

## ENGINEERING OF EXTRACELLULAR MATRIX SCAFFOLDS VIA HOLLOW FIBER CELL CULTURE

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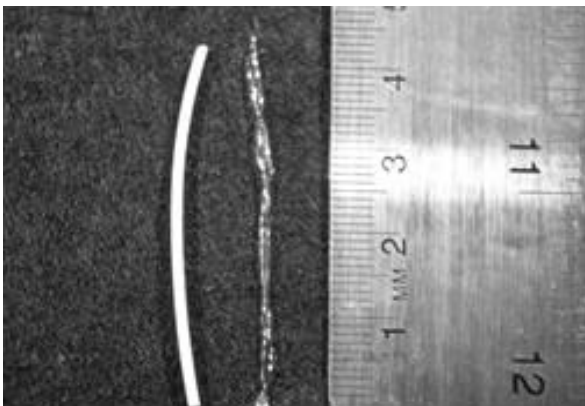
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Extracellular matrix (ECM) tissue scaffolds are seeing increased use for clinical applications, as they significantly decrease the time course of healing for injured tissues; however, the use of animal-sourced matrix for these scaffolds introduces xenogeneic epitopes into the patient toward which deleterious immune responses are directed, decreasing the effectiveness of the scaffold. ECM scaffolds produced *in vitro* have potential to minimize the foreign body response, as ECM can be cultured using human cell lines and decellularized to produce an allogeneic scaffold with high biocompatibility. The primary challenge of producing ECM-based therapeutics *in vitro* is fabricating such material in a manner which approximates the composition and architecture of native matrix while maintaining high yield and ease-of-handling. In previous work, we have demonstrated that sacrificial open-cell foams can be used for the production of ECM scaffolds with properties approximating those of native tissues.<sup>1</sup> Herein we demonstrate a novel approach for the production of continuous threads of extracellular matrix by statically culturing ECM-secreting fibroblasts in the lumina of mesoporous hollow fiber membranes (HFMs). This approach exploits the fact that mesoporous HFMs prevent cross-membrane transport of high molecular weight proteins produced by cells in their lumina, while allowing for diffusion of low molecular weight cell medium components.



*Figure 1 – Polysulfone HFM (left) and cell-derived matrix (right) following cell culture and dissolution of membrane.*

Mesoporous polysulfone (35 kD) hollow fiber membranes were manufactured using a common dry-jet wet spinning process and rinsed in DI water to remove residual solvent. Prepared membranes were treated with fibronectin to promote cell adhesion, and seeded with 100,000 NIH/3T3 fibroblasts per 6 cm fiber using a sterile syringe. Seeded HFMs were cultured for up to three weeks in DMEM-F12 with FBS, ascorbic acid, and TGF- $\beta$ . Cultured HFMs were rinsed in n-methyl-2-pyrrolidone to dissolve the membrane, leaving behind the sequestered matrix (Figure 1, right). Immunofluorescence directed toward key ECM proteins, ATR-FTIR, and tandem mass spectrometry indicated the presence of several ECM constituents.

Reseeding of isolated material with NIH/3T3 fibroblasts demonstrated no noticeable cytotoxicity, with cell cultures reaching confluency within 72 hours post-seeding. Results presented appear to indicate that this material may have application toward the repair of fibrous tissues. Results

suggest that production of ECM-based tissue engineering scaffolds can be achieved via ubiquitous hollow fiber membrane culture systems.

1. Jeffrey C. Wolchok, Patrick A. Tresco, The isolation of cell derived extracellular matrix constructs using sacrificial open-cell foams, *Biomaterials*, Volume 31, Issue 36, December 2010, Pages 9595-9603