

# Hyaluronic acid Hydrogels Synthesized by Thiol-ene Step Growth Polymerization

William M. Gramlich, Jason A. Burdick.

Department of Bioengineering, University of Pennsylvania

**Statement of Purpose:** Hyaluronic acid (HA) hydrogels have a myriad of uses from matrices to control cell differentiation to scaffolds for regenerative medicine.<sup>1</sup> These gels have been synthesized through both chain growth and step growth polymerization of functional groups pendant to the HA backbone. Chain growth polymerizations typically proceed through a radical polymerization mechanism initiated by light which allows for control of the extent of reaction (i.e. crosslink density) and spatial control. However, these polymerized side groups aggregate and lead to non-polar regions within the gel that can be difficult to degrade.<sup>2</sup> Step growth gel formation occurs through a one-to-one coupling of pendant groups, usually using a small molecule reactant to link the chains through reactions such as Michael-addition or aldehyde-hydrazide coupling. Here, degradation can be introduced into the crosslinkers, but there is no spatial control over reaction and these step growth reactions tend towards completion (i.e. not controllable). To obtain the benefits of both systems, we investigated the radically mediated thiol-ene gelation of HA. We synthesized a norbornene functionalized HA (Nor-HA) which reacted with dithiols to form hydrogels with a controllable range of mechanical properties.

**Methods:** Nor-HA was synthesized through the di-tert-butyl dicarbonate (BOC<sub>2</sub>O) activation of HA hydroxyl groups and subsequent reaction with 5-norbornene-2-carboxylic acid (Figure 1). Degree of functionalization was quantified by proton nuclear magnetic resonance (<sup>1</sup>H NMR) spectroscopy. For gel synthesis, Nor-HA was dissolved in phosphate buffered saline (PBS) with dithiothreitol (DTT) and I2959 (UV radical initiator) at various ratios and concentrations. To generate gels, 50  $\mu$ L of reaction solution was placed in the tip of a syringe, covered with a piece of glass cover slip to exclude air, and irradiated with a mercury arc UV light source filtered to 320 – 390 nm for various times. The mechanical properties of the gels were measured by constant strain rate (10%/min) compressive tests using dynamic mechanical analysis. Moduli of the materials were calculated from the slopes (10 – 20% strain) of the stress strain curves. Gels were degraded in hyaluronidase and analyzed by <sup>1</sup>H NMR spectroscopy to determine the amount of unreacted norbornene groups.

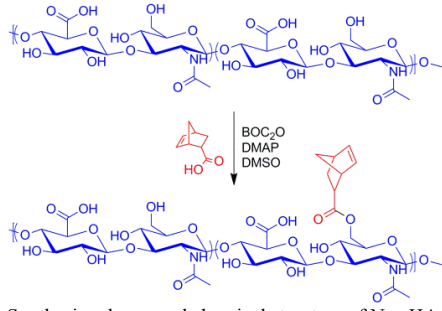


Figure 1. Synthesis scheme and chemical structure of Nor-HA

**Results:** A 4 wt% Nor-HA solution in PBS with a 0.5 molar ratio of thiol (DTT) to norbornene gelled in less than 15 s (Figure 2a). Figure 2a details the compressive modulus of this solution as a function of UV irradiation time. After 10 min of irradiation, the modulus plateaued around 60 kPa. Further irradiation did not change the modulus significantly, suggesting the formation of a stable gel. We also investigated how changing the ratio of thiol to norbornene (4 wt% Nor-HA) in the gel precursor solution affected the modulus of the final hydrogel (Figure 2b) after the modulus plateaued (45 min irradiation). At ratios (thiol:norbornene) lower than 0.6, an increase in thiol concentration yields a higher modulus as more crosslinks are made. For ratios between 0.6 and 1.0, the same modulus (ca. 70 kPa) is observed, indicating that additional crosslinks are not being formed. At ratios higher than 1.0, more thiols than norbornenes exist in the reaction mixture resulting in competition between crosslinking and reactions that give dangling thiols. As a result, the observed modulus decreases. <sup>1</sup>H NMR analysis of enzymatically degraded gels with a ratio less than 0.5 indicates that norbornenes remain, allowing for subsequent thiol-ene reactions in the gels.

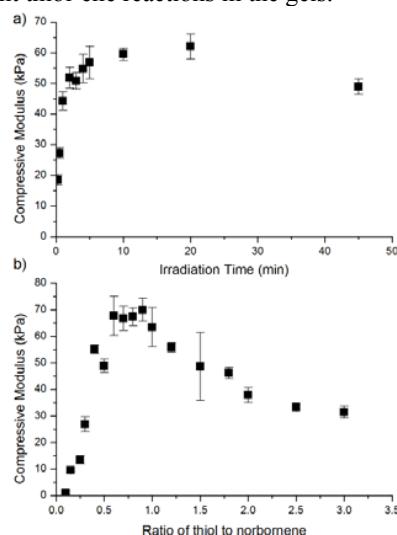


Figure 2. Compressive modulus of 4 wt% Nor-HA gels (a) at a thiol:norbornene ratio of 0.5 irradiated for various times and (b) at various thiol:norbornene ratios irradiated for 45 min.

**Conclusions:** HA was functionalized with norbornene groups to give Nor-HA that underwent a one-to-one thiol-ene reaction with DTT under UV irradiation to give hydrogels. The modulus of the material was controlled by varying both irradiation time and thiol to norbornene ratio. Ongoing experiments are to further characterize the range of mechanical properties attainable, as well as patterning of gels that form at low thiol to norbornene ratios and leave unreacted norbornenes for further modification.

**References:** <sup>1</sup>Burdick JA. Adv Mater 2011;23:H41–H56. <sup>2</sup>McCall JD. Biomacromolecules 2012;13:2410–2417.