Ca^{2+} and cAMP Signaling in Chondrocytes is Sensitive to Hydrogel Composition and Permeability

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Statement of Purpose: Among various applications, hydrogels are used to promote chondrogenesis in progenitor cells and maintain chondrocyte phenotype and differentiated activity. These functions can be enhanced by altering hydrogel composition to incorporate polymers that promote adhesion, activate cell receptors, and bind biofactors (e.g. growth factors, cytokines), and by modifying hydrogel concentration and crosslink density to control diffusion of nutrients and cell secreted factors. These hydrogel properties ultimately modulate intercellular signaling and thereby cell differentiation and tissue growth. To optimize hydrogel composition, we have developed a system with which to screen material effects on transport and signaling of biofactors within hydrogels in real-time. With a microfluidic permeability assay platform to observe biofactor transport within hydrogels, and fluorescence and Förster resonance energy transfer (FRET) imaging we monitor the real-time signaling response of cells within these hydrogels. Here we utilize hydrogels of polyethylene glycol (PEG), a PEG and gelatin blend, and Matrigel to test compositional effects on chondrocyte response to small molecular weight agonists of Ca^{2+} and cAMP signaling.

Methods: *Fabrication:* PEG (4000 MW, Sigma Aldrich) and gelatin (45000 MW, Sigma Aldrich) were acrylated with methacrylic anhydride (Sigma Aldrich) [2,3,4]. Solutions of 10 w/v% PEGDA and a 4:1 ratio (w/w%) of PEGDA to GELMA were prepared in Hank's balanced salt solution (HBSS). The initiator lithium phenyl-2,4,6-trimethyl-benzoylphosphinate (LAP) was synthesized according to Majima et al. [5]. Matrigel was used as received and prepared according to the manufacturer.

Permeability: We created a chamber consisting of glass slides enclosing a polydimethylsiloxane mold to examine the permeability of the hydrogels. PEGDA containing hydrogels were photocrosslinked at the center of the circular chamber at an exposure of 3.2 J/cm^2/mm UVA (λ=365 nm). Diffusion over time of fluorescent biofactors was captured using time lapse cinematography with a Nikon Eclipse TiE epifluorescent microscope (Nikon Instruments Inc.) and NIS-Elements AR v4software. To study hydrogel permeability of molecules at physiologic size, a range of fluorescent surrogate biofactors, such as Nile Red (NR, MW= 318), salbutamol (SALB, MW= 800), and Dextran 3000 (DEX3, MW=3000) were diluted in HBSS and added to the chamber.

Real-time Signaling in 3-D: Primary bovine chondrocytes were transfected with a cAMP activity probe (EPAC CFP/YFP) over 48hrs or loaded with Fura2-AM for 45min. Chondrocytes were suspended in the aforementioned hydrogel solutions and polymerized as cylinders. After 24hrs, cells were stimulated with either forskolin (cAMP agonist) or ionomycine (Ca^{2+} agonist) and their signaling responses were observed.

Results: Solely radial diffusion was achieved through the bonding of the hydrogel cylinder to the glass, which inhibited diffusion from the cylinder ends. The hydrogel permeability depended on biomolecule and hydrogel composition. The smaller molecules similar in size to forskolin and ionomycine showed rapid diffusion and a positive partition coefficient relative to the larger Dextran (see Figure 1), which showed a negative partition effect. Signaling experiments showed a delay in the EPAC and FURA response time in both PEGDA containing hydrogels compared to Matrigel. This correlated

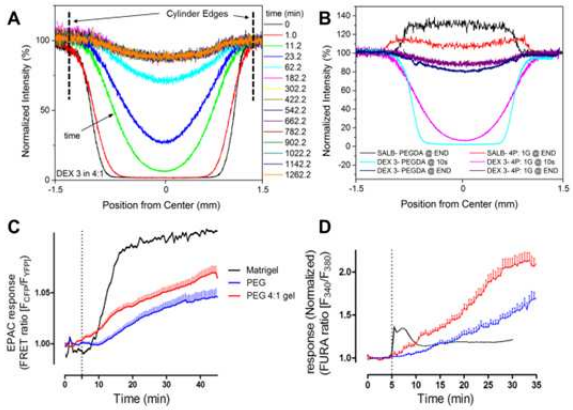

Figure 1

positively to the difference in biomolecule diffusion and partition, with PEGDA showing a lower diffusion rate and higher partition coefficient than the 4:1 (PEGDA: GELMA) blend hydrogel (Fig. 1B). However, the magnitude of the response depended on the interaction of the agonist with the hydrogel type. PEGDA containing gels showed a lower EPAC response, but greater FURA response than Matrigel (Fig.1C and 1D).

Conclusions: Here we developed a novel system for characterizing hydrogel diffusion and partition coefficients with relevant biomolecules. We showed that the permeability of hydrogels used in current tissue engineering approaches strongly modulate not only diffusion of biomolecules, but actual cellular signaling in 3-D in real-time. Differences in hydrogel density and porosity alone do not account for the opposite pattern of peak signal between EPAC and FURA among the hydrogel types, indicating material modulation of the cell biology. These findings support that tailoring material permeability to multiple factors is critical in regenerating and mimicking structures and functions of native tissues, such as in the growth plate, where several cell phenotypes communicate through different intracellular signaling factors. This also demonstrates that the characterization of permeability is necessary for understanding results of pharmacological studies in more relevant 3-D cultures.

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