Fabrication of nanofiber reinforced protein structures for tissue engineering

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Introduction: Natural extracellular matrices (ECMs), such as collagen, gelatin, laminin and fibronectin, promote cell attachment, survival, and growth. However, these materials are structurally weak, which greatly limits their potential use for guiding tissue regeneration. In addition, ECMs without appropriate alignment may not offer directional guidance to attached cells. On the other hand, degradable electrospun nanofibers support cell attachment, and provide excellent directional guidance to the attached cell [1]. Like ECMs, individual degradable nanofibers are also very fragile and hard to handle unless a relatively dense mat of nanofibers is formed. However, densely packed nanofibers provide a barrier for cell penetration and thus prevent the use of 3D structures of aligned nanofibers as effective tissue engineering scaffolds. Interestingly, a combination of ECMs and loose degradable nanofiber arrays may help to address the weakness of each component as a scaffold. Nanofibers can offer directional cues and structural integrity to the ECMs, and ECMs can immobilize individual nanofibers and serve as spacers, thus creating nanofiber arrays with low packing densities that also provides adequate structural integrity. Since ECMs may degrade very fast in the body, the resulting spaces will allow cells to populate.

Material/Methods: Polycaprolactone (PCL, M_n=80,000, Sigma) was dissolved in 3:1 dichloromethane/ dimethylformamide (Sigma) at 18% w/v. The solutions were feed through a 23 gauge needle at 0.015-0.020 ml/min and a voltage of 8kV was applied to the needle tip to initiate electrospinning. Aligned and crisscrossed fiber arrays were collected across a rack using a parallel mobile track device designed in our lab [2]. The racks holding the nanofiber arrays were then coated in a 1 wt% gelatin aqueous solution (Bovine skin type B, Sigma) and allowed drying. Some composite films were cross-linked with 1-Ethyl-3-(3-dimethylaminopropyl) carbodimide (EDC, TCI America) or genipin (Wako).

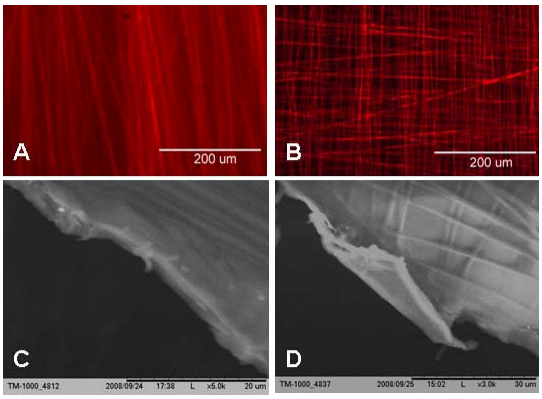

Fig. 1: Aligned (A,C) and crisscrossed (B,D) composite films imaged with fluorescent (A,B) and SEM (C,D) microscopy.

Results/Discussion: The surface tension of the gelatin solution caused the formation of a thin film over both aligned and bi-directional nanofiber arrays. A thin hybrid film remained intact after drying. Fluorescent and SEM microscopy confirmed that the nanofibers remained embedded in the gelatin films in their original configurations (Fig. 1). The average thickness of the films was 2.73 um with a standard deviation of 0.28. It was estimated that the volume fraction of PCL nanofiber films was around 3%. Hybrid films had the strength and structural integrity for manipulation into complex shapes. The elastic modulus of hydrated films with aligned embedded fibers was 392% (p=0.05) higher in the direction of the fibers. The contribution of embedded fibers to the mechanical strength of these composites is displayed in biaxial loading data obtained from samples with aligned and crisscrossed fiber orientations (Fig. 2). PCL nanofiber/gelatin hybrid thin films were maintained their integrity under cell culture conditions and promoted the attachment of 3T3 fibroblast cells. Since the membrane is very thin, cells were able to sense the embedded PCL nanofibers. Fibroblast cells elongated in the direction of the aligned nanofibers on unidirectional hybrid films.

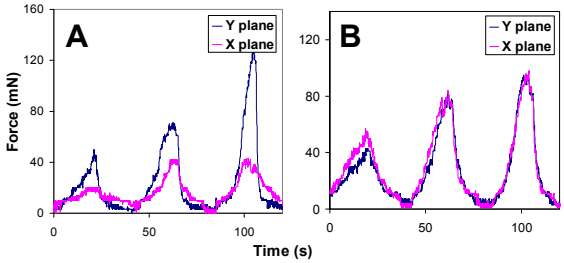

Fig. 2: Aligned (A) and crisscrossed (B) composite films loaded bi-axially with increasing strain.

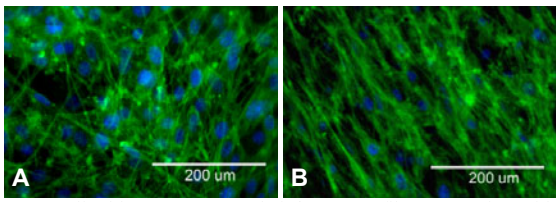

Fig. 3: Fibroblasts grown on gelatin/PCL nanofiber composites with (A) bi-directional and (B) unidirectional fiber orientations.

Conclusions: A method for fabricating nanofiber and protein hybrid thin membranes was developed. Hybrid membranes could be fabricated with a relatively consistent thickness. The hybrid films were structurally robust enough for handling and manipulation into complex structures. Hybrid membranes could be fabricated with unidirectional and bi-directional embedded fibers, and cross-linking with EDC strengthened the films significantly. Increased strength of films in the direction of unidirectional nanofibers suggests that an embedded volume fraction of PCL nanofibers as low as 3% can improve the mechanical properties of gelatin significantly. In vitro culture of fibroblasts confirmed that these composite films supported cell attachment, survival, and growth, and that embedded nanofibers could provide directional guidance to cells growing on the hybrid membranes. It was shown that nanofibers and protein matrices can be easily combined into a composite structure that has improved properties as a tissue engineering scaffold when compared to either component alone. This versatile technique can be used to develop tissue scaffolds that are biodegradable and biocompatible; promote cell attachment, survival, and growth; provide directional guidance; and possess the structural stability required for practical handling.

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References:

1.) Yang F. et al. Biomaterials 2005; 26(15):2603-10.
2.) Beachley V, Wen X. Society for Biomaterials Meeting 2007; Chicago, IL, USA.