Diffusion of Surrogate Biomolecules in Photopolymerizable Hydrogels

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Statement of Purpose: The growth plate is a critical component of developing limbs. With substantial occurrences of traumatic injuries, epiphyseal dysplasia, and growth plate fractures, an effective, efficient, and less invasive approach to growth plate repair is needed.

The growth plate has a stratified architecture consisting of zones with cells at different differentiation states. The spatiotemporal progression of cells though these states is maintained through the organized signaling of biomolecules, which regulate chondrocyte proliferation, terminal differentiation, and longitudinal length [1].

Implanting an engineered construct created by patterning cells in hydrogels, which mimics the growth plate structure and function, is a promising way to address these deficits. However, before such a hydrogel can be implemented the diffusive properties, which are critical in biomolecule signaling, must be characterized. Here, we examine two types of 10w/v% hydrogels: polyethylene glycol diacrylate (PEGDA) and a 4:1 ratio of PEGDA to methacrylated gelatin (GELMA).

Methods: PEGDA hydrogels were fabricated from polyethylene glycol (4000 MW, Sigma Aldrich), which was acrylated with methacrylic anhydride (Sigma Aldrich), according to Lin-Gibson et al. [2]. Through the reaction of methacrylic anhydride with gelatin (45000 MW, Sigma Aldrich), GELMA was produced according to work by Van Den Bulcke and Nichol [3, 4]. PEGDA (10 w/v%) solutions were prepared in Hank's balanced salt solution (HBSS). In a similar manner, solutions consisting of both PEGDA and GELMA were prepared at a 4:1 ratio. The initiator lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) was synthesized according to Majima et al. [5].

To characterize diffusion, we created a 1.0 mm high chamber consisting of a glass microscope slide bottom, a PDMS mold, and a microscope cover glass (see Figure 1). Both the microscope slide and the cover glass were pretreated with O_2 gas plasma and then silanated with 3-(trimethoxysilyl) propyl methacrylate (Sigma Aldrich) to bond the hydrogel to the glass. A photomask was then used to pattern a 2 mm diameter hydrogel at an exposure of 32 J/cm^3 (λ=365 nm) in the center of the circular chamber created with the PDMS mold and the glass. Unpolymerized solution was rinsed out with HBSS. Following this, fluorescent "surrogate" biomolecules of different molecular weights including Sulforhodamine 101 (SFR) (606 Da MW, Invitrogen) and dextrans (Dex 3 = 3 kDa MW, Dex 7= 7kDa MW, and Dex 10 = 10 kDa MW, Invitrogen) were used to examine the permeability of the two types of hydrogels (PEGDA and 4:1 PEGDA:GELMA). A solution of a surrogate biomolecule in HBSS was added to the chamber, thereby immersing the hydrogel. Time lapse cinematography was then performed to observe the radial diffusion into the hydrogel over time until equilibrium using a Nikon Eclipse TiE epifluorescent microscope (Nikon Instruments Inc.) and NIS-Elements AR v4software (Laboratory Imaging s.r.o.). Images were corrected for possible photo-bleaching, as well as for any inhomogeneous source excitation through the use of a calibration well.

Results: Solely radial diffusion was achieved through the silanation treatment of the glass, which enabled covalent bonding of the hydrogel to the glass microscope slide below and cover glass above. Based on the choice of surrogate biomolecules, the permeability varied in the both hydrogels. In PEGDA hydrogels, we find that the diffusion is more rapid with the decreasing molecular weight of biomolecules. Additionally, the partition coefficient decreases with increasing molecular weight (Dex3 >Dex10 >Dex70) (see Figure 1d). Moreover, the hydrophobicity is also influential, as seen with SFR.

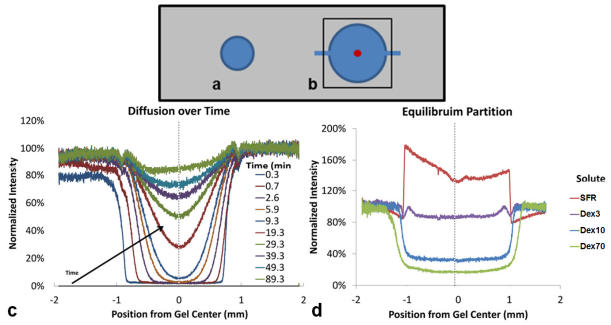

Figure 1. Schematic of system showing a) calibration well and b) diffusion chamber consisting of the cylindrical hydrogel (red circle) in the center. Intensity profiles of c) diffusion of Dex 3 and d) partitioning of four surrogate biomolecules, shown here for PEGDA (10w/v%).

Conclusions: These results showed that the molecular weight and hydrophilicity of the surrogate biomolecules influence the diffusion and partition coefficients. Through the complete characterization of biomolecules, the appropriate hydrogel composition can be chosen and the optimum hydrogel for growth plate regeneration can be used. Furthermore, not only can the data be applied to other regenerative needs where the hydrogel's permeability is critical to its application, but also the system can be used to determine the permeability of other hydrogel carrier systems.

References: [1] Lanske, B. Science.1996;273:663-666. [2]Lin-Gibson, S. Biomacromolecules.2004;5:1280-1287. [3]Van Den Bulcke,A. Biomacromolecules.2000;1:31-38. [4]Nichol, J. Biomaterials.2010; 31: 5536-5544. [5]Majima, T. Makromol. Chem.1991;192:2307–2315.