Delivery of bFGF with Injectable pH- and Temperature-responsive Hydrogel Promotes Revascularization and Improves Cardiac Function Post-infarct

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Statement of Purpose: Physical hydrogels that are capable of reversible gel formation in response to clinically-relevant stimuli have significant potential as drug delivery systems (DDS). In particular, polymers that are soluble at 37˚C and pH 7.4 but form gels under conditions of intermediate acidity (pH 6.0-7.0) at 37°C may be able to target regions of local acidosis, as found in ischemia. With drug delivery and tissue healing, these hydrogels can subsequently dissolve as the tissue returns to physiologic pH, facilitating elimination from the body. Polymers containing N-isopropylacrylamide and propylacrylic acid have been shown to be sharply responsive to changes in pH and temperature.[1] In this study, we evaluated the ability of a novel pH- and temperature-responsive injectable hydrogel system, poly(N-isopropylacrylamide-co-propylacrylic acid-co-butyl acrylate) (p[NIPAAm-co-PAA-co-BA]), to deliver basic fibroblast growth factor (bFGF), promote angiogenesis and improve cardiac function in a rat model of myocardial infarct.

Methods: Poly(NIPAAm-co-PAA-co-BA) (10 mol % PAA, 10 mol % BA in the feed) was synthesized by reversible addition fragmentation chain transfer (RAFT) polymerization. Chilled polymer solutions were combined with bFGF by bulk mixing. Permanent occlusion of the left anterior descending (LAD) coronary artery in male Fischer 344 rats was used as an animal model of myocardial infarct.[2] Polymer (40 µL, 8 wt %) containing 5 µg bFGF was injected into infarcted myocardium. Hydrogel retention of biotinylated bFGF *in vivo* at 0-7 days post-injection into infarcted rat myocardium was quantified by western blot in homogenized tissues. Arteriolar densities were quantified by smooth muscle α-actin (α-SMA) staining in histological sections of infarcted myocardium at 4 weeks post-injection of bFGF with hydrogel or saline vehicle. Fractional shortening (FS%) as a measure of cardiac function was determined by echocardiography at 2, 14, and 28 days post-injection.

Results: P(NIPAAm-co-PAA-co-BA) (28 kDa) was successfully synthesized by RAFT polymerization. This polymer exists as a liquid at pH 7.4 and 37 ˚C but rapidly forms a gel at pH 6.8 and 37 ˚C following injection into infarcted myocardium. Delivery of bFGF-biotin with the hydrogel *in vivo* provided improved retention of bFGF between 0–7 days post-injection vs. saline vehicle (Figure 1). After 4 weeks of treatment *in vivo*, there was a 30% increase in arteriolar density in rats treated with polymer+bFGF compared to all control groups ($p<0.001$) (Figure 2). FS% was determined by echocardiography to be significantly higher ($p<0.05$) in rats treated with polymer+bFGF (30±1.4%) compared to saline (25±1.2%) and polymer alone (25±1.8%) (Figure 3).

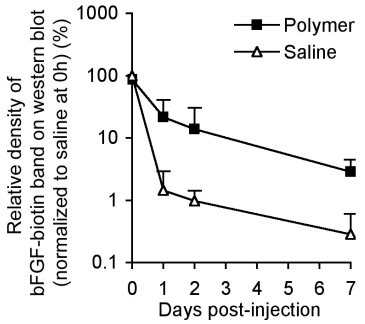

Figure 1. Quantification of bFGF-biotin retention by western blot following injection with saline or polymer into infarcted rat myocardium.

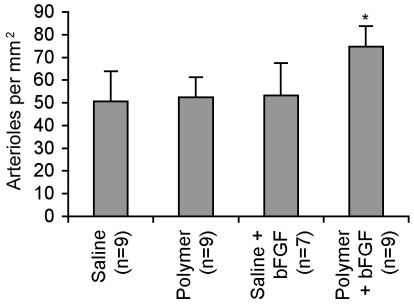

Figure 2. Arteriolar density evaluated by smooth muscle α-actin (α-SMA) staining after 4 weeks of treatment *p<0.001 vs saline, polymer, and saline+bFGF.

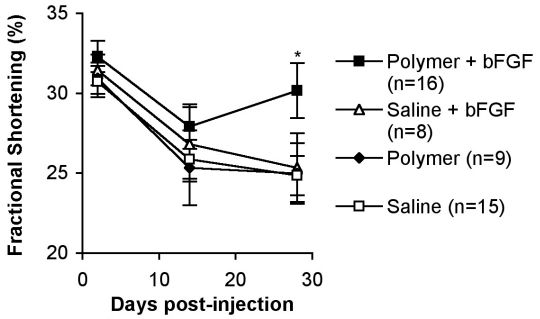

Figure 3. Fractional shortening (mean±SEM) at 2, 14, and 28 days post-surgery in rats injected with polymer+bFGF, saline+bFGF, polymer, or saline. *p<0.05 versus saline and polymer at 4 weeks.

Conclusions: By responding to local changes in pH and temperature in an animal model of ischemia, this hydrogel system was able to provide sustained, local delivery of bFGF, improve angiogenesis, and improve cardiac function. Future work might combine use of this hydrogel with other sustained release approaches to provide sequential delivery of multiple growth factors.

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References: [1]Yin X. Biomacromol. 2006;7:1381-1385.
[2]Huang et al. Nat Protoc. 2006;1:1596-1609.