Reversibly Crosslinked Gold Nanoparticle - Hyaluronan Hydrogels for Automated Vessel Construct Bioprinting

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Statement of Purpose: Bioprinting, or organ printing, is a promising area of tissue engineering. It is particularly relevant today as the need for organs for transplantation and testing will likely increase as population and lifespans increase. However, the development of functional organs in the laboratory remains a distant goal, and despite recent advances, there has been little fusion between nanotechnology and true tissue engineering with viable tissue as the end product. Here, we present new hyaluronic acid (HA) hydrogels crosslinked with gold nanoparticles (AuNPs) that are suitable for bioprinting. We use these extrudable, biocompatible, degradable, and recrosslinkable hydrogels to print viable matrix-producing vessel-like tissue constructs using the automated Fab@Home printing system (NextFab, Albuquerque, New Mexico).

Methods: AuNPs (14 nm) were synthesized using a citrate reduction reaction. AuNP-crosslinked hydrogels were prepared by dissolving CMHA-S and gelatin-DTPH (thiolated HA and gelatin) in a 3:1 ratio to make a 2% w/v solution in PBS before sterile filtration. AuNPs (10.7 nM) were added to the hydrogel solution in a 1:15 volume ratio and allowed to crosslink for 24 hours. For cell-free hydrogels gel-DTPH was excluded. Hydrogel stiffnesses were determined using rheological shear stress sweep tests. Biocompatibility was determined by LIVE/DEAD assays on NIH 3T3, HEPG2 C3A, and Intestine 407 cells cultured on hydrogels using Extracel (Glycosan Biosystems, Salt Lake City, Utah) as a control. Tubular vessel-like constructs were bioprinted by a series of stacked NIH 3T3-containing rings with cell-free cores and outer rings for support using the Fab@Home printer. Printed constructs were imaged with light and fluorescent microscopy, and cultured for 4 weeks before being processed for histology. Sections were stained for collagen with Masson Trichrome and procollagen using an IHC protocol.

Results: Rheological experiments showed that G' varied with several factors, but most importantly increased with crosslinking time. At 24 hours gelation time, the hydrogel was appropriate for printing at ~200 Pa. Cell culture showed viabilities of over 95% for all cell types. Bioprinted tubular constructs were mechanically sound after allowing printed layers to recrosslink for 1 hour. Over the culture period, the constructs became increasingly opaque, likely from cell proliferation and extracellular matrix (ECM) production, allowing increased visualization of the vessel walls and lumen. Construct sections stained positive for collagen and procollagen, further indicating ECM production.

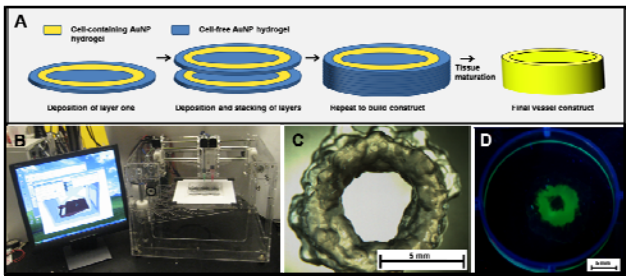

Figure 1. A) The stacked ring printing approach. B) The Fab@Home printing system. C) A construct without the central core and outer support hydrogel. D) A complete construct, supplemented with HA-Bodipy for fluorescent visualization.

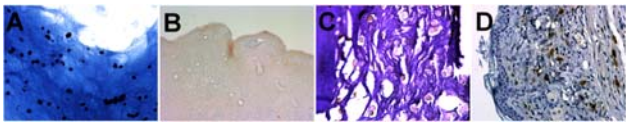

Figure 2. Trichrome stain of A) a construct illustrating cell nuclei (black) and collagen matrix (blue), and B) a negative control hydrogel, confirming that gelatin does not result in a false positive stain. IHC stain for procollagen (brown, indicated by arrows) in C) a construct and D) in a control skin sample illustrating specificity.

Conclusions: Bioprinting is an attractive tissue engineering method because the combination of precise deposition with rapid prototyping allows it to address intricacies in viable tissues. AuNP hydrogels are mechanically sound and able to reversibly crosslink after the printing process – characteristics that we believe are present from the many nanoscale crosslinks in the network and the multivalent nature of the AuNPs for thiols. By printing a simple tube, we show that it is feasible to print a blood vessel or a duct structure that one might find in a number of organs. More complex tissues and organs can be represented with multiple tubes or containers in various orientations, some of them embedded within cell-containing hydrogels. One staple of living tissue is its ability to remodel its environment. Our cells began in a simple HA matrix, but produced a collagen-rich matrix, essentially having transformed from synthetically-organized groups of cells to actual tissue. By incorporating nanotechnology into familiar biomaterials, we can create versatile systems for bioprinting of organs and tissues, which may become future commodities in high demand.

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