

Effect of molecular weight and degree of methacrylation on hyaluronan hydrogel mechanical properties

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Statement of Purpose: Methacrylation of polymers is used as a method to form hydrogels through free radical-induced crosslinking; the resulting gels have been shown to be amenable to drug delivery and tissue engineering applications.^{1,2,3} Anhydrous methacrylation of hyaluronan (HA-MA) allows for tunable control over the degree of polymer modification, and thus the ability to achieve varied mechanical properties. This control could allow for broad applications in tissue engineering and wound care.

Methods: HA-MA was synthesized through an anhydrous reaction resulting in varying degrees of modification (DOM). Briefly, HA (100kDa and 700kDa, Lifecore) was initially rendered amphiphilic through an aqueous ion exchange with hexadecyltrimethylammonium bromide (Sigma) using Dowex® 50WX8-400 ion exchange resin. The resulting polymer was lyophilized and subsequently placed in solution with dimethyl sulfoxide (1% w/v). The solution was then reacted for 24h at RT with methacrylic anhydride (MA, Sigma) using dimethylaminopyridine (Sigma) as a catalyst. Three degrees of methacrylation were achieved for each molecular weight by varying the ratio of MA molecules to HA hydroxyl groups. The solution was then hydrolyzed through extensive dialysis and periodic pH adjustment. The DOM was determined using ¹H-NMR (Bruker AVANCE III 500 MHz). Polymer solutions of varying HA-MA concentration were UV-crosslinked with 0.05% (w/v) Irgacure D2959 (Ciba). An MTT cell viability assay was performed using primary human mesenchymal stem cells (hMSCs). Mechanical and rheological testing was performed on AR2000 series rheometer (TA Instruments); these tests included compression, viscosity, and gelation experiments.

Results: DOM was determined to be 71, 81, 90% and 43, 54, 68% for the 100kDa and 700kDa HA-MA respectively (Fig.1). The crosslinked hydrogels were found not to be cytotoxic to hMSCs. The compressive moduli of the hydrated HA-MA hydrogels were found to increase with molecular weight, DOM and polymer concentration (Fig.2). Viscosity was found to decrease as DOM increased. Gelation testing revealed varied gel points for each of the polymers tested (Fig.3), which were identified as the point at which tan δ reached a maximum for each polymer; tan δ was then found to proceed to zero as the polymer continued its crosslinking to termination.

Conclusions: The anhydrous reaction used for HA modification was found to successfully control DOM, however higher molecular weight HA was found to react less efficiently, potentially due to increased viscosity. Altering molecular weight, DOM and polymer concentration in the synthesis of HA-MA hydrogels allows for mechanical property optimization. Future work will concentrate on fabrication of multi-phasic scaffolds exhibiting a gradient of compressive moduli.

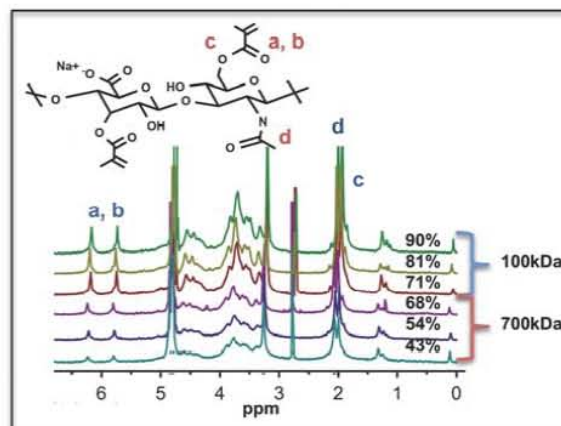


Fig.1. ¹H-NMR spectra of 100kDa and 700kDa HA-MA denoting methacryloyl/methyl peaks and DOM.

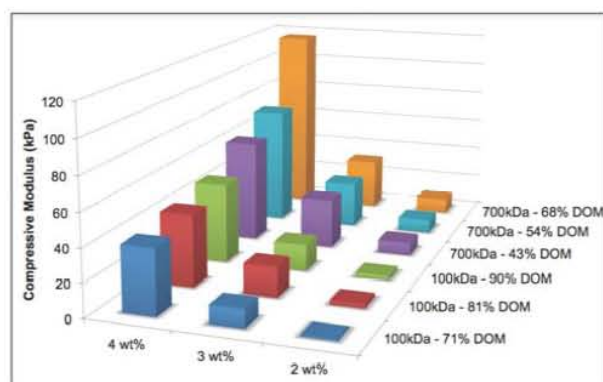


Fig.2. Average compressive modulus values (kPa) for HA-MA hydrogels varying MW, DOM and wt%.

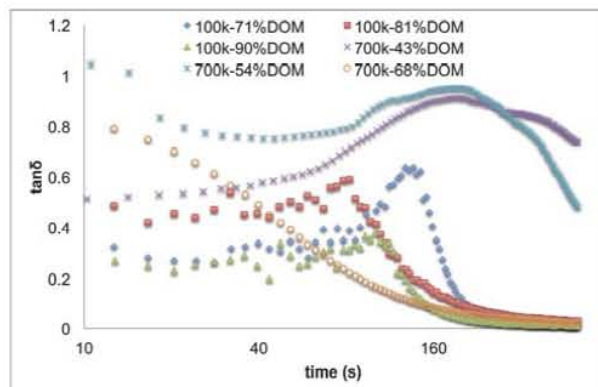


Fig.3. tan δ values for HA-MA gelation sweeps performed using 10% strain for 8.5 min.

References: 1. Bencherif, S. et al. *Biomaterials*, 2008. 2. Burdick, J. A. et al. *Biomacromolecules*, 2005. 3. Oudshoorn, M. et al. *Polymer*, 2007.