Injectable Guest-Host Hydrogels for Cell and IL-10 Co-Delivery to the Heart

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Statement of Purpose: Previous work showed that the delivery of endothelial progenitor cells (EPCs) with an injectable hydrogel carrier improves functional and remodeling outcomes after myocardial infarction (MI)[1]. However, cells are delivered to a hostile infarct environment that is ischemic, hypoxic, and inflammatory. Interleukin-10 (IL-10) is an anti-inflammatory cytokine that can modulate inflammation at the injection site and has been shown to promote EPC survival, retention, and vascularization potential[2]. Thus, co-delivery of EPCs and IL-10 using an injectable hydrogel carrier could alter inflammation at the implantation site, which could enhance viability and retention of delivered cells. Further, the encapsulation of IL-10 in microgels can decouple IL-10 release from degradation of the bulk gel for temporal control over IL-10 delivery (Fig. 1A).

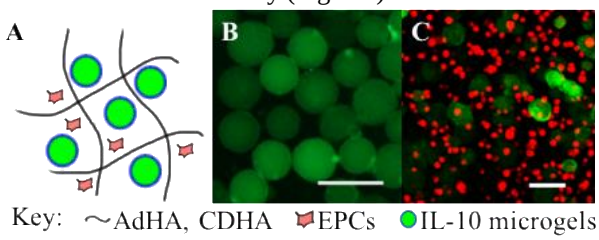

Figure 1. Schematic of injectable therapy for co-delivery of IL-10 and EPCs within a guest-host hydrogel carrier (A) and FITC-dextran encapsulated microgels after rehydration (B) and with CellTracker Red-labeled cells in a bulk guest-host hydrogel (C). Scale bar is 100 um.

Methods: Hyaluronic acid (HA) was modified either with adamantane (AdHA) or ß-cyclodextrin (CDHA) as previously described to form an injectable guest-host hydrogel[3]. Separately, HA was modified with norbornene groups (NorHA) to allow for covalent crosslinking with di-thiols and photoinitiator (I2959) via UV light[4]. Microgels were formed by combining NorHA, pentaerythritol tetrakis(mercaptoacetate) (PETMA), I2959, thiolated heparin, and IL-10 (1.5 ng/uL), forming droplets in a PDMS microfluidic device[5], and crosslinking with UV light. Microgels were precipitated and washed in acetone then IPA, dried overnight under vacuum, and rehydrated for use (Fig. 1B-C). For microgel characterization, releasate was collected for 2 weeks with hyaluronidase degradation at the final time point. Degradation was quantified with uronic acid assay and IL-10 release with ELISA.

Bone marrow-derived GFP+ EPCs isolated from syngeneic adult male Wistar rats and IL-10 microgels were co-encapsulated in an injectable guest-host hydrogel and injected into the border zone region of an infarct in a rat MI model. Injection of EPCs and guest-host hydrogel alone served as a control. EPC retention was evaluated at 48 hours via immunostaining (DAPI, GFP).

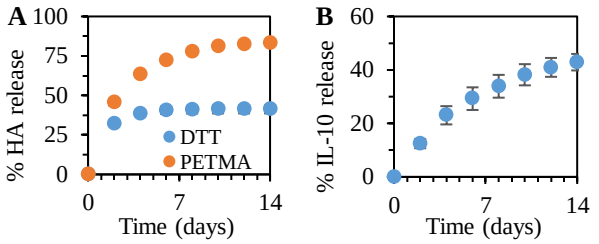

Figure 2. Degradation of microgels crosslinked with hydrolysable (PETMA) or stable (DTT) crosslinker (A) and IL-10 release from microgels over two weeks (B). Values are mean ± SE (n=3).

Results: Microgels were fabricated from HA with heparin included to sequester IL-10 and with either hydrolytically unstable (PETMA) or stable (DTT) crosslinks (Fig. 1B). The degradation of microgels crosslinked with hydrolysable PETMA was faster than degradation of microgels crosslinked with stable DTT, and PETMA microgels were >80% degraded by two weeks (Fig. 2A). IL-10 was detectable in microgels even after washing and drying, and a release profile of IL-10 from microgels showed steady release over 2 weeks (Fig. 2B). EPCs were co-encapsulated with IL-10 and delivered to the heart and GFP+ cells were identified in tissues both with and without IL-10 (Fig. 3A,B). Preliminary results show an increase in the number of GFP+ EPCs in the +IL-10 treatment group (Fig. 3C).

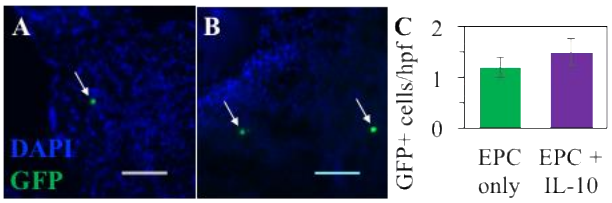

Figure 3. GFP+ EPCs (arrows) in myocardium after 48 hours in control (A) and +IL-10 (B) treatment groups and quantification of number of GFP+ cells per high power visual field (C) (mean ± SE). Scale bar is 100 um.

Conclusions: An injectable hydrogel system for co-delivery of EPCs and IL-10 microgels was developed. Microgels were hydrolytically degradable and released IL-10. *In vivo* results show that co-delivery of IL-10 can improve cell retention and further studies are needed to investigate cell survival, which is important to enhance the efficacy of injectable cell therapies for cardiac repair, as well as improve functional outcomes after MI.

References:

[1]Gaffey AC et al. J Thorac Cardio Surg. 2015;150:1268-76. [2]Krishnamurthy P et al. Circ Res. 2011;109:1280-9. [3]Rodell CB et al. Biomacromolecules. 2013;14:4125-34. [4]Gramlich WM et al. Biomaterials. 2013;34:9803-11. [5]Jeong H et al. Lab Chip. 2015;15:4387-92.