In Situ Forming Hydrogels to Localize Stem Cell Recruitment

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Statement of Purpose: Exogenously delivered bone marrow derived stem cells (BMSCs) have been extensively explored to treat myocardial infarction (MI). While there is evidence that delivering these cells provides some functional benefit to the injured heart, transplanted cell retention and survival in the post-MI environment is extremely low[1]. As an alternative to cell delivery, we have developed an in situ forming biomaterial system to localize and sustain the release of a chemoattracting cytokine, stromal-derived factor-1 alpha (SDF-1α), to recruit endogenous BMSCs. SDF-1α is critical for the retention of BMSCs in the bone marrow, but can also induce directional chemotaxis of BMSCs to injured tissues[2]. In our system, the crosslink density of a hyaluronic acid (HA) hydrogel controls the diffusion of SDF-1α with changes in macromer concentration and extent of reactive group modification. In addition, the hydrogel delivery system is formed in situ to promote integration with the myocardium via a visible light initiated photopolymerization, and is degraded into potentially angiogenic molecules.

Methods: 74kDa HA (Lifecore) was modified with a hydroxyethyl methacrylate (HEMA) group to incorporate photoreactivity and hydrolytic degradation. Briefly, HEMA was reacted with succinic anhydride via a ring opening polymerization in the presence of N-methylimidazole to obtain HEMA-COOH, which was then coupled to a tetrabutylammonium (TBA) salt of HA in the presence of 4-dimethylaminopyridine. By varying the ratio of HEMA-COOH to HA-TBA, the number of HA units modified with a HEMA group can be controlled. HEMA-HA hydrogels were polymerized with a cytocompatible visible light (VL) -initiated system consisting of 0.02 wt% eosin Y, 225 mM triethanolamine, 37mM vinylpyrrolidone and a commercially available dental curing lamp[3]. For release kinetics, recombinant human SDF-1α was encapsulated into HEMA-HA hydrogels with two different crosslink densities (n=3 per group) during photopolymerization and released into a buffer over two weeks. Released SDF-1α was quantified using ELISA and reported as percent cumulative release. The ability of the released SDF-1α to chemoattract BMSCs was quantified with a Boyden chamber assay[4]. The number of migrated human mesenchymal stem cells (hMSCs) after 16 hr incubation in response to serial dilutions of SDF-1α released during the first 24 hrs was reported as a fold increase compared to 0 ng/ml SDF-1α.

Results/Discussion: Hydrogels photopolymerized on murine hearts adhered to the tissue (Figure 1). In addition, the VL photocrosslinking of the HA hydrogels was rapid, reaching maximal crosslinking within minutes (rheometry data not shown). This photopolymerization system allows us to localize the therapy to the infarct zone in situ without further damaging the vasculature with multiple injections. To direct SDF-1α diffusion into the tissue, a PEG hydrogel with a much higher crosslink density was easily photopolymerized on top of the HEMA-HA hydrogel. HEMA-HA hydrogels showed a minimal burst release of SDF-1α despite the molecule's small size (~8kDa). Release was sustained for two weeks from both hydrogel formulations, while the release kinetics were controlled by varying macromer concentration and percent modification of the HA macromer (Figure 1). Controlling SDF-1α release kinetics is important to sustain an optimal gradient of the chemoattractant from the myocardium to the bone marrow (through the circulation) to recruit and potentially engraft BMSCs in the injured heart. To this end, the in vitro Boyden migration assay confirmed the activity of released SDF-1α (Figure 1). hMSCs were recruited in a dose dependent manner towards higher concentrations of released SDF-1α.

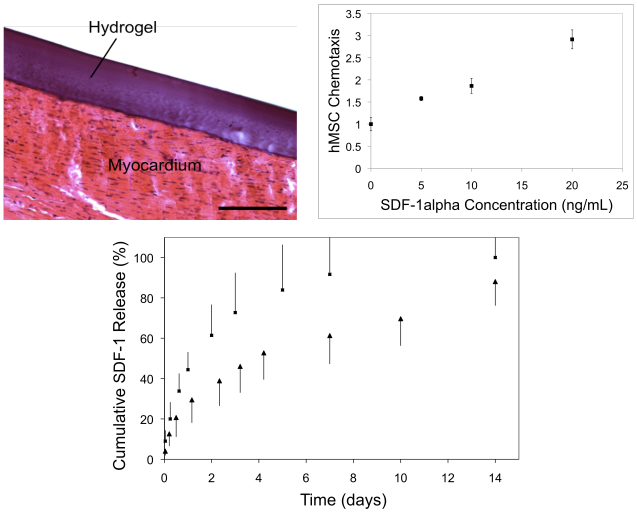

Figure 1. H&E histology of adherent HEMA-HA hydrogel on murine myocardium (top left, scale bar = 100μm), activity of released SDF-1α (top right), and release kinetics from 3wt%, 7% modified (squares) and 6 wt%, 20% modified (triangles) (bottom).

Conclusions: Our photopolymerizable biomaterial system represents a useful vehicle to sustain the delivery of SDF-1α to the injured myocardium. In addition to ultimately providing a therapeutic benefit post-MI, this system represents a useful platform to study the recruitment of BMSCs through the circulation to injured tissues in vivo. Ongoing work is investigating the distribution of SDF-1α delivered from the patch into the adjacent myocardium as well as optimal concentrations of SDF-1α required to recruit and engraft BMSCs in explanted hearts with an ischemia reperfusion model.

References: 1) Passier R. *Nature* 2008; 453:322-329. 2) Kucia M. *Blood Cells, Molecules, and Diseases* 2004; 32:52-57. 3) Pathak CP. *J. Am. Chem. Soc.* 1992; 114:8311-8312. 4) Goncharova EA. *Nature Protocols* 2006; 1:2933-2939.