Fiber-based microcarriers for enhanced proliferation of hydrogel-encapsulated cells

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Statement of Purpose: Natural or synthetic polymers have been used to make hydrogels that mimic the extracellular milieu in order to organize cells into a three-dimensional environment and present stimuli that direct the growth and formation of a desired tissue. Hydrogels offer a number of important advantages including delivery via minimally invasive injection and in-situ crosslinking under mild conditions compatible with cells and biomolecules. However, many synthetic and hybrid hydrogels are predominately amorphous networks, while a number of recent studies have highlighted that structural, topographic features of materials affect cell morphology, proliferation, and differentiation. Previously, capillary channel polymer (CCP) fibers with micrometer-scale parallel surface grooves have been shown to support the polarized alignment of multiple cells types.[1,2] The objective of this study was to fabricate micrometer-length 'staples' from CCP fibers that can serve as topographic microcarriers within injectable hydrogel formulations. We show that CCP staples can be fabricated in the ~100 µm length range and support fibroblast adhesion, alignment, and proliferation within 3D hydrogels.

Methods: Poly-L-lactide (PLLA) was used to fabricate CCP fibers by melt extrusion technique through custom spinnerets. Fiber yarns were embedded in OCT compound and cut with a cryotome to fabricate fiber staples. The OCT was removed by sonication in water and the staples recovered by centrifugation. Fibers and staples were imaged by scanning electron microscopy. Complete removal of OCT from the staples was confirmed using ATR-FTIR. The staples were sterilized in 70% ethanol and coated with fibronectin (FN) (20µg/ml) overnight at room temperature, followed by washing in phosphate buffered saline (PBS). NIH3T3 fibroblasts were seeded onto them for 48 hrs prior to encapsulation in photocrosslinked semi-interpenetrating polymer networks composed of 6% w/v of a hydrolytically degradable PEG-diacrylate with 0.36% w/v native hyaluronic acid (1.5MDa) and 1mM acrylate-PEG-GRGDS that degrade by a combination of hydrolysis and cell-mediated enzymatic remodeling [3]. Live/Dead staining was done with calcein/ethidium homodimer at Day 7, 14, 21 and 28 and imaged by confocal microscopy. Macroscopic images were captured with a digital camera.

Results: CCP fiber staples of 100-200 µm length were made by OCT embedding and cryostat sectioning (Fig.1). NIH3T3 fibroblasts attached and spread within the grooves when seeded on FN-coated staples (Fig.2a). Beginning on day 14, large clusters of dividing cells began to clearly expand around the staples within the 3D volume of the gels (Fig.2b). The 3D nature of clusters was confirmed by confocal imaging and 3D reconstruction (data not shown). These cellular clusters progressively expanded over 28 days in culture, becoming increasingly visible at the macroscopic level (Fig.3).

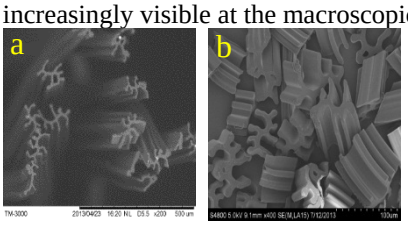

Fig.1. SEM image of (a).CCP fibers (b). CCP fiber staples sized 100µm.

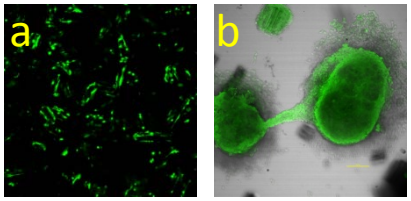

Fig.2. Calcein AM stained NIH3T3 fibroblasts (a). Cell spreading on CCP fiber staples at 48 hrs post-seeding prior to encapsulation (b). Cells proliferating to form large clusters around staples at Day 14 post-encapsulation.

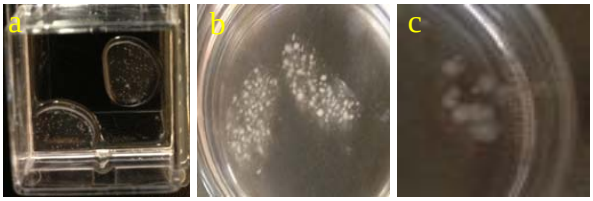

Fig.3. Macroscopic digital photographs of 3T3 fibroblasts proliferating on staples inside hydrogels at (a). Day 7 (b). Day 21 (c). Day 28

Conclusions: Fibronectin-coated CCP fiber staples promote directional contact guidance of fibroblasts and support cell survival, sustained cell division, and the formation of expanding macroscopic clusters within degradable 3D hydrogel networks. Ongoing studies are investigating the effect of staple density and quantitatively assessing increases in cell number. Future studies will investigate the applicability of this model system for topography-mediated induction of neuronal differentiation from NSCs for the treatment of neurodegenerative disorders.

References:

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