Fabrication of cell-laden macroporous hydrogel scaffolds using sacrificial microparticles

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Statement of Purpose: Volumetric muscle loss (VML) injury results in permanent deficit of skeletal muscle function and drastic reduction in quality of life. Regenerative medicine approaches utilizing porous scaffolds can improve functional outcomes over nanoporous hydrogels by promoting easier cell infiltration from surrounding tissue, pro-regenerative immunomodulation, and improved angiogenesis[1]. Therefore, development of a macroporous (pores > 100 µm diameter) hydrogel system combining the benefits of hydrogels (water-swollen ECM-like structure, simple fabrication process) and the porosity commonly found in scaffolds would be useful for VML repair. Such a system would also have the added benefit of being amenable to cell encapsulation during scaffold preparation and implantation, which is critical for cell-rich tissues like skeletal muscle. We have repurposed a sacrificial microparticle system previously used to support bioprinted constructs[2] into a sacrificial porogen to easily fabricate cell-laden macroporous hydrogels.

Methods: Microparticles were synthesized through single emulsion of acidic water and complex coacervate of gelatin and hyaluronic acid (HA). An aqueous solution of gelatin and HA at pH of 5.5 was heated to 50C. The solution was continuously mixed and allowed to cool to room temperature overnight. Porous hydrogels were fabricated by suspending microparticles in an aqueous solution of norbornene-modified HA (NorHA), dithiol crosslinker, and LAP photoinitiator. After exposure to UV light, the resultant gels were incubated at 37C in PBS overnight. A similar method was followed for cell culture studies with the following exceptions: an MMP-degradable dithiol peptide crosslinker was used, and C2C12 mouse myoblasts were incorporated before crosslinking at a concentration of 400,000 cells/mL. C2C12s were cultured in growth media for 2 days, and media was changed to low serum differentiation media afterward. Mechanical characterization was performed on an oscillatory shear rheometer. Rhodamine phalloidin and DAPI were used to image the cell F-actin cytoskeleton and nuclei respectively on a confocal microscope.

Results: Macroporous hydrogels of porosities up to 80% could be fabricated (Figure 1A). Maximum achievable porosity was dependent on polymer weight percent and crosslinking density of the hydrogel. At 1 wt% and 60% crosslinking efficiency, a NorHA hydrogel could only reach a maximum porosity of 60%, while a 2 wt% NorHA hydrogel with 80% porosity could be fabricated. Apparent elastic modulus of increasingly porous hydrogels followed the predictions from cellular solids materials theory[3]. Linearity between the square of relative density and the relative modulus was observed in 1, 1.5, and 2 wt% NorHA hydrogels with porosities varying from 0% to 80% (Figure 1B). A 2 wt% NorHA hydrogel with 60% porosity was chosen for cell culture due to its relatively robust apparent modulus (E = 1 kPa) and physiologically-relevant microenvironmental stiffness (E = 3.5 kPa). Other formulations did not form stable gels (low apparent modulus) or formed hydrogels with excessively stiff microenvironments. In growth media, C2C12s exhibited larger cell areas in macroporous hydrogels compared to those cultured in nanoporous hydrogels. After a week in differentiation media, C2C12s in macroporous hydrogels exhibited muscle cell-like morphologies.

Conclusions: A simple macroporous hydrogel fabrication process amenable to cell encapsulation was developed through the use of gelatin microparticles as sacrificial porogens. We characterized the mechanical properties of hydrogels at different stiffnesses and porosities, and identified a 2% NorHA gel at 60% porosity as ideal for cell culture due to its robust bulk mechanical properties and relatively low microenvironmental stiffness. At early time points, C2C12s seemed to have enhanced spreading in the macroporous hydrogel condition.

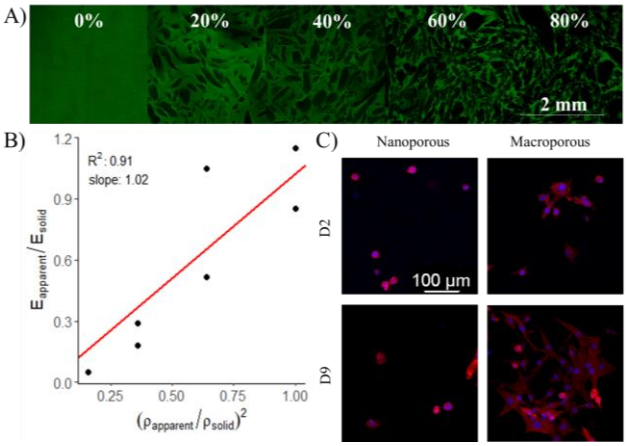

Figure 1. A) Fluorescent imaging of FITC-labeled NorHA hydrogels with varying degrees of porosity. B) The square of the relative density of macroporous hydrogels (1.5 wt%) is linearly related to their respective relative moduli as predicted by cellular solids theory. C) Maximum intensity projections of C2C12s (F-actin (*red*) and nuclei (*blue*)) cultured within nanoporous and macroporous hydrogels. Two days post-encapsulation, cells within macroporous hydrogels exhibited greater cytosolic volume and reduced circularity compared to the nanoporous condition. After an additional seven days in differentiation media, cells within macroporous hydrogels showed significant changes in morphology compared to the nanoporous condition.

References:

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