

# FGF2-Targeted Adenovirus Encoding Platelet-Derived Growth Factor-B Enhances *de Novo* Tissue Formation

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Gene therapy has yet to achieve reproducible clinical efficacy, due to inadequate gene delivery, inadequate gene expression, or dose-limiting toxicity. We have developed a gene therapy technology for tissue repair and regeneration that employs a structural matrix for DNA delivery. The matrix holds the DNA vector at the treatment site and provides a scaffolding for in-growth and accumulation of repair cells and efficient DNA transfection. We now report, for the first time, matrix-mediated delivery of targeted DNA vectors for soft tissue repair. A collagen matrix was used to deliver an adenoviral vector encoding platelet-derived growth factor-B (AdPDGF-B), resulting in efficient transgene expression *in vitro* and *in vivo*. Increases in the overall levels of expression and in the relative amounts of secreted PDGF-BB were achieved when AdPDGF-B was conjugated to fibroblast growth factor (FGF2) such that the virus was targeted for cellular uptake via FGF receptors. Matrix-mediated delivery of AdPDGF-B enhanced wound healing responses *in vivo*, and FGF2 targeting generated effects comparable to nontargeted vectors at significantly lower doses. Therefore, matrix-mediated delivery in combination with FGF2 targeting overcomes some of the safety and efficacy limitations of current gene therapy strategies and is an attractive therapeutic approach for tissue repair and regeneration.

**Key Words:** matrix-mediated gene delivery; targeted adenovirus; tissue repair; growth factor; gene therapy.

## INTRODUCTION

Normal wound healing proceeds via an ordered sequence of events including inflammation, cell migration, angiogenesis, provisional matrix synthesis, collagen deposition, and reepithelialization (1). These processes are mediated by specific growth factors and cytokines. In chronic wounds such as lower extremity neuropathic ulcers occurring in patients with diabetes, the wound-healing response is impaired. This impairment is due in part to a deficiency of endogenous growth factors (2, 3). Therefore, significant effort has been put into the development of protein growth factors and cytokines as wound-healing therapeutics. Unfortunately, most growth factors have not produced clinically significant

improvements in wound healing (4, 5). In fact, only recombinant human platelet-derived growth factor-BB (rhPDGF-BB) has been commercialized for the treatment of diabetic ulcers, and even it yields only marginal improvements in ulcer healing (6). The overall lack of success with protein growth factors has been attributed in part to ineffective delivery and poor retention in the wound defect, an environment known to contain high levels of protease activity (7).

In recent years, gene therapy has been proposed and evaluated as an alternative approach for wound therapy (5). For example, *ex vivo* modification of keratinocytes to express PDGF-A followed by transplantation to the skin has been shown to enhance healing (8). Plasmid DNAs encoding EGF, TGF- $\beta$ 1, PDGF-A, and PDGF-B have also been shown to enhance wound healing when introduced into wounds using gold particle-mediated DNA transfer (9–11). In general, these approaches have generated limited success and appeal due to low gene transfer efficiencies and labor-intensive methodologies.

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We have addressed the current limitations of gene therapy for tissue repair by using biocompatible/biodegradable matrices to deliver DNA vectors. Previous tissue repair studies have demonstrated the benefits of matrices for the delivery of therapeutic proteins such as EGF, angiotensin II, FGF2, and TGF $\beta$  (12–15). By applying matrix-mediated delivery to gene therapy, the DNA vector as well as the transgene product is retained within the defect site. Therefore, there is increased opportunity for achieving a maximal therapeutic response with decreased likelihood of vector dissemination to normal tissue. Fibroblasts, endothelial cells, and other repair cells, which originate in viable tissue surrounding a wound site, proliferate and migrate into the matrix and then take up and transiently express the therapeutic transgene (16). Once transfected, cells act as *in vivo* bioreactors, producing the encoded proteins that stimulate wound repair. We have demonstrated successful repair of critical bone defects using a collagen matrix containing a plasmid encoding parathyroid hormone (16, 17). In addition, an enhancement of granulation tissue formation and vascularization has been observed in rats for 2–4 weeks following subcutaneous implantation of a poly(lactide–coglycolide) (PLG) matrix containing a plasmid encoding PDGF-BB (18). In contrast, direct injection of an aqueous formulation of the plasmid yielded no significant increases in granulation tissue or vessel number. Therefore, formulation of the plasmid in the matrix was required for sustained transgene delivery and expression and induction of a tissue-repair response (18).

Adenoviral vectors establish high but transient transgene expression and do not integrate into the genome of transduced cells. These properties are well suited to wound-healing applications since the presence of therapeutic molecules is required during the healing process, but not during the long-term remodeling phase. Adenovirus normally enters cells through coxsackie adenovirus receptors (CAR), which are variably expressed on quiescent and proliferating cells. Therefore, modification of adenovirus (Ad) to improve specificity and transduction efficiency is desirable. In addition, due to potential toxicity at higher doses, methods for minimizing Ad doses without compromising overall levels of transgene expression are needed. We have developed modified Ad vectors that exhibit these improvements. Specifically, we have complexed basic fibroblast growth factor (FGF2) with the adenoviral knob protein, thus retargeting delivery of Ad vectors into cells via fibroblast growth factor receptors (FGFR) (19, 20). FGF2 binds cells via a cooperative association with low-affinity heparan sulfate proteoglycans (HSPG) and high-affinity tyrosine kinase receptors, resulting in a final binding affinity much greater than most other cellular ligands ( $K_d \sim 2$  pM) (21, 22). FGFRs are expressed at sites of normal and pathological tissue repair, and on most solid tumors, but not on quiescent cell populations (23–27). In numerous *in vitro* and *in vivo* studies, we have demonstrated that FGF2 retar-

geting provides three enhancements to Ad-based gene therapy for cancer: neutralization of normal viral tropism (decreased toxicity); retargeting to an upregulated, high-affinity cellular receptor (increased specificity); and enhanced transgene expression (increased potency) (28–31).

In this report, we investigate the feasibility of combining matrix-mediated gene delivery and FGF2-targeting technologies to the gene therapy of soft tissue repair. Specifically, we evaluate FGF2-retargeted adenovirus encoding PDGF-BB (AdPDGF-B) delivered in collagen gels. Given that FGFRs are overexpressed on tissue repair cells, this work was a natural extension of our previous findings that targeted vectors show improved activity as anticancer agents. This novel approach offers the potential to significantly increase the potency and specificity of wound-healing gene therapeutics.

## MATERIALS AND METHODS

**Adenovirus vectors.** E1- and E3-deleted human adenovirus type 5 vectors encoding firefly luciferase (AdLuc, gift of David Curiel, University of Alabama at Birmingham) or the 160-amino-acid open reading frame of human PDGF-B (AdPDGF-B) were prepared as purified stocks with defined viral particle (PN) and plaque-forming unit (PFU) concentrations (PN:PFU < 100), as previously described (32).

***In vitro* association of AdPDGF-B and collagen.** AdPDGF-B ( $8 \leftrightarrow 10^{11}$  PN) was mixed with 0.2 ml collagen and added to a 2-ml flat-bottom polypropylene tube. Eight hundred microliters of water was added, and the tubes were centrifuged at 1000 rpm for 5 min, followed by gentle rocking at 37°C. Fifty-microliter aliquots were taken at 30 min and 48 h, diluted 10-fold, and lysed in 0.1% SDS for 15 min at 56°C. Adenovirus particle concentrations in the lysed samples were determined using the PicoGreen DNA quantitation reagent (Molecular Probes, Eugene, OR).

**FGF2–adenovirus conjugates.** The conjugation of FGF2 with the Fab fragment of a neutralizing anti-adenoviral fiber mAb has previously been described (19). In the studies reported here, a Fab' fragment containing one free cysteine was used for ease in conjugation and provided for increased homogeneity of the conjugate. The purified conjugate (FGF2–Fab') was bound to adenoviral vectors by incubation at room temperature for 30 min, yielding FGF2–Ad. The numbers of FGF2–Fab' molecules and viral particles used were adjusted so as to achieve a final FGF2–Fab':fiber monomer ratio of 10:1.

**Cell culture.** WS1 normal human dermal fibroblasts and WI-38 human lung fibroblasts were obtained from the American Type Culture Collection (Manassas, VA). Ad in collagen was prepared on ice according to the following formulation: 1 mg/ml purified bovine dermal type I collagen (CellPrime, Cohesion Technologies, Palo Alto, CA),  $1 \leftrightarrow$  MEM (GIBCO/BRL, Grand Island, NY), 2.2 mg/ml NaHCO<sub>3</sub>, 10% FBS, and Ad in buffer. Following gentle pipetting to adjust the pH to ~7.4, collagen solutions were aliquoted into 24-well cluster plates at 500  $\mu$ l/well and gelled by incubation at 37°C for 30 min. Gels were then seeded with  $5 \leftrightarrow 10^4$  cells resuspended in 1 ml complete medium in order to achieve the desired multiplicity of infection (m.o.i.). Culture supernatants were collected after 48 h and clarified by centrifugation. Cells were recovered by digesting collagen gels for 60 min at 37°C with 500  $\mu$ l of type IV collagenase solution (200 U/ml in DME, Worthington Biochemical, Freehold, NJ), centrifuging the digest, and rinsing the cell pellets  $3 \leftrightarrow$  with PBS. Culture media, collagen digest supernatants, and cell lysates were analyzed for PDGF-BB expression as described below.

**PDGF-B transgene expression.** For detection of PDGF-BB expression, 96-well ELISA/RIA plates (Costar, Cambridge, MA) were coated overnight with 50 pg/well rhPDGF R $\beta$ /Fc chimera (R & D Systems, Minneapolis, MN). Wells were then rinsed with TBS containing 0.05% Tween 20

(TBST) and blocked for 2 h in TBST containing 2% BSA. Experimental samples and a standard curve constructed from rhPDGF-BB (GIBCO/BRL) were then added as 50- $\mu$ l volumes. After a 30-min incubation, wells were rinsed with TBST, and bound human PDGF-BB was detected using a biotinylated polyclonal anti-PDGF-BB antibody (R & D Systems) followed by alkaline phosphatase-streptavidin and *p*-nitrophenyl phosphate. Substrate conversion was determined at a detection wavelength of 405 nm and a reference wavelength of 490 nm. Total cellular protein levels were determined by bicinchoninic acid (BCA) assay (Pierce, Rockford, IL).

**In vivo polyvinyl alcohol sponge model.** All *in vivo* studies were approved by the Selective Genetics Institutional Animal Care and Use Committee and conformed to the guidelines set forth in the Guide for the Care and Use of Laboratory Animals (National Research Council, Washington, DC).

Male Sprague-Dawley rats were anesthetized, and three bilateral sets of 5-mm full-thickness longitudinal incisions were made through the skin and panniculus carnosus, spaced 20 mm from midline and 10 mm apart. One sterile polyvinyl alcohol (PVA) sponge (1.2 cm diameter, 3 mm thick, grade 3, M-PACT, Eudora, KS) was placed per incision (six sponges total per animal) and the incisions were closed with wound clips. On day 4 postimplantation, collagen solution (1.45 mg/ml CellPrime collagen, 0.5 $\times$  MEM, 1.8 mg/ml NaHCO<sub>3</sub>, pH 7.4) with or without Ad was injected percutaneously into sponges (200  $\mu$ l/sponge). PVA sponges were harvested on day 6 posttreatment (day 10 postimplantation) and dissected from the surrounding tissue with the capsule intact. For histochemical analyses, PVA sponges were bisected, fixed for 4 h at 4°C in 4% paraformaldehyde, and paraffin-embedded. Masson's trichrome-stained cross-sections were used to determine the area of new granulation tissue (defined as containing fibroblasts and blood vessels) present within sponge voids.

To quantify PDGF-BB expression, sponges were snap-frozen in liquid nitrogen, minced, and placed at 0.2 g/ml in modified RIPA buffer (20 mM Tris, 100 mM NaCl, 1 mM EDTA, 0.5% NP-40, 0.5% sodium deoxycholate, 10 mM NaI, pH 7.8) containing a cocktail of protease inhibitors (Complete Tablets, Roche Molecular Biochemicals, Indianapolis, IN). Samples were then agitated for 20 s using a FastPrep system (BIO101, Vista, CA) set at 5.5 m/s. Extracts were freeze-thawed and centrifuged. Extract supernatants were analyzed for PDGF-BB expression as described above. Hydroxyproline contents were determined as an indicator of total collagen content. Sponges were dried under vacuum, and following acid hydrolysis, ninhydrin-reactive hydroxyproline was quantified at the Stanford University Medical Center Protein and Nucleic Acid Facility (Stanford, CA) using a Beckman 6300 amino acid analyzer (33).

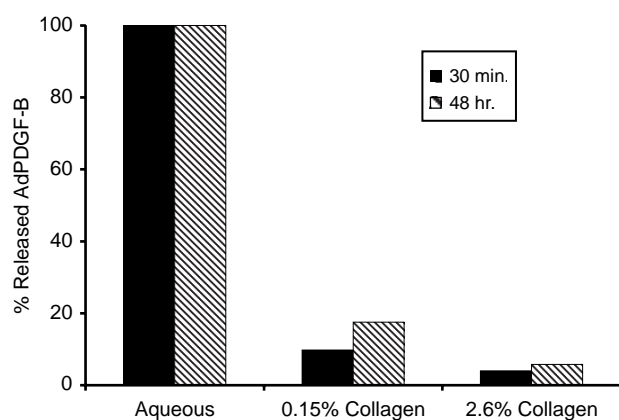


FIG. 1. AdPDGF-B associates with collagen matrices.  $8 \times 10^{11}$  PN AdPDGF-B were mixed with 0.2 ml collagen and added to a 2-ml flat-bottom polypropylene tube. Eight hundred microliters of water was added, and the tubes were centrifuged followed by gentle rocking at 37°C. Aliquots were taken at 30 min and 48 h and diluted 10-fold, and adenovirus particle concentrations were determined.

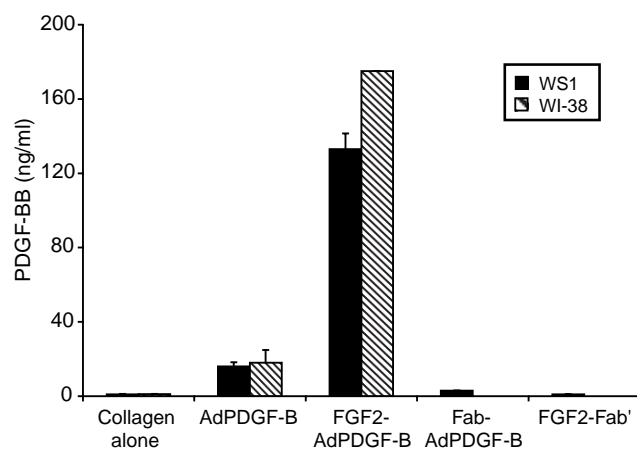


FIG. 2. PDGF-BB production following adenovirus transduction from collagen gels *in vitro*. WS1 and WI-38 human fibroblasts were plated onto collagen gels containing the following: no treatment (collagen alone), AdPDGF-B, FGF2-AdPDGF-B, Fab'-AdPDGF-B (WS1 only), or FGF2-Fab' (WS1 only). All adenovirus-containing gels were formulated so as to establish a theoretical m.o.i. of 150. After a 48-h culture period, PDGF-BB levels in culture supernatants were determined by ELISA. Data are presented as means  $\pm$  SD ( $n = 3$ ).

**Statistical analyses.** Groups of three or more means were compared using one-way ANOVA and Fisher's procedure for least-significant differences (StatView software, Abacus Concepts, Berkeley, CA).

## RESULTS

**AdPDGF-B associates with collagen matrices.** Prolonged availability of the DNA vector at the site of tissue injury is likely to provide maximal benefit for soft tissue repair. *In vitro* studies were conducted to determine the kinetics of release from collagen matrices of an adenoviral vector encoding human PDGF-BB (AdPDGF-B). Specifically, AdPDGF-B was formulated in 0.15 or 2.6% collagen, and the amount of vector released into an aqueous environment at 37°C was assessed at 30 min and 48 h. For each formulation, the amount of AdPDGF-B detected in the supernatant increased slightly from 30 min to 48 h (Fig. 1). For the 0.15% collagen formulation, ~80% of the vector remained associated with the collagen after 48 h. For the 2.6% collagen formulation, >90% of the vector remained associated with the collagen after 48 h. The association of AdPDGF-B with collagen supports the use of collagen as a matrix that will provide sustained availability of AdPDGF-B to infiltrating tissue repair cells and will limit dissemination of the vector from the treatment site. Furthermore, one can manipulate the relative amounts of released and collagen-associated vector (i.e., AdPDGF-B exhibits prolonged association with a 2.6% collagen paste compared to a thinner, 0.15% collagen gel).

**FGF2 targeting enhances matrix-mediated gene expression *in vitro*.** An *in vitro* assay system was developed in order to evaluate transgene expression following exposure of cells to collagen matrices containing AdPDGF-B.

Briefly, gels containing bovine type I collagen, culture medium, and AdPDGF-B were formed in the bottom of multiwell tissue culture plates. Cells were seeded onto the surface of the collagen gels, subsequently migrated into the collagen, and became transduced with adenovirus. Forty-eight hours after cell plating, culture medium was harvested and analyzed for transgene expression. As shown in Fig. 2, WS1 human dermal fibroblasts and WI-38 human lung fibroblasts secreted PDGF-BB following culture on collagen gels containing AdPDGF-B. The amount of PDGF-BB secreted by each cell type was in excess of the mitogen's ED<sub>50</sub> of ~3 ng/ml (34). When AdPDGF-B delivery was retargeted to FGF2 receptors (FGF2-AdPDGF-B), PDGF-BB production was increased ~8-fold in WS1 cells and ~10-fold in WI-38 cells when compared to nontargeted virus. Complexation of AdPDGF-B with the neutralizing anti-fiber antibody used to construct FGF2-Fab' (Fab-AdPDGF-B) resulted in a marked reduction in PDGF-BB production, consistent with ablation of the virus' natural tropism. Neither AdLuc (not shown), FGF2-AdLuc (not shown), nor FGF2-Fab' induced PDGF-BB expression. Thus, the observed increase in PDGF-BB was due to enhanced transgene expression as opposed to a nonspecific response to viral transduction or FGF2 stimulation. Therefore, FGF2 targeting, in combination with matrix-mediated gene delivery, significantly increased *in vitro* transgene expression.

*FGF2 targeting of AdPDGF-B leads to increased levels of secreted PDGF-BB.* Exon 6 of PDGF-B mRNA encodes a highly basic sequence that mediates electrostatic interactions with the extracellular matrix (35–37). Exon 6-encoded amino acids may be removed by proteolytic processing to generate a more freely diffusible isoform. Following transduction of WS1 cells with AdPDGF-B/collagen, 40% of the PDGF-BB produced was cell associated, and 60% was present in secreted compartments (i.e., the culture media and collagen digest supernatants) (Table 1). Following transduction with FGF2-AdPDGF-B/collagen, the overall level of PDGF-BB expression increased approximately fourfold, and the relative amount of secreted PDGF-BB increased to 87% of the total. This result indicates inherent differences in PDGF-BB processing and/or secretion following transduction of cells via

the native receptors of adenovirus versus the FGF receptor pathway. The increase in the overall level of PDGF-BB expression and the increased ratio of secreted vs cell-associated protein observed with FGF2 targeting could lead to improved tissue repair responses *in vivo* by maximizing the potential for autocrine and paracrine stimulation of repair fibroblasts and endothelial cells.

*FGF2 targeting enhances matrix-mediated gene expression in vivo.* The PVA sponge implant model has been utilized in the evaluation of numerous potential therapeutics for soft tissue repair (38, 39). PVA sponges are implanted subcutaneously into rats, providing a scaffolding for infiltration by inflammatory and stromal cells with subsequent formation of granulation tissue within the void spaces of the sponge. This granulation tissue remodels over time, producing mature granulation tissue such as that found in healing dermal wounds. Since PDGF-BB is an essential component of the natural tissue repair process, it was predicted that delivery of AdPDGF-B would enhance the formation of granulation tissue within the treated sponges. This hypothesis was tested using 1.45 mg/ml (0.15%) type I collagen as the delivery vehicle. Sponges were injected with test articles 4 days after implantation. By waiting 4 days, a modest foreign body response will have initiated, and a small influx of inflammatory cells and fibroblasts will have begun, thus resembling a wound environment. A representative example of the effects of AdPDGF-B in the PVA sponge model is shown in Fig. 3. Throughout the AdPDGF-B-treated sponge (Fig. 3C), void spaces were replaced with new granulation tissue. In contrast, there was minimal new tissue formation in the collagen-alone (Fig. 3A) or AdLuc-treated (Fig. 3B) sponges and only at the very edges of the sponges. The lack of a response with AdLuc confirmed the role of the PDGF-B transgene in stimulating the formation of granulation tissue. Importantly, no excess inflammation was associated with the administration of AdPDGF-B. By immunohistochemistry, we have detected human PDGF-BB for 6 days following injection of AdPDGF-B/collagen into PVA sponges (unpublished observations). The signal intensity increased from days 1 to 4, and by day 6 the major location of PDGF-BB was extracellular, thus establishing a depot and perhaps prolonging the availability of the

TABLE 1  
FGF2-AdPDGF-B Induces PDGF-BB Expression and Secretion

Treatment	PDGF-BB in media (ng/well)	PDGF-BB in collagen (ng/well)	PDGF-BB in cell lysate (ng/well)	Secreted PDGF-BB (% of total)
Collagen	2 ± 0	0.7 ± 0.1	2 ± 1	NA
AdPDGF-B/collagen	38 ± 2	7 ± 0.3	30 ± 7	60
FGF2-AdPDGF-B/collagen	237 ± 37	12 ± 0.7	36 ± 11	87

Note. WS1 human fibroblasts were plated onto collagen gels containing the following: no treatment, AdPDGF-B, or FGF2-AdPDGF-B (moi 150). After a 48-h culture period, culture media were harvested, gels were digested with collagenase and centrifuged, and the cell pellets were lysed in detergent. Data are presented as ng PDGF-BB/well (means ± SD, n=3). For culture media and collagen digests, all groups differ by p < 0.0001. NA, not applicable.

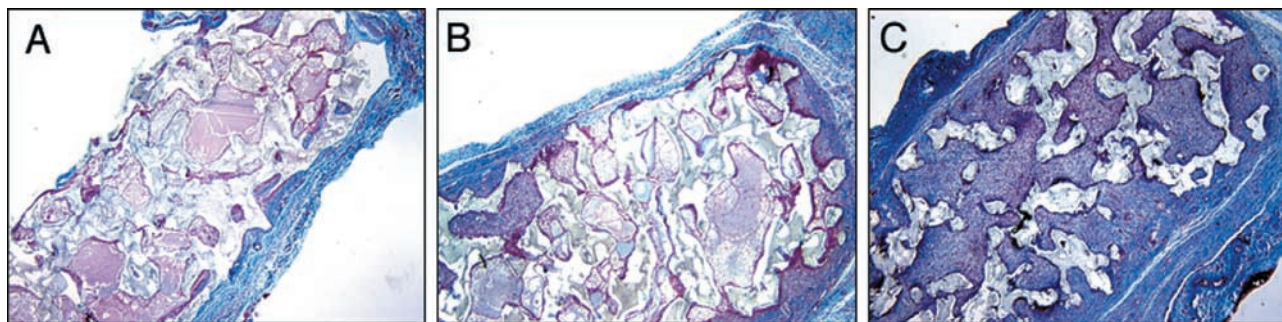
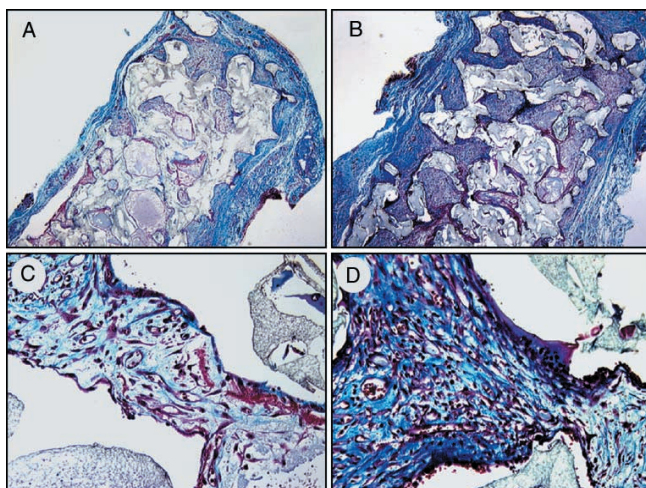


FIG. 3. AdPDGF-B in collagen induces increased granulation tissue *in vivo*. PVA sponges were implanted subcutaneously into rats and injected 4 days later with one of the following treatments formulated in CellPrime collagen: no treatment (collagen alone, A), AdLuc ( $6 \times 10^{10}$  PN, B), or AdPDGF-B ( $3.6 \times 10^{10}$  PN, C). Sponges were removed 6 days later and processed for Masson's trichrome staining.

therapeutic protein. This observation was not unexpected since PDGF-BB contains collagen binding motifs (40).

Given that FGF2 targeting resulted in enhanced transgene expression *in vitro* (Fig. 2), the effects of FGF2 targeting on *in vivo* PDGF-B transgene expression and induction of granulation tissue were assessed. PVA sponges were implanted subcutaneously in rats and injected 4 days later with Ad vectors ( $\pm$ FGF2-Fab') suspended in collagen. Sponges were harvested 6 days post-treatment for evaluation of PDGF-BB expression by ELISA. Treatment with AdPDGF-B resulted in the expression of  $\sim 20$  ng PDGF-BB per sponge (Fig. 4). Therefore, in agreement with the *in vitro* results, delivery of AdPDGF-B *in vivo* resulted in production of PDGF-BB at levels in excess of the protein's  $ED_{50}$  (34). When AdPDGF-B was targeted with FGF2, PDGF-BB production was enhanced nearly twofold. In contrast, PDGF-BB expression was not detected in the AdLuc- or FGF2-AdLuc-treated controls. Therefore, as observed *in vitro*, AdPDGF-B formulated in collagen leads to PDGF-BB production by transduced cells *in vivo*, and FGF2 retargeting enhances this response.



To detect histochemical differences between targeted and untargeted AdPDGF-B, the virus was delivered to PVA sponges at a log lower dose than that employed in Figs. 3 and 4. As expected with this lower dose, AdPDGF-B treatment generated sparse granulation tissue, with large void spaces remaining in the sponges (Figs. 5A and 5C). In contrast, PVA sponges injected with an equivalent dose of FGF2-AdPDGF-B contained well-vascularized and highly cellular granulation tissue (Figs. 5B and 5D). Again, there was no excess inflammation associated with administration of AdPDGF-B or FGF2-AdPDGF-B. The results were quantified by measuring the area of newly formed granulation tissue within treated sponges. When delivered at  $2.3 \times 10^9$  PN/sponge, FGF2-AdPDGF-B, but not AdPDGF-B, significantly increased granulation tissue area when compared to the collagen-treated control (Fig. 6A). Only when AdPDGF-B was delivered at the higher dose of  $2.3 \times 10^{10}$  PN/sponge were responses significantly greater than the controls observed. The ability of FGF2-AdPDGF-B to enhance these responses was not due to actions of FGF2, since FGF2-Fab' alone had no effect relative to the controls. Furthermore, the FGF2 dose delivered by retargeted FGF2-AdPDGF-B (140 ng) was well below the 500–2000 ng required for wound-healing effects (39, 41, 42).

Another reliable measure of the collagen content of granulation tissue is hydroxyproline, which is a major component of mature collagen (43). As shown in Fig. 6B, the hydroxyproline content of sponges treated with  $2.3 \times 10^9$  PN AdPDGF-B/sponge was comparable to that of the sponges treated with collagen alone. In contrast,  $2.3 \times 10^9$  PN FGF2-AdPDGF-B/sponge generated an increase in the hydroxyproline content of the sponges that was

FIG. 5. FGF2-AdPDGF-B induces increased granulation tissue formation *in vivo*. PVA sponges were implanted subcutaneously into rats and 4 days later injected with  $2 \times 10^9$  PN/sponge of either AdPDGF-B (A and C) or FGF2-AdPDGF-B (B and D) formulated in CellPrime collagen. PVA sponges were removed 6 days posttreatment and processed as Masson's trichrome stains, representative areas of which are shown (A and B, total magnification  $20\times$ ; C and D, total magnification  $200\times$ ).

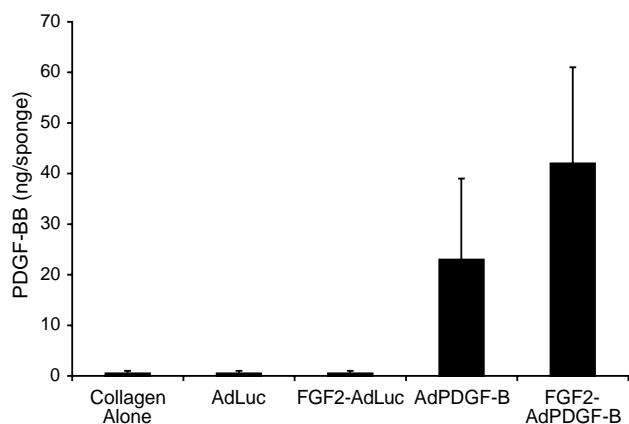


FIG. 4. PDGF-BB production following transduction from collagen gels *in vivo*. PVA sponges were implanted subcutaneously into rats and injected 4 days later with one of the following treatments formulated in CellPrime collagen: no treatment (collagen alone,  $n = 9$ ), AdLuc ( $n = 3$ ), FGF2-AdLuc ( $n = 6$ ), AdPDGF-B ( $n = 6$ ), or FGF2-AdPDGF-B ( $n = 3$ ). AdPDGF-B ( $\pm$ FGF2) was delivered at  $2 \times 10^{10}$  PN/sponge, and AdLuc ( $\pm$ FGF2) was delivered at  $6 \times 10^{10}$  PN/sponge. PVA sponges were removed 48 h later, dissected free of overlying capsules, and solubilized in lysis buffer. PDGF-BB levels in lysates were then determined by ELISA. Data are presented as means  $\pm$  SD. The AdPDGF-B and FGF2-AdPDGF-B groups differ from all others and from each other by  $P \leq 0.05$ .

comparable to the response generated by nontargeted AdPDGF-B at  $2.3 \times 10^{10}$  PN/sponge. Taken together, the data presented in Fig. 6 demonstrate the ability of FGF2-AdPDGF-B delivered in a collagen matrix to enhance granulation tissue formation at substantially reduced vector concentrations compared to nontargeted vectors.

## DISCUSSION

The ability to physically localize protein and DNA therapeutics to application sites has been identified as an important point of consideration for the effective treatment of chronic wounds. Improved tissue-repair responses have been observed with matrix-mediated delivery of therapeutic proteins such as EGF, angiotensin II, FGF2, and TGF- $\beta$  when compared to aqueous delivery systems (12–15). Direct injection of an adenovirus vector encoding PDGF-BB (AdPDGF-B) into wound margins has generated improved healing responses in ischemic rabbit ears (32). However, the study employed a model in which cartilage was left intact at the base of the wound defect, allowing for a prolonged residence time of the vector. Also, in chronic wounds, repair cells originate from the wound bed as well as from the wound margins. For maximum transfection of repair cells, it is therefore desirable to employ a formulation that exposes all surfaces of a wound to the gene therapeutic and for a prolonged period of time. Indeed, Shea *et al.* demonstrated that *in vivo* matrix-mediated delivery of plasmid DNA encoding PDGF-BB enhanced granulation tissue formation, whereas direct injection of aqueous plasmid had no effect (18).

In this report, we demonstrate that AdPDGF-B delivered in a collagen matrix induces PDGF-BB production *in vitro* and *in vivo* as well as *de novo* tissue formation *in vivo*. The tissues formed in response to AdPDGF-B are well developed as indicated by their high degree of cellularity, vascularity, and extracellular matrix content. The demonstration that AdPDGF-B associates with collagen *in vitro* supports the hypothesis that the matrix prolongs the availability of the vector to incoming repair cells. By PCR, we have demonstrated persistence of AdPDGF-B in treated wound beds for at least 1 week, without dissemination to the general circulation (unpublished observations).

Inflammatory reactions are of a general concern when considering the clinical use of adenoviral vectors. In this report, at 6 days posttreatment with AdPDGF-B there is no evidence for inflammatory reactions other than that which is part of the normal wound-healing response. Similarly, no late inflammatory reactions have

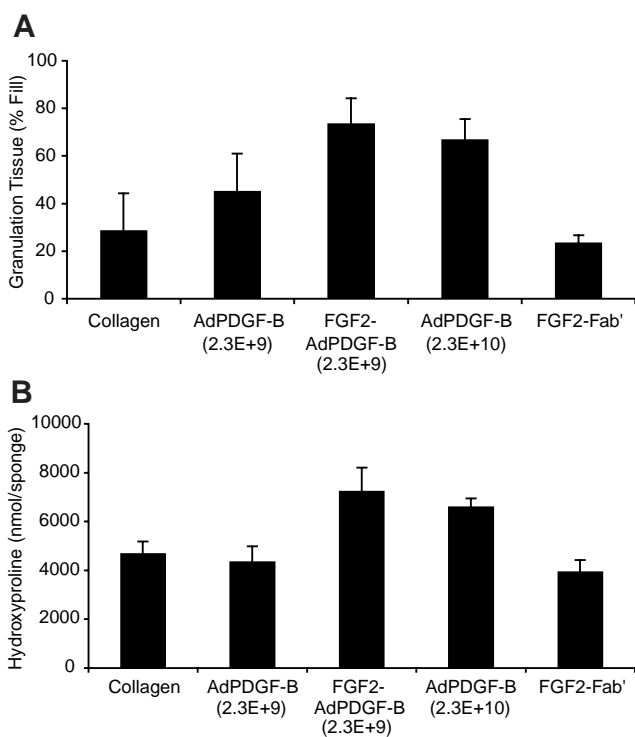


FIG. 6. FGF2-AdPDGF-B induces increased granulation tissue formation *in vivo*. PVA sponges were implanted subcutaneously into rats and 4 days later injected with one of the following treatments formulated in CellPrime collagen: collagen alone, AdPDGF-B ( $2.3 \times 10^9$  or  $2.3 \times 10^{10}$  PN/sponge), or FGF2-AdPDGF-B ( $2.3 \times 10^9$  PN/sponge). Sponges were removed 6 days post-treatment and processed by Masson's trichrome staining for determination of granulation tissue areas (A). Alternatively, following acid hydrolysis, ninhydrin-reactive hydroxyproline was quantified using an amino acid analyzer (B). The FGF2-AdPDGF-B and AdPDGF-B ( $2.3 \times 10^{10}$  PN/sponge) groups differ from all others by  $P < 0.04$  for hydroxyproline content and  $P \leq 0.02$  for granulation tissue areas. Data are presented as means  $\pm$  SEM ( $n = 3$ ).

been observed (unpublished observations). Sylvester *et al.* (44) recently reported studies evaluating the inflammatory response to adenovirus in a human skin/SCID mouse chimera wound-healing model. Despite an increased acute inflammatory response following injection of adenovirus into the wounded skin graft, the normal sequence and quality of wound healing were unaffected. Therefore, this study in combination with our own data supports the feasibility of adenovirus-mediated gene therapy for tissue repair.

Repeat dosing with AdPDGF-B/collagen would likely be required in order to achieve complete healing of a chronic wound. Several studies have addressed the potential inhibitory effects of circulating antibodies on the efficacy of repeat administrations of adenoviral vectors. When delivered to a localized tissue site (i.e., intramuscular or intratumoral injection), successful and effective repeat administration of adenoviral vectors has been demonstrated (45–47). Therefore, for local gene therapy applications, the antigenicity of adenovirus can be overcome. In preliminary studies employing the PVA sponge model, we have demonstrated increased granulation tissue formation upon repeat administration of AdPDGF-B/collagen and in the presence of anti-adenovirus antibodies (unpublished observations).

To achieve enhanced responses to AdPDGF-B, and to potentially increase its margin of safety, we have used FGF2 as a targeting ligand. This approach was based on our previous work demonstrating enhanced efficacy of targeted adenovirus for anticancer applications and the knowledge that FGF receptors are expressed on proliferating cell types involved in tissue repair, such as endothelial cells, fibroblasts, and macrophages (20, 24, 31, 48, 49). *In vitro* transduction with FGF2-Ad can result in increased levels of transgene expression per cell, increased numbers of transduced cells, and expression equivalent to nontargeted vector at markedly reduced doses (28–31). The data presented here demonstrate the broader utility of this targeting strategy. Retargeting AdPDGF-B delivery through high-affinity FGFRs greatly increases the production of PDGF-BB by transduced normal tissue repair cells. In addition, the relative amount of secreted PDGF-BB is increased when AdPDGF-B is targeted with FGF2. As a result, tissue formation *in vivo* is enhanced over that seen using nontargeted vector.

This is the first report in which molecular (i.e., FGF2) targeting has been coupled with localized, matrix-mediated gene delivery. FGF2 retargeting was not required for an *in vivo* wound-healing response. However, in combination with matrix-mediated gene delivery, FGF2-AdPDGF-B did enhance granulation tissue formation at a given vector dose and allowed for responses comparable to the nontargeted vector at significantly lower doses. Therefore, matrix-mediated delivery in combination with FGF2 targeting generates improved therapeutic outcomes and potentially overcomes some of the safety and efficacy limitations of current gene therapy strategies for tissue repair and regeneration.

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