Bioskiving: Bioinspired Fabrication of Nanostructures from Tissue Slices

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Introduction: Nanoskiving is a technique for the fabrication of nanostructures that combines the deposition of thin films on flat or topographically patterned polymeric substrate using physical vapor methods with sectioning using an ultramicrotome. Nanoskiving requires three steps: 1) deposition of a metallic, semiconducting, ceramic, or polymeric thin film onto an epoxy substrate; 2) embedding of the film in epoxy, to form an epoxy block with the film as an inclusion; and 3) sectioning the epoxy block into slabs with an ultramicrotome.(1)
Nature has evolved a variety of clever ways to create nanoscale entities with remarkable complexity. Many biological specimen have extremely well-defined shapes and chemical activities, such as collagen fibers in tendon, muscle fibers in muscle, cornea, bone, diatoms, wood.. Tendon for example, comprises bundles of well-aligned of collagen nanofibers.
Recently, we used nature-derived materials as a substrate for sectioning, and demonstrated the fabrication of novel nanostructured constructs using a combination of sectioning, stacking and rolling, a process as we call "Bioskiving"(Figure 1A). Using decellularized tendon, we demonstrated the fabrication and characterization of various nanostructured constructs. The tendon-derived constructs are biocompatible, biodegradable and have good mechanical properties. More importantly, they provide nanotopographic cues which could offer contact guidance for oriented cell growth. These novel structures could be useful for a number of biomedical applications.

Methods: *Fabrication:* Bovine Achilles tendon was decellularized in 0.1% SDS buffer. The decellulurized tendon was either directly sectioned using a cryo-microtome or fixed and sectioned using a regular microtome. *Mechanical property characterization:* Mechanical properties were determined by tensile testing until failure of the sheets and by calculating the burst pressure of the tubular structures. *Cell culture*: various cell types, including smooth muscle cells, fibroblasts, and chick nerve explants were grown on the surface of the tendon slices to monitor the surface-guided oriented cellular growth.

Results and Discussion: Figure 1B and C are SEM images showing the well-aligned collagen nanofibers in the sections of the decellularized bovine Achilles tendon sectioned using a microtome in a direction parallel to the longitudinal orientation of the collagen nanofibers in the tendon.(2)
Stacking multiple tendon slices with a 90º rotation of each layer would create a prosthetic construct with high mechanical strength in both directions. We built 2-D multilayered constructs by stacking such sections on top of each other (Figure 1D). Sheets with aligned fibers were made by stacking ten sheets on top of each other while sheets with alternating fiber directions were created by stacking sheets with the fiber orientations at 90º. We studied the tensile strength of the stacked constructs from tendon slices, which proved to be much stronger than collagen constructs made from other methods, including reconstituted collagen or electrospun collagen fibers. We successfully fabricated 3-D tubular constructs from tendon sections by wrapping the stacked tendon constructs around PTFE rods.(Figure 1E and F) The burst pressure of such 3D tubular constructs was measured.(3)
We further demonstrated the nanostructured tendon slices comprising aligned collagen nanofibers could serve as a scaffold for guiding directional cell growth. Various cells including fibroblasts, smooth muscle cells, and neuronal cells have been cultured on the tendon slices. We observed high cellular alignment in all these cases.

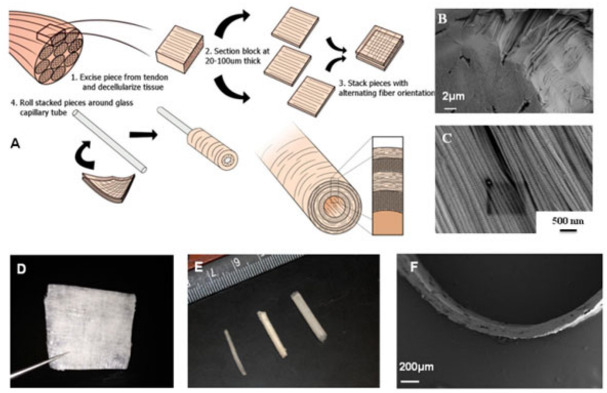

Figure 1. (A) Scheme illustration of the fabrication of multilayered stacked constructs from tendon slices using bioskiving. (B, C) SEM image showing the aligned nanofiber bundles within the collagen sections. (D) Photograph of non-crosslinked, 10-layer 2D constructs with 50 μm thick layers. (E) The resulting tubular constructs made from tendon sections. (F) The SEM showing the cross-section of the tubular constructs.

Conclusion: We demonstrated the capability of manufacturing various nanoscale constructs using bioskiving. The tissue sections could be useful as a novel substrate for tissue engineering.

References:

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