Directional Cell Migration Induced by Electrospun Silk Nanofibers

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Statement of Purpose: Cell migration is one of the critical factors that facilitate wound healing. The migration of cells *in vivo* can be guided by constituent fibrils of natural extracellular matrix (ECM), a physical cue that is absent on flat surfaces used in routine two-dimensional (2D) cell culture [1-3]. To construct fibrous matrices that can recapitulate this *in vivo* topography *in vitro*, electrospinning offers a convenient platform technology to fabricate fibers from a variety of polymers with diameters ranging from nanometer to micrometer. Such fibers can direct *en mass* cell migration from tissue explants or agarose droplets as previously shown on poly(ε-caprolactone) (PCL) [4] and poly(methyl methacrylate) (PMMA) [3], respectively. In this study, we fabricated nanofibers from naturally derived silk fibroin (SF) into random and aligned patterns, and used live cell imaging to investigate the collective emigration of dermal fibroblasts from microcarrier beads onto the patterns. Data of this study will provide a fundamental understanding of how cells interact with nanofibers and evidence that nanofiber coating can be applied on biomaterials intended for wound healing treatment.

Methods: SF protein was extracted from cocoons of Thai silkworms *Bombyx mori* (variant Nangnoi Si Sa Ket) and reconstituted in hexafluoroisopropanol solution for electrospinning as previously described [5]. An aluminum plate and stainless steel rods with a 3-cm air gap were used to collect electrospun nanofibers into random and aligned orientation, respectively. Subsequently, the fibers were transferred onto glass coverslips and stabilized by immersion in methanol. To prepare microcarrier beads coated with cell monolayers, dermal fibroblasts were co-cultured with dextran beads (diameter ~ 180 μm) at a concentration of 3×10^6 cells per 1.5×10^3 beads. After gentle resuspension, the mixture was incubated in tissue culture media for 48 h to obtain confluent cell monolayers covering the bead surface. To observe cell migration in real-time, the cell monolayer-coated beads were fluorescently labeled and seeded onto coverslips coated with these two nanofibrous patterns. Live images of outward cell migration onto nanofibers were captured every 15 min by time-lapse fluorescence microscopy for durations of up to 4 h. Selected samples were then fixed and stained for actin filaments and cell nuclei.

Results: Electrospun SF nanofibers of 775 ± 26.9 nm (n = 150) diameter were prepared on bare glass coverslips in random or aligned patterns (Fig. 1) to serve as topographic cues for cell migration. Data from live cell imaging showed that individual cells emigrating from microcarrier beads preferentially extended processes and migrated along the contour of individual nanofibers with migration velocities of 12-15 μm/h (n = 30) on both

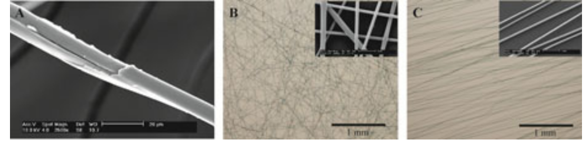

Figure 1. Silk as (A) natural fiber, (B) random and (C) aligned electrospun nanofibers.

patterns. However, the collective movement of the cells in a uniform direction was observed only on the aligned pattern. As a consequence, the mats of cells that populated the area around the beads were polarized into a rectangular shape (Fig. 2A), whereas there was no coherent structure observed for the mats on the random pattern (Fig. 2C). When the cells were allowed to grow to confluence a uniform elongated cell morphology was obtained on the aligned pattern in contrast to the random orientation of cells on the surface patterned with random nanofibers (Fig. 2B and 2D). Our data suggest that nanotopographic cues were able to guide directional migration of individual cells albeit the directional *en mass* migration depended on the assembly of nanofibers.

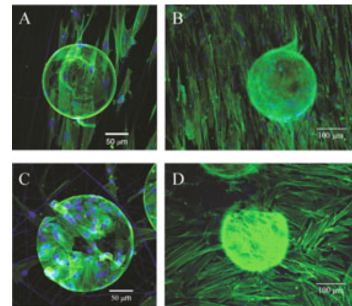

Figure 2. Fluorescence images of cells on aligned (top panel) and random patterns (lower panel) at (A) and (C) the end of live cell imaging, and (B) and (D) at confluence.

Conclusions: In this report we have demonstrated that cell-coated microcarrier beads are a suitable cell source for the study of *en mass* cell migration and that a coating of electrospun SF nanofibers can provide topographic cues capable of guiding a uniform, collective movement of the cells. Our data warrant future studies in animal models to further investigate a potential effect of these nanofibers on the rate of wound closure.

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