Enhancing the vascularization of three-dimensional porous alginate scaffolds by incorporating controlled release basic fibroblast growth factor microspheres

Anat Perets,[1] Yaacov Baruch,[2] Felix Weisbuch,[2] Gideon Shoshany,[3] Gera Neufeld,[4] Smadar Cohen[1,5]
[1]*Department of Biomedical Engineering, Ben-Gurion University of the Negev, Beer Sheva, Israel*
[2]*Liver Unit, Medical Center and The Bruce Rappaport Faculty of Medicine, Haifa, Israel*
[3]*Department of Pediatric Surgery, Rambam Medical Center and The Bruce Rappaport Faculty of Medicine, Haifa, Israel*
[4]*Faculty of Biology, Technion-Israel Institute of Technology, Haifa, Israel*
[5]*Dept. of Biotechnology Engineering and The Institute for Applied Biosciences, Bldg. 39, Room 222 Ben-Gurion University of the Negev, Beer Sheva, Israel 84105*

Received 3 May 2002; revised 5 August 2002; accepted 13 August 2002

Abstract: Site-specific delivery of angiogenic growth factors from tissue-engineered devices should provide an efficient means of stimulating localized vessel recruitment to the cell transplants and would ensure cell survival and function. In the present article, we describe the construction of a novel porous alginate scaffold that incorporates tiny poly (lactic-*co*-glycolic acid) microspheres capable of controlling the release of angiogenic factors, such as basic fibroblast growth factor (bFGF). The microspheres are an integral part of the solid alginate matrix, and their incorporation does not affect the scaffold porosity or pore size. *In vitro*, bFGF was released from the porous composite scaffolds in a controlled manner and it was biologically active as assessed by its ability to induce the proliferation of cardiac fibroblasts. The controlled delivery of bFGF from the three-dimensional scaffolds accelerated the matrix vascularization after implantation on the mesenteric membrane in rat peritoneum. The number of penetrating capillaries into the bFGF-releasing scaffolds was nearly fourfold higher than into the control scaffolds (those incorporating microspheric BSA and heparin but not bFGF). At day 10 posttransplantation, capillary density in the composite scaffolds was 45 $\pm$ 3/mm^2 and it increased to 70 $\pm$ 7/mm^2 by day 21. The released bFGF induced the formation of large and matured blood vessels, as judged by the massive layer of mural cells surrounding the endothelial cells. The control over bFGF delivery and localizing its effects to areas of need, may aid in the wider application of bFGF in therapeutic angiogenesis as well as in tissue engineering. © 2003 Wiley Periodicals, Inc. J Biomed Mater Res 65A: 489–497, 2003

Key words: bFGF; controlled release; PLGA microspheres; alginate scaffolds; vascularization; tissue engineering

INTRODUCTION

Tissue engineering has evolved to address the increasing need in tissues and organs for transplantation. The neotissue is constructed by seeding functional cells, originated from either donors or differentiated stem cells, within three-dimensional (3D) polymeric scaffolds, which provide the biomechanical support until the cells develop into a functioning tissue.[1,2] Thus, an ideal scaffold should be fabricated from materials conducive to the seeded cells, such as polysaccharides resembling the glyco-components of the extracellular matrix. It should be highly porous to accommodate a large number of cells and should possess pores with diameters >50 μm to allow vascular ingrowth after device implantation.

To meet these requirements, we developed porous scaffolds from calcium-crosslinked alginate solutions by a freeze-dry method.[3,4] Alginate, a polysaccharide derived from brown seaweed, is a biocompatible polymer widely used as a material for cell transplantation.[5,6] The alginate scaffolds were fabricated to possess 90% porosity and pore diameters of 100–150 μm.[3] The hydrophilic nature of the alginate scaffolds en-

Correspondence to: Prof. Smadar Cohen; e-mail: scohen@bgumail.bgu.ac.il
Contract grant sponsor: Israel Science Foundation; contract grant number: 52/99-1
Contract grant sponsor: Israel Ministry of Science
Contract grant sponsor: Niedersachsen; contract grant number: 180039

abled high cell loading and the seeded cells maintained viability and function in culture.[7,8] 3D cardiac muscle constructs based on alginate scaffolds are capable of preventing the deterioration in cardiac function after an extensive myocardial infarction in rats.[9] Histological cross sections in the 1-mm thick implanted grafts, 55 days postimplantation, revealed an extensive vascular ingrowth into the implanted biograft from neighboring coronary vessels and well-formed striated cardiac fibers within the biograft.

In thicker implants, however, nutrient and waste transport to and from the transplanted cells may be a major concern. Cells that are more than 0.2–0.5 mm from a blood vessel suffer from hypoxia and limitation of other nutrients.[10] Although capillary ingrowth is often noted in the implanted thick cellular constructs over time, the vascularization is either too slow or limited to provide sufficient nutrient transport for most of transplanted cells. Thus, stimulation of blood vessel ingrowth into the implant would assure tissue survival and function. One way of achieving this goal is by incorporating angiogenic factors into the scaffold, such as the potent basic fibroblast growth factor (bFGF), an 18-kDa heparin-binding protein, which induces the proliferation of endothelial cells, fibroblasts, smooth muscle cells, and hepatocytes.[11–14]

Yet, the incorporation of bFGF into open-pore matrices imposes a great challenge because of the inherent inability of these matrices to efficiently entrap and control the release of macromolecules. Several groups attempted to overcome this challenge by attaching the bFGF to heparinized matrices[15–17] or by incorporating bFGF containing microspheres into the matrix.[18] In the present work, we explore and develop alginate composite scaffolds, which incorporate 3-μm diameter microspheres without interfering the porous structure of the scaffold. These microspheres are capable of controlling the release of the angiogenic factor, bFGF. After implantation on the mesenteric membrane in rat peritoneum, the extent of matrix vascularization, and capillary morphology were evaluated and compared with that observed in the control composites, that is, scaffolds containing microspheric bovine serum albumin and heparin but no bFGF.

MATERIALS AND METHODS

Materials

Recombinant human basic fibroblast growth factor (rHu bFGF) was produced in *Escherichia coli* using the T7-based expression vector pet-8C and was affinity-purified on heparin-Sepharose columns.[15] Bovine serum albumin (BSA), poly (vinyl alcohol), and sodium heparin were from Sigma Chemical Co. (St Louis, MO). Poly (D,L-lactide-co-glycolide) (PLGA; 0.2 dL/g, RG 502H) was from Boehringer Ingelheim (Ingelheim, Germany). Sodium alginate (Protanal LF120), with 70% guluronic acid (G) content, was from Pronova Biopolymers (Drammen, Norway). The media and supplements were purchased from Biological Industries, Kibbutz Beit Ha'Emek (Israel), unless otherwise specified. All chemicals were analytical grade.

Encapsulation of bFGF within PLGA microspheres

PLGA microspheres containing bFGF (0.5 μg/mg polymer), heparin (0.5 μg/mg polymer), and BSA (50 μg/mg polymer) or BSA and heparin but no bFGF (control) were prepared by the solvent extraction method based on a double emulsion.[19,20] The co-encapsulation of albumin and heparin intended to stabilize bFGF during encapsulation and release. Microsphere size was manipulated by changing the extent of homogenization during the second emulsification.[21] The size was analyzed using a laser light-quenching particle size analyzer (Galai Cis-1, Israel). Enzyme-linked immunosorbent assay (ELISA; Quantikine Human FGF Basic Immunoassay; R&D Systems, Minneapolis, MN) determined the percentage of bFGF loading into the microspheres after dissolving them in dichloromethane and extracting the protein into phosphate buffer, pH 7.

Preparation of composite alginate scaffolds

The alginate composite scaffolds were fabricated as described in Perets et al.[22] Briefly, sodium alginate was dissolved in water, to a final concentration of 2 % (w/v) and filtered through 1.2-, 0.45-, and 0.2-μm nylon membrane filters. Basic FGF- or BSA-containing microspheres were dispersed in the sterile alginate solution (40 mg microspheres/mL alginate), and the alginate was crosslinked with calcium ions by adding an equal volume of 0.4% (w/v) calcium gluconate solution. One mL/well of the crosslinked alginate solution was placed in 24-well plate, frozen in liquid nitrogen (−196°C) and lyophilized at <10 mbar until dry.

Scanning electron microscopy

The morphology of alginate composite scaffolds and PLGA microspheres was observed by scanning electron microscopy (SEM, JEOL JSM-35CF). PLGA microspheres or thin sections (20 μm) of the composite scaffolds were attached to sample stubs with conductive paint and sputter-coated with an ultrathin (100 Å) layer of gold in a Polaron E 5100 coating apparatus. The samples were viewed under SEM at an accelerating voltage of 15 kV.

Release studies and bFGF analysis by ELISA

Alginate composite scaffolds containing bFGF were placed in Dulbecco's buffered saline (pH 7.2) and incubated

on a rotating incubator at 37°C. The buffer was replaced daily and the amount bFGF in releasing media was determined by an ELISA. ELISA was performed according to the procedure of the kit (Quantikine Human FGF Basic Immunoassay; R&D Systems, Minneapolis, MN).

Western blot analysis

For determination of the physical state and immunoreactivity of the released bFGF, releasing media from different time intervals were subjected to Western blot analysis. The samples were run on 15% polyacrylamide gels and transferred electrophoretically (Trans-Blot SD, Bio-Rad, Hercules, CA) to nitrocellulose membranes. An equal loading and transfer efficiency were verified by staining with 2% Ponceau S. The membranes were blocked overnight with PBS/5% fat-free skim milk and then incubated for 4 h at room temperature with a 1:500 diluted monoclonal anti human bFGF (R&D). For detection, we used horseradish peroxidase-linked anti human IgG antibody (1:200, 1 h at room temperature; R&D) and enhanced chemiluminescence substrate (Amersham).

Bioassay for bFGF

The biological activity of the released bFGF was determined by testing its ability to stimulate the proliferation of cultured neonatal rat cardiac fibroblasts. The cells were isolated, purified and cultured on two-dimensional culture dishes as previously described.[8] The fibroblasts were plated at a density of 5000 cells/cm^2 on tissue culture dishes and overnight incubated in Dulbecco's modified eagle medium at 37°C in 5% CO_2. After cell adhesion, the culture supernatant was replaced by a fresh medium and 100 μL of the releasing medium was added into each well. After incubation for 96 h, the cells were detached with EDTA/trypsin and the cell number was counted using a hemocytometer.

In vivo angiogenesis

Female Lewis rats (6 to 8 weeks old) were purchased from Harlan Laboratories. The study was performed with the approval and according to the guidelines of the Institutional Animal Care and Use Committee. The rats were anesthetized with inhalational methoxyflurane, prepped and draped. After a midline incision, the alginate composite scaffolds containing bFGF (20 μg; $n = 3$ rats per time point) or the control composites ($n = 3$ rats per time point) were implanted on the mesenteric membrane using 5-0 Prolene, with one polymer construct per animal. At days 5, 10, and 21 days postimplantation, the rats were sacrificed and the scaffolds were retrieved, fixed in formalin, paraffin embedded, and sectioned for histology and stained with hematoxylin-eosin (H&E).

The number of capillaries in 15 different fields for each time point, randomly selected from captured images of three H&E slides from each animal, was determined using ImagePro 3. The results of capillary density are reported as means ± standard deviations. Statistical analysis of data was performed by analysis of variance single factor, using Excel Software 2000.

Immunohistochemistry

Sections (4 μm thick) were cut from the paraffin blocks of the scaffold samples and placed onto precoated slides. The samples were deparaffinized and rehydrated gradually. The endogenous peroxidase was blocked with 0.6% (v/v) hydrogen peroxide in methanol. Immunological detection was achieved with commercially available polyclonal antibodies to Von Willebrand factor (VWF) (Dako) and α-smooth muscle actin (α-SMA; Sigma).

RESULTS

Fabrication of the alginate composite scaffold

The alginate scaffolds are characterized by high porosity (>90%) with an average pore size of 130 μm in diameter and high degree of pore interconnectivity.[3,4] These matrix properties should enable the penetration of blood vessel into alginate scaffolds and their efficient vascularization after implantation. To maintain the scaffold structural properties, it was necessary to fabricate microspheres with particle size that would not interfere with the pore structure of the scaffold, following their entrapment in the scaffold. Thus, RG502H microspheres with an average diameter of 1–3 μm were made by the solvent evaporation-double emulsion method, using an extensive homogenization during the second emulsification.[21] According to size measurements [Fig. 1(a)], 98% of the microspheres were less than 10 μm in diameter. The microspheres displayed an intact surface as judged by SEM [Fig. 1(b)]. The incorporation efficiency of bFGF into the microspheres was 90%, as determined by ELISA.

The incorporation of these tiny PLGA microspheres during fabrication of the scaffolds had no significant effect on the scaffold internal morphology, as shown by the SEM micrograph [Fig. 1(c)]. The alginate composite scaffolds maintained their 90% porosity and the open-pore structure with an average pore size of 130 μm. The incorporated PLGA microspheres were uniformly distributed throughout the alginate matrix. In a higher magnification picture, the microspheres appeared to be an integral part of the solid alginate matrix [Fig. 1(d)]. In contrast, the incorporation of PLGA microspheres to preformed alginate scaffolds

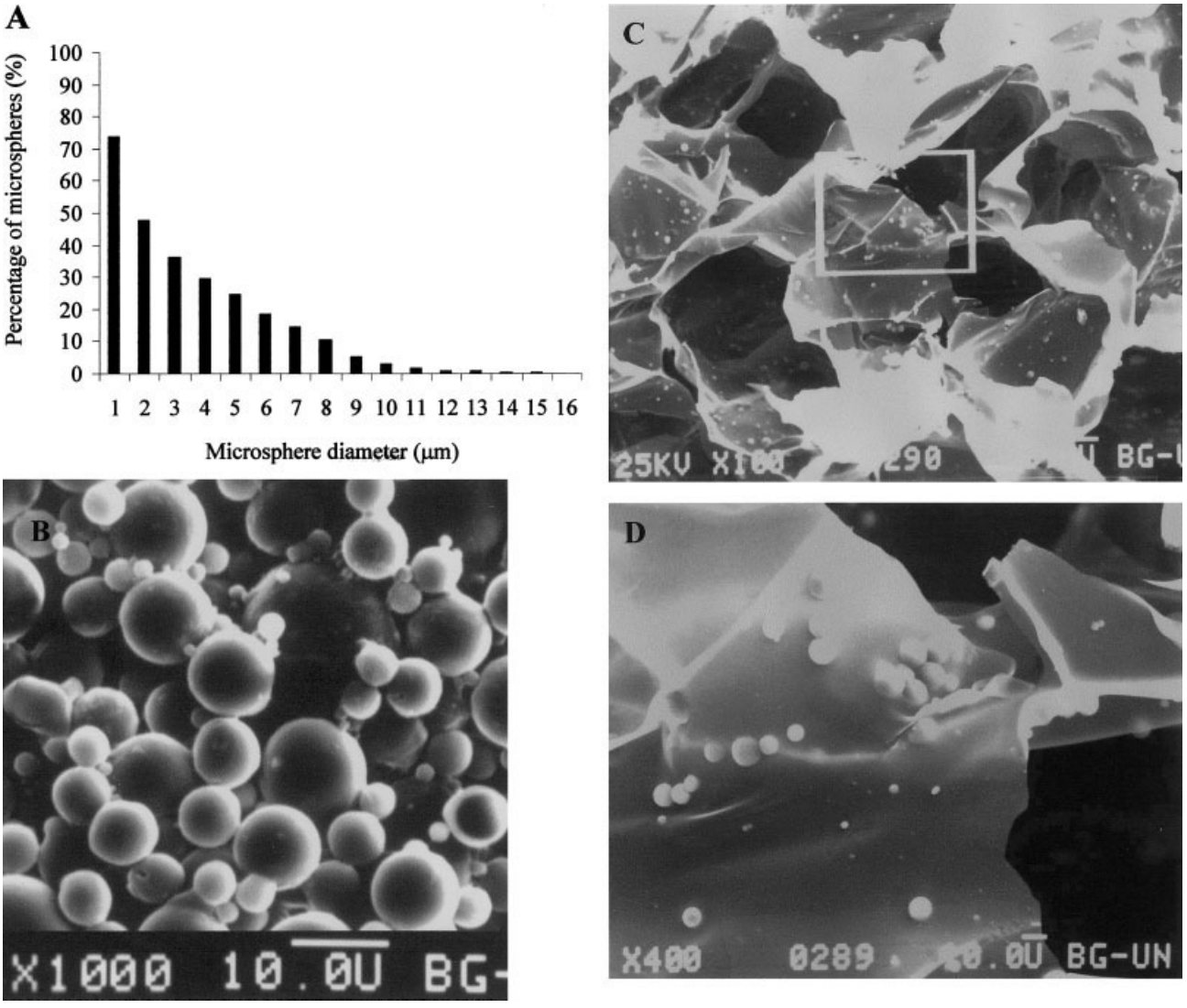

Figure 1. Morphology of alginate composite scaffolds. (A) Size distribution of the incorporated PLGA (RG502H) microspheres, as determined by Galai Cis-1 (Israel); (B) SEM picture of the microspheres; (C) SEM picture of the alginate composite scaffold; and (D) higher-magnification, SEM picture of the composite scaffold.

resulted in the accumulation of most microspheres on the surface of the scaffold (data not shown).

Kinetics of bFGF release and bioactivity

The release rate of bFGF from the alginate composite scaffolds was fairly constant after an initial burst of 40%, and the cumulative release increased linearly with time, as judged by ELISA [Fig. 2(a)]. By day 8 of the release study, approximately 90% of the encapsulated growth factor was released to the external medium. The release profile was similar to that obtained from bFGF-containing microspheres not incorporated into the alginate scaffolds. In contrast, incorporating bFGF and heparin directly onto the alginate scaffolds resulted in a rapid release of the growth factor from the scaffolds (data not shown).

Western blotting of the releasing media from bFGF-containing matrices showed one major band with a molecular weight of 17.8 kDa, which corresponds to monomeric bFGF (standard bFGF, lane 6), and a minor band at a higher molecular weight, between 31 and 41 kDa [Fig. 2(b)]. The minor band probably represents bFGF dimer and it is found in the original bFGF solution (standard bFGF, lane 6). Thus, it appears that the encapsulation of bFGF within PLGA microspheres had no affect on the physical state of the growth factor. Monomeric bFGF was detected in the releasing medium by western blotting for 12 days, indicating the ability of PLGA microspheres to sustain the release of bFGF.

The released bFGF was further assessed for its abil-

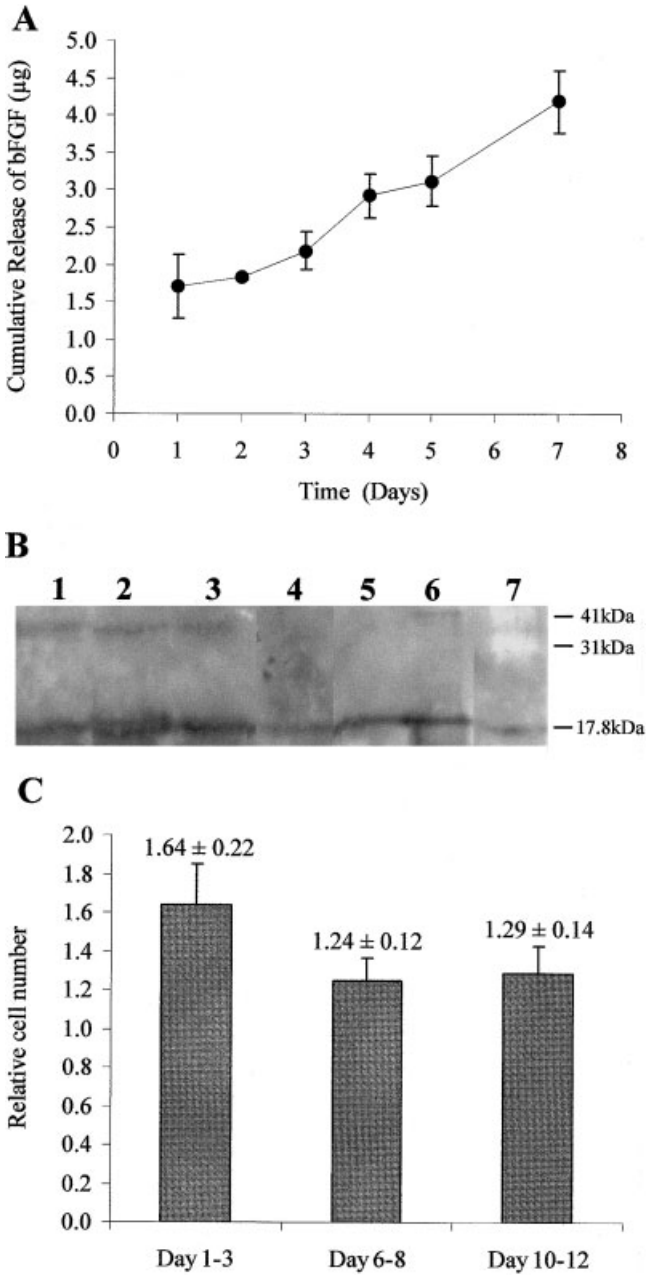

Figure 2. Basic FGF release profile and activity from alginate composite scaffolds. (A) Cumulative bFGF release by ELISA; (B) Western blot analysis of releasing media at days 0.5, 1, 2, 4, and 12, lanes 1–5, respectively, as compared with original bFGF solution (lane 6) and size markers (lane 7); (C) bioactivity of the released bFGF. The bioactivity was determined using cardiofibroblast proliferation assay. Y-axis is the relative cell proliferation resulting from the addition of bFGF released from the microspheres at days 1–3, 6–8, and 10–12, as compared with controls. Values represent mean and standard deviation ($n = 3$).

ity to stimulate fibroblasts proliferation *in vitro*. The growth factor was collected at three time points during the 12-day release study. We focused our attention on this time period because angiogenesis must occur within this time frame to maintain survival of the transplanted cells. The results presented in Figure 2c reveal that the releasing media collected from bFGF-containing matrices during the 12-day study stimulated the proliferation of cardiac fibroblasts *in vitro*. The highest proliferation response was obtained when the cells were exposed to the cumulative releasing media from days 1–3 of the experiment. This result is in accordance with the ELISA results, which revealed the highest amount of released bFGF at initial days (days 1–3). Comparison of the effective concentration of bFGF from the proliferation assay with the amount released by ELISA [Fig. 2(a)] shows that the released growth factor is more than 90% active at all times.

In vivo angiogenesis

Five days after implantation of the bFGF-releasing alginate composite scaffolds on the mesenteric membrane in the peritoneal cavity, a thin layer of transparent tissue that was enriched with blood capillaries, surrounded the scaffolds. H&E of cross sections in the scaffolds at this time point showed minimal host tissue penetration into the perimeter of the scaffolds. At day 10 post-implantation, a significant host tissue ingrowth onto the composite scaffolds was noted. The tissue was nourished by a large number of blood vessels, 45 ± 3 capillaries/mm^2 (Fig. 3). In the absence of bFGF, the density of penetrated capillaries was significantly smaller, 9 ± 1 capillaries/mm^2. It thus appears that incorporating controlled release bFGF

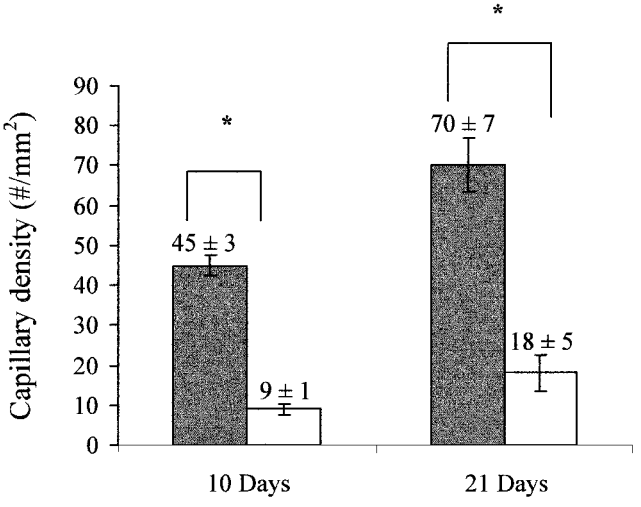

Figure 3. Kinetics of scaffold vascularization after implantation on the mesenteric membrane in rat peritoneum. Blood vessel density (number per mm^2) was determined in 15 different fields per each time point, randomly selected from captured images of three H&E slides per one animal, using Image Pro 3.0. Each group consisted of three animals. *The difference between the two groups for each time is significant (analysis of variance, single factor; $p = 0.0000013$ for $p < 0.01$).

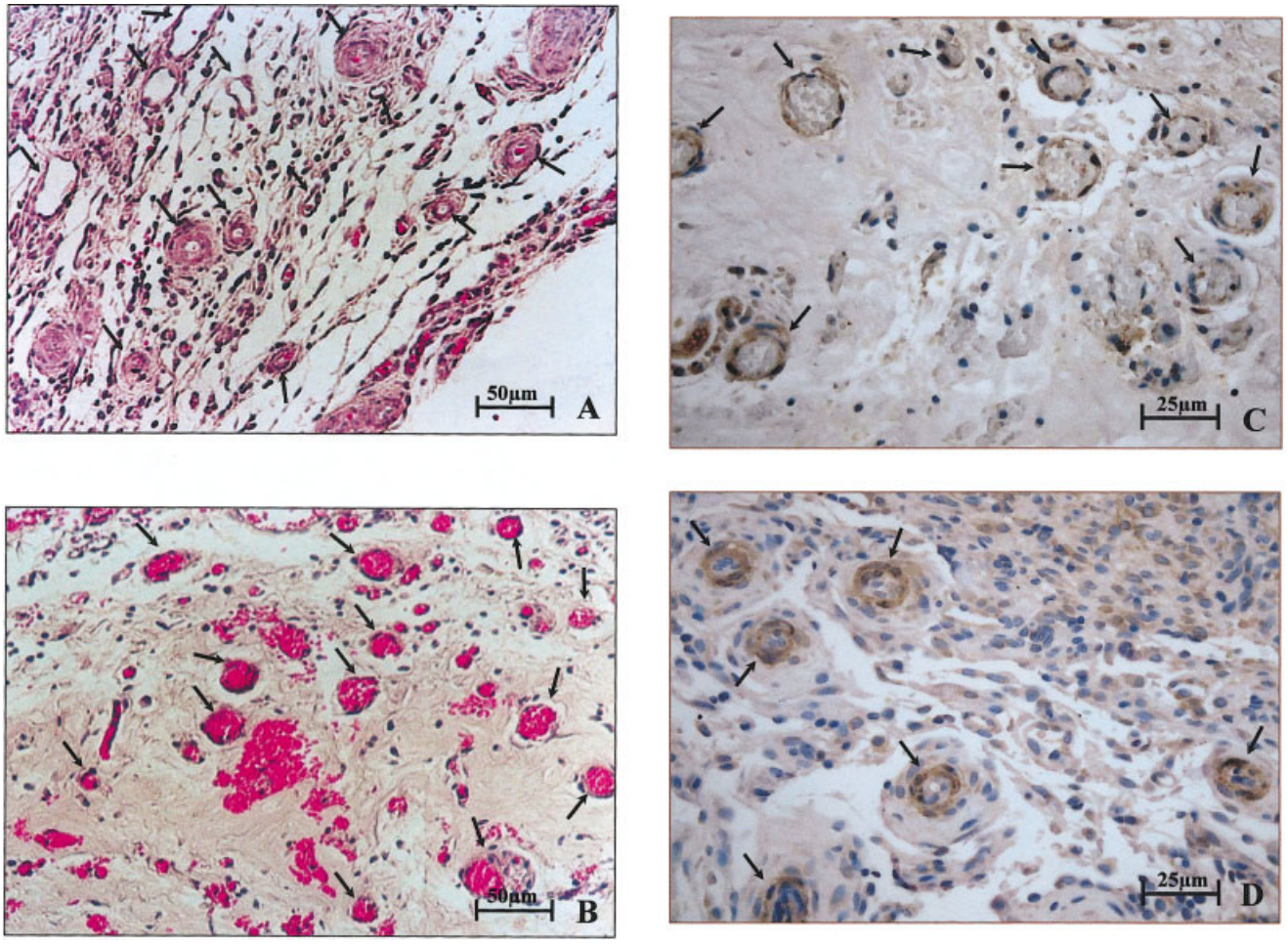

Figure 4. Histological cross sections (5 μm-thick) of tissues implanted with alginate composite scaffolds, 21 days after implantation on the mesenteric membrane in rat peritoneum. (A-D) Cross-sections in tissues implanted with composites releasing bFGF. (A, B) micrographs of H&E-stained sections taken at the scaffold and membrane interface (A) and in scaffold (B). (C) Von Willebrand factor and (D) α-SMA staining of the bFGF-releasing composite scaffolds. (E-G) Cross-sections in tissues implanted with control scaffolds; (E, F) micrographs of H&E-stained sections taken at the scaffold and membrane interface (E) and in scaffold (F), (G) Von Willebrand factor staining of the scaffolds. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

into the 3D scaffolds enhances the rate and extent of matrix vascularization. The blood vessel density increased with time and by day 21, the bFGF releasing composites contained 70 ± 7 capillaries/mm^2, whereas in the absence of bFGF, they contained only 18 ± 5 capillaries/mm^2. The increase in the number of blood vessels indicates that the composite scaffolds slowly release their bFGF contents over that time period.

Most of the blood vessels in the membrane surrounding the implant as well as those penetrating the bFGF-releasing composites, at day 21 after implantation, appeared to be large (>10 μm in diameter) and matured [Fig. 4(a,b)]. The endothelial cells lining the blood vessels created a consistent layer [Fig. 4(c), staining for VWF] that was surrounded by cells positively stained for α-SMA [Fig. 4(d)]. No significant inflammatory and immune response was observed in the implanted alginate composites, following the initial phase of wound healing.

In the control composites, on the other hand, the majority of the blood vessels were micro-capillaries (<10 μm in diameter) [Fig. 4(e,f)]. Slightly larger capillaries were seen in the membrane surrounding the implants [Fig. 4(g)], yet they were much smaller in size and number than those seen in the membrane surrounding the FGF-releasing scaffolds.

DISCUSSION

One of the challenges in engineering a thick tissue construct is to provide adequate nutrients and oxygen to the transplanted cells within a few hours after implantation. The surroundings at the implant site is

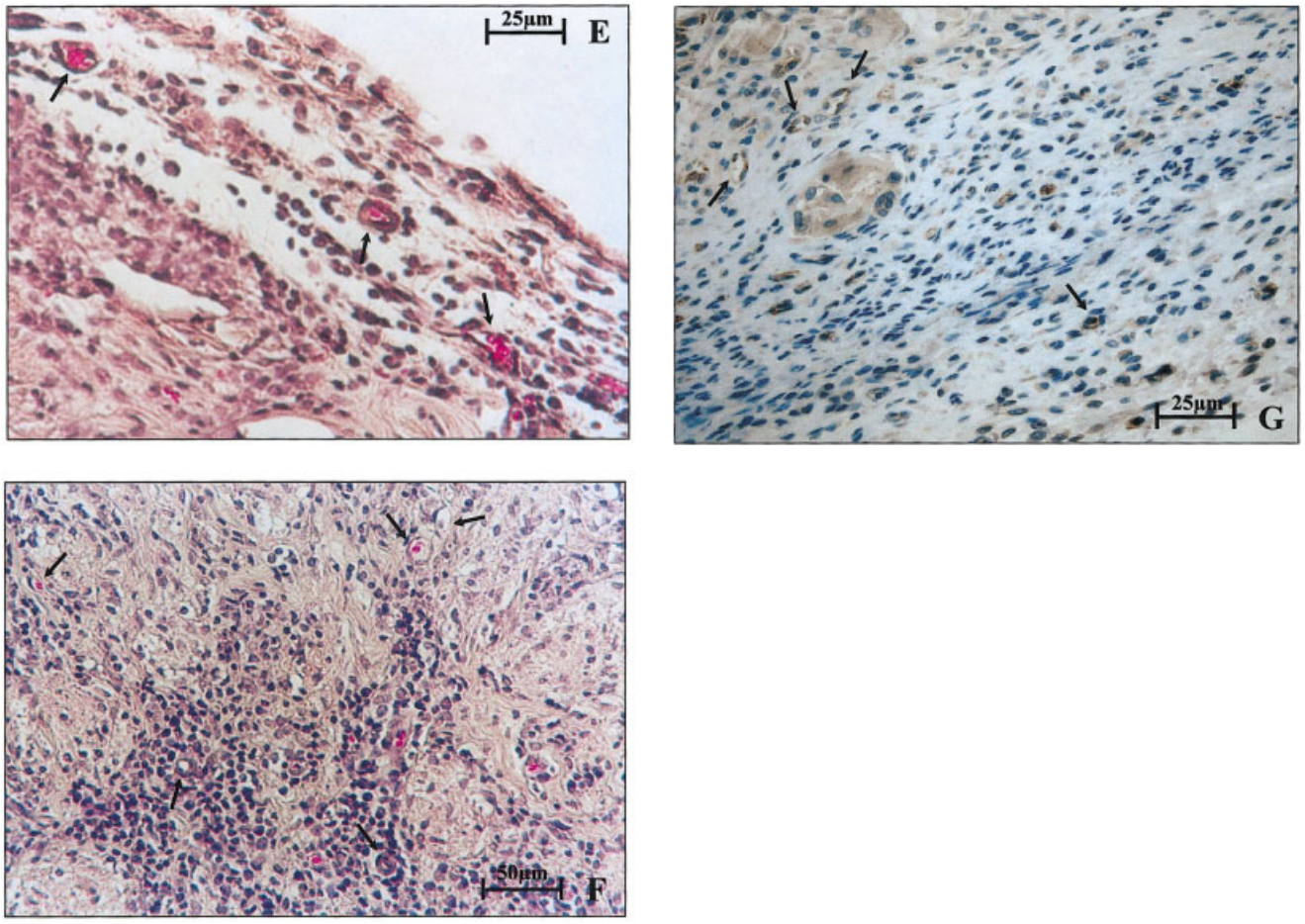

Figure 4. (*Continued from the previous page*)

usually nutrient-poor by virtue of being several cell layers away from the nearby capillaries, and is likely to remain so until angiogenesis occurs. Thus, the simultaneous delivery of potent angiogenic agents, such as bFGF, from the tissue-engineered biograft may allow the vascularization to be enhanced.

In the present article, we aimed at exploring and developing 3D alginate scaffolding for tissue engineering with the capacity for the controlled delivery of bFGF. Basic FGF was first encapsulated within 3-μm diameter, biodegradable PLGA microspheres, which were then incorporated onto the scaffold during its fabrication. The incorporated microspheres did not interfere with the porous structure of the alginate scaffold and they were integral part of the continuous solid wall of the matrix. Such scaffold morphology should enable the penetration of blood vessels throughout the matrix volume, without the bottleneck of interfering microspheres. Furthermore, the retention of 90% porosity in the scaffold should enable a large space for accommodating high-density cell cultures.

Basic FGF was co-entrapped with heparin and BSA in the polymeric microspheres to enhance the stability of the growth factor during encapsulation and release. At physiological pH and temperature, the *in vitro* half-lifetime of bFGF activity is approximately 12 h.[23] Its binding to heparin induces a conformational change in the bFGF molecule resulting in an increased resistance against thermal denaturation and enzymatic degradation, and a reduced activation at acidic pH.[24,25] Our studies reveal that the entrapment of bFGF-heparin, 1:1 weight ratio, enabled the stabilization of bFGF within the microspheres, in agreement with the stoichiometry of heparin binding to bFGF.[26]

The release profile of bFGF from the PLGA microspheres was nearly linear after the burst, and the growth factor was biologically active as assessed by its ability to induce the proliferation of cardiac fibroblasts. The release of the entrapped growth factor was mainly controlled by microsphere degradation. According to GPC measurements, the RG502H polymer (M_W 12 kDa; 50% glycolic acid content; H for hydrophilic) losses 50% of its initial molecular weight within a week at 37°C (data not shown). Consequently, while the microsphere matrix erodes, the encapsulated protein is released. Most probably, the majority of the entrapped bFGF was released while it is still bound to

heparin, due to the high affinity binding between the two molecules. This interaction appears also to govern the behavior of the complex within the alginate scaffolds. When the bFGF-heparin complex was incorporated directly onto the alginate scaffolds, it was rapidly released. Thus, it appears that the negatively charged alginate can not compete with the highly-specific interactions between bFGF and heparin; those are mediated by ionic interaction between both *2-O*-sulfate groups and *N*-sulfate groups of heparin molecules[27] and certain lysine and arginine residues in bFGF.[28] Because binding of bFGF to heparin or heparan sulfate facilitates the binding of bFGF to high-affinity cell membrane receptor, the fact that bFGF is released while in complex with heparin may explain its enhanced biological activity on the cardiac fibroblasts.

The local, sustained bFGF release over time has been shown to enhance the rate and extent of vascularization of the alginate scaffolds, when implanted in rat peritoneum on the mesenteric membranes. The continuous increase in the number of capillaries penetrating the scaffolds from day 10 to 21 after implantation indicated that bFGF is continuously presented at the implantation site over that time period. In general, the soluble bFGF has a short half-life, ~3 min, and is rapidly degraded *in vivo*.[29,30] Thus, its prolonged effect, as seen in this study, may be the result of its slow release *in vivo*. It has been shown before that controlled release of bFGF over time is preferred over the bolus administration of the same dose of growth factor; the sustained release of bFGF was up to threefold more potent at increasing vascular endothelial and smooth muscle cell proliferation than bolus administration.[31]

The continuously released bFGF induced the formation of matured and larger blood capillaries within the implanted 3D alginate scaffolds as compared with those formed in control scaffolds, which contain microspheric BSA and heparin but no bFGF. The extensive positive staining for α-actin, a marker for smooth muscle cells, indicated the formation of matured blood vessels. The ability of bFGF to induce matured blood vessels is attributed to its wide mitogenic effects on the different cells that build a matured vessel, that is, endothelial and mural cells. Furthermore, it appears that bFGF may induce the expression of vascular endothelial factor (VEGF), which is a specific mitogen to endothelial cells.[32] Masaki et al.[32] have found that overexpression of bFGF boosted the expression of endogenous VEGF in murine model of critical limb ischemia, and that the therapeutic effects of FGF-2 were completely diminished by anti-VEGF neutralizing antibody *in vivo*. Thus, it appears that bFGF is a critical player in therapeutic angiogenesis. In comparison with bFGF, therapeutic angiogenesis protocols based on VEGF requires the participation of additional growth factors, such as Angiopoietin-1[33] and platelet-derived growth factor-BB,[34] which recruit the mural cells to induce the formation of matured blood vessels. The growth factors need to be delivered at different developmental stages of the matured blood vessels, as they can have antagonistic actions. Recently, the dual delivery of VEGF and platelet-derived growth factor-BB from a polymeric system, each with a distinct kinetics, resulted in the formation of matured blood vessels,[35] thus proving the advantageous of incorporating controlled delivery system in therapeutic angiogenesis.

It is expected that controlled delivery systems, which can localize the angiogenic effect of bFGF to a desired site while minimizing its systemic undesired effects, may facilitate the use of bFGF in therapeutic angiogenesis. In tissue engineering, its incorporation into the cell delivery vehicle would ensure tissue survival and function.

The authors thank Ms. Parvin Zerin and Ms. Larisa Shenkar for histology.

References

1. Langer R, Vacanti JP. Tissue engineering. Science 1993;260: 920–926.
2. Vacanti JP, Langer R. Tissue engineering: The design and fabrication of living replacement devices for surgical reconstruction and transplantation. Lancet 1999;354:32–34.
3. Shapiro L, Cohen S. Novel alginate sponges for cell culture and transplantation. Biomaterials 1997;18:581–590.
4. Zmora S, Glicklis R, Cohen S. Tailoring the pore architecture in 3-D alginate scaffolds by controlling the freezing regime during fabrication. Biomaterials 2002;23:4087–4094.
5. Atala A, Cima LG, Kim W, Paige KT, Vacanti JP, Retik AB, Vacanti CA. Injectable alginate seeded with chondrocytes as a potential treatment for vesicoureteral reflux. J Urol 1993;150: 745–747.
6. Lanza RP, Ecker DM, Kühtreiber WM, Marsh JP, Ringeling J, Chick WL. Transplantation of islets using microencapsulation: studies in diabetic rodents and dogs. J Mol Med 1999;77:206–210.
7. Glicklis R, Shapiro L, Agbaria R, Merchuk JC, Cohen S. Hepatocyte behavior within three-dimensional porous alginate scaffolds. Biotechnol Bioeng 2000;67:344–353.
8. Dar A, Shachar M, Leor J, Cohen S. Cardiac tissue engineering: Optimization of cardiac cell seeding and distribution in porous alginate scaffolds. Biotechnol Bioeng 2002;80:305–312.
9. Leor J, Aboulafia-Etzion S, Dar A, Shapiro L, Barbash IM, Granot Y, Battler A, Cohen S. Bioengineered cardiac grafts to repair the infarcted myocardium and attenuate heart failure. Circulation 2000;02:III-56–III-61.
10. Colton CK. Implantable biohybrid artificial organs. Cell Transplant 1995;4:415–436.
11. Baird A, Walicke PA. Fibroblast growth factors. Br Med Bull 1989;45:438–452.
12. Burgess WH, Maciag T. The heparin-binding (fibroblast) growth factor family of proteins. Ann Rev Biochem 1989;58: 575–606.
13. Neufeld G, Gospodarowicz D. Identification of the fibroblast growth factor receptor in human vascular endothelial cells. J Cell Physiol 1988;136:537–42.

14. Baruch Y, Shoshany G, Neufeld G, Enat R. Basic fibroblasts growth factor is hepatotropic for rat liver regeneration. J Hepatol 1995;23:328–32.
15. Edelman ER, Mathiowitz E, Langer R, Klagsbrun M. Controlled and modulated release of basic fibroblast growth factor. Biomaterials 1991;12:619–626.
16. Wissink MJB, Beernink R, Pieper JS, Poot AA, Engbers GHM, Beugeling T, van Aken WG, Feijen J. Binding and release of basic fibroblast growth factor from heparinized collagen matrices. Biomaterials 2001;22:2291–2299.
17. Tanihara M, Suzuki Y, Yamamoto E, Noguchi A, Mizushima Y. Sustained release of basic fibroblast growth factor and angiogenesis in a novel covalently crosslinked gel of heparin and alginate. J Biomed Mater Res 2001;56:216–21.
18. Tabata Y, Miyao M, Yamamoto M, Ikada Y. Vascularization into a porous sponge by sustained release of basic fibroblast growth factor. J Biomater Sci Polym Ed 1999;10: 957–968.
19. 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;8:713–720.
20. Chen L, Apte R, Cohen, S. Determinants of release rate of interleukin-1 from biodegradable polyester microspheres. J Control Release 1997;43:261–272.
21. Mullerad J, Cohen S, Voronov E, Apte RN. Macrophage activation for the production of immunostimulatory cytokines by delivering IL-1 via biodegradable microspheres. Cytokine 2000;12:1683–1690.
22. Perets A, Baruch Y, Spira G, Cohen S. Fabrication of alginate composites containing vascular endothelial growth factor to enhance scaffold vascularization. In: Park K, Potts RO, editors. Proceedings of the 25th International Symposium on Controlled Release Bioactive Materials CRS; 1998. p 225–226.
23. Westal FC, Rubin R, Gospodarowicz D. Brain derived fibroblast growth factor: a study of its inactivation. Life Sci 1983;33: 2425–2459.
24. Gospodarowicz D, Cheng J. Heparin protects basic and acidic FGF from inactivation. J Cell Physiol 1986;128:475–484.
25. Gospodarowicz D, Ferrara N, Schweigerer L, Neufeld G. Structural characterization and biological functions of fibroblast growth factor. Endocr Rev 1987;8:95–114.
26. Arakawa T, Wen J, Philo JS. Stoichiometry of heparin binding to basic fibroblast growth factor. Arch Biochem Biophys 1994; 308:267–273.
27. Rusnati M, Coltrini D, Caccia P, Dell'Era P, Zoppetti G, Oreste P, Valsasina B, Presta M. Distinct role of *2-O-*, *N-*, and *6-O-*sulphate groups of heparin in the formation of the ternary complex with basic fibroblast growth factor and soluble FGF receptor-1. Biochim Biophys Res Commun 1994;203:450–458.
28. Erikson AE, cousins LS, Weaver LH, Matthews BW. Three-dimensional structure of human basic fibroblast growth factor. Proc Natl Acad Sci USA 1991;88:3441–3445.
29. Whalen GF, Sing Y, Folkman J. The fate of intravenously administered bFGF and the effect of heparin. Growth Factors 1989;1:157–164.
30. Edelman ER, Nugent MA, Karnovsky MJ. Perivascular and intravenous administration of basic fibroblast growth factor: vascular and solid organ deposition. Proc Natl Acad Sci USA 1993;90:1513–1517.
31. Dinbergs ID, Brown L, Edelman ER. Cellular response to transforming growth factor-β1 and basic fibroblast growth factor depends on release kinetics and extracellular matrix interactions. J Biol Chem 1996;271:29822–2934.
32. Masaki I, Yonemitsu Y, Yamashita A, Sata S, Tanii M, Komori K, Nakagawa K, Hou X, Nagai Y, Hasegawa M, Sugimachi K, Sueishi K. Angiogenic gene therapy for experimental critical limb ischemia: acceleration of limb loss by overexpression of vascular endothelial growth factor 165 but not of fibroblast growth factor-2. Circ Res 2002;90:966–973.
33. Thurston G, Suri C, Smith K, McClain J, Sato TN, Yancopoulos GD, McDonald DM. Leakage resistant blood vessels in mice transgenically overexpressing angiopoietin-1. Science 1999;286: 2511–2514.
34. Benjamin LE, Hemo I, Keshet E. A plasticity window for blood vessel remodeling is defined by pericyte coverage of the preformed endothelial network and is regulated by PDGF-B and VEGF. Development 1998;125:1591–1598.
35. Richardson TP, Peters MC, Ennett AB, Mooney DJ. Polymeric system for dual growth factor delivery. Nat Biotech 2001;19: 1029–1034.