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POLYMER-BIOACTIVE GLASS COMPOSITE SCAFFOLD FOR BONE TISSUE ENGINEERING: MATRIX DESIGN AND *IN VITRO* EVALUATIONS

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INTRODUCTION

Alternative bone grafts are needed due to limitations associated with autografts, currently the clinically preferred grafting material for osseous repair and replacement. Biodegradable polymers such as polylactide (PLA), polyglycolide (PGA) and their co-polymers (PLAGA) have been used widely in orthopaedics as suture materials and fixation devices. Bone bioactive materials such as 45S5 bioactive glass (BG) have been shown to stimulate the formation of new bone, while developing a continuous interface with bone through a surface calcium phosphate (Ca-P) layer.(1,2)

Our approach is to develop a polymer-ceramic composite that is biocompatible, biodegradable, able to support bony growth (osteoconductive) and is also bone bioactive (able to form a calcium phosphate layer on its surface). This composite will build on the strength of the parent phases, while reducing known limitations associated with each material.

The objective of this study is to examine the response of human osteoblast-like cells to the PLAGA-BG composite, and to evaluate the ability of the composite to form a surface Ca-P layer. We hypothesize that the constituting BG phase will promote the formation of Ca-P deposits, and the PLAGA-BG composite will enhance osteoblastic growth and function.

MATERIALS AND METHODS

Synthesis of polymer-ceramic composite

PLAGA-BG composites were fabricated in both disc and microsphere forms. Composite discs were formed via a standard solvent-casting technique, while the microspheres were synthesized by modifying a method used by Laurencin et al.(3) Here, PLAGA 50:50 granules were first dissolved in methylene chloride, and BG granules (<40μm) were added to achieve a 25% mixture. The mixture was then poured into a 1% polyvinyl alcohol solution. The suspension was stirred and the spheres allowed to harden. The 3-D construct (0.5x1.0 cm) was made by heating the microspheres at 70°C for 20 hours in a stainless steel mold.

Characterization of polymer-ceramic composite

Porosity- Mercury porosimetry (Micromeritics Autopore III)
Bioactivity- Scanning electron microscopy (SEM) and energy dispersive x-ray analysis (EDXA) were used to monitor the formation of a Ca-P layer on composite films immersed in a simulated body fluid (SBF) with ion concentration similar to that of blood plasma.
Mechanical properties- The composite was tested under compression using the Instron Servohydrolic System 8500, at the ramp speed of 0.020 cm/sec, n=6.

In vitro evaluation of the composite

Human osteosarcoma cells (Saos-2) were cultured in M199 with 10% fetal bovine serum, L-glutamine, and 1% antibiotics at 37°C and 5% CO_2. Saos-2 cells were seeded on the composite at the density of 50,000 cells/cm2, and were cultured for up to 3 weeks. Two weeks after culture, 3mM β-glycerophosphate and ascorbic acid were added to the medium. PLAGA 50:50 and tissue culture plastic (TCPS) served as control groups. Type I collagen synthesis was quantified using a modified ELISA developed in our laboratory. Cell morphology and the formation of mineralized nodules were examined by SEM-EDXA. Mineralization was further ascertained through Alizarin red staining for calcium.

RESULTS

Characterization of polymer-ceramic composite

The PLAGA-BG composite measured an average porosity of 43%, a median pore diameter of 89 μm while the PLAGA control had 31% porosity and 116 μm median pore diameter, n=3.

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The PLAGA-BG composite measured a higher elastic modulus (51.336±6.080 vs. 26.479±3.468 MPa), while exhibiting a lower compressive strength than the PLAGA control (0.417±0.054 vs. 0.533±0.068 MPa), n=6.

Formation of a surface calcium phosphate layer

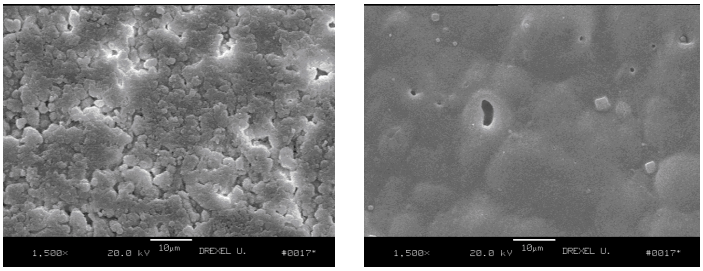

Figure 1. Polymer-ceramic composite (left, x1,500) formed a surface Ca-P layer after 14 days of immersion in a simulated body fluid (Ca, P presence confirmed by EDXA) . No such layer was found on PLAGA surface without 45S5 bioactive glass.(right, x1,500)

***In vitro* evaluation of the composite**

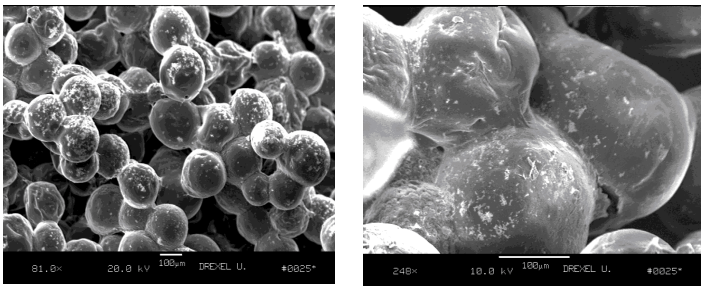

Figure 2. Saos-2 cells cultured on porous PLAGA-BG composite for 3 weeks.(left=80x, right=248x) The spheres were covered with a cellular layer; note the formation of surface Ca-P nodules, unobserved on PLAGA controls.

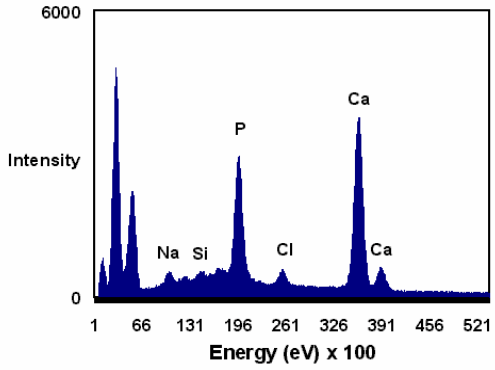

Figure 3. EDXA spectra of polymer-ceramic composite cultured with Saos-2 cells for 7 days. The presence of Ca and P peaks indicate the formation of calcium phosphate minerals in the presence of cells.

The constructs also stained positive for alkaline phosphatase. The formation of a mineralized matrix was confirmed by positive staining for Ca using an Alizarin red assay. Stain intensity in both assays increased with culturing time. After 2 weeks, the synthesis of type I collagen was found to be the highest on PLAGA-BG composite (0.146±0.006 mg), as compared to PLAGA (0.132±0.006 mg) and TCPS (0.073±0.005 mg) controls.

DISCUSSION

The ideal bone graft should be biodegradable, porous, possessing mechanical properties matching that of human bone and able to stimulate new bone formation. We have developed a microsphere-based, porous, PLAGA-BG composite, and have demonstrated here its ability to support the growth and mineralization of human osteoblast-like cells.

The composite exhibited a higher elastic modulus, but measured a lower compressive strength than the PLAGA control. The disparity in compressive strength can be attributed to the higher porosity of the composite structure. Moreover, the addition of BG particles to PLAGA has resulted in a particle-reinforced and harder structure, as reflected in its higher elastic modulus. Mechanical properties of the structure can be further modified by controlling the ratio of the two phases, microsphere size, and the sintering process. Recently, Borden reported a compressive modulus of 297 MPa for PLAGA 85/15 microspheres sintered for 4 hours.(4)

The PLAGA-BG composite was also found to form an amorphous surface calcium phosphate layer in a simulated body fluid as well as in the presence of osteoblast-like cells. SEM and quantitative EDXA revealed the formation of calcium phosphate nodules on the composite surface after only 3 days of culture. In time, the Ca-P nodules increased in size and formed larger aggregates. The Ca/P ratio of the deposits decreased as a function of culturing time. These results collectively suggest that by forming a calcium phosphate layer *in vitro*, the composite is potentially bioactive *in vivo*.

CONCLUSIONS

We have successfully fabricated and characterized a three-dimensional, porous polymer-ceramic composite, applicable as a scaffold for bone tissue engineering. This microsphere-based, 3-D scaffold demonstrates significant potential as a bone replacement material. It was found to support the growth, differentiation and mineralization of human osteoblast-like cells *in vitro*.

This 3-D construct was found to be bioactive, as it formed surface calcium phosphate deposits in the presence of cells and serum proteins *in vitro*. The formation of this Ca-P layer may promote its integration with bone tissue *in vivo*. Future work will focus on evaluation of the 3-D composite in an *in vivo* model.

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