JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 9 (1998) 819–823

Development of a new synthetic bone graft

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A process for the replication of bovine cancellous bone in synthetic bioceramic materials for use as artificial bone graft substitutes is described. The process detailed here may be easily implemented to allow production of large numbers of blocks of material, even on a laboratory scale. The graft material has a pore morphology and interconnectivity identical with that of the original cancellous bone used as a starting material. Strength of the material is adequate, and at lower porosity levels it meets the FDA requirements for coralline materials for spinal applications. The synthetic graft is also shown to have excellent fluid-retention characteristics, making it a potential carrier for morphogenic agents such as solutions of bone morphogenic protein.

1. Introduction

Cancellous bone grafts are required in a number of surgical procedures, such as reconstructive surgery, filling of voids left after removal of diseased bone, and in spinal fusion. The graft material of choice is autograft, but harvesting of this causes additional trauma, and there is not always sufficient volume of material available. Allograft, which provides most of the desirable tissue characteristics of autograft, is increasingly unpopular due to the risk of transmitting HIV and hepatitis. Xenograft, typically bovine in origin (e.g. Kiel bone), is similarly considered problematic owing to the possibility of transmitting BSE prions. Thus, entirely synthetic grafts offer the best possibility of augmenting or replacing autograft. Existing synthetic materials fall into two main categories:

1. materials produced by conversion of an existing, naturally occurring matrix, e.g. hydrothermally converted coralline materials [1, 2];
2. entirely synthetic materials where porosity is introduced by artificial means into the structure, e.g. porous hydroxyapatites [3, 4].

Neither of these routes produce the same scale, volume, morphology, and interconnectivity of porosity which is found in cancellous bone, and this has implications for subsequent revascularization of the graft. If the porosity fails to match that in the host bone, then not only may larger blood vessels fail to penetrate the graft, but finer vessels, needed to maintain the function of the larger vessels, will also be unable to form. In this situation, the graft remains as inert matter within the bone, serving purely as a space filler. A highly porous graft with the correct type of porosity will strongly encourage tissue ingrowth [5–9].

Thus, there is a need for a graft material which exhibits the porosity which is characteristic of natural cancellous bone, but which is entirely synthetic, removing the possibility of disease transmission. A synthetic material should also allow adjustment of resorption rates by alteration of the phase composition of the material. The biological response may also be modified by the addition of, for example, growth factors to the implant material, and there is considerable interest in suitable carriers for bone morphogenic proteins (BMP). Because these are usually added in liquid form, it is therefore advantageous if synthetic grafts also show good fluid-retention characteristics.

The aim of this work was to produce an entirely bone-like structure in synthetic calcium phosphate-based materials. To this end, a process was developed, loosely based on that of White *et al.* [10]; here bovine bone was used as the prototype for a synthetic graft. An earlier version of the present process has been described elsewhere [11]. This paper describes the further development of this new synthetic bone graft with a cancellous bone-like pore system, and presents data relating to strength, and suitability as a carrier for BMP.

2. Materials and methods

2.1. Preparation of graft material

2.1.1. Harvesting of cancellous bone

Blocks, approximately 10 mm × 10 mm × 7 mm, were cut from bovine femoral condyles. Freshly harvested femurs were used, although femurs frozen within 24 h of harvesting have also been used successfully. Care was taken to avoid the inclusion of defects or growth plates in the blocks.

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2.1.2. Removal of inter-trabecular matter

To minimize the quantity of 1,2-diaminoethane (ethylenediamine, ED) required in later stages, fat and marrrow were removed from the bone by boiling for 10–15 min in water to which a small quantity of detergent had been added, using a domestic pressure cooker.

2.1.3. Removal of intra-trabecular matter

To remove the collagen and non-collagenous proteins from within the trabeculae, a procedure adapted from that described by Losee [12] was used. This involved treatment of the bone blocks with ED in a soxhlet apparatus for 48 h, with approximately three distillation–drain cycles per hour. After this treatment, the bone blocks were washed in deionized water to ensure removal of all ED. Analysis of the bone for residual nitrogen using a Carlo Erba 1106 CHN analyzer showed the nitrogen content to be below the detection threshold of the instrument (0.05%), indicating complete deproteinization; this was in agreement with the findings of Wheeler and Hyatt [13]. The material at this stage is referred to as anorganic bone mineral (ABM).

2.1.4. Pretreatment

Prior to infiltration of the anorganic bone with wax, it was necessary to seal the microporosity present in the trabeculae (Fig. 1). Failure to do so made subsequent leaching of the bone mineral impossible. The preferred technique involved boiling the ABM blocks for 2–3 min in 14% aqueous NaOCl followed by drying in air at 80–100 °C for 16 h. This resulted in the deposition of crystals of NaCl and $NaClO_3$ within the micropores, sealing them, whilst leaving the macroporosity unaffected [11].

2.1.5. Wax infiltration

The pretreated ABM blocks were infiltrated under vacuum with A7 machining wax (Blayson Olefins, London) approximately 20 °C above the wax melting point (T_m = 80–85 °C). It was found that the infiltration time should be kept below 5 min, otherwise subsequent decalcification was found to be impaired. Following infiltration, the samples were allowed to cool in air, and excess wax removed using a scalpel or by grinding, exposing the bone mineral. Full infiltration was verified using X-radiography. Note that the previously reported procedure [11] required Castylene B492 wax. This is no longer manufactured, and A7 was found to be a suitable alternative.

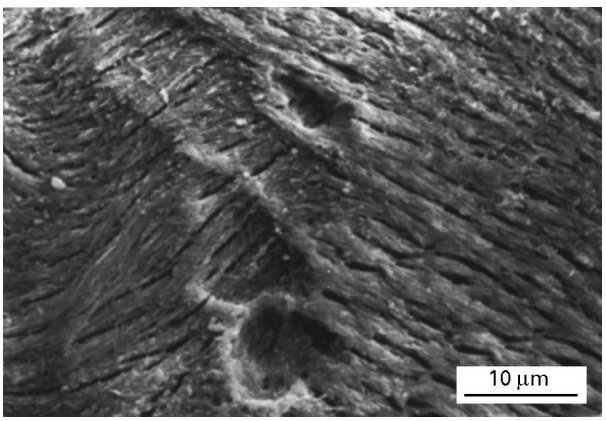

Figure 1 Surface of trabecula in inorganic bone mineral showing microporosity in structure which must be filled prior to wax infiltration. (Courtesy of K. U. O'Kelly.)

2.1.6. Decalcification

The infiltrated blocks were immersed in 10% HCl (20 ml acid per 1 ml sample) for 24 h at room temperature, resulting in complete decalcification of the blocks. Use of an ultrasonic bath or increased temperatures was found to lead to degradation of the wax. Following decalcification, the wax negatives were washed in flowing water for 1 h, and allowed to dry overnight at room temperature, supported on absorbent paper. Complete decalcification was checked using X-radiography.

2.1.7. Infiltration with ceramic slip

The preparation of aqueous hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) slips with adequate solids contents has been described by Tancred *et al.* [11]. The slip was de-aired, the wax negatives immersed in it, and kept immersed using a plastic or stainless steel mesh. The container holding the slip and negatives was placed in a vacuum desiccator, which was then evacuated to better than 100 mbar. After 2 min, the desiccator was brought back up to atmospheric pressure, the samples turned upside-down in the slip, and the procedure repeated. Gentle vibration using a Fritsch Analysette was found to enhance slip penetration for thicker samples. The samples were then dried for 16 h in an oven at 30–35 °C in air. Again, radiography was used to check that complete infiltration had occurred.

2.1.8. Wax removal and sintering

Wax was removed by slow heating to above the melting point (80–85 °C) in air, with the samples supported on a porous refractory (e.g. Rath Altraboard KVS). Too high a heating rate led to fracture of the ceramic body. Suitable conditions were found to be: 1 °C min^{-1} to 100 °C, hold for 1 h, 1 °C min^{-1} to 350 °C, then 4 °C min^{-1} up to the sintering temperature. The sintering temperature depended on the ceramic used, typically 1000–1200 °C, and sintering times of 3 h were normal. Following sintering, the samples were cooled at 4 °C min^{-1} to room temperature.

2.2. Compressive strength

Ultimate compressive strengths of blocks were measured using a Lloyd 6000S universal testing machine

with 500 N and 5 kN load cells and a crosshead speed of 0.5 mm min^{-1}. Self-aligning platens were used to prevent localized crushing when the top and bottom faces were not perfectly parallel. Sample dimensions were measured using vernier calipers to better than ± 0.1 min, and the blocks were tested with the shortest dimension parallel to the compressive loading direction.

2.3. Fluid retention

To determine the ability of the synthetic graft to retain fluid (e.g. a solution containing BMP), tests were carried out using deionized water. Samples were individually weighed, dry, then immersed in water for 1 min. The block of material was then removed from the water, and allowed to drain for 15 s, followed by reweighing. Subtraction of the two readings gave the mass of water retained by the graft material.

3. Results

3.1. Replication

The replication process described above has previously been shown, using image analysis, to produce a structure extremely similar to cancellous bone in terms of macroporosity [14]. Fig. 2 shows the structures formed at different stages of replication: (a) shows the anorganic bone material, (b) the wax negative, and (c) the replicated bone, in HA. Note that the photographs do not represent the same sample at each stage. Fig. 3 shows sections through anorganic bone and a replica in Al_2O_3, emphasizing the similarity in pore morphology.

The scale of the porosity is generally slightly smaller than for the bovine starting material, due to linear shrinkage on sintering, but this is typically about 10%, and always somewhat less than 20%. The process has successfully been used to make large quantities (up to 200 blocks per batch) of HA, HA : 30 wt % β-TCP, β-TCP : 30 wt % HA, and β-TCP samples [11], and has also been used to make rather smaller numbers of samples in an experimental grade of Bioglass, Bioglass: HA, and in HA : phosphate glass materials of the type developed by Knowles and co-workers [15–17].

3.2. Compressive strength

Twenty-eight blocks of β-TCP (Fluka Chemica, Purum Grade), sintered at 1200 °C, were tested as described above and the results are presented in Fig. 4. At porosities less than about 65%, the strength exceeds the FDA requirements for coralline materials for spinal work of 2.2 MPa. At all porosity levels, a consultant orthopaedic surgeon [18] deemed the materials to be sufficiently strong to withstand handling during surgery, whilst still being easily shapeable with a scalpel.

For comparison, blocks of anorganic bone mineral were also tested, and the results are presented in Fig. 5. As can be seen, the ABM is considerably stronger than the β-TCP replicas, possibly due to lower intratrabecular porosity, more strongly bonded particles of calcium phosphate, or preferred orientation of the calcium phosphate in the ABM. This is in broad agreement with earlier strength measurements on HA-Bioglass-based replicas [14].

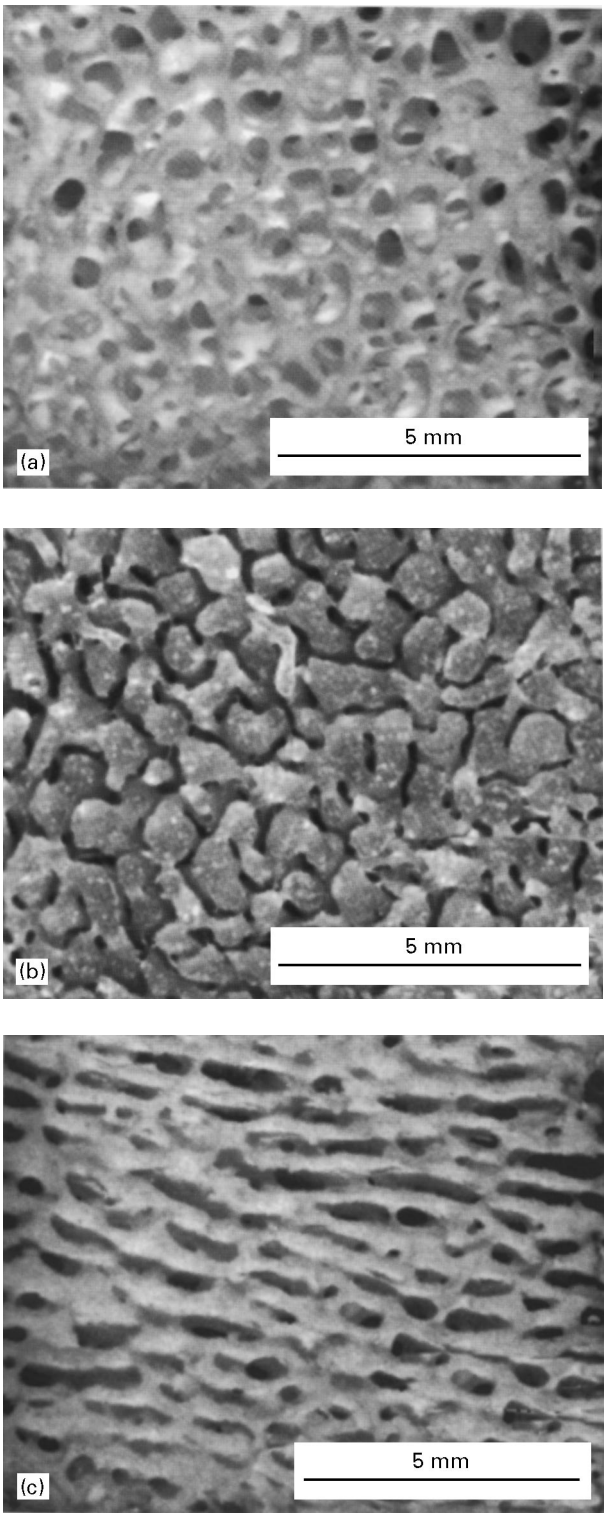

Figure 2 Stages in replication of cancellous bone: (a) anorganic bone, (b) wax negative, (c) replica in hydroxyapatite.

3.3. Fluid retention

In order that a bone graft material may be used as a carrier for BMP, it must be able to retain a solution containing BMP within its structure. Tests were carried out using β-TCP-based replicas, using deionized

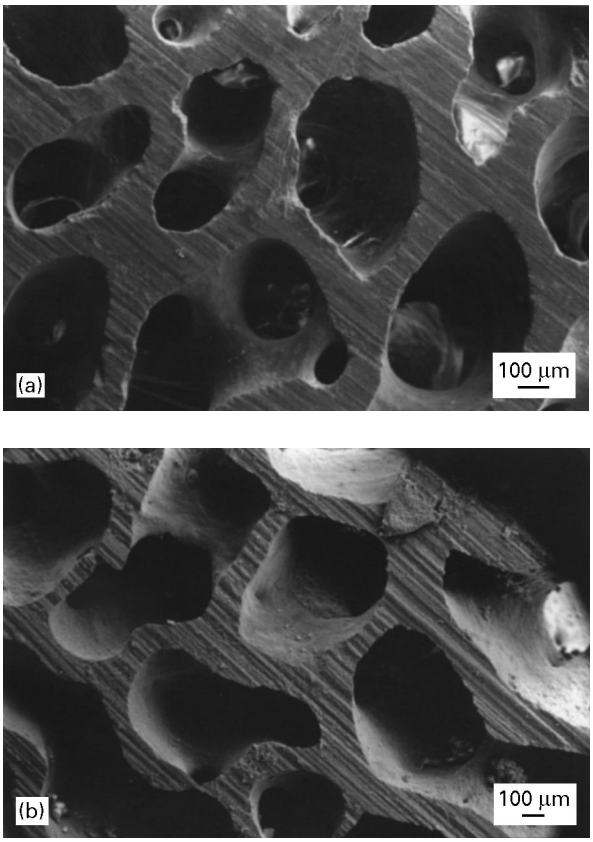

Figure 3 Sections through (a) anorganic cancellous bone, and (b) a replica of cancellous bone in Al_2O_3.

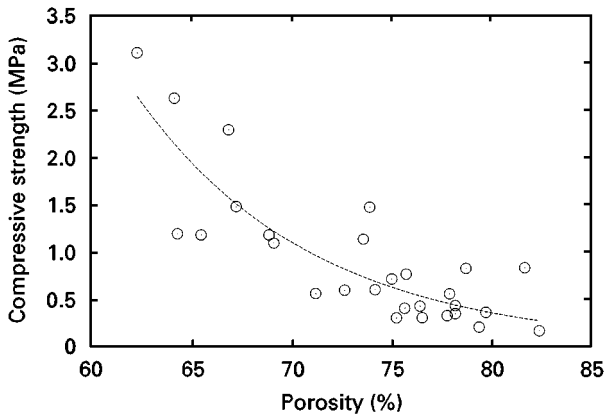

Figure 4 Ultimate compressive strengths of porous β-TCP replicas of bovine cancellous bone.

water, and the results are given in Fig. 6. As can be seen, these materials were capable of retaining water at the level of at least 50 wt % of the mass of the mineral, at less than 65% porosity, to about 150 wt % of the mass of the mineral at 80%–85% porosity.

4. Discussion

It has been shown that it is possible to reproduce precisely the structure of bovine cancellous bone, using bioceramics such as HA and β-TCP. These replicas had a pore morphology and interconnectivity almost identical to the original bovine cancellous bone, and this should encourage revascularization. The process is relatively simple to implement, and on

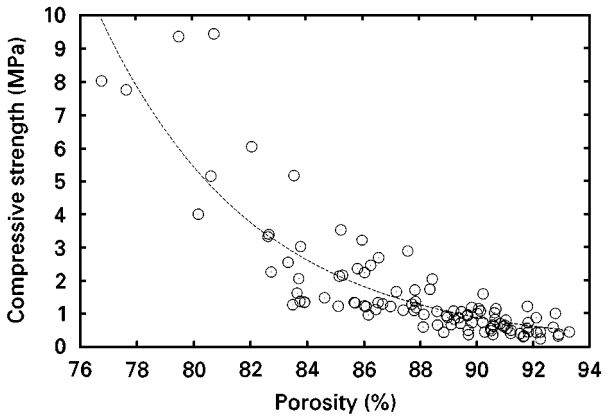

Figure 5 Ultimate compressive strengths of anorganic bovine cancellous bone.

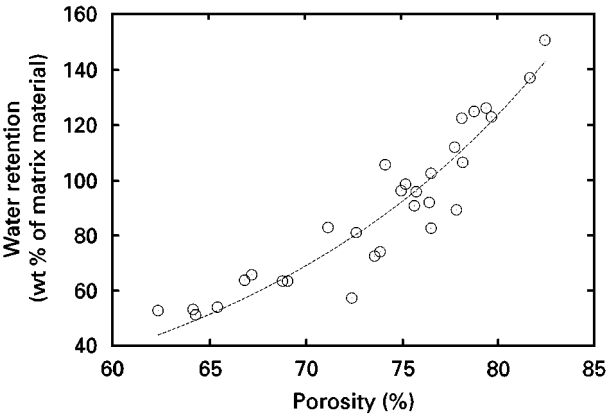

Figure 6 Water retention characteristics of porous β-TCP replicas of bovine cancellous bone.

a laboratory scale, batches of up to 200 blocks have been produced in HA, β-TCP, and composites of these two bioceramics. It has been shown that it is possible to replicate the cancellous bone structure in other materials, such as Al_2O_3 and Bioglass.

It was found that the strength of the replicated materials was somewhat reduced, compared to anorganic bovine cancellous bone mineral, and this is in agreement with earlier findings [14]. It is possible that small additions of phosphate glass to the HA or β-TCP may significantly increase the strength of the replicas, as has been shown for fully dense materials [19]. Blocks prepared from β-TCP had strengths sufficient to meet FDA requirements at porosities less than about 65%, and at all porosity levels were considered adequately strong and shapeable by a consultant orthopaedic surgeon [18].

Fluid retention capabilities were shown to be excellent, indicating that the replicated structures could be useful as a carrier for osteogenic agents such as BMP.

5. Conclusions

1. It is possible to reproduce the structure of cancellous bone in entirely synthetic materials, and laboratory trials have shown that large-scale production is relatively simple to implement.

2. These replicated materials have a pore morphology and interconnectivity which is almost identical to

that of the original cancellous bone, and this should serve to encourage revascularization of the graft.

3. Strengths of replicas in β-TCP were found to be low compared to anorganic bone mineral with equivalent porosity levels. However, material with a porosity less than 65% satisfies FDA requirements for the coralline materials for spinal implantation. Additionally, the materials were deemed sufficiently strong, and suitably shapeable by a consultant orthopaedic surgeon.

Acknowledgments

The authors thank Forbairt (the Government Agency for Enterprise, Innovation, Investment, and Development) for financially supporting this research. They also thank Mr K. U. O'Kelly for permission to use the photograph reproduced in Fig. 1, and Mr J. Colville (Blackrock Clinic, Co. Dublin) for initiating the project, and for advice on surgical requirements.

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Received 7 May
and accepted 4 June 1998