Protease Triggered Release of Macromolecules from MMP-sensitive Hyaluronic Acid Hydrogels

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Statement of Purpose: Hydrogel design is evolving for a wide range of applications, such as towards the delivery of therapeutic molecules. Often, molecule release is controlled through simple diffusion or hydrolytic degradation; however, it may be beneficial to release molecules in a feedback process, where the presence of a local trigger initiates gel degradation and coincident molecule release. As an example application, 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 extracellular matrix (ECM) breakdown and subsequent infarct expansion following MI[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 and hydrazide-aldehyde reactivity is incorporated to allow in situ gel formation.

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 benzotriazol-1-yloxytris(dimethylamino)-phosphonium hexafluorophosphate to produce a maleimide functionalized HA (MAHA). An MMP-cleavable peptide was synthesized using solid-state peptide synthesis with the following sequence: GCGQGWIGQPGGG-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-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. *Hydrogel formation/degradation*. HA-ald macromers (30μL, 5wt%) and HA-MMP-hyd macromers (20μL, 8 wt%) were individually dissolved in PBS and then mixed together to form gels. Gelation characteristics were quantified by monitoring the storage (G') and loss (G'') moduli with time using an AR2000ex Rheometer (TA Instruments) at 37°C under 1% strain and 1 Hz. For release studies, gels were incubated in cylindrical molds for 1hr 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 groups. After gel formation, gels were placed into 1mL TCC buffer (50mM Tris-HCL pH 7.4, 1mM CaCl2, 0.05% triton X-100) and incubated at 37°C. Buffers were refreshed every 2 days and analyzed for gel degradation products with a uronic acid assay. 40nM of active recombinant human MMP-2 was added to +MMP groups with each buffer refresh. After 8 days, intact gels were degraded with hyaluronidase (1mg/mL).

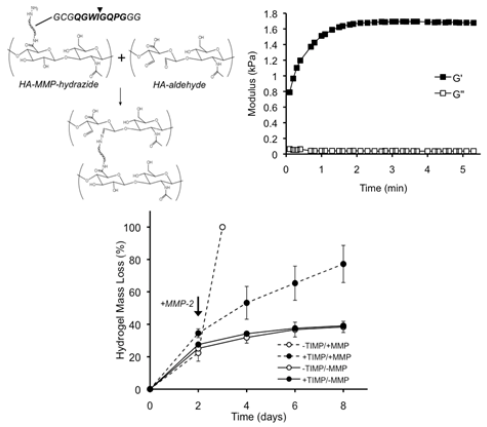

Figure 1. Formation of MMP-sensitive hydrogels through incorporation of MMP-cleavable peptides and hydrazide-aldehyde reactivity is instant upon mixing (top). MMP-dependent degradation of hydrogels either with or without encapsulated rTIMP-3 (n=3 gels per group) (bottom).

Results/Discussion: Extent of maleimide functionalization was determined to be ~35% via ^{1}H NMR. Peptide coupling to MAHA through cysteine residue functionality was complete within 2 hrs. The absence of characteristic maleimide peaks in the ^{1}H NMR spectra of HA-MMP-hyd indicated complete reaction of maleimide groups with cysteine terminated peptides, indicating hydrazide functionalization of ~35%. Extent of aldehyde modification was determined to be 17% with a colorimetric TNBS assay[2]. By utilizing hydrazide and aldehyde functionalities, we are able to form gels simply by mixing the two complimentary macromers in physiological buffers, forming a hydrazone bond. Gelation occurred within seconds and reached a plateau within 5 min (Fig 1, top). The crosslinking reaction does not require cytotoxic initiators and water is the only reaction byproduct, so the hydrogels are well suited for injection into living tissues. Hydrogel crosslinking was most efficient (i.e., greatest mechanics) when a molar ratio of 1:1 hydrazide:aldehyde was maintained during gelation. After initial mass loss due to unreacted macromer (~25-30%), the networks were stable in the absence of MMPs and degraded rapidly in the presence of rMMP-2, illustrating an MMP-specific degradation mechanism (Fig.1, bottom). When rTIMP-3 was encapsulated in these gels, MMP-specific degradation decreased, demonstrating activity of the encapsulated and released TIMPs.

Conclusions: An injectable hydrogel system was designed that rapidly gels using a two-component system and delivers encapsulated macromolecules in response to elevated MMP activity. Ongoing work is to investigate the feedback mechanism of gel degradation and rTIMP-3 release in a porcine MI model.

References: [1]Wilson et al., Circulation, 2003;107:2857-63. [2]Bouhadir et al., Polymer, 1999;3575-3584.