**Modularly assembled hyaluronic acid hydrogels with tunable stimuli responsiveness**

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**Statement of Purpose:** Hydrogels have traditionally been static materials that provide physical bulk and diffusion controlled drug delivery, but as these materials can also closely mimic the body's native tissues and the extracellular matrix (ECM), considerable recent research has been undertaken to develop hydrogel systems that are as stimuli responsive, spatially patterned, and varied as the ECM.[1] To closely mimic the nature of the ECM, new hydrogel systems introduce all this complexity with orthogonal reactions so that mechanical properties, ligands, degradability, etc. can be introduced independently, but normally require numerous different reaction types and functional groups. Recently, a norbornene functionalized hyaluronic acid (NorHA) hydrogel system was developed that utilized only the thiol-norbornene reaction to independently pattern molecules and mechanical properties with significant control, simplifying this process.[2] As the NorHA hydrogel system is modular, it could also serve as a matrix material to generate stimuli responsive hydrogels through introducing a stimuli responsive crosslinker. Here, we created a temperature responsive hydrogel by crosslinking the NorHA with a dithiol-functionalized poly(N-isopropylacrylamide) (PNIPAM) crosslinker (Figure 1). Through straightforward modifications of the PNIPAM crosslinker and its concentration, the magnitude of the thermal response and hydrogel modulus was controlled.

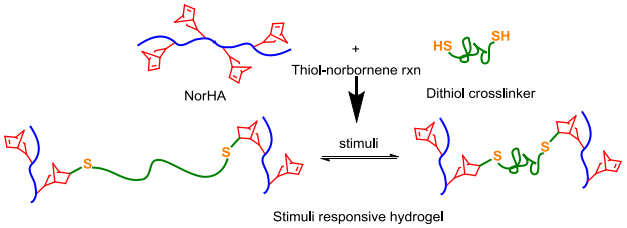


**Figure 1**. Reaction of norbornene functionalized HA (NorHA) with a dithiol-functionalized PNIPAM through UV light initiated thiol-norbornene reaction, generating a stimuli responsive hydrogel.

**Methods:** NorHA was synthesized through a literature reported procedure.[2] PNIPAM was synthesized using reversible addition-fragmentation chain transfer (RAFT) polymerization with a ditrithiolcarbonate chain transfer agent (CTA) to give a CTA terminated PNIPAM that was converted to a thiol-terminated polymer with subsequent aminolysis. Two PNIPAMs were synthesized with degrees of polymerization of 64 (long) and 29 (short). To synthesize hydrogels, NorHA and PNIPAM were dissolved in phosphate buffered saline (PBS) with I2959 (UV radical initiator) at various thiol to norbornene ratios and 6 wt% solids. To synthesize hydrogels, 50 µL of reaction solution was covered with glass to exclude air, and irradiated with a UV light source filtered to 320 – 390 nm (10 mW/cm$^2$) for 45 or 90 s. The moduli of the hydrogels were calculated from the slope of the stress/strain curve of a constant strain rate (10%/min) compressive test at r.t. The swelling degree (SD) was calculated by $(m_1-m_0)/m_0$, where $m_0$ is the initial mass of the gel equilibrated at 24 h at 4 °C in PBS and $m_1$ is the mass after 24 h at the indicated temperature in PBS. Significance was calculated through ANOVA with $p<0.05$ and $n\geq3$.

**Results:** The PNIPAM successfully reacted with NorHA to synthesize hydrogels using UV light in 45 s for the long PNIPAM. The short PNIPAM required 90 s of irradiation to yield a testable hydrogel. The SD at different temperatures and moduli of the hydrogels could be varied without synthesizing a new matrix polymer (Figure 2). Heating all the hydrogels with PNIPAM led to a decrease in mass while a control hydrogel without PNIPAM had a constant mass at all temperatures. For a set thiol to norbornene ratio (0.5), solids concentration, and irradiation time (90 s), halving the length of the PNIPAM crosslinker significantly reduced the SD reduction, while yielding the same modulus (Figure 2a). Changing the thiol to norbornene ratio from 0.5 to 0.3 for the long PNIPAM led to the SD decreasing more upon heating (Figure 2b). However, a significant change in modulus was observed. For all responsive hydrogels, the temperatures could be cycled at least three times without a significant difference in the measured SD upon heating.

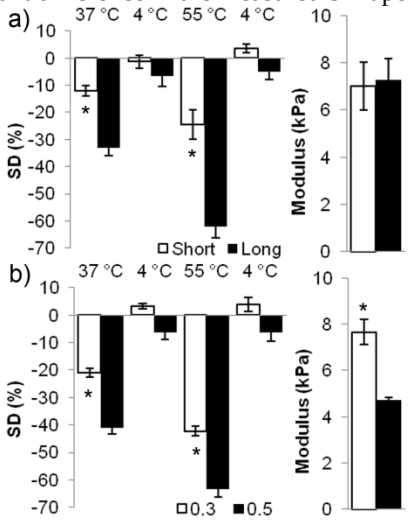


**Figure 2.** Comparison of swelling degree (SD) and modulus of PNIPAM/NorHA hydrogels with (a) short/long PNIPAM crosslinkers and (b) 0.3/0.5 thiol to norbornene ratios. Asterisk denotes statistical significations ($p<0.05$) of short PNIPAM and 0.3 values as compared to long PNIPAM and 0.5 values, respectively.

**Conclusions:** NorHA was crosslinked with a dithiol-functionalized PNIPAM to create a thermally responsive HA hydrogel. The SD and therefore the magnitude of the thermal response could be tuned by changing the length of the PNIPAM crosslinker and the ratio of thiol to norbornene groups without synthesizing a new matrix polymer. Ongoing experiments are to spatially pattern the thermal responsive nature into HA hydrogels as well as introduce other stimuli responsive crosslinkers.

**References:** [1]Burdick JA.; Murphy WL. Nat Commun 2012;3:1269. [2]Gramlich WM, Kim IL, Burdick JA. Biomaterials 2013;34:9803.