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Biomaterials 21 (2000) 2521–2527

Biomaterials

Sustained release of vascular endothelial growth factor from mineralized poly(lactide-co-glycolide) scaffolds for tissue engineering

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Abstract

Strategies to engineer bone tissue have focused on either: (1) the use of scaffolds for osteogenic cell transplantation or as conductive substrates for guided bone regeneration; or (2) release of inductive bioactive factors from these scaffold materials. This study describes an approach to add an inductive component to an osteoconductive scaffold for bone tissue engineering. We report the release of bioactive vascular endothelial growth factor (VEGF) from a mineralized, porous, degradable polymer scaffold. Three dimensional, porous scaffolds of the copolymer 85 : 15 poly(lactide-co-glycolide) were fabricated by including the growth factor into a gas foaming/particulate leaching process. The scaffold was then mineralized via incubation in a simulated body fluid. Growth of a bone-like mineral film on the inner pore surfaces of the porous scaffold is confirmed by mass increase measurements and quantification of phosphate content within scaffolds. Release of ^{125}I-labeled VEGF was tracked over a 15 day period to determine release kinetics from the mineralized scaffolds. Sustained release from the mineralized scaffolds was achieved, and growth of the mineral film had only a minor effect on the release kinetics from the scaffolds. The VEGF released from the mineralized and non-mineralized scaffolds was over 70% active for up to 12 days following mineralization treatment, and the growth of mineral had little effect on total scaffold porosity.

Keywords: Drug delivery; Tissue engineering; VEGF; Mineralization; Bone; Poly(lactide-co-glycolide)

1. Introduction

Orthopaedic tissue engineering strategies have often focused on the use of natural or synthetic, degradable materials as scaffolds for cell transplantation (cell-based strategies) [1–3] or as a means to guide regeneration by native osteogenic cells (conductive strategies) [4]. The degree of success of these tissue engineering methods is highly dependent on the properties of the scaffold. Basic scaffold design requirements include degradability, biocompatibility, high surface area/volume ratio, osteoconductivity, and mechanical integrity. A biocompatible scaffold material that is degradable over a controllable time scale into non-toxic degradation products may disappear in concert with new tissue formation, leaving a natural tissue replacement. A high surface area/volume ratio allows for mass transport between cells within the scaffold and the surrounding host tissue, and provides space for ingrowth of fibrovascular tissue. Osteoconductivity is important for binding and migration of transplanted and/or native osteogenic cells, and mechanical integrity is required to withstand cellular contractile forces during tissue development [5] to ensure maintenance of the initial shape of the scaffold.

The degradability, biocompatibility, and large surface area/volume ratio of scaffolds can be accomplished by the appropriate choice of synthetic or natural material and processing approach. Poly(lactic acid), poly(glycolic acid), and their copolymers have been widely used in tissue engineering applications because they undergo controllable hydrolytic degradation into natural metabolites [6,7], and can be processed into highly porous scaffolds by a variety of methods [8–10]. Osteoconductivity, and mechanical integrity may be enhanced by the presence of a continuous bone-like mineral film on the inner pore surfaces of a scaffold. It has been shown

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0142-9612/00/$ - see front matter
PII: S0142-9612(00)00120-4

previously that a single-step, room temperature process can be used to grow a continuous bone-like mineral (BLM) layer on the inner pore surfaces of a porous poly(lactide-co-glycolide) (PLG) scaffold [11]. This layer significantly increases the mechanical resistance of the scaffold, indicated by a 5-fold increase in compressive modulus after mineral film growth. In addition, a BLM layer is considered a prerequisite to conduction of osteogenic cells onto the surface of orthopaedic and dental implant materials in vivo [12,13], so the presence of the BLM layer is expected to enhance the osteoconductivity of the PLG scaffold surface.

Although conductive and cell transplantation strategies have enjoyed success in bone tissue engineering [14–16], they exert minimal biochemical control over the process of tissue regeneration. The process of new tissue formation may be augmented by the delivery of bioactive factors (e.g. growth factors) that induce cells to behave in a specific manner [17]. Several factors have been identified which induce chemotaxis, proliferation, differentiation, and matrix synthesis of specific cell types. Although several systems have been developed for factor delivery [18], macroporous tissue engineering matrices have only recently been used as vehicles for delivery of bioactive factors [19–22]. Sustained delivery of plasmid DNA encoding for platelet derived growth factor from a 95% porous PLG scaffold has been recently achieved [21]. This release led to transfection of a large number of cells in vivo and induced increases in matrix deposition and blood vessel formation. Vascular endothelial growth factor (VEGF) has also been delivered from porous PLG scaffolds in a sustained manner [22], and the released factor retained 90% of its bioactivity. The inclusion of bioactive factors into a scaffold may exert a higher level of control over cell function within and adjacent to a scaffold construct, and allow one to address specific limitations in conductive and cell-based tissue engineering methods.

A major limitation in engineering of many tissue types, including bone tissue, is the inability to induce rapid vascular ingrowth during tissue development [23]. The viability of transplanted cells and/or host cells that migrate into the scaffold from the native tissue is highly dependent on transport of nutrients and waste products between the cells and the host tissue. Transport is initially due solely to diffusion, and cells more than several hundred microns from blood vessels in the surrounding tissue either fail to engraft or rapidly die due to oxygen deprivation [24]. Studies indicate that blood vessels will infiltrate a macroporous scaffold to provide enhanced transport to engineered tissues, but the process occurs at a rate of < 1 mm per day and it typically takes 1–2 weeks for complete penetration of vascular tissue into relatively thin (e.g. 3 mm thick) scaffolds [25,26].

This study was undertaken to develop a scaffold that combines the degradability, biocompatibility, and osteoconductivity of a mineralized PLG scaffold with the tissue inductive properties of a bioactive polypeptide growth factor. Specifically, the goal was to grow bone-like mineral on the inner pore surfaces of a scaffold containing a growth factor without compromising factor bioactivity or scaffold porosity. Vascular endothelial growth factor was specifically chosen for these studies because it is a potent mitogen for human micro and macrovascular endothelial cells, but does not exhibit mitogenic effects on other cell types [27]. This specific action may result in the induction of neovascularization concurrent with engineering of bone tissue. Enhanced vascular tissue formation during tissue development may lead to enhanced viability of native and/or transplanted osteogenic cells within a scaffold, potentially enabling the engineering of a larger volume of bone tissue.

2. Materials and methods

2.1. Sample preparation

Poly(lactide-co-glycolide) pellets with a lactide : glycolide ratio of 85 : 15 were obtained from Medisorb, Inc. (I.V. = 0.78 dl/g) and ground to a particle size between 106 and 250 μm. 7 mg of ground PLG particles were then combined with 250 μl of a 1% alginate (MVM, ProNova; Oslo, Norway) solution in ddH_2O, and 3 μg of vascular endothelial growth factor (VEGF, Intergen; Purchase, NY), and vortexed. These solutions were lyophilized, mixed with 100 mg of NaCl particles ($250\ \mu m < d < 425\ \mu m$), and compression molded at 10 MPa for 1 min in a 4.2 mm diameter die. This yielded 2.8 mm thick disks with a diameter of 4.2 mm. Disks were then exposed to 5.9 MPa CO_2 gas in an isolated pressure vessel and allowed to equilibrate for 20 hr. The pressure was decreased to ambient in 2 min, causing thermodynamic instability, and subsequent formation of gas pores in the polymer particles. The polymer particles expand and conglomerate to form a continuous scaffold with entrapped alginate, VEGF, and NaCl particles. After gas foaming, the disks were incubated in 0.1 M $CaCl_2$ for 24 h to leach out the salt particles and induce gellation of the alginate within the polymer matrix. Alginate was included in the scaffolds because it has been shown to abate the release of VEGF from PLG scaffolds [20].

Certain scaffolds were mineralized via a 5 day incubation in a simulated body fluid (SBF). Simulated body fluid (SBF) was prepared by dissolving the following reagents in deionized H_2O: NaCl — 141 mM, KCl — 4.0 mM, $MgSO_4$ — 0.5 mM, $MgCl_2$ — 1.0 mM, $NaHCO_3$ — 4.2 mM, $CaCl_2$ — 2.5 mM, and KH_2PO_4 — 1.0 mM. The resulting SBF was buffered to pH = 7.4 with Trisma-HCl and held at 37°C during the incubation periods. The SBF solutions were refreshed daily to ensure

adequate ionic concentrations for mineral growth. The porosity of scaffolds was calculated before and after mineralization treatment using the known density of the solid polymer, the known density of carbonated apatite [28], the measured mass of mineral and polymer in the scaffolds, and the volume of the scaffold (defined by the outside dimensions of the scaffold, and measured using Vernier calipers).

2.2. Characterization of mineral growth

To analyze mineral growth on gas foamed PLG scaffolds, sets of three scaffolds were incubated in SBF for periods ranging from 0 to 10 days. Samples were removed from solution, lyophilized to dryness, and analyzed after 0, 2, 4, 8, and 10 day incubation periods. The dry mass of each scaffold was measured before and after incubation in SBF, and percent increases in mass were calculated and compared using ANOVA, and a Student's *t*-test to reveal significant differences in mass for different SBF incubation times.

The amount of phosphate present in the scaffolds after the aforementioned incubation times was determined using a previously described colorimetric assay [11]. The phosphate mass data were also compared using ANOVA, and a Student's *t*-test to reveal significant differences in mass for different SBF incubation times. To estimate the amount of apatite on the scaffold after a 6 day incubation, the measured mass of phosphate was multiplied by the known ratio of mass of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$, f.w. = 1004.36 g] to mass of phosphate in hydroxyapatite (569.58 g). This is a conservative estimate, since it assumes that all phosphate is being incorporated into stoichiometric hydroxyapatite. This mineral mass estimate increases if one assumes increasing substitution of carbonate into the mineral crystal.

2.3. VEGF release measurements

In order to assess the incorporation efficiency of VEGF into the PLG scaffolds and to track the VEGF release kinetics from the scaffolds, receptor-grade ^{125}I-labeled human VEGF (90 μCi/μg; Biomedical Technologies Inc.; Stoughton, MA) was utilized as a tracer. In place of the 3 μg VEGF in the normal sample preparation, 0.5 μCi of radiolabeled VEGF was added to each matrix. To assess VEGF incorporation efficiency, the total incorporated activity was compared to the activity of the initial ^{125}I VEGF sample prior to incorporation into the scaffolds.

To determine the effects of mineral growth on factor release, release kinetics were measured both in SBF during mineral formation and in phosphate buffered saline (PBS). Scaffolds prepared with radiolabeled VEGF were placed in 4 ml of SBF or PBS and held at 37°C. At various set times, the scaffolds were removed from solution and their radioactivity was assessed using a gamma counter. After each analysis, solutions were refreshed and scaffolds were placed back into solution. The amount of radiolabeled VEGF released from the scaffolds was determined at each time point by comparing the remaining ^{125}I VEGF to the total originally loaded into each scaffold. The percent release of VEGF from scaffolds incubated in SBF was compared to that of scaffolds incubated in PBS at each time point via a Student's t-test to reveal significant differences in cumulative release.

2.4. In vitro analysis of the biological activity of released VEGF

The biological activity of VEGF incorporated into, and released from, polymer matrices was determined by testing its ability to stimulate the growth of cultured human dermal microvascular endothelial cells isolated from neonatal dermis (HMVEC(nd), Cascade Biologics; Portland, OR). HMVEC(nd) were cultured to passage 7 in MCDB 131 media (Cascade Biologics) supplemented with Cascade Biologics' microvascular growth supplement (5% fetal bovine serum, hydrocortisone, human fibroblast growth factor, heparin, human epidermal growth factor, and dibutyryl cyclic AMP) prior to use. Cells were plated at a density of 5×10^3 cells/cm^2 on 12 well tissue culture dishes (Corning; Cambridge, MA) which were precoated with 1 μg/cm^2 human plasma fibronectin (Life Technologies, Grand Island, NY). The cells were allowed to attach for 24 h, and the media in each well was replaced then with 3 ml of serum-free media (Cell Systems; Kirkland, WA) supplemented with 50 μg/ml gentamicin (Life Technologies). A 12 mm transwell (3 μm pore diameter, Corning) containing either mineralized, or non-mineralized, VEGF releasing matrix was placed in each experimental well ($n = 5$ for each group), while mineralized matrices containing no VEGF were placed in the control wells ($n = 5$). To determine the dose response to known concentrations of VEGF, additional wells ($n = 4$ per concentration) were supplemented with 40, 20, 10, and 5 ng/ml of soluble VEGF which had not been incorporated into matrices. After 72 h all of the cells in the experimental and control wells were removed with a solution of 0.05% trypsin/0.53 mM EDTA (Life Technologies), and counted using a ZM Coulter counter (Coulter; Miami, FL). The transwells containing the matrices were immediately transferred to new fibronectin-coated (1 μg/cm^2) wells that had been seeded with cells (5×10^3 cells/cm^2) 24 h before, and allowed to incubate for an additional 72 h before the cells were removed and counted. A new set of VEGF dose response wells were also set up concurrent with the transfer of the transwells. The 72 h cycles were continued for 12 days. Cell counts in experimental wells were compared to cell counts in control wells for each 72 h interval using a Student's *t*-test to reveal significant differences in HMVEC proliferation.

3. Results

Incubation of gas foamed 85 : 15 poly(lactide-co-glycolide) scaffolds containing VEGF resulted in the growth of bone-like mineral on the inner pore surfaces. Analysis of variance showed that differences in percent mass gain with SBF incubation time were significant ($p < 0.05$). The scaffolds showed an increase in mass with incubation time, with a $6 \pm 1\%$ mass gain after a 4 day incubation in SBF (Fig. 1). The scaffold mass subsequently remained relatively constant. The increase in mass between 2 and 4 day incubation times was significant ($p < 0.05$), while there was no significant difference in percent mass gain between the 4 day incubation time and the longer incubation times ($p > 0.05$). A previous study on scaffolds fabricated with the same 85 : 15 PLG used in this study demonstrated that there is no significant mass loss due to polymer degradation during a 16 day incubation in *Tris*-HCl (pH = 7.4) [11]. Thus polymer degradation was not expected to effect the mass measurements in this study. To verify that the increase in mass was caused by the deposition of an apatitic mineral, the mass of phosphate in the scaffolds was analyzed. Phosphate content within scaffolds increased with SBF incubation time (Fig. 2). Analysis of variance showed that differences in phosphate content with SBF incubation time were significant ($p < 0.05$). The difference in phosphate content between the 2 and 6 day incubation times was significant ($p < 0.05$), while there was no significant difference between the phosphate mass of the six day incubation time and longer incubation times ($p > 0.05$). We have previously shown that the increase in mass and phosphate content in these scaffolds indicates growth of a continuous bone-like mineral film on the inner pore surfaces [11]. The total porosity of the scaffolds after a 10 day incubation in SBF was $92 \pm 1\%$, which is similar to the initial scaffold porosity ($93 \pm 1\%$).

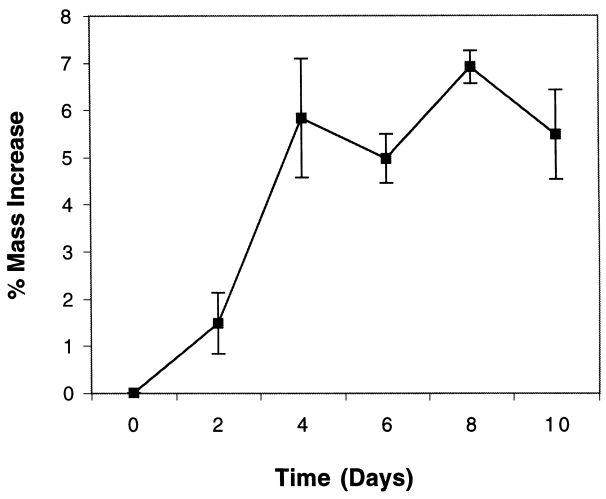

Fig. 1. Percent mass increase vs. incubation time for PLG scaffolds incubated in SBF. Values represent mean and standard deviation ($n = 3$).

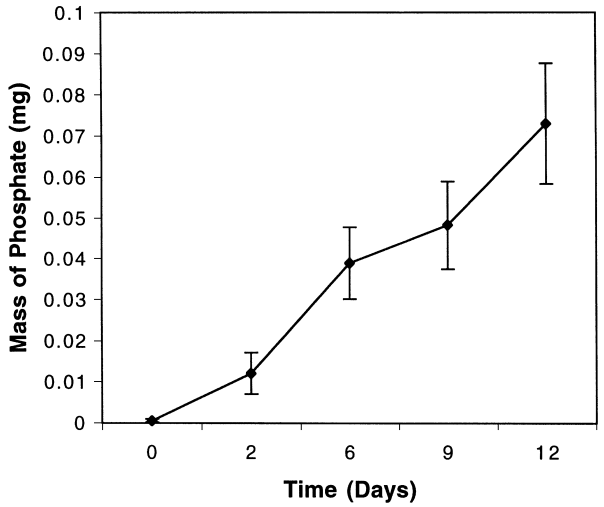

Fig. 2. The mass of phosphate present in scaffolds vs. incubation time in SBF. Values represent mean and standard deviation ($n = 3$).

After a 6 day incubation, estimation of the mass of mineral on the scaffold using phosphate mass data gives 0.10 mg of hydroxyapatite, while the measured mass increase of the scaffold is 0.39 ± 0.03 mg. The fact that the measured value is larger than the estimated value suggests significant carbonate substitution in the mineral crystal.

Vascular endothelial growth factor was incorporated into PLG scaffolds with an efficiency of $44 \pm 9\%$ and released in a similar manner over a 15 day period in SBF and PBS solutions. An initial burst release of the incorporated growth factor was observed over the first 36 h followed by a sustained release for the remainder of the experiment (Figs. 3a and b). The cumulative release from mineralizing scaffolds incubated in SBF was significantly smaller than release from scaffolds incubated in PBS from 3–10 days ($p < 0.05$). At time points beyond 10 days there is no significant difference in cumulative release from scaffolds incubated in SBF versus those incubated in PBS ($p > 0.05$).

Vascular endothelial growth factor released from mineralized and non-mineralized scaffolds had a mitogenic effect on human dermal microvascular endothelial cells (HMVECs). Cells were grown in wells containing three different scaffold types: (1) Mineralized, VEGF-containing scaffolds (MV scaffolds); (2) non-mineralized, VEGF containing scaffolds (NV scaffolds); and (3) mineralized control scaffolds without VEGF (MC scaffolds). Cells grown in wells containing MV and NV scaffolds demonstrated significantly increased proliferation when compared with cells grown in wells containing MC scaffolds (Fig. 4a). Cell counts were significantly higher in wells containing MV and NV scaffolds for all time intervals

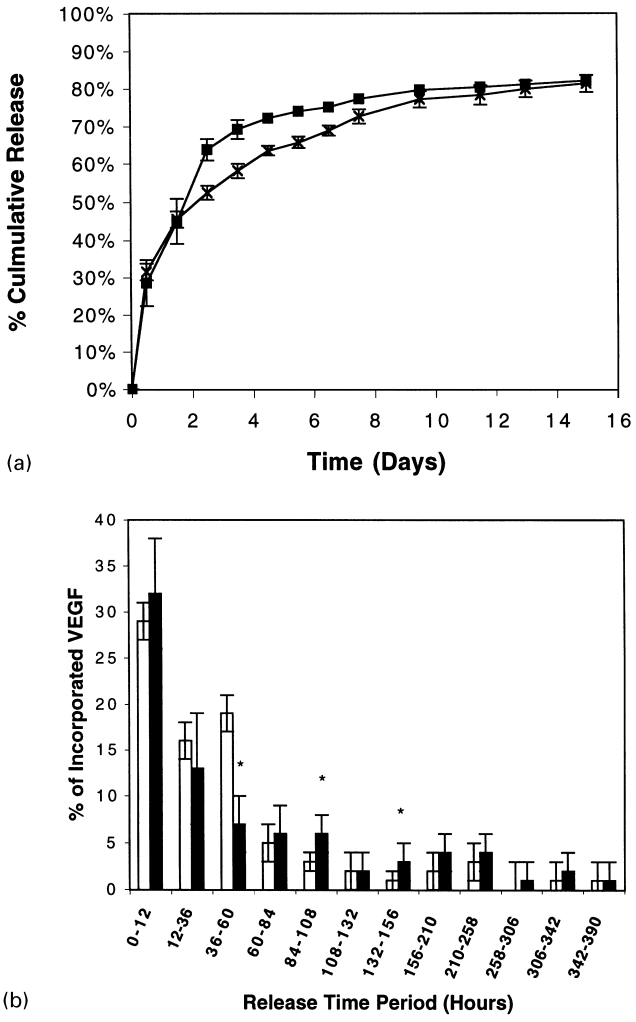

Fig. 3. (a) Cumulative release of VEGF from mineralized (X) and non-mineralized (■) scaffolds. Values represent mean and standard deviation ($n = 5$). (b) Daily release of incorporated VEGF from mineralized (■) and non-mineralized (□) scaffolds. Values represent mean and standard deviation ($n = 5$), and *'s denote statistically significant difference ($p < 0.05$) in release between mineralized and non-mineralized scaffolds.

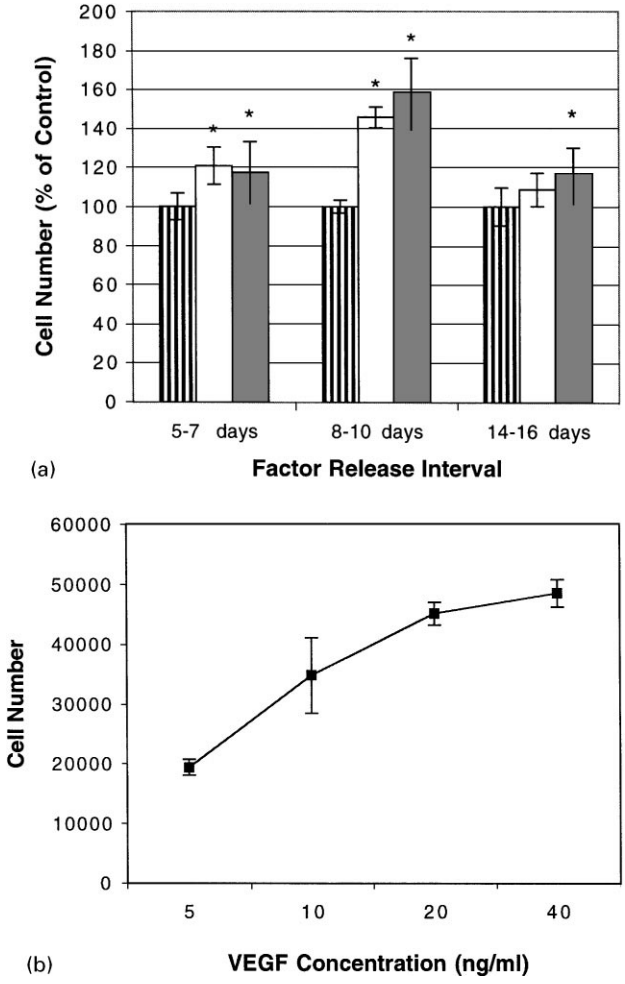

Fig. 4. (a) Stimulatory effect of VEGF release from mineralized (■) and non-mineralized (□) scaffolds on human dermal microvascular endothelial cells. Cell counts for each release time interval are given as percents of the control value (striped column) for that interval. Values that are significantly larger than their corresponding control are indicated by *'s. Values represent mean and standard deviation ($n = 5$). (b) Sample dose–response curve demonstrating the mitogenic effect of VEGF on human dermal microvascular endothelial cells. Values represent mean and standard deviation ($n = 5$).

($p < 0.05$) with the exception of the wells containing NV scaffolds over the 14–16 day factor release interval. During the 8–10 day factor release interval, MV scaffolds showed a significantly greater mitogenic effect on HMVECs than NV scaffolds ($p < 0.05$). There was no significant difference in the stimulatory effect of MV scaffolds versus NV scaffolds for any other time interval ($p > 0.05$).

A dose-response curve (Fig. 4b) generated for the HMVECs was used to calculate an effective concentration for the released growth factor. Comparison of this effective concentration with the amount of VEGF known to be released during each time interval (Fig. 3) indicates that the released VEGF is over 70% active for all time intervals.

4. Discussion

Bone-like mineral can be formed on the inner pore surfaces of PLG scaffolds using a five day incubation in simulated body fluid, and the mineral does not lead to an appreciable decrease in total scaffold porosity. Sustained release of a bioactive factor was achieved from PLG scaffolds during the mineralization process. The mineral presence slightly slowed the release of the growth factor from the scaffold, resulting in release of less VEGF during the 36–60 h. release interval, and a greater amount of factor during the 84–108 h, and the 132–156 h. release intervals. The mineral layer had a minimal effect on the bioactivity of vascular endothelial growth factor released from the scaffold.

A 5 day incubation in SBF is sufficient for growth of bone-like mineral on the inner pore surfaces of a porous PLG scaffold. Quantification of percent mass gain and phosphate content suggests that the majority of mineral growth occurs between days 2 and 4 of incubation. Previous studies of bone-like mineral formation on polyester surfaces have shown varying mineral growth results after 5 day incubations in SBF. Incubation of poly(methyl methacrylate), and poly(ethylene terephthalate) in a solution with 1.5 times the ionic concentrations of SBF for 6 days resulted in no trace of mineral formation [29]. Incubation of poly(L-lactic acid) (PLLA) scaffolds in SBF for 6 days resulted in nucleation and growth of a low density of hydroxyapatite particles [30]. Similar to the current study, 85 : 15 PLG scaffolds prepared via a solvent casting/particulate leaching technique showed nucleation and growth of bone-like mineral crystals, but after a 6 day incubation the mass increase was only $3 \pm 1\%$ [11]. We hypothesize that 85 : 15 PLG prepared in this study via the gas foaming/particulate leaching technique has more surface carboxylic acid groups than other polyester materials subjected to incubation in SBF, and this greater surface functionalization leads to more rapid nucleation and growth of apatitic mineral. In addition, the particulate leaching of scaffolds used in this study was done in a 0.1 M $CaCl_2$ solution prior to incubation in SBF, which may have facilitated chelation of Ca^{2+} ions and thus more rapid bone-like mineral nucleation.

Sustained release of bioactive VEGF from PLG scaffolds can be achieved in the presence of a bone-like mineral. During the foaming process the factor and a factor-containing alginate hydrogel component are incorporated into the PLG scaffold as previously shown [22]. The incorporation efficiency ($44 \pm 9\%$) in this process was reasonable, but it indicates that a relatively large amount of factor is released during the particulate leaching step, which suggests that there is a large amount of VEGF bound to the inner pore surfaces of the scaffold after the foaming process. Both the scaffolds incubated in SBF and the scaffolds incubated in PBS display a burst release in the first 1.5 days followed by sustained release of factor for up to 15 days. In each case, the initial burst effect is likely due to release of VEGF bound to the inner pore surfaces of the scaffold, while the subsequent sustained release is likely due to diffusion of the incorporated VEGF out of the polymer scaffold and the alginate hydrogel component within the scaffold [22]. Polymer degradation is not expected to be a substantial mechanism of factor release in this study, since a previous study shows no significant mass loss from 85 : 15 PLG scaffolds ($IV = 0.78$) over a 16 day time period [11]. Release of a bioactive factor from a mineralized scaffold is an exciting result for bone tissue engineering because it combines the osteoconductive qualities of a bone-like mineral with the tissue inductive qualities of a protein growth factor. VEGF release could be specifically useful in the induction of vascular tissue ingrowth for tissue engineering, and this system could potentially be used with a variety of other inductive protein growth factors.

The growth of mineral on the surface of porous PLG does not change the overall shape of the VEGF release profile when compared with control samples incubated in PBS, although there are slight differences in release characteristics. The release profiles from both mineralized and non-mineralized scaffolds display a sustained release of VEGF for up to 15 days in vitro (Fig. 3a), however, there was a significantly smaller amount of factor released from scaffolds incubated in SBF during the 36–60 h. release interval (Fig. 3b). This time interval correlates with the onset of mineral growth according to the mass increase data (Fig. 1) and the phosphate mass data (Fig. 2). The net effect of the difference in release over the 36–60 h time interval is that a significantly larger amount of VEGF is released during two later time intervals (84–108 h, and 132–156 h) (Fig. 3b). Achieving significantly greater factor release at later release intervals is a substantial goal in polymeric drug delivery, and it has been addressed in a variety of ways in previous studies using various poly(α-hydroxy acid) formulations [31–37].

Formation of a mineral layer within the pores of PLG scaffolds had little effect on the ability of released growth factor to stimulate proliferation of human dermal microvascular endothelial cells. The possibility of protein denaturation and aggregation upon exposure to moisture is a justified concern in the controlled release of proteins from polymer systems [38]. In this case, the protein is clearly bioactive for eleven days after mineralization treatment (16 days after sample preparation). The 11 day time scale was chosen for analysis in this study because a large percentage of transplanted cells fail to engraft and die within this time period without the development of a vascular supply to augment mass transport [23,38]. Sustained release over this time scale induces increased proliferation of endothelial cells in vitro, and may lead to angiogenesis during the initial stages of osseous tissue development in vivo.

In this study, a system has been developed for sustained release of polypeptide growth factors from mineralized PLG scaffolds for orthopaedic tissue engineering. The presence of a bone-like mineral has been shown to be beneficial for conduction of osteogenic cells into various porous synthetic constructs [39,40], and so the mineral has been associated with increased bioreactivity [41]. Therefore, the mineral grown in the present study is expected to provide enhanced osteoconductivity in addition to the inductive angiogenic effect of protein release. The growth of the mineral is accomplished via a simple single step, room temperature process which, importantly, does not compromise growth factor bioactivity, or total scaffold porosity.

Acknowledgements

The authors are grateful for financial support from the NIH (R01 DE13033). They also would like to acknowledge the NIH for a cellular biotechnology training Grant (T32 GM 08353) to WLM and the Whitaker Foundation for a graduate fellowship to MCP.

References

[1] Langer R, Vacanti JP. Tissue Engng Sci 1993;260:920–6.
[2] Crane GM, Ishaug SL, Mikos AG. Bone Tissue Engng Nat Med 1995;1:1322–4.
[3] Putnam AJ, Mooney DJ. Tissue engineering using synthetic extracellular matrices. Nat Med 1996;2:824–6.
[4] Minabe M. A critical review of the biologic rationale for guided tissue regeneration. J Periodontol 1991;62:171–9.
[5] Kim BS, Mooney DJ. Engineering smooth muscle tissue with a predefined structure. J Biomed Mater Res 1998;41:322–32.
[6] Gilding DG. Biodegradable Polymers. In: Williams DF, editor. Biocompatibility of Clinical Implant materials. Boca Raton, FL: CRC Press, 1981. p. 209–32.
[7] Li SM, Garreau H, Vert M. Structure property relationships in the case of the degradation of massive poly(α-hydroxy acids) in aqueous media. J Mater Sci Mater Med 1990;1:123–39.
[8] Harris LD, Kim BS, Mooney DJ. Open pore biodegradable matrices formed with gas foaming. J Biomed Mater Res 1998; 42:396–402.
[9] Lo H, Ponticiello MS, Leong KW. Fabrication of controlled release biodegradable polymer foams by phase separation. Tissue Engng 1995;1:15–28.
[10] Mikos AG, Thorsen AJ. Preparation and characterization of poly(L-lactic acid) foams. Polymer 1994;35:1068–77.
[11] Murphy WL, Kohn DH, Mooney DJ. Growth of continuous bone-like mineral within porous poly(lactide-co-glycolide) scaffolds in vitro. J Biomed Mater Res 2000;50:50–8.
[12] Hench LL. Bioceramics: from concept to clinic. J Am Ceram Soc 1991;74:1487–510.
[13] Kokubo T. Recent progress in glass-based materials for biomedical applications. J Ceram Soc Japan 1991;99:965–73.
[14] Ripamonti U. Calvarial reconstruction in baboons with porous hydroxyapatite. J Craniofac Surg 1992;3:149–59.
[15] Ishaug-Riley SL, Crane GM, Gurlek A, Miller MJ, Yaszemski MJ, Yasko AW, Mikos AG. Ectopic bone formation by marrow stromal osteoblast transplantation using poly(DL-lactic-co-glycolic acid) foams implanted into the rat mesentery. J Biomed Mater Res 1997;36:1–8.
[16] Shea LD, Wang D, Francheschi RT, Mooney DJ. Bone formation from pre-osteoblasts on 3-d scaffolds. Tissue Engng, in press.
[17] Giannobile WV. Periodontal tissue engineering by growth factors. Bone 1996;19:23S–37S.
[18] Langer R. New methods of drug delivery. Science 1990;249: 1527–32.
[19] Whang K, Tsai DC, Nam EK, Aitken M, Sprague SM, Patel PK, Healy KE. Ectopic bone formation via rhBMP-2 delivery from porous bioabsorbable polymer scaffolds. J Biomed Mater Res 1998;42:491–9.
[20] Wheeler DL, Chamberland DL, Schmitt JM, Buck DC, Brekke JH, Hollinger JO, Joh S, Suh K. Radiomorphometry and biomechanical assessment of recombinant human bone morphogenetic protein 2 and polymer in rabbit radius ostectomy model. J Biomed Mater Res 1998;43:365–73.
[21] Shea LD, Smiley E, Bonadio J, Mooney DJ. DNA delivery from polymer matrices for tissue engineering. Nat Biotech 1999; 17:551–4.
[22] Sheridan M, Shea LD, Peters MC, Mooney DJ. Bioabsorbable polymer scaffolds for tissue engineering capable of sustained growth factor delivery. J Control Rel 2000;64:91–102.
[23] Mooney DJ, Sano K, et al. Long term engraftment of hepatocytes transplanted on biodegradable polymer sponges. J Biomed Mater Res 1997;37:413–20.
[24] Colton CK. Implantable biohybrid artificial organs. Cell Transplant 1995;4:415–36.
[25] Mikos AG, Sarakinos G, Ingber DE, Vacanti J, Langer R. Prevascularization of porous biodegradable polymers. Biotech Bioengng 1993;42:716–23.
[26] Mooney DJ, Kaufmann PM, Sano K, McNamara KM, Vacanti JP, Langer R. Transplantation of hepatocytes using porous, biodegradable sponges. Transplant Proc 1994;26:3425–6.
[27] Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. Science 1989;246:1306–9.
[28] Weast RC, Astle MJ. In: CRC Handbook of Chemistry and Physics. 63rd ed. Boca Raton, FL: CRC Press, 1983. p. B228.
[29] Tanahashi M, Yao T, Kokubo T, Minoda M, Miyamoto T, Nakamura T, Yamamuro T. Apatite coated on organic polymers by biomimetic process: improvement in adhesion to substrate by glow discharge treatment. J Biomed Mater Res 1995;29:339–47.
[30] Zhang R, Ma PX. Porous poly(L-lactic acid)/apatite composites created by biomimetic process. J Biomed Mater Res 1999; 45:285–93.
[31] Cohen S, Yoshioka T, Lucarelli M, Hwang LH, Langer R. Controlled delivery systems for proteins based on poly(lactic/glycolic acid) microspheres. Pharm Res 1991;8:713–20.
[32] Kwong AK, Chou S, Sun AM, Sefton MV, Goosen MFA. In Vitro and in vivo release of insulin from poly(lactic acid) microbeads and pellets. J Control Rel 1986;4:47–62.
[33] Pekarek KJ, Jacob JS, Mathiowitz E. Double-walled polymer microspheres for controlled drug release. Nature 1994;367:258–60.
[34] Kreitz MR, Webber WL, Galletti PM, Mathiowitz E. Controlled delivery of therapeutics from microporous membranes: II. In vitro degradation and release of heparin-loaded poly(D, L-lactide-co-glycolide). Biomaterials 1997;18:597–603.
[35] Athanasiou KA, Singhal AR, Agrawal CM, Boyan BD. In vitro degradation and release characteristics of biodegradable implants containing trypsin inhibitor. Clin Orthop Rel Res 1995;315: 272–81.
[36] Okada H, Doken Y, Ogawa Y, Toguchi H. Preparation of three month depot injectable microspheres of leuprorelin acetate using biodegradable polymers. Pharm Res 1994;11:1143–7.
[37] Sah H, Toddywala R, Chien YW. The influence of biodegradable microcapsule formulations on the controlled release of a protein. J Control Rel 1994;30:201–11.
[38] Ishaug-Riley SL, Crane-Kruger GM, Yaszemski MJ, Mikos AG. Three-dimensional culture of rat calvarial osteoblasts in porous biodegradable polymers. Biomaterials 1998;19:1405–12.
[39] Hench LL. Bioceramics: from concept to clinic. J Am Ceram Soc 1991;74:1487–510.
[40] Kokubo T. Recent progress in glass based materials for biomedical applications. J Ceram Soc Japan 1991;99:965–73.
[41] LeGeros RZ, Daculzi G. In vivo transformation of biphasic calcium phosphate ceramics: Ultrastructural and physicochemical characterizations. In: Handbook of Bioactive Ceramics. Boca Raton, FL: CRC Press, 1990. p. 17–28.