Three-dimensional, bioactive, biodegradable, polymer–bioactive glass composite scaffolds with improved mechanical properties support collagen synthesis and mineralization of human osteoblast-like cells *in vitro*

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Abstract: In the past decade, tissue engineering-based bone grafting has emerged as a viable alternative to biological and synthetic grafts. The biomaterial component is a critical determinant of the ultimate success of the tissue-engineered graft. Because no single existing material possesses all the necessary properties required in an ideal bone graft, our approach has been to develop a three dimensional (3-D), porous composite of polylactide-*co*-glycolide (PLAGA) and 45S5 bioactive glass (BG) that is biodegradable, bioactive, and suitable as a scaffold for bone tissue engineering (PLAGA-BG composite). The objectives of this study were to examine the mechanical properties of a PLAGA-BG matrix, to evaluate the response of human osteoblast-like cells to the PLAGA-BG composite, and to evaluate the ability of the composite to form a surface calcium phosphate layer *in vitro*. Structural and mechanical properties of PLAGA-BG were measured, and the formation of a surface calcium phosphate layer was evaluated by surface analysis methods. The growth and differentiation of human osteoblast-like cells on PLAGA-BG were also examined. A hypothesis was that the combination of PLAGA with BG would result in a biocompatible and bioactive composite, capable of supporting osteoblast adhesion, growth and differentiation, with mechanical properties superior to PLAGA alone. The addition of bioactive glass granules to the PLAGA matrix resulted in a structure with higher compressive modulus than PLAGA alone. Moreover, the PLAGA-BA composite was found to be a bioactive material, as it formed surface calcium phosphate deposits in a simulated body fluid (SBF), and in the presence of cells and serum proteins. The composite supported osteoblast-like morphology, stained positively for alkaline phosphatase, and supported higher levels of Type I collagen synthesis than tissue culture polystyrene controls. We have successfully developed a degradable, porous, polymer bioactive glass composite possessing improved mechanical properties and osteointegrative potential compared to degradable polymers of poly(lactic acid-glycolic acid) alone. Future work will focus on the optimization of the composite scaffold for bone tissue-engineering applications and the evaluation of the 3-D composite in an *in vivo* model. © 2003 Wiley Periodicals, Inc. J Biomed Mater Res 64A: 465–474, 2003

Key words: polymer; bioactive glass; composite; bone; tissue engineering; osteointegration; bioactivity; biodegradable

INTRODUCTION

Bone is the most commonly replaced organ of the body, with over 500,000 bone repair procedures performed per year in the United States alone.[1] Currently, both biological and synthetic grafts have been used for bone repair. Biological grafts include autografts, allografts, and xenografts. Allografts consist of bone tissue donated by another individual or procured from cadavers, and xenografts are bone tissue obtained from other species. The wider application of allografts and xenografts is limited because of disadvantages such as histoincompatibility and the possible transfer of infectious diseases. The autograft, often taken from the iliac crest of the patient, has a success rate of 80–90%, with minimal risks of immune infection, rejection, or disease transfer.[2] Consequently, it is the clinically preferred grafting material for bone repair and regeneration. However, autografts are limited in supply, restricted by anatomical incompatibilities, and are often associated with donor site morbidity.

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The aforementioned disadvantages of autografts have prompted significant interest in the development of alternative bone grafts. Ideally, a bone graft should be biocompatible, able to support abundant bone formation (osteoconductive), able to induce bone formation (osteoinductive), able to form a continuous interface with surrounding bone tissue (osteointegrative), able to support angiogenesis, and able to be structurally and mechanically compatible with bone tissue. In the past decade, tissue engineering-based bone grafting has emerged as a viable alternative to biological and synthetic grafts, presenting a possible solution to problems currently associated with autografts. Tissue engineering can be defined as the application of biological, chemical, and engineering principles toward the repair, restoration, or regeneration of living tissues using biomaterials, cells, and factors alone or in combination. Tissue-engineered bone grafts are attractive because they can be designed to possess the many advantages of autogenous grafts, without associated limitations. First, because they initially are populated with a patient's own cells and composed of biocompatible materials, biological rejection of tissue-engineered grafts may be obviated. Moreover, with the addition of appropriate growth factors, the grafts can be engineered to be osteoinductive and support the initiation of bone formation. Lastly, by using a biomaterial substrate whose physical and structural properties mimic those of biological bone, an osteoconductive and osteointegrative construct can be engineered.

To date, a variety of three-dimensional, porous scaffolds based primarily on either biodegradable polymers or glass and glass ceramics have been investigated as bone tissue engineering grafts, with varying degrees of success.[3–8] Degradable poly (α-hydroxy) esters are Food and Drug Administration-approved polymers for clinical applications, available as either polylactide (PLA), polyglycolide (PLG), or various molar ratios of their co-polymers (PLAGA), and these materials have been used as surgical sutures and orthopedic fixation devices with extended success.[9–17] The poly (α-hydroxy) esters are highly versatile materials because polymer bulk and surface, physical, and chemical properties can be readily manipulated during both the synthesis and processing stages.

Similarly, glass and glass ceramic-based matrices have been used in a variety of medical applications; as dental implants, bone fixation devices, and implant coatings.[18–21] Bioactive glasses and hydroxyapatite are well suited for osseous repair because these materials are bioactive, that is, they actively interact with the biological environment and can chemically integrate with surrounding bone tissue *in vivo*. In this case, osteointegration is achieved by forming a surface calcium phosphate (Ca-P) layer, which is later modified by bone cells, and it is through this layer that the implant is chemically fixed to surrounding bone tissue.[5,18,19] 45S5 bioactive glass is the most bone-bioactive material known to date.[19] In addition to being osteointegrative, the biocompatibility, osteoconductive, and osteoinductive nature of 45S5 bioactive glass have been well documented.[18,19,21–26] However, despite its osteoconductive potential and superior ability to bond to bone, the direct application of bioactive glass in load-bearing situations has been limited. Although existing bioactive materials possess high compressive strength, they are unfortunately very brittle and have inherently poor tensile and torsional properties.[18,27,28]

The appropriate selection of the biomaterial component of the tissue-engineered scaffold is a critical step in determining the ultimate success of the engineered graft. Scaffold surface chemistry and physical properties will direct biological response such as cell adhesion and differentiation. Material selection is especially important in bone tissue engineering because a supporting substrate is critical for maintaining mechanical strength, structural support, and providing the optimal culturing environment for bone formation during the early stages of the regenerative process.

Because no single existing material possesses all the necessary properties required in an ideal bone graft, there is a growing interest in composite materials. Composites are formed to improve the properties of existing materials, resulting in a superior material for the intended application. The promise of combined advantages of the composite phases, as well as the inherent ease in optimization where desired material properties can be accentuated in a well-controlled manner, have made composite materials attractive for biomedical applications. From a biomimetic standpoint, various types of biological tissue, including bone, are composites in nature. Bone tissue can be viewed as a composite with organic and inorganic phases, made up of bone-forming cells, bone-resorption cells, extracellular matrix, and inorganic bone mineral.

Our approach was to develop a biodegradable, bioactive, porous composite of polylactide-*co*-glycolide (PLAGA) and 45S5 bioactive glass (BG), which integrates the advantages of the parent phases while minimizing known limitations associated with each phase. A biodegradable scaffold is attractive because it may be eventually replaced by host tissue while at the same time providing the initial structural strength and integrity necessary for regeneration of critical size bone defects. 45S5 bioactive glass granules are combined with the polymeric matrix to produce a bioactive and biodegradable composite substrate. The addition of bioactive glass granules can serve to reinforce and stiffen the polymeric matrix. Moreover, the degradation kinetics of the polymer and polymerization

shrinkage can be modulated by the concentration of the bioactive glass particulate. Through hydrolysis reactions, PLAGA degrades into glycolic and lactic acid, the release of which can cause a biologically significant decrease in local pH. Through dissolution reactions, BG releases alkaline ions, which produce an elevated local pH. By forming a composite of PLAGA and BG, the acidic and basic degradation products may be neutralized, and potentially, a physiological pH can be maintained in the growth environment.

We report here the development and *in vitro* characterization of a composite scaffold (PLAGA-BG) based on biodegradable polylactide-*co*-glycolide (PLAGA) and 45S5 bioactive glass (BG), with intended application as a bone tissue engineering scaffold. The objective of this study was 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 calcium phosphate layer *in vitro*. The underlying hypothesis is that the combination of PLAGA with BG will result in a biocompatible and bioactive composite, capable of supporting osteoblast adhesion, growth and differentiation.

MATERIALS AND METHODS

Fabrication of polymer–ceramic composite

Polymer–ceramic composite discs

Polylactide-*co*-glycolide 50:50 co-polymer (PLAGA, Mw ~ 50,000, American Cyanamide, Sunnyvale, CA) and 45S5 bioactive glass (BG, MO-SCI Corporation, Rolla, MO) granules were used to fabricate the composite (PLAGA-BG) discs and microspheres. Figure 1 is a schematic of the synthesis process of the two forms of PLAGA-BG composite used in this study. Specifically, PLAGA-BG discs were formed through the traditional solvent-casting process, where PLAGA and BG granules were first mixed according to a polymer to ceramic weight ratio of 1:3 and dissolved in methylene chloride. The solution was then slowly poured into a Teflon® mold and allowed to cool overnight in a −20°C freezer. The resultant polymer-ceramic film was bored into 1-cm wide and 0.1-mm thick discs. The discs were then dried overnight to remove any residual solvent (Lyph-lock 12, Labconco, Kansas City, KS).

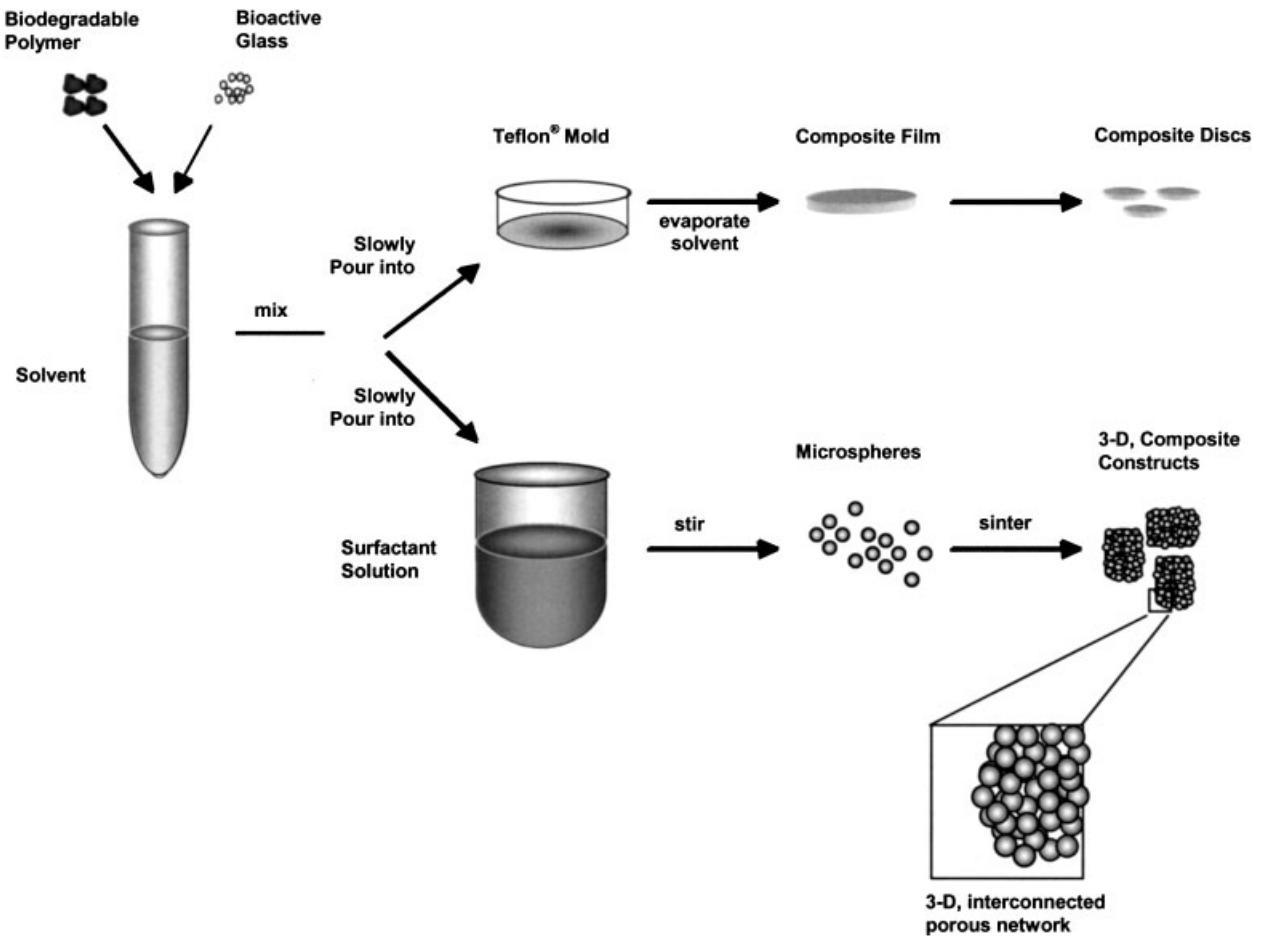


Figure 1. A schematic depicting the fabrication process of a composite (PLAGA-BG) of PLAGA and BG. The composite was prepared in thin film form, and as a 3-D, porous scaffold.

Three dimensional (3-D), microsphere-based, polymer-ceramic composite scaffolds

PLAGA-BG composite microspheres were formed through a water-oil-water emulsion, modifying a method developed by Laurencin et al.[4] Specifically, PLAGA granules were first dissolved in methylene chloride, and BG particles (<40 μm) were added to achieve a 25% mixture. The mixture was then poured into a 1% polyvinyl alcohol (Polysciences, Warrington, PA) solution. The suspension was stirred constantly, and the spheres were allowed to harden in the polyvinyl alcohol solution. The resultant microspheres were then washed, vacuum filtered, and dried at room temperature. Next, the composite microspheres were sifted using a mechanical sifter to a final size range of 100–200 μm. The cylindrical construct, averaging 0.5 cm in width and 1.0 cm in height, was fabricated by heating the microspheres at 70°C for 20 h in a stainless-steel mold.

Characterization of polymer-ceramic composite

Before *in vitro* evaluations, the morphology, porosity and mechanical properties of the PLAGA-BG construct were determined. Pore interconnectivity, morphology, and the bonding of microspheres within the construct was examined by scanning electron microscopy (SEM, Amray 1830-D4), at an acceleration voltage of 20 keV. Elemental composition of the composite surface was determined by energy-dispersive X-ray analysis (EDXA). Mercury porosimetry (Micromeritics Autopore III, Micromeritics, Norcross, GA) was used to measure the porosity, average pore diameter, and total surface area of the composite construct. In this method, the construct porosity was determined by measuring the volume of mercury infused into the structure during analysis. In addition, the construct ($n = 6$) was tested under compression using the Instron Servohydrolic System 8500 (Instron, Canton, MA), with a ramp speed of 0.02 cm/s. The compressive strength and elastic modulus of the construct were determined. PLAGA scaffolds without BG served as controls.

In vitro bioactivity of the polymer-ceramic composite

The composite discs were immersed for 1, 7, and 14 days in a simulated body fluid (SBF) whose ion concentration is similar to that of extracellular fluid.[36] PLAGA discs without BG served as controls. A surface area to volume ratio of 1.0 cm^{-1} was maintained for all immersions. The pH of the solution as a function of immersion time was measured. Perfect sink conditions were maintained during the immersion study. SEM (Amray 1830-D4) and EDXA were used to monitor the formation of a Ca-P layer on composite films.

In vitro evaluation of the polymer-ceramic composite

Cell and cell-culturing conditions

Human osteosarcoma cells (SaOS-2), a gift of Dr. Elliot Levine of Wistar Institute, were cultured in Medium 199 (M199, Sigma Chemicals, St. Louis, MO), supplemented with 10% fetal bovine serum (Life Technologies, Rockville, MD), L-glutamine, and antibiotics. The cells were grown to confluence at 37°C and 5% CO_2. Under these conditions, the osteoblastic phenotype of SaOS-2 was maintained for up to at least four weeks of culture, with positive expression of alkaline phosphatase, type I collagen, osteocalcin, and formation of mineralized cultures.

Culture of SaOS-2 cells on PLAGA-BG composites

SaOS-2 cells were seeded on the porous, PLAGA-BG scaffolds ($n = 3$) at the density of 5×10^4 cells/cm^2, and were cultured in 12-well plates (Fisher Scientific, Fair Lawns, NJ) for up to 3 weeks. PLAGA alone and tissue culture polystyrene (TCPS) served as control groups. Once the cells have grown to confluence, at two weeks from the start of culture, mineralization medium containing 3.0 m*M* of β-glycerophosphate and 10 μg/ml of L-ascorbic acid were added to the culture.

Growth and differentiation of SaOS-2 cells on PLAGA-BG composites

Cell adhesion and growth morphology on the 3-D construct were monitored using SEM (20 keV). Alkaline phosphatase staining was performed at each culturing time point, using a standard histochemical assay. The samples were incubated for 30 min with Napthol AS-Bi (Sigma), phosphate salt, *N,N*-dimethyl formamide (Sigma), and Fast Red (Sigma) at 37°C. The samples were then fixed in 2% paraformaldehyde for 30 min at 4°C. The synthesis of type I collagen by SaOS-2 cells was quantified using a modified ELISA developed by El-Amin et al.

The formation of mineralized nodules was examined by SEM-EDXA. Mineralization was further ascertained using Alizarin Red S staining for calcium. Briefly, the samples were washed with deionized H_2O, fixed with 2% paraformaldehyde and incubated in 2% Alizarin Red S solution for 5 min. The samples were then washed with deionized water and viewed under the microscope.

Statistical analysis

Data in the graphs are presented in the form of mean ± standard deviation (mean ± SD), with n equal to the number of samples analyzed per immersion treatment. One-way analyses of variance (ANOVA) and the Student's t-test were used to compare the mechanical testing data ($n = 6$), porosimetry results ($n = 3$), as well as the collagen synthesis data ($n = 3$). Statistical significance was evaluated at the $p < 0.05$.

RESULTS

Characterization of polymer-ceramic composite

SEM examination of the PLAGA-BG discs revealed a homogenous distribution of the BG particles within

TABLE I
Summary of Structural and Mechanical Properties of the PLAGA-BG Composite as Compared to the PLAGA Control

Scaffold Type	Average Porosity	Pore Diameter	Elastic Modulus (MPa)	Compressive Strength (MPa)
PLAGA	31%	116 μm	26.48 ± 3.47	0.53 ± 0.07
PLAGA-BG	43%	89 μm	51.34 ± 6.08	0.42 ± 0.05

BG particle-reinforcement of the PLAGA structure resulted in a near two-fold increase in compressive modulus. The structural and mechanical properties of the scaffold can be systematically optimized by varying microsphere and scaffold fabrication parameters.

the PLAGA phase. In addition, the composites in disc form as well as microsphere form were visually more opaque than PLAGA alone, largely because of the addition of BG. Sintering of the microspheres resulted in a well-integrated structure, with the microspheres joined at the contact necks. SEM analysis revealed that a 3-D, interconnected porous network was found throughout the composite construct. Elemental analysis using EDXA showed that the composite surface was largely made up of C, Na, Si, Ca, and P before any immersions.

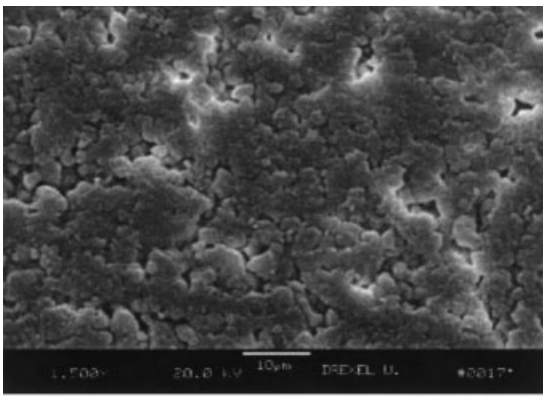
Table I summarizes the result from structural characterizations of the as-fabricated composite scaffold. Porosimetry analysis revealed that the 3-D composite measured an average porosity of 43%, with a mean pore diameter of 89 μm. The PLAGA control scaffold exhibited 31% total porosity and a mean pore diameter of 116 μm. The PLAGA-BG composite possessed a higher elastic modulus (51.336 ± 6.080 MPa vs 26.479 ± 3.468 MPa) than the control PLAGA scaffold. Although the means were different, the compressive strength of the composite at 0.417 ± 0.054 MPa was not statistically different from that of the PLAGA control (0.533 ± 0.068 MPa), at $p < 0.05$.

In vitro bioactivity of the polymer-ceramic composite

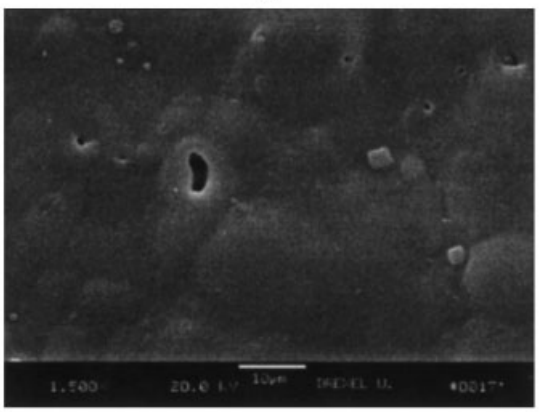
The bioactivity of the composite was determined by monitoring the formation of a calcium phosphate layer on the composite discs in a SBF. The composite was found to be bioactive because it formed a calcium phosphate layer on its surface after immersion in SBF for 7 days. SEM-EDXA results showed that an amorphous calcium-phosphate layer was found on the composite surface after 7 days of immersion, whereas no such layer was detected on the control polymer without bioactive glass particles for the same duration. As shown in Figure 2(a), the composite surface was covered with calcium phosphate nodules after 14 days of immersion, while the PLAGA control surface began to exhibit surface pores formed due to the degradation of the polymer [Fig. 2(b)]. As seen in Figure 3, the composite surface still contained C, Si, Ca, and P, whereas the Cl peak was detected after immersion in SBF.

Growth and mineralization of SaOS-2 cells on PLAGA-BG composites

The microsphere-based, porous, PLAGA-BG composite supported the growth and phenotypic expres-



(a)



(b)

Figure 2. Polymer-ceramic composite (PLAGA-BG) immersed in simulated body fluid (SBF) for 14 days formed a surface calcium phosphate layer (Ca, P presence confirmed by X-ray analysis in Fig. 3). No such layer was found on the PLAGA control without 45S5 bioactive glass. (a) PLAGA-BG surface after immersion for 14 days in SBF. The composite formed a surface calcium phosphate layer. (b) PLAGA control surface after immersion for 14 days in SBF did not form a calcium phosphate layer. The pores observed on the polymer surface were a result of polymer degradation.

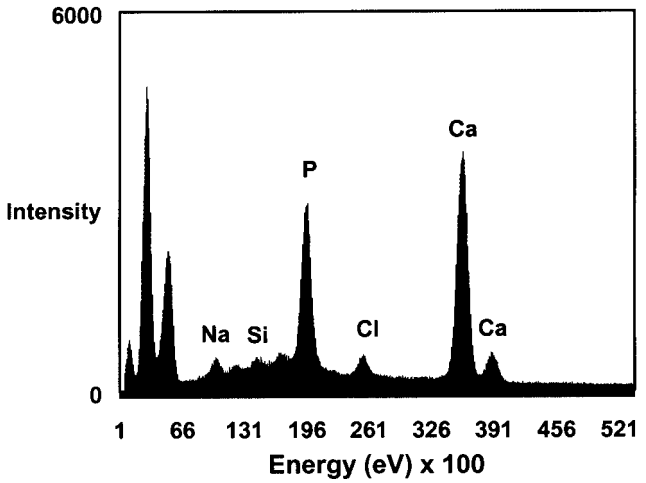


Figure 3. EDXA spectra of the PLAGA-BG composite immersed in a SBF for 14 days. Note the presence of Ca, P, and Cl on the surface, and the Cl peak appeared after immersion in solution. A surface calcium phosphate layer has formed on the PLAGA-BG composite surface, as seen in Fig. 2(a). The Ca and P peaks were not found in the spectra of PLAGA controls.

sion of human osteoblast-like cells. Media pH variation was measured for the full duration (3 weeks) of cell culture with PLAGA-BG and PLAGA, and physiological pH (7.3–7.7) was maintained in all cultures for up to 3 weeks. There was no significant change in solution pH after 2 weeks of culture with osteoblast-like cells, and culture media was exchanged every other day to remove metabolic products and supply fresh nutrients to the cells. As seen in Figure 4, extensive cellular growth was detected on the scaffold surface as well as within the PLAGA-BG composite. In many areas, cellular growth had bridged two or more microspheres while maintaining the porous structure. SEM analysis revealed the synthesis of collagen-like fibers by the SaOS-2 cells.

All cultures stained positively for the synthesis of alkaline phosphatase, although a much higher intensity of stain was observed in cultures with the PLAGA-BG scaffold than for PLAGA cultures. As shown in Figure 5, the synthesis of type I collagen by SaOS-2 cells increased with culturing time, with the highest amount found on PLAGA-BG composite (0.146 ± 0.006 μg), as compared to PLAGA (0.132 ± 0.006 μg), and TCPS controls (0.073 ± 0.005 μg). The expression of type I collagen by SaOS-2 cells cultured on the composite was significantly higher than cells grown on TCPS controls, ($p < 0.05$). There was a trend towards higher Type I collagen synthesis on the PLAGA-BG composite compared to PLAGA alone, but this was not found to be significant.($p = 0.06$) The formation of a mineralized matrix was confirmed by positive staining with Alizarin Red S and elemental analysis in which Ca and P were detected on PLAGA-BG scaffolds cultured with SaOS-2 cells. Alizarin stain intensity increased with culturing time. The mineralized nodules were not observed on PLAGA or TCPS controls after 2 weeks of culture, before the addition of the mineralization medium. After 1 week of culturing with the mineralization medium, mineralization as reflected in staining intensity, was much less on the control substrates than on PLAGA-BG.

As shown in Figures 4 and 6, SEM and EDXA analyses confirmed the formation of calcium phosphate nodules on the composite surface after only 3 days of culture, before the addition of the mineralization medium. These calcium phosphate nodules are similar in size and shape as observed on PLAGA-BG discs in the SBF. In time, the Ca-P nodules increased in size and formed larger aggregates, indicating that the PLAGA-BG composite was bioactive *in vitro*. The relative Ca to P peak ratio of the deposits decreased as a function of culturing time. These results collectively suggest that

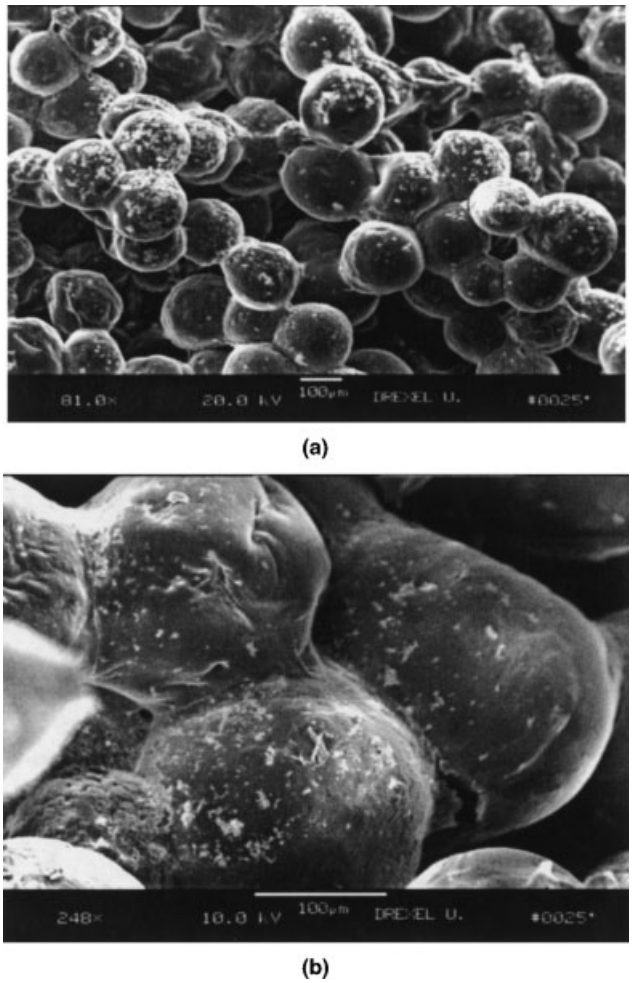


Figure 4. Human osteoblast-like cells (Soas-2) cultured on porous PLAGA-BG composite for 3 weeks. (b) Note the extensive coverage of the spheres by cell growth and the formation of surface Ca-P nodules, as shown in the higher magnification frame (magnification ×240). In addition, the porous network of the scaffold was maintained even after 3 weeks of culture.

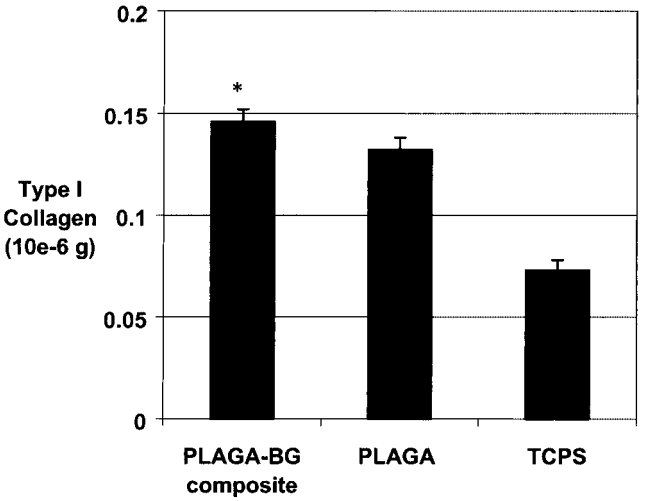


Figure 5. After 2 weeks, the expression of type I collagen by human osteoblast-like cells (SaOS-2) cultured on the PLAGA-BG composite was significantly higher than cells grown on TCPS controls, ($p < 0.05$, denoted with *). There was a trend towards higher Type I collagen synthesis on the PLAGA-BG composite compared to PLAGA alone, but this was not found to be significant ($p = 0.06$).

the composite was bioactive, and was capable of forming a surface calcium phosphate layer.

DISCUSSION

In this study, a 3-D, microsphere-based, porous, polymer bioactive glass composite (PLAGA-BG) possessing improved mechanical properties and osteointegrative potential has been described. The PLAGA-BG composite was developed for several purposes. First, our goal was to augment the mechanical properties of the parent phases, and this was achieved through BG particulate-reinforcement of a polymeric matrix. Improved mechanical properties and optimized structures will ultimately promote bone formation and implant healing. It was found here that the PLAGA-BG composite measured a higher compressive modulus than PLAGA alone. The addition of BG particles to PLAGA has resulted in a particle-reinforced and stiffer structure, as reflected in the significantly higher elastic modulus. The small disparity in compressive strength can be attributed to the higher porosity of the composite structure compared to PLAGA alone, and it may also be a function of the sintering process. Although the mechanical properties of the PLAGA-BG composite was similar to those of weak trabecular bone, the design goal is to optimize the structure so that its mechanical properties can be tailored to the bony defect to be replaced or repaired. This can be achieved by controlling the polymer to bioactive glass ratio, co-polymer ratios, microsphere diameter, as well as sintering parameters such as heating rate, temperature, and duration. The microsphere-based scaffold system described here is a versatile platform upon which a series of controlled optimization can be systematically carried out to achieve functional properties. Recently, Borden et al.[30] reported a compressive modulus of 297 MPa for a porous, PLAGA 85:15 microsphere-based composite by varying polymer composition, microsphere size and sintering duration.

More importantly, the composite was developed to improve the overall biological response to the scaffold by creating a bioactive substrate that could ultimately integrate with bone tissue. The long term outcome of total joint replacement is determined by the degree of implant loosening and tissue ingrowth, and a significant cause of implant failure is the lack of osseous integration or a mechanically functional interface between the implant and bone tissue. Consequently, osteointegration is a critical factor controlling the successes of viable or non-viable bone grafts. The objective was to take advantage of the unique ability of bioactive glass to form a surface calcium phosphate layer and, consequently, develop a bioactive composite that may bond to bone *in vivo*. Here, PLAGA and 45S5 bioactive glass composite discs were fabricated using the traditional solvent-casting method, and 3-D, porous scaffolds using polymer-bioactive glass composite microspheres. The bioactivity of the composite was demonstrated by immersing the composite discs in SBF with ion concentration similar to that of extracellular fluid, while solution pH was maintained within physiological ranges. An amorphous calcium-phosphate layer was formed on the composite discs

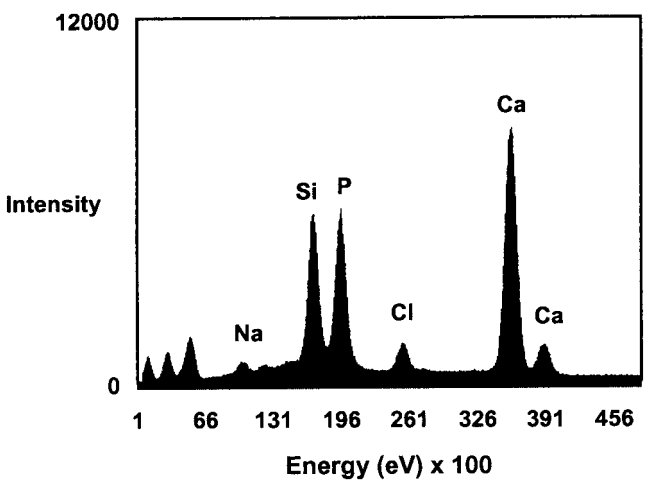


Figure 6. EDXA spectra of PLAGA-BG composite cultured with human osteoblast-like cells (SaOS-2) for 7 days. Ca, P, and Cl are found on the surface, whereas the Si and Na peaks observed on unreacted specimen have significantly diminished. It is likely that Si and Na leached from the composite. Calcium phosphate minerals were observed in the presence of cells.

after 14 days of immersion, whereas no such layer was found on the control polymer without bioactive glass particles.

Next, our goal was to engineer a structure that may minimize the disadvantages associated with the parent phases. Specifically, the objective was to combine PLAGA with BG in an effort to neutralize possible detrimental effects of the acidic polymer degradation products of the polymer. PLAGA degrades to glycolic and lactic acids when exposed to an aqueous environment, the release of which can cause a biologically significant decrease in local pH and may lead to undesirable responses. Through a series of surface dissolution and precipitation reactions, 45S5 bioactive glass releases alkaline ions, which produce an elevated local pH. By forming a composite of PLAGA and bioactive glass, the acidic and basic degradation products may be neutralized and physiological pH can be maintained. In this study, a lower solution pH was measured for the PLAGA scaffold than for the PLAGA-BG scaffold, during both bioactivity and cell culture experiments. Within the 3-week period, the cultures with PLAGA-BG maintained pH variations within physiological ranges. The next step will be to optimize this response by matching the kinetics of polymer degradation with BG dissolution, in order to achieve a more efficient system.

In addition, cellular response and bioactive potential of the 3-D, porous composite were evaluated *in vitro* by culturing the scaffold with human osteoblast-like cells (SaOS-2) for up to 3 weeks. It was found that the microsphere-based composite scaffold supported the synthesis of alkaline phosphatase, type I collagen, in addition to the adhesion and proliferation of human osteoblast-like cells. Statistically significant differences in type I collagen synthesis were observed between PLAGA-BG composite and the tissue culture polystyrene controls, and between PLAGA and the culture controls. The PLAGA-BG composite also formed surface calcium phosphate deposits in the presence of cells and serum proteins, which suggest that it could integrate with bone tissue *in vivo* and promote a continuous interface between implant and bone. The human osteoblast-like cells produced mineralized matrix on PLAGA-BG. Moreover, mineralization was only observed on the composite structure, not on PLAGA or TCPS controls during the first 2 weeks of culture. The mineral detected on the composite scaffold during surface analysis is likely a combination of osteoblast-mediated mineralization, as well as a result of the bioactive nature of the composite PLAGA-BG scaffold. Currently, there is no reliable, quantitative method to distinguish these two processes, in particular on bioactive materials. The kinetics and the chemistry of the calcium phosphate layer formed on 45S5 bioactive glass in electrolyte solutions differ significantly from the surface layers formed in the presence of serum proteins and during cell culture. El-Ghannam et al.[37] demonstrated that the formation of a bone equivalent apatite on a bioactive material was observed only after culturing with osteoblast-like cells.

A significant advantage of the composite over the polymeric matrix is that it is a bioactive material, that is, a material that is able to form a calcium phosphate layer *in vitro*. No such calcium phosphate layer was detected on PLAGA alone, and currently, bioactivity is deemed a critical factor in facilitating the chemical fixation of a biomaterial to bone tissue, and ultimately the *in vivo* success of the bone grafting material. The second advantage of the scaffold is that the addition of bioactive glass granules to the PLAGA matrix resulted in a structure with higher compressive modulus than PLAGA alone. The compressive properties of the composite approached those of trabecular bone, and a successful tissue-engineering scaffold must exhibit mechanical properties similar to those of the tissue to be replaced. Therefore, in addition to being bioactive, the PLAGA-BG would lend great functionality i*n vivo* compared to the PLAGA matrix alone. Finally, the combination of the two phases served to neutralize both the acidic byproducts produced during polymer degradation and the alkalinity due to the formation of the calcium phosphate layer. The resultant structure maintained a physiological pH range for up to 3 weeks of culture. Future studies will address the effects of co-polymer ratio and effects of composition on bioactivity and *in vivo* healing potential of the scaffold.

Several groups have begun to explore the potential of combining bioactive glass and polymers to form composite materials for bone tissue engineering.[31–35] The form of polymer-bioactive glass composite under research for bone repair has been predominantly particle-reinforced, with the exception of Marcolongo et al., who fabricated composite rods of polysulfone and bioactive glass fibers and implanted them in rabbit femora. The authors reported that after 6 weeks, interfacial strength between the composite and tissue was more than twice that of polysulfone alone, likely the result of the formation of a calcium-phosphate interface between the composite and surrounding bone. This study clearly demonstrated the bone healing potential of the bioactive composite *in vivo,* although polysulfone is a non-degradable polymer and thus may not be replaced by host tissue. In 1997, Niederauer et al. reported at the Society for Biomaterials meeting on the development of polylactide-*co*-glycolide/Bioglass® composites, where various ratios of PLAGA were combined with 45S5 bioactive glass particles, and porous structures were manufactured using a dissolution/precipitation technique. The synthesis parameters were not fully described, as no detailed or follow-up study has been published. In the abstract, the authors reported that by increasing the concentration of bioactive glass particles in the composite, a calcium phosphate layer was formed on the composite after six weeks of immersion in SBF. More-

over, an increase in storage modulus of the composite was measured as the polymer to bioactive glass volume% increased, but it is unclear how the storage modulus was determined. There also seemed to be a compositional dependence on both molecular weight and glass transitional temperature of the composite.

Qiu et al.[33] reported on the fabrication of bioactive, resorbable, polylactide-bioactive glass microcarriers for culturing in the bioreactor under simulated microgravity conditions. In this study, the bioactive glass granules were first treated in SBF to form a surface calcium phosphate layer. It was reported that the resulting polylactide-bioactive glass microcarriers were bioactive, as they formed surface calcium phosphate deposits when immersed in SBF. Later, these carriers were found to support the growth and phenotypic expression of rat marrow stromal cells,[35] and these results corroborate with our findings with human osteoblast-like cells. In this approach, the carriers were not processed further to form a scaffold, rather they were individually cultured with cells, and random aggregates of cells and beads were formed after three weeks of culture. Consequently, the random aggregates did not yet have the structural integrity or the mechanical strength necessary in a bone tissue-engineering scaffold. In addition, the buffering effect of bioactive glass against acidic degradation products of the polylactide would be diminished as pre-treated bioactive glass particles with a pre-formed surface calcium phosphate layer were used.

We have demonstrated here the potential of a PLAGA-BG composite as a bone tissue engineering scaffold. This composite system is versatile, and can be readily optimized in a controlled manner, resulting in structures possessing functional properties similar to those of bone. Currently, we are investigating methods to optimize the microsphere-based, porous PLAGA-BG scaffold described in this study. Future studies will be aimed at determining the relative benefits of the bioactive glass component, and compositional-dependence of structural and mechanical properties will be investigated. The degradation kinetics of PLAGA must match the formation kinetics of the surface calcium phosphate layer on BG, as alkaline ions are released. Methods need to be developed to improve the mechanical properties of the PLAGA-BG composite, without compromising its bioactivity. Future work will focus on the *in vivo* evaluation of the bone bonding and bone repair potential of the composite, as compared to PLAGA or BG alone.

CONCLUSIONS

We have described here the development of degradable, porous, polymer bioactive glass composite possessing improved mechanical properties and osteointegrative potential compared to degradable polymers of poly(lactic acid-glycolic acid) alone. The addition of bioactive glass granules to the PLAGA matrix resulted in a structure with higher compressive modulus than PLAGA alone. This scaffold has been shown to be a bioactive material, as it formed surface calcium phosphate deposits *in vitro*, and in the presence of cells and serum proteins. In addition to supporting the adhesion, growth and mineralization of human osteoblast-like cells *in vitro*, PLAGA-BA composite measured higher amount of Type I collagen synthesis than tissue culture polystyrene controls. Future work will focus on the optimization of these composites to further determine the relative benefits of the bioactive glass component, as well as the evaluation of the 3-D composite in an *in vivo* model.

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