Fibroblast-derived, ECM-enriched biomaterials for use in nerve repair.

Michael B. Christensen, Patrick A. Tresco.
University of Utah

Statement of Purpose: A variety of experimental models using peripheral nerves require an entubulation or cuff device to guide regeneration of the damaged nerve fibers or to anchor devices to the underlying nerve. However, man-made or synthetic materials currently in use for such applications elicit a chronic foreign body reaction, which includes persistent activation of macrophages at the device interface that is accompanied by changes in morphometric parameters in the underlying nerve associated with the inflammatory sequelae. In an effort to mitigate this reaction, we are developing an approach to make such devices out of cell derived biomaterials. Our approach makes use of a sacrificial, open-celled, foam to capture the extracellular matrix shed from pure populations of cells that is then removed by solvent treatment to isolate the cell derived biomaterial[1]. The bulk material can be fabricated in a variety of ways to address several applications including use as a nerve cuff.

Methods: Porous, rectangular, polyurethane foams were custom fabricated using a phase inversion method, incubated in a fibronectin solution (20ug/ml) overnight, seeded (2 million cells/cm^3) with either fibroblasts harvested from human donors (HFs) or harvested from rat dermis (DFs), and cultured long term in a growth medium. Following the culture period, the foam was removed by solvent treatment and the remaining cell derived material was rinsed in DI water, frozen to -80°C, and lyophilized (Figure 1). Extracted material from LFs was tested for cytotoxicity by reseeding the material with LFs (50k cells/scaffold) and maintaining the reseeded material in growth medium for 48 hours. Viability was tested using calcein AM and propidium iodide. *In vivo* implantations were conducted in male Spague-Dawley rats by implanting sterilized DF derived material around the left sciatic nerve.

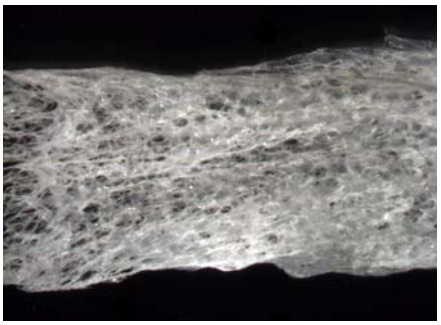

Figure 1. Example of a DF derived scaffold. Bulk material is collected in sheet form of sufficient quantity to handle and test.

Results: We have found that the approach yields a sufficient quantity of biomaterial to be easily handled and wrapped around an explanted nerve (Figure 2). Cytotoxicity studies also revealed that our cell-derived materials support cell adherence and growth after 48 hours (Figure 3).

Figure 2. Fibroblast derived biomaterial can be harvested in sufficient quantities to be easily handled and wrapped around a nerve.

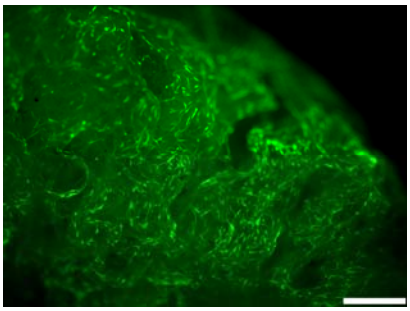

Figure 3. Cytotoxicity tests revealed that our cell-derived material supports cell adhesion and growth.

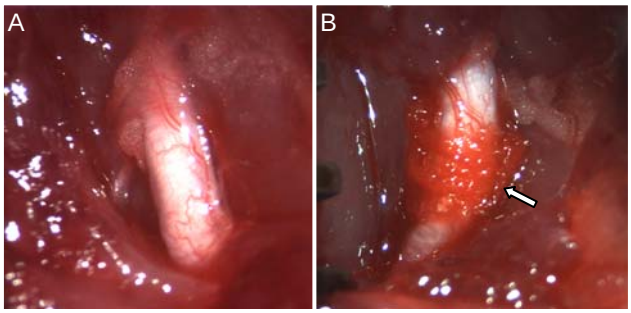

Figure 4. A) Example of an exposed rat sciatic nerve prior to implantation of a DF derived ECM nerve cuff (arrow in B).

Conclusions: Our data indicates that naturally derived, cell-based biomaterials are non-cyto-toxic and can be harvested in sufficient quantity to be easily handled and tested. Studies in progress are evaluating the foreign body response to these materials in a nerve cuff model which will be reported at the meeting.

References:

1. Wolchok, J.C., Tresco, P.A., *Biomaterials* **31**(36)