Highly Elastic Nanofibrous Scaffolds for Skin Tissue Engineering Applications

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Statement of Purpose: Skin is a viscoelastic material with complex material properties comprised of both the elastic properties of solids and the viscous properties of fluids[1]. Electrospun polymeric nanofibers have gained increasing attention for use as artificial skin substitutes due to their morphological similarities to the native collagen fibers in skin tissue. Multiple polymers are being investigated for such applications. Polyurethane (PU) holds promise given its good barrier properties, oxygen permeability and elastic properties and, accordingly, has been evaluated for wound healing applications. However, cell adhesion is typically diminished on PU relative to many other biomaterials. Many studies have focused on improving cytocompatibility of PU nanofibers via blending with other cytocompatible polymers. Polylactic acid (PLA) is a FDA approved, biocompatible, and bioresorbable polymer that has been extensively used in our lab for over a decade. We have previously shown that a variety of different cell types facilely attach and proliferate on PLA scaffolds[2,3]. The goal of this study was to determine if a blend of PLA and PU would provide a polymeric system with appropriate physical properties and cytocompatibility for skin tissue engineering. We further investigated the effect of nanofiber orientation on scaffolds' mechanical properties and cell morphology. Human adipose derived stem cells (hASCs) were used in this study due to their potential participation in the wound healing process[4].

Methods: PLA was dissolved in chloroform and dimethylformamide (DMF) (3:1) to achieve an 11 wt% solution. Similarly, PU was dissolved in DMF to obtain a 14 wt% solution. To prepare the PLA/PU blend solution, PLA and PU solutions were mixed together to obtain a solution with 1:1 (w/w) ratio of PLA to PU. Solutions were electrospun using a custom electrospinning system. Random and oriented nanofibers were collected on a static collector or a rotating mandrel (rpm = 2500), respectively. Scanning electron microscopy (SEM) was used to visualize nanofiber morphology. Tensile properties were determined using a Q-Test/5 Universal Testing Machine and ASTM D882 testing method (n=8). Human adipose derived stem cells were cultured on nanofibers in complete growth medium. Fluorescent microscopy was used to image the attached cells on the nanofibrous scaffolds.

Results: SEM images (**Figure 1**) confirmed successful formation of blended PU/PLA nanofibers. Nanofibers collected on a rotating mandrel oriented along the rotating axis. Fiber diameter measurements revealed smaller fiber diameters for oriented nanofibers (485±182nm) compared to random fibers (764±259 nm). Material property testing of the random and oriented nanofibers indicated that ultimate tensile strength and strain of the oriented fibers along their orientation direction was significantly higher that random nanofibers. **Figure 2(a)** shows a representative stress-strain curve for random and oriented nanofibers.

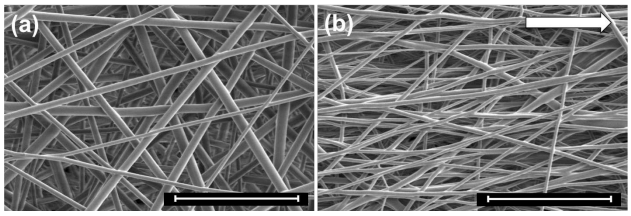

Figure 1. SEM micrographs of random (a) and oriented (b) PLA/PU nanofibers. Arrow shows orientation direction of nanofibers. Scale bar = 10 μm.

Viability and proliferation analyses of hASCs seeded on PLA/PU nanofibers indicated that hASCs remained viable and proliferated throughout the two-week experimental duration (data not shown). Phalloidin (actin) and DAPI (nuclei) staining indicated that hASCs oriented along the nanofiber direction **(Figure 2(b)).**

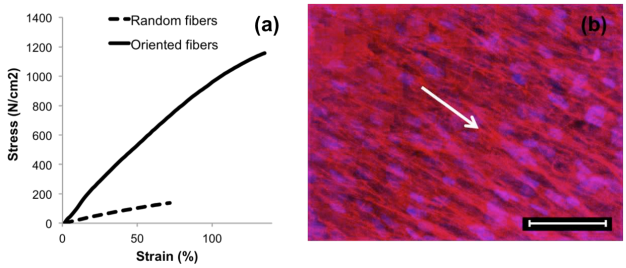

Figure 2. Stress-strain curve of random and oriented nanofibers (a). Phalloidin and DAPI staining of hASCs seeded on nanofibers (b). Red: Actin; Blue: Nuclei. Arrow indicates nanofiber direction. Scale bar = 50 μm.

Conclusions: Biocompatible, elastomeric nanofibrous scaffolds can be developed by blending a highly elastic polymer (PU) and a cytocompatible polymer (PLA) via an electrospinning system equipped with a rotating mandrel. The average tensile strength of the developed oriented nanofibers was approximately 12 MPa, close to the average tensile strength of skin (20 MPa), which varies greatly depending on the location of the skin in the body[5]. We further showed that hASC morphology can be greatly controlled by changing the physical topography of the scaffolds. The newly developed PLA/PU nanofibers hold promise for applications not only in skin tissue engineering but also in development of other tissues that require elastomeric properties, such as tendon and ligaments.

References: 1. Wilkes GL. CRC Crit Rev Bioeng. 1973;1:453-95; 2. Mohiti-Asli M. Macromol Biosci. 2012;12:893-900; 3. Mohiti-Asli M. Acta Biomater. 2014;10: 2096-2104; 4. Chen M. Crit Rev Biomed Eng. 2009;37:399–421; 5. Annaidh AN. IFMBE Proceedings. 2010;31:1000-1003.

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