In vitro evaluation of novel bioactive composites based on Bioglass®-filled polylactide foams for bone tissue engineering scaffolds

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Abstract: Highly porous poly(DL-lactic acid) (PDLLA) foams and Bioglass®-filled PDLLA composite foams were characterized and evaluated *in vitro* as bone tissue engineering scaffolds. The hypothesis was that the combination of PDLLA with Bioglass® in a porous structure would result in a bioresorbable and bioactive composite, capable of supporting osteoblast adhesion, spreading and viability. Composite and unfilled foams were incubated in simulated body fluid (SBF) at 37°C to study the *in vitro* degradation of the polymer and to detect hydroxyapatite (HA) formation, which is a measure of the materials' *in vitro* bioactivity. HA was detected on all the composite samples after incubation in SBF for just 3 days. After 28 days immersion the foams filled with 40 wt % Bioglass® developed a continuous layer of HA. The formation of HA for the 5 wt % Bioglass®-filled foams was localized to the Bioglass® particles. Cell culture studies using a commercially available (ECACC) human osteosarcoma cell line (MG-63) were conducted to assess the biocompatibility of the foams and cell attachment to the porous substrates. The osteoblast cell infiltration study showed that the cells were able to migrate through the porous network and colonize the deeper regions within the foam, indicating that the composition of the foams and the pore structures are able to support osteoblast attachment, spreading, and viability. Rapid formation of HA on the composites and the attachment of MG-63 cells within the porous network of the composite foams confirms the high *in vitro* bioactivity and biocompatibility of these materials and their potential to be used as scaffolds in bone tissue engineering and repair. © 2003 Wiley Periodicals, Inc. J Biomed Mater Res 67A: 1401–1411, 2003

Key words: tissue engineering scaffolds; degradable polymers; bioactive glass; composite materials; bone regeneration

INTRODUCTION

Current clinical technologies such as autografting and allografting cancellous (spongy) bone and applying vascularized grafts suffer from cost and anatomical limitations and donor site morbidity or tissue rejection.[1–3] Tissue engineering presents a promising alternative to permanent implants in the repair of damaged tissue. The underlying principle involved is the regeneration of living tissue, where a loss or damage has occurred as a result of injury or disease.[1] The scientific challenge encompasses understanding the cells themselves, their mass transport requirements and biological environment as well as the development of suitable scaffold materials, usually porous, that act as templates for cell adhesion, growth, and proliferation.[1–3]

Certain bioactive ceramics such as tricalcium phosphate (TCP) and hydroxyapatite (HA), and selected compositions of glass-ceramics and silicate glasses, e.g., Bioglass® (US Biomaterials, Alachua, FL), react with physiological fluids to form tenacious bonds to hard (and in some cases soft) tissue.[4] Thus porous bioactive ceramics are being considered for clinical applications, including scaffolds for tissue engineering.[5] These bioactive materials are however relatively stiff, brittle, and difficult to form into complex shapes. Conversely synthetic resorbable polymers are more easily fabricated into complex structures yet are too weak to meet the demands of orthopedic surgery.[6] Synthetic polymers such as poly(α-hydroxyesters),

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polyanhydrides, polyorthoesters, and polyphosphazenes have found application in orthopedic devices, as implantable drug delivery systems and in tissue engineering.[6–8] Chemical hydrolysis as opposed to enzymatic reactions are responsible for degradation of polymeric chains; thus degradation does not vary from patient to patient. However these polymers may elicit inflammatory response in the host tissue because of the release of acidic degradation products.[7,8] The degradation of poly(α-hydroxyesters) depends on molecular weight, composition, configurational structure, and crystallinity, because these factors control water accessibility to ester linkages.[9–11] Composites combining these degradable polymers with inorganic bioactive phases are of particular interest as tissue engineering scaffolds[12,13] as tailored physical, biological, and mechanical properties can be obtained. Bioactive and resorbable composites have therefore a variety of further biomedical applications including bone-filling materials, guided bone regeneration, and in drug delivery systems.[14,15]

Diverse approaches to the development of bioresorbable and bioactive composites for bone tissue engineering are being investigated, including combinations of polylactide (PLA), polyglycolide (PGA), and other resorbable polymers with HA, tricalcium phosphate (TCP), or bioactive glasses or glass-ceramics in various scaffold architectures.[12,13,16–31] Besides imparting bioactivity to a polymer scaffold, the addition of bioactive phases to a bioresorbable polymer may be used to alter polymer degradation behavior. Bioactive phases in bioresorbable polymers allow rapid exchange of protons in water for alkali in the glass or ceramic, which should provide a pH buffering effect at the polymer surface, reducing acceleration of acidic degradation of the polymer.[19] A further advantage of using bioactive inorganic particles in biodegradable polymers relates to mechanical properties. Stiff Bioglass® particles as filler can be used to enhance the elastic constants, mechanical strength, and structural integrity of porous polymer constructs.[12] Thus by combining bioactive materials with bioresorbable polymers, composites of tailored physical, biological, and time-dependant mechanical properties can be produced, allowing new tissue, as it grows naturally, to take over its load-bearing capability.

In recent studies Bioglass® particles have been incorporated into poly(DL-lactic acid) (PDLLA) foams to create bone tissue engineering scaffolds.[12,20] Bioglass® is a Class A bioactive material exhibiting both osteoinductive and osteoconductive properties. Thus, by combining Bioglass®-filled PDLLA foam composites with graded Bioglass® coatings, scaffolds of controlled tailored and variable porosity, resorption, mechanical properties, and enhanced bioactivity may be produced, proving ideal for bone tissue engineering.[12,21,29]

In the present work the *in vitro* response of PDLLA foams and novel Bioglass®-filled PDLLA composite foams was comprehensively assessed. The materials are intended as scaffolds for bone tissue engineering applications; therefore, the degree of *in vitro* bioactivity was investigated by studying HA formation on the surface of samples after incubation in simulated body fluid (SBF). The attachment of MG-63 human osteosarcoma cells to the foam surface was also analyzed to assess the biocompatibility of the foams. These cells were chosen because of their extensive characterization with regard to the osteoblastic phenotype[32] and their extensive use in the investigation of osteoblast responses to various biomaterials.[33–35]

EXPERIMENTAL

Materials

The bioactive material used as filler for PDLLA/Bioglass® composite foams was melt-derived Bioglass® powder (grade 45S5, US Biomaterials, Alachua, FL) with a mean particle size < 5 μm and composition (in weight percentage): 45% SiO_2, 24.5% Na_2O, 24.5% CaO, and 6% P_2O_5, which is the original composition developed by Hench and coworkers in 1971.[36] The preparation of Bioglass®-filled PDLLA foams (of diameter 8 mm and height 3–5 mm) involved a thermally induced phase separation (TIPS) process, which has been described elsewhere.[20,37] Purasorb (PDLLA) was used in the foam fabrication, with an inherent viscosity of 1.62 dL/g (obtained from Purac Biochem, Goerinchem, The Netherlands). The polymer was dissolved in dimethylcarbonate to give a polymer weight to solvent volume ratio of 5%. The mixture was stirred overnight to obtain a homogeneous polymer solution. For the composite foams, determined quantities of Bioglass® powder were added to the solution, to result in either 5 or 40 wt % Bioglass® concentration. The PDLLA/Bioglass® mixture was transferred to a lypholization flask and sonicated for 15 min to improve the dispersion of the Bioglass® into the polymer solution. The flask was immersed in liquid nitrogen and maintained at −196°C for 2 h. The frozen mixture was then transferred into an ethylene glycol bath at −10°C and connected to a vacuum pump (10^{-2} Torr). The solvent was sublimed at −10°C for 48 h and then at 0°C for 48 h. The sample was completely dried at room temperature in a vacuum oven until reaching a constant weight.

In vitro studies and characterization

In vitro studies in SBF were based on the composition and method described by Kokubo et al.[38] Composite and unfilled PDLLA foams were immersed individually in 50 mL of SBF in clean conical flasks, which had been previously rinsed with hydrochloric acid and deionized water. Foam samples were placed inside stainless steel mesh cages to

prevent them from floating, due to the high hydrophobicity of PDLLA. Lids were placed on the flasks to form an airtight seal, preventing contamination. The flasks containing the specimens were placed inside an orbital shaker (C24 Incubator Shaker, New Brunswick Scientific), which maintained a temperature of 37°C and rotated at 175 rpm. The PDLLA foam/Bioglass® composites and unfilled foams were left in immersion in SBF for varying time periods of 3, 7, 21, and 28 days. The SBF was changed every 3 days because the cations concentration decreases during the experiments because of the chemical change of the samples as discussed below. After immersion in SBF the samples were gently rinsed with deionized water before drying for 48 h in a desiccator cabinet (Townsen & Mercer, Cheshire, UK) that maintained a temperature of 37°C and controlled relative humidity of ~60%. Samples were subsequently placed in desiccator jars awaiting characterization by scanning electron microscopy (SEM), X-ray diffraction (XRD), and Raman spectroscopy.

PDLLA foams and PDLLA/Bioglass® composite samples were characterized by using SEM before and after incubation in SBF. The cylindrical foams were sectioned axially to permit examination of the interior morphology. Samples were frozen in liquid nitrogen and fractured using a razor blade, thus avoiding compression damage. Samples were gold-coated and observed at an accelerating voltage of 15–25 kV.

As-received and *in vitro* (SBF) tested foams were analyzed using XRD to verify the formation and crystallization of HA on their surfaces. A Philips PW 1700 Series Automated Powder Diffractometer, employing Cu kα radiation (at 40 kV and 40 mA) with a secondary crystal monochromator was used.

Raman spectroscopy (Renishaw Raman System 2000) was performed to confirm the results of SEM and XRD and to provide a more quantitative analysis of HA formation. A 785 nm laser with a power of 100 mW was used (magnification was ×50) and measurement time was 20 s. Five points in different regions of each sample were analyzed because the development of HA was thought to be heterogeneous. The areas under the polymer and HA characteristic peaks were measured to estimate the ratio of HA to PDLLA, which serves to indicate the level of apatite formation and polymer degradation with increasing time in SBF.

Cell culturing studies

Cell culture studies using a commercially available (ECACC) human osteosarcoma cell line (MG-63) were conducted to assess the biocompatibility of the foams and cell attachment to the porous substrates. Foams were sterilized with ultraviolet radiation and incubated for 24 h in Dulbecco's modified Eagle's medium (DMEM) to prevent the sudden release of high ion concentrations from the Bioglass®, which occurs within the first few hours of immersion in simulated body fluids, as described in the literature.[5] MG-63 cells were cultured in flasks in complete DMEM, containing 10% fetal bovine serum (FBS), 5% penicillin/streptomycin, 5% glutamine, and 0.25 mg ascorbic acid [dissolved in 2 mL of phosphate-buffered saline (PBS) and filtered to 0.2 μm]. Foam specimens were incubated at 37°C in a humidified incubator with 5% CO_2. MG-63 cells were cultured using the aforementioned DMEM to subconfluency and subcultured using trypsin/EDTA. Cells were seeded on pure polymer, composite foams, and Thermanox discs as positive controls at a density of 80,000 cells/cm^2. A droplet of cells suspended in DMEM was placed on the foam and allowed to sink into the foam before addition of more DMEM to a final volume of 1 mL to ensure good cell penetration. Cells were cultured on the foams for periods up to 4 weeks in a humidified incubator with 5% CO_2. Foams were washed with PBS and fixed with 1.5% glutaraldehyde (in phosphate buffer) for 30 min at 4°C, rinsed gently in PBS and stored in PBS before freeze-drying for SEM analysis. Prior to the SEM preparation procedure described, foams cultured with MG-63 cells were coated in isopentane and frozen in liquid nitrogen. Saturating the foam with isopentane reduces the formation of a shell of frozen material on the outside of the foam. Specimens were placed in a freeze-dryer (Edwards Modulyo Pirani 10) for 48 h.

For staining of the cell nuclei, samples were rinsed gently in PBS and fixed with 4% paraformaldehyde for 10 min at room temperature. Foams were carefully bisected with a scalpel and stained with 10 μg/mL propidium iodide at room temperature, rinsed in PBS, and mounted on coverslips using Vectashield (Vector Laboratories Ltd, Peterborough, UK). Samples were then viewed under a Bio-Rad confocal microscope. Cells were counted after 8 days in culture, and counting was performed over nine fields of view on two axially sectioned samples of each composition (0%, 5 wt % and 40 wt % Bioglass® content). A one-way ANOVA with Tukey-Kramer post-test, following standard a procedure,[39] was carried out to assess the statistical significance of the results.

RESULTS AND DISCUSSION

Materials characterization

As-received pure polymer and composite foams are shown in Figure 1(a–d). The foams exhibit high porosities and possess two distinct pore sizes, macropores ~100 μm average diameter, and interconnected micropores of 10–50-μm diameter. The tubular macropores are highly orientated as a result of the unidirectional cooling process. This porous structure is typical of foams prepared by the phase separation process.[20,21,37] The composite foam structures with Bioglass® particles show a similarly well-defined tubular and interconnected porous structure, although the 40 wt % Bioglass®-filled foam has more irregular pore morphology, as evident by comparing Figure 1(a,b) and Figure 1(c,d). All these foams have sufficient pore volume and pores of given controlled size and orientation for cell proliferation as required in tissue engineering scaffolds.[12,13,40,41]

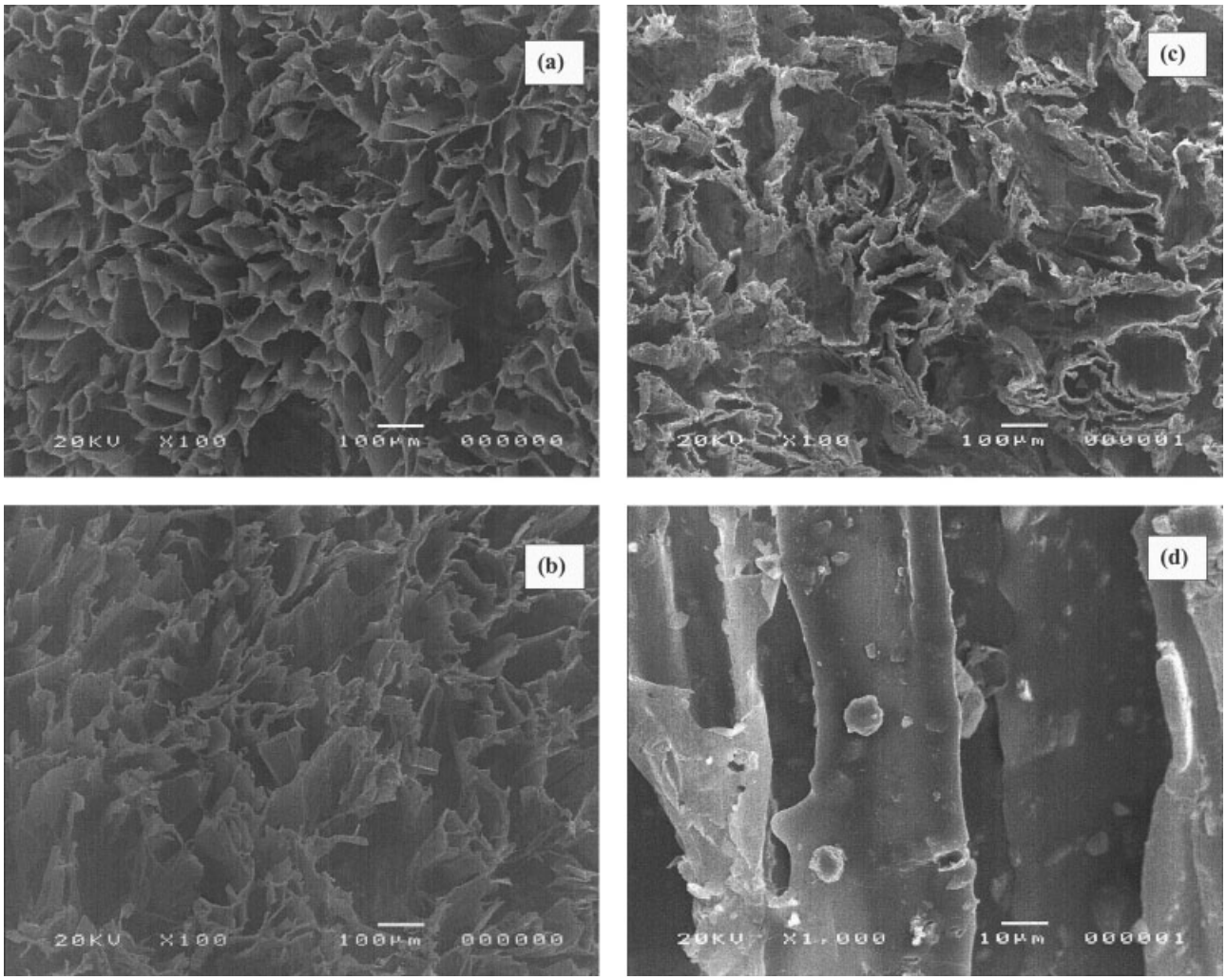

Figure 1. SEM micrographs showing the microstructure of (a) a pure PDLLA foam (orthogonal to the pore direction); (b) a PDLLA/Bioglass®-filled composite foam (5 wt % Bioglass®; orthogonal to the pore direction); (c) a PDLLA/Bioglass®-filled composite foam (40 wt % Bioglass®; orthogonal to the pore direction); and (d) a PDLLA/Bioglass®-filled composite foam (40 wt % Bioglass®; parallel to the pore direction).

In vitro studies in SBF

The response of PDLLA foam/Bioglass® composites and unfilled PDLLA foams in contact with SBF was analyzed using SEM, XRD, and Raman spectroscopy. After 3 days' immersion in SBF, small crystals developed on the surface of the foams, in regions close to Bioglass® particles. The HA crystal morphology was similar to that reported recently for HA formed on dense Bioglass® discs immersed in SBF and tris buffer.[42] In the present composites, however, continuous layers of HA formed by coalescence of large crystals after the third week of incubation in SBF. SEM micrographs shown in Figure 2(a–d) depict the gradual development of the HA layer in the interior of the 40 wt % filled foam. The composite with 5 wt % Bioglass® exhibits more discrete patches of HA formation localized to the Bioglass® inclusions as shown in Figure 3 in comparison to the 40 wt % filled foam, which developed a continuous layer of HA up to 7 μm thick after 28 days in SBF. Flake-like particles were evident on the pure polymer foam after 7 days in SBF. XRD analyses indicate the formation of crystalline HA on the pure polymer foam after 15 days in SBF, which is in agreement with recent findings on similar materials.[43] However, in comparison with the composite foams the development of HA on the PDLLA foam is limited. This HA formation on pure PDLLA foams is somewhat surprising, but it may be explained in the light of findings reported in the literature. For example, Zhang et al.[44] conducted experiments involving composites prepared from highly porous poly(L-lactic acid) (PLLA) foams and apatite. Bone-like apatite was "grown" on the surfaces of pore walls throughout the PLLA foams during immersion in SBF at 37°C. This method of preparing composites by growing apatite on PLLA substrates by immersion in SBF for prolonged periods of time supports the finding in this

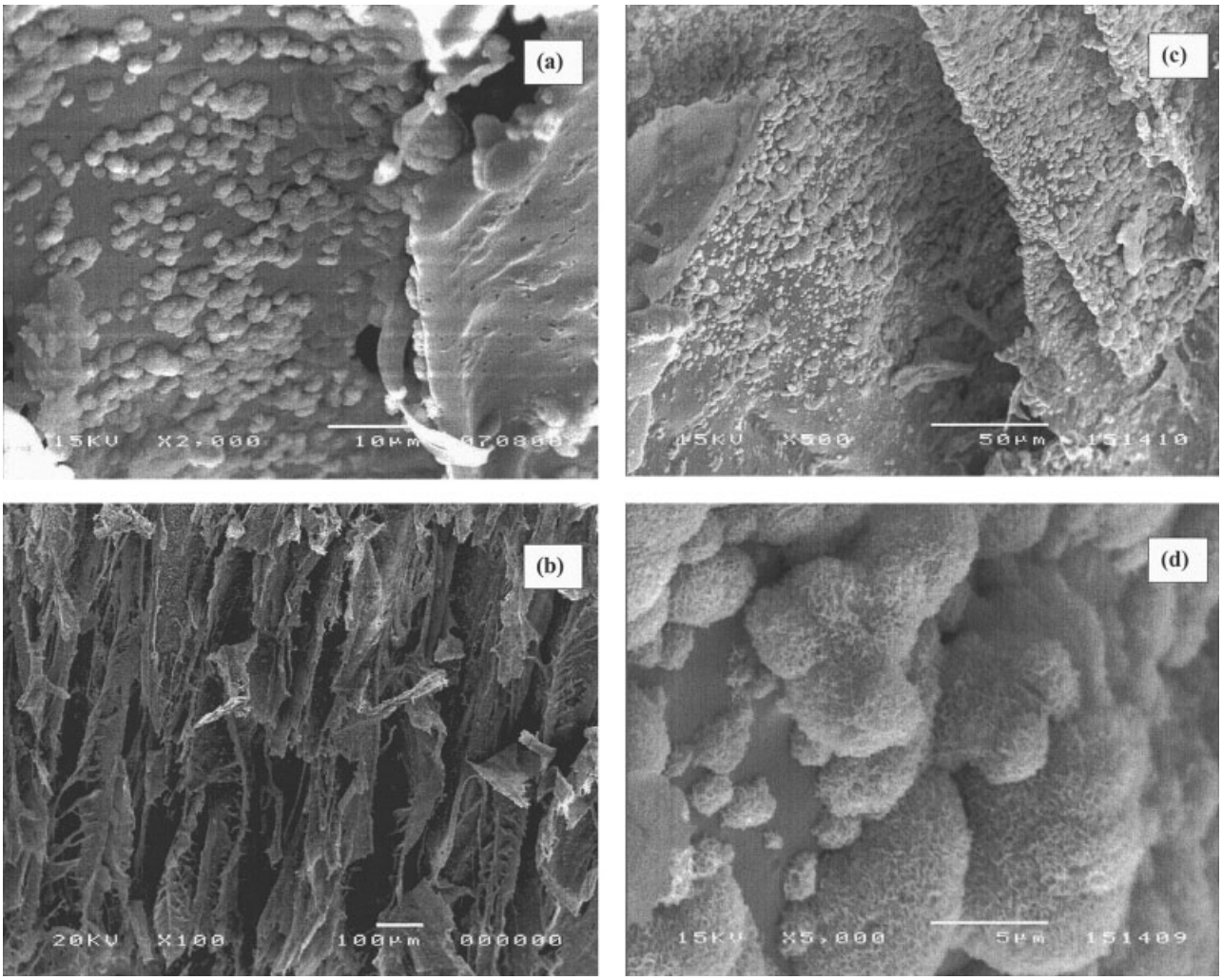

Figure 2. SEM micrographs of the PDLLA/Bioglass®-filled composite foams (40 wt % Bioglass®; parallel to the pore direction) after immersion in SBF for (a) 7 days, (b) 14 days, (c) 28 days at low magnification and (d) 28 days at high magnification. The micrographs reveal the progressive formation of HA crystals and the development of a surface HA layer.

study of formation of HA on the PDLLA foams; however, a more detailed investigation of the mechanism of HA formation on pure PDLLA foams should be undertaken.

XRD analysis of the 5 wt % filled composite foam shown in Figure 4 demonstrates the gradual crystallinity of the HA forming on the foam surface with increasing time in SBF; similar results were obtained for the 40 wt % samples.However, because the size and morphology of the samples varied, a quantitative analysis indicting the amount of HA formation in relation to the period of immersion in SBF could not be conducted using XRD. Raman spectroscopy was used to give a more quantitative analysis. As shown in Figure 5 the characteristic peak for HA (at a wavelength of 960 cm^{-1})[45] intensifies, and the polymer characteristic peak (at a wavelength of 875 cm^{-1})[46] reduces with increasing incubation time in SBF. The ratio of HA to PDLLA given by the ratio of peak areas increases with the period of immersion, as shown in

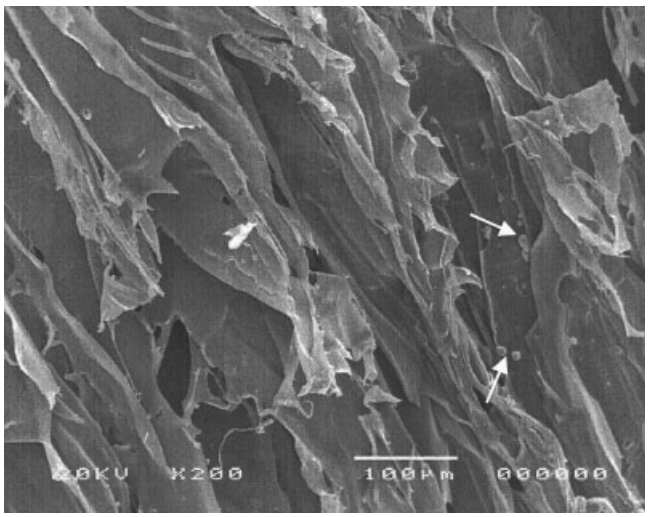

Figure 3. SEM micrograph of a PDLLA/Bioglass®-filled composite foam (5 wt % Bioglass®; parallel to the pore direction) after 28 days in contact with SBF showing localized formation of HA at the Bioglass® particles (marked by arrows).

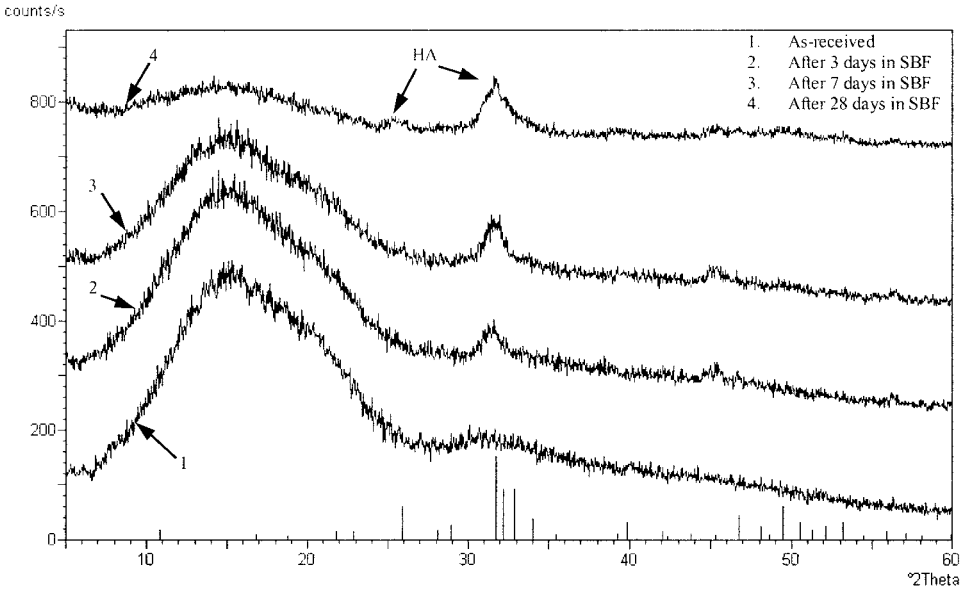

Figure 4. XRD patterns of PDLLA foam/Bioglass® (5 wt % Bioglass®) foams after immersion in SBF for different times, showing the formation of crystalline HA. Note the intensity is given in arbitrary units, i.e., the relative height of the peaks does not correlate with the amount of HA present in different samples. The XRD standard pattern of stoichiometric crystalline HA is also shown.

Figure 6. The data shown in Figure 6 are related to the kinetics of *in vitro* HA formation and polymer degradation in bioresorbable composites exhibiting bioactive properties.

Degradation of PDLLA implants is known to be heterogeneous and divided into a fast degrading center and a slowly degrading outer layer, which stays intact and retains degradation products until the swelling of the implants or mechanical failure cause it to break, after about 32 weeks *in vitro*.[47] In a previous study, the degradation of similar PDLLA foams and composites containing up to 40 wt % of Bioglass® was investigated *in vitro* by immersion in PBS.[20] It was shown that the pure polymer foams and the composite foams retained their structural integrity until the end of the experiment (i.e., 16 weeks), which means that the degradation process was still in its early stage. The disruption of the outer layer was not observed, and therefore the release of the acidic residues did not occur, which explains why no significant lowering of pH was measured.[20] Moreover, it was shown that the PDLLA molecular weight started to decrease after 6 weeks of incubation in PBS, i.e., before the mass decrease of the PDLLA foam, indicating that the autocatalysis in the interior of the sample might have initiated.[20] This phenomenon has been reported also using other polyester devices.[48]

In the Bioglass®-filled foams, this phenomenon is though to be delayed or even hindered because of the buffering effect of the Bioglass®. The measurement of pH variation in the incubation medium also indicated that dissolution of alkaline ions from the Bioglass® particles was counteracting the effect of the acidic

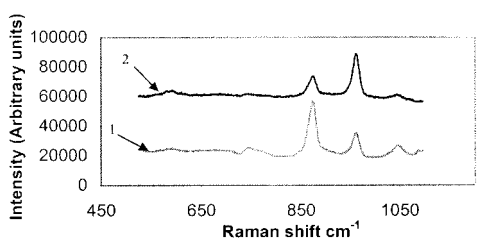

Figure 5. Raman spectra of PDLLA foams filled with 40 wt % Bioglass®, showing characteristic peaks for HA (at a wavelength of 960 cm^{-1})[45] and polymer (at a wavelength of 875 cm^{-1}).[46] 1, after 7 days immersion in SBF; 2, after 28 days immersion in SBF.

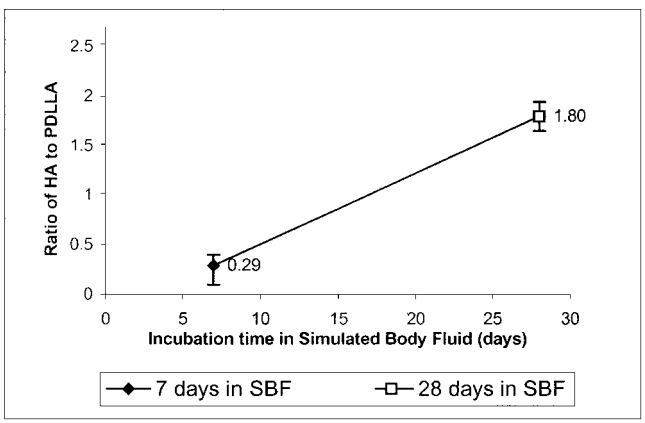

Figure 6. Ratio of HA to PDLLA in PDLLA/Bioglass® composite foams filled with 40 wt % Bioglass®, as a function of immersion time in SBF, determined by the ratio of areas under the peaks in Figure 5.

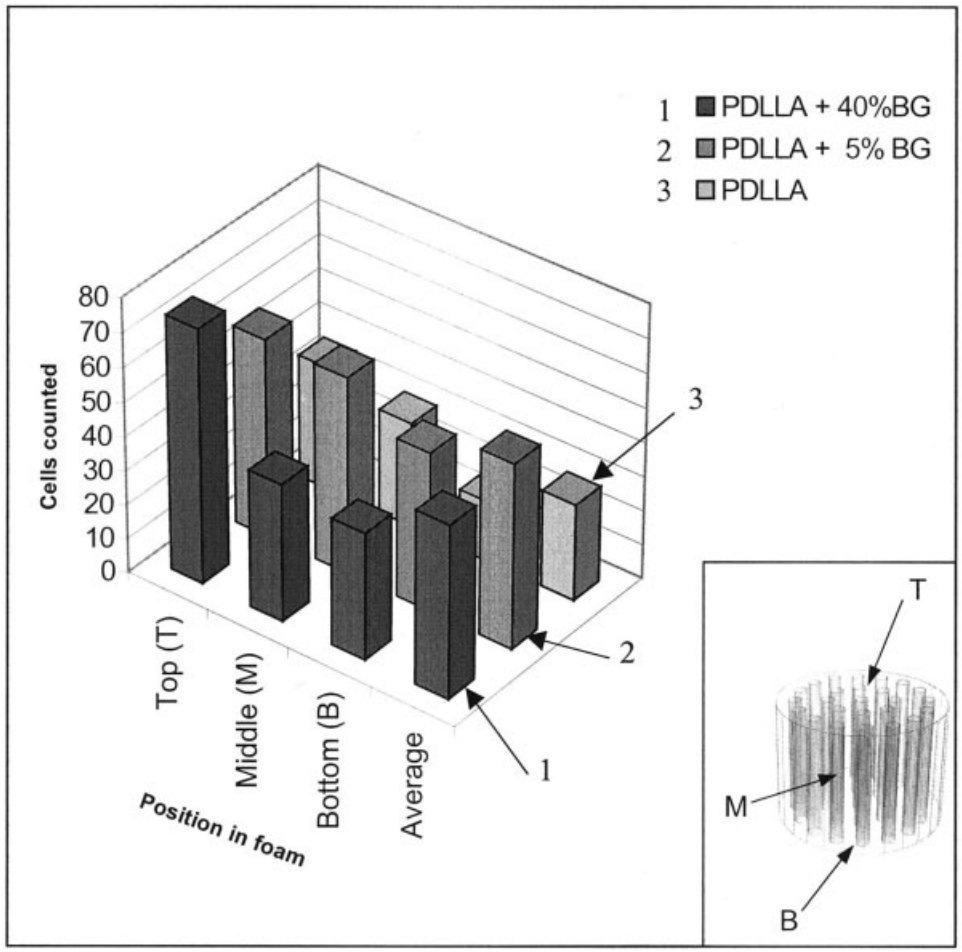

Figure 7. Osteoblast cells counted using confocal microscopy on the inside of the different foams at various positions (as shown in the inset) after 8 days in culture. The maximal relative error of the counts was 20%. (BG, Bioglass®).

products of the polymer degradation.[20] A detailed study of the effect of Bioglass® content on the time-dependant rates of PDLLA degradation and HA formation in composite scaffolds during immersion in SBF is the focus of current research. The matching of the kinetics of polymer degradation and HA formation, which is related to the rate of Bioglass® dissolution, is very important for the application of the composites in bone tissue engineering. This is because one of the advantages of composite scaffolds is that acidic and basic degradation products of polymer and Bioglass®, respectively, can be neutralized and physiological pH can be maintained.[12,13] The further elaboration of Raman spectroscopy results, such as those presented in Figure 6, may prove to be very useful in this context.

Cell culturing

Cell counts were performed using confocal microscopy on axially bisected foams at several positions inside the foam; cells were counted in the upper, middle, and lower sections of the foams after 8 days in culture. The results are shown in Figure 7, which confirm the infiltration and migration of osteoblasts deep into the porous network of the foams. Significantly more cells were evident on the Bioglass®-filled foams compared with the unfilled PDLLA foams, as determined by a one-way ANOVA with Tukey–Kramer post-test.[39] However there was no significant difference between the 5 and 40 wt% Bioglass®-filled PDLLA foams. In all samples, cells were more evident on the highly porous (top) surface of the foams than the deeper interior, possibly because of the cell seeding method used, which involved placing a cell suspension on top of the samples. Current research is focussed on improving the cell culture technique to enhance the spreading of cells within the porous network and obtaining more quantitative cell counts through proliferation assays. The higher cell density in the PDLLA/Bioglass® composites compared with the pure PDLLA foams after 8 days in culture confirms the enhanced bioactivity of these materials. These re-

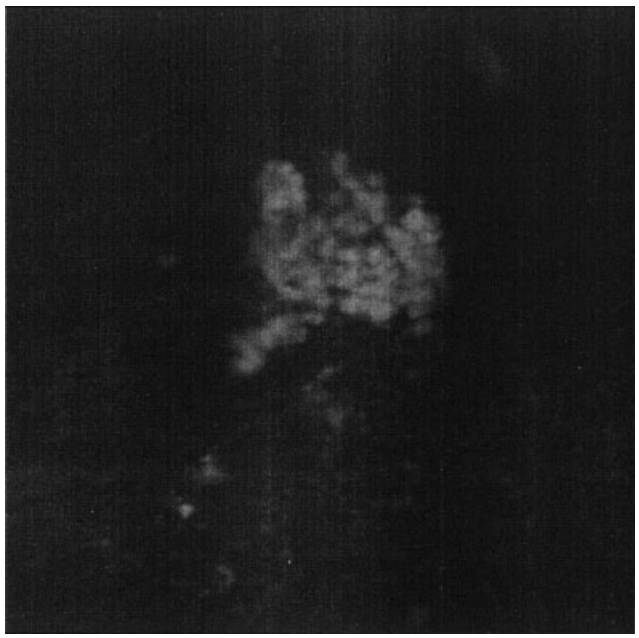

Figure 8. Confocal image of propidium iodide-stained MG-63 cells cultured on PDLLA/5 wt % Bioglass® composite foam after 8 days. The pocket of cells observed is possibly due to proliferation within a pore.

sults are in broad agreement with a previous study, where Bioglass® particles were added as a coating on PDLLA foams.[31] Figure 8 shows a representative confocal image of propidium iodide stained cells cultured on PDLLA/Bioglass® (5 wt % Bioglass®) after 8 days. A pocket of cells was observed, possibly due to proliferation within the pore. Ongoing studies should confirm proliferative activity of these cells and primary human osteoblasts within the porous network.

A qualitative analysis of cell adhesion and proliferation was carried out by SEM observation of samples at different time points of cell culture. Figure 9 shows

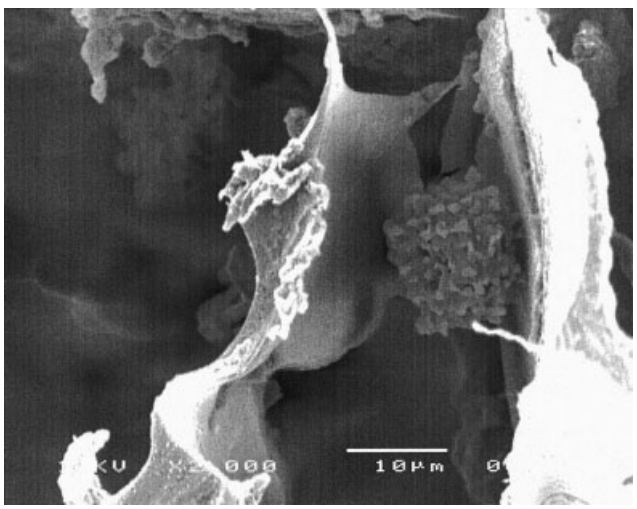

Figure 9. SEM micrograph showing an MG-63 cell adhering to the wall of a PDLLA pore after 90 min in culture.

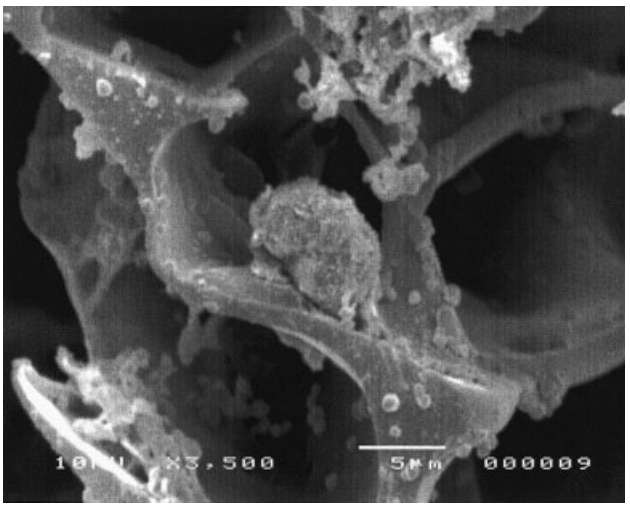

Figure 10. SEM micrograph showing an MG-63 cell adhering to the pore wall of a PDLLA/5% Bioglass® composite foam after 90 min in culture.

an MG-63 cell adhering to the wall of a PDLLA pore after 90 min of culture. The cell is typically rounded after this short culture period and exhibits plasma membrane dorsal ruffles. Similar cellular morphology was observed after 90 min of culture on the PDLLA/5 wt % Bioglass® composite, as shown in Figure 10. After 24 h of culture in the PDLLA/40 wt % Bioglass® composite, cells were observed to have a well-spread and flattened morphology as shown in Figure 11. Cellular processes can be clearly seen attaching to the walls of the pore. Figure 12 shows an MG-63 cell attaching and spreading on the wall of a pore in a PDLLA foam after 5 days of culture. The cell is well spread and flattened in morphology. When cells were restricted within a smaller architecture of pore size and shape, they adopted a more rounded morphology

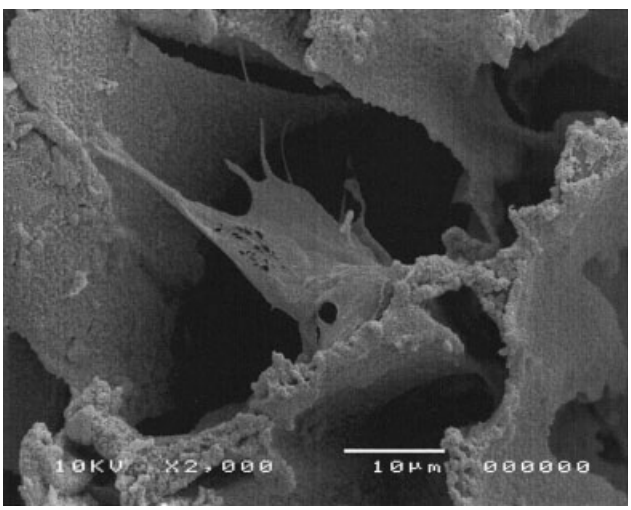

Figure 11. SEM micrograph showing the well-spread and flattened morphology of an MG-63 cell adhering to PDLLA/40 wt % Bioglass® foam after 24 h in culture.

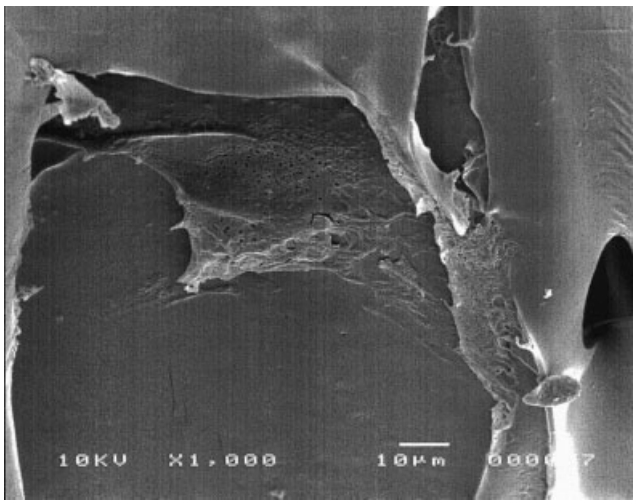

Figure 12. SEM micrograph of an MG-63 attaching and spreading on the wall of a pore in a PDLLA foam after 5 days in culture.

but still possessing cellular projections reaching out to the pore walls, as shown in Figure 13 where a cell cultured for 5 days in a PDLLA/5 wt % Bioglass® composite foam is shown. Because osteoblasts *in vivo* have a more three-dimensional cuboidal morphology rather than a very flattened one, this may be advantageous to the osteoblast phenotype and therefore bone formation. After 8 days of culture, more osteoblasts were observed under scanning electron microscopy and Figure 14 shows cells bridging pores in a PDLLA/40 wt % Bioglass® composite foam. Figure 15 shows a PDLLA/40 wt % Bioglass® composite foam cultured for 11 days where highly fibrous material was observed. It is possible that this is extracellular matrix (ECM) produced by the osteoblasts. MG-63 cells are known to rapidly produce large amounts of

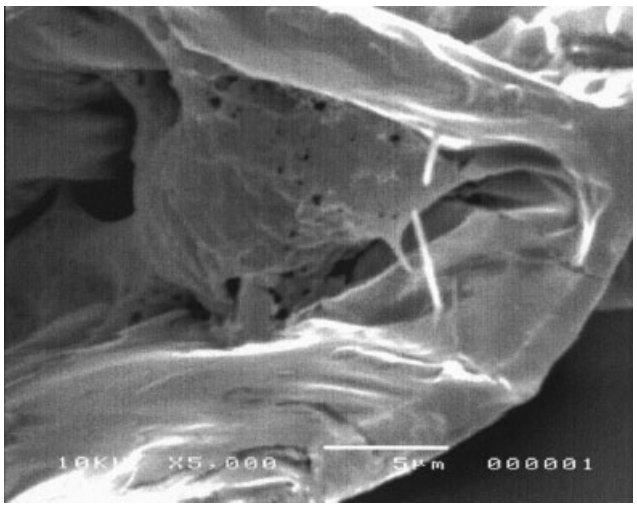

Figure 13. SEM micrograph of an MG-63 cell after 5 days in culture on a PDLLA/5 wt % Bioglass® foam.

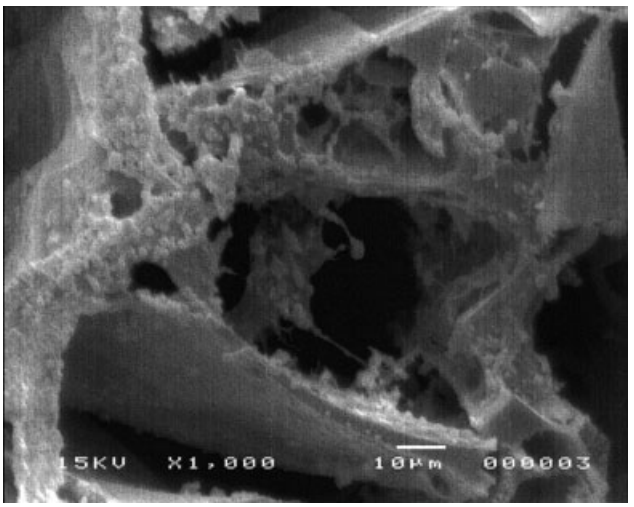

Figure 14. SEM micrograph showing cells bridging pores in a PDLLA/40 wt % Bioglass® foam after 8 days in culture.

ECM.[49] On going studies are directed to determine whether this is so.

CONCLUSIONS

PDLLA foam/Bioglass® composites and unfilled PDLLA foams were characterized, and their potential as bone tissue engineering scaffolds was assessed by means of *in vitro* testing using SBF and cell culture with a commercially available (ECACC) human osteosarcoma cell line (MG-63). SBF tests confirmed the high *in vitro* bioactivity of the foams documented by the ability of HA to form on the foam surfaces. HA crystals appeared to form on the surface of the 40 wt %

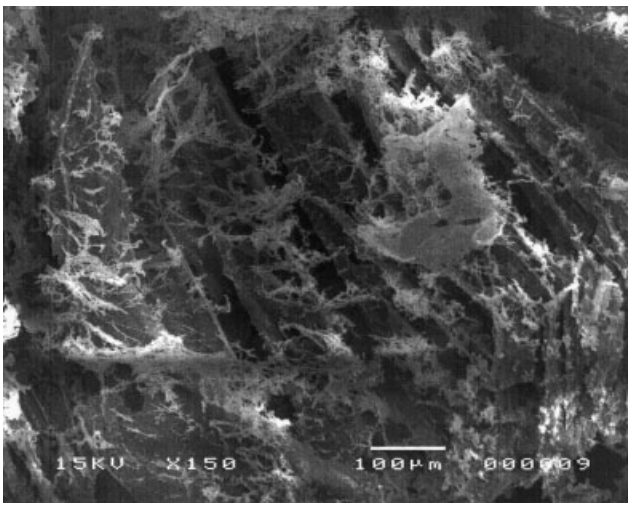

Figure 15. SEM micrograph of a PDLLA/40 wt % Bioglass® foam cultured for 11 days. Fibrous material (possibly extracellular matrix produced by the osteoblasts) can be observed.

Bioglass® composites within 3 days in SBF, and after 28 days' incubation HA had formed continuous layers up to ~7 μm thick. MG-63 cells exhibited an affinity to attach to the foam substrates within the porous interior, indicating the biocompatibility of the scaffolds. The osteoblast cell infiltration study has shown that the cells were able to migrate through the porous network and colonize the deeper regions within the foam, indicating that the composition of the foams and the pore structures are able to support osteoblast attachment, spreading and viability.

Current research is focussed on the *in vivo* evaluation of these scaffolds using a rat model and on characterising the mechanical properties of foams as-received and after immersion in SBF. Future work should encompass the development of gradient porosity scaffolds to produce bioactive and resorbable composites with graded mechanical, resorption, and bioactive properties. Such composites could mimic the properties and morphology of cortical (compact) and cancellous (spongy) bone. Graded porosity could be produced by coating or blocking the pores with a third body, i.e., Bioglass® particles of varying size or by mechanically deforming foams.

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