Effects of FGF-1 and PEG Matrix Properties on 3D Fibroblast Invasion

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Statement of Purpose: The ability to precisely control cell-substrate interactions in three dimensions has been an important factor in engineering scaffolds for regenerative medicine. Synthetic poly(ethylene glycol) diacrylate (PEGDA) hydrogels have been widely used as scaffolds in tissue engineering since they offer an environment that allows for facile tuning of matrix mechanical properties as well as the integration of biochemical cues of the natural extracellular matrix. One of the key regulators in supporting cellular proliferation and migration of cells including fibroblasts and endothelial cells in processes such as wound healing and angiogenesis is acidic fibroblast growth factor (FGF-1). However, there is limited literature on the use of FGF-1 in PEGDA hydrogels to stimulate cell invasion in 3D. In this study we exploited the effect of soluble FGF-1 on 3D fibroblast cell (FC) invasion in PEGDA hydrogels modified with proteolytically degradable collagenase sensitive (GGLGPAGGK) crosslinks, and YRGDS as the bioactive peptide. The rate of FC invasion was studied as a function of the physical and mechanical properties of the gels and varying concentration of soluble FGF-1.

Materials and Methods: PEGDA hydrogels with degradable crosslinks were conjugated with single (SBlock8000 or SBlock16000 MW) or multiple (MBlock) (theoretical MW 16000) degradable crosslinks. For cell invasion studies, 3T3 (FC) spheroids formed with carboxymethylcellulose were encapsulated within these hydrogels containing 15mg/ml acryl-PEG-YRGDS, 0, 50, or 100ng/mL FGF-1 (RD systems) and 5U/mL Heparin and monitored for 15days. Invasion was measured by subtracting the projected area of the spheroid on day 1 from the cell area over time. The swelling ratio was obtained from the mass of the swollen over dried hydrogels. Compression studies were conducted to obtain the Young's modulus of the hydrogels.

Results: As shown in Figure 1, a wide range of both physical and mechanical properties of the hydrogels regardless of the MW of the PEG can be obtained. FC invasion, however only resulted in conditions where the Young's Modulus was ~ ≤1100Pa and the Qw was ~ ≥40. Variations in soluble concentrations of FGF-1 encapsulated in hydrogels indicate that FC invasion increases in a dose-dependent manner for the various PEG types investigated. (Fig.2). As shown in Fig.3, S Block 16000 PEG hydrogels with the lowest Young's modulus resulted in the highest FC invasion. Alterations in the PEG macromer molecular weight demonstrated that S Block 8000 hydrogels had the lowest FC invasion but slightly higher Young's modulus than S Block 16000. The range of mechanical/physical properties where FC invasion resulted within the S Block PEG types (S Block 8000 vs. S Block 16000) was smaller (200-500 Pa add corresponding Qw) than that obtained with the M Block PEG. In addition, small changes in the properties of the gel resulted in drastic changes in FC invasion. FC invasion with M Block PEG can be obtained over a larger range of Young's moduli (500-1100 Pa) with small changes in the rate of FC invasion. At a Young's modulus of ~1100 Pa, FC invasion resulted in M Block PEG while FCs failed to invade in S Block PEG gels at this condition. For all cases that demonstrated FC invasion the addition of soluble FGF-1 resulted in increased invasion as compared to gels encapsulated in the absence of FGF-1

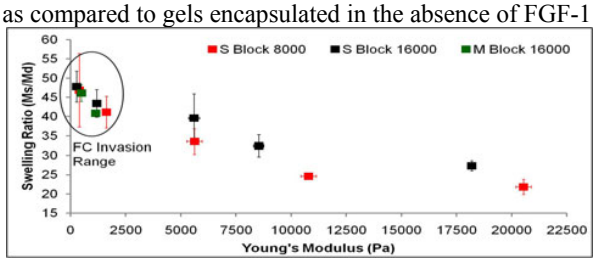

Fig. 1. Swelling ratio as a function of Young's modulus.

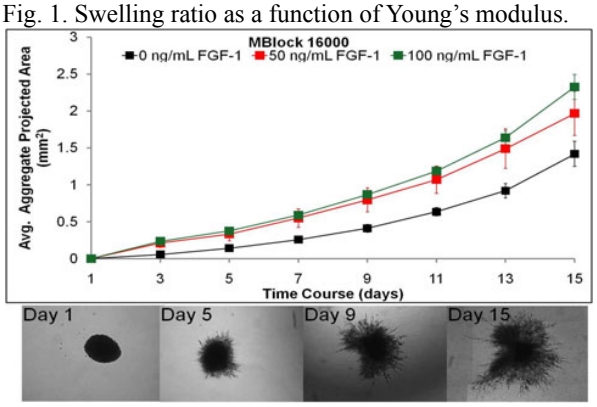

Fig. 2. Time course of FC invasion with varying [FGF-1]. Aggregate images over time course with 0ng/mL FGF-1.

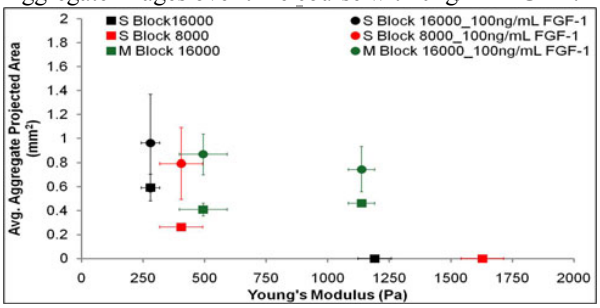

Fig. 3. Day 9 FC invasion as a function of Young's Moduli comparing PEG types.

Conclusions: An important factor in designing scaffolds for tissue regeneration is the ability to finely control the cell-scaffold interaction. In this study, the results show that a variety of factors can be used to control cell invasion including the molecular weight of the macromer, the number of the degradable crosslinks within the network and growth factors as well as the inclusion of soluble FGF-1. This study gives great insight into FC migration, an important process for understanding wound healing and angiogenesis.