Recapitulating Physical Changes in the Extracellular Matrix with Dynamic Hydrogels

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Statement of Purpose: The extracellular matrix is a dynamic environment that undergoes cycles of stiffening and softening, especially during disease [1]. However, many in vitro cell culture platforms have static moduli, requiring one to replate cells to study physiologically-relevant changes in stiffness. A platform that recapitulates these dynamic changes will lead to better models that may shed light on the impact of matrix mechanics on disease mechanisms. Here, we present a strategy to reversibly control the modulus of hyaluronic-acid (HA) based hydrogels with light exposure through the use of photoresponsive crosslinkers. Two different crosslinkers were incorporated onto the HA backbone such that orthogonal wavelengths of light led to matrix softening followed by matrix stiffening, leading to a unique platform that reversibly alters mechanics on demand in the presence of attached cells (Figure 1A) [2].

Methods: Hyaluronic acid polymers were functionalized with α-nitrobenzyl acrylates (14 mol%) and methacrylates (40 mol%), as well as short RGD peptides for cell adhesion (1 mol%). Initially stiff gels were formed using a Michael-type addition reaction between HA and dithiothreitol crosslinkers. These gels were softened upon exposure to 365 nm light due to photodegradation of the α-nitrobenzyl moieties. After the desired timepoint, gels were re-stiffened with a photoinitiator and 400-500 nm light. The mechanical properties of the hydrogels were measured using shear rheology with a light accessory for stimulus or with atomic force microscopy (AFM). Human mesenchymal stem cells (hMSCs) were seeded on the gels at a density of 3000 cells/cm², and changes in cell morphology and YAP/TAZ nuclear localization were monitored upon changes in bulk stiffness.

Results: At a formulation of 14% acrylate and 40% methacrylate, gels were formed with an initial elastic modulus of 14.8 kPa, as measured by AFM. These gels could be softened to 3 kPa upon exposure to 365 nm light (Stiff → Soft, Figure 1B). Subsequent re-stiffening with a photoinitiator and visible light led to an elastic modulus of 27.7 kPa (Soft → Stiff, Figure 1B). These moduli span a range of active mechanosensing for hMSCs. For this reason, hMSCs were seeded onto the dynamic HA substrates and monitored for changes in cell morphology and YAP/TAZ nuclear localization at each stiffness condition. Specifically, 24 hours after seeding, the cells were spread and largely contained YAP in the nucleus (Figures 1C-1E). Softening occurred at 24 hours, and the hMSCs were assessed 48 hours later. Compared to the cells on Initial Stiff gels, the cells on softened gels decreased in spread area and exhibited a lower YAP/TAZ nuclear/cytosolic ratio (Figures 1C-1E). Finally, stiffening occurred at 72 hours, and again, cells were assessed 48 hours later. Cell spread area and YAP/TAZ nuclear localization again increased back to similar levels seen on the Initial Stiff gels (Figures 1C-1E). Several controls were run to ensure that the changes seen in cell morphology or YAP nuclear localization were the result of dynamic changes in stiffness.

Figure 1. A. Reversible control of hydrogel crosslinking density using photoresponsive linkers in the presence of attached cells. B. Elastic modulus as measured by AFM shows Initial Stiff gels are 14.8 kPa, Stiff → Soft gels are 3.5 kPa, and Soft → Stiff gels are 27.7 kPa. C. Cell spread area starts high on the Initial Stiff gels, decreases on the softened gels, and increases again on the re-stiffened gels. D. YAP/TAZ nuclear/cytosolic ratio decreases on the softened gels and increases again on the re-stiffened gels. E. Representative images of cells on Initial Stiff, Soft, and Soft → Stiff gels stained for F-actin (red), nuclei (blue), and YAP/TAZ (green). (**: P<0.01, *: P<0.05, n > 70 cells, scale = 50 microns).

Conclusions: Incorporating two orthogonal photoresponsive crosslinkers into HA hydrogels allows for the reversible control of gel modulus with light. Seeded hMSCs responded in a reversible fashion to these changes in modulus while attached to the substrates. The theoretical moduli limits of the gels are tunable with acrylate and methacrylate modifications of the HA polymers, and further tunability could be achieved with partial degradation or photopolymerization. Due to their tunability and non-invasive stimulus, these innovative materials may be broadly useful for probing the effect of dynamic stiffness on many cell types.

References: