to the site of hydrogel formation, or they may be included and intended to be retained in the hydrogel, for example, to encourage tissue regeneration in the hydrogel.

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In an embodiment, patient specific mesenchymal stem cells, in particular adipose derived mesenchymal stem cells, are used in conjunction microparticles which have controlled release of growth factors, thus avoiding the complications of supraphysiological growth factor doses.

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Preferably the alginate hydrogel and components used to make the hydrogel are biodegradable and biocompatible. Preferably at least 50% of the gel will have biodegraded within 2 years, 1 year, or 6 months of implantation in a subject

25 Reference to biocompatible is intended to refer to the fact that none of the components raise any significant immune response when implanted into a subject. Preferably the components and hydrogel gel formed are not toxic to a host and are able to function in a human or animal subject.

30 Preferably the hydrogel is a temporary substitute in a cavity or void which is intended

to selectively promote tissue repair or regeneration, in particular bone regeneration and/or repair.

5 The method or composition of the invention may be used in bone repair and/or regeneration.

10 In an embodiment the hydrogel may be for use in the treatment of vertebral compression fractures. The alginate containing composition may be injected into a vertebral stent located in the spine of human or animal subject. In an embodiment the alginate containing composition of the invention may be injected into a vertebral stent where it crosslinks to form a hydrogel and at least 50%, 60%, 70%, 80%, 90% or more of the alginate containing composition remains in the stent. The stent may have a lattice mesh structure and preferably only minimal alginate containing composition leaves the stent. Preferably the alginate containing composition can be injected down a
15 cannula into the stent prior to the hydrogel forming. The cannula is preferably between 5 and 50cm long, more preferably between 5 and 30cm long. The alginate containing composition must retain sufficient viscosity to pass down the cannula in order for it to be administered to a locus.

20 The method or composition of the invention may also be used as a prophylactic treatment for osteoporosis, trauma, or the like by replacing weakened bone with a stronger synthetic bone substitute using minimally invasive surgical procedures. The weakened bone may first be surgically removed from the affected site, thereby forming a cavity. The cavity may then be filled with an alginate containing composition
25 according to the invention and allowed to gel to form a hydrogel. The alginate hydrogel may then promote new bone growth which may provide structural reinforcement and lessen the risk of fracture of the affected bone.

30 The method or composition of the invention may also be used to treat bone cancer and avascular necrosis both of which are initially surgically treated by removing diseased tissue which creates a tissue cavity, the cavity may then be filled with an alginate

containing composition according to the invention, in this way bone regrowth can be encouraged in a minimally invasive manner.

5 The method or composition of the invention may be used for the treatment or prevention of a condition selected from: neurodegeneration disorders (e.g. post stroke, Huntington's, Alzheimer's disease, Parkinson's disease), bone-related disorders (including osteoarthritis, spinal disk atrophy, bone cavities requiring filling, bone fractures requiring regeneration or repair), burns, cancers, liver disorders (including hepatic atrophy), kidney disorders (including atrophy of the kidney), disorders of the bladder, 10 ureter or urethra (including damaged ureter or damaged bladder requiring reconstruction, prolapse of the bladder or the uterus), diabetes mellitus, infertility requiring IVF treatment, muscle wasting disorders (including muscular dystrophy), cardiac disorders (e.g. damaged cardiac tissue post myocardial infarction, congestive heart disease), eye disorders (e.g. damaged or diseased cornea), damaged vasculature requiring regeneration or repair, ulcers, and damaged tissue requiring regeneration or reconstruction (including 15 damaged organ requiring regeneration or reconstruction, and damaged nerves requiring regeneration or reconstruction).

20 According to a further aspect the invention provides a method for treating vertebral compression fractures the method comprising:

locating a stent at the site of compression;

delivering an alginate containing composition according to the invention to the stent via a hollow cannula connected to the stent

25 allowing the alginate containing composition to form a hydrogel inside the stent, wherein at least 50% of the delivered alginate containing composition forms a hydrogel and is retained in the stent.

The stent may be arranged such that it is located at the site of compression and then opened or inflated to create a void, this void may then be filled by the alginate 30 containing composition

The stent may have a mesh like structure. The stent may be made of metal and may be retained at the site of implantation. Alternatively the stent may be made of a biodegradable material that will degrade overtime, preferably once bone has regenerated in the hydrogel to provide the physical support needed.

According to another aspect, the invention provides a kit for use in forming a hydrogel at a locus in a human or animal body, wherein the kit comprises an alginate solution with a viscosity in the range of 200-3000 centipoise and a divalent ion containing solution, together with instructions to mix the two solutions in a certain ratio and to administer to a human or animal.

The kit may include a syringe for use in injecting the mixed composition. The alginate solution and the divalent ion containing solutions may be provided in preloaded syringes, ready for use. Preferably the kit also contains a connector to connect the two syringes and allow the two solutions to be mixed prior to administration. Preferably the kit can be stored either refrigerated or at room temperature.

The skilled man will appreciate that preferred features of any one embodiment and/or aspect of the invention may be applied to all other embodiments and/or aspects of the invention.

The present invention will be further described in more detail, by way of example only, with reference to the following figures in which:

Figure 1 – is a table illustrating stents containing hydrogels formed from an alginate containing composition comprising either 2% or 3% sodium alginate in PBS and 0.25% or 0.3% CaCl_2 in dH_2O .

Figure 2 –illustrate stents containing hydrogels formed from an alginate containing composition comprising either 2% or 3% sodium alginate in calcium

free cell medium and 0.25% CaCl_2 in dH_2O .

Figure 3 – illustrates the effect of an alginate/ECM solution on bone repair, and compares this to no agent and a bone graft.

Figure 4 – illustrates the effect of various alginate solutions on bone repair, and compares them to no agent and a bone graft.

A 2% or 3% low viscosity sodium alginate solution was prepared by the addition 2g or 3g of low viscosity sodium alginate to 100 ml of PBS or calcium free medium, the solution was stirred. The 2% or 3% low viscosity sodium alginate solution was then rocked, rotated or stirred for 24 hours at 37°C to produce a more viscous solution which was still pourable and had a viscosity between 200 – 3000. Calcium chloride solutions were produced in deionised water. 0.25g or 0.3 g of calcium chloride was added to 100 ml of dH_2O and mixed to produce a 0.25% or a 0.3% w/v solution. A 1% w/v calcium chloride solution was also prepared (1 g CaCl_2 added to 100 ml dH_2O). Stents (provided by Alphatec spine) were pre-soaked in the 1% CaCl_2 solution for 2 – 10 minutes

Sodium alginate solution and calcium chloride (ratio of 3ml:1ml of 2% sodium alginate to 0.25% or 0.3% calcium chloride, and a ratio of 2ml:1ml of 3% sodium alginate to 0.25% or 0.3% calcium chloride) were mixed in two syringes connected by a syringe connector with 20 passes back and through the syringes. The crosslinked sodium alginate solution was then drawn up into one syringe and injected into the top of a 20cm cannula and into the stent. The photographs in Figure 1 and 2 illustrate the alginate hydrogel formed within the open mesh stent under the conditions described above. It is clear that there is no or only minimal ‘ballooning’ through the holes in the mesh of the stent. Similar results were seen (but are not illustrated) when 2.8% alginate was used.

To demonstrate the efficacy of alginate hydrogels according to the invention in inducing bone repair studies were undertaken on ovine femurs. An ovine femoral condyle defect was created in thirty female non-pregnant skeletally mature (age 2-4 years, weight 60-80 kgs) English Mule sheep. The surgical site was incised and a drill hole was made in the cancellous bone of the medial femoral condyle in the left and right hind leg. The

- cylindrical hole was 8mm diameter x 15mm deep and created with a custom bone corer to create a standardised defect. In the ovine cancellous bone defect model a critical size defect has been published at a diameter of 5.1mm – 10mm. The sheep were then divided into three test groups, one where the defect was left empty, one where an alginate hydrogel according to the invention comprising ECM was applied to the defect, and one where a bone graft was applied to the defect. The animals were sacrificed after 8 weeks using an overdose of barbiturate (Pentobarbital solution 20 %, 0.7 mg/kg, Pharmasol Ltd., Andover, UK). The femoral condyle was then removed and fixed in alcoholic formalin for 5 days at room temperature. The percentage bone fill was then determined and images were taken using micro CT. The results and images are shown in Figure 3. Form the results it can be seen that after 8 weeks defects treated with an alginate hydrogel according to the invention had started to show bone growth, with at least 30% bone fill being observed.
- Figure 4 shows the results when the ovine experiment described above was repeated using alginate hydrogels according to the invention with various combinations of ECM, BMP2 and stem cells/bone progenitor cells.

CLAIMS

1. A method of forming an alginate hydrogel in a locus in the human or animal body, comprising
 - 5 i) mixing an alginate solution with a viscosity in the range of 200 – 3000 centipoise with a quantity of divalent ions to make an alginate containing composition;
 - ii) administering the alginate containing composition to a locus in the human or animal body; and
 - 10 iii) forming an alginate hydrogel in the locus wherein at least 50% of the alginate containing composition is retained in the locus.
2. An alginate solution with viscosity in the range of 200 – 3000 centipoise for use in the treatment of a human or animal subject, wherein the treatment comprises administering the alginate solution with a quantity of divalent ions to a locus in the
15 subject wherein the alginate crosslinks to form a hydrogel.
3. A quantity of divalent ions for use in the treatment of a human or animal subject, wherein the treatment comprises administering the quantity of divalent ions with an alginate solution with viscosity in the range of 200 – 3000 centipoise to a locus in the
20 subject wherein the alginate crosslinks to form a hydrogel.
4. An alginate containing composition for use in the treatment of a human or animal subject, wherein the treatment comprises administering the alginate containing composition which comprises a quantity of divalent ions and an alginate solution with
25 viscosity in the range of 200 – 3000 centipoise to a locus in the subject, and wherein the alginate crosslinks to form a hydrogel.
5. The composition of claim 2, 3, or 4 wherein at least 50% of the alginate solution remains at the locus after administration.

6. The method of claim 1 or the composition of any of claims 2 to 5 wherein the alginate is sodium alginate.

5 7. The method or composition of any preceding claim wherein between about 2% and about 3% w/v alginate solution is used.

8. The method or composition of any preceding claim wherein the divalent cation is Ca^{2+} .

10

9. The method or composition of any preceding claim wherein the cation is provided at a concentration of between about 0.07% and about 1.4%.

10. The method or composition of any preceding claim wherein the alginate solution
15 is mixed with a solution containing divalent cations at a ratio of between about 2:1 and 3:1.

11. The method or composition of any preceding claim wherein the alginate
containing composition is of sufficient viscosity when it reaches the delivery locus that
20 it does not immediately dissipate, as water would, but instead takes the form of the site where it is administered and crosslinks to form a hydrogel.

12. The method or composition of any preceding claim wherein the resulting
hydrogel comprises one or more additional agents in addition to the alginate and
25 divalent cations.

13. The method or composition of claim 12 wherein the additional agent may be a

therapeutic agent.

14. The method or composition of any preceding claim wherein the resulting hydrogel further includes cells.

5

15. The method or composition of claim 14 wherein the cells are adipose derived mesenchymal stem cells

10 16. The method or composition of any preceding claim wherein the hydrogel is intended to promote tissue repair or regeneration.

17. The method or composition of any preceding claim for use in the treatment of vertebral compression fractures.

15 18. The method or composition of any preceding claim for use a prophylactic treatment for osteoporosis, trauma, or the like by replacing weakened bone with a stronger synthetic bone substitute.

20 19. The method or composition of any preceding claim for use in the treatment of bone cancer or avascular necrosis or both.

25 20. The method or composition of any preceding claim for use in the treatment or prevention of a condition selected from: neurodegeneration disorders (e.g. post stroke, Huntington's, Alzheimer's disease, Parkinson's disease), bone-related disorders (including osteoarthritis, spinal disk atrophy, bone cavities requiring filling, bone fractures requiring regeneration or repair), burns, cancers, liver disorders (including hepatic atrophy), kidney disorders (including atrophy of the kidney), disorders of the bladder, ureter or urethra (including damaged ureter or damaged bladder requiring

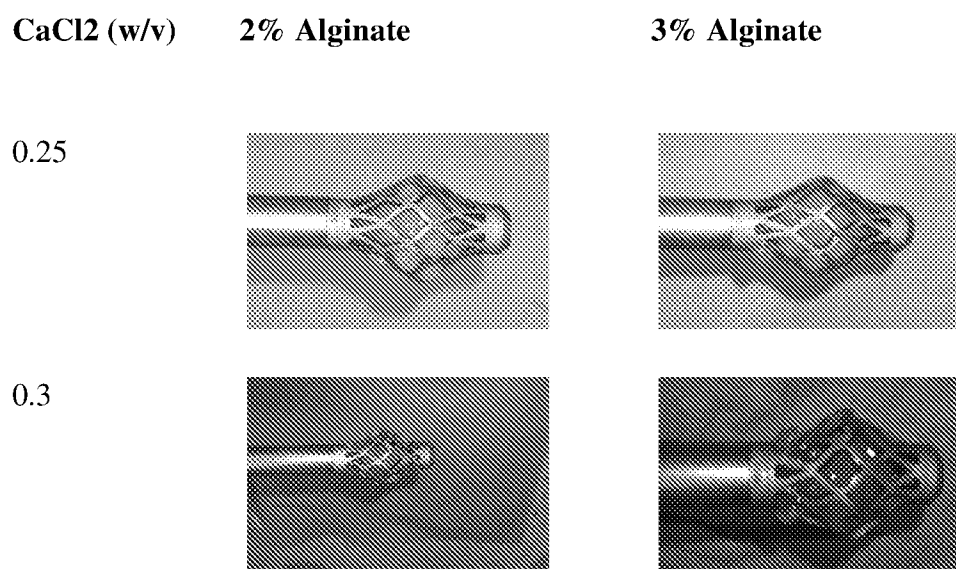
reconstruction, prolapse of the bladder or the uterus), diabetes mellitus, infertility requiring IVF treatment, muscle wasting disorders (including muscular dystrophy), cardiac disorders (e.g. damaged cardiac tissue post myocardial infarction, congestive heart disease), eye disorders (e.g. damaged or diseased cornea), damaged vasculature requiring regeneration or repair, ulcers, and damaged tissue requiring regeneration or reconstruction (including damaged organ requiring regeneration or reconstruction, and damaged nerves requiring regeneration or reconstruction).

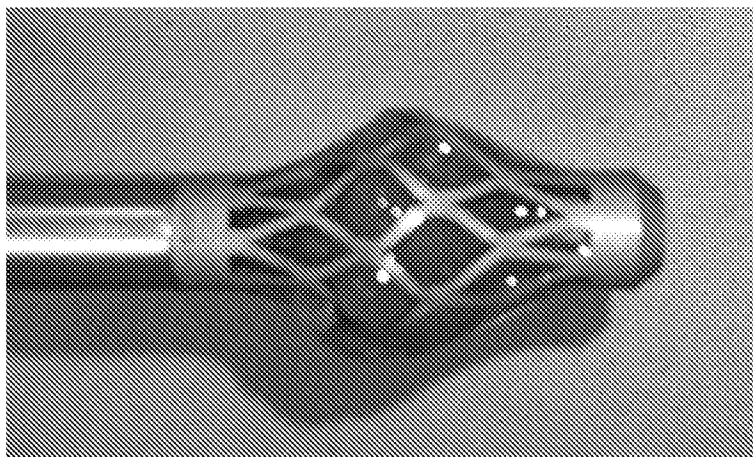
21. A method for treating vertebral compression fractures the method comprising:

- 10 locating a stent at the site of compression;
- delivering an alginate containing composition according to the invention to the stent via a hollow cannula connected to the stent;
- allowing the alginate containing composition to form a hydrogel inside the stent, wherein at least 50% of the delivered alginate containing composition forms a
- 15 hydrogel and is retained in the stent.

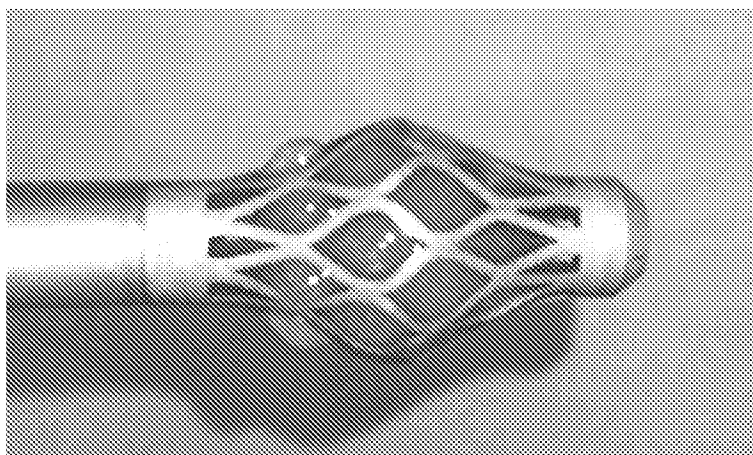
22. A kit for use in forming a hydrogel at a locus in a human or animal body, wherein the kit comprises an alginate solution with a viscosity in the range of 200-3000 centipoise and a divalent ion containing solution, together with instructions to mix the

20 two solutions in a certain ratio and to administer to a human or animal.

**Figure 1**



2% Alginate 0.25% CaCl_2



3% Alginate 0.25% CaCl_2

Figure 2

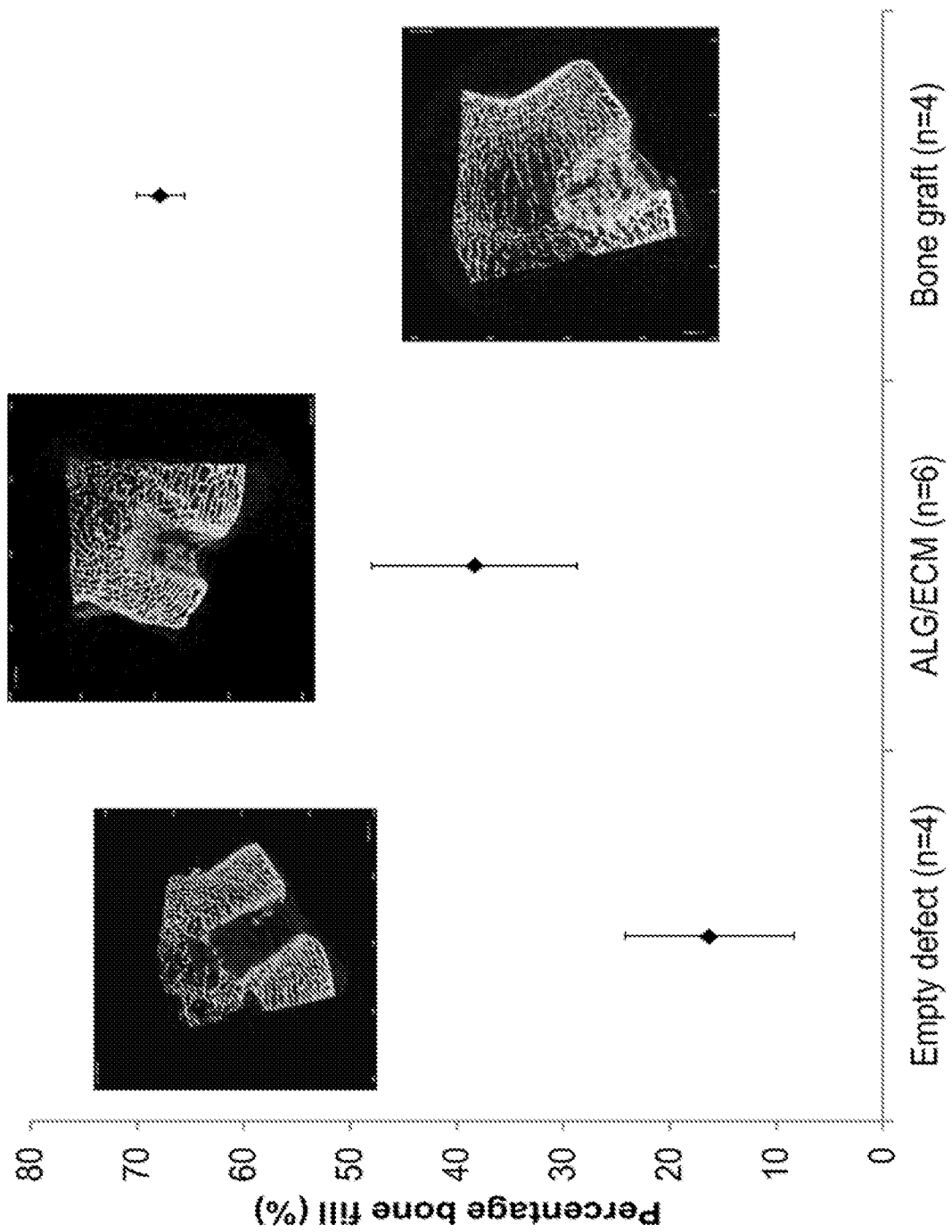
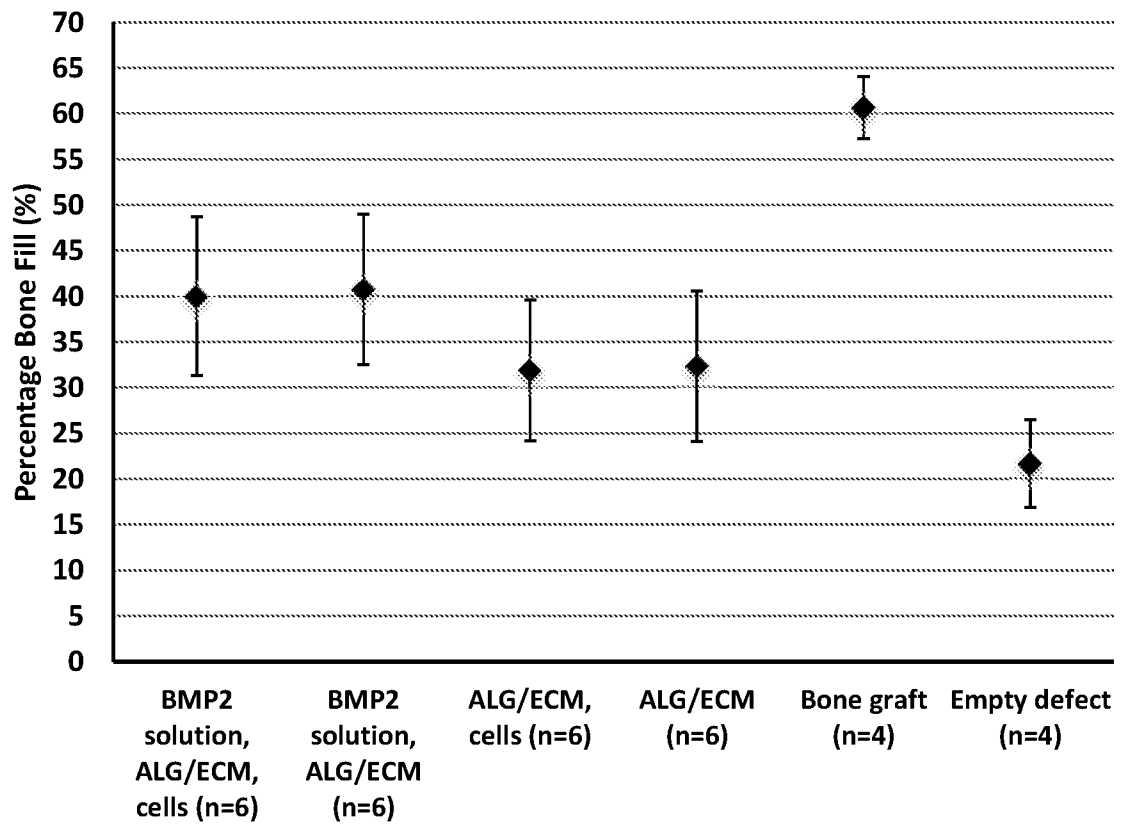


Figure 3

**Figure 4**

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2015/051988

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61L27/20 A61L27/38 A61L27/52 A61L27/54
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61L

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)

EPO-Internal, WPI Data

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Further documents are listed in the continuation of Box C.



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"&" document member of the same patent family

Date of the actual completion of the international search

10 September 2015

Date of mailing of the international search report

16/09/2015

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INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2015/051988

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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International application No

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