Bioskiving: Fabrication of Tendon-derived Collagen Nerve Guidance Materials

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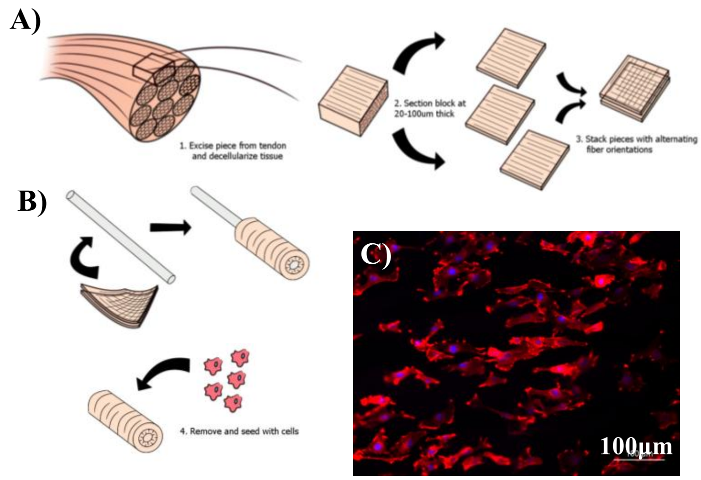
Statement of Purpose: Bioskiving is a technique developed by our group that allows the construction of nanostructured substrates from natural materials. Many natural materials have well aligned nanostructures such as the collagen fibers in tendon or the muscle fibers in muscle. We utilize these naturally occurring features in decellularized tendon, combined with a slicing, stacking and rolling technique to create flat 2D, and tubular 3D scaffolds.(1) These scaffolds have good mechanical properties as well as nanotopographic features in the form of the highly aligned collagen fibers, that provide cell guidance cues.(2) Recently, we have explored the potential of these constructs as a scaffold for nerve guidance.



Following damage, the peripherial nervous system has been shown to regenerate over small distances but must be surgically repaired over longer distances. One method of this repair is via insertion of a nerve guidance conduit that bridges the two ends of the broken nerve stump. There are a number of materials currently used for this including reconstituted type 1 collagen; however these conduits lack the structural guidance cues found in the aligned fibers of native collagen. Using bioskiving we are able to fabricate tubular structures and have demonstrated aligned growth and proliferation of several neuron cell types and nerve ganglia explants.

Methods: *Fabrication*: Bovine Achilles tendon is decellularized in a 1% SDS buffer. It is then frozen, sectioned on a cryomicrotome and either stacked into 2D (Figure 1A) structures of rolled around PTFE rods into 3D tubular structures (Figure 1B). *Cell Culture*: Multiple cell types including Schwann cell, PC12, neuronal stem cell and chick dorsal root ganglia (DRG) explants have been cultured on the tendon. *Cell Adhesion*: Adhesion and proliferation of several cell types were also tested via alamarBlue assay over one to seven days. *Cell Staining*: Cell structure has been observed through several methods including phalloidin staining of actin fillaments (Figure 1C) and immunohistochemistry with anti-β-III-tubulin staining.

Results: The bioskiving process shown in Figure 1A has been used to generate both flat two dimensional, and tubular three dimensional structures. These structures that have excellent mechanical properties when compared to reconstituted collagen gels. (1) The reorientation of the fibers in adjacent sheets provides strength in multiple directions making the material easy to handle and manipulate. Additionally, given that these constructs are composed entirely of collagen, they are biodegradable and biocompatible. These factors, combined with the nanotopographical surface of the sheets makes these scaffolds good candidates for tissue engineering. In particular neural tissue engineering and nerve regeneration as there are a number of commercially available nerve guidance conduits comprised of collagen gels. To confirm our scaffolds are also viable for neuronal growth, Schwann cells (Figure 1C), Rat pheochromocytoma (PC12), DRG and neural stem cells were cultured on the scaffolds. These cells show good alignment along the fiber direction. Additionally, cells cultured on the tendon samples have shown good adhesion and proliferation rates compared to tissue culture plastic and PLGA substrates.

Conclusions: These constructs have shown good promise for neural tissue engineering and nerve growth. Individual cell lines as well as primary cells and ganglia explants all appear to grow well and in an aligned manner along the collagen's fibers. Future work will include coculture of multiple cell types relevant to this application as well as biodegradation studies and in vivo implantation as an attempt to bridge a critical size defect gap with the tubular structures in a rodent model.

References:

1. Alberti, K. A. and Xu, Q. B. (2012) Slicing, Stacking and Rolling: Fabrication of Nanostructured Collagen Constructs from Tendon Sections, *Adv. Healthcare Mater.* in press.
2. Dai, X. S., and Xu, Q. B. (2011) Nanostructured substrate fabricated by sectioning tendon using a microtome for tissue engineering, *Nanotechnology 22,* 494008.