J Mater Sci (2006) 41:5127–5133
DOI 10.1007/s10853-006-0449-1

Microspheres as building blocks for hydroxyapatite/polylactide biodegradable composites

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Received: 12 December 2005 / Accepted: 28 December 2005 / Published online: 18 July 2006

Abstract A new generation of organic/inorganic composites is offering a promising approach for creating biocompatible and biodegradable materials with mechanical properties that match that of human bone better than traditional metallic implants. Here, we report a novel technique whereby hydroxyapatite powder is encapsulated in polylactide-based microspheres, processed by an emulsion-solvent evaporation method, and then used as the building blocks to produce dense, microstructurally-uniform composites through a hot pressing route. The mechanical properties of these composites—both ab initio and after in vitro degradation in a simulated environment- were subsequently characterized. Although despite in vitro degradation remains an issue, the Young's modulus, bending strength and fracture resistance were higher than the corresponding minimum values for human cortical bone. These results suggest that the hot-pressing of hydroxyapatite/polylactide microspheres can be a viable route for the synthesis of load-bearing bone-replacement materials.

Introduction

The number of orthopedic surgeries performed worldwide is growing steadily due, in part, to the combination of longer average life spans and more active lifestyles. Just in the United States, 152,000 total hip replacement and 299,000 knee replacements were performed in 2000 and 59,000 revisions of hip and knee replacements were performed the same year [1–3], leading to over $50 billion being spent on orthopedic related conditions annually [4–7]. As these procedures are becoming more popular, and patients desire to lead more active lives are common, these rates are expected to increase. These numbers emphasize the need for a coordinated research effort to provide patients and doctors with better and more durable implant materials.

While bone grafts—either allografts or autografts—were traditionally used to correct bone defects and injuries, the paucity of supply of usable tissue led to the increasing reliance on man-made materials. The search for stronger implant compounds has initiated the use of materials developed for other, more traditional engineering applications such as stainless steel, cobalt–chromium or titanium alloys and ceramics such as alumina or zirconia that are not only stronger but also much stiffer than the bone they replace. This difference in stiffness is the genesis of many failed implants; living bone is responsive to its environment and implants that are stiffer than bone bear a greater proportion of the load, shielding the surrounding tissue from its normal stress levels [8] and promoting osteoporosis [9]. The result is that the surrounding tissue is resorbed and the implant becomes loose over time, often requiring revision surgery. Although these materials are biocompatible, unlike natural bone, they cannot be resorbed over time. Hydroxyapatite and calcium phosphates are of particular interest as they chemically resemble apatite, the main inorganic component of bone [10, 11] and are believed to aid bone formation in vivo [12]. Polymers based on lactic and glycolic

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acids are also now in wide use owing to their biodegradability [13], but their low stiffness and in some cases strength, limits their use predominantly to a limited number of non-load bearing applications [14, 15].

Over the last few decades, there has been increasing interest in organic/inorganic composites that would combine the flexibility, toughness and bioresorbability of a polymer with the stiffness, strength and osteoconductivity of a ceramic. One of the most commonly studied hybrid material is hydroxyapatite (HA)/polylactide (PLA) composites. Several studies in archival literature have looked at various aspects of synthesis and characterization of these materials (e.g., [16–22]); however, detailed studies on optimizing the microstructure to attain the required mechanical properties are somewhat lacking. To address this, in a previous study [22], we examined HA/PLA composites prepared using a conventional hot-pressing route[1] with a fine-grained (average particle size ~5 μm) commercial powder and with coarser HA whiskers [23] (~25–30 μm long, ~5 μm diameter). Both microstructures showed similar mechanical properties—sufficient to replace bone ab initio, but progressively degraded in a simulated environment. One problem that was observed was the agglomeration of HA particles, which is expected to be deleterious to the properties. To address this issue, we examined another processing route where HA particles are encapsulated in PLA microspheres that serve as the building blocks for the fabrication of dense, homogeneous composites. While polymer- and polymer-ceramic-based microspheres have been developed as substrates for cell cultures, drug delivery vehicles etc. (e.g., [24–27]), here we use them to build dense composites that could be utilized in load-bearing implants.

Materials and methods

Materials

HA powder obtained from a commercial vendor (Alfa Aesar, Ward Hill, MA) (Fig. 1a) was calcined at 1100 °C for 1 h and sieved to a particle size lower than 35 μm. The ''as-received'' powders from Alfa Aesar consist of ~100 nm crystallites that form porous agglomerates a few microns in size. After the heat treatment process, the agglomerates sinter to form grains with a small amount of residual porosity and a bimodal size distribution. HA was then encapsulated into PLA spheres using an emulsion-solvent evaporation method [25, 28]. Specifically, 2–3 g of PLA (Purasorb PL, Purac America, Lincolnshire, IL; inherent viscosity = 2.39 dL/g) were dissolved in 200 mL of methylene chloride for about 1 h. HA powder (7–8 g) was then added to the polymer solution, with continuous stirring for 15 min with a magnetic stirrer. The HA/PLA solution thus formed was added drop by drop to 600 mL of a 0.5 wt.% polyvinyl alcohol (PVA)—water solution (Fig. 1b), while stirring at ~300 rpm. The stirring was then accelerated to ~600 rpm and maintained for 2 h to allow the HA/PLA microspheres to harden by evaporation of the solvent[2]. Finally, the microspheres were isolated by vacuum filtration, washed, air-dried and sieved to remove any residual PVA. The spheres were then hot pressed in air using a uniaxial press (Carver Inc., Wabash, IN) with a stainless steel die (13 mm in diameter) at 190 °C for 30 min under 90 MPa pressure.

Physical and microstructural characterization

The theoretical density of the compacts was determined using the law of mixtures and compared to measurements performed by the Archimedes' method. The microstructures of the microspheres and the hot pressed compacts (as prepared and after in vitro degradation) were analyzed by X-ray diffraction (D500 diffractometer, Siemens AG, Munich, Germany), optical microscopy (Axiotech microscope, Carl Zeiss AG, Oberkochen, Germany) and by environmental-scanning electron microscopy (ESEM: S-4300SE/N, Hitachi, USA) with associated energy dispersive spectroscopy (EDS). In order to obtain a three-dimensional perspective of the microstructure, i.e., both at the surface and within the bulk, to determine if the samples made were homogeneous, synchrotron X-ray computed tomography was performed at the Advanced Light Source (ALS, Berkeley, CA). Imaging was performed with 16 keV monochromatic x-rays on the microspheres and the composites. The tomography data were reconstructed into three-dimensional images by a Fourier-filtered back-projection algorithm as described in detail elsewhere [29].

[1] The conventional route mentioned here has been detailed elsewhere [22] and involved dissolving PLA in methylene chloride and adding HA powders/whiskers to the solution. The slurry was dried to remove the residual solvent and the dried pellets hot pressed under various conditions (time, temperature and pressure).

[2] A stirring time of 2 h was employed to obtain uniform spheres as it was noticed that with longer times, the microspheres began to disintegrate.

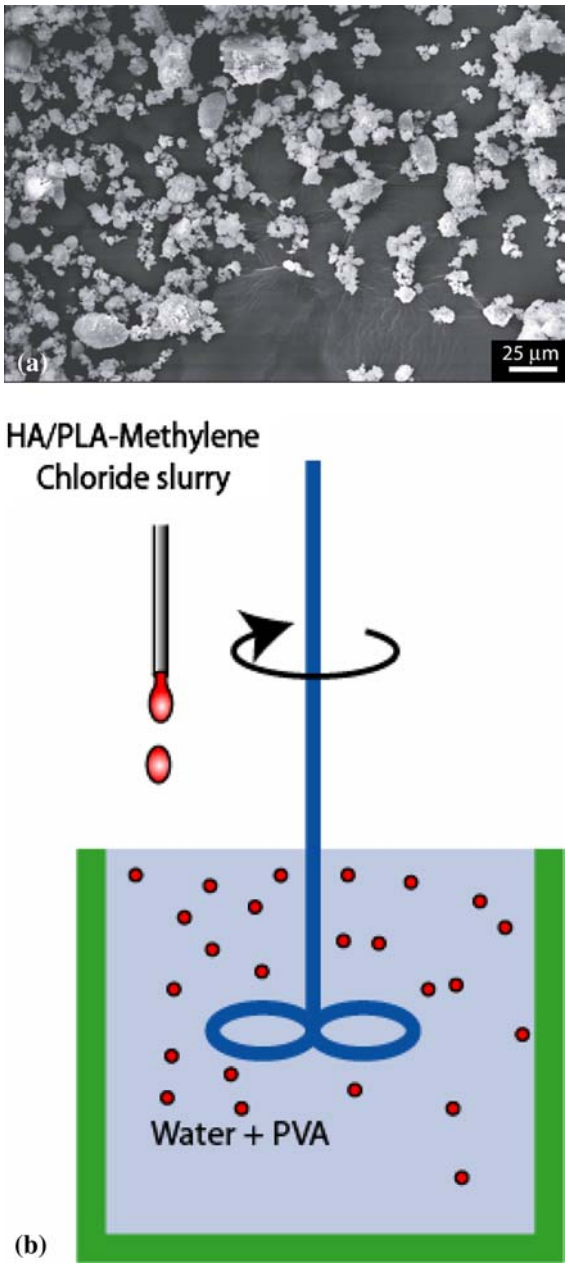

Fig. 1 (**a**) Scanning electron micrograph of the calcined hydroxyapatite powder used in this work. (**b**) Schematic of the emulsion solvent-evaporation method used to prepare the HA/PLA microspheres

Mechanical characterization

Mechanical properties of the composites, specifically Young's modulus, bending strength and fracture toughness, were evaluated both in the as-received condition and following degradation in vitro. The modulus was measured using an instrumented micro-indentation technique with a Vickers indentor attached to the actuator of a mechanical testing machine (ELF® 3200 series, EnduraTEC Inc., Minnetonka, MN). The procedure involved engaging the indenter with the surface of polished specimens while loading at a constant displacement rate of 0.01 mm/sec and then unloading at the same rate. The unloading load-displacement data were used to determine the elastic modulus based on classical indentation theory, the so-called Oliver-Pharr method (reviewed in ref. [30]), simplified by Sneddon [31], and generalized by Pharr et al. [32]). At least three such measurements were made on each composite in four conditions—as received, after storage in a simulated environment, Hanks' Balanced Salt Solution (HBSS) for 1, 10 and 20 days.

Bending strength tests were conducted in the four conditions mentioned above. Unnotched, nominally flaw-free, beams ~1 × 2.5 × 8 mm were sectioned from the composites and loaded to failure at a rate of ~0.01 mm/sec under three-point bending (center-to-end loading span = 5.15 mm) using the ELF® 3200 series voice-coil mechanical testing machine. The load-displacement data thus obtained were analyzed to assess the ultimate bending strength.

Fracture toughness testing was performed in general accordance with ASTM Standard E-399 for Plane-Strain Fracture Toughness [33] under the four conditions mentioned. Beams ~1 × 2.5 × 8 mm were sectioned, and notched to a depth of ~1.25 mm using a slow speed saw and then sharpened with a razor blade. The samples were loaded to failure at a rate of ~0.01 mm/sec under three-point bending using the mechanical testing machine. The load–displacement data obtained were analyzed to assess the fracture resistance which was reported in terms of the strain energy release rate, G.

Results and discussion

Physical and microstructural characterization

Using a solvent-evaporation method, HA-containing microspheres with a diameter ranging between 50 and 150 µm were produced (Fig. 2). PLA concentration in methylene chloride was observed to control the sphere size- an increase in PLA concentration resulted in an increase of the mean microsphere size. Micron-scale porosity was also observed on the surface of the spheres (Fig. 2, inset). The spheres consist of a homogeneous mixture of HA and PLA (Fig. 3a). During the solvent-evaporation process, a small fraction of the starting hydroxyapatite powder that is not embedded into the spheres remains in suspension and does not enter into the final composite. Consequently, the HA

content of the composite is always slightly smaller that the one of the starting suspension. To measure the final hydroxyapatite content in the composites, hot pressed samples have been fired at 600 °C for 2 h in order to burn the PLA and weighted before and after the thermal treatment. The measurements showed that starting from suspension containing 70 wt.% of HA and 30 wt.% of PLA it is possible to fabricate composites with a hydroxyapatite content of 60 wt.%.

Scanning electron micrographs showing the typical microstructures obtained for the composites made by hot pressing the microspheres are shown in Fig. 3b. It can be observed that during hot pressing the spheres soften and densify and that few small hydroxyapatite agglomerates remain. To examine the composites three-dimensionally, X-ray computed tomography was also performed on both the microspheres and the subsequent hot pressed composites; Fig. 4 shows typical results that were obtained. It can be observed from the tomography images that the composites formed were very dense, with a homogeneous microstructure, though there were some instances of HA agglomerations. Indeed, the relative density of the bulk composites as measured by Archimedes' method was greater than 90%, which was comparable to our previous composites obtained by the more conventional hot pressing route [22].

Mechanical characterization and degradation

For the composites made by hot pressing, the microspheres had an average modulus of 12 GPa as measured by instrumented microindentation (Fig. 5), which is higher than the 10 GPa that is considered the lower limit for human cortical bone [34]. One of the important conditions for an ideal bone substitute, in particular those that are to be used for load bearing applications, is to have mechanical properties that match those of the bone tissue that they are intended to substitute for [9]. Matching the elastic modulus, as we have been able to achieve here, is very important since one of the main factors that limit the life of current metallic implants is the mismatch of elastic modulus with the surrounding bone and the resulting stress shielding that ends causing tissue necrosis leading to osteoporosis [9] and implant failure [8]. Furthermore, the moduli measured previously for the conventionally-processed composites (also shown in Fig. 5) with 70 wt.% of HA were some 40% lower than those obtained in this study. This improvement in the modulus may be the result of the more uniform microstructure, with fewer loose HA agglomerates that can lower the overall modulus. As with the previous processing route, degradation in a simulated body

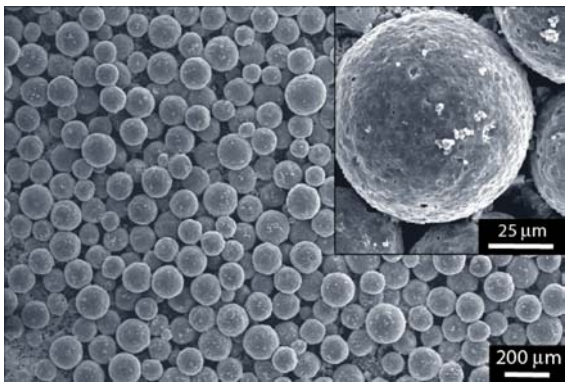

Fig. 2 Scanning electron micrographs of the microspheres obtained by solvent-evaporation. The inset shows a single microsphere at a higher magnification. Note the evidence of some porosity on the surface

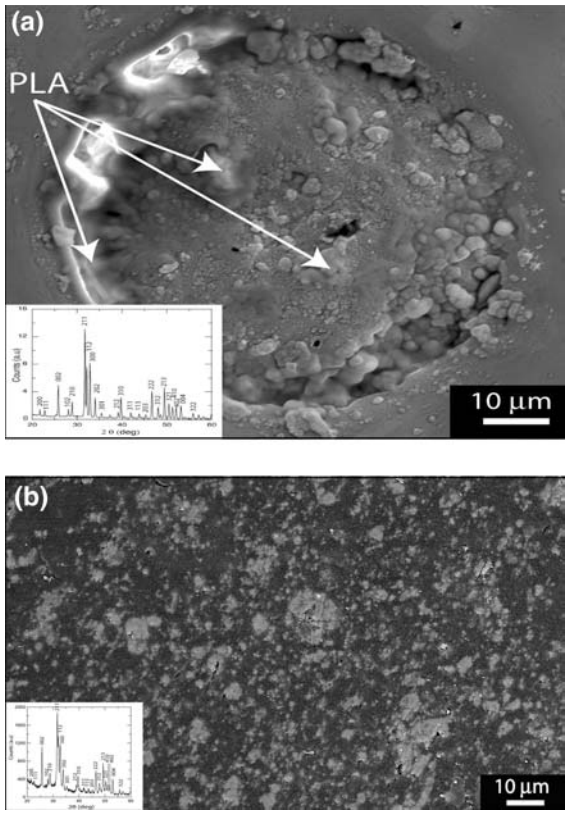

Fig. 3 Scanning electron images and X-ray diffraction patterns of the starting HA/PLA microspheres and the composites obtained after hot pressing at 190 °C for 30 min under 90 MPa pressure. In both cases, the diffraction patterns correspond to pure hydroxyapatite. (**a**) Secondary electron imaging of the cross section of a HA/PLA sphere mounted in epoxy resin and polished. (**b**) Backscatter electron images of a composite polished surface showing a typical microstructure. The hydroxyapatite particles (white phase) are well dispersed in the polymer matrix

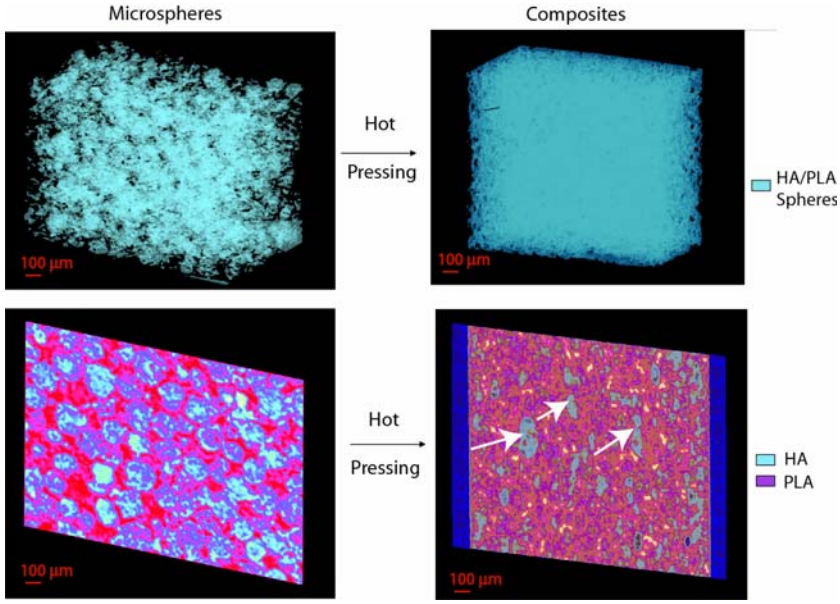

Fig. 4 Three-dimensional reconstructed images (top) and two-dimensional cross-sectional ''slices'' taken at the center of the images (bottom) obtained by synchrotron X-ray computed tomography of the HA/PLA microspheres (left) and of the composite obtained by hot pressing them at 190 °C for 30 min under 90 MPa pressure (right). Note that though overall, the microstructure of the composites was quite homogeneous; there were some instances of HA agglomeration as shown by white arrows in bottom right panel

environment was quite minimal; indeed, the modulus decreased by 4% after 20 days in HBSS, but still remained higher than the minimum needed to match human cortical bone.

The bending strengths and fracture resistance in terms of the strain energy release rates, G, were also measured. The microsphere-based composites generally showed as-received strengths and the strain energy release rates comparable to that of human cortical bone, with initial strengths of 126 MPa and toughness values of 137 J/m^2, as compared to lower-bound values of 35 MPa and 100 J/m^2 for human cortical bone [34]). While the strengths were comparable to those obtained for the conventionally-processed composites (with 70 wt.% of HA), the toughness values were some 30% lower, reflective of the higher elastic modulus of the present materials. However, as with the previous composites, they typically showed some degradation upon exposure to HBSS. Data for the in vitro degradation are included in Fig. 6 and reveal that the strength and toughness appear to approach the values of 70 MPa and 115 J/m^2 over a period of 20 days in HBSS, which are higher than the corresponding lower limits for human cortical bone [34].

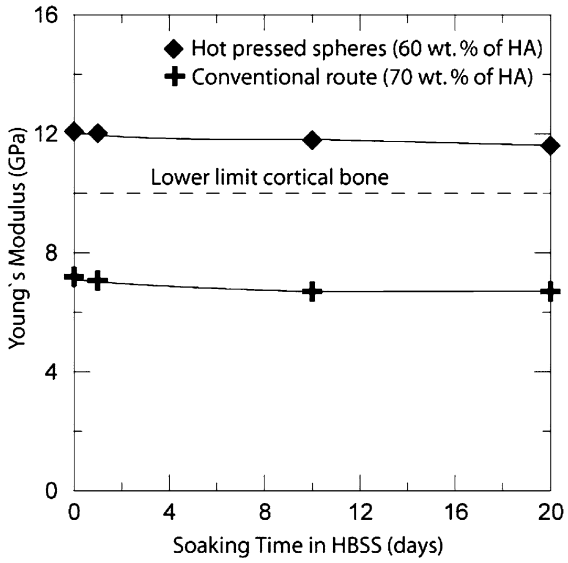

Fig. 5 Evolution of the composite Young's modulus with soaking time in HBSS. For comparison, data previously obtained for a conventionally processed composite with 70 wt. % of HA is also shown [22]

Examination of the fracture surfaces suggests that while the homogeneity of the microstructure is improved as compared to our previous composites [22], it remains susceptible to environmental degradation in HBSS (Fig. 7). The choice of PLA as the polymer phase is dictated by its bioresorbability and the need for the composite to be slowly replaced by bone. Unfortunately, this very property leads to the degradation in strength and the toughness. The modulus, on the other hand, is primarily dictated by the ceramic component and is relatively unaffected by environmental degradation. It is therefore important to design microstructures where the degradation rate matches the rate at which bone is formed at the implant site so that the implant remains mechanically stable during its useful life.

As stated previously, biodegradable polymer- and polymer-ceramic based microspheres are extensively used as drug delivery vehicles. In this study we have used HA/PLA-based microspheres as building blocks

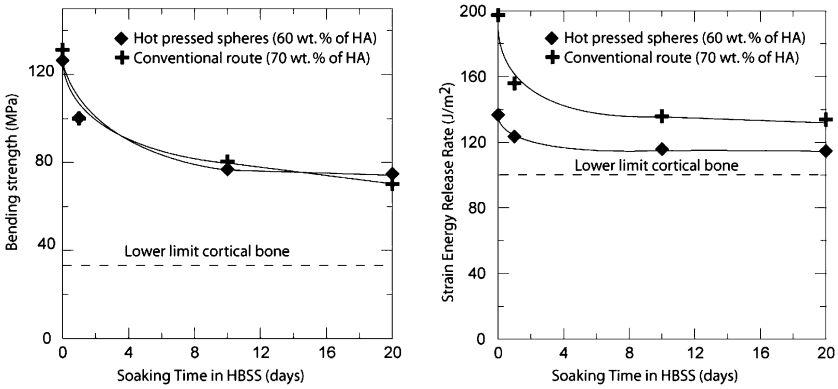

Fig. 6 Evolution of the bending strength and the strain energy release rate after soaking time in HBSS. The properties were observed to degrade with time, but remained higher than the levels needed to match those of cortical bone

for making dense composites. Clearly, these composites show great promise for the development of implant materials that match closely the properties of bone. Future work will concentrate on optimizing the composition and the processing parameters to obtain better in vitro strength and fracture properties, particularly in order to match the degradation behavior of the composites with in vivo resorption kinetics, including examining the effects of using alternative organic and inorganic components. The HA powder used here as a precursor for the microspheres was not fully defect-free and improving its quality would allow for the use of a lower ceramic burden content to achieve the same modulus levels; According to our results the degradation rate can be manipulated by varying the polymer content of the composite. The HA could be also replaced with bioactive glasses along the lines of the work of Qiu et al. [35]; such bioglasses are known to bond better with surrounding bone than traditional bioceramics [36]. The polymer used here, polylactide, could be replaced with an isomer, polyDlactide, that has similar mechanical properties, but shows much better adhesion to HA (Neuendorf et al. unpublished work) and slower hydrolysis [37]. Improved adhesion of the polymer to the ceramic component, together with slower environmental degradation, should help in developing materials with much better resistance in vitro.

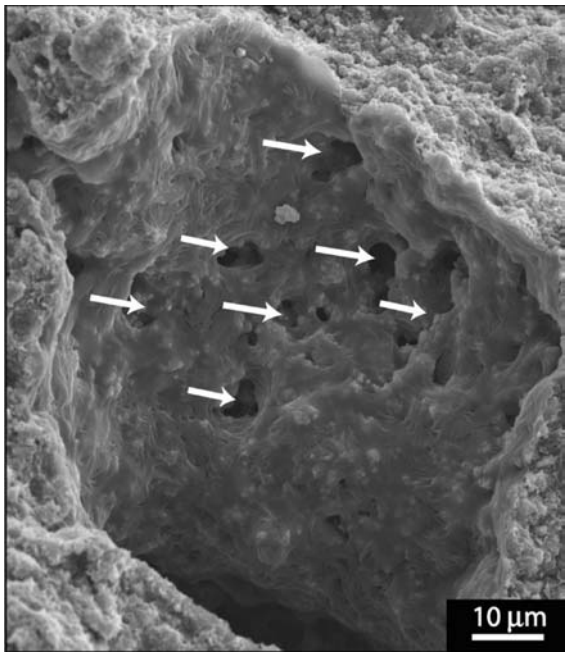

Fig. 7 Scanning electron micrographs of the composite fracture surface showing the degradation of the polymer after 20 days of immersion in HBSS. White arrows indicate zones where the polymer has started to dissolve

Conclusions

Microspheres of hydroxyapatite powder encapsulated in polylactide were synthesized through an emulsion-solvent evaporation method and used as building blocks to produce dense and homogeneous composites through a hot pressing route. The mechanical properties, specifically, the modulus, strength and the fracture toughness, of these materials were comparable to those of human cortical bone. While the elastic modulus was relatively unaffected by in vitro degradation, both the strength and the fracture toughness degrade with immersion in a simulated environment, presumably due to the degradation of the polymer phase. Modifications of the fabrication route that seek to manipulate the microstructure in order to improve the resistance to environmental degradation are being investigated.

Acknowledgements This work was supported by the National Institutes of Health (NIH) under Grant No. 5R01 DE015633. We acknowledge the support of the dedicated tomography beamline (BL 8.3.2) at the Advanced Light Source (ALS), supported by the Department of Energy under Contract No. DE-AC03-76SF00098. The authors also wish to thank Drs. Alastair MacDowell, John H. Kinney and Robert O. Ritchie for many helpful discussions.

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