

Local Delivery of Basic Fibroblast Growth Factor (bFGF) Using Adsorbed Silyl-heparin, Benzyl-bis(dimethylsilylmethyl)oxycarbamoyl-heparin

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A growth factor delivery system was developed that is based on the use of silyl-heparin, a chemically modified analogue of heparin. The silyl-heparin was adsorbed onto surfaces by hydrophobic interaction via the prosthetic unit and can then be used as a solid-phase adsorbent for bFGF. All the coating steps were performed by adsorption, a process that allowed preparation of surfaces by immersion or "dip-coating". In this study a series of silyl-heparins were synthesized and each of the analogues found to function similar to unmodified heparin relative to their binding of antithrombin III and also the binding of bFGF. The silyl-heparins were found to be adsorbed onto a wide variety of substrates including polystyrene and lactide:glycolide copolymer. Enzyme-linked immunosorbent assay (ELISA) was used to establish that bFGF was readily bound to surface adsorbed silyl-heparin, and that the amount bound was directly related to amount offered for binding. Once adsorbed the silyl-heparin/FGF was able to induce capillary tube formation of endothelial cells and to increase the growth of endothelial cells. When coated onto suture material and implanted in muscle, the FGF/silyl-heparin coating caused an increased density of mesenchymal cells in the area of the implant. This coating method could prove to be useful in a number of tissue engineering applications for the local delivery of FGF and other growth factors.

INTRODUCTION

The objective of this research was to develop a growth factor delivery system for use in tissue repair that would provide a localized release of bFGF and related growth factors to enhance the tissue repair process.

Fibroblast growth factors comprise a large family of developmental and physiological signaling molecules (Szebenyi and Fallon, 1999). All FGFs have a high affinity for the glycosaminoglycan heparin and for cell surface heparan sulfate proteoglycans. The affinity of FGF for heparan sulfate limits its diffusion and restricts its release into the extracellular matrix. The binding of FGFs to heparin/HS results in the formation or stabilization of dimers and higher order oligomers along the proteoglycan chain (Herr et al., 1997; Mach et al., 1993; Moy et al., 1997). Heparin/heparan sulfate also play a role in the formation of an active FGF/FGF receptor signaling complex, and heparin/HS increases the affinity and half-life of the FGF/FGFR complex (Flaumenhaft et al., 1990; Szebenyi and Fallon, 1999). bFGF is mitogenic in many cell types including fibroblasts, endothelial cells, smooth muscle cells, and osteoblasts among others (Nugent and Iozzo, 2000). It is angiogenic and can protect cells from toxic insult.

Heparin-containing delivery systems for locoregional application of growth factors have been described by several investigators (Edelman et al., 1992; Gosselin et al., 1996; Nimni, 1997; Sakiyama-Elbert and Hubbell,

2000b; Wissink et al., 2000; Zarge et al., 1997). When heparin is covalently bound to a scaffolding, the release of growth factors has been suggested to be influenced by enzymatic factors such as heparinase or plasmin (Sakiyama-Elbert and Hubbell, 2000a), and by diffusion-based release. The current work presents a delivery system that is based on the use of heparin conjugated to silyl-type prosthetic units. The resulting conjugate, benzyl-bis-(dimethylsilylmethyl)oxycarbamoyl-heparin (silyl-heparin), is amphipathic and is readily adsorbed onto hydrophobic surfaces, including biodegradable polymers. The adsorbed silyl-heparin can then be used as a solid-phase adsorbent for growth factors such as bFGF. A key advantage of the current method is that all the coating steps are performed by adsorption thereby allowing preparation of surfaces by "dip-coating".

METHODS

A schematic for the synthesis of silyl-heparins is provided in Figure 1, and the various steps are detailed below.

Synthesis of Benzyl(chloromethyl)dimethylsilane, 3 ($n = 1$). Under an atmosphere of nitrogen, chloro-(chloromethyl)dimethylsilane (100 g, 0.685 mol) was added by syringe to THF (500 mL) and solution cooled to 0 °C with an ice/acetone bath. Benzylmagnesium chloride (2.0 M solution, 400 mL, 0.8 mol) was added dropwise over 2 h. Care was taken to maintain the temperature below 10 °C until all the reagent was added. Thereafter, the ice bath was allowed to warm to room temperature and the reaction mixture stirred overnight. Hexane (300 mL) was added and saturated aqueous NH_4Cl (300 mL) added dropwise. The reaction mixture was transferred to a 2 L separatory funnel with more hexane

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was dissolved in CH_2Cl_2 (200 mL) and stirred at 0 °C under N_2 in a 1-L three-necked flask equipped with thermometer, dropping funnel and N_2 inlet. Na_2CO_3 (65 g, 0.6 mol) was added followed by $\text{Bn}(\text{SiMe}_2\text{CH}_2)_n\text{OH}$ (0.13 mol dissolved in 200 mL of CH_2Cl_2) dropwise over 30 min. Thereafter, the ice/acetone bath was allowed to come to room temperature. The reaction mixture was stirred overnight and filtered through a sintered glass funnel, and the reaction vessel was rinsed with PhCH_3 (200 mL). The solution was concentrated under vacuum to give a colorless oil. The oil was dissolved in CH_2Cl_2 and stirred in an ice bath under N_2 . *N*-Hydroxysuccinimide (30 g, 0.26 mol) was added followed by Et_3N (40 mL, 0.28 mol) dropwise over 15 min and the resulting cloudy mixture stirred at room temperature for 1 h. The reaction mixture was diluted with hexane (600 mL), washed with saturated aqueous NH_4Cl (3×100 mL), and the combined aqueous phases were backwashed with hexane (2×200 mL). The combined organic phases were dried over MgSO_4 and concentrated under vacuum to give an amber oil. The amber oil was chromatographed with silica gel in a 6.5×100 cm (height of silica 40 cm) flash column and eluted with 20–50% EtOAc /hexane to give an amber syrup. Yields are generally greater than 75%. ^1H NMR spectrum for $\text{Bn}(\text{Me}_2\text{SiCH}_2)_2\text{OCO}_2\text{N}(\text{COCH}_2)_2$: δ 7.0–7.2 (m, 5H, arom Ph); 4.0 (s, 2H, SiCH_2OCO), 2.7 (s, 4H, $\text{N}[\text{COCH}_2]_2$), 2.1 (s, 2H, SiCH_2Ph), 0.0 and 0.1 (2 s, $2 \times 6\text{H}$, SiCH_3), -0.2 (s, 2H, SiCH_2Si). Quantitative organic microcombustion analysis for $\text{Bn}(\text{Me}_2\text{SiCH}_2)_2\text{OCO}_2\text{N}(\text{COCH}_2)_2$: theoretical C 54.9%, found 54.7%; theoretical H 6.9%, found 7.0%; theoretical N 3.6%, found 3.5%. TLC in 20% $\text{EtOAc}/80\%$ hexane, $R_f = 0.2$.

Conjugation of Heparin with $\text{Bn}(\text{SiMe}_2\text{CH}_2)_n\text{OCO}_2\text{N}(\text{COCH}_2)_2$ 5_n To Give 6_{n,x}. Heparin (ammonium ion-free, average molecular weight 10000; 100 g, 10 mmol) was dissolved in H_2O (500 mL) in a 2-L flask with stirring. DMF (400 mL) was added followed by DMAP ($1.6 \times \text{g}$, $13 \times \text{mmol}$). $\text{Bn}(\text{SiMe}_2\text{CH}_2)_n\text{OCO}_2\text{N}(\text{COCH}_2)_2$ 5_n ($10 \times \text{mmol}$), in DMF (100 mL) was added, and the resulting milky mixture was allowed to stir at room temperature for >24 h. The reaction mixture was concentrated by evaporating most of the water followed by trituration with acetone (1 L). The white suspension was filtered through a sintered glass funnel to give a white solid residue. This crude material was purified by Soxhlet extraction with acetone overnight to give a white powder. The yields were generally greater than 95%.

The average number of prosthetic units per molecule of heparin was estimated based on a comparison of the molar ratios of the hydrolyzed prosthetic unit benzyl-tri-(dimethylsilylmethyl)-OH and heparin as determined by use of dimethyl methylene blue (Groves et al., 1997). Heparin was detected using a commercially available enzyme-linked assay that measures the heparin-induced inhibition of antithrombin/factor Xa as measured with a factor Xa specific chromogenic substrate (Teien and Lie, 1977; Teien et al., 1976).

The silyl-heparin was used as a coating solution at 1% solution (w/v) in 60% aqueous ethanol. Materials were coated with silyl-heparins for 15 min at 37 °C. The coated materials were then rinsed extensively in water, air-dried, and stored until use.

Comparison of Silyl-heparins with Heparin. Comparisons were made using competitive binding to heparin-agarose (end-point attachment, Sigma Chemical Co.) generally following the methods of Ishihara (Ishihara et al., 2000). Heparin agarose was rinsed in phosphate-buffered saline and equilibrated in PBS containing 1% bovine serum albumin (PBS/BSA). Aliquots were added

to microfuge tubes and the volume adjusted to 0.5 mL with 0.5 mL of PBS/BSA. An additional 0.5 mL of Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (FBS) containing heparin or silyl-heparin-5135 was added. An aliquot of 5 μL of bFGF (50 ng/mL) was added and the solutions incubated for 30 min with mixing. The unbound bFGF was removed by multiple centrifugations and rinsing in PBS. Rabbit anti-bFGF (1:500) was added in 0.5 mL of PBS/BSA and the solution incubated with mixing for 1 h. After rinsing donkey anti-rabbit (1:500) in PBS/BSA was added and incubated 1 h. After rinsing and transfer to new vials, chromogen (ABTS One-Step, Pierce Chemical Co.) was added and the reaction developed and subsequently read at 595 nm.

Detection of Adsorbed Silyl-heparin. Adsorbed heparin was detected by staining with dimethyl methylene blue (Groves et al., 1997). Stains were filtered through 0.2 μm filters prior to use. The stain associated with the test materials was solubilized by addition (1:1) of methanol and an aqueous solution of 0.5 M Tris, pH 6.8, containing 0.4% sodium dodecyl sulfate (SDS). Heparin was also detected by an enzyme-linked assay (Sigma Chemical Co., St. Louis) that measures the heparin-induced inhibition of antithrombin/factor Xa as measured with a factor Xa specific chromogenic substrate (Teien and Lie, 1977; Teien et al., 1976). The assay was performed following the directions of the manufacturer.

ELISA Detection of bFGF Bound to Silyl-heparins. Wells of polystyrene microtiter plates were coated with 30 μL of silyl-heparin for 15 min at 37 °C. The surface area of the well was 0.32 cm^2 . The wells were rinsed extensively in water and air-dried. bFGF (Sigma Chemical Co.) in PBS/BSA was added in 150 μL aliquots and in doubling dilutions (final volume 150 μL). The plate was incubated at 37 °C for 1 h and rinsed $5 \times$ in PBS. A 150 μL aliquot of PBS/BSA containing 1:500 rabbit anti-FGF antibodies was added, incubated 1 h, and rinsed. In some cases an irrelevant rabbit antibody (rabbit anti-TGF-receptor II, Santa Cruz Biotechnology) was used at a similar concentration. An aliquot of PBS/BSA containing 1:500 HRPO-conjugated donkey anti-rabbit IgG was added, and after incubation and rinsing ABTS-chromogen was added. All antibody solutions were filtered through 0.2 μm filters prior to use. The absorbance was monitored at 405 nm.

Attachment Molecules. The following were used as heparin-complexing materials: murine type IV collagen, human fibrinogen, and bovine fibronectin isolated from plasma. The proteins were used at a concentration of 100 $\mu\text{g}/\text{mL}$ in PBS/BSA. The wells were coated adding 100 μL followed by a 1 h incubation at 37 °C. Thereafter, the plates were washed extensively in water. To the wells was added bFGF in PBS/BSA in 150 μL aliquots. The plate was incubated at 37 °C for 1 h and processed as above using dilutions of 1:200 and 1:500 for the primary and secondary antibody, respectively.

Capillary Tube Formation. Bovine aorta endothelial cells were seeded into low-attachment 96-well plates. The wells had been pretreated with saline or silyl-heparin and/or fibrinogen and FGF. The silyl-heparin coating was applied as above, and the fibrinogen was applied as a 0.1% solution in PBS and uncomplexed material washed off with PBS. FGF (100 ng/mL) was allowed to attach for 30 min to the silyl-heparin in PBS containing 1% bovine serum albumin and the unbound material rinsed off with PBS. Endothelial cells (5×10^3 cells) were seeded into the wells in DMEM containing 10% FBS. The cells were allowed to grow for 4 days and the cultures examined by phase contrast microscopy.

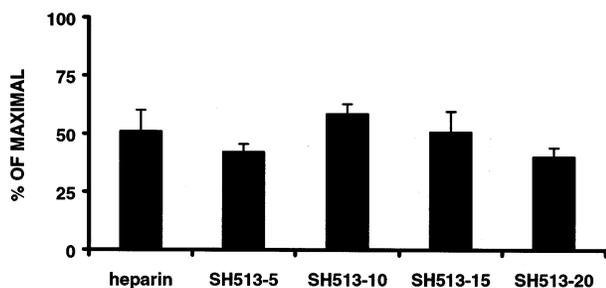


Figure 2. Comparison of the inhibition of bFGF binding to heparin-agarose by either heparin or silyl-heparin analogues as determined by immunochemical methods. The analogues nominally contained 5, 10, 15, and 20 prosthetic groups per heparin. A serial assay was used to establish the EC_{50} for heparin and then the comparison of the silyl-heparins made using identical concentrations. The data represents the average \pm SD.

Stimulation of Endothelial Cell Growth. Six-well cluster plates were seeded with 5×10^4 bovine aorta endothelial cells. Cell culture inserts (25 mm diameter) with 3 μ m pores in the bottom were placed in each well. Suture material (10 cm) with or without coatings was placed in the well and medium added sufficient to cover the suture (3 mL total). One set of inserts containing an uncoated suture was spiked with 50 ng of FGF by adding the FGF directly to the medium and was used as a positive control. The cells were allowed 4 days to grow, fixed, and stained with crystal violet. Thereafter, the stain was eluted in aqueous methanol and 0.4% SDS and monitored at 595 nm.

Implants. Vicryl suture material was immersed in water, silyl-heparin, or silyl-heparin followed by FGF for 15 min at 37 °C. Vicryl, a synthetic absorbable surgical suture composed of a copolymer made from 90% glycolide and 10% L-lactide. The suture material was then rinsed extensively, air-dried, mounted on 16-gauge needles, and stored until use, typically overnight. The batch receiving FGF was immersed in PBS/BSA containing 100 ng/mL FGF (15 min, 37 °C). The sutures were passed through the thigh muscle of adult rats under anesthesia and secured with knots and surgical clips. After two weeks the animals were euthanized and the sutured area removed, fixed in formalin, and processed by standard histological methods.

RESULTS

FGF Binding by Silyl-heparins and Heparin. The inhibition of binding of bFGF to heparin-agarose was

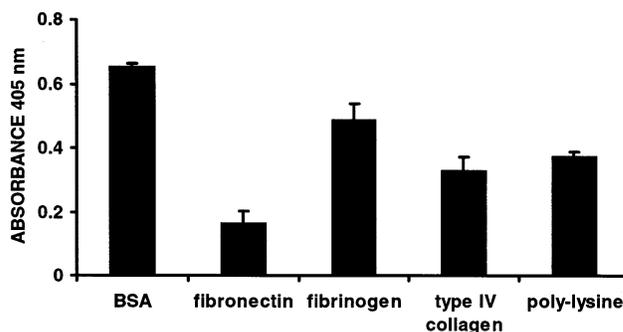
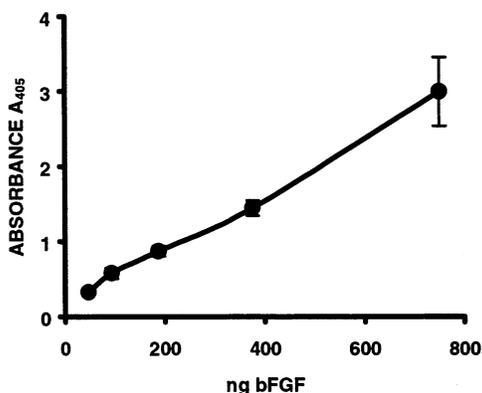


Figure 4. Effect of precomplexing adhesion molecules to silyl-heparin on the subsequent binding of bFGF. All adhesion molecules were complexed to silyl-heparin at a concentration of 100 μ g/mL and unbound material rinsed out prior to use in experiments. The data represents the average \pm SD. All treatments were statistically significant as indicated by $p < 0.05$ by Student's *t*-test relative to treatment with bovine serum albumin (BSA).

used to compare silyl-heparins with heparin. The heparin used in this assay was the same as was used in the synthesis of the silyl-heparins. In this assay silyl-heparin 5135 provided essentially the same EC_{50} as unmodified heparin (Figure 2) when used on equal weight basis. One of the silyl-heparins, SH513-5, was selected for further study, as the EC_{50} was similar for each of the silyl-heparin variants examined.

Adsorption of Silyl-heparin onto Polymeric Substrates. Heparin-specific assays and dimethylene blue dye uptake were used to establish that silyl-heparin could be adsorbed to a variety of materials including polystyrene, hydrogel-coated polystyrene, polycarbonate, polyurethane, polycaprolactone, poly(vinyl chloride), stainless steel, titanium, platinum wire, and glow discharge-deposited siloxane (over stainless steel). The loading efficiency of silyl-heparin was generally in the range of 10 mIU/cm², but did vary by type of substrate.

Silyl-heparin Coated onto Polystyrene. Staining with crystal violet and dimethyl methylene blue, stains known to react with heparin verified the presence of silyl-heparin-5135 on the polystyrene. The bound stain could be seen visually as a light coloration on the bottom of the wells. The stains were solubilized in 1:1 methanol/0.1% SDS; this slightly increased the stain intensity. Little if any stain was detected on plates to which 1 mg/mL heparin was added or which were uncoated. Wells coated with silyl-heparin-5135 were positive for bioactive heparin as determined by monitoring the heparin-induced inhibition of antithrombin/factor Xa. Wells simi-

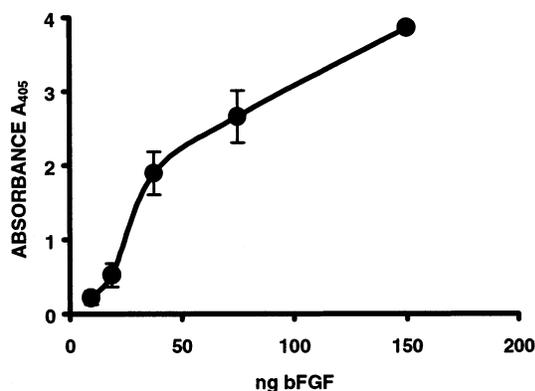


Figure 3. Effect of bFGF loading concentration the amount of bFGF bound to silyl-heparin. Left panel. The primary and secondary antibodies, as described in the Methods section were used at a dilution of 1:500. Right panel. The primary and secondary antibodies were used at a dilution of 1:250. The data represents the average \pm SD.

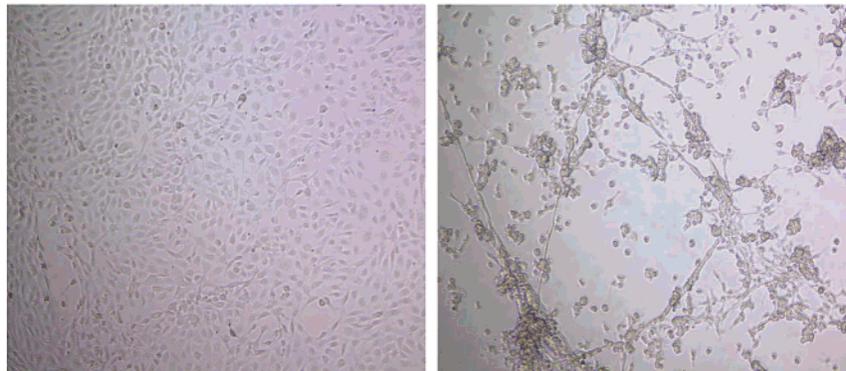


Figure 5. Capillary tube formation induced by growth of bovine aorta endothelial cells on FGF-silyl-heparin. The silyl-heparin was precomplexed to fibrinogen to increase the level of endothelial cell attachment. The panel on the left is a phase contrast micrograph of endothelial cells after 4 days on silyl-heparin/fibrinogen. The panel on the right illustrates endothelial cells grown on FGF-silyl-heparin/fibrinogen. Note the tubelike structures in the right panel and the typical endothelial cell morphology in the left panel.

larly coated with heparin or left untreated did not demonstrate such an inhibition.

bFGF Detection. bFGF was detected over a wide range of concentrations from as much as 750 ng to as low as 20 ng (Figure 3). The color development was linear at high concentrations indicating that under the conditions of the assay the upper saturation limit of the silyl-heparin had not been reached.

As controls, when anti-TGF-betaRII (irrelevant antibody directed against the transforming growth factor receptor) was used instead of FGF and at the same anti-dilutions, no bFGF was detected. When TGF-beta (40 ng) was used as an alternative to bFGF and with anti-bFGF antibodies, no activity was detected.

Effect of Adhesion Molecules on FGF Binding. Type IV collagen, fibronectin, and fibrinogen are known to complex to heparin, and therefore the effect of pre-complexing these extracellular matrix molecules to silyl-heparin was evaluated (Figure 4). Fibronectin was found to considerably decrease the binding of bFGF to silyl-heparin, whereas type IV collagen and fibrinogen decreased the signal less. Immunochemical analysis of fibronectin indicated that it was bound to the silyl-heparin on the plate and had not undergone spurious sloughing.

Capillary Tube Formation. Bovine aorta endothelial cells underwent morphological changes consistent with the formation of capillary tube formation when cultured on a preformed coating of silyl-heparin plus fibrinogen and bFGF (Figure 5). Fibrinogen was used to help the endothelial cells attach to the silyl-heparin. It should be pointed out that the only source of bFGF was that which was bound to the silyl-heparin. Tubes did not form when the cells were cultured in wells coated with silyl-heparin, silyl-heparin and fibrinogen, fibrinogen, fibrinogen and FGF, or FGF. On silyl-heparin plus fibrinogen the cells formed essentially confluent monolayers.

Endothelial Cell Growth. FGF from the FGF/silyl-heparin coating was able to transit a separating membrane and act regionally to stimulate the growth of endothelial cells at the end of 4 days (Figure 6). In these experiments, FGF was complexed via silyl-heparin to suture material and separated from endothelial cells by a permeable membrane. The suture-derived FGF increased cell numbers to a similar extent as that observed in control cultures spiked with soluble FGF. Suture material coated with just silyl-heparin did not result in an increase in cell number.

Suture Implant. Coated sutures implant in rat muscle was selected as a simple model of an implant that could

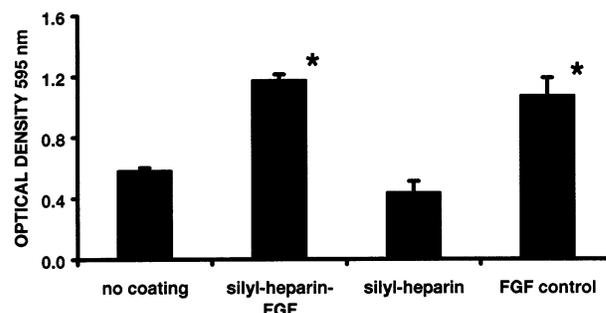


Figure 6. Trans-pore effect of FGF-silyl-heparin from coated sutures on the growth of bovine aorta endothelial cells after 4 days as determined by crystal violet staining. Cells were seeded into cluster plates and with 3 μ m pores in the bottom placed in each well. Sutures with or without coatings were placed in the well and medium added sufficient to cover the suture. One set of inserts, containing an uncoated suture, was spiked by adding the FGF directly to the medium (positive control). The cells were allowed 4 days to grow, fixed, and stained with crystal violet. Thereafter, the stain was eluted in aqueous methanol and 0.4% SDS and monitored at 595 nm. The data are presented as the average absorbance \pm SD. The asterisk indicates statistical significance greater than 0.05 as determined with Student's *t*-test.

be histologically monitored. At two weeks after implant, sutures coated with FGF/silyl-heparin had a marked increase in cell density in the area surrounding the implant compared to the uncoated suture or the suture coated with silyl-heparin (Figure 7). The diameter of the granulation tissue surrounding the implant coated with FGF/silyl-heparin was approximately 2-fold larger than that of the uncoated material or silyl-heparin coated material (data not shown). The tissue surrounding the implant coated with FGF/silyl-heparin had numerous blood vessels (Figure 8) suggestive of angiogenesis.

DISCUSSION

FGFs have a well-characterized role in fibroblast and endothelial cell growth and are involved in angiogenesis, wound healing, tissue repair, and tissue regeneration (Powers et al., 2000). One of the defining features of the FGF family is a strong affinity for heparin and heparan sulfate proteoglycans. This affinity for heparin is shared by a number of other growth factors including VEGF, TGF- β , NGF, and PDGF, among others. The broad physiological impacts of growth factors on wound healing has led to efforts to deliver FGF, and other growth factors, from implant material or wound dressings (Nimni, 1997). A variety of approaches have been used for this

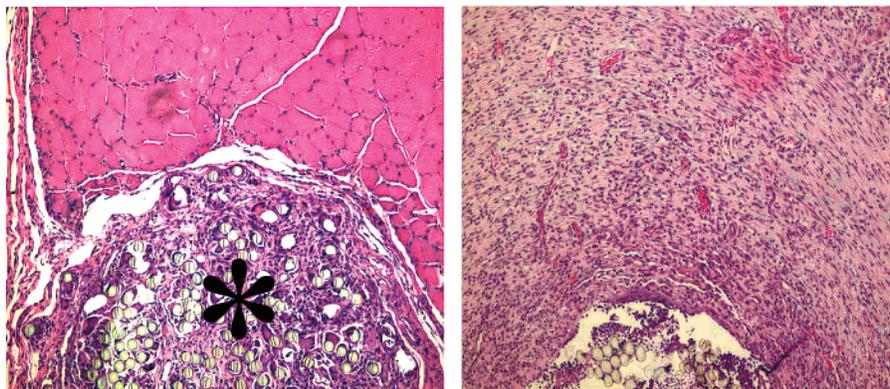


Figure 7. Morphology of tissue surrounding suture material with a silyl-heparin coating (left), and with FGF/silyl-heparin (right). Note the increased cellularity of in the tissue surrounding the suture coated with FGF/silyl-heparin. The suture marked in the panel on the left with an asterisk, and left unmarked (center bottom) in the panel on the right. Original magnification $\times 100$, hematoxylin and eosin stain.

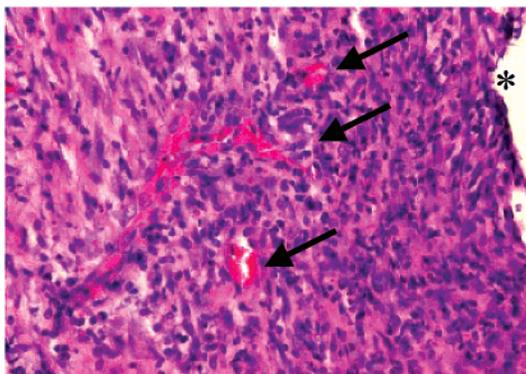


Figure 8. Micrograph of tissue surrounding a suture material coated with FGF/silyl-heparin. Note the presence of blood vessels (arrows) indicative of angiogenesis. The suture is marked with an asterisk. Original magnification $\times 400$, hematoxylin and eosin stain.

purpose including using conjugation of FGF (Sakiyama-Elbert and Hubbell, 2000b), FGF embedded in matrixes (Soparkar et al., 2000; Wang, 1996), FGF complexed to heparin-conjugates (Bos et al., 1999; Wissink et al., 2000), and the use of genetic engineering approaches to producing FGF-secreting cells (Sosnowski et al., 1999).

Because of the potential wide application of FGF to implants ranging from cardiovascular (Post et al., 2001) to osteocartilagenous applications and the variety of matrixes and surfaces that might be involved, our approach was to develop a simple coating approach that could apply FGF to a large number of substrates. To that end, we have synthesized heparins with prosthetic groups that promote heparin adsorption. The bound heparin can then be used to further complex FGF. The overall method is simple and requires a low level of technical skill, as all of the adsorption steps are performed by immersion.

FGF bound to adsorbed silyl-heparin as determined by ELISA, and the amount of FGF bound was directly related to the amount offered for binding. This correlation should allow for predictable loading of FGF on implant surfaces. And while the focus of this report is on FGF, similar approaches have been used for complexing vascular endothelial growth factor (VEGF) and transforming growth factor-beta (TGF- β).

Heparin is known to bind to a variety of extracellular matrix molecules including fibronectin, laminin, and type IV collagen (Kreis, 1993). Additionally, it can bind fibrinogen and fibrin (Mohri et al., 1994; Odrliin et al., 1996). All the aforementioned proteins have been used

to support cell attachment on various experimental implants. We evaluated the binding of FGF to silyl-heparin following an initial complexation with silyl-heparin, as this might be a desirable format for certain applications where cell attachment is desired. At the concentration of extracellular matrix protein used, fibronectin was found to substantially reduce the subsequent binding of FGF. Fibrinogen had the least effect.

One interesting observation was that endothelial cells cultured directly on FGF-silyl-heparin that had been precomplexed to fibrinogen underwent capillary tube formation. This appears to be similar to those formed when endothelial cells are cultured on extracellular matrix (Matrigel) in the presence of FGF (Garrido et al., 1995; Zimrin et al., 1995). This clearly indicated that the FGF-silyl-heparin was having a local effect on the endothelial cells. In our studies, capillary tubes rarely developed in cultures grown on silyl-heparin-fibrinogen without FGF. On Matrigel, the formation of capillary tubes is widely used as an *in vitro* model of angiogenesis.

One prediction of the use of FGF-silyl-heparin was that the complex should elute from an implant surface and effect cells in the general region. The trans-pore studies support that hypothesis as endothelial cell growth was stimulated when implant material was coated with FGF/silyl-heparin and supplied to the cells across a membrane barrier.

The suture model was also used to evaluate the effect of the FGF-silyl-heparin coating on wound repair. Following implant, the granulation tissue surrounding the implant had a larger diameter and a higher cell density than uncoated suture material, clearly suggesting a local effect of the FGF bound to the suture material. A fuller examination of the dose-response characteristics of this response is planned.

Collectively, the results of the current study suggest that silyl-heparin could be a useful vehicle for the local delivery of FGF and other growth factors.

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