



(43) International Publication Date
28 June 2012 (28.06.2012)

(10) International Publication Number
WO 2012/084214 A1

- (51) **International Patent Classification:**
A61L 27/42 (2006.01) *A61L 27/56* (2006.01)
- (21) **International Application Number:**
PCT/EP2011/006457
- (22) **International Filing Date:**
21 December 2011 (21.12.2011)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
EP10015924 22 December 2010 (22.12.2010) EP
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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, BR, BW, BY, BZ,

CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO,
DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN,
HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR,
KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME,
MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ,
OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, 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, SZ, TZ,
UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU,
TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE,
DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU,
LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK,
SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to the identity of the inventor (Rule 4.17(i))
- as to applicant's entitlement to apply for and be granted a
patent (Rule 4.17(ii))

Published:

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

(54) **Title:** BONE SUBSTITUTE MATERIAL

(57) **Abstract:** The invention relates to: - a porous biphasic calcium phosphate/hydroxyapatite (CAP/HAP) bone substitute material comprising a sintered CAP core and at least one uniform and closed epitactically grown layer of nanocrystalline HAP deposited on top of the sintered CAP core, whereby the epitactically grown nanocrystals have the same size and morphology as human bone mineral, i.e. a length of 30 to 46 nm and a width of 14 to 22 nm, which is impregnated with collagen fibers at a weight ratio of said collagen fibers to said porous biphasic calcium phosphate/hydroxyapatite (CAP/HAP) bone substitute material of at least 2%, - a process of preparing the above porous CAP/HAP bone substitute material, which comprises (a) mixing a slurry of collagen fibers and a porous biphasic calcium phosphate/hydroxyapatite (CAP/HAP) bone substitute material comprising a sintered CAP core and at least one uniform and closed epitactically grown layer of nanocrystalline HAP deposited on top of the sintered CAP core, whereby the epitactically grown nanocrystals have the same size and morphology as human bone mineral, i.e. a length of 30 to 46 nm and a width of 14 to 22 nm, and (b) eliminating the water by vacuum suction, - an implant which comprises a porous collagen matrix surrounding and impregnating particles or granules of porous biphasic calcium phosphate/hydroxyapatite (CAP/HAP) bone substitute material comprising a sintered CAP core and at least one uniform and closed epitactically grown layer of nanocrystalline HAP deposited on top of the sintered CAP core, whereby the epitactically grown nanocrystals have the same size and morphology as human bone mineral, i.e. a length of 30 to 46 nm and a width of 14 to 22 nm, - the use of the above bone substitute material as implant or prosthesis for bone formation, bone regeneration, bone repair and/or bone replacement at a defect site in a human or animal.



BONE SUBSTITUTE MATERIAL

The invention relates to a new biphasic bone substitute material with a bilayer structure based on calcium phosphate/hydroxyapatite (CAP/HAP) which is
5 impregnated with collagen fibers, a process for preparing that biphasic bone substitute material and the use thereof as implant or prosthesis to support bone formation, bone regeneration, bone repair, bone remodeling and/or bone replacement at a defect site in a human or animal.

Defects in bone structure arise in a variety of circumstances, such as trauma,
10 disease, and surgery and there is still a need for effective repair of bone defects in various surgical fields.

Numerous natural and synthetic materials and compositions have been used to stimulate healing at the site of a bone defect. A well known natural, osteoconductive bone substitute material that promotes bone growth in periodontal and maxillofacial
15 osseous defects is Geistlich Bio-Oss®, commercially available from Geistlich Pharma AG. That material is manufactured from natural bone by a process described in US Patent No. 5,167,961, which enables preservation of the trabecular architecture and nanocrystalline structure of the natural bone, resulting in an excellent osteoconductive matrix which is not or very slowly resorbed.

20 Tricalcium phosphate / hydroxyapatite (TCP/HAP) systems and their use as bone substitute materials are described, for example, in US-6,338,752 disclosing a process for preparing a biphasic cement of α -TCP/HAP by heating a powder mixture of ammonium phosphate and HAP at 1200-1500°C.

European Patent EP-285826 describes a process for the production of a layer of
25 HAP on metallic and non-metallic bodies for implants by application of a layer of α -TCP and completely converting the α -TCP layer into HAP by reaction with water of

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pH 2 to 7 at 80-100 °C. The product obtained is a metallic or non metallic body covered with a layer of HAP.

WO 97/41273 describes a process for coating a substrate such as notably hydroxyapatite (HAP) or other calcium phosphates (CAP) with a coating of carbonated hydroxyapatite, i.e. hydroxyapatite wherein phosphate and/or hydroxyl ions are partially replaced by bicarbonate ions, by a process comprising (a) immersing the substrate in a solution of pH 6.8 to 8.0 containing calcium ions, phosphate ions and bicarbonate ions at a temperature lower than 50 °C, (b) heating the portion of the solution in contact with the substrate to a temperature of 50 to 80 °C until having a pH greater than 8, (c) maintaining the substrate in contact with the alkali solution obtained in step (b) to form a carbonated hydroxyapatite coating, and (d) taking the substrate off the solution and subjecting the coating to drying. The bicarbonate ions are disclosed to act as inhibitors of hydroxyapatite crystal growth, resulting in non-stoichiometric crystals containing defects and having rather small dimensions, namely 10-40 nm in length and 3-10 nm in width (see page 7, lines 1-7).

The components of calcium phosphate / hydroxyapatite (CAP/HAP) systems, especially TCP/HAP systems differ in their thermodynamic stability. Due to this difference, when CAP/HAP systems are implanted into a mammal, in particular a human patient, the solubility of TCP and other calcium phosphates is higher in the body fluid than the solubility of HAP. The difference in solubility between calcium phosphates and HAP causes a breakdown of the unordered sinter structure of the CAP/HAP system because the better soluble compound CAP (e.g. TCP) is removed quicker than HAP. The sintered interconnection between CAP and HAP produced at high temperatures will also make a remarkable contribution to higher solubility of the device in the physiological environment. Two different types of reactions dominate accelerated in-vivo degradation of such ceramics: Chemical dissolution and biological resorption by cells. Both processes cause dissolution of the ceramic

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material which furthermore causes a local oversaturation of calcium ions, whereby there are more calcium ions released than calcium ions adsorbed. The natural equilibrium of calcium ions no longer exists, neither in the extracellular matrix nor in the tissue surrounding of the implant. The local disturbance of the natural calcium
5 equilibrium in terms of oversaturation of calcium ions leads to an increased osteoclast activity and therefore to an accelerated ill-controlled resorption of the ceramic material and a risk of adverse inflammation reactions, especially when using a large amount of synthetic bone substitute material.

When bone substitute material Geistlich Bio-Oss® is implanted into a human
10 patient, the natural calcium equilibrium is practically not affected, the concentration of calcium ions on the surface of the material and within the local environment thereof remaining almost constant. Biological resorption of the material hence does not take place or proceeds at a very slow rate without the risk of adverse inflammation reactions.

The objective of the invention disclosed in international patent application No.
15 PCT/EP2010/003590 was to provide a calcium phosphate/hydroxyapatite (CAP/HAP) bone substitute material which, like bone substitute material Geistlich Bio-Oss®, after being set in vivo enables the concentration of calcium ions on the surface of the material and within the local environment thereof to remain almost constant and thus does not
20 lead to an increased osteoclast activity.

Indeed, the natural calcium equilibrium which is necessary for optimal bone regeneration should not be disturbed or destroyed. Moreover the natural calcium concentration equilibrium should be lastingly supported by the bone substitute material until the regeneration process is completed. When those conditions are met
25 there is no increase of osteoclast activity, hence no risk of adverse inflammation reactions.

The above international patent application reports that the above objective was attained by a new biphasic nanocrystalline CAP/HAP bone substitute material with an exactly defined biomimetic bilayer structure obtained under specific conditions as described therein.

5 Indeed, as shown by observation under fluorescence light microscopy of that new biphasic nanocrystalline CAP/HAP bone substitute material implanted into a mammal, there is no detectable increase of osteoclast activity in the neighborhood of the implant, which indicates the absence of a rise in the calcium ion concentration on the surface of the material and within the local environment thereof.

10 The new biphasic nanocrystalline CAP/HAP bone substitute material shows very interesting in vivo properties.

The invention of international patent application No. PCT/EP2010/003590 thus relates to a biphasic calcium phosphate/hydroxyapatite (CAP/HAP) bone substitute material comprising a sintered CAP core and at least one uniform and closed
15 epitactically grown layer of nanocrystalline HAP deposited on top of the sintered CAP core, whereby the epitactically grown nanocrystals have the same size and morphology as human bone mineral, i.e. a length of 30 to 46 nm and a width of 14 to 22 nm.

The sintered CAP core may comprise tricalcium phosphate (TCP), notably α -
20 TCP (α -Ca₃(PO₄)₂) or β -TCP (β -Ca₃(PO₄)₂), and/or tetracalcium phosphate (TTCP) Ca₄(PO₄)₂O.

According to a frequently used embodiment the sintered CAP core essentially consists of TCP, α -TCP being preferred.

The epitactically grown layer of nanocrystalline HAP is structurally and
25 chemically nearly identical to the natural human bone mineral.

The epitactically grown layer of nanocrystalline HAP generally has a thickness of at least from 15 to 50 nm, preferably at least from 20 to 40 nm, more

preferably at least from 25 to 35 nm. That minimum thickness corresponds to one layer of HAP nanocrystals in epitaxial orientation.

The epitactically grown layer of nanocrystalline HAP may comprise a single or multiple layers of HAP nanocrystals in epitaxial orientation. The thickness of the epitactically grown layer of nanocrystalline HAP, which is related to the number of such layers of HAP nanocrystals in epitaxial orientation, will be selected according to the intended application of the bone substitute material as implant or prosthesis in differently loaded parts of the body. The bone substitute material of the invention is indeed designed to function in vivo as a living-like system progressively transforming the sintered CAP core into hydroxyapatite similar in size and morphology to human bone mineral, the rate of that transformation being dependent on the rate of calcium release by the sintered CAP core, which is to a large extent controlled by the thickness of the epitactically grown layer of nanocrystalline HAP.

The properties of the CAP/HAP bone substitute material are to a large extent controlled by the thickness of the epitactically grown layer of crystalline HAP. The term "properties" includes the ability of the CAP/HAP bone substitute to release a constant concentration of calcium ions to the local environment in vitro and in vivo.

The thickness of the epitactically grown layer of nanocrystalline HAP is related to the ratio of the sintered CAP core material to HAP, said ratio being generally between 5:95 and 95:5, preferably from 10:90 to 90:10.

The CAP/HAP bone substitute material may be a particulate or a granulate, the particles or granules having a desired size and shape. Generally the particles or granules are approximately spherical and have a diameter of 1 to 5000 μm , preferably 10 to 5000 μm , in particular 250 to 5000 μm .

The CAP/HAP bone substitute material may also be a shaped body, e.g. a screw, a nail, a pin or a structure having the profile of an osseous body part such as notably a hip, a clavicle, a rib, a mandible or a skull part. Such a screw, a nail or a pin

may be used in reconstructive orthopedic surgery for fixing a ligament to a bone, for example in the knee or the elbow. Such a structure having the profile of an osseous body part may be used in orthopedic surgery as prosthesis for replacing a missing or defective bone or bone part.

5

The invention of international application No.PCT/EP2010/003590 further relates to a process of preparing the above defined CAP/HAP bone substitute material comprising the steps of

- a) preparing a sintered CAP core material,
- 10 b) immersing the sintered CAP core material in an aqueous solution at a temperature between 10°C and 50°C to start the transformation process of CAP to HAP, whereby a uniform and closed epitactically grown layer of nanocrystalline hydroxyapatite is formed on the sintered CAP core material surface, the epitactically grown nanocrystals having the same size and
- 15 morphology as human bone mineral,
- c) stopping the transformation by separating the solid material from the aqueous solution at a time when a uniform and closed coating of at least one nanocrystalline layer of HAP is present but before the transformation process is finished completely,
- 20 d) optionally sterilizing the separated material coming from step c).

The sintered CAP core material may comprise tricalcium phosphate (TCP), notably α -TCP (α -Ca₃(PO₄)₂) or β -TCP (β -Ca₃(PO₄)₂), and/or tetracalcium phosphate (TTCP) Ca₄(PO₄)₂O.

According to a frequently used embodiment the sintered CAP core material

25 essentially consists of TCP, α -TCP being preferred.

The preparation of the sintered CAP core material may be performed by methods known in the art comprising first mixing powders of calcium hydrogen phosphate

(CaHPO₄), calcium carbonate and/or calcium hydroxide, then calcining and sintering the mixture within an appropriate temperature range, thereby giving a bulk sintered CAP core material (see e.g. Mathew M. et al., 1977, Acta. Cryst. B33: 1325; Dickens B. et al., 1974, J. Solid State Chemistry 10, 232; and Durucan C. et al., 2002, J. Mat. Sci., 37:963).

A bulk sintered TCP core material may thus be obtained by mixing powders of calcium hydrogen phosphate (CaHPO₄), calcium carbonate and/or calcium hydroxide in stoichiometric ratio, calcining and sintering the mixture at a temperature in the range of 1200-1450°C, preferably about 1400°C.

A bulk sintered TTCP core material may also be obtained by the above described process.

The bulk sintered CAP material prepared by such methods may be porous with a porosity of 2 to 80 vol% and a wide distribution of pores. The porosity parameters will be selected according to the intended application of the CAP/HAP bone substitute material.

The sintered CAP core material used in step b) may be

- the bulk sintered CAP core material prepared as described above,
- a particulate or granulate of sintered CAP core material obtained from the bulk sintered CAP core material prepared as described above, by using conventional methods such as crushing, grinding and/or milling, and sieving, or
- a preform of sintered CAP core material having a desired shape and size, e.g. a screw, a nail, a pin or a structure having the profile of an osseous body part.

Such a preform of any desired shape and size may be obtained from the bulk sintered core material prepared as described above, by using well known

prototyping techniques such as CNC milling or 3D printing (see for example Bartolo P. et al., 2008, Bio-Materials and Prototyping Applications in Medicine, Springer Science New York, ISBN 978-0-387-47682-7; Landers R. et al., 2002, Biomaterials

23(23), 4437; Yeong W.-Y. et al., 2004, Trends in Biotechnology, 22 (12), 643; and Seitz H. et al., 2005, Biomed. Mater. Res. 74B (2), 782).

The aqueous solution of step b) may be pure water, a simulated body fluid or a buffer. Important is that the pH value of the immersing solution of step b) is nearly neutral and remains stable throughout the transformation process, preferably within
5 a pH range from 5.5 to 9.0.

The buffer may be any buffer in the above pH range but is preferably a phosphate buffer with or without calcium, magnesium and/or sodium.

The term "simulated body fluid" refers to any solution that mimics a body
10 fluid. Preferably, the simulated body fluid has an ion concentration similar to that of blood plasma.

The temperature range in step b) is generally between 10°C and 50°C, preferably between 25 and 45 °C, more preferably between 35°C and 40°C.

The immersing step b) induces in a first phase a first-order phase transition of
15 the CAP core material and therefore the nucleation of HAP nanocrystal precursors. During the second phase the resulting HAP precursors from the first phase will grow and establish a closed (i.e. completely coating) epitactic nanocrystalline composite layer. The first HAP nanocrystal layer must be uniform and closed and epitaxially connected to the sintered CAP core material throughout its external
20 surface, including, in the case of the sintered CAP core material being porous, in the pores thereof.

During a third phase the first-order phase transition may proceed within the newly formed bilayer composite to further transform the sintered CAP core material (TCP or TTCP) into nanocrystalline HAP. During this third step of phase transition
25 calcium ions will be released for a controllable time by a slow diffusion controlled process until a part of the sintered CAP core material has been transformed into

nanocrystalline HAP. The transformation time and therefore the rate of calcium release can be controlled by variation of the thickness of the HAP layer.

The epitactically grown nanocrystalline HAP layer of appropriate thickness will be prepared in-vitro, the transformation of CAP into HAP being stopped before
5 it is completed.

As soon as the CAP/HAP bone substitute material is set in vivo the transformation process of CAP into HAP will be reactivated by contact with the body fluids and the bone substitute material will function as a living-like system forming new hydroxyapatite similar in size and morphology to humane bone mineral.
10 During the in vivo phase transformation process the transported calcium ions will be released into the local environment supporting the local calcium equilibrium which is important and beneficial for bone regeneration processes.

Due to different regeneration times of bone defects in differently loaded regions of the body it is important that the rate of calcium release can be controlled.
15 This can be achieved by variation of the thickness of the epitactically grown layer of hydroxyapatite .

Step c) is therefore a very critical step. The exposure time in the aqueous solution of step b) is based upon the thickness of the HAP layer desired. At least one layer of nanocrystalline HAP in epitaxial orientation is necessary. It is essential that
20 the transformation of CAP into HAP is not finished.

The proper exposure time according to the thickness desired can be calculated by using several thermodynamic differential equations well known to the skilled person in the art of calcium phosphates and cement and concrete chemistry.

See for example: Pommersheim, J.C.; Clifton, J.R. (1979) Cem. Conc. Res.;
25 9:765; Pommersheim, J.C.; Clifton, J.R. (1982) Cem. Conc. Res.; 12:765; and Schlüssler, K.H. Mcedlov-Petrosjan, O.P.; (1990): Der Baustoff beton, VEB Verlag Bauwesen, Berlin.

Transferring the solution of the above mentioned differential equations to the CAP/HAP system enables the prediction of the phase transition of CAP into HAP and the thickness of the layer such that the epitactic layer of HAP can be prepared in a stable and reproducible manner.

5 Separating the solid material from the aqueous solution is usually performed
by filtration and drying, using techniques well known in the art.

The optional sterilizing step d) may be performed by techniques well known in the art such as gamma-irradiation.

Advantages of the CAP/HAP bone substitute material of the above invention

10 The epitactically grown HAP nanocrystals surrounding the sintered CAP core material are identical in size and morphology to the apatite crystals of natural human bone mineral as shown in Table 1 below. Thus the CAP/HAP bone substitute material of the invention successfully mimics the composite or microstructure of bone and is representing a biomimetic material of human bone mineral.

15 Table 1

Comparison of the HAP crystal size and morphology for the CAP/HAP bone
substitute of the invention and human bone mineral

Crystallographic axes (hexagonal space group P6 ₃ /m)	CAP/HAP of the invention prepared at physiological temperature. Crystal size* [nm]	natural human bone mineral Crystal size* [nm]
a (1,0,0)	18 (±4)	15-21
b (0,1,0)	18 (±4)	15-21
c (0,0,1)	38 (±8)	34-45

* Crystal size analysis has been performed by using TEM (transmission electron microscopy), SPM (scanning probe microscopy techniques) as well as refinement of X-ray diffraction data by using the Bragg method.

5 The constant concentration of calcium ions results in an improved adhesion of osteoblasts and osteoclasts to the HAP surface in the correct ratio for the osteogenesis and thus to a steady state in the cycle of bone regeneration. A surface is provided to which osteoblasts and osteoclasts readily attach in the correct ratio for bone regeneration.

10 Furthermore due to its highly controllable surface properties the above CAP/HAP bone substitute material can function as a matrix for bioactive molecules such as extracellular matrix proteins such as notably growth factors for bone regeneration.

15 It has now been found that the adhesion of osteoblasts and osteoclasts to the HAP surface in the correct ratio for the osteogenesis can be further improved by impregnating the above defined porous CAP/HAP bone substitute material with collagen fibers such as to further mimic the in vivo natural processes of bone formation, bone regeneration, bone repair, bone remodeling and bone replacement
20 by creating an environment closer to that of bone or damaged bone, namely a hydroxyapatite part and an organic matrix comprising collagen fibers.

 The collagen fibers are generally fibers of collagen type I, collagen type III or a mixture thereof, or fibers of collagen type II.

 Interesting results have been so far obtained in particular with fibers derived
25 from pig skin which are a mixture of collagen I and collagen III, collagen I being predominant.

The present invention thus concerns a porous biphasic calcium phosphate/hydroxyapatite (CAP/HAP) bone substitute material comprising a sintered CAP core and at least one uniform and closed epitactically grown layer of nanocrystalline HAP deposited on top of the sintered CAP core, whereby the epitactically grown nanocrystals have the same size and morphology as human bone mineral, i.e. a length of 30 to 46 nm and a width of 14 to 22 nm, which is impregnated with collagen fibers at a weight ratio of said collagen fibers to said porous biphasic calcium phosphate/hydroxyapatite (CAP/HAP) bone substitute material of at least 2%.

Generally the impregnation with collagen fibers takes place throughout the external surface of the uniform and closed epitactically grown layer of nanocrystalline HAP, including inside the pores, as shown by scanning electron microscopy.

The weight ratio of said collagen fibers to said porous biphasic calcium phosphate/hydroxyapatite (CAP/HAP) bone substitute material is preferably at least 5%, in particular at least 8%. That weight ratio is generally from 2 to 80 %, preferably from 5 to 50 %, in particular from 8 to 25 %.

According to an interesting embodiment the collagen fibers are fibers of collagen type I, collagen type III or a mixture thereof.

The epitactically grown layer of nanocrystalline HAP generally has a thickness of at least from 15 to 50 nm, preferably at least from 20 to 40 nm, more preferably at least from 25 to 35 nm. That minimum thickness corresponds to one layer of HAP nanocrystals in epitaxial orientation.

The thickness of the epitactically grown layer of nanocrystalline HAP is related to the ratio of the sintered CAP core material to HAP, said ratio being generally between 5:95 and 95:5, preferably from 10:90 to 90:10.

According to a frequently used embodiment the sintered CAP core material essentially consists of TCP, α -TCP being preferred.

The present invention further relates to a process of preparing the above
5 porous biphasic calcium phosphate/hydroxyapatite (CAP/HAP) bone substitute material comprising the steps of

(a) mixing a slurry of collagen fibers and a porous biphasic calcium phosphate/hydroxyapatite (CAP/HAP) bone substitute material comprising a sintered CAP core and at least one uniform and closed epitactically grown layer of
10 nanocrystalline HAP deposited on top of the sintered CAP core, whereby the epitactically grown nanocrystals have the same size and morphology as human bone mineral, i.e. a length of 30 to 46 nm and a width of 14 to 22 nm, and

(b) eliminating the water by vacuum suction.

The porous biphasic calcium phosphate/hydroxyapatite (CAP/HAP) bone
15 substitute material may be a shaped body of porous CAP/HAP bone substitute material or a particulate or granulate of porous CAP/HAP.

According to a preferred embodiment the porous (CAP/HAP) bone substitute material is a particulate or a granulate of porous CAP/HAP bone substitute material, the impregnated collagen fibers then also acting as a binder between the particles or
20 granules, thus providing a porous collagen matrix surrounding and impregnating particles or granules of porous CAP/HAP bone substitute material. Pieces cut from that matrix show some elastic properties which are of advantage for their use as implants.

The mixing of step (a) is generally a vigorous mixing, the latter being
25 conveniently performed in the case of the above preferred embodiment in a mixer having a rotating blade.

Preferably the above process comprises the additional step of drying in a vacuum oven.

The present invention also relates to an implant which comprises a porous collagen matrix surrounding and impregnating particles or granules of porous biphasic calcium phosphate/hydroxyapatite (CAP/HAP) bone substitute material comprising a sintered CAP core and at least one uniform and closed epitactically grown layer of nanocrystalline HAP deposited on top of the sintered CAP core, whereby the epitactically grown nanocrystals have the same size and morphology as human bone mineral, i.e. a length of 30 to 46 nm and a width of 14 to 22 nm.

The present invention also concerns the use of the above defined CAP/HAP bone substitute material, generally in the form of a particulate or a shaped body as an implant or prosthesis for supporting bone formation, bone regeneration, bone repair and/or bone replacement at a defect site in a human or animal.

The present invention also relates to a method of promoting bone formation, bone regeneration and/or bone repair at a defect site in a human or animal by implanting the above defined CAP/HAP bone substitute material, generally in the form of a particulate or a shaped body.

The following examples illustrate the present invention without limiting its scope.

Example 1 Preparation of a bulk sintered material of α -TCP

For a mixture of 500 g (dry weight), 360 g dicalcium phosphate anhydrous powder, 144 g calcium carbonate powder and 220 ml deionized water were mixed for 7 minutes at 500 rpm using a laboratory stirrer. The slurry from the mixing process was immediately transferred into a high temperature stable platinum cup. The filled platinum cup was placed in a cold furnace. The furnace was heated to

1400°C by using a heating rate of 60°C per hour. The heating process was stopped after 72 hours by switching off the furnace. The sample was cooled down to room temperature within the furnace. The bulk sintered material (phase pure α -Ca₃(PO₄)₂) was removed from the furnace and the platinum cup. The bulk product from the sintering process had a weight of 420 g (weight loss 16.7%).

The control of phase purity was performed using powder X-ray diffraction analysis.

Example 2 Preparation of porous granules of sintered α -TCP with a particle size between 0.25 and 2 mm

The bulk product from example 1 was crushed by using a jaw crusher (slot size 4 mm). The coarse granules were sieved by using a sieving machine and sieve inserts with mesh aperture 2 mm and 0.25 mm. After sieving the granule fractions were rinsed 2 times by using purified water for separating fine powder residuals adsorbed to the granules. The porous granules were dried for 10 hours at 120°C in a cabinet dryer. The control of particle size distribution was done by using laser diffraction technology. The cleanness of the particle surfaces after rinsing was controlled by surface observation using scanning electron microscopy.

Example 3 Preparation of porous cylinders (length 10 mm, diameter 6 mm) of sintered α -TCP by CNC Milling

The bulk product from example 1 was grinded to a cuboidal work piece with edge lengths a=3 cm, b=2 cm, c=2 cm using a grinding machine. The work piece was placed and fixed into a 4-axis CNC milling machine equipped with a round-head hard metal milling cutter tool with a diameter of 3 mm. The cylinders were milled by using a helical milling route with a radius of 3 mm and a slope of 0.25 mm. The main speed of the work piece during the CNC milling process was 1700 rotations per minute, the maximum rotation speed of the helical milling route was calculated by

an integral process within the CNC equipment and averages 10 rotations per minute. After milling the cylindrical preforms were rinsed 2 times by using purified water for separating fine powder residuals adsorbed to the cylinder surface. The porous cylinders were dried for 10 hours at 120°C in a cabinet dryer. The cleanness of the preform surface after rinsing was controlled by surface observation using scanning electron microscopy. The correctness of the preform dimensions was controlled by using a slide gauge.

Example 4 Preparation of an epitactically grown nanocrystalline HAP coating on the granules of sintered α -TCP from Example 2

A buffered solution (1000ml) adequate for the coating and phase transformation process was prepared by using 1.82 mol/l sodium, 4.68 mol/l hydrogen, 0.96 mol/l phosphorus, 5.64 mol/l oxygen, 0.01 mol/l calcium and 0.71 mol/l chlorine. The solution will be adjusted to a pH of 7.4 at a temperature of 40°C.

The granules produced according to example 1 and 2 were immersed into the prepared solution and stored within a well tempered water bath (40°C) for a time calculated according a layer thickness at an average of 250 nm (10 hours) which equates to a phase composition of (w/w) 75 % α -TCP and 25% hydroxyapatite. After immersing the granules were rinsed 3 times by purified water to remove residuals from the buffered solution. The porous granules were dried for 4 hours at 120°C in a cabinet dryer. The phase composition of the granules were analyzed by Rietveld analysis of powder X-ray diffraction data, the crystal sizes of crystalline phases obtained by the coating process were analyzed by size-strain refinement of X-ray diffraction data according to the Bragg technique. The porosity of the granules was controlled by using mercury intrusion porosimetry, the surface morphology after coating was controlled by using scanning electron microscopy.

Example 5 Preparation of an epitactically grown nanocrystalline HAP coating on the cylinders of sintered α -TCP from Example 3

A buffered solution (1000 ml) adequate for the coating and phase transformation process was prepared by using 1.82 mol/l sodium, 4.68 mol/l hydrogen, 0.96 mol/l phosphorus, 5.64 mol/l oxygen, 0.01 mol/l calcium and 0.71 mol/l chlorine. The solution was adjusted to a pH of 7.4 at a temperature of 40°C. The porous cylinders produced according to example 1 and 3 were immersed into the prepared solution and stored within a well tempered water bath (40°C) for a time calculated according a layer thickness at an average of 20 μ m (60 hours) which equates to a phase composition of approximately 85% (w/w) alpha-TCP and 15% (w/w) hydroxyapatite. After immersing the cylinders were rinsed 3 times by purified water to remove residuals from the buffered solution. The porous cylinders were dried for 10 hours at 120°C in a cabinet dryer. The phase composition of the cylinders were analyzed by Rietveld analysis of powder X-ray diffraction data, the crystal sizes of crystalline phases obtained by the coating process were analyzed by size-strain refinement of X-ray diffraction data according to the Bragg technique. The epitaxial growth were analysed by using reflectance-difference (RD) spectroscopy. The porosity of the cylinders was controlled using mercury intrusion porosimetry, the surface morphology after coating was controlled using scanning electron microscopy. The layer thickness was controlled using reflection high-energy electron diffraction (RHEED) and/or photoelectron spectroscopy (XPS).

Example 6 Influence of the immersing time on the layer thickness and the phase composition

Tables 2 and 3 show experimental data for an example showing the influence of the immersing time on the layer thickness and the phase composition, respectively, for porous α -TCP particles with nearly spherical geometry and size from 10 to 20 μ m, a

porosity 25-40 vol.-%, a specific (inner) surface area of 50-60 m²/g, a bulk density of 0.6-0.8 g/ml.

Table 2:

Influence of the immersing time on the layer thickness

5

Immersing time [min]	Layer thickness* [nm]
0	--
15	37 (±10)
30	112 (±4)
60	121 (±9)
600	238 (±8)

* Epitaxy, chemical composition of the layer and layer thickness analysis were determined using RHEED (reflection high-energy electron diffraction) and XPS (photoelectron spectroscopy)

Table 3:

10

Influence of the immersing time on the phase composition

Immersing time [h]	TCP** [weight%]	HAP** [weight%]
0	100	--
0.5	86.6 (±1)	13.4 (±2)
1	85.8 (±1)	14.2 (±3)
2	83.5 (±1)	16.4 (±3)
5	78.1 (±1)	21.9 (±3)
7.5	75.3 (±1)	24.7 (±3)
10	74.2 (±5)	25.8 (±2)
12	58.8 (±6)	41.2 (±7)
24	44.8 (±9)	55.2 (±6)

48	35.8 (± 6)	64.2 (± 3)
72	--	100

** Quantitative phase analysis was performed using Rietveld refinement of powder X-Ray diffraction data.

*** Experimental data were evaluated on a system with following parameters: Liquid

5 Phase: PBS buffered saline liquid, 20x, temperature 40°C.

Example 7 Preparation of a slurry of collagen fibres derived from pig skin.

Porcine hides were ground in a meat grinder to pieces of 1 to 20 mm. The water was
 10 removed using a water soluble solvent such as an alcohol or a ketone. The collagen fibres were defatted using a chlorinated hydrocarbon such as dichloroethane or methylene chloride or a non-chlorinated hydrocarbon such as hexane or toluene. After removing the solvent the collagen was treated with a strong inorganic base at a pH above 12 for a period of 6 to 24 hours and treated with a strong inorganic acid at
 15 a pH of 0 to 1 for a period of 1 to 12 hours. The excess acid was removed by rinsing with water and the suspension was homogenized to a 0.5 to 2 % homogenous suspension of collagen fibres in the presence of a swelling regulator such as an inorganic salt. The suspension was dried by freeze-drying and the dry collagen fibres were successively cleaned with different organic solvents such as alcohols, ethers,
 20 ketones and chlorinated hydrocarbons, the solvents being then evaporated under vacuum to a solvent residue of less than 1 %. A slurry of collagen fibres was prepared by mixing the cleaned dry fibres obtained above with water.

Example 8 Preparation of a plate of porous (CAP/HAP) bone substitute material
 25 impregnated with collagen fibers

- 20 -

594.2 g of the slurry of collagen fibers (corresponding to about 14.855 g of dry collagen) prepared in Example 7 and 119.7 g of porous granules of (CAP/HAP) bone substitute material prepared as described in Example 5 and having a particle size between 0.5 and 1.0 mm were thoroughly mixed during 30 minutes and the mixture
5 was poured into a suction filter and dried by suction. The plate of (CAP/HAP) bone substitute material impregnated with collagen fibers was carefully removed from the suction filter and dried in a vacuum oven at about 35 °C for 48 hours. Pieces of that plate were cut for in vivo testing.

CLAIMS

1. Porous biphasic calcium phosphate/hydroxyapatite (CAP/HAP) bone substitute material comprising a sintered CAP core and at least one uniform and closed epitactically grown layer of nanocrystalline HAP deposited on top of the sintered CAP core, whereby the epitactically grown nanocrystals have the same size and morphology as human bone mineral, i.e. a length of 30 to 46 nm and a width of 14 to 22 nm, which is impregnated with collagen fibers at a weight ratio of said collagen fibers to said porous biphasic calcium phosphate/hydroxyapatite (CAP/HAP) bone substitute material of at least 2%.
5
2. A porous CAP/HAP bone substitute material according to claim 1 wherein the weight ratio of said collagen fibers to said porous biphasic calcium phosphate/hydroxyapatite (CAP/HAP) bone substitute material is at least 5%.
10
3. A porous CAP/HAP bone substitute material according to claim 2 wherein the weight ratio of said collagen fibers to said porous biphasic calcium phosphate/hydroxyapatite (CAP/HAP) bone substitute material is at least 8%.
15
4. A porous CAP/HAP bone substitute material according to claim 1 wherein the weight ratio of said collagen fibers to said porous biphasic calcium phosphate/hydroxyapatite (CAP/HAP) bone substitute material is from 8 to 25%.
5. A porous CAP/HAP bone substitute material according to any of claims 1 to 4, wherein the collagen fibers are fibers of collagen type I, collagen type III or a mixture thereof.
20
6. A porous CAP/HAP bone substitute material according to any of claims 1 to 5, wherein the epitactically grown layer of nanocrystalline HAP generally has a thickness of at least from 15 to 50 nm.
7. A porous CAP/HAP bone substitute material according to any of claims 1 to 6 wherein the ratio of sintered CAP core material to HAP is between 5:95 and 95:5.
25

8. A porous CAP/HAP bone substitute material according to any of claims 1 to 6 wherein the ratio of sintered CAP core material to HAP is between 10:90 and 90:10.
9. A porous CAP/HAP bone substitute material according to any of claims 1 to 8 wherein the sintered CAP core essentially consists of α -TCP.
- 5 10. A porous CAP/HAP bone substitute material according to any of claims 1 to 9 which comprises a particulate or a granulate of porous CAP/HAP bone substitute material impregnated with collagen fibers.
11. A porous CAP/HAP bone substitute material according to any of claims 1 to 9 which comprises a shaped body of porous CAP/HAP bone substitute material
- 10 impregnated with collagen fibers.
12. A process of preparing a porous CAP/HAP bone substitute material according to any of claims 1 to 11, which comprises
- (a) mixing a slurry of collagen fibers and a porous biphasic calcium phosphate/hydroxyapatite (CAP/HAP) bone substitute material comprising a
- 15 sintered CAP core and at least one uniform and closed epitactically grown layer of nanocrystalline HAP deposited on top of the sintered CAP core, whereby the epitactically grown nanocrystals have the same size and morphology as human bone mineral, i.e. a length of 30 to 46 nm and a width of 14 to 22 nm, and
- (b) eliminating the water by vacuum suction.
- 20 13. A process of claim 12 which further comprises the additional step of drying in a vacuum oven.
14. Use of a CAP/HAP a bone substitute material according to any of claims 1 to 11 as implant or prosthesis for bone formation, bone regeneration, bone repair and/or bone replacement at a defect site in a human or animal.
- 25 15. An implant which comprises a porous collagen matrix surrounding and impregnating particles or granules of porous biphasic calcium phosphate/hydroxyapatite (CAP/HAP) bone substitute material comprising a

sintered CAP core and at least one uniform and closed epitactically grown layer of nanocrystalline HAP deposited on top of the sintered CAP core, whereby the epitactically grown nanocrystals have the same size and morphology as human bone mineral, i.e. a length of 30 to 46 nm and a width of 14 to 22 nm.

- 5 16. A method of promoting bone formation, bone regeneration and/or bone repair at a defect site in a human or animal by implanting a CAP/HAP bone substitute material according to any of claims 1 to 11.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2011/006457

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

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

1. ☒ Claims Nos.: 14, 16
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

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

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2011/006457

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61L27/42 A61L27/56
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 practical, search terms used)

EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2007/190083 A1 (SCIFERT JEFFREY L [US] ET AL) 16 August 2007 (2007-08-16) example 1	1-13,15
A	----- JOSEPH E. ZERWEKH ET AL: "Fibrillar collagen-biphasic calcium phosphate composite as a bone graft substitute for spinal fusion", JOURNAL OF ORTHOPAEDIC RESEARCH, vol. 10, no. 4, 1 July 1992 (1992-07-01), pages 562-572, XP55018030, ISSN: 0736-0266, DOI: 10.1002/jor.1100100411 page 563, left-hand column, last paragraph - right-hand column, paragraph 1 ----- -/-	1-13,15



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not
considered to be of particular relevance

"E" earlier document 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 docu-
ments, 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

15 March 2012

Date of mailing of the international search report

23/03/2012

Name and mailing address of the ISA/

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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2011/006457

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6 338 752 B1 (LIN FENG-HUEI [TW] ET AL) 15 January 2002 (2002-01-15) claims 1-12 -----	1-13,15
A	WO 97/41273 A1 (FLAMETAL S P A [IT]; CHI BO S R L [IT]; 3C RESEARCH S R L [IT]; CREMAS) 6 November 1997 (1997-11-06) page 2, line 18 - page 3, line 19 -----	1-13,15
A	EP 0 285 826 A2 (BENCKISER KNAPSACK GMBH [DE]; MTU MUENCHEN GMBH [DE] MTU MUENCHEN GMBH) 12 October 1988 (1988-10-12) claims 1-6 -----	1-13,15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2011/006457

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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			EP 2010237 A2 07-01-2009
			JP 4663026 B2 30-03-2011
			JP 2009533165 A 17-09-2009
			US 2007190083 A1 16-08-2007
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			EP 0285826 A2 12-10-1988
			JP 1025869 A 27-01-1989
			US 4871578 A 03-10-1989

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.1

Claims Nos.: 14, 16

Claims 14 and 16 relate to a subject-matter considered by this Authority to be covered by the provision of rule 39.1(iv)/67.1(iv) PCT. The patentability can be dependent upon the formulation of the claims. The EPO, for example, does not recognise as patentable claims to the use of a compound in medical treatment, but may allow claims to substances or compositions for use in a first or further medical treatment. However, first or further medical use claims are not allowable for implants/medical devices (only for substances or compositions) and therefore, claims 14 and 16 can not be reformulated. Consequently, no examination will be carried out on the subject-matter of claims 14 and 16.