Characterization of and Cellular Response to Changes in Composition of a Hyaluronic Acid-Based Hydrogel

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Statement of Purpose: Hyaluronic acid (HA)-based hydrogels have been under investigation as promising scaffold materials for tissue engineering and wound healing applications. This stems from the importance of HA in many tissues, as well as its role in development and wound healing processes. Although HA alone has poor mechanical properties and is rapidly degraded, covalently crosslinked HA-based hydrogels can solve these drawbacks. However, biological and mechanical properties of these hydrogels need to be optimized and tailored for different applications and cell types in order to achieve functional tissue. Here we have used a 3-component system to create a family of hydrogels with modified HA, modified gelatin, and poly(ethylene glycol) diacrylate (PEGda). The hydrogels were characterized in terms of gelation time, compressive strength, and degradation. Cells were also seeded within the hydrogels and monitored for metabolic activity.

Methods: Hydrogels were created by combining thiolated carboxymethyl hyaluronic acid (CMHA-S) with thiolated gelatin (Gtn-S) and crosslinking with PEGda. Table 1 gives the formulations for the 6 materials used here.

For gelation time, a test tube inversion assay was used, and gelation was determined as the point at which the material would no longer flow with gravity.

For degradation studies, materials were mixed and placed in a 96-well plate until gelled. Gels were then removed and placed in either phosphate-buffered saline (PBS) alone or PBS with hyaluronidase (HAase). Gels were monitored over time for changes in mass.

For compressive strength, gels were created in molds and tested under confined compression in an Instron to determine the force required to rupture the hydrogels.

For cell studies, CMHA-S, Gtn-S, and PEGda solutions were filter-sterilized through a 0.22-μm filter prior to use. Human dermal fibroblasts (HDFs) or human mesenchymal stem cells (MSCs) (final density of $2x10^5$ cells/ml) were gently mixed in to the materials prior to gelation. Following gelation, medium was placed on top of the gels and incubated for up to 15 days. Gels were periodically removed and examined for metabolic activity using an MTS assay (Promega).

Table 1. Formulations for HA-based hydrogels.

Material	PEGda (mg/ml)	CMHA-S (mg/ml)	Gtn-S (mg/ml)	Thiol:Acrylate
1	8	10	12	2.83 : 1
2	8	7	16	2.83 : 1
3	8	13	8	2.83 : 1
4	12	10	12	1.89 : 1
5	16	10	12	1.42 : 1
6	12	7	16	1.89 : 1

Results: In order to understand how component changes to an HA-based hydrogel affect properties of the hydrogel and subsequent cellular response, we have created a family of hydrogels by varying the modified HA, modified gelatin, and crosslinker (PEGda) concentrations. The total thiol concentration in the material was always held constant at 13.53 μmol/ml. These materials crosslink primarily via Michael-type addition of the thiol and acrylate; however, excess thiols could also crosslink over longer periods forming disulfide bonds. The gelation time of the hydrogels varied from 15 min (#5) to 27 min (#3), with the time depending mostly on the thiol:acrylate ratio. Some hydrolytic degradation of these materials occurred over the course of a month due to the ester bond formed during crosslinking. In the presence of enzyme, hydrogel degradation was faster than with hydrolytic degradation alone (as fast as 9d for 5 U/ml and 2.5d for 50 U/ml HAase vs >>30d for hydrolytic). Due to the higher concentration of HA, material #3 degraded fastest in the presence of HAase.

Studies on the compressive strength of these hydrogels indicate that an increase in CMHA-S results in a decrease in compressive strength, whereas an increase in Gtn-S leads to an increase in compressive strength. Further, an increase in PEGda concentration results in a higher compressive strength. Previous work has shown that the elastic modulus also changes with composition changes[1].

Following encapsulation in the hydrogels, the metabolic activity of HDFs drops over the course of 7 days before recovering and subsequently increasing by day 15. Similar results were observed with the MSCs, although longer term studies need to be done to confirm this trend. As an example of the differential response of the cell types to the different materials, Figure 1 shows the activity at day 4 relative to Gel #1 for each cell type. As seen in Figure 1, metabolic activity is highest for HDFs in Gel #1 and in Gel #3 for MSCs.

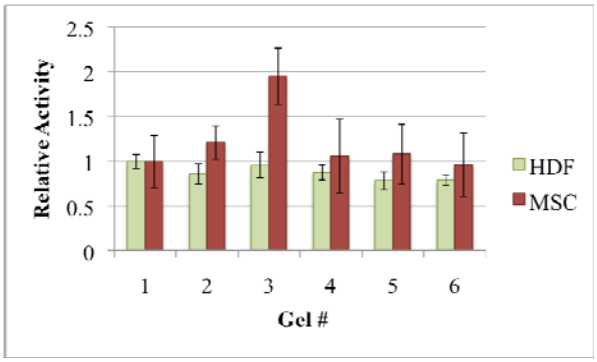

Figure 1. Relative metabolic activity of cells at day 4 in CMHA-S/Gtn-S/PEGda hydrogels.

Conclusions: In order to understand how changes in the physical properties and biological activity of HA-based hydrogels affect subsequent cell behavior, we have developed a family of hydrogels that were examined. Variations in hydrogel composition affected gelation time, degradation, compressive strength, and metabolic activity of two different cell types. Further work will focus on longer term studies with the cells, examining multiple aspects of cell behavior, including differentiation of the MSCs and new ECM deposition.

References: Vanderhooft JL. Macromol Biosci. 2009;9:20-28.