Extracellular matrix hydrogel as growth factor delivery system for prolonged release and enhanced effect of a novel engineered HGF mimic in a small animal model of myocardial infarction

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Statement of Purpose: The goal of this work was to establish the potential of a novel hepatocyte growth factor (HGF) mimic delivered in an extracellular matrix (ECM) hydrogel as a treatment for myocardial infarction (MI). Immobilization of growth factors has been shown to have a beneficial effect *in vivo*. Previously, we showed a heparin-binding growth factor could bind to an injectable ECM hydrogel derived from pericardial tissue (PPM) via the sulfated polysaccharide content [1]. Recently, an engineered HGF mimic was developed and demonstrated to be a strong agonist of the c-Met receptor, approaching activity levels similar to full-length human recombinant HGF (rh-HGF) [3]. rh-HGF has been shown to be anti-fibrotic, pro-angiogenic, and cardioprotective [2], though it is expensive to manufacture and unstable, hindering widespread clinical use. Here, we sought to evaluate the *in vivo* potential for delivery of this novel HGF mimic post-MI using an injectable ECM hydrogel.

Methods: Elution of HGF mimic from a heparin column with NaCl was performed. Also, 10 µg of HGF mimic, which has a His tag, was loaded into 100 µL PPM and collagen gels and release quantified with Ni-HRP. In Sprague Dawley rats, MI was induced via 25 minute occlusion-reperfusion of the left coronary artery, and one week later, infarcted animals were injected with PPM alone (n=10), PPM with the HGF mimic (n=9), HGF mimic in saline (n=8), or saline alone (n=12). Four weeks post-injection, a final echo was performed before euthanasia. Resected hearts were sectioned and stained.

Results: Elution from a heparin column was seen with 1.4M NaCl; *in vitro* release from PPM gels was prolonged compared to release from equivalent collagen gels (Fig.1). Complete release from PPM gels was only seen after the addition of NaCl, which has been shown to dissociate heparin-binding interactions. As expected, saline-injected control animals worsened with respect to multiple measures of cardiac function, specifically LV volumes and areas (Table 1). There were no significant changes in these parameters for animals treated with PPM, PPM+HGF, or HGF mimic alone. Functional improvement was supported by a significant increase in fractional area change (FAC) only with delivery of PPM+HGF mimic. Similar trends were seen with ejection fraction (EF). With respect to cellular response, delivery of PPM+HGF mimic significantly increased arteriole density (36.4±8.9 arterioles/mm^2) in the infarct region compared to saline, PPM, and HGF mimic alone (11.7±1.1, 10.5±0.8, and 15.6±5.2, respectively). A slight, non-significant increase over the controls was observed with delivery of the HGF mimic alone. Interstitial fibrosis, as measured by percent collagen content in the remote myocardium did not vary significantly, though it trended lower in animals treated with PPM+HGF mimic.

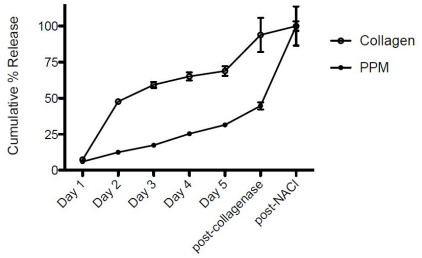

Figure 1: *In vitro* release. Diffusion of HGF mimic is rapid from collagen (open circles) and slow from PPM (solid circles). Nearly complete release from collagen is seen post-collagenase incubation, while 1.5M NaCl is needed to dissociate the HGF dimer from PPM.

Conclusions: Here, we demonstrate *in vivo* potency of a novel engineered HGF mimic, opening the potential to use this protein, in conjunction with an ECM hydrogel, as an injectable therapy for MI. We have shown this HGF mimic can be sequestered by an ECM hydrogel via interactions with sulfated sugars in the biomaterial; small animal studies indicate delivery of the HGF mimic with PPM may provide benefit post-MI by increasing neovascularization, reducing interstitial fibrosis, and preserving LV geometry. This HGF mimic and ECM hydrogel are a promising therapeutic combination with potential benefits of reduced cost, improved stability, and improved delivery compared to treatment with rh-HGF. Future studies evaluating dose response will also allow further investigation of the mechanisms by which positive outcomes are achieved and optimize therapeutic benefit.

References: 1. Seif-Naraghi SB. Acta Biomat. 2012.
2. Jin H. Curr Pharm Des, 2004.10(20):2525-33.
3. Jones DS. Proc Natl Acad Sci,2011.108(32):13035-40.

Table 1: Functional parameters	Saline		PPM		PPM+HGF mimic		HGF mimic in Saline	
	Pre-	Post-	Pre-	Post-	Pre-	Post-	Pre-	Post-
ESV (mL)	0.107±0.031	*0.142±0.057	0.124±0.031	0.131±0.018	0.112±0.021	0.125±0.033	0.168±0.093	0.332±0.063
EDV (mL)	0.274±0.101	*0.325±0.090	0.305±0.040	0.339±0.047	0.290±0.068	0.347±0.046	0.289±0.086	0.185±0.115
LVAs (cm)	0.372±0.074	*0.430±0.083	0.403±0.051	0.420±0.037	0.391±0.051	0.410±0.054	0.469±0.140	0.439±0.047
LVAd (cm)	0.652±0.112	*0.710±0.119	0.672±0.042	0.731±0.073	0.660±0.100	0.731±0.044	0.649±0.122	0.723±0.072
EF (%)	59.50±7.70	54.601±14.52	59.65±8.43	60.42±6.11	60.13±5.65	64.45±5.91	60.00±8.51	56.61±9.23
FAC	0.416±0.027	0.443±0.055	0.400±0.425	0.425±0.011	0.404±0.017	*0.44±0.017	0.383±0.035	0.373±0.033