Human Stem Cell Growth within Superporous Poly(ethylene glycol)-based Hydrogels

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Statement of Purpose: Superporous PEGDA hydrogel scaffolds have the potential to overcome the problems of difficulty seeding cells into the scaffold and delayed angiogenesis that are seen with current scaffolds [1]. The interconnected pore network of these hydrogels [2] not only allows cells to attach to the interior porous structure but also facilitates nutrient exchange within cells in the construct. As the initial step in surface modifications of PEGDA hydrogels, it is necessary to incorporate a bioactive peptide sequence that interacts with cell-surface receptors. To serve this purpose, we have chosen the well known RGD sequence [3]. Acrylated peptide, acryl-GRGDSG, and the scrambled control, acryl-GDGRSG, were copolymerized with the PEG diacrylate. The response of human mesenchymal stem cells (hMSCs) to this surface modification was studied.

Methods: All chemicals were purchased from Sigma-Aldrich Chemical Company as reagent grade and used without purification unless otherwise indicated. Poly (ethylene glycol) diacrylate (PEGDA) was synthesized from poly(ethylene glycol) 4000 as previously described by Sawhney et al.[4]. To make the PEGDA SPH, polymer solution, foam stabilizer (Pluronic® F127), water, the initiator pair, N,N,N′,N′-tetra-methylethylenediamine (TEMED) and ammonium persulfate (both from Fisher Scientific, Pittsburgh, PA), were added sequentially to a 4 mL vial. Citric acid (Spectrum Chemicals, Gardena, CA) was used for pH adjustment. Sodium bicarbonate, 200 mg (Fisher Scientific, Pittsburgh, PA), was added with constant stirring to evenly distribute the salt and evolving gas. For the PEGDA-peptide gels, the peptide (Protein Research Laboratory, UIC, IL) was dissolved in water and added to the polymer precursor solution to give a final concentration of 5mM. The remainder of the copolymerization procedure remained the same. The SPHs were then removed from the tube and allowed to swell in water before dehydrating them in ethanol and drying in a food dessicator. HMSCs, obtained from commercially available whole bone marrow (AllCells, Inc., Berkely CA) were grown in DMEM supplemented with 10% fetal bovine serum and 1% penicillin/ streptomycin. Cells were seeded within the partially dehydrated SPH by immersion within a fresh cell suspension. Visualization of live cells was done using calcein AM®. Actin staining of the cells was done using Alexa Flour 546®. Both reagents were purchased from Invitrogen Coorporation, Carlsbad, CA.

Results/Discussion: As expected there was rapid uptake of hMSCs by the SPH. By the end of week 1, hMSCs in the DGR gels were nonadherent while the ones seeded in the RGD modified gel were spread and attached, **Figure 1**. Cells on the RGD-containing hydrogels also stained positive for actin which was more prominent at the end of the third week, **Figure 2**. The cell to cell connections were evidently larger and denser by then.

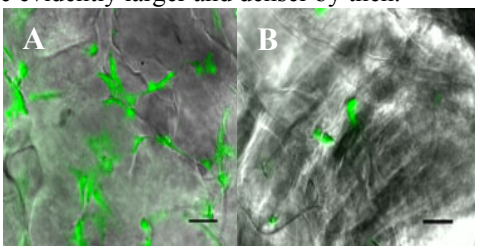

Figure 1. Week 1, fluorescence microscopy images of live hMSCs seeded on PEGDA-RGD SPH (A) and on PEGDA-DGR SPH (B). For both images the magnification was 10X and the scale bar indicates 100 µm.

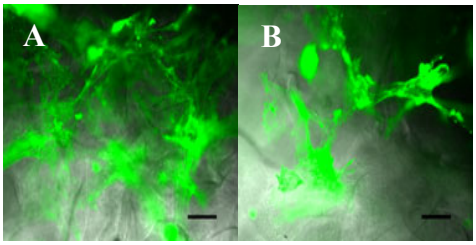

Figure 2. Week 3, fluorescence microscopy images of actin filaments observed with hMSCs seeded in PEGDA-RGD SPH (A and B). For both images the magnification was 10X and the scale bar indicates 100 µm.

Conclusions: We have successfully shown that superporous PEGDA hydrogels are capable of rapid hMSC uptake. These hydrogels can be surface modified with celladhesive peptide sequences for enhanced hMSC attachment. While we know that the hMSCs not only stay alive in the gels for over 3 weeks but also start attaching via actin filaments, further studies for cell differentiation will test the full potential of these modified PEGDA superporous hydrogels as scaffolds for tissue engineering.

References:

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