A Novel Perfusion Based Approach to 3D Gradient Generation in poly(ethylene glycol) diacrylate Hydrogels

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Statement of Purpose: Hydrogels with embedded biological signals have been widely investigated as a means of supporting the development of 3D tissue scaffolds. Poly (ethylene glycol) diacrylate (PEGDA) based hydrogels are particularly attractive because they are biocompatible, resistant to cell adhesion in the absence of signaling molecules and easily modified for specific applications. While most studies have focused on fabricating scaffolds with homogeneously distributed properties, cell migration is naturally guided by extracellular gradients of proteins. As such, the goal of this work is to develop scaffolds with 3D gradients of physical properties and biological signaling moieties. To accomplish this, a novel perfusion based system in which the photoinitiator is sequentially added to one face of the growing polymer network is proposed. It is hypothesized that by systematically feeding the initiator to the lower surface of the growing hydrogel, a gradient of initiator, and therefore rate of initiation, will be established. Since the rate of polymerization is related to both the crosslink density of the network and the degree of incorporation of tethered biological moieties, this technique will result in hydrogels containing physical gradients as well as potential gradients of immobilized biofunctional cues to which cells will respond.

Methods: PEGDA hydrogels were formed by the sequential addition of the photoinitiator, eosin Y, throughout polymerization. This was accomplished using a custom made chamber in which a glass frit filter (10-16 µm pore size; Adams and Chittenden Scientific Glass, Berkeley, CA) separates the PEGDA pre-gel solution from a reservoir of the photoinitiator (figure 1). The photoinitiator was added under pressure to the reservoir and forced through the glass frit at a constant rate using a syringe pump. After passing through the glass frit, the photoinitiator was exposed to light (λ = 514 nm), commencing polymerization.

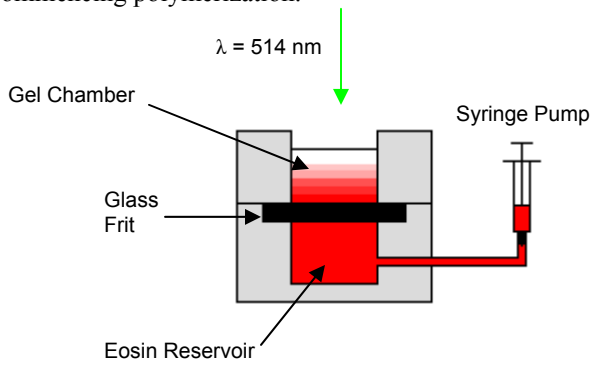

Figure 1: Schematic of glass frit perfusion chamber.

The standard pre-gel solution contained 25% PEGDA (w/v), 225 mM TEA and 37 mM NVP in ddH_2O and was polymerized using 0.1 mM eosin Y added at 20 µL/min for 15 minutes with exposure to 10 mW/cm^2 visible light. To characterize the structural properties of the resultant hydrogels, swelling analysis was performed. Briefly, the hydrogels were polymerized, sectioned into 2 mm slices and the swelling ratio ($Q_w = m_s/m_d$) of each segment normalized to the base section of the gel was obtained with statistical analysis performed using a paired t-test.

Results: Preliminary results indicate that gels formed using the perfusion based glass frit system exhibit a gradient of swelling properties, and in turn crosslink density. As shown in figure 2, the standard precursor conditions resulted in a gel with a 38 ± 13 % decrease in swelling ratio by 8 mm ($p < 0.05$). Furthermore, varying the pre-gel concentrations of NVP and TEA to 110 mM and 100 mM (respectfully) increases the rate of polymerization and the decrease in swelling ratio becomes 16 ± 16 % by 8 mm.

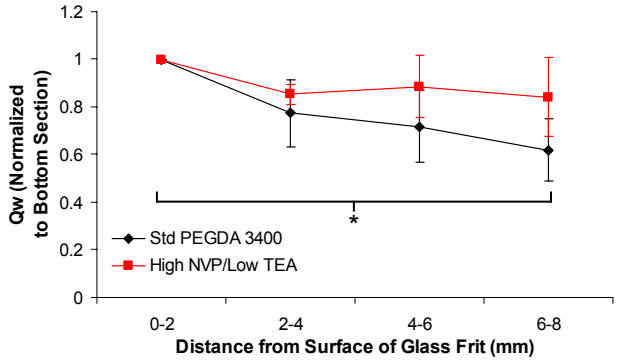

Figure 2: Spatial variation of the swelling ratio of PEGDA hydrogels formed using the glass frit perfusion chamber under standard as well as more rapidly polymerizing conditions (n = 3; * = $p < 0.05$).

Conclusions: Based on the results, it is evident that the proposed technique results in hydrogels with tunable gradients. Increases in the rate of polymerization, decrease the steepness of the gradient and vice versa. Furthermore, the glass frit system offers a reliable alternative to more complex microfluidic systems. Future studies will evaluate this phenomenon using thinner sections (< 500 µm) under a wider range of parameters, including altered photoinitiator flow rate and pre-gel characteristics. Additionally, radiolabeling will be used to track the spatial incorporation of an adhesion moiety, such as YRGDS, into the polymer network. Lastly, the effect of these gradients on cell response will be evaluated. Ultimately, the ability to produce gradients of biological and physical properties will lead to advances in directed cell migration and eventually angiogenesis.

References:

1. Peppas NA. Adv. Mater. 2006; 18:1345-1360.
2. Zaari N. Adv. Mater. 2004; 16:2133- 2137.