Engineering a Muscle Mimetic ECM Biomaterial

Shiloh Hurd, Ben Kasukonis, Katelin Cherry, Shahryar Ahmadi, Jeff Wolchok.
University of Arkansas

Statement of Purpose: This abstract describes the fabrication and in-vivo testing of a muscle regenerative implant made from the extracellular matrix (ECM) secreted by living cells. The performance of implantable biomaterials derived from decellularized tissues suggests that the ECM derived from native tissue has promising regenerative potential. Yet, the supply of biomaterials derived from donated human tissues will always be limited, which is why the *in-vitro* fabrication of ECM biomaterials that mimic the properties of native tissue ECM deserves research investment. Towards this end our group has developed a novel method to collect the ECM that cells secrete and form them into implantable scaffolds. To do so we have engineered a sacrificial foam and demonstrated that when cells are seeded onto these foams and grown in culture they secrete a multitude of ECM molecules that accumulate within the foam's open spaces. Our group is interested using this approach to engineer ECM scaffolds targeting the repair of damaged skeletal muscle.

Methods *Sacrificial Foam Preparation:* Open celled sacrificial foams were fabricated from medical grade polyurethane (Tecoflex SG-80, Thermedics) using a sugar scaffolding process. Molds were filled with a moistened sugar slurry. Polyurethane (PU) pellets were dissolved in dimethyacetamide (DMAC) (10% w/v) at 60°C and pipetted into the sugar filled molds. Prepared molds were immersed in a DI water bath, which both precipitated the PU solution and dissolved the sugar scaffold. The PU foams contained a network of pores consistent with the prior location of the sugar scaffold.

Cell Seeding and Culturing: PU foams were coated overnight in a fibronectin solution (20ug/ml) and then seeded (2 million cells/scaffold) with skeletal muscle myoblasts (L6, ATCC, Manasas VA). Samples were cultured for four weeks in a growth medium consisting of DMEM F12 supplemented with 10% FBS, gentamicin, and 1mM ascorbic acid. Media was changed every 2-3 days. Some samples were stimulated with TF-Beta1 (4ng/ml) to stimulate ECM production.

Material Extraction: Samples (n=12) were lyophilized, weighed, and then soaked in the solvent DMAC for 72 hours at 37°C to remove the PU foam. The extracted material was rinsed, lyophilized, and weighed. Yield was calculated relative to initial PU foam weight.

Material Testing: ECM materials were resin embedded and histological sectioned to examine ECM biomaterial architecture. . Biochemical analysis was conducted to detect the presence of known muscle ECM components. In-vitro cyto-compatibility (reseeding of ECM biomaterials with 3T3 cells) and in-vivo biocompatibility (subcutaneous implant in a rat) were examined.

Results:

- On average 2 mg of biological material can be isolated for each cm^3 of sacrificial foam seeded with cells.
- The material consists of an interconnected network of cell derived material that takes the shape of the previously present sacrificial foam (Figure 1).
- Residual cellular debris can be removed using standard decellularization procedures;
- The material contains various extracellular matrix constituents, including collagen and fibronectin;
- The cell derived material supports the growth of cultured cells.
- Explanted tissue sections surrounding the implant site showed evidence of active remodeling without evidence of a foreign body reaction.

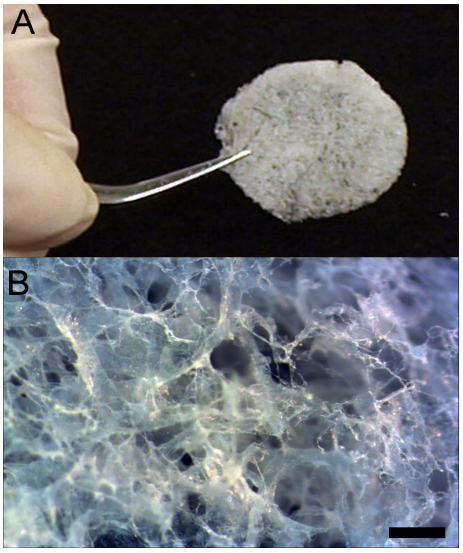

Figure 1: An ECM biomaterial prepared using muscle myoblast cells (L6 cells) shown in bulk form (A) and magnified (B) to show surface texture and porosity

Conclusions: Traditionally biomedical materials consist of synthetic polymers or coatings that activate the innate immune system producing chronic inflammation. As an alternative, we are exploring ways of making materials from the ECM secreted by living cells. Using this approach we have synthesized a biological material from muscle derived cells The results suggest that this material might be suitable for the repair of damaged muscle tissue. Currently we are exploring the regenerative potential of this material using a muscle defect repair model in a rat.

References

Wolchok, JC & Tresco, PA. (2010).. Biomaterials, 31(36), 9595-9603.