Sustained Release of SDF-1α Polypeptide Analogue from Hyaluronic Acid Hydrogels for Cardiac Repair

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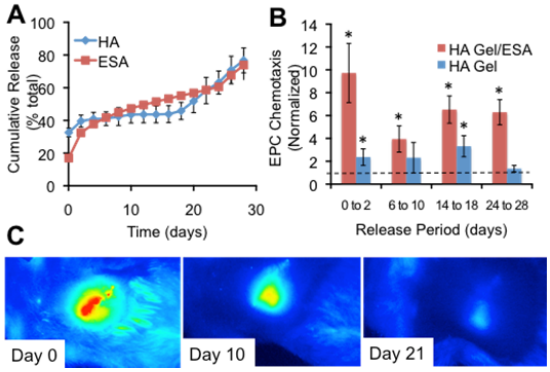
Statement of Purpose: Exogenous delivery of the SDF-1α chemokine is being explored to recruit endogenous cells to the heart for myocardial repair following a myocardial infarction (MI). While this approach has been shown to attenuate adverse post MI remodeling, the sustained delivery of active SDF-1α in the myocardium over long time-scales (days to weeks) has been challenging. To this end, an engineered SDF-1α polypeptide analogue (ESA) was recently reported with improved stability and activity compared to recombinant SDF-1α [1]. The goal of this work was to develop in situ forming and degradable hyaluronic acid (HA) hydrogels to encapsulate and sustain the release of ESA in the myocardium following MI.

Methods: *ESA synthesis.* ESA was produced using solid phase peptide synthesis as previously reported[1]. For in vitro detection, ESA was labeled with HiLyte Fluor TR, and for in vivo detection, ESA was labeled with HiLyte Fluor 750 (Anaspec). *HA modification*. 74kDa sodium hyaluronate (Lifecore) was modified with hydroxyethyl methacrylate (HEMA) to incorporate free radical initiated crosslinking and hydrolytic degradation[2]. 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. 15% of the HA repeat disaccharides were modified with HEMA as determined by ^{1}H NMR. *Hydrogel formation and molecule release.* HEMA-HA macromer was dissolved in PBS at 4wt% with 25μg ESA per 100μL sample volume. For gelation, ammonium persulfate and TEMED initiators were added to the polymer solution at a final concentration of 10mM each. For in vitro release kinetics, 50uL gels were formed in cylindrical molds for 30 min at 37°C then placed in 1mL PBS supplemented with 1% BSA and incubated at 37°C. Buffers were refreshed every 2 days and ESA was quantified with fluorescence measurements, while HA content was quantified with a uronic acid assay. The activity of released molecules was assessed using rat endothelial progenitor cells (EPCs) in a boyden chamber assay. *MI model.* Adult male Wistar rats were subjected to permanent occlusion of the LAD, and 100 μl of saline (n=8), hydrogel (4wt% macromer, n=10), or hydrogel+ESA (4wt% macromer, 25μg ESA, n=9) was injected into the borderzone of the infarct at 4 injection sites immediately following ligation. ESA was imaged using transthoracic fluorescence imaging (Li-core Pearl), and left ventricle (LV) geometry and function were evaluated with echocardiography 4 weeks post MI.

Results/Discussion: ESA release was sustained for 28 days in vitro when encapsulated in crosslinked HEMA-HA hydrogels (Fig 1A). After an initial burst release, ESA was steadily eluted for over 20 days. As the hydrogel hydrolyzed and HA polymers were released from the gels, the release rate of encapsulated ESA increased, indicating a temporal increase in the mesh size of the gel. The ESA and HA released from the gel remained active out to 28 days as evidenced by significant increases in EPC chemotaxis (Fig 1B). Previous work suggests that the observed chemotaxis is driven by CXCR4 and CD44 receptor signaling after ESA and HA binding, respectively[2]. In vivo, fluorescently tagged ESA was detectable in the heart for over 20 days when encapsulated within the in situ forming HA gels (Fig 1C). 4 weeks following MI, both hydrogel and hydrogel+ESA groups showed similar and statistically significant decreases in left ventricular end diastolic volume (LVIDd) compared to saline control (Fig 1D). However, only the hydrogel+ESA group showed significant increases in ejection fraction compared to saline control. Further, the hydrogel+ESA group showed significant increases in ejection fraction and cardiac output compared to the hydrogel group (Fig 1D). Taken together, these results indicate a combined effect of the gel in reducing stress and preventing infarct expansion, as well as a biological effect of the released chemokine in improving LV function.



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	Cardiac Output (cc/min)	Ejection Fraction (%)	LVIDd (cm)
Hydrogel + ESA *(n=9)*	*46.1 ± 10.4	*62.2 ± 6.1	*0.635 ± 0.113
Hydrogel *(n=10)*	*34.9 ± 8.5	49.7 ± 13.9	*0.692 ± 0.201
Saline Control *(n=8)*	27.0 ± 8.6	39.7 ± 14.2	0.767 ± 0.126

Figure 1. Encapsulated ESA release and HA gel degradation over 28 days in vitro (A) (n=3 gels), chemotactic activity of released ESA and HA in vitro (B) (n=5-6 samples per release period), representative imaging of ESA encapsulated within HA gels in the rat heart in vivo (C), and functional outcomes in rats at 4 weeks post MI (D) (*p<0.05 versus control, student's t-test).

Conclusions: Our injectable HA gels represent a useful vehicle to deliver ESA locally for myocardial repair. ESA release was sustained for over 3 weeks in vivo, resulting in improved LV function. Ongoing work will further investigate mechanisms of the HA gel and ESA mediated repair post MI.

References: [1]Hiesinger et al., Circulation, 2011;S18-S26. [2]Purcell et al., Biomaterials, 2012;7849-57.