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In Vivo Evaluation of Hydroxyapatite Foams

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Porous hydroxyapatite manufactured by foaming of aqueous ceramic suspensions and setting via gelcasting of organic monomers was tested for *in vivo* biocompatibility in rabbit tibia for a period of 8 weeks. The foams provide tortous frameworks and large interconnected pores that support cell attachment and organisation into 3D arrays to form new tissue. The HA foam implants were progressively filled with mature new bone tissue and osteoid after the implanted period, confirming the high osteoconductive potential and high biocompatibility of HA and the suitability of foam network in providing good osteointegration. No immune or inflammatory reactions were detected.

Keywords: *porous hydroxyapatite, gelcasting of foams, biocompatibility, rabbit tibia, bone grafts*

1. Introduction

When a significant loss of bony tissue occurs as a result of trauma or by the excision of diseased or tumourous tissue, healing requires the implantation of bone grafts. Synthetic hydroxyapatite (HA) and other calcium-phosphate based materials have attracted considerable interest because of the similarities with the mineral fraction of bone, high biocompatibility with living tissues and high osteoconductive potential[1-4].

Attempts to synthesise materials that mimic the mineral structure of bone have been made for many years, with the appreciation that nature can be hardly ever reproduced. Bone has a complex morphology; it is a specialised connective tissue composed of a calcified matrix and an organic matrix. The tissue can be organised in either the dense (compact) or spongy form (cancellous), with pore sizes within the wide range of 1-100 μm [2]. The mineral fraction of bone consists of significant quantities of non-crystalline calcium phosphate compounds and predominantly of a single phase that closely resembles that of crystalline hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) [1,2].

Many processing technologies have been employed to obtain porous ceramics with hierarchical structures for bone repair. Recently, the method of gelcasting foams has shown suitability to manufacture strong and reliable macro-porous ceramics that have great potential to replace bone tissue. The process yields non-cytotoxic compounds in various porosity fractions, optimised strength and open spherical pores, as shown in previous works[5-7]. In tissue repair applications, the macropores and the highly interconnected network are required to provide the means of access for ingrowth of surrounding host tissues, facilitating further deposition of newly formed tissue in the spherical cavities. Additionally, the intricate shape of the walls provides a framework that supports the organisation of growing tissue, improving biological fixation and avoiding drawbacks that may result from implant mobility[8].

This work reports the *in vivo* evaluation of bone-like structures fabricated with hydroxyapatite foams. These porous ceramics have potential for use as aids in reconstitution and substitution of damaged bone tissue and various other biomedical fields such as membranes for enzyme cultivation, carriers for controlled delivery of drugs and matrices for tissue engineering.

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2. Material and Methods

Hydroxyapatite foam preparation and characterisation

Macroporous bodies of biomedical-grade hydroxyapatite (Plasma Biotal Ltd., U.K.) containing 85-90 vol% porosity were produced by foaming of ceramic suspensions and setting through gelcasting[7]. Sintering of the specimens was applied at 1350 °C for 2 h for matrix consolidation. The pore size distribution was evaluated by mercury porosimetry. Scanning electron microscopy (SEM, Leica-Stereoscan 440) of gold-coated specimens was carried out for observation of the pore morphology.

In vivo evaluation procedure

Porous HA cylinders measuring 3 mm in diameter were cut, sterilised, and implanted into the tibia of six adult albino New Zealand male rabbits, under general anaesthetic conditions and antibiotic protection[9]. After a healing period of 8 weeks, the animals were sacrificed and the tibial bone parts containing the implant was prepared for histological analysis. The area of implantation was fixed, dehydrated and embedded in methyl methacrylate resin. Slices were cut perpendicularly to the tibial axis, ground, polished for observation under light and scanning electron microscopy.

3. Results and Discussion

A representative specimen of the hydroxyapatite foams tested in this work is shown in Fig. 1, at various levels of magnification. The structure is highly porous (Fig. 1a) typically composed of large spherical pores and interconnecting windows (Fig. 1b) enclosed by a compact framework of polycrystalline hydroxyapatite (Fig. 1c). The pore size in foams can be varied according to the volume of foam produced, with pores in different size ranges. SEM observation revealed the presence of large pores ranging within 100-500 µm and smaller interconnecting windows between 20 and 300 µm, as depicted in Fig. 2, by the increase in volume of mercury intruded through the specimens at the specified range.

The foam structure resembles that of trabecular bone. In tissue repair applications, an open porous network is required to promote extensive cell attachment and organisation of cells in the 3D form that leads to tissue ingrowth with vascularization, and good implant integration. The tissue ingrowth rates depend greatly upon the pore morphology, the degree of pore connectivity and pore volume. It has been reported that pores larger than 100 mm are seen as necessary to allow blood and nutrient supply access for bone mineralization within the graft[2].

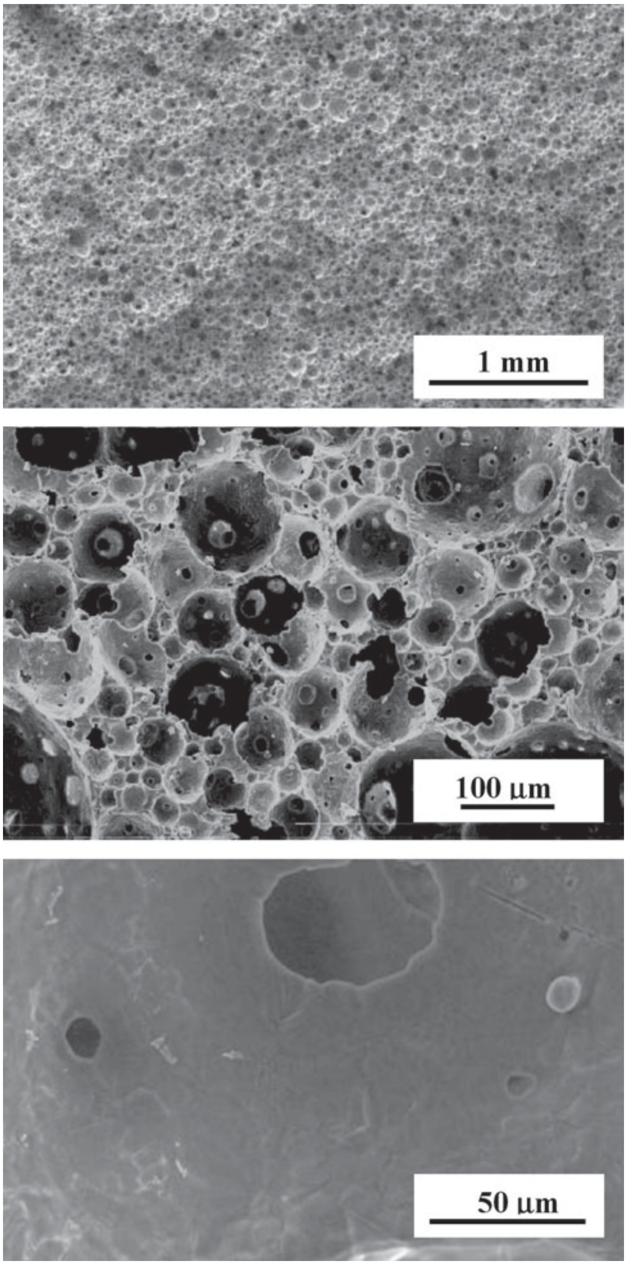

Figure 1. SEM micrographs of hydroxyapatite foams, at various levels of magnification.

The suitability of the porous network of HA foams to promote tissue regeneration was confirmed by *in vi*vo experiments. All animals survived the 8 weeks study period without evidence of inflammation or infection at the implantation site. The results revealed that the HA foam structure was filled almost entirely with newly formed trabecular bone within the 8 weeks of implantation, confirming the high osteonconductive behaviour of HA and the ability of the porous network to promote tissue ingrowth. Fig. 3a illustrates bone-implant integration, new bone filling the foam

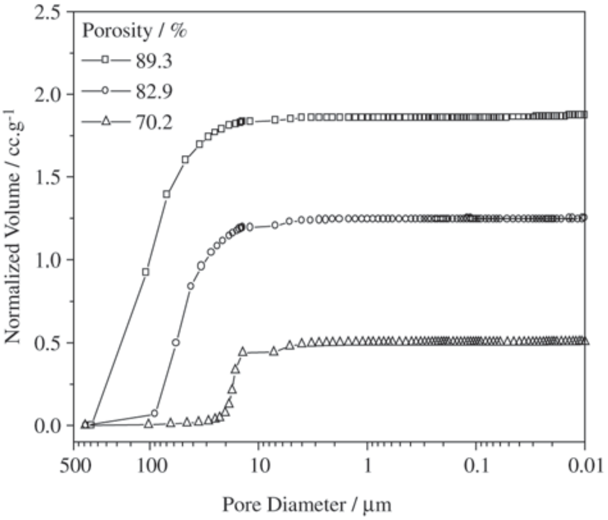

Figure 2. Mercury porosimetry intrusion curves for three different foam densities.

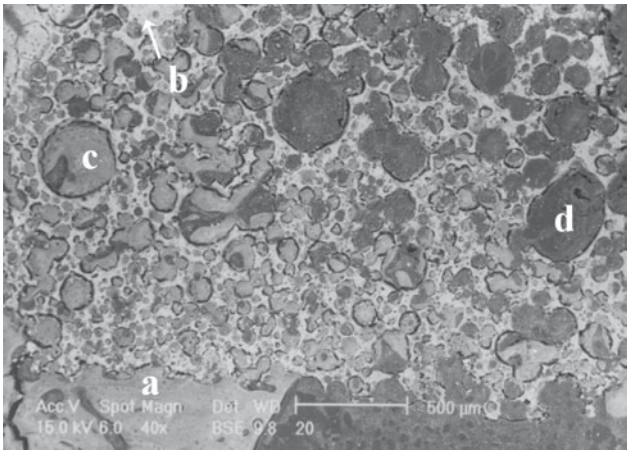

Figure 3a. Section of HA foam implant after 8 weeks, showing osteointegration and extensive bone ingrowth in within the pore and connections of the structure. The original bone is shown in (a), the porous implant appear in light coloured areas (b), whereas new bone tissue appears in light grey (c) and collagen in dark grey (d).

structure progressively, from areas of neighbouring old bone towards the inner part of the implant. In all implants, bone tissue deposition occurred mainly in the form of layers with trabecular architecture and a significant number of osteocyte lacunae were noticed. Newly formed bone tissue at various stages of maturation were also detected. Figure 3b gives an example of mature bone with regular osteon structure surrounded by areas where the new bony tissue was still in early stages of maturation. The new bone tissue appears within the pores and communicates through the interconnections.

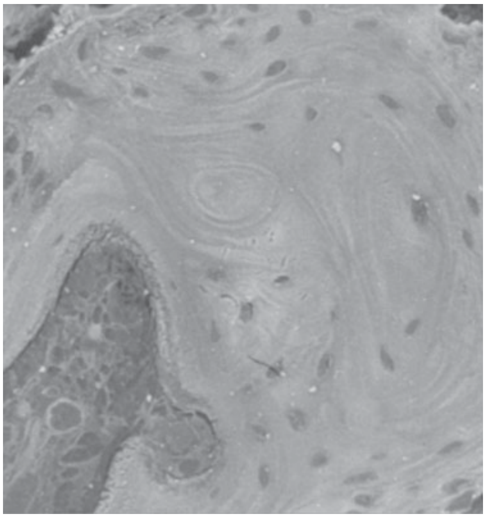

Figure 3b. Detail of Fig. 3a showing the remodelling in mature bone.

Despite providing an adequate porous structure for large osteointegration, foaming as a technique to incorporate porosity into ceramics has many advantages, including its simplicity, the regular pore shape and the ability to tailor pore size range. Although a precise replica of cancellous bone structure has not been synthesised up to date, as synthetic materials differ from bone in terms of mineralogy, pore shape and size distribution, interconnectivity and porosity levels, the results reported herein show that foamed materials provide a good alternative for bone grafts.

4. Conclusions

Hydroxyapatite foams manufactured by the gelcasting of foams technique provide an structure that resembles that of trabecular bone, with large spherical pores connected by smaller windows. The framework of HA foams provide the scaffold for extensive cell attachment and tissue ingrowth allowing good osteointegration in bone repair applications. This type of material has potential for applications in the biomedical field as filling of bone defects, craniofacial reconstructions and arthroplasty revision surgery.

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