Injectable and Degradable Sulfated Hyaluronic Acid Hydrogels for Sustained Protein Delivery

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Statement of Purpose: Matrix metalloproteinases (MMPs) play a large role in remodeling the extracellular matrix (ECM). MMP activity is controlled endogenously by interaction with tissue inhibitors of metalloproteinases (TIMPs). However, under pathological conditions, excessive expression of MMPs without a concomitant expression of TIMPs leads to tissue destruction and pathological progression.[1] Therefore, one way to restore the balance between MMPs and TIMPs is to exogenously deliver TIMPs at the site of remodeling through the use of injectable biomaterials. Injectable hydrogels based on hyaluronic acid (HA) have recently been developed with controlled degradation, making them well-suited for localized macromolecule delivery.[2]

Of the four known TIMPs, TIMP-3 is known as the "ECM-bound" TIMP due to its high binding affinity for glycosaminoglycans (GAGs) in the ECM.[3] In particular, TIMP-3 binds to sulfated GAGs through electrostatic interaction.[3] HA is unique among GAGs because it is exclusively non-sulfated. However, sulfating HA has been shown to increase affinity to proteins, with higher degrees of sulfation leading to enhanced interactions.[4,5] Therefore, the goal of this work was to introduce sulfate groups into injectable and degradable HA hydrogels to further sustain encapsulated TIMP-3 delivery. It is hypothesized that sulfation of HA will enhance TIMP-3 binding and enable better tuning of TIMP-3 release profiles.

Methods: Briefly, hydroxy-ethyl methacrylated HA (HeMA-HA; Figure 1A) was synthesized by coupling 2-hydroxyethylmethacryl-succinate (HeMA-COOH) to HA-tetrabutylammonium salt (HA-TBA) using ditertbutyl-dicarbonate (BOC_2O) and 4-dimethylaminopyridine.[2] Sulfated HeMA-HA (HeMA-S-HA; Figure 1A) was synthesized by first synthesizing HeMA-HA as previously described.[2] To restore solubility in organic solvents, the remaining cations in HeMA-HA were converted again to TBA to generate HeMA-HA-TBA. HeMA-HA-TBA was dissolved in anhydrous DMF and reacted with SO_3/DMF at a molar ratio of 20:1 SO_3:HA at room temperature for 1 hour to generate HeMA-S-HA. Both macromers were purified by dialyzing against deionized H_2O and then lyophilized. The extent of HA modification with HeMA was assessed with ^{1}H-NMR.

Preliminary affinity binding studies were conducted with each macromer and bovine serum albumin (BSA) as a model protein. Macromers were titrated into a 2μm BSA in HEPES buffer, pH 7.4 and intrinsic BSA fluorescence (280/350nm excite/emit) was monitored with a plate reader (TECAN). Dissociation constants were calculated by nonlinear regression of the fluorescence titrations.[6]

For gel formation, HA macromers were dissolved in PBS at 4 wt%, and hydrogel crosslinking was initiated by adding 10mM APS and 10mM TEMED. Copolymer hydrogels were formed by mixing HeMA-HA and HeMA-S-HA macromers at 90% and 10% mass, respectively. Gelation onset (*n =3*) was quantified with a AR2000ex Rheometer (TA Instruments) by monitoring the storage (G') and loss (G'') moduli over time at 37°C under 1% strain and a frequency of 1 Hz in a cone-plate geometry (1°, 20 mm diameter).

To assess differences in TIMP-3 release, 50μL hydrogels (*n=3* per group) containing 10 μg of rTIMP-3 (Amgen) were formed and placed at 37°C in 1 mL PBS with 1% BSA. HA degradation was monitored using an uronic acid assay. TIMP-3 release was quantified using an ELISA (R&D Systems).

Results: Both HeMA-HA and HeMA-S-HA were successfully synthesized with the same modification of HeMA groups on HA (~15% modified). HeMA-S-HA exhibited a higher binding affinity to BSA (K_d = 2 μM) compared to HeMA-HA (K_d = 13 μM). Rheology studies (data not shown) were used to select a single copolymer ratio to compare TIMP release with pure HeMA-HA hydrogels. The 90:10 copolymer ratio was picked because the G' (6000 ± 700 Pa) was not significantly different from G' (6800 ± 400 Pa) of pure HeMA-HA hydrogels (*p = 0.135*). Similarly, the degradation profiles of both formulations were not significantly different (Figure 1B).

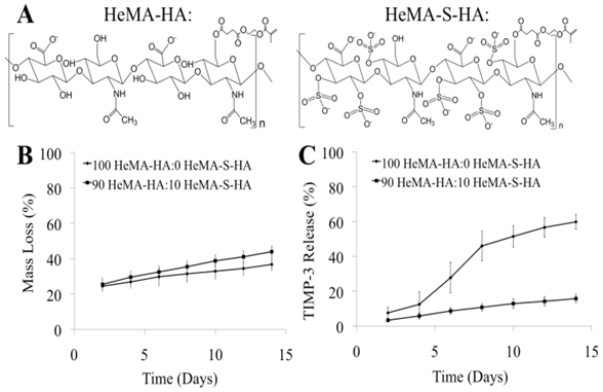

Figure 1: Chemical structures of HeMA-HA and HeMA-S-HA (A). Degradation rates (B) and release of TIMP-3 (C) from two selected hydrogel formulations over 2 weeks. Data presented as mean ± SD.

Conversely, the release of TIMP-3 from the sulfated copolymer hydrogels was significantly lower than the release from the pure HeMA-HA hydrogels for all time points (Figure 1C). Ongoing *in vitro* studies are being performed to examine long-term TIMP-3 release from hydrogels with varying rates of degradation.

Conclusions: We have successfully designed a sulfated HA macromer and examined its ability to control TIMP-3 release from hydrogels. These results indicate that sulfating HA hydrogels helps retain TIMP-3 in the gels for longer periods of time, providing sustained release due to enhanced binding interactions. This approach provides a better mimic of protein interactions with ECM, as well as another hydrogel variability to control release profiles.

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