pH- and Temperature-Responsive Hydrogel for Delivery of Angiogenic Growth Factors

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Statement of Purpose: A new pH- and temperature-responsive hydrogel system was designed for delivering drugs to regions of local acidosis, as seen in infection, tumors, or ischemia. The random copolymer, poly(N-isopropylacrylamide-co-propylacrylic acid) (p[NIPAAm-co-PAA]), is soluble at pH 7.4 and 37˚C but forms a physical hydrogel at 37˚C under conditions of moderate acidity (pH < 6). This polymer will gel at acidic pH values following injection into injured tissue. Furthermore, this hydrogel is designed to gradually dissolve as the tissue heals in response to sustained drug delivery and the wound site returns to physiological pH.

Methods: P(NIPAAm-co-PAA) was synthesized by reversible addition fragmentation chain transfer (RAFT) polymerization.[1] To tune the sol-gel pH response above pH 6, the hydrophobic monomer, butyl acrylate (BA), was added to the feed in some polymers to synthesize p(NIPAAm-co-PAA-co-BA). To quantify hydrogel erosion *in vitro* and to facilitate visualization following injection *in vivo*, polymers were covalently tagged with a BODIPY-FL hydrazide fluorescent label (Molecular Probes, Eugene, OR). Solutions of polymers doped with 0.1 wt % BODIPY-labeled p(NIPAAm-co-PAA) were mixed in bulk with vascular endothelial growth factor (VEGF) then heated to promote gel formation. Release buffer (PBS) adjusted to pH 7.4, 6, or 5 was added on top of the gel. Hydrogel erosion and VEGF concentration released from the hydrogel at various time points were quantified by a fluorescence plate reader and by enzyme-linked immunosorbent assay (ELISA), respectively. Permanent occlusion of the left anterior descending (LAD) coronary artery in male Sprague-Dawley rats was used as an animal model of myocardial infarct. Polymer was visualized by hematoxylin and eosin (H&E) and by fluorescence microscopy of cryosections of the heart.

Results: Hydrogel erosion of p(NIPAAm-co-PAA) was dependent upon the pH of the surrounding environment. In pH 7.4 release buffer, the hydrogel dissolved within 4 days, while in pH 6 buffer, more than 70% of the polymer remained in the gel phase at 28 days (Figure 1a). Furthermore, P(NIPAAm-co-PAA) hydrogels were also capable of pH-dependent sustained delivery of VEGF over a period of at least three weeks *in vitro* (Figure 1b). Butyl acrylate added to the p(NIPAAm-co-PAA) hydrogels facilitated gel formation above pH 6 (data not shown). P(NIPAAm-co-PAA-co-BA) hydrogels were injected into infarcted rat myocardium. Immediately after injection as a chilled liquid, the polymer was visible as a white mass in the myocardium. Figure 2 shows an H&E-stained section and a serial fluorescence image of the polymer injected into rat heart then harvested within 30 minutes of injection.

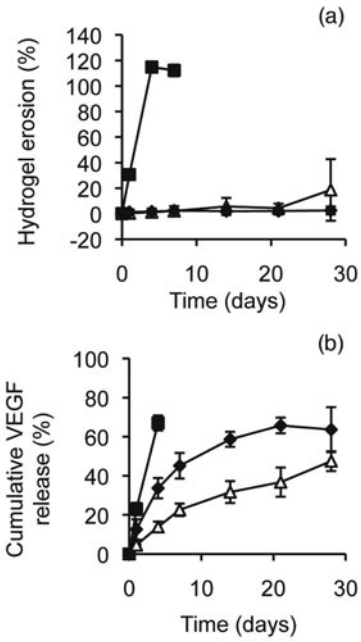

Figure 1. (a) Erosion and (b) cumulative VEGF release from p(NIPAAm-co-PAA) (37 kDa, 17 mol % PAA) hydrogel (5 wt % in DPBS). Release buffer adjusted to pH 7.4 (■), pH 6 (▲), or pH 5 (◆) was added on top of the hydrogel.

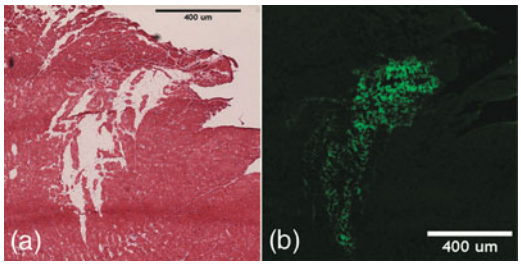

Figure 2. P(NIPAAm-co-PAA-co-BA) (28 kDa, 10 mol% PAA in feed, 10 mol % BA in feed) injected into infarcted rat myocardium (5 wt % polymer). (a) H&E viewed with light microscopy. (b) BODIPY-FL fluorescence viewed in unfixed serial cyrosection with FITC filter.

Conclusions: Aqueous solutions of p(NIPAAm-co-PAA) are capable of sustained release of angiogenic growth factor. The pH-response can be tuned with the hydrobopic monomer, butyl acrylate. This hydrogel system can be injected into regions of ischemia *in vivo* and is designed to undergo dissolution as the tissue returns to physiological pH with wound healing. This system has significant potential as a minimally-invasive delivery system to regions of local acidosis.

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References:

[1]Yin X. Biomacromolecules. 2006;7:1381-1385.