Cell-laden gelatin hydrogels formed by orthogonal thiol-ene photo-crosslinking

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Statement of Purpose: Gelatin hydrogels are a popular choice for 3D cell culture due to their inherent bioactivity and cytocompatibility. While gelatin hydrogels can be prepared by means of temperature-induced physical interactions, chemical cross-linking methods (e.g., photopolymerization) can produce a more tunable and mechanically more stable gel network. For example, gelatin-methacrylamide (GelMA) can be covalently cross-linked into insoluble gelatin hydrogels via a chain-growth polymerization mechanism.[1] However, hydrogels prepared from chain-growth polymerization often contain heterogeneous and non-degradable poly(meth)acrylate kinetic chains that may not be ideal for certain biological applications.[2] Here, we present an alternative gelatin hydrogel system based on orthogonal step-growth thiol-ene photochemistry. Gelatin-norbornene (GelNB) and dithiol-containing linker were used to form an orthogonal network via radical-mediated thiol-ene photopolymerization. In addition to evaluating the biophysical properties of GelNB hydrogels, we also demonstrated the cytocompatibility of this orthogonally cross-linked gelatin hydrogels using in situ encapsulation of human mesenchymal stem cells (hMSCs).

Methods: Norbornene-functionalized gelatin (GelNB) was prepared by reacting type B gelatin with carbic anhydride in aqueous buffer at room temperature for 70 hours. The degree of functionalization was characterized via Ellman's assay. Gelatin methacrylamide (GelMA) was synthesized according to a published protocol. The concentration of methacrylic anhydride was adjusted in order to obtain a similar degree of functionalization (ca. ~45-50%) between the two gelatin derivatives.[1] GelNB hydrogels at desired wt. content were formed via radical-mediated step-growth photopolymerization with dithiothreitol (DTT) as the cross-linker (1.5 mM per wt.% GelNB) and lithium arylphosphonate (LAP, 1mM) as the photoinitiator. Gelation kinetics and gel points were characterized by *in situ* photorheometry. Chain-growth polymerized GelMA hydrogels were obtained with a similar polymerization setup except that DTT was not added in the precursor solution. Hydrogel properties were characterized by measuring gel stiffness (via rheometrical testing) and swelling ratio. Human mesenchymal stem cells (hMSC) were encapsulated in GelNB-DTT or GelMA hydrogels for comparing the cytocompatibility of the two gel systems.

Results: GelNB was synthesized successfully after 70 hrs of reaction under slightly basic conditions (pH8). The degree of functionalization was determined to be between 45% and 50%. Using DTT as a cross-linker, GelNB was rapidly cross-linked into hydrogels with a gel point comparable to that of chain-growth cross-linked GelMA hydrogels (Fig. 1A, 16+1 and 13+1 s for GelNB and GelMA hydrogel, respectively). Fig. 1B shows that an inverse relationship between gelation weight content and hydrogel swelling and no significant difference was found between the GelNB-DTT and GelMA hydrogels. While GelNB-DTT and GelMA hydrogels were similar in hydrogel properties, we found that orthogonal GelNB-DTT hydrogels showed a higher degree of hMSC viability following cell encapsulation (~90% and ~98% for GelMA and GelNB, respectively. Fig. 2A). After extended in vitro culture, encapsulated hMSC had enhanced cell spreading in orthogonal GelNB-DTT hydrogels as compared to chain-growth GelMA hydrogels.

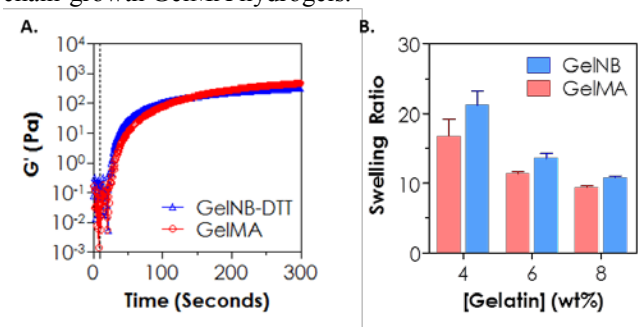

Figure 1. (A) *In situ* photorheometry of GelMA and GelNB-DTT hydrogels ([Gelatin]=5wt%). (B) Effect of GelNB/GelMA concentration on hydrogel equilibrium swelling ratio.

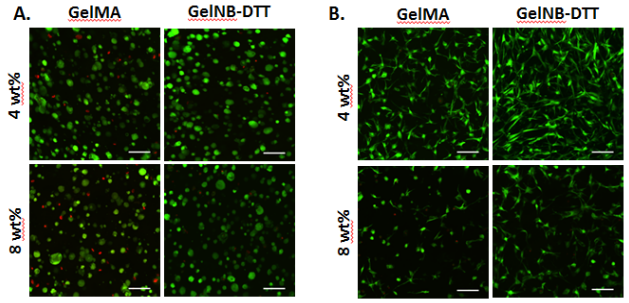

Figure 2. Live/dead staining of hMSC encapsulated in GelMA and GelNB-DTT hydrogels at Day-1 (A) and Day-13 (B) post-encapsulation. (Scales: 100 μm)

Conclusions: In summary, we have successfully synthesized norbornene-functionalized gelatin (GelNB) and used it to prepare orthogonally cross-linked gelatin hydrogels. The GelNB hydrogel system exhibits comparable gelation and mechanical properties to chain-growth GelMA hydrogels but possesses a higher degree of network homogeneity due to the orthogonal crosslinks. The stiffness and swelling of GelNB hydrogels could be easily tuned and the network structure did not contain dense hydrophobic regions generated from the chain-growth reaction. GelNB hydrogels are high cytocompatibility and potentially can serve as a convenient material platform for 3D cell culture.

References:

[1] Nichol et al. Biomaterials. 2010; 21:5536-5544.
[2] Lin et al. Biomaterials. 2011; 32:9685-9695.