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ScienceDirect
Biomaterials 28 (2007) 1862–1870
Biomaterials
www.elsevier.com/locate/biomaterials

Modulation of protein delivery from modular polymer scaffolds

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Received 24 August 2006; accepted 1 December 2006
Available online 20 December 2006

Abstract

Growth factors are increasingly employed to promote tissue regeneration with various biomaterial scaffolds. In vitro release kinetics of protein growth factors from tissue engineering scaffolds are often investigated in aqueous environment, which is significantly different from in vivo environment. This study investigates the release of model proteins with net-positive (histone) and net-negative charge (bovine serum albumin, BSA) from various scaffolding surfaces and from encapsulated microspheres in the presence of ions, proteins, and cells. The release kinetics of proteins in media with varying concentrations of ions (NaCl) suggests stronger electrostatic interaction between the positively charged histone with the negatively charged substrates. While both proteins released slowly from hydrophobic PCL surfaces, plasma etching resulted in rapid release of BSA, but not histone. Interestingly, although negatively charged BSA released readily from negatively charged collagen (col), BSA released slowly from col-coated PCL scaffolds. Such electrostatic interaction effects were abolished in the presence of serum proteins and cells as evidenced by the rapid release of proteins from col-coated scaffolds. To achieve sustained release in the complex environment of serum proteins and cells, the model proteins were encapsulated into poly(D,L-lactic-*co*-glycolic acid) (PLGA) microspheres, which were embedded within col-coated PCL scaffolds. Protein release from microspheres was modulated by changing the lactide-to-glycolide ratio of PLGA polymer. BSA adsorbed to col released faster than histone encapsulated in microspheres in the presence of serum and cells. Collectively, the data suggest that growth factor release is highly influenced by scaffold surface and the presence of ions, proteins, and cells in the media. Strategies to deliver multiple growth factors and studies which investigate their release should consider these important variables.

Keywords: Growth factors; Controlled release; Scaffolds; Collagen; PLGA microspheres

1. Introduction

The delivery of multiple growth factors at different rates may be required to guide complex tissue regeneration [1–3]. For example, epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) are known to stimulate the growth of the intestinal mucosa [4,5]. However, transforming growth factor β (TGF-β) inhibits the proliferation of intestinal epithelial cells and induces them into differentiation pathway [6]. Thus, temporal control over the delivery of these factors may be important for the regeneration of the functional intestine.

Scaffolds provide not only a surface for cell attachment but also may serve as a carrier to deliver bioactive molecules. The release of growth factors from scaffolds has been achieved by incorporating or immobilizing molecules into polymer scaffold during fabrication [2,7,8], or from different components of prefabricated scaffolds. However, the final release kinetics from all strategies may be influenced by scaffold materials, fabrication methods, and the biological environments.

In vitro release kinetics of protein growth factors from tissue engineering scaffolds are often investigated in aqueous environment [9,10], which is quite different from the in vivo environment. Since ions, proteins, and cells

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doi:10.1016/j.biomaterials.2006.12.006

[11–13] can influence protein–scaffold interactions, this study investigates the release of model proteins with net-positive (histone) and net-negative charge (bovine serum albumin, BSA) from various scaffolding strategies in the presence of proteins and cells.

The release profiles in phosphate buffered saline (PBS) were determined for the release of model proteins with net-positive (fluorescent-labeled histone; p*I* 10.9) and net-negative charge (fluorescent-labeled BSA; p*I* 4.9) from four substrates: collagen (col), polycaprolactone (PCL) surfaces, plasma-etched PCL surfaces (pe-PCL), and col-coated PCL (col-PCL), and from poly(D,L-lactic-*co*-glycolic acid) (PLGA) microspheres. The effects of ions (NaCl), serum (fetal bovine serum; FBS), and cells (smooth muscle cells, SMC) on release kinetics of these model proteins were determined.

2. Materials and methods

2.1. Materials

PCL (intrinsic viscosity 1.04 dL/g), PLGA (lactide:glycolide ratio 85:15, intrinsic viscosity 0.61 dL/g; 50:50, intrinsic viscosity 0.67 dL/g) were purchased from Birmingham Polymers (Birmingham, AL). Alexa Fluor conjugated BSA (A13100, A34786) and histone (H13188) were obtained from Molecular Probes (Eugene, OR). Type I col solution purified from bovine skin was obtained from Vitrogen (Palo Alto, CA). Polyvinyl alcohol (PVA) (87–89% hydrolyzed, Mw 31,000–50,000) and polyvinyl pyrolidone (PVP) (Mw 10,000), chloroform (C2432), and methanol (M3641) were obtained from Sigma (St. Louis, MO). SMC (CRL 2018) were purchased from ATCC (Manassas, VA). $VEGF_{165}$ (293-VE) was purchased from R&D systems (Minneapolis, MN).

2.2. Incorporation of proteins into the col gel

Type I col solution (3 mg/ml) was neutralized with 0.1 N NaOH and mixed with 3 μg of BSA or histone at 4 °C. The mixture was incubated for 1 h at 37 °C for gelation, and the gel was lyophilized on a freeze drier (SP Industries, Inc., Warminster, PA) overnight. The freeze dried gel was immersed in 1 ml of 10 mM PBS (pH 7.4) at 37 °C. The whole incubating solution was removed and replaced with 1 mL of fresh solution at various time points, and the fluorescence of the sample was measured with a spectrofluorometer at excitation and emission wavelength (Fluoromax-3, Jobin Yvon Inc., NJ). To evaluate the effect of the ionic strength, col gels were incubated in different concentrations of NaCl (0.15, 0.5, and 2 N) in distilled water (DW).

2.3. Preparation of porous polymer scaffolds

Polymer scaffolds were fabricated by the solvent casting and particulate leaching technique [14]. Briefly, PCL (20%, w/w) was dissolved in a mixture of chloroform and methanol (67/33, w/w). Sucrose particles were sieved to range 100–150 μm in diameter. Polymer solution mixed with sucrose (polymer/sucrose ratio 5/95, w/w) was cast into Teflon molds (inner diameter: 8 mm, height: 2 mm). Scaffolds were dried in a fume hood and solvents were removed by freeze drying at 100 mTorr and −110 °C (SP Industries, Inc., Warminster, PA) overnight. Sucrose was removed by immersing scaffolds in 1 l of deionized water overnight. Scaffolds were sterilized in 70% ethanol for 20 min, and ethanol was removed by rinsing in deionized water three times for 30 min each.

2.4. Adsorption of protein to the polymer scaffolds

Proteins were adsorbed onto PCL scaffolds by placing 100 μl PBS containing 3 μg of BSA or histone. To avoid leakage, 25 μl of the protein solution was dropped onto the scaffolds at a time at room temperature for 20 min. This process was repeated four times until all of the protein solution was delivered. The protein-adsorbed scaffolds were immersing in 1 ml of PBS at 37 °C. For plasma-etched PCL scaffolds, prior to the adsorbing process, scaffolds were subjected to glow discharge plasma (Harrick Scientific, NY) to make the surfaces more hydrophilic.

2.5. Coating of scaffolds with protein-loaded col and in vitro release of protein

Coated scaffolds were prepared by using col solution containing proteins. Three micrograms of BSA or histone was mixed with 100 μl of neutralized 0.025% col solution at 4 °C. Twenty-five microliters of the mixture was dropped onto PCL scaffolds at room temperature for 20 min. This process was repeated four times until all of the protein-containing col solution was delivered. The coated scaffolds were immersed in 1 ml of PBS at 37 °C. In other experiments, the coated scaffolds were immersed in 0.5% or 5% BSA in PBS or in 10% FBS in Dulbecco's modified Eagle's medium (DMEM). The effect of cells was evaluated by seeding SMC on col-coated scaffolds. SMC were cultivated in DMEM with low glucose, 10% FBS, 10 μg/ml insulin, and 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco, Gaithersburg, MD). Col-coated scaffolds were seeded with 0.5 ml of 1×10^6 cells/ml SMC cell suspension, and an additional 0.5 ml of culture medium was added to the 24-well plates in 37 °C, 10% CO_2, humidified incubators.

2.6. Scanning electron microscopy

Scaffolds were fixed using 2.5% glutaraldehyde (EM Sciences, PA) and dehydrated using a serial graded ethanol (50%, 70%, 95%, and 100%). After dehydration, the specimens were immersed in hexamethyldisilazane (HDMS) (EM Sciences, PA) and air dried. The scaffolds were mounted on aluminum stubs and gold coated with a sputter coater at 20 mA under 70 mTorr for 90 s. Scaffold pore morphology was observed with scanning electron microscopy (SEM, Philips/FEI XL30) at 5 kV.

2.7. Encapsulation of protein into PLGA microspheres

Protein-loaded PLGA microspheres were prepared by a double emulsion solvent evaporation method [15,16]. Briefly, 100 μl of protein solution containing 10 μg of BSA, histone, or VEGF in PBS was emulsified with 500 μl of 0.5% solution of 50/50 or 85/15 PLGA in chloroform using sonicator with a microtip (Sonic Dismembrator 500, Fisher Scientific, NH) for 1 min on ice to get the primary emulsion. The primary emulsion was then emulsified with 2 ml of 2% PVA solution using sonicator for 1 min on ice to produce a water/oil/water emulsion. The resulting double emulsion was then transferred into 40 ml of 0.5% PVA/PVP solution and was magnetically stirred overnight at room temperature to evaporate the solvent. The microspheres were collected by centrifugation at 4500*g* for 30 min and were washed three times with DW.

2.8. Preparation of composite scaffolds and in vitro release of protein

Protein-loaded PLGA microspheres were dispersed in 100 μl neutralized 0.025% col solution at 4 °C, and 25 μl of the mixture was dropped onto PCL scaffolds at room temperature for 20 min. This process was repeated four times until all of microsphere-containing col solution was delivered. Uniform distribution of microspheres was assessed using Alexa Fluor 488 conjugated BSA. Images of composite scaffolds were captured in RGB color digital camera (Optronics, Goleta, CA) with a Leica DM

IRB light microscope (Leica Microsystems Inc., Bannockburn, IL). The internal morphology of composite scaffolds was observed using SEM. Release experiments were carried out in the presence of SMC. To assess ability of this system to deliver two different factors with distinct release kinetics, Alexa Fluor 555 conjugated BSA was incorporated into col layer and Alexa Fluor 488 conjugated histone was incorporated into microspheres of composite scaffolds and the release profile was examined in the presence of SMC.

2.9. Integrity of encapsulated VEGF

Integrity of encapsulated protein was evaluated using VEGF. Freeze-dried microspheres (5 mg) containing VEGF was dissolved in 1 ml of chloroform/acetone (50/50 ratio). The solution was vortexed for 1 min and centrifuged at 14,000*g* for 5 min. The pellet was washed with chloroform/acetone two more times. Following evaporation of organic solvents, the pellet was dissolved in PBS. The protein samples were resuspended in SDS sample buffer, heated at 95 °C for 10 min, and subsequently resolved by SDS-PAGE and transferred to nitrocellulose membranes. The membranes were blocked with 5% milk in Tris-buffered saline containing 0.1% Tween 20 (TBS/T) for 1 h at room temperature, followed by 1 h incubation at room temperature with the rabbit anti-VEGF (1:1000) [17]. After washing with TBS/T, the membranes were then probed with horseradish peroxidase conjugated stabilized anti-rabbit IgG for 1 h at room temperature (1:5000, all dilutions in TBS/T, 5% dried milk). After washing with TBS/T, blots were visualized by chemiluminescence (ECL kit, Pierce, Rockford, IL).

2.10. Biological activity of released VEGF

Ability of release VEGF to induce VEGFR2 phosphorylation of endothelial cells was evaluated to assess the biological activity of released VEGF. VEGF or VEGF-loaded PLGA microspheres were incorporated into col-coated PCL scaffolds. Scaffolds were incubated in Ham's F-12 medium supplemented with 1% BSA for 24 h, and the incubating medium was collected for the assay. Incubating media from scaffolds not containing VEGF and media with soluble VEGF served as negative and positive controls, respectively. Porcine aortic endothelial (PAE) cells (provided by Dr. Gera Neufeld, Technicon, Israel) were grown in Ham's F-12 medium supplemented with 10% FBS. Sub-confluent cells were incubated overnight in serum-free media and were subsequently pre-incubated for 5 min with 0.1 mM Na_3VO_4 (Sigma) to inhibit phosphatase activity. Cultures were then washed once and were incubated with 1 ml of the collected incubating media for 5 min at 37 °C. The incubation was terminated by the removal of the media and washing with cold PBS/0.2 mM Na_3VO_4. Cells were solubilized in lysis buffer (1% Triton X-100, 10 mM Tris-HCl, pH7.6, 150 mM NaCl, 30 mM sodium pyrophosphate, 50 mM sodium fluoride, 2.1 mM sodium orthovanadate, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 2 μg/ml of aprotinin) at 4 °C for 15 min. Insoluble material was removed by centrifugation at 4 °C for 30 min at 14,000*g*. Equal amounts of the soluble cell lysate were separated by SDS-PAGE and were transferred to nitrocellulose membranes. Phosphorylated proteins were detected by immunoblotting using antiphosphotyrosine antibodies (4G10, Upstate, Charlottesville, VA) followed by secondary antibodies coupled with horseradish peroxidase and visualized by chemiluminescence (ECL kit, Pierce). Protein-loading control was assessed by Western blot using anti-VEGFR2 (55B11, Cell Signaling, Danvers, MA).

3. Results and discussion

3.1. In vitro release of proteins from col gels

Col is a popular carrier material because it is a major component of the extracelluar matrix (ECM) known to be a reservoir of endogenous growth factors, and col coating is often used to improve cell affinity to synthetic polymers [18–20]. The immobilization and release of growth factors using col has been employed previously, but the factors that influence their release are not well understood [9,21]. Electrostatic or hydrophobic interaction between growth factors and col, specific binding to col, col degradation rate, and other environmental factors may all influence the release of the incorporated growth factors.

In this study, the electrostatic interaction between proteins and col was evaluated using oppositely charged proteins, BSA or histone, mixed with col. Release profile was observed from col gels to exclude other possible interactions between proteins and substrate, which may exist and mask electrostatic interaction when it is tested on the hydrophobic PCL scaffolds. BSA was released from col gels with an initial burst that was higher than the initial burst release of histone from col gels (Fig. 1(a)). At pH 7.4, histone carries a net positive charge and can interact electrostatically with col, which carries a net negative charge. The electrostatic interaction was further studied by increasing the ionic strength in the incubating medium. The release of BSA was higher in 0.15 N NaCl as compared to water, but there was no significant increase at higher concentrations of NaCl (Fig. 1(b)). In contrast, the addition of NaCl beyond 0.15 N resulted in the significantly increased release of histone, suggesting that positively charged proteins have stronger electrostatic interaction with col (Fig. 1(c)).

Tabata et al. demonstrated electrostatic interaction between protein and polymer using positively or negatively charged gelatin and oppositely charged protein [9]. Slow release of bFGF was observed when it was incorporated into a negatively charged gelatin, in contrast to the release from a positively charged gelatin.

3.2. In vitro release of proteins from polymer scaffolds

Effect of surface hydrophilicity on protein release was evaluated on the PCL scaffolds using BSA or histone as model proteins. Plasma treatment of the PCL scaffolds modified the hydrophobicity of the PCL surface [22]. The release of both BSA and histone was slow from the hydrophobic PCL surface, but the release of BSA was increased on the plasma-treated PCL surface (Fig. 2), indicating hydrophobic interaction of proteins with PCL surface. The slow release of histone from the plasma-treated PCL surface may be due to strong ionic interaction between the positively charged histone and the negatively charged polar groups (such as hydroxyl groups and carboxyl groups) on the plasma-treated PCL surface [22].

3.3. In vitro release of proteins from col-coated scaffolds

Three-dimensional porous PCL scaffolds were coated with a mixture of model proteins incorporated into col. A smooth surface was observed in the scaffolds prior to col

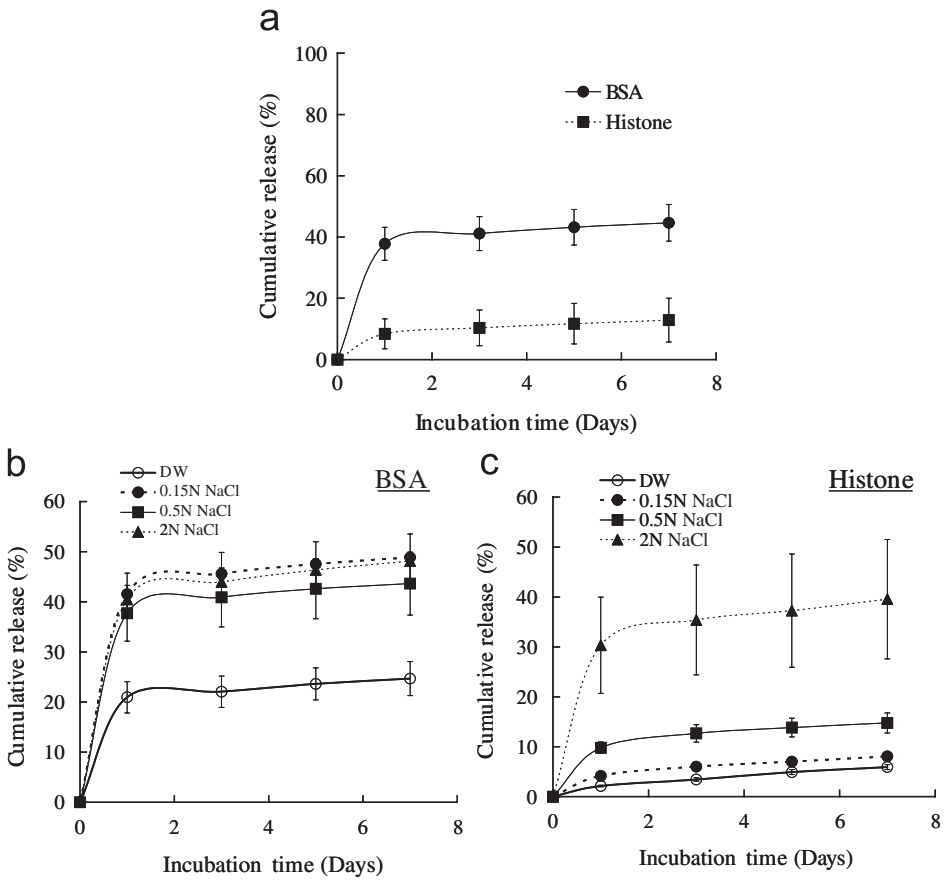

Fig. 1. In vitro release of proteins from collagen gels in PBS (a). The effect of ionic strength on release profile of BSA (b) and histone (c). Collagen gel was incubated in different concentrations of NaCl. Significant change in histone release was observed by the addition of NaCl ($n = 3$, mean ± SD).

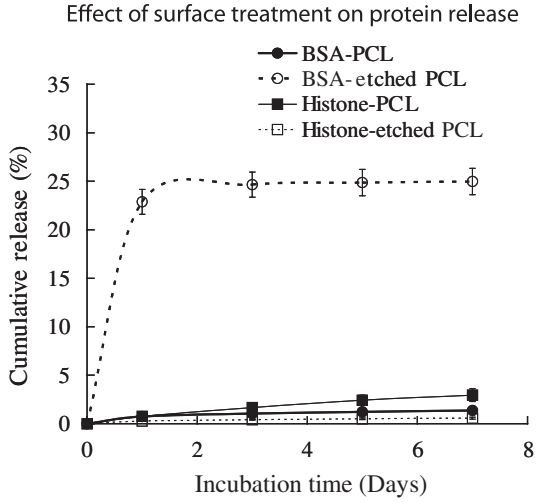

Fig. 2. In vitro release of protein from the PCL (●, ■) or plasma-etched PCL (○, □) scaffolds in PBS. Release of BSA was increased on the plasma-etched PCL surface ($n = 3$, mean ± SD).

coating (Figs. 3(a) and (b)), and a ruffled surface covered with fibers (arrow) was observed after coating with col (Figs. 3(c) and (d)).

The release of proteins from col-coated PCL scaffolds was assessed in the presence of serum and serum proteins which may better represent the physiological conditions, in addition to examining the protein release in the presence of only PBS which is commonly employed in the literature [23,24]. The release of BSA and histone from col-coated scaffolds in PBS was similar to their release in DMEM, a more complex culture medium without protein additives. As compared to their release rates in PBS, the release of both BSA and histone was higher in the media containing serum or additional serum proteins (BSA) (Fig. 4). This is presumably due to the protein displacement by additional proteins in serum resulting in reducing the electrostatic interaction between protein and col. Similar observations of increased release in the presence of serum have been reported using VEGF loaded alginate bead [25].

An additional complexity for protein release in vivo is the presence of cells that may degrade col. In the presence of cells, the release profile was observed for a longer duration than that in the presence of only ions or proteins because release profile with seeded cells reached a plateau slowly. In the presence of SMC seeded on the col-coated scaffolds, the release of BSA and histone was further accelerated as compared to their release in the presence of serum (Fig. 5). It is possible that proteolytic enzymes secreted from cells digested col and resulted in the more rapid release of proteins. Holland et al. [26] demonstrated

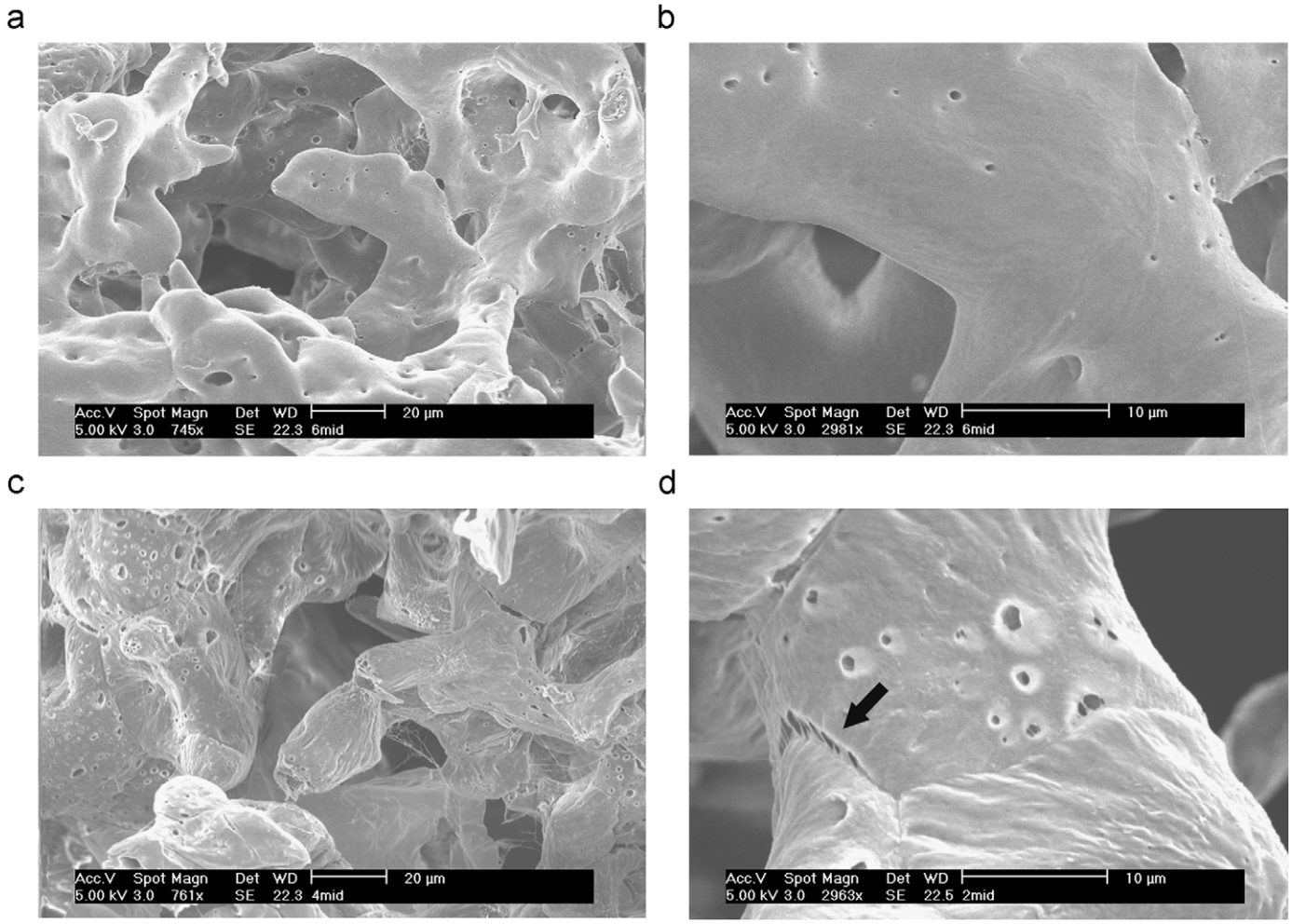

Fig. 3. SEM images of PCL scaffolds prepared with solvent casting and particulate leaching before (a), (b) and after collagen coating (c), (d).

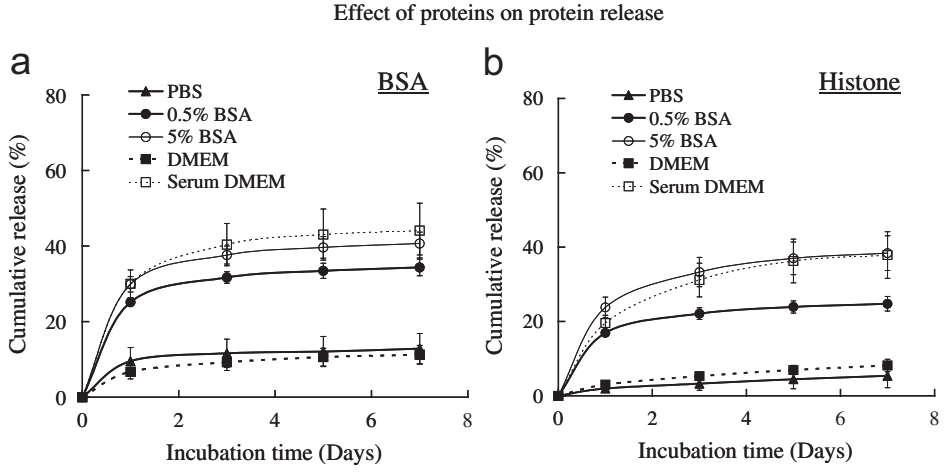

Fig. 4. In vitro release of BSA (a) or histone (b) from the collagen-coated scaffolds. BSA or histone was mixed with collagen and coated onto PCL scaffolds. Coated scaffolds were incubated in PBS (▲) or PBS supplemented with 0.5%, 5% BSA (●, ○) and in DMEM or DMEM supplemented with 10% serum (■, □). Rapid release of both BSA and histone was observed in the media containing BSA or serum ($n = 3$, mean ± SD).

that release of growth factors from the gelatin microparticles was increased by the addition of collagenase in the incubating medium.

These results suggest that delivery systems using electrostatic interaction between protein and carrier polymers may not be suitable for the sustained release of proteins

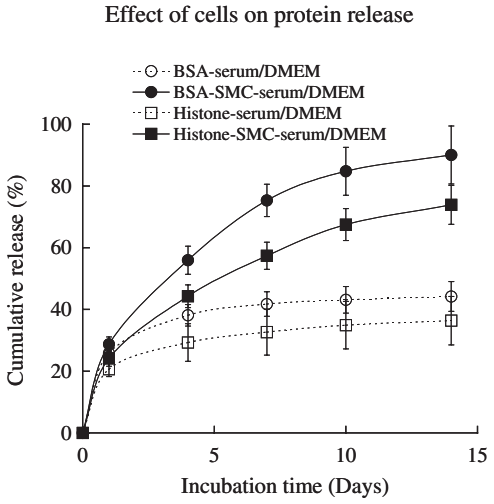

Fig. 5. In vitro release of proteins from the collagen-coated scaffolds cultured with rat vascular smooth muscle cells (●, ■). Release was compared to scaffolds incubated in serum media (○, □) without cells ($n = 3$, mean ± SD). Protein release was further accelerated in the presence of cells.

because more dominant in vivo factors would abolish the electrostatic interaction.

3.4. Preparation of composite scaffolds

The delivery of proteins using biodegradable polymers such as PLGA microspheres has been widely studied [15,16]. PLGA microspheres can be formulated by multiple emulsion methods, and the desired properties such as the size can be obtained by changing processing conditions employed during preparation [27,28]. In this study, BSA or histone was encapsulated into PLGA microspheres using a double emulsion solvent evaporation method. These microspheres were subsequently incorporated into col for coating porous PCL scaffolds. Microspheres with submicron size were fabricated for the stable incorporation into col layer without obstructing the pores of prefabricated scaffolds by changing the extent of homogenization during the second emulsion step (Fig. 6(a)). Fluorescently tagged proteins encapsulated by microspheres mixed with col were used to coat scaffolds and microspheres were uniformly incorporated over the porous scaffolds (Fig. 6(b)). The internal microstructure of microsphere/col-coated scaffolds demonstrated uniform distribution of microspheres on the wall of pores without obstructing the porous structure of the scaffolds (Figs. 6(c) and (d)).

3.5. In vitro release of proteins from the composite scaffolds

The release of proteins from PLGA microspheres depends on the degradation rate of the microspheres, which is a function of the polymer composition, molecular weight, hydrophilicity, and crystallinity [16]. In this study, proteins were encapsulated into PLGA with different lactide content (lactide:glycolide ratio 50:50 or 85:15). In the presence of cells seeded on microsphere/col-coated PCL scaffolds, the 85/15 PLGA microsphere showed a more sustained release of BSA with a lower initial burst release (Fig. 7(a)). Approximately 25% of the initially loaded BSA was released after 14 d. In contrast, BSA was released more rapidly from 50/50 PLGA microspheres under the same conditions, with a higher initial burst release as compared to 85/15 PLGA microspheres. Thus, the rate of protein release from microspheres was modulated by changing the polymer composition even in the presence of cells and serum proteins.

The delivery of multiple growth factors at distinct stages may be necessary to promote complex tissue regeneration [1–3]. Dual growth factor delivery systems were recently described for angiogenesis and cartilage repair [26,29]. To evaluate the feasibility of delivering multiple factors with distinct release profile, we have characterized two practical methods of incorporation under the influence of cells. Histone was encapsulated into 85/15 PLGA microspheres, mixed with col and differently labeled BSA to coat PCL scaffolds. The BSA release from the col coating layer was higher than the observed release of histone from the microspheres in the presence of cells seeded on such composite scaffolds (Fig. 7(b)). Approximately 80% cumulative release of BSA was observed after 14 d, as compared to 25% cumulative release of histone. The release profile of BSA from the composite scaffold was similar to the release of BSA from col-coated scaffold without the microsphere (Fig. 5), indicating that neither microspheres nor the released histone altered the release of BSA.

3.6. Bioactivity of VEGF in composite scaffolds

The integrity of proteins encapsulated within microspheres was confirmed by using VEGF. Proteins extracted from VEGF-loaded PLGA microspheres exhibited a dominant band with a molecular weight of 22 kDa, which is identical to the nonencapsulated VEGF (Fig. 8(a)). A minor band was observed with a lower molecular weight, but this was also observed with the nonencapsulated VEGF. This indicated that the emulsion process used to entrap proteins within the microspheres did not alter the size of the encapsulated VEGF.

The biologic activity of the released VEGF was investigated by assessing its ability to activate vascular endothelial growth factor receptor 2 (VEGFR2) on endothelial cells [30]. The incubating media collected from VEGF/col-coated scaffolds or composite scaffolds containing VEGF-loaded PLGA microspheres induced VEGFR2 phosphorylation (Fig. 8(b)), indicating that the biologic effect of VEGF was preserved during the incorporation procedure either into col or PLGA microspheres. The incubating media collected from col-coated scaffolds induced stronger phosphorylation of VEGFR2 as compared to that collected from the composite scaffolds,

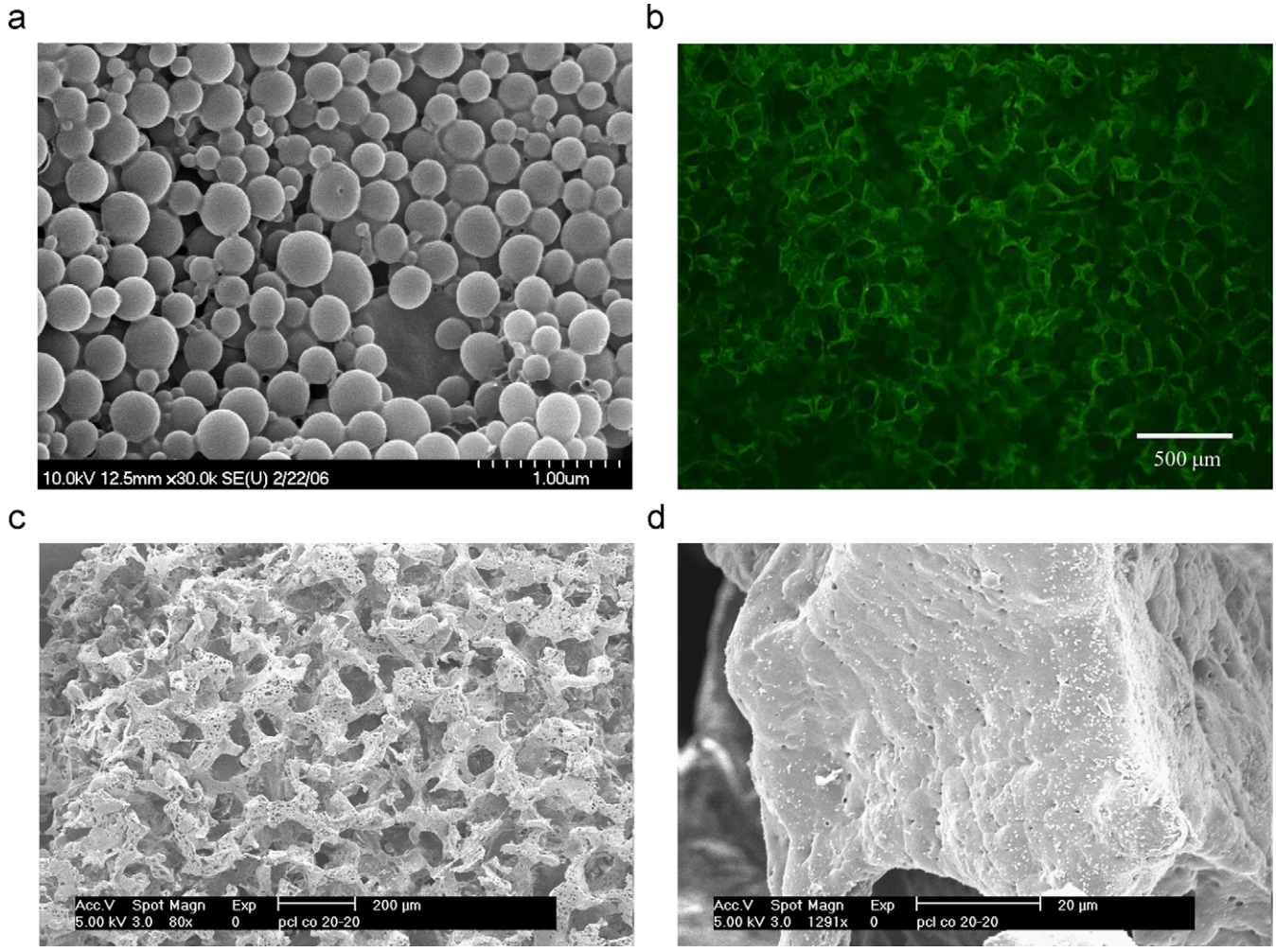

Fig. 6. (a) SEM images of PLGA microspheres and (b) fluorescent image of composite scaffolds showing uniform coating using fluorescent BSA-containing microspheres. Internal morphology of composite scaffolds by SEM showing open pore structure (c) and uniform distribution of microspheres on the wall of the pores (d).

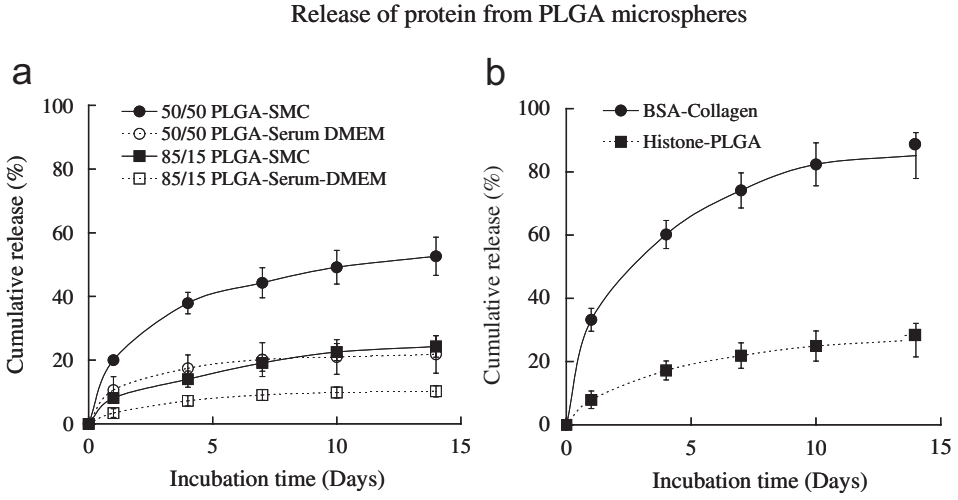

Fig. 7. (a) In vitro release of BSA encapsulated in 50/50 or 85/15 PLGA microspheres of composite scaffolds in serum media (○, □) or in the presence of rat vascular smooth muscle cells (●, ■). (b) In vitro simultaneous release of BSA (●) and histone (■) from the dual delivery system cultured with rat vascular smooth muscle cells. Histone-loaded 85/15 PLGA microspheres and BSA were incorporated into collagen-coated PCL scaffolds ($n = 3$, mean $\pm$ SD). BSA release from the collagen coating was relatively faster than the observed release of histone from microspheres.

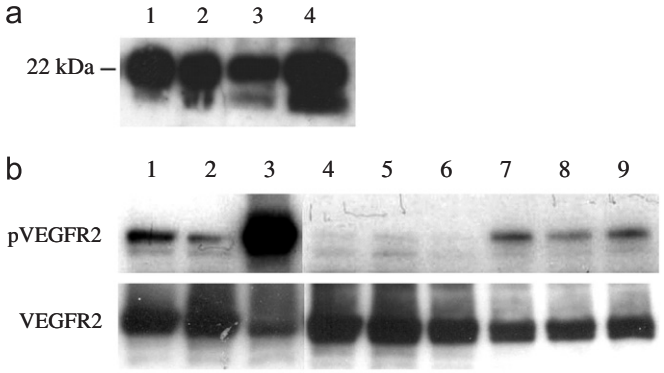

Fig. 8. (a) Western blot analysis of VEGF extracted from PLGA microspheres. Lanes 1 and 2: standard VEGF; lanes 3 and 4: VEGF extractred from 50/50 or 85/15 PLGA microsphere respectively. (b) Bioactivity of VEGF released from 50/50 PLGA microsphere (lane 1), 85/15 PLGA microsphere (lane 2), and collagen (lane 3) of composite scaffolds. VEGF or VEGF-loaded PLGA microspheres were incorporated into collagen-coated PCL scaffolds and incubated for 24 h. Porcine aortic endothelial (PAE) cells were exposed to the collected incubating media for 5 min and VEGFR2 phosphorylation of PAE was evaluated. Release media from the same formulations without VEGF served as negative controls (lanes 4–6) and release media supplemented with VEGF served as positive controls (lanes 7–9). Inset shows VEGFR2 levels of the same samples resolved in a parallel Western blot.

consistent with the expected higher release of VEGF from col as compare to the release from PLGA microspheres. Similar results have been reported by incorporating VEGF directly into polymer scaffold or pre-encapsulated in PLGA microspheres [31]. Release of VEGF directly incorporated into scaffolds was higher than that of VEGF pre-encapsulated in microspheres and more sustained release was observed when incorporated into 85/15 PLGA microspheres compared to 50/50 PLGA microspheres. Other studies reported greater release of VEGF from col matrices compared to that of VEGF pre-encapsulated in microspheres in vivo test [32].

4. Conclusions

Collectively, the data suggest that growth factor release is highly influenced by scaffold surface, and the presence of ions, proteins, and cells in the media. The sustained release in this complex environment was demonstrated by encapsulating proteins into microspheres and their release was further controlled by changing the composition of microspheres. Furthermore, the material-processing steps did not reduce the biologic activity of the released growth factors. Strategies to deliver multiple growth factors and studies which investigate their release should consider these important variables.

Acknowledgments

This work was supported by the Fubon Foundation.

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