Cytocompatible Covalently Adaptable Networks to Probe Biophysical Behavior of Encapsulated Cells

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Statement of Purpose: Covalently crosslinked synthetic hydrogels are especially suitable as tissue engineering scaffolds due to their well-defined and easily tunable biochemical and biophysical properties. In order to enable complex cell functions like ECM deposition, motility, and spreading, a mechanism for crosslink degradation must be engineered into the material; however, the presence of a degradation trigger can complicate the cellular biophysical microenvironment. Furthermore, covalently crosslinked polymers typically produce a predominantly elastic material, while native tissues are complex viscoelastic structures. Here, we present a step-growth poly(ethylene glycol) (PEG) hydrogel crosslinked by reversible hydrazone bonds. The macromer components are readily synthesized from commercially available precursors, and the resulting gels form rapidly under physiological conditions and provide a non-toxic matrix that is suitable for cell culture. This material is capable of mimicking aspects of the viscoelastic properties of native tissues, and the dynamic stress relaxing crosslinks permit complex cellular functions to occur while retaining the benefits of traditional covalently crosslinked hydrogels. Taken together, these attributes make hydrazone crosslinked hydrogels a unique tool for designing viscoelastic scaffolds and studying cellular responses to scaffold elasticity.

Methods: PEG-octa-amino-oxy, -hydrazine, and aldehyde were easily synthesized in 1-2 steps from inexpensive, commercially available reagents.[1] Mechanical material properties were characterized using shear rheology. C2C12 myoblasts were cultured under standard conditions, porcine chondrocytes were isolated from juvenile porcine knees, and motor neurons were differentiated from mouse embryonic stem cells using standard techniques.[2] Motor axon extension was visualized using real-time DIC microscopy and results were analyzed in MetaMorph and Mathematica. Chondrocyte matrix deposition was stained through histological and immunofluorescence techniques, imaged using confocal microscopy, and quantified in ImageJ.

Results: Exploiting the dynamism of the covalently adaptable hydrogel, we showed the ability to recapitulate many of the viscoelastic properties of native muscle tissue and the cytocompatibility of the platform using C2C12 mouse myoblasts. Furthermore, the myoblasts were able to stress the gel until relaxation and spread out in the adaptable gel while they remained rounded in the static control (Figure 1a). Because the mechanical properties of the hydrogel are well understood, the material was then used as a tool to explore the biophysical pressures cells are able to transmit to their environment. Motor axon extension was monitored in real time (Figure 1b) and calculations were performed to determine the exact force, power, and energy expended by the growth cone to navigate through this complex viscoelastic environment reminiscent of native nerve tissue. Finally, studies are underway with primary chondroctyes to investigate the effect of a viscoelastic microenvironment and potential benefits in promoting matrix deposition in 3D.

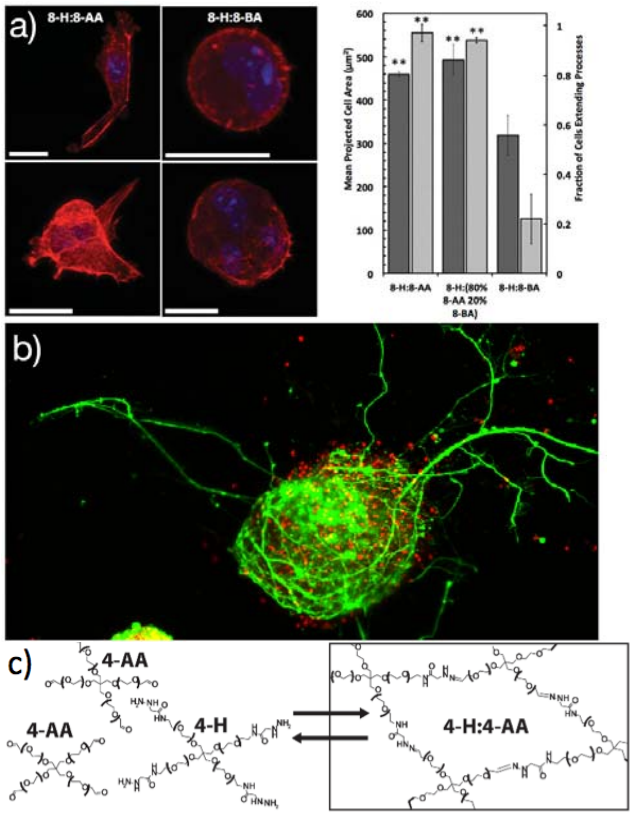

Figure 1: a) C2C12 myoblasts spread and extend filapodia into the covalently adaptable gel (8-H:8-AA), but not into the static gel (8-H:8-BA). Mean cell projected cell area is significantly higher in the adaptable gel indicating material relaxes on a time scale relevant to myoblast spreading. Scale bar 10 μm. F-actin is shown in red and the nuclei in blue. b) Motor neurons extend axons through the well-characterized covalently adaptable gel, allowing calculation of the force of extension. Live cells are shown in green and dead in red. c) Chemical structures of hydrazine- and aliphatic aldehyde-terminated PEG macromers showing covalent adaptable gelation.

Conclusions: Our results show the strength of a synthetic covalently adaptable network in both functional tissue engineering and fundamental bioscience applications. Using this system, we demonstrated the simulation of the biophysical properties of muscle tissue, the cytocompatibility of the hydrazone chemistry, and myriad cell responses to this unique scaffold.

References:

1. McKinnon, DD. *Adv. Mater*. 2013.
2. Wichterle, H. *Cell*. 2002; 110:385-397.

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