Characterization and Evaluation of Electrospun Tropoelastin and Collagen Bioscaffolds in support of Closing Full-Thickness Wounds

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Statement of Purpose: Repairing damaged tissues and organs often requires the use of replacement tissues or biomaterials. In the case of biomaterials, they must undergo biocompatibility testing prior to their clinical use. For example, biomaterials must appropriately interact with living cells as well as mimic the native biology and mechanics of the recipient tissue or organ. By electrospinning various concentrations of tropoelastin (TE) and collagen blends a mechanically adjusted or "tuned" cellular delivary device can be manufactured. Human adipose-derived stem cells (hADSC) were cultured on electrospun scaffolds and evaluated for structural architecture using scanning electron microscopy (SEM). *In vitro* screening suggests that these scaffolds would support *in vivo* implantation and cellular delivery. Therefore, a full-thickness, (SCID mouse), dermal wound model was used to evaluate the regenerative potential of hADSC-seeded scaffolds. Post-wound healing comparisons using TE and collagen bioscaffolds seeded with hADSC showed improvements in wound closure compared to controls.

Methods: *Tropoelastin:* Human tropoelastin was supplied by Protein Genomics, Inc. TE is the precursor protein to elastin, which is a durable protein found in nearly every organ in the body. The TE is manufactured using recombinant techniques.

Electrospinning process: A 5ml syringe was connected to a syringe pump, programmed for specific flow rates to advance the slurry through a small diameter tube connected to an 18 gauge blunt tip needle. The electric field (V/m) was adjusted to create a Taylor cone, directed at the aluminum foil target.

Porosity measurements: Porosity was measured using a computational method using an SEM of the scaffolds to eliminate some of the depth of view and measure relative percent porosity based on the amount of black vs. white pixels.

Mechanical characterization: The scaffolds were cut into 1cm wide strips with a 1mm gauge length and used to measure the stress and strain of the materials under the following conditions: hydrated vs. dry conditions, cross-linked vs. noncrosslinked, and 100% tropoelastin vs. 1:1 tropoelastin/collagen.

Biocompatibility evaluations: For *in vitro* biocompatibility testing of electro-spun tropoelastin, human adipose derived stem cells (hADSC), human dermal fibroblasts (hDFn) and porcine endothelial cells (pEC) were cultured and grown on 2 and 4mm scaffolds. Samples were processed for SEM.

A hairless SCID model was used to perform full thickness dermal wounds. The wounds were excised at day six post implantation and the tissue was processed for histology. The samples were reacted with anti vWF to assess neovascularization. The measurements and counts were performed on digital scans using Aperio's Imagescope software.

Results: Linear stress-strain data demonstrate the need to perform experiments in a more natural environment, as well as demonstrate the change in characteristics based on crosslinking and blending of the proteins. *In vitro* cell assays demonstrate normal cellular morphologies of various cell lines grown on electrospun bioscaffolds. *In vivo* data, demonstrate that full thickness wounds close more quickly when treated with hADSC on electrospun scaffolds compared to control treatment.

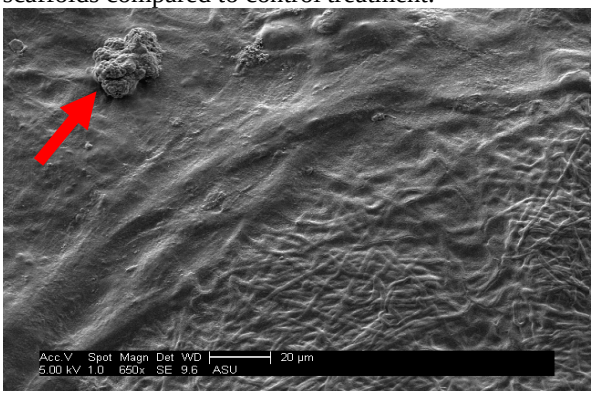

Figure 1: *SEM of the tropoelastin scaffold seeded with hADSC. The cells demonstrate normal morphology with evidence of cellular division taking place (Red Arrow) and native extracellular matrix production.*

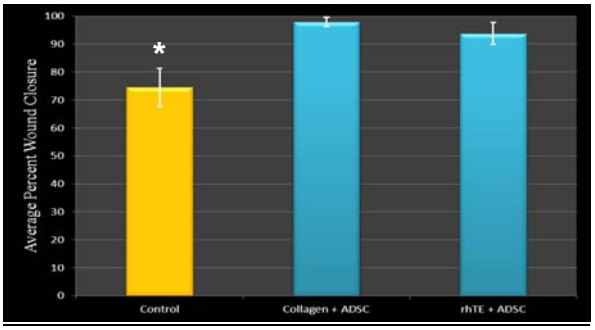

Figure 2: *Average percent wound closure - collagen and TE yielded higher percent wound closure rates. $p \leq 0.05$*

Conclusions: Electrospinning affords the ability to develop novel scaffolds that closely represent the native ECM of the target tissue, with similar physical and mechanical characteristics. These scaffolds are biocompatible with various cell lines and can be created with unique resorption characteristics by altering crosslinking or scaffold blends. *In vivo*, the tropoelastin and collagen scaffolds with hADSC demonstrate the capacity to close full thickness wounds more quickly than control treatments.