Sequential Delivery of Angiogenic Cytokines from Fibrin Gels in a Murine Critical Limb Ischemic Model

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Statement of Purpose: Therapeutic angiogenesis approaches that are based on the controlled delivery of angiogenic cytokines at the site of ischemia are promising alternatives to current invasive therapies (e.g. vein grafting and endarterectomy) for the treatment of critical limb ischemia (CLI) [1,2]. A number of exciting studies have shown enhanced revascularization at the site of ischemia by locally delivering angiogenic factors (i.e. VEGF, bFGF, PDGF-BB) [1,2,3]. We have synthesized gelatin and fibrin-based hydrogels that deliver at controlled rates bFGF and G-CSF and assessed their angiogenic potential in a murine CLI model [3]. Herein, we report on the effect of sequentially delivering bFGF and G-CSF, their order of release and loading concentrations on the rate of revascularization in *in vitro* and *in vivo* studies.

Methods: Anionic gelatin-polyglutamate (gelatin-PLG) hydrogels were synthesized by reacting gelatin (gelatin B from bovine skin, bloom 225, 0.1 g/ml) with poly-L-glutamic acid (0.01 g/ml) in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) at molar ratios of 2:1 for 10 min at 4 °C. bFGF and G-CSF were added during the polymerization reaction at loading concentrations of 100 ng of each growth factor per hydrogel implant (300 µl). Fibrin gels were synthesized by combining fibrinogen (4 mg/ml) with thrombin (20 NIH U/ml) and calcium chloride (10 mM) at a 5:1 ratio and crosslinked for 3 hours at 37 °C. bFGF and G-CSF were added at various ratios during polymerization. Ionic albumin microspheres were prepared in an oil/water emulsion in the presence of poly-L-glutamic acid or poly-L-lysine (PLL) and precipitated using acetone. The collected microspheres were crosslinked in 100 mM EDC solution, washed using double-distilled water, filtered, and freeze-dried for 48 hours. 100 ng of bFGF (in microspheres with PLG) or G-CSF (in microspheres with PLL) was absorbed into 25 mg of microspheres overnight at 4 °C and were sandwiched between two fibrin layers. Degradation characteristics of the gel scaffolds and release kinetics of growth factors from gelatin, fibrin, and microsphere loaded hydrogels were determined using an ELISA. Balb/C mice (age 6-7 weeks) underwent surgery to induce CLI by ligation of the profunda femoris and the common femoral artery and all arteries between were excised and removed. Hydrogels loaded with bFGF and/or G-CSF (either fibrin or gelatin; 400 µL) were placed directly on top of the quadriceps muscle of the mouse and Doppler perfusion ratios were collected at various time points post-surgery. Immunohistochemistry using CD-31 and smooth muscle α-actin antibodies was performed at 2, 4, and 8 weeks post-surgery on the ischemic muscle.

Results: Co-delivery of bFGF and G-CSF from ionic, gelatin-based hydrogels resulted in a significant increase in reperfusion (0.80 ± 0.04), capillary sprouting (1278 ± 92 vessels mm^{-2}), and mature vessel formation (317 ± 24 SMA vessels mm^{-2}) compared to controlled release treatment of a single growth factor ($p < 0.05$) after 8 weeks. Alternatively, sequential delivery of bFGF and G-CSF from fibrin hydrogels resulted in 93% blood flow recovery (± 3.4%) eight weeks post ligation which is significantly greater ($p < 0.05$) than the co-delivery of bFGF and G-CSF (Figures 1, 2).

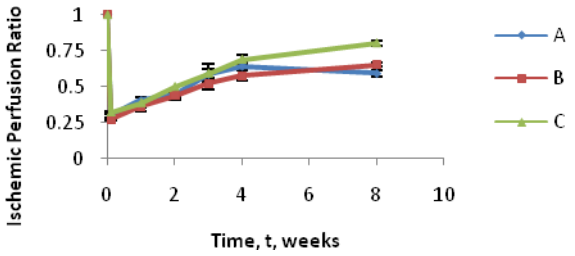

Fig 1. Delivery of 100 ng bFGF (A), 100 ng G-CSF (B), or both (C) from crosslinked fibrin gels over eight weeks in a mouse CLI model (3 mice/per time point per group).

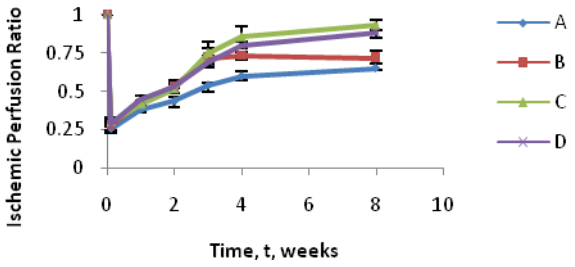

Fig 2. Sequential delivery of bFGF and G-CSF from fibrin gels over eight weeks. (A) fibrin gels containing physically entrapped bFGF and G-CSF loaded albumin microspheres; (B) fibrin gels containing physically entrapped G-CSF and bFGF loaded albumin microspheres; (C) fibrin gels containing physically entrapped bFGF and G-CSF loaded cationic albumin microspheres; (D) fibrin gels containing physically entrapped G-CSF and bFGF loaded anionic albumin microspheres.

Conclusions: We have demonstrated that the co-delivery of bFGF and G-CSF resulted in significant reperfusion, increased capillary density, and robust vessel formation compared to single factor delivery. In addition, incorporating one growth in ionic microspheres and then loading them into the hydrogels that contained the other growth factor sequential delivery was achieved and a significant increase in blood flow reperfusion was observed compared to co-delivery of the same growth factors.

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References:

1. Cao R. Nat Med 2003; 9:604-613.
2. Hackam DG. Am Heart J 2005; 150:35-40.
3. Layman H. Acta Biomaterialia 2008; in press.