Shear-Thinning Hydrogels with Secondary Michael-type Crosslinking to Modulate Properties *In Situ*

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Statement of Purpose: Self-assembling hydrogels continue to gain interest as scaffolds for biomolecule delivery and tissue engineering due to their injectability.[1] While covalent crosslinking results in materials incapable of flow, crosslinks based on physical associations enable shear-thinning and self-healing, affording injectable delivery. We have recently reported on shear-thinning hydrogels based on the guest-host interaction of modified hyaluronic acid (HA).[2] While these systems show ease of initial delivery and retention at the target site, they provide limited control over the properties of the hydrogel following injection. For example, one limitation of physically crosslinked hydrogels is that they typically have low mechanical strength.[3] To overcome this, we engineered HA hydrogels which undergo both guest-host assembly for retention at the target site and additional secondary covalent crosslinking via Michael-addition to increase moduli following injection.

Methods: To obtain hydrogel precursors capable of self-assembly, three macromer pairs were synthesized. For guest-host assembly, HA was modified either by coupling of 1-adamantane acetic acid (guest, Ad-HA) via BOC2O mediated esterification or aminated β-cyclodextrin (host, CD-HA) via BOP mediated amidation.[2] To afford Michael-addition crosslinking, HA was modified by esterification with 3,3'-dithiodipropionic acid followed by reduction with DTT to yield thiolated HA (HA-SH) or Michael-acceptors: methacrylates (MeHA), acrylates (AHA), or vinyl sulfones (VSHA). For dual crosslinking, thiolated Ad-HA and methacrylated CD-HA were prepared by modular synthesis to allow combination of physical and covalent bonding. Continuous flow experiments and oscillatory rheology (AR2000) were used to characterize hydrogel properties, including shear-thinning and reassembly time. Crosslinking kinetics were monitored by time-sweeps (1Hz, 0.5% strain) following mixing of the two macromers. Guest-host hydrogels (5wt%) were injected into explanted porcine hearts and imaged by MRI (3T or 9.4T; day 0 or day 7).

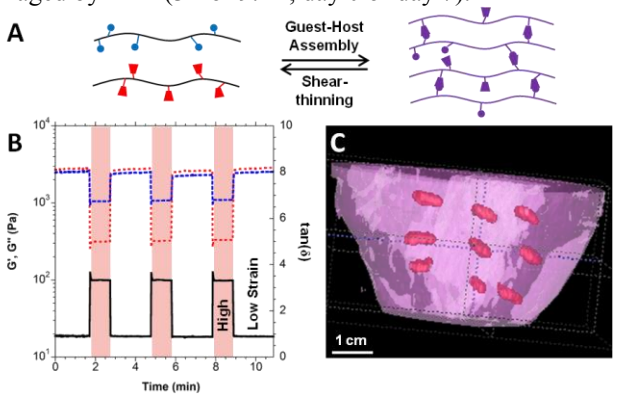

Figure 1. (A) Schematic of guest-host assembly. (B) Storage (red), loss modulus (blue), and loss tangent (black) of hydrogel (7.5wt%) under cyclic deformation of 0.5 (low) and 250% strain (high, shaded) at 10Hz. (C) 3D reconstruction of nine 300μL guest-host hydrogel injections (red) in a porcine whole heart explant (pink); imaged at 3T.

Results: Mixing of Ad-HA and CD-HA resulted in rapid formation of a hydrogel composed of non-covalent bonds. The guest-host hydrogels exhibited shear-thinning for ease of injection and rapid reassembly (Fig 1A,B) enabling retention at the target site. MRI of injections into cardiac explants confirmed materials were retained at the delivery site (Fig 1C) and maintain morphology at 1 week post-injection with minimal swelling (24.4±2.1%).

For Michael-addition crosslinking, real-time rheological observation was used to identify hydrogel parameters which provided necessary time for ease of delivery after mixing (i.e. without premature gelation). Gel times were observed to decrease with increasing reactivity of the Michael-acceptor (Fig 2B), pH, and polymer concentration. MeHA was determined to be a viable Michael-acceptor for clinical use, as it exhibited gel times greater than 45 minutes if polymer concentration and pH were properly controlled. Shear-thinning guest-host macromers containing these covalently crosslinking groups retained their native mechanical properties, and subsequent covalent crosslinking via Michael-addition (Fig 2A) afforded a more rigid viscoelastic solid with drastically increased shear modulus (Fig 2C). This dual crosslinking mechanism also modulated the hydrogel frequency dependence, creep, and relaxation behavior.

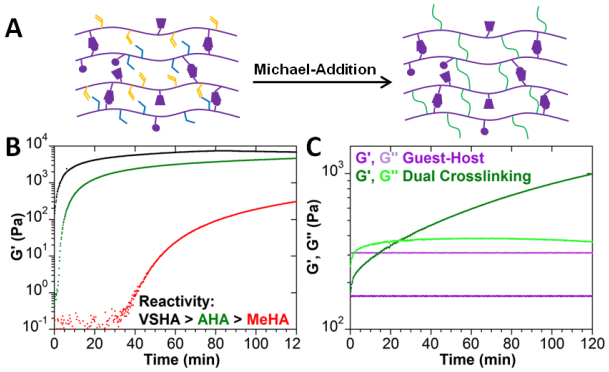

Figure 2. (A) Schematic of dual crosslinking. (B) Crosslinking kinetics of various Michael-acceptors with HA-SH (3wt%, pH 6). (C) Time-sweep of guest-host and dual crosslinking hydrogels.

Conclusions: Results demonstrate the ability of the materials developed to form a shear-thinning hydrogel upon mixing, flow for injection delivery, and recover at the target site. Furthermore, biophysical properties of the injected hydrogel are controllable through the incorporation of secondary covalent crosslinking which is tailored to proceed at a clinically relevant time-scale. Ongoing work includes examination of hydrogel degradation *in vivo*, as well as implementing towards specific biomedical applications.

References: [1]Kretlow JD, et al. Adv Drug Delivery Rev. 2007:59:263-73. [2]Rodell CB, et al. Biomacromolecules. *In Press*. [3]Appel AE, et al. Chem Soc Rev. 2012:41:6195-6212.