Sustained Release of Short Peptides Using Guest-Host Complex Formation in Injectable Hydrogels

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Statement of Purpose: The use of small molecule inhibitors of matrix metalloproteinases (MMPs) has been effective in attenuating maladaptive tissue remodeling caused by elevated profiles of MMPs after myocardial infarction (MI)[1]. In particular, several short peptide sequences have been developed as inhibitors to specific MMPs, including the sequence APP-IP (ISYGNDALMP), which selectively inhibits two of the most highly upregulated MMPs after MI [2]. Systemic delivery of MMP inhibitors frequently has deleterious off-target outcomes. Providing local and sustained MMP inhibition using a drug delivery system is one strategy that could reduce these effects. As delivery systems to address this spatiotemporal control, injectable hydrogels offer high biocompatibility, mechanical support, and clinical translatability; however, they fail to adequately sustain the release of small molecules like APP-IP due to their highly swollen state. In this work, we engineer self-assembled, injectable "guest-host" hydrogels formed with adamantane-modified hyaluronic acid (AD-HA) and β-cyclodextrin-modified hyaluronic acid (CD-HA) to provide sustained release of short peptides. The formation of guest-host complexes between cyclodextrin and aromatic amino acids increases retention - and subsequently sustains release - of short peptides in these systems [3]. Here, we show that we can tune the release of short peptides by engineering both payload chemistry and gel formulation.

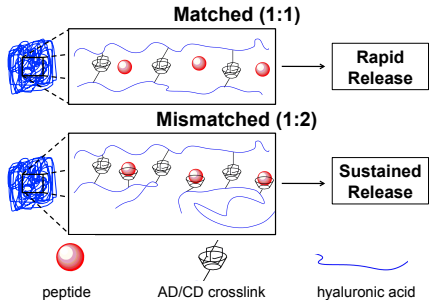

Figure 1. Tuning guest-host gels for sustained peptide release.

Methods: AD-HA and CD-HA were synthesized with 90kDa HA using previously published protocols [4]. Loaded gels were formed through mixing solutions of AD-HA and CD-HA containing dissolved payloads at various concentrations and ratios. Fluorescence recovery after photobleaching (FRAP) was used to determine the effective diffusivity (D_E) of fluorescein labeled polymers and peptides that were synthesized using standard solid phase peptide synthesis. Briefly, a 30μm spot was bleached using the 488 nm laser line of a confocal microscope, and single plane images were recorded. Recovery profiles were then fit using non-linear regression analysis to determine D_E. Bulk release kinetics of peptides from gels were determined through release assays in phosphate buffered saline, conducted at 37°C. APP-IP inhibitory activity on MMP-2 was assessed through the use of a fluorogenic substrate.

Results: ^{1}H-NMR confirmed successful functionalization of HA with AD and CD, both at ~25%. AD-HA and CD-HA were combined in 1:1, 1:2, and 1:3 molar ratios of AD:CD, while maintaining a constant weight percent of AD-HA. FRAP analysis shows that D_E for a FITC labeled, cyclodextrin-binding peptide containing tryptophan (3W peptide, GKWEWKWE-FITC) decreases with increasing cyclodextrin content. Bulk release kinetics follow a similar trend, with more sustained release of the 3W peptide in gels containing higher amounts of CD-HA (Figure 2A). Additionally, for a constant weight percent, changing the ratio of AD-HA to CD-HA from 1:1 to 1:2 (decreasing crosslink density at a constant macromer concentration) results in lower D_E and more sustained profiles. Furthermore, peptide chemistry functions as a secondary handle by which peptide release may be controlled from these systems (Figure 2B). The 3W peptide, containing the aromatic tryptophan group, had significantly lower D_E and slower release kinetics than the analogous sequence, where tryptophan had been replaced with glycine (NoW peptide, GKGEGKGE-FITC). Using a 1:2 ratio of CD-HA to AD-HA at 7.8%, APP-IP had a sustained release for ~3 weeks from gels *in vitro* and APP-IP activity was confirmed at multiple time points through fluorogenic assays (results not shown).

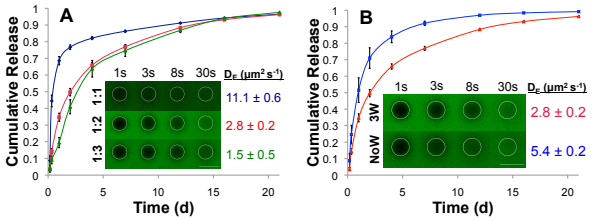

Figure 2. Fluorescence recovery data and cumulative release profiles for fluorescein labeled peptides in gels with increasing CD-HA macromer weight percent (A) and increasing cyclodextrin affinity (B). Scale bar represents 50 μm.

Conclusions: Both gel formulation and payload chemistry can be leveraged to provide sustained release of short peptides in self-assembled hyaluronic acid hydrogels. Cyclodextrin content and peptide chemistry dramatically alter micro-scale and bulk kinetics of short peptides, suggesting polymer affinity is a powerful tool for controlling small molecule release from these systems. Towards translational therapies, APP-IP sustained release from these gels could lead to improved cardiac outcomes. Ongoing studies in rat models of MI will assess therapeutic benefit of injectable gels designed for the sustained release of APP-IP.

References: 1. Mukherjee R. Circulation. 2003;107(4):618-25. 2. Higashi S. J. Biol. Chem. 2003;278:14020-28. 3. Matsuyama, K. Drug Dev. Ind. Pharm. 1987;13:2687-91. 4. Rodell CB. Biomacromolecules. 2013;14: 4125-34.