J Mater Sci (2007) 42:4183–4190
DOI 10.1007/s10853-006-0636-0

In situ synthesized ceramic–polymer composites for bone tissue engineering: bioactivity and degradation studies

Yusuf M. Khan · Emily K. Cushnie · John K. Kelleher · Cato T. Laurencin

Received: 7 March 2006 / Accepted: 5 July 2006 / Published online: 2 March 2007

Abstract As an alternative to current bone grafting strategies, a poly-lactide-*co*-glycolide/calcium phosphate composite microsphere-based scaffold has been synthesized by the direct formation of calcium phosphate within forming microspheres. It was hypothesized that the synthesis of low crystalline calcium phosphate within forming microspheres would provide a site-specific delivery of calcium ions to enhance calcium phosphate reprecipitation onto the scaffold. Both polymeric and composite scaffolds were incubated in simulated body fluid (SBF) for 8 weeks, during which time polymer molecular weight, scaffold mass, calcium ion concentration of SBF, pH of SBF, and calcium phosphate reprecipitation was monitored. Results showed a 20% decrease in polymeric scaffold molecular weight compared to 11–14% decrease for composite scaffolds over 8 weeks. Composite scaffold mass and SBF pH decreased for the first 2 weeks but began increasing after 2 weeks and continued to do so up to 8 weeks, suggesting interplay between pH changes and calcium phosphate dissolution/reprecipitation. Free calcium ion concentration of SBF containing composite scaffolds increased 20–40% over control values within 4 h of incubation but then dropped as low as 40% below control values, suggesting an initial burst release of calcium ions followed by a reprecipitation onto the scaffold surface. Scanning electron micrographs confirm calcium phosphate reprecipitation on the scaffold surface after only 3 days of incubation. Results suggest the composite scaffold is capable of initiating calcium phosphate reprecipitation which may aid in bone/implant integration.

Y. M. Khan · J. K. Kelleher · C. T. Laurencin (✉)
Department of Orthopaedic Surgery, School of Medicine, The University of Virginia, 400 Ray C. Hunt Drive, Suite 330, Charlottesville, VA 22903, USA
e-mail: ctl3f@virginia.edu

Y. M. Khan · C. T. Laurencin
Department of Biomedical Engineering, The University of Virginia, Charlottesville, VA 22904, USA

E. K. Cushnie · C. T. Laurencin
Department of Chemical Engineering, The University of Virginia, Charlottesville, VA 22904, USA

Introduction

Current strategies for healing traumatic bone injury include autografts, tissue transplanted from one region of the patient to another, and allografts, tissue donated from a cadaver and transplanted into the patient. However, both autografts and allografts have their limitations including donor-site morbidity and risks of disease transmission, respectively. Several materials have been examined as candidates for bone graft substitutes using both natural and synthetic polymers, ceramics, and composites of the two. One strategy that incorporates these materials and continues to show promise is bone tissue engineering.

Tissue engineering has been 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,'' [1]. At the core of the tissue engineering approach to bone repair is the development of a suitable scaffold. A well-designed scaffold will have a porous, interconnected structure onto which newly attached cells can proliferate and migrate,

and will support the differentiation and mineral deposition by these same cells. The choice of materials used for the scaffold should reflect these requirements, and perhaps encompass other applications such as the delivery of factors from the scaffold material to further encourage the differentiation and accelerated healing of the bone. Additionally, as the newly forming bone tissue incorporates the scaffold material, the scaffold itself should be biodegradable either through cellular events or as a result of the surrounding environment.

Several degradable polymers, both natural and synthetic, have been investigated as scaffold materials for bone tissue engineering substrates, including collagen [2, 3], chitosan [4, 5], poly(caprolactones) [6, 7], poly(propylene fumarate) [8, 9], and polyesters such as polylactide, polyglycolide, and their copolymer poly (lactide-*co*-glycolide) (PLAGA) [10, 11]. In addition to degradable polymers, resorbable ceramics have been used as well, both alone and in combination with polymers. Tricalcium phosphate, a degradable form of calcium phosphate has found favor in both the research and clinical side of bone repair, given its resorbability and predilection to remodeling via osteoclasts [12]. However, tricalcium phosphate is considerably more crystalline than native bone itself and its overall resorption and remodeling characteristics can be different than those of native bone. Studies examining ceramics with varied crystallinities have shown a positive correlation between reduced crystallinity and healing success [13]. One reason for this positive correlation is thought to be due to the resorbability of the ceramic and the resulting reprecipitation of calcium phosphate on the surface of the resorbed calcium phosphate. This reprecipitation has been shown to enhance both healing and strength at the bone/implant interface [13]. This suggests that a low crystalline material may yield better healing than a more crystalline material [13].

Our laboratory has developed a microsphere-based composite scaffold of PLAGA and a low crystalline calcium phosphate in which the calcium phosphate is synthesized in situ within the forming polymeric microspheres, resulting in composite microspheres that form the larger scaffold [14]. The precipitated calcium phosphate has been shown through x-ray diffraction analysis (XRD), Fourier transform infrared spectroscopy (FTIR) and energy dispersive spectroscopy (EDS) to be similar to trabecular bone in crystal structure, crystallinity, and calcium/phosphorus ratio [14]. The composite scaffold is porous and has a three-dimensional interconnected structure through which cells can migrate, proliferate, and differentiate.

We hypothesize that the design of this scaffold, a degradable polymeric scaffold that incorporates a low crystalline calcium phosphate similar to native bone, will provide benefits to healing beyond those of a purely polymeric scaffold. Specifically, the addition of calcium phosphate will encourage the precipitation of new calcium phosphate through the dissolution of calcium ions at the implant site that may enhance bone/implant bonding during healing. This ion dissolution may serve to alter the pH of the immediate environment.

The goal of this study was to examine and gain an understanding of the degradation characteristics of this scaffold through assessment of the following parameters: polymer molecular weight change, scaffold mass change, calcium ion release into the degradation solution, pH of the degradation solution, and calcium phosphate reprecipitation.

Materials and methods

Scaffold preparation

Scaffolds were prepared as described in detail previously [14]. Briefly, PLAGA/calcium phosphate composite scaffolds were formed by creating an emulsion of a calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$) solution and ammonium hydrogenphosphate ($(NH_4)_2HPO_4$) solution in a separate solution of PLAGA (85/15 MW 101 kDa) (Alkermes, Cambridge, MA) in methylene chloride. This suspension was added dropwise to 1% polyvinyl alcohol (PVA) (Sigma-Aldrich, St. Louis, MO) (M_w: 30,000–70,000) and allowed to mix at 4°C for 24 h after which the formed composite microspheres were isolated from the PVA via vacuum filtration and dried at room temperature for 48 h. Microspheres were then lyophilized for an additional 48 h. Three types of microspheres were formed; pure polymeric microspheres, high polymer/ceramic ratio microspheres that contain approximately 17% calcium phosphate, and low polymer/ceramic ratio microspheres that contain approximately 27% calcium phosphate. After drying, microspheres ranging from 355 to 600 μm were isolated using stainless steel sieves (Fisher Scientific, Suwanee, GA) and poured into a stainless steel mold to form cylinders measuring 5 mm in diameter and 10 mm in thickness. Microspheres were then heated at 90 °C for 105 min to sinter neighboring microspheres together, and subsequently allowed to cool slowly over several hours, resulting in either a pure polymeric or polymer/ceramic composite scaffold with a porous, interconnected structure.

Degradation media

Scaffolds were incubated in 10 mL of simulated body fluid (SBF) that was maintained at 37 °C and changed once per week for up to 8 weeks. The w/v ratio of scaffolds:SBF was at least 1:70, to maintain perfect sink conditions. SBF was based on Kokubo et al. [15] and contained the following ions in the following concentrations (Table 1).

Polymer molecular weight change

Samples were examined for molecular weight change of the polymer after 2 weeks, 4 weeks, and 8 weeks. Samples were removed from the SBF and rinsed twice with distilled, deionized water (DDH_2O), and lyophilized for 48 h to ensure complete water removal. Samples were then dissolved in methylene chloride to form a final polymer concentration of 1%. Composite scaffolds were dissolved in methylene chloride while the calcium phosphate was allowed to settle to the bottom. The polymer solution from the composite scaffolds was then decanted and adjusted to 1% concentration in methylene chloride. Samples were analyzed for molecular weight using a gel permeation chromatographer (Agilent, Palo Alto, CA) at a flow rate of 1 mL/min and a column temperature of 40 °C. Weight average molecular weight was recorded for each sample, and four samples were analyzed per experimental group.

Scaffold mass change

Scaffolds were weighed once prior to the degradation study, and once again after the following time points: 1 day, 3 day, 5 day, 1 week, 2 weeks, 4 weeks, 6 weeks, and 8 weeks. At each time point, samples were permanently removed from SBF, rinsed twice with DDH_2O, and lyophilized for 48 h to ensure complete removal of water, and weighed. Samples were not re-inserted for subsequent time points after measurement. Six samples were weighed from each group.

Table 1 Content of simulated body fluid (adapted from Kokubo et al. [15])

Ion	Concentration (μmol/L)
Na^+	142.0
K^+	5.0
Ca^{2+}	2.5
Mg^{2+}	1.5
Cl^-	148.8
HCO_3^-	4.2
HPO_4^{2-}	1.0
SO_4^{2-}	0.5

Calcium ion release

Calcium ion release studies were conducted as previously described [16]. SBF solution was analyzed for calcium ion content at the following time points: 1 h, 4 h, 12 h, 24 h, 3 days, 5 days, 1 week, 2 weeks, 4 weeks, 6 weeks, and 8 weeks. SBF was analyzed for calcium ion concentration using a calcium reagent set (Pointe Scientific Inc., Canton, MI). At the appropriate time points, 20 μL of SBF was removed and added to 1.0 mL of reagent, and analyzed for optical density using a spectrophotometer (Shimadzu, Columbia, MD) at a wavelength of 570 nm. Optical density was normalized to a known calcium ion concentration and reported as mg/dL. SBF that had not been exposed to scaffolds was used as a control. Six samples were analyzed for calcium ion concentration from each group.

pH of simulated body fluid

The pH of the SBF containing scaffolds was analyzed at the following time points: 1 h, 4 h, 12 h, 24 h, 3 days, 5 days, 1 week, 2 weeks, 4 weeks, 6 weeks, and 8 weeks. Samples of SBF were analyzed for pH using an Accumet pH meter (Fisher Scientific, Pittsburgh, PA). Deviations of SBF containing scaffolds from SBF not exposed to scaffolds were reported. Six samples from each group were analyzed.

Calcium phosphate reprecipitation

Calcium phosphate reprecipitation was qualitatively evaluated at 3 days and 6 weeks using scanning electron micrographs (JEOL JSM 6700F) at an acceleration voltage of either 3 or 15 kV. The samples were coated with gold/palladium prior to analysis.

Statistics

Six samples were analyzed for each scaffold type at each time point for mass change, calcium ion release, and pH change while four samples were analyzed for each scaffold type at each time point for molecular weight change. Data within each scaffold group was examined between time points and was analyzed using a one-way analysis of variance (ANOVA) with statistical significance at $p < 0.05$. Post-hoc analysis for any statistical differences was performed using the Tukey test.

Results

Polymer molecular weight change

The degradation of the PLAGA in the scaffolds was reported as weight average molecular weight and was noted to decrease over the 8-week degradation (see Fig. 1). Molecular weight of the scaffolds with polymer alone was seen to decrease by 20% while scaffolds from either the low or high polymer/ceramic ratio were seen to decrease by only 11.5% or 13.5%, respectively, after 8 weeks. There was no statistical difference seen between the low and high polymer/ceramic composite molecular weights after 8 weeks, but there were differences seen at 2 and 4 weeks between both the low and high polymer/ceramic ratios. At all time points, the PLAGA molecular weight was significantly lower than either the low or high polymer/ceramic composite scaffold molecular weights.

Scaffold mass change

The mass of pure PLAGA scaffolds was observed to decrease slightly up to 2 weeks of incubation, but statistical significance was only noted between 1 and 2 weeks (see Fig. 2). There was a plateau at 4 weeks and a statistically significant gain in mass from 4 to 6 weeks. Similar trends were seen for both the low and high polymer/ceramic ratio scaffolds, with a gradual decrease in mass up to 2 weeks and then a gradual mass increase after 4 weeks that continued until 8 weeks. However, there was a difference in mass between the three groups, with the composite scaffolds losing more mass after 2 weeks than the pure polymeric scaffolds, although statistically significant differences were only seen at 1, 2, and 6 week time points for the low polymer/ceramic ratio and no statistical differences between time points for the high polymer/ceramic ratio. Between the low and high polymeric scaffolds the trends seen suggested that the low polymer/ceramic scaffold underwent more dramatic shifts in mass, with greater extremes of mass loss and gain than either the high ratio or pure PLAGA scaffolds. It therefore appeared from this data that the greater the calcium phosphate content, the greater the overall mass loss and gain.

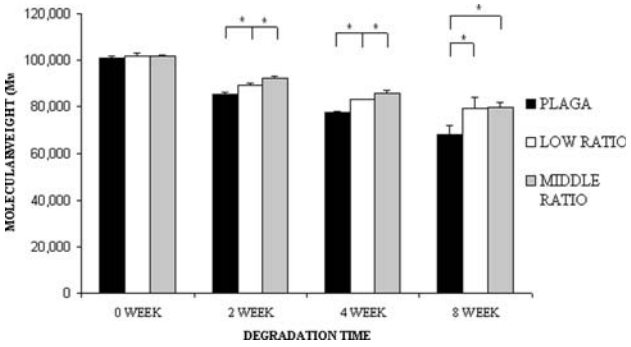

Fig. 1 Molecular weight analysis (weight average) of PLAGA in pure polymeric scaffolds and composite scaffolds. Statistical differences were noted between all three groups after 2 and 4 weeks of degradation, while after 8 weeks of degradation the composite scaffolds were statistically similar to each other but different than pure polymer scaffolds ($p < 0.05$)

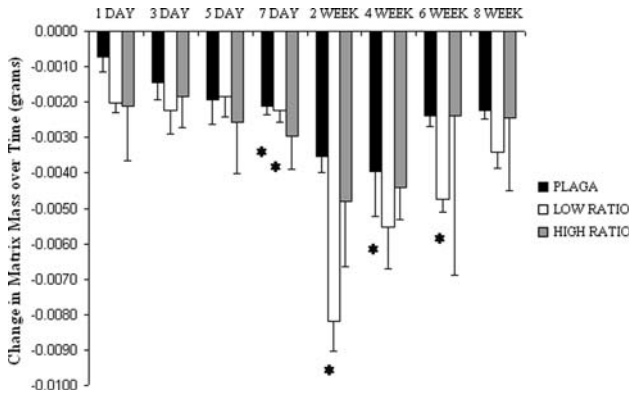

Fig. 2 Mass loss of scaffolds over time. Composite scaffolds showed greater fluctuations in mass over time, suggesting that calcium phosphate was dissolving but also reprecipitating. Mass change was most dramatic in the low ratio composite scaffold, the scaffold with the greatest original calcium phosphate content

Calcium ion release

The concentration of free calcium ions in the SBF underwent fluctuations depending on the duration of incubation of the composite scaffolds. For both the low and high composite scaffolds, the SBF in which they were incubating showed an initial increase in free calcium ion concentration over the control SBF after both the 1 and 4 h time point (see Fig. 3). However, after 12 h of incubation a sharp and statistically significant decrease in free calcium ions was noted for SBF containing both scaffold types below the calcium ion concentration of the control SBF [16]. This decreased calcium ion concentration remained below that of the control SBF for the duration of the study, but a slight increase in concentration was seen between the 2 and 4 week time point. This slight increase coincided temporally with the change in scaffold mass noted above.

pH of simulated body fluid

The pH of the simulated body fluid containing either pure PLAGA or composite scaffolds was reported as

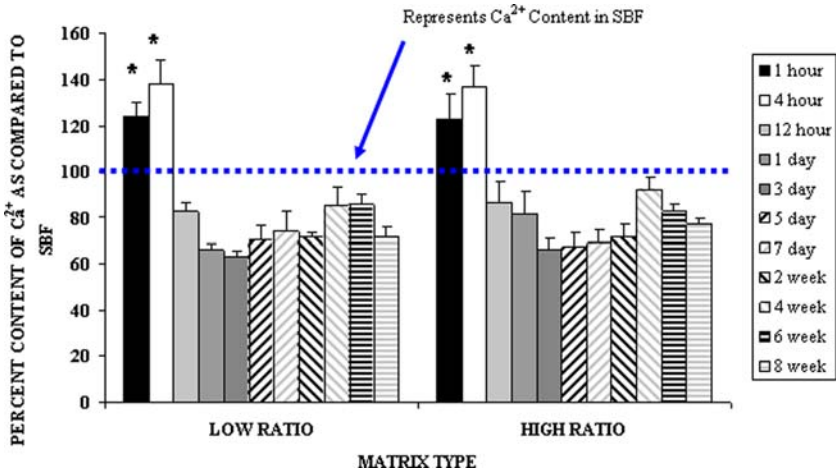

Fig. 3 Calcium ion concentration in simulated body fluid containing both low and high ratio composite scaffolds is represented as percent of control solution. An initial burst of calcium ions is quickly reversed, suggesting that after an initial dissolution of calcium ions, calcium phosphate began to reprecipitate on the polymer surface

deviations from control SBF that was incubated at 37 °C but contained no scaffolds (see Fig. 4). Therefore any changes noted in SBF pH were due to the scaffold it contained. The pH of SBF containing pure polymeric scaffolds was not seen to deviate considerably from that of control SBF; however, SBF containing composite scaffolds underwent a decrease in pH steadily from 1 h until 2 weeks of incubation. After 2 weeks there was a sharp increase in pH almost up to control SBF levels for the 4, 6, and 8 week time points. Differences between the low and high polymer/ceramic ratio scaffolds were minimal, with the largest difference seen at 2 weeks, where the low polymer/ceramic ratio scaffolds showed a greater decrease in pH levels than high polymer/ceramic ratio scaffolds.

Calcium phosphate reprecipitation

SEM images of pure polymeric microspheres showed minimal to no evidence of calcium phosphate precipitation (see Fig. 5a). However, composite scaffolds incubated in SBF after only 3 days show evidence of calcium phosphate precipitation on microsphere surfaces. After 6 weeks of incubation, pure polymeric microspheres still show minimal to no precipitation (Fig. 5c), while extensive calcium phosphate mineralization is seen on the surface of the composite matrices (Fig. 5d).

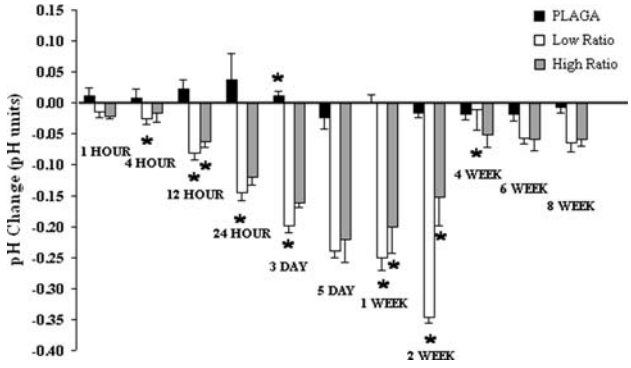

Fig. 4 Changes in SBF pH during degradation. Pure polymer scaffolds had little to no effect on SBF pH but composite scaffolds induced a drop in pH that abruptly reversed after 2 weeks of incubation

Discussion

Often the success of an orthopaedic implant is dependent on how well the host bone is integrated with or grows onto the implant surface. Several approaches have been undertaken to increase the incorporation of the two materials including texturing the surface of titanium implants to allow host bone to form a mechanical interlock with the implant [17], releasing growth factors from the material to encourage host bone growth onto the implant surface [18], and coating the implant material with ceramics such as hydroxyapatite or tricalcium phosphate to encourage implant integration [19]. Certain success has been achieved through the use of ceramic coatings, largely because of calcium and phosphorus ions leeching from the calcium phosphate coating on the implant material. These ions are released into the surrounding milieu and provide a site-specific delivery of some of the necessary building blocks for new mineral formation. Through a complicated sequence of events, the ions released from the mineral coating reprecipitate onto the implant surface and begin new mineral formation, encouraging the integration of the host bone with the implant material [13]. The process of reprecipitation on existing hydroxyapatite has been theorized to occur through exposed phosphate and hydroxyl groups from the hydroxyapatite that elicit a negative charge on the

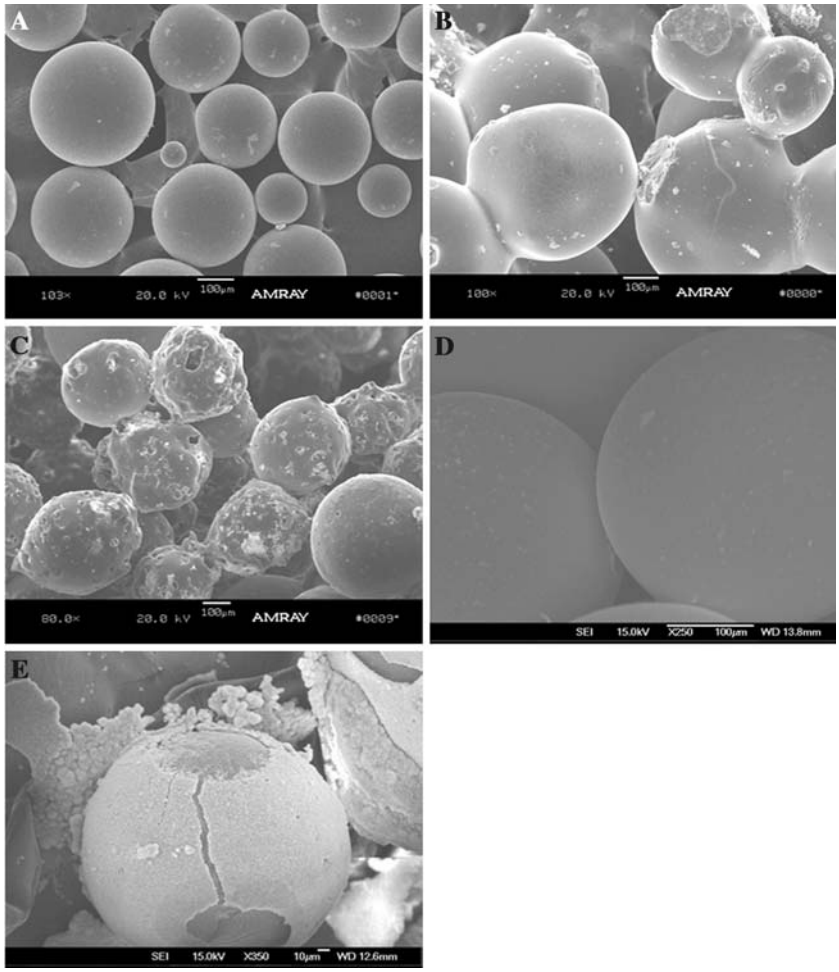

Fig. 5 Scanning electron micrographs of (**a**) pure polymeric scaffolds, (**b**) low ratio scaffolds, and (**c**) high ratio scaffolds prior to SBF incubation. Scanning electron micrographs of (**d**) pure polymeric (**e**) low ratio scaffolds after 6 weeks of incubation. Calcium phosphate reprecipitation begins as early as 3 days for composite scaffolds and continues up to 6 weeks, while pure polymeric scaffolds show no evidence of reprecipitation after 3 days and 6 weeks

surface of the material [20]. This negative charge attracts the liberated, positively charged calcium ions to the surface which in turn attract the negatively charged free phosphate ions, resulting in precipitation.

However, the material used to coat implants is often a highly crystalline hydroxyapatite which, by nature, degrades very slowly if at all. Also, with the high sintering temperatures necessary to form crystalline hydroxyapatite, it has been observed that the negatively charged groups that are exposed to the surface and initiate reprecipitation are reduced in number [20], suggesting that a less crystalline material may result in enhanced reprecipitation. Studies examining the relationship between calcium phosphate crystallinity (and therefore ion dissolution rate) and defect healing have shown an inverse correlation between crystallinity and healing. That is, as crystallinity decreases, healing increases [13]. Therefore one can surmise that the delivery of calcium and phosphorus ions may have an influence on overall healing.

Given this, the use of a low crystalline calcium phosphate in an implant for bone repair is well reasoned. The polymer/ceramic composite scaffold described herein was formed with a degradable polymer and a low crystalline calcium phosphate that resembled bone in both crystallinity and calcium/phosphorus ratio [14]. The work described here supports the theory behind this design.

The molecular weight of the polymer was seen to decline over 8 weeks for all scaffold types. However, the overall decline was noted to be almost twice as much for the pure polymeric scaffold as compared to the composite scaffolds. PLAGA is a degradable polyester that undergoes hydrolysis in aqueous environments but will also undergo accelerated degradation in acidic environments, which can be brought about by the degradation products of PLAGA (lactic and glycolic acid). One possible reason for the accelerated degradation of the pure polymer scaffolds may be that the environment was more acidic than that of the composite scaffolds. This may have also led to the slight but measurable loss in mass over the first 2 weeks of the study. Other studies have shown that amorphous or low crystalline calcium phosphate can

act as a buffer due to the dissolution of ions [21]. However, an examination of the pH of solution over the 8 week degradation time shows that the SBF containing the pure polymeric scaffolds was actually the most stable of all three scaffold types, with the composites showing much larger fluctuations in pH. Another explanation for the slower rate of degradation with the composite scaffolds may be that the calcium phosphate was reprecipitating on the surface of the polymer and preventing the aqueous solution from coming in contact with the polymer as readily as it could with the pure polymeric scaffold, thereby hindering the hydrolytic degradation of the polymer in the composite scaffolds. Scanning electron micrographs confirm that much of the surface of the microspheres was covered with newly precipitated calcium phosphate (Fig. 5e).

The noticeable drop in pH seen by the SBF containing composite scaffolds may be explained by the dissolution of calcium, phosphate, and hydroxyl ions from the scaffolds or the reprecipitation of calcium phosphate onto the surface of the scaffolds. The composite microspheres were synthesized at pH 10 and 4°C, which dictates the formation of hydroxyapatite ($Ca_{10}(PO_4)_6OH_2$) and thus the potential availability of hydroxyl ions, and a low crystalline material, respectively [14]. Further, the formation of a low crystalline hydroxyapatite within the microspheres has been previously confirmed [14]. As the calcium ions were released, they began to reprecipitate as early as 12 h, as suggested by the reduction in free calcium ions, and thus potentially were binding with hydroxyl ions to form hydroxyapatite on the surface of the scaffold, as this is the most stable form of calcium phosphate reprecipitation in SBF [22]. Further, the free calcium ions may have been simultaneously forming $CaOH_2$ as a precipitate. This decrease in OH^- ions would result in a net increase in H^+ ions and therefore a decrease in pH. The PO_4^{3-} ions that were being released would likely form phosphoric acid (H_3PO_4), which has been noted elsewhere [23].

This decrease in pH was shown to continue until the 2-week time point for both composite scaffold types, at which point there was a noticeable increase in pH. The decrease and subsequent increase in pH matched a decrease and subsequent increase in the scaffold mass that followed the same time course. These two events may be linked. The change in solution pH may have been brought on by the precipitation of calcium phosphate onto the scaffold. As the pH continued to drop there was increased precipitation of calcium phosphate when examining SEM images and mass change data. As pH dropped, this acidic environment may have induced the breakdown of encapsulated calcium phosphate, higher availability of Ca^{2+} and PO_4^{3-}, and subsequent reprecipitation, as calcium phosphate is known to degrade in acidic conditions [24]. As the pH continued to drop, calcium phosphate continued to reprecipitate onto the surface of the scaffold, as evidenced by SEM images in Fig. 5 showing calcium phosphate precipitation as early as 3 days and as late as 8 weeks, and calcium ion concentration data showing reductions in free calcium ion concentration as early as 12 h. The dissolution of encapsulated calcium phosphate continued up to the 2- and 4-week time points, when the mass of the composite scaffolds began to increase. One explanation for this may be that the amount of encapsulated calcium phosphate had been reduced through dissolution and therefore the amount of free calcium ions added to the SBF was reduced, causing the pH to rise. As the pH rose, the solubility of any remaining synthesized calcium phosphate was reduced [25] and the rate of precipitation of new calcium phosphate dominated over dissolution. It is likely that prior to the 2-week time point reprecipitation was taking place but the dissolution of encapsulated calcium phosphate occurred at a greater rate than the reprecipitation of new calcium phosphate, resulting in a net reduction in pH and scaffold mass. The similar trend in mass change seen in the pure polymeric scaffolds was perhaps due to a mass loss from the accelerated degradation as indicated by the molecular weight analysis initially, followed by minimal calcium phosphate precipitation on the surface of the scaffold. Although SEM analysis shows no evidence of calcium phosphate precipitation, small amounts may have precipitated within the pore structure of the scaffold, which would be difficult to image using SEM.

The capacity for a scaffold to induce the reprecipitation of calcium phosphate onto its surface may indeed lead to better bone/implant integration or possibly more robust mineralization of the scaffold once implanted in vivo. Further, the released calcium ions may have beneficial effects on osteoblasts in terms of proliferation or differentiation [26–28]. The scaffold evaluated here holds promise as a bone graft substitute. Cell studies confirming the biocompatibility and suitability of this scaffold as a support for osteoblast viability and differentiation have confirmed that promise (manuscript in preparation).

Conclusions

The formation of a composite scaffold utilizing a low crystalline calcium phosphate was undertaken in the hopes that the calcium phosphate would encourage the

apposition of new bone and the incorporation of the implant material into the surrounding bone. Close examination of the degradation and ion dissolution of the composite scaffolds suggest that the release of calcium ions and resulting precipitation of calcium phosphate would promote good bone/implant integration. The alteration in pH, although minimal overall, was noted only in composite scaffolds and may be due to interplay between calcium phosphate dissolution and reprecipitation. Future studies will examine the compatibility of the composite scaffolds with osteoblast-like cells.

Acknowledgements This work was funded by The National Science Foundation (BES-0201923). The authors would also like to thank Swaminathan Sethuraman, Ph.D. for his assistance with scanning electron microscopy.

References

1. Laurencin CT, Ambrosio AA, Borden MD, Cooper JA (1999) In: Yarmush ML (ed) Annual review of biomedical engineering. Annual Reviews Inc., Palo Alto, p 19
2. Niemeyer P, Krause U, Fellenberg J, Kasten P, Seckinger A, Ho AD, Simank HG (2004) Cells Tissues Organs 177:68
3. Yang C, Hillas PJ, Baez JA, Nokelainen M, Balan J, Tang J, Spiro R, Polarek JW (2004) BioDrugs 18:103
4. Li Z, Ramay HR, Hauch KD, Xiao D, Zhang M (2005) Biomaterials 26:3919
5. Ho MH, Wang DM, Hsieh HJ, Liu HC, Hsien TY, Lai JY, Hou LT (2005) Biomaterials 26:3197
6. Rai B, Teoh SH, Ho KH, Hutmacher DW, Cao T, Chen F, Yacob K (2004) Biomaterials 25:5499
7. Schantz JT, Teoh SH, Lim TC, Endres M, Lam CX, Hutmacher DW (2003) Tissue Eng 9:S113
8. Lewandrowski KU, Bondre SP, Wise DL, Trantolo DJ (2003) Biomed Mater Eng 13:115
9. Vehof JW, Fisher JP, Dean D, van der Waerden JP, Spauwen PH, Mikos AG, Jansen JA (2002) J Biomed Mater Res 60:241
10. Lu HH, Kofron MD, El-Amin SF, Attawia MA, Laurencin CT (2003) Biochem Biophys Res Commun 305:882
11. Karp JM, Shoichet MS, Davies JE (2003) J Biomed Mater Res A 64:388
12. Bucholz RW (2002) Clin Orthop 395:44
13. Ducheyne P, Qiu Q (1999) Biomaterials 20:2287
14. Khan Y, Katti DS, Laurencin CT (2004) J Biomed Mater Res 69A:728
15. Kokubo T, Kushitani H, Sakka S, Kitsugi T, Yamamuro T (1990) J Biomed Mater Res 24:721
16. Khan Y, Katti DS, Laurencin CT (2005) In: Laurencin CT, Botchwey EA (eds) Nanoscale materials science in biology and medicine. Mater. Res. Soc. Symp. Proc., Warrendale, PA, p 63
17. Wiskott HW, Belser UC (1999) Clin Oral Implants Res 10:429
18. Salata LA, Franke-Stenport V, Rasmusson L (2002) Clin Implant Dent Relat Res 4:27
19. Dumbleton J, Manley MT (2004) J Bone Joint Surg Am 86A:2526
20. Kim HM, Himeno T, Kokubo T, Nakamura T (2005) Biomaterials 26:4366
21. Schiller C, Epple M (2003) Biomaterials 24:2037
22. Lu X, Leng Y (2005) Biomaterials 26:1097
23. Prabhakar RL, Brocchini S, Knowles JC (2005) Biomaterials 26:2209
24. Krieger NS, Frick KK, Bushinsky DA (2004) Curr Opin Nephrol Hypertens 13:423
25. Godley R, Starosvetsky D, Gotman I (2004) J Mater Sci Mater Med 15:1073
26. Shu R, McMullen R, Baumann MJ, McCabe LR (2003) J Biomed Mater Res 67A:1196
27. Dvorak MM, Riccardi D (2004) Cell Calcium 35:249
28. Dvorak MM, Siddiqua A, Ward DT, Carter DH, Dallas SL, Nemeth EF, Riccardi D (2004) PNAS 101:5140