In vitro Testing of Tropoelastin and Collagen Electrospun Scaffolds

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Introduction

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.

The Specific Aim of the current study was to create electrospun tropoelastin and collagen scaffolds which can be mechanically adjusted or "tuned" to support cellularization.

Human adipose-derived stem cells (hADSC), human neonatal fibroblasts (hDFn), and porcine endothelial cells (pEC) 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.

Materials and Methods

Tropoelastin:

Human tropoelastin (TE) 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 a 18 gauge blunt needle. The electric field (V/m) was adjusted to create an appropriate Taylor cone, directed at the aluminum foil target.

Porosity measurements:

Porosity was measured using MatLab software. Code was written to threshold an SEM image of the scaffolds; targeting the superficial layers of the scaffold for the evaluation of relative percent porosity . This was calculated based on the amount of black vs. white pixels.

Mechanical characterization:

Scaffolds were cut into 1cm wide strips with a 1mm gage length and used to measure the stress and strain of the materials under the following conditions: hydrated vs. dry conditions, crosslinked vs. noncrosslinked, and 100% tropoelastin vs. 1:1 tropoelastin/collagen conditions.

Biocompatibility evaluations:

For *in vitro* biocompatibility testing of electrospun 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 analysis.

Electrospinning Process

Electrospinning is a process in which an electric field is used to propel a solubilized, charged material onto a grounded collecting plate, forming continuous, solid fibers with diameters in the submicron range. This is an ideal process for the manufacture of biomaterial scaffolds, will the ability to produce a material with physical and spatial properties similar to the topography of the native extracellular matrix of many tissues.

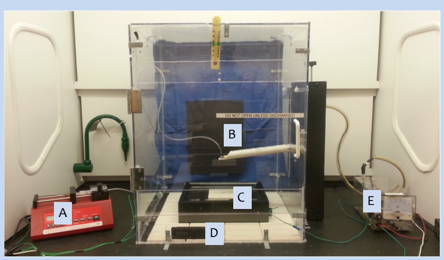

Figure 1. Electrospinning apparatus set-up inside a fume hood. A – Syringe pump, B – Cathode with blunt-tipped needle, C – Anode target surface, D – Hygrometer, E – High voltage power supply

Porosity Measurement Results

Measuring the porosity of scaffolds allows for the native extracellular matrix of the target tissue to be mimicked.

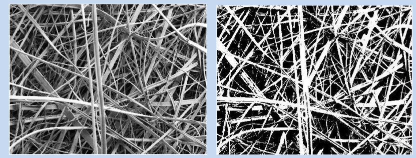

Figure 2. ***(Left)*** SEM of bare electrospun collagen scaffold, ***(Right)*** Threshold image of the scaffold for porosity measurements at 1000x. The black area is summed for a relative percent porosity. This specific example yields a relative porosity value of 54.7%.

Mechanical Characterization Results

Hydrated materials were soaked in phosphate buffer for 60 sec. prior to and throughout testing. The effect of hydration had a three order of magnitude change in the elastic modulus emphasizing the need to test the materials in an end-use environment. Blending of the scaffolds can yield "tunable" materials which can be used to compliance match various tissues or organs. Tropoelastin may have the flexibility to be used as a resorbable material and the degree of crosslinking may be able to influence the half-life of the resorption, as the material demonstrated mechanical resilience in the absence of crosslinking. Whether in cardiac, dermal, or other applications, tissue scaffolds may be subject to both static and dynamic loads, as they will be attached to the tissues of a dynamic, living organism. As such, the strain-rate dependence of the mechanical characteristics of electrospun tropoelastin and collagen may also be a necessary consideration when designing scaffolds for specific tissue uses.

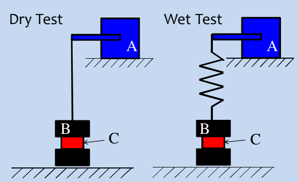

Figure 3. Schematic of the mechanical characterization testing apparatus - dry testing used a chain, wet testing used a calibrated elastomeric module. A. Servo motor, B. Custom grips, C. Material being tested

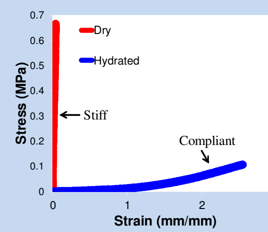

Figure 4. Effect of hydration on tropoelastin scaffolds

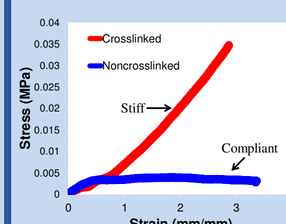

Figure 5. Effect of crosslinking on tropoelastin scaffolds

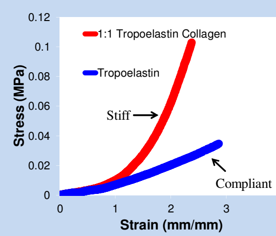

Figure 6. Comparison of crosslinked tropoelastin to 1:1 tropoelastin-collagen blends.

Biocompatibility Evaluation Results

A variety of cells were grown on the scaffolds to determine the cellular compatibility of the electrospun scaffolds. Human dermal fibroblasts (hDFn) were used to represent the use of the scaffolds in a skin environment. Porcine endothelial cells (pEC) were used to determine if the scaffolds could be used in vascular applications. Human adipose derived stem cells (hADSC) were used to determine if the scaffolds could be used as delivery devices for stem cells into target organs or tissues. The images below show representative scanning electron micrographs (SEM) of the cells living in a bio-friendly environment.

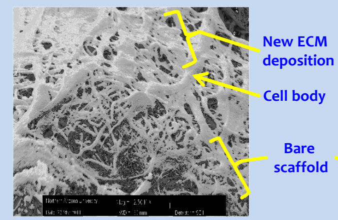

Figure 7. hDFn growing onto 100% tropoelastin scaffold

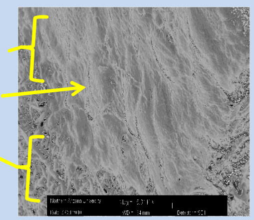

Figure 8. pEC growing onto 100% tropoelastin scaffold

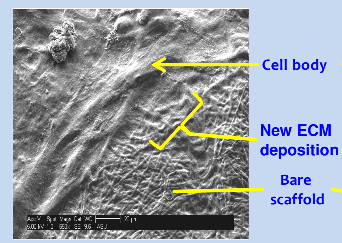

Figure 9. hADSC growing onto 100% tropoelastin scaffold

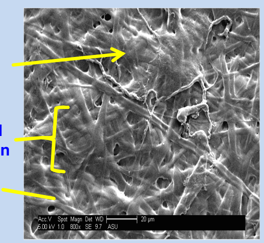

Figure 10. hADSC growing onto 100% collagen scaffold

Discussion

Human recombinant tropoelastin is an elastic biocompatible material with unique mechanical characteristics. Tropoelastin can be blended with other proteins (e.g. collagen) or synthetic materials to create novel scaffolds that offer specific solutions to various tissues. The ability to create "tunable" biocompatible scaffolds will assist in the future development of novel bioengineered solutions for medical challenges.

Conclusions

- Electrospinning allows us to create "tunable" scaffolds to match the native ECM
- Scaffolds can be created with various resorption characteristics
- Electrospun scaffolds are biocompatible and offer numerous clinical utilities

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