Methacrylated Hyaluronic Acid Hydrogels for Two-Step Photocrosslink-Mediated Bioprinting

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Statement of Purpose: Tissue engineering is a field full of therapeutic and academic potential, but fraught with obstacles. Tissues and organs are complex, and mirroring this complexity in the laboratory has proven difficult. Bioprinting, the deposition of cells and materials into an organized scheme, offers an attractive solution, given that there is a selection of appropriate printable materials. Here, we show the synthesis of methacrylated hyaluronic acid (HA-MA) and gelatin (gel-MA) that are components in a bioprintable hydrogel system. With the Fab@Home printing system (NextFab, Albuquerque, New Mexico), we implement a two-step photocrosslinking protocol by which tubular tissue constructs can be bioprinted that maintain viability in culture and remodel their environment, producing their own collagen, a substantial extracellular matrix component. Photocrosslinkable HA and gelatin hydrogels may hold potential for reaching the ultimate goal in bioprinting and tissue engineering – building a complex functional organ in the laboratory.

Methods: HA-MA was synthesized using a common methacrylic anhydride reaction. Gel-MA was synthesized by hydroxylation of gelatin with ethanolamine, followed by methacrylation. Hydrogels were prepared by dissolving HA-MA at 1.5% w/v, sterile filtering the solution, adding 10 µl acetophenone in NVP (300 mg/ml) per ml hydrogel solution, and photocrosslinking at 365 nm UV for several minutes. For cell-containing hydrogels 20% gel-MA was supplemented to the HA-MA solution. UV time dependent stiffness was determined using shear stress sweep tests with a rheometer. *In vitro* biocompatibility was determined by MTS assay of encapsulated NIH 3T3, HEPG2 C3A, and Intestine 407 cells during 3-D culture. *In vivo* biocompatibility was assessed in a nude mouse model. Cell-free HA-MA/gel-MA and Extracel control (Glycosan Biosystems, Salt lake City, Utah) hydrogels were injected subcutaneously. At 2 and 4 weeks mice were sacrificed, tissues were excised, and examined with H&E.

Tubular vessel-like constructs were printed by the Fab@Home system in a series of stacked NIH 3T3-containing rings with cell-free hydrogel cores and outer rings for support. A secondary UV exposure was used after each bioprinted layer to fuse the layers together. After bioprinting, constructs were maintained in culture for 3 weeks before being fixed, embedded, and sectioned. Sections were stained for collagen with Masson Trichrome and procollagen using an IHC protocol.

Results: Rheological experiments showed that G', the storage modulus, increased with UV exposure from about 10 to 90 Pa over the course of 6 minutes. At 2-3 minutes, G' was approximately 50 Pa, just greater than G", and appropriate for printing. *In vitro* culture showed increases in cell number between time points, not significantly different than the control, indicating sufficient biocompatibility. *In vivo*, H&E-stained tissues showed no signs of inflammatory response to both the injected HA-MA and control hydrogels at either time point. Bioprinted tubular constructs were mechanically sound after printing, allowing them to be easily maintained in culture. Over the culture period, the constructs became increasingly opaque, likely from cell proliferation and extracellular matrix (ECM) production. Construct sections stained positive for collagen and procollagen, further indicating ECM production.

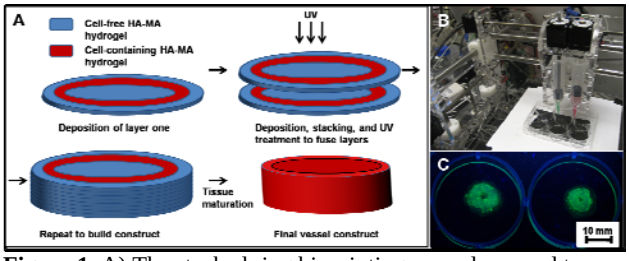

Figure 1. A) The stacked ring bioprinting procedure used to build our tubular tissue construct. B) The dual syringe deposition tool of the Fab@Home Model 1 printer. C) Bioprinted tissue constructs. Visible is the cell-containing ring (fluorescent green due to HA-Bodipy supplement) and the clear cell-free core and outer ring.

Conclusions: Combining established biomaterials with creative processing and manipulation protocols may be one way to reach the goal of a true engineered organ. By using methacrylated hyaluronic acid and gelatin and applying a multiple step photocrosslinking method we were able to tailor the materials for bioprinting. We have illustrated the ability to extrude hydrogels and cells to build a tubular tissue construct that matured during culture to the point where it established its own secreted extracellular matrix. One can imagine taking this work several steps further, resulting in a multilayered tissue tube construct that could be matured into a functional blood vessel.

References:

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