On-demand Delivery of TIMP-3 from Injectable and MMP Degradable Hydrogels for Infarct Repair

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Statement of Purpose: Hydrogel design is evolving for biomedical applications, such as towards therapeutic delivery. Often, molecule release is controlled through diffusion or hydrolytic degradation; however, it may be beneficial to release molecules in a feedback process, where the presence of a trigger initiates gel degradation and coincident molecule release. As an example, the tissue remodeling response following a myocardial infarction (MI) causes global changes to ventricle geometry that are detrimental to heart function. The induction of matrix metalloproteinases (MMPs) in the infarct tissue is largely responsible for ECM breakdown and subsequent infarct expansion[1]. One approach to attenuate matrix breakdown is the local delivery of tissue inhibitors of MMPs (e.g., TIMP-3). In this work, we developed *in situ* forming hydrogels containing MMP degradable crosslinks where the local presence of MMPs controls the release of encapsulated TIMP-3.

Methods: *HA-MMP-hydrazide synthesis*. 74kDa sodium hyaluronate (NaHy, Lifecore) was converted to a tetrabutylammonium (TBA) salt of HA and then reacted with N-(2-aminoethyl)maleimide trifluoroacetate salt (Sigma) in the presence of BOP to produce a maleimide functionalized HA (MAHA). An MMP-cleavable peptide was synthesized using solid-state peptide synthesis with the following sequence: GCNSGGRM↓SMPVSNGG-hyd, where "hyd" is hydrazinoacetic acid. The sequence was coupled to MAHA via maleimide-cysteine reaction by mixing peptide and MAHA for 2 hr in PBS at a molar ratio of 4:1 cysteine:maleimide. *HA/DS-aldehyde synthesis.* 350kDa NaHy was reacted with sodium periodate ($NaIO_4$) at a molar ratio of 2:1 NaHy:$NaIO_4$ in DI H_20 for 2 hrs. Dextran sulfate (DS) was reacted with $NaIO_4$ at a molar ratio of 1:2 DS:$NaIO_4$ in DI H_20 for 5 hrs. *Hydrogel formation/degradation*. To form hydrogels, ALD (2.4% HA-ald, 1.4% DS-ald (w/v)) and HYD (3.2% (w/v) HA-MMP-hyd) modified polymers were dissolved in PBS, mixed 1:1 (v/v) for 1:1 ALD:HYD. Gels were incubated in cylindrical molds for 30 min at 37°C after mixing the two macromers. Recombinant human TIMP-3 (10μg per gel) was added to the HA-MMP-hyd solution prior to gel formation for +TIMP-3 groups. After gel formation, gels were placed into physiologic buffer and incubated at 37°C. Buffers were refreshed every 2 days and analyzed for gel degradation products with a uronic acid assay. 20nM of active recombinant human MMP-2 was added to +MMP groups with each buffer refresh. *Post MI rTIMP-3 delivery.* MI was induced in adult pigs randomized to receive 9x100μL MI region injections of gel/rTIMP-3 (20μg rTIMP-3/injection, n=7), gel alone (n=7) or saline (MI only, n=6). Sham operated pigs (no MI, n=3) served as controls. Serial echos were measured after 1,3,7 and 14 days and *in vivo* interstitial MMP activity was measured after 14 days with microdialysis.

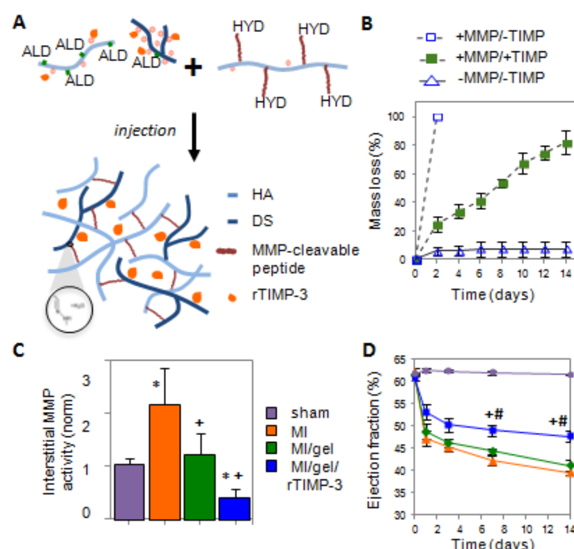

Figure 1. (A) Injectable MMP-sensitive hydrogels through inclusion of MMP-cleavable peptides and aldehyde (ALD)/hydrazide (HYD) reactivity onto hyaluronic acid (HA) and dextran sulfate (DS) polymers. (B) Hydrogels degraded rapidly in the presence of 20nM rMMP-2. Encapsulated rTIMP-3 slowed rMMP-2 degradation over 14 days *in vitro* (n=3 gels per group). (C) Hydrogel delivery of rTIMP-3 significantly inhibited MMP activity in the infarct and (D) attenuated the decline in heart function (mean±SEM; pairwise t-test with Bonferroni; *p<0.05 vs. sham, +p<0.05 vs. MI, #p<0.05 vs. MI/hydrogel).

Results/Discussion: By utilizing HYD and ALD functionalized polymers, we are able to form gels through hydrazone bond formation by simply mixing the complimentary macromers in buffers, (Fig. 1A). Gelation occurred within seconds and reached a plateau within 5 min (data not shown). The networks were stable in the absence of MMP activity and limited encapsulated rTIMP-3 release to less than 20% over 14 days *in vitro* through rTIMP-3 binding to sulfated polymers (data not shown). In the presence of rMMP-2 the gels degraded rapidly (Fig. 1B); however, with rTIMP-3 included, MMP-specific degradation declined, demonstrating sustained activity of the encapsulated and released TIMPs. Gels were formed *in situ* by blending ALD/HYD polymers upon injection through a dual barrel syringe. When applied following experimental MI in pigs, injected gels significantly inhibited elevated MMP activity in the MI region, potentially by providing a substrate for MMPs, reducing MMP activity elsewhere (Fig. 1C). rTIMP-3 encapsulation further inhibited MMP activity post MI. Finally, localized rTIMP-3 delivery from the gels significantly attenuated loss of heart function (Fig. 1D).

Conclusions: An injectable hydrogel system was designed that delivers encapsulated MMP inhibitors in response to elevated MMP activity. This system effectively inhibited elevated MMP activity within the MI region and attenuated adverse LV remodeling following experimental MI in a large animal model.

References: [1]Wilson et al., Circulation, 2003;107:2857-63.

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