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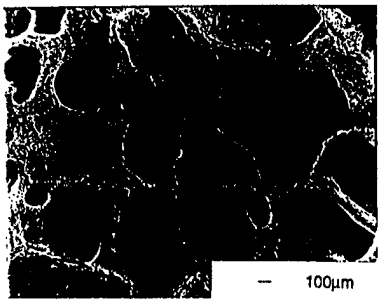
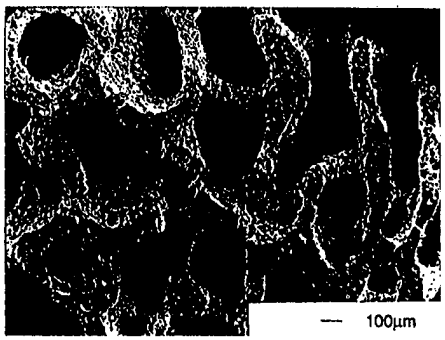
(54) Title: BONE REPLACEMENT MATERIALS WITH INTERCONNECTING PORE SYSTEM

(57) Abstract

The invention concerns synthetic bone replacement materials having a macroporous structure similar to that of natural cancellous bone with a pore volume of 70–95 % and fully interconnecting pores having a diameter from 10 μm to 1 mm. Preferred materials are calcium phosphates, bioglass, poly–L–lactic acid and titanium.





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BONE REPLACEMENT MATERIALS WITH INTERCONNECTING PORE SYSTEM

This invention relates to bone replacement materials. In particular, it relates to synthetic bone replacement materials having the macroporous structure of natural cancellous bone.

The replacement of damaged bone or bone tissue with synthetic bone replacement materials is well known. Materials such as macroporous hydroxyapatite (HA) and beta-tricalcium phosphate (beta-TCP) are widely used due to their high biocompatibility and osteoconductive properties. The porosity of the bone replacement material, its pore size distribution, pore morphology, and the degree of pore interconnectivity significantly influence the extent of bone ingrowth. Ideally, the macrostructure of the bone replacement material is similar in morphological characteristics to the inorganic matrix of the bone it is replacing. While macroporous bone replacement materials have been produced based on coralline skeletal and other generic macrostructures, it has not so far been possible to produce suitable synthetic bone replacement materials having a macroporous structure similar to that of natural cancellous bone.

Cancellous bone consists of a network of interconnecting rods and plates called trabeculae which form a sponge-like matrix. Individual trabeculae are typically 50-150 μm thick. Fully interconnecting spaces, ranging from tens of μm to 1 mm, the majority of which are 400-600 μm in diameter, run throughout the lattice. The porosity of natural cancellous bone poses

considerable difficulties in forming synthetic replicas thereof having the requisite characteristics to allow their use in non-load bearing applications. Such replicas should be easy to handle and shape without damage, have the required mechanical strength and allow bone regeneration throughout their porous structure. Replacement materials for cancellous bone are generally used in non-load bearing situations so that their mechanical strength need not be very high. The primary mechanical function of cancellous bone replacement materials is to prevent prolapse of soft tissue into the defect site during bone regeneration. An implant which can fulfil this function would possess sufficient strength as a bone filler.

It is an object of the invention to provide a synthetic bone replacement material having a macroporous structure similar to that of natural cancellous bone suitable for use as a bone graft replacement material in non-load bearing applications.

According to the present invention there is provided a synthetic bone replacement material having a macro-porous structure similar to that of natural cancellous bone, characterized by a pore volume of 70-95%, fully interconnecting pores having a diameter in the range of from about 10 μm to 1 mm.

The synthetic bone replacement material of the invention may be any suitable biomaterial, examples of which include bioceramic materials such as hydroxyapatite (HA), a calcium phosphate such as α- and ß-tricalcium phosphate (TCP), bioglass, HA/α-or ß-TCP composites, HA/glass or bioglass composites and alumina; polymeric materials such as poly-L-lactid

acid; and metals such as titanium. Preferred biomaterials are HA, ß-TCP, HA/ß-TCP composites, bioglass and HA/glass or bioglass composites.

Bioglasses are well known, a number of which are suitable for use in the bone replacement materials of the invention, either as the sole component or as a component of a HA composite. Suitable bioglasses are described in *An Introduction to Bioceramics*, Vol. 1, Hench, L.L. and Wilson, J. (ed.), World Scientific, Singapore, 1993. A preferred bioglass has the following composition, the percentages being mol.% of the total composition: 13.1% CaO; 4.7% P_2O_5; 52.6% SiO_2; 25.8% Na_2O; 0.5% Al_2O_3; and 3.0% B_2O_3.

The amount of HA in HA/TCP composites can range from 5% to 95% by weight, suitably from 20% to 70% by weight. In HA/glass composites, the glass is preferably a phosphate glass containing about 50 mol.% CaO and about 50 mol.% P_2O_5 and is preferably present in the composite at a concentration of from 2.5 to 10% by weight.

The synthetic bone replacement material of the present invention may be used as a carrier matrix for a bone inductive material, such as a bone morphogenetic protein (BMP) or a non-collagenous protein (NCP) in the stimulation of bone formation.

The invention also provides the use of cancellous bone in the preparation of a synthetic bone replacement material having a macroporous structure similar to that of natural cancellous bone.

A synthetic bone replacement material of the present

invention may be obtained by a process comprising the following steps:

(a) providing a cancellous bone sample;

(b) removing the organic material from the cancellous bone sample to give the anorganic bone mineral (ABM);

(c) optionally treating the ABM of the cancellous bone to seal its trabecular micropores;

(d) impregnating the ABM with a suitable inert material, such as a wax, to form a mould, followed by decalcification;

(e) impregnating the mould with a bioceramic material or metal; and

(f) removing the mould and sintering the resulting product if appropriate to produce the desired synthetic bone replacement material.

The resulting product is a positive replica of the original cancellous bone matrix.

A synthetic bone replacement material of the present invention may also be obtained by a process comprising the following steps:

(a) providing a cancellous bone sample;

(b) removing the organic material from the cancellous bone sample to give the anorganic bone mineral ABM);

(c) optionally treating the ABM of the cancellous bone to seal its trabecular micropores; and

(d) impregnating the ABM with a biomaterial, followed by decalcification.

The resulting product is a negative replica of the original cancellous bone matrix.

The above processes may be carried out by cutting cancellous bone samples to the desired shape. For example, the samples may be in particulate form wherein individual particles are about 1-3 mm in diameter, or block form wherein individual blocks preferably have a thickness of up to about 12 mm. The organic material is then removed in such a way that the ABM remains intact. This may be accomplished by, for example, thermal treatment (calcination) wherein all of the organic material is burnt off by heating to a sufficiently high temperature (typically 800°C or higher). Alternatively, the organic material may be removed by chemical extraction with a suitable solvent, such as ethylenediamine or formamide, using a soxhlet extraction apparatus. Chemical extraction of the organic material is preferred.

Because of the particular morphology of cancellous bone, the anorganic bone mineral (ABM) should preferably be treated prior to its infiltration so as to seal its trabecular microporosity without blockage of the macroporous structure to be replicated. A preferred treatment involves boiling the ABM for several minutes in sodium hypochlorite and drying the treated product without rinsing. A crystalline residue

is thus formed which blocks the trabecular microporosity without alteration or blockage of the trabecular macrostructure. Alternatively, the ABM may be coated by treatment with a suitable polymeric substance, for example, Mowiol (Trade Mark of Hoechst) prior to its infiltration.

Where a positive replica of the bone matrix is required, the ABM is then impregnated with wax under vacuum at about 20°C above the melting temperature of the wax. Any suitable wax may be used. Suitable waxes are those which are tough, have a moderate viscosity permitting full infiltration of the ABM, solidify without excessive shrinkage resulting in pore formation within the casting, and resist degradation following exposure to the decalcifying agent. Preferred waxes are Castylene B271 and Techniwax 9210 (Dussek Campbell, U.K.) and A7 machining wax (Blayson Olefines Ltd., U.K.). Following wax solidification, the wax castings are decalcified by, for example, immersion at a temperature ranging from about 20°C to 50°C for about 24 hours to 7 days in dilute acid, for example HCl.

The negative mould is then infiltrated with the relevant biomaterial, such as a bioceramic slip. A suitable slip is one which is capable of being rendered sufficiently fluid by vibration to penetrate the wax mould completely while having as high a solids content as possible. A bioceramic slip is formed by dispersing the bioceramic in powder form in a carrier fluid having a pH in the range of 7 to 13. The carrier fluid may comprise water and optionally a deflocculant. The deflocculant is preferably present at a concentration up to about 4% by weight of the slip. The bioceramic powder preferably constitutes about 75% by weight of

the slip. The slip may be infiltrated into the wax mould by applying a suitable vibratory action. Alternatively, where the slip is sufficiently fluid, slip infiltration may be performed by application of a vacuum. In this case, the prepared slip is placed in a glass container within a vacuum chamber to which a full vacuum may be applied. Wax negative samples are immersed and secured below the surface of the slip. The vacuum chamber is then sealed and a vacuum of at least -0.9 bar applied and held for a period of about 2 minutes. The system is then allowed to back-fill to atmospheric pressure and samples are removed. Mould infiltration may also be achieved by centrifugal casting.

Following slip penetration, samples are recovered and dried slowly, for example at 30-35°C for 24 hours. The wax is removed by slow-melting to prevent destruction of the delicate slip-infiltrated structure. Following wax removal, the product is sintered by increasing the temperature step-wise, for example by increasing the temperature by 1-15°C/min., to sintering temperature where it is held for three hours followed by step-wise cooling to room temperature. The resulting product is a positive replica of the original cancellous bone.

Where a negative replica of the bone matrix is to be prepared, the ABM is impregnated with a viscous melt of a suitable biomaterial, for example a polymeric material. A vacuum may be applied to aid full infiltration of the porous matrix by the biomaterial. Following infiltration, the samples are allowed to cool to room temperature and solidify. Decalcification is then carried out as described above, the resulting product being a negative replica of the original

cancellous bone.

The cancellous bone may be derived from any suitable source, bovine cancellous bone being preferred. Cancellous bone suitable for replication should be of similar porosity to the bone being replaced and should not include any cortical bone, growth plate or defects. Furthermore, variations in porosity should be minimal within a block. Bovine bone is a suitable source for the supply of bone samples due, not only to its availability and the relatively large size of each bone, but also due to its close structural similarity to human cancellous bone. Bovine cancellous bone from the distal femoral condyles, below the growth plate, was found to be particularly useful in the present invention.

Samples of bone used preferably have a maximum thickness of about 12 mm. Preferably, several smaller blocks are used for a large defect, rather than a single large block.

The invention is illustrated in the following Examples.

EXAMPLE I

(a) Bioceramic Powders

Synthetic hydroxyapatite (HA) powder of medical grade (Captal (Trade Mark) Plasma Biotal Ltd. Tideswell, UK) and synthetic ß-tricalcium phosphate (ß-TCP) powder from Fluka chemika (Purum grade) were used.

Two glasses in powder form were also prepared - a Hench-type bioglass and a phosphate glass. The composition of each glass was as follows (mol.%):

Glass	CaO	P_2O_5	SiO_2	Na_2O	Al_2O_3	B_2O_3
Bioglass	13.1	4.7	52.6	25.8	0.5	3.0
Phosphate	50	50	--	--	--	--

(i) Bioglass Powder

Reagent grade silica, sodium carbonate, calcium carbonate, phosphorous pentoxide, alumina, and boric acid were used. The source and purity of raw materials used is shown in Table 1.

All chemicals with the exception of P_2O_5 were weighed out to provide the appropriate molar ratios and were added to a 2 litre polypropylene bottle (Nalgene, Trade Mark). Powders were premixed by rotating on ball-mill rollers (without media) for approximately one hour. P_2O_5 was weighed, added, and hand-mixed by vigorous shaking.

Mixed powders were transferred to a dense, fine-grained, sintered mullite crucible (Zedmark Refractories, Earlsheaton, Dewsbury, U.K.) fitted with a lid. This was placed in a furnace preheated to 1370°C and held for two hours to allow melting and homogenisation of the glass. The resulting melt was quenched by pouring into water. The glass frit was collected and dried in a conventional oven at 100°C. Raw materials to give a theoretical batch yield of 800 g were used.

The glass frit was crushed and dry-milled in a five litre Al_2O_3 milling pot, with 12.5 mm diameter cylpeb magnesia-stabilised zirconia milling media. Powders

were dry-milled for one hour. The powder was graded by passing through 250, 106, and 45 μm sieves. Particles above 45 μm were returned for further ball-milling.

(ii) Phosphate Glass Powder

Calcium carbonate and phosphorous pentoxide were weighed and premixed to give a 50:50 $CaO:P_2O_5$ molar ratio. Powders were placed in a mullite crucible with lid, and transferred to a muffle furnace preheated to 1070°C for two hours. Quenching, recovering, and milling of the glass were carried out as described above for bioglass.

Table 1: Source and purity of raw materials used

Procedure	Raw materials	Source	Purity
Manufacture of glasses	Silica, SiO_2	Tilcon Industrial Minerals, Stoke-on Trent, U.K.	99.9% GPR
	Sodium carbonate, Na_2CO_3	BDH Ltd., Poole, England	99.5% GPR
	Phosphorous pentoxide, P_2O_5	Merck, Darmstadt, Germany	97% GPR
	Boric acid crystals, H_3BO_3	Merck (as above)	99.8% GPR
	Calcium carbonate, $CaCO_3$	Aldrich Chemical Co. Ltd., Dorset, U.K.	98% GPR
	Alumina, Al_2O_3	British Alcan Chemicals, Buckinghamshire, U.K.	99.5% GPR

GPR = General Purpose Reagent

(b) Preparation of Bioceramic Slips

Bioceramic slips were prepared from the bioceramic powders described above having the composition shown in Table 2, in which the following abbreviations are used:

HA	:	Hydroxyapatite
ß-TCP	:	ß-Tricalcium Phosphate
BG	:	Bioglass
PG	:	Phosphate glass

Composites are referred to according to the nomenclature HA*XY*, where *X* is ß-TCP, PG or BG and *Y* is the glass or ß-TCP addition (in wt.%). Table 2 also shows the sintering temperature range for each material and the actual sintering temperature used.

Table 2

Bioceramic No.	Bioceramic Composition	Sintering Temperature Range (°C)	Actual Sintering Temperature (°C)
1	HA	1150-1350	1200
2	ß-TCP	1150-1350	1150
3	BG	600-800	750
4	HA/ß-TCP90	1100-1350	1200
5	HA/ß-TCP75	1100-1350	1200
6	HA/ß-TCP50	1100-1350	1200
7	HA/PG02.5	1150-1350	1350
8	HA/PG05	1150-1350	1350
9	HA/PG10	1150-1350	1300
10	HA/BG10	1100-1250	1200
11	HA/BG25	1050-1200	1100
12	HA/BG50	600-900	600

The powders of bioceramics 1-12 were subjected to a wet milling process in which 60 g of each powder or composite powder were added to 500 ml wide-mouth polypropylene bottles (Nalgene, Trade Mark), together with 180 ml isopropyl alcohol (IPA) and approximately 650 g of 7 mm diameter cylpeb magnesia-stabilised zirconia milling media. Milling bottles were shaken vigorously to disperse the powder and then wet milled on ball-mill rollers at approximately 140 rpm for 1 hour.

Slips were recovered and passed through a 38 μm sieve to remove possible contamination resulting from chipping of the milling media. Each slip was placed in an evaporating dish and allowed to dry to a powder cake in a fume hood. Final drying to a constant weight was done in a vacuum oven at approximately 70°C. The dry powders were passed through a 106 μm sieve to break up any agglomerates formed on drying. Powders were stored in a desiccator until required.

(c) Replication of Cancellous Bone

Bovine cancellous bone from the distal femoral condyles, below the growth plate, was harvested and cut into blocks of 8-10 mm thick using a butcher's bandsaw. Frozen bone was used, although fresh bone may also be used. All removable soft tissue was cut or scraped free to prevent entanglement around the blade. Contamination due to metal inclusion or from other sources was avoided as such contamination may not be removed subsequently. The organic matrix of the bone samples was removed by treating them with ethylenediamine (ED) (approx. 95% pure) in a Wheaton 1 litre soxhlet apparatus. Samples were placed in the soxhlet chamber

and treated for 48 hours, at a rate of approximately three cycles per hour. Small or delicate samples may be placed in a soxhlet thimble plugged with cotton wool. It was necessary to lag the soxhlet chamber during extraction to maintain the solvent temperature close to boiling point and prevent solidification of fat-laden ethylenediamine within the apparatus.

Depending on the percentage of organic material in the charge, it may be necessary to change the ethylenediamine once during the extraction period. A large soxhlet apparatus of the type used is capable of producing 60-70 cubic specimens of 10 mm side length per charge.

On removal, the bone mineral was rinsed in flowing water for three hours. It was then replaced into the soxhlet extractor for about 18 hours and cycled with deionised water. Following treatment, the bone matrix was chalky white and extremely brittle. Measurement by microanalysis showed there was no detectable residual nitrogen (i.e. organic material) present (below 0.05%) following ethylenediamine treatment.

The resulting anorganic bone mineral (ABM) specimens were immersed in an aqueous solution of 14% sodium hypochlorite in a wide mouth, round bottom flask, fitted with a condenser, at a ratio of 20 ml solution to 1 cm^3 of ABM. The temperature was raised to boiling point and held for two minutes. The samples were removed and placed directly (without rinsing) onto absorbent paper to allow draining, and were then dried in a conventional oven at approximately 70°C and stored in a desiccator until required.

The treated bone samples were then impregnated with the wax Castylene B271 (Dussek Campbell, Kent, U.K.). This wax has a casting temperature of about 105°C, is of medium viscosity, infiltrates without void formation and is hard and shapeable.

Impregnation was carried out in a vacuum oven at 100-110°C as follows. The wax was melted in a glass container. The bone samples were placed on the wax surface and allowed to sink under their own weight, expelling air from the voids as the wax penetrated the structure. Following full immersion, a vacuum of at least 600 mm Hg was applied and held for one minute. The vacuum was released and the oven allowed to back-fill to atmospheric pressure.

Next, the samples were transferred into a flexible container (e.g. weighing boat of high impact polystyrene) and allowed to cool submerged to their own depth in wax. This provided a reservoir of molten wax during the period of solidification which prevented surface pore formation due to retraction of wax from the surface pores in a manner analogous to pipe formation in casting. When solidified, the samples were removed from the container and trimmed by scalpel to expose bone mineral on all sides. If required, shaping was possible at this stage as the wax-filled inorganic matrix had reasonable mechanical integrity.

The samples with exposed bone mineral were then placed in 10% HCl at room temperature (about 25°C) so as to decalcify the wax castings. Evidence of dissolution of bone mineral was apparent due to gas evolution at the specimen surface. Full dissolution from a standard sized block 8-10 mm thick required approximately 48

hours without agitation of solution, which would speed decalcification. Following full decalcification, samples were rinsed thoroughly in flowing tap water and dried. The resulting negative wax casting of bone mineral was fragile and required careful handling. Complete decalcification was verified by radiography. As the wax was almost transparent to X-rays, areas remaining to be decalcified were observed as a light core at the centre of the sample on the radiograph. Samples found to be incompletely decalcified were returned to the acid for completion.

For slip infiltration of the wax mould, each dried bioceramic powder prepared as described in (b) above was redispersed in a carrier fluid as described below, to form a ceramic slip. This was done by rotating at 60 rpm in an airtight glass container on ball-mill rollers for 24 hours. All slips were prepared to contain 75 wt.-% bioceramic powder with the remainder consisting of carrier fluid, i.e. 300 g of powder in 100 ml of fluid. For materials 1-6 of table 2, the carrier fluid consisted of deionised water to which 3 wt.-% deflocculant (Darvan 811, R.T. Vanderbilt Company, Inc., USA) was added. The pH of the carrier solution was adjusted to 10 by addition of ammonia solution. For materials 7-12, the carrier fluid was deionised water without additives.

Prepared slips were placed in a stainless steel container to a depth just sufficient to allow full submersion of the samples. Each slip was rendered fluid by vibration. A suitable action was found to be produced by a Fritsch Analysette 3E. This machine allowed control of the amplitude of vibration, and consequently the shear rate within the slip.

Application of strong vibrations should produce a slip sufficiently fluid to penetrate the trabecular structure fully. The container was strapped to the Fritsch Analysette during the slip infiltration process.

Samples were placed on the surface of each slip and pressed into the slip, with the aid of gentle vibratory action, sufficiently to allow adherence to the surface. Stronger vibration was then applied and the amplitude of vibration adjusted so as to render the slip fluid. Penetration of the slip into the pores occurred and the sample was allowed to gradually submerge as the slip penetrated and expelled air from the porosity. Rapid submersion should be avoided as, if all surface pores are blocked, trapped air may not be able to escape, resulting in internal voids.

Following slip infiltration, samples were removed and any excess adhering slip was removed from the surface. Samples were then dried slowly at 30-35°C for 24 hours in air.

Following drying, the samples were placed on a piece of refractory brick for removal of the wax. Wax removal must be accomplished very gently, by slow melting, to prevent destruction of the delicate unsintered slip-cast structure. If the temperature is increased too fast, the wax may melt quickly and the subsequent hot flow may destroy the delicate trabecular structure.

The first stage of dewaxing was done in a conventional oven. The temperature was raised at 1°C per minute to 75°C where it was held for 15 minutes. It was then raised in increments of 15°C, using a 1°C per minute

ramp rate, up to 150°C, where it was held for 1 hour. Each 15°C increment was followed by a dwell of 15 minutes. The samples at this stage still contained a residual solid wax content, degraded due to the thermal treatment. Consequently they still retained some strength. The samples were allowed to cool and were removed from the oven. The samples were not moved from the refractory brick as handling may damage the slip infiltrated structure. They were placed centrally in a muffle furnace and heated at a rate of 1°C/min to 350°C where they were held for 1 hour, to allow burnoff of remaining wax. The temperature was then increased at 4°C per minute to sintering temperature (dependent on the composition of the composite powder used), held for 3 hours and cooled to room temperature at 4°C per minute.

All sintering was performed in a muffle furnace in air at atmospheric pressure. The furnace was built at University College, Dublin, according to a design by Kanthal (Stoke-on-Trent, U.K.) and as described in the Ph.D. thesis of David Tancred, University College, Dublin, National University of Ireland.

Fig. 1 is a scanning electron micrograph (SEM) of the macroporous structure of the ABM of a bovine cancellous bone sample used in the above Examples.

Fig. 2 is an SEM of the macroporous structure of a sintered replica based on bioglass only (Bioceramic No. 3 of Table 2).

Fig. 3 is an SEM of the macroporous structure of a sintered replica based on HAPG05 (Bioceramic No. 8 of Table 2).

Fig. 4 is an SEM of the macroporous structure of a sintered replica based on HABG50 (Bioceramic No. 12 of Table 2).

Fig. 5 is an SEM of the macroporous structure of a sintered replica based on ß-TCP (Bioceramic No. 2 of Table 2).

Fig. 6 is an SEM of the macroporous structure of a sintered replica based on HA (Bioceramic No. 1 of Table 2).

Samples for scanning electron microscopy were mounted on an aluminium stud with conductive carbon cement and sputter-coated with gold. A JEOL 35C scanning electron microscope operating at 25 kV was used.

The prepared replicas based on bioceramics 1-12 of Table 2 are precise cast structural replicas (except for a shrinkage factor due to sintering, typically 15-20%) of the original starting cancellous bone.

EXAMPLE II

Preparation of a polylactic acid negative bone replica

30 g of poly-L-lactid acid (available from Hycail, Holland) were heated in a conventional oven to a temperature of about 180°C to produce a viscous melt. An anorganic bone mineral specimen prepared as described in Example I was placed on the surface of the melted polymer. The melted polymer infiltrated the porous structure, expelling air, and caused the sample to sink under its own weight. A vacuum of 600 mm Hg was applied during infiltration to aid full

infiltration of the porous matrix by the polymer.

Following infiltration, the specimen was removed, shaped and trimmed as appropriate, and decalcified according to the procedure described in Example I.

All replicas prepared had adequate compressive strength (typically 1-3 MPa), were easy to handle and shape without damage, and had good biocompatibility, thus making them particularly suitable for use as bone graft substitutes in non-load bearing applications.

CLAIMS:

1. A synthetic bone replacement material having a macroporous structure similar to that of natural cancellous bone, characterised by a pore volume of 70-95%, fully interconnecting pores having a diameter in the range of from about 10 μm to 1 mm.

2. A synthetic bone replacement material according to claim 1, wherein the bone replacement material is a biomaterial which is a bioceramic material, a polymeric material or a metal.

3. A synthetic bone replacement material according to claim 2 wherein the bioceramic material is hydroxyapatite (HA), a calcium phosphate such as α-or ß-tricalcium phosphate (TCP), bioglass, a HA/α-or ß-TCP composite, a HA/glass or bioglass composite or alumina; the polymeric material is poly-L-lactid acid; and the metal is titanium.

4. A synthetic bone replacement material according to any of claims 1 to 3 for use as a carrier matrix for a bone inductive material, such as a bone morphogenetic protein or a non-collagenous protein, in the stimulation of bone formation.

5. A process for preparing a synthetic bone replacement material according to any of claims 1 to 3, the process comprising the following steps:-

(a) providing a cancellous bone sample;

(b) removing the organic material from the cancellous bone sample to give the anorganic bone

mineral (ABM);

(c) optionally treating the ABM of the cancellous bone to seal its trabecular micropores;

(d) impregnating the ABM with a suitable inert material, such as a wax, to form a mould, followed by decalcification;

(e) impregnating the mould with a bioceramic material or metal; and

(f) removing the mould and sintering the resulting product, if appropriate.

6. A process for preparing a synthetic bone replacement material according to any of claims 1 to 3, the process comprising the following steps:-

(a) providing a cancellous bone sample;

(b) removing the organic material from the cancellous bone sample to give the anorganic bone mineral (ABM);

(c) optionally treating the ABM of the cancellous bone to seal its trabecular micropores; and

(d) impregnating the ABM with a biomaterial, followed by decalcification.

7. A process according to claim 5 or 6 wherein step (c) is included.

8. A process according to any of claims 5 to 7

wherein in step (a) bovine cancellous bone is used; and/or in step (b) the organic material is removed with an organic solvent such as ethylenediamine or formamide; and/or in step (c) the ABM is boiled in sodium hypochlorite to seal its trabecular micropores; and/or in step (d) decalcification is carried out by immersion in dilute HCl.

9. A process according to claim 5 or 7 or claim 8 when dependent on claim 5, wherein in step (f) sintering is carried out by increasing the temperature step-wise to sintering temperature and subsequent step-wise cooling to room temperature.

10. Use of cancellous bone in the preparation of a synthetic bone replacement material according to any of claims 1 to 3.

Fig.1

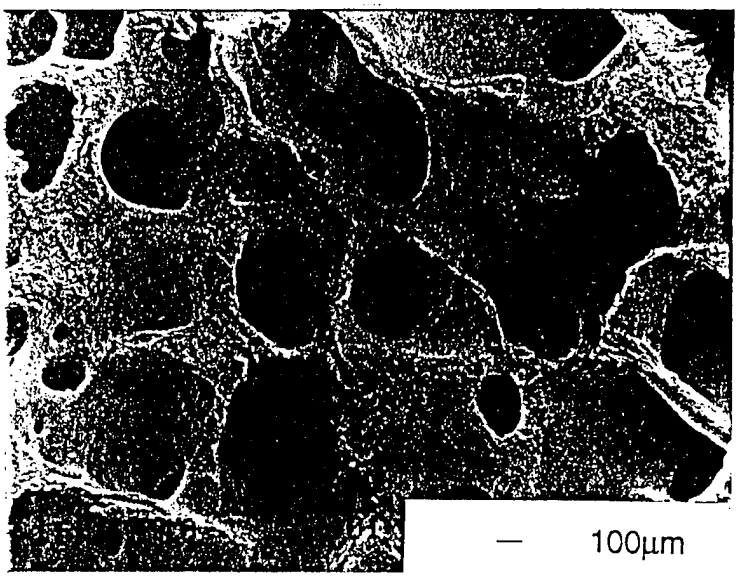

Fig.2

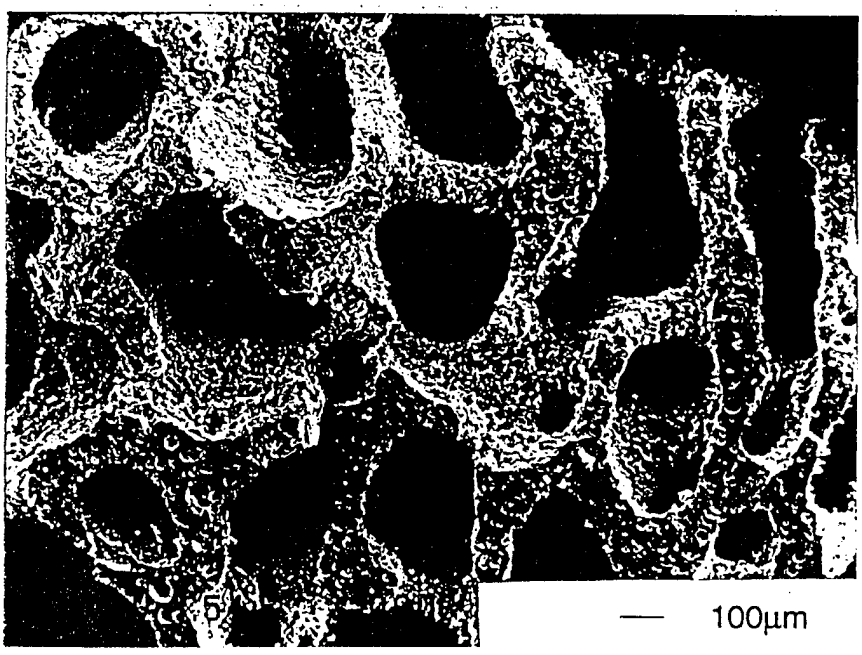

Fig.3

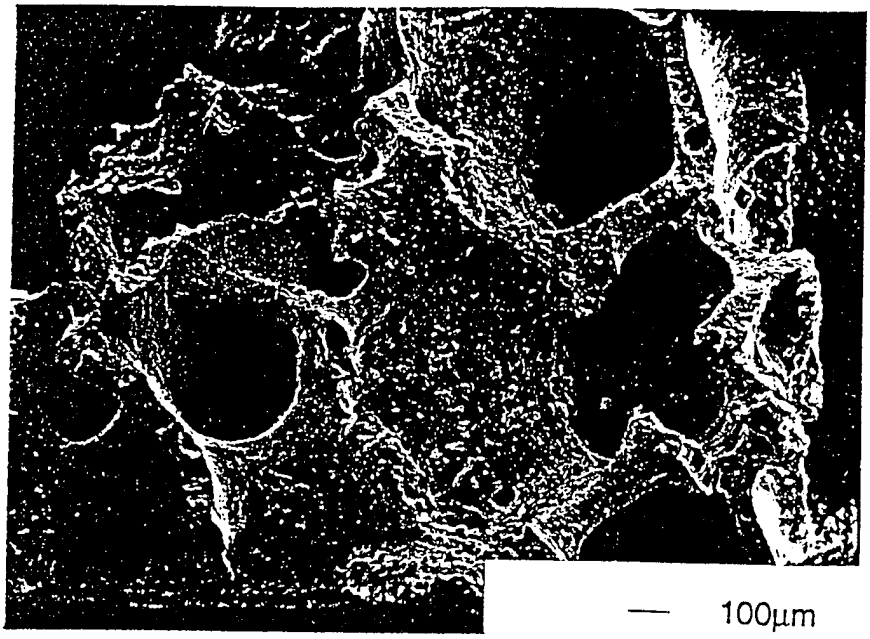

Fig.4

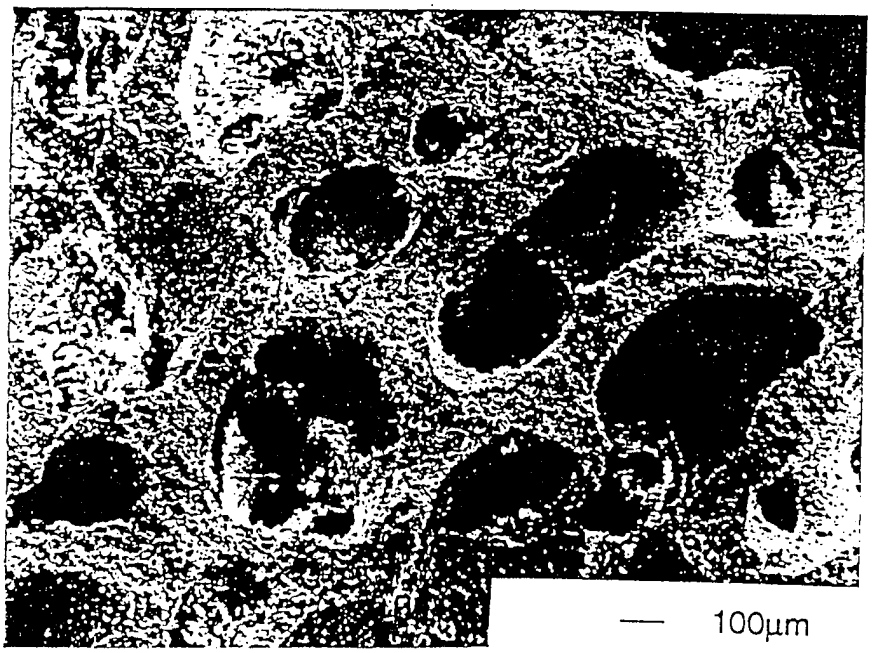

Fig. 5

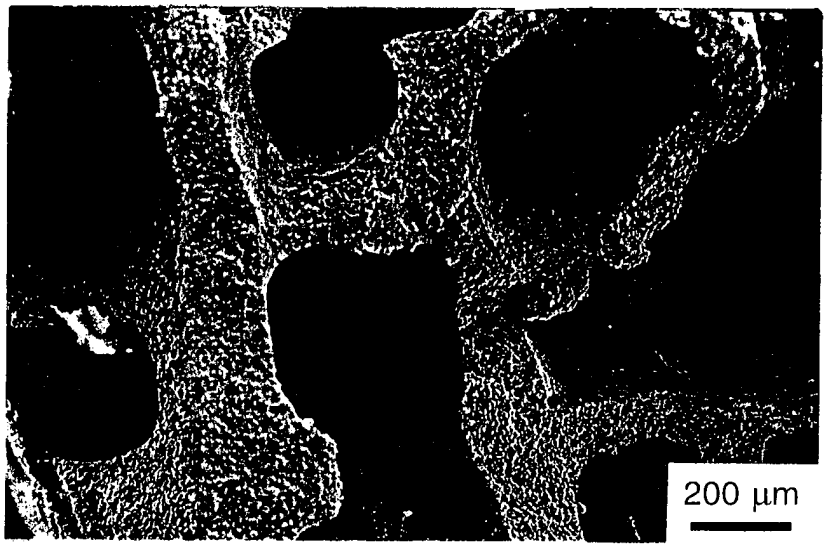

Fig. 6

INTERNATIONAL SEARCH REPORT

Intc onal Application No
PCI/IE 97/00068

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61L27/00 C08K5/00 C08K5/435

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61L C08K

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)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 92 21302 A (LUCOCER AB) 10 December 1992 see page 3, line 20 - line 26 see page 4, line 19 - line 32 see claims 1,2,4,5,7,9,10 ---	1-4
X	US 5 492 697 A (BOYAN BARBARA D ET AL) 20 February 1996 see column 5, line 43 - line 50 see column 6, line 46 - line 57 see column 7, line 11 - line 28 see claims 1,3,7,8 --- -/--	1-4

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in 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

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"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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 January 1998	02/02/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Heck, G

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INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/IE 97/00068

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	GB 2 078 696 A (MITSUBISHI MINING & CEMENT CO) 13 January 1982 see abstract see page 1, right-hand column, line 128 - page 2, left-hand column, line 30 see claims 1,2	1-3
Y	DE 44 03 509 A (DRAENERT KLAUS) 10 August 1995 see column 3, line 5 - column 4, line 11 see examples 1,2	5,6
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INTERNATIONAL SEARCH REPORT

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PCT/IE 97/00068

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