Cell Derived Biomaterials for Biomedical Applications

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Statement of Purpose: As an alternative to autograft and allograft reconstructive approaches, synthetic materials have been developed from permanent and degradable polymers. While synthetics activate the innate arm of the immune system, animal derived tissues have been shown to activate adaptive immune mechanisms. What appears to be needed is a human cell or tissue derived alternative that closely mimics the gold standard for reconstruction. Towards this end, we have developed new approaches to produce extracellular matrix that can be fabricated into sheets, powders and various 3-D geometries. One *in vitro* approach uses a polymeric open celled foam to support cell growth and capture the biologically derived extracellular matrix (ECM) from different cell types The synthetic component is then removed and the remaining material is decellularized to produce unique and complex, biologically derived materials that can be used in a variety of biomedical applications including stem cell delivery, wound healing and surgical reconstruction.

Methods: Tecoflex SG-80 pellets (Thermedics) (4.1G) were dissolved in dimethylacetamide (39.1 ml) (DMAC) overnight at 60°C. Pluronic 10R5 (18.95 ml) (BASF) was added and the solution thoroughly mixed. The solution was cooled to 46°C and pipetted into plastic molds. Loaded molds were cooled on a dry ice/ethanol bath for 2.5 minutes and precipitated in a DI water bath. Residual solvent was removed with DI water, and the solid was frozen, and lyophilized.

Cell Seeding and Culturing: Open celled foams were coated overnight in a fibronectin solution (20ug/ml) and seeded (2 million cells/scaffold) with one of the following cell types including human laryngeal fibroblasts (LF), rat dermal fibroblasts (DF), human mesenchymal stem cells (MSC), rat astrocytes, or rat glial restricted precursors (GRP). Samples were cultured for three weeks in a growth medium consisting of DMEM F12 supplemented with 10% FBS and 1mM ascorbic acid.

Characterization: Samples (n=4/cell type) were lyophilized, weighed, and then soaked in the solvent DMAC for 72 hours at 37°C to remove the PU foam and then decellurized. The remaining material was rinsed, lyophilized, and weighed. Yield was calculated relative to the initial polyurethane foam weight. Light and Immuno-histochemical analysis was conducted for known ECM components.

Results: Bulk extracellular matrix was collected from each of the cell types evaluated. Yield ranged from 18mg to 55 mg of material per gram of foam at a cost of approx. $30-50/mg (Figure 1). The lacy material consisted of a porous network of fibrillar ECM components containing collagen, fibronectin, laminin and glycosaminoglycans (Figure 2), which could be ground into powder and resuspended into an injectable viscous liquid.

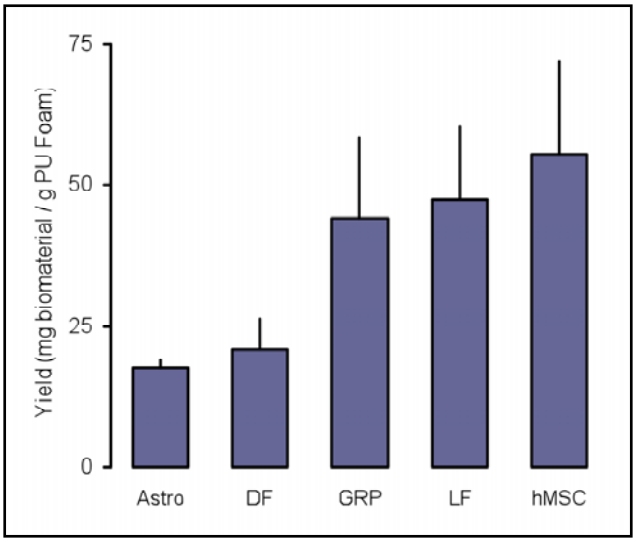

Figure 1. ECM yield as function of cell type.

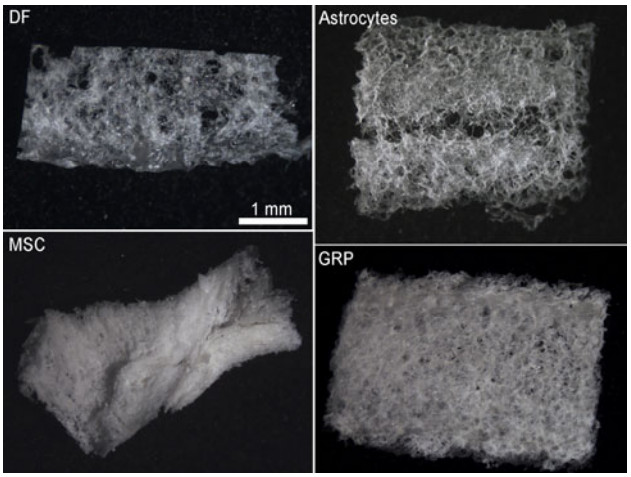

Figure 2. Representative light micrographs of cell-derived ECM in bulk sheet form. Cell sources are indicated in the upper left had of each panel.

Conclusions: Most biomedical materials consist of synthetic polymers or coatings that activate the innate immune system producing chronic inflammation that surrounds the implant over its lifetime. As an alternative, we are exploring ways of making materials from living cells. As such they have the potential to be tailored to the medical or research application by selecting the cell source and the growth conditions to produce unique materials with inherent, biologically specific activity. Early studies show such an approach is feasible and may represent a new class of biomaterials with broad biomedical application.