# Directing Cell Migration by Electrospun Fibers

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## ABSTRACT

In this study, aligned poly (methyl methacrylate) (PMMA) fibers designed as guidance structure were produced by electrospinning and tested in en masse cell migration assays. We have shown that migration of fibroblasts on the planar surface results in a radial outward trajectory, and a spatially dependent velocity distribution decreases exponentially in time towards the single cell value. If the cells are plated on the surface of aligned electrospun fibers above 1 micro in diameter, they become polarized along the fiber. The velocity of the cells on the fibrous scaffold is lower than that on the planar surface. Contrary to expectations, the rate at which cells migrate within the construct is mostly dependent on the orientation of the fibers and nearly independent on the size of the holes. These results indicate that electrospun fibers can be used to regulate cell migration and therefore provide controlled cell delivery systems.

***Keywords***: electrospinning, polymer fibers, cell migration, wound healing

## 1 INTRODUCTION

Cell migration into the wound is an essential part of all wound healing in mammals [1, 2]. Even though cell migration mechanisms and regulation of cell migration have been studied extensively on numerous substrates, the surfaces were mostly planar [3]. The research on fibers may be more relevant to understand the process involved in the wound healing process, since in vivo cells migrate on the extracellular matrix (ECM), which is fibrillar [4].

In this study, we chose to study human dermal fibroblasts migration on flat and fibrillar poly (methyl methacrylate) (PMMA) substrates. Here we show that the cell migration rate can dependent on the microstructure of the morphology of the substrate, such that on the flat surfaces the speed decreases with increasing incubation time, whereas on the aligned fibrous scaffold, cells can migrate for very long time with constant velocity. These results are critical important to fulfill our long-term goal, which is to develop an artificial implant as a conduit for cell migration and skin regeneration after skin injury.

## 2 MATERIALS AND METHODS

### 2.1 Fabrication and Characterization of PMMA Aligned Scaffold

Solutions of PMMA ($M_w$=*120 kDa*, $M_w/M_n$=*3*, Sigma-Aldrich Inc., St. Louis, MO) were made in toluene (Fisher Scientific, Pittsburgh, PA) at a concentration of 30 mg/ml and spun cast onto the clean glass coverslips using a photoresist spinner with a frequency of 25,000 PRM. The film thickness was determined by ellipsometry to be approximately 100 nm. The polymer-coated coverslip were then annealed at 120$^o$C in a vacuum of $10^{-7}$ Torr for 12 h to remove the residual solvent. Similar substrates were also prepared as targets for the electrospun fibers. This served as a two folds purpose: (a) the small amount of residual solvent plasticized the film and allowed the fibers to adhere; (b) placing the fibers onto a chemical identical substrate allowed for a direct comparison of the cell structure on flat and fibrillar surfaces.

The procedure to fabricate PMMA fibers is shown in figure 1. In the electrospinning process, PMMA solutions were loaded in a 5 ml glass syringe (Popper and Sons Inc., New Hyde Park, NY) attached to a syringe pump (KDS200, KD Scientific Inc., New Hope, PA) which provided a steady solution flow rate of 20 μl/min. A high-voltage power supply (Gamma High Voltage Research, Ormond Beach, FL) was employed to apply a potential of 15 kV between a 25 gauge blunt end needle (I.D. 0.26 mm, Popper and Sons Inc.) and a metal target which was horizontally placed 10 cm away from the tip of the needle a counter electrode. Highly aligned PMMA fibrous scaffolds were obtained by collecting the fibers with rotating drum at a speed of 6750 r/min. In further to fabricate the cross-aligned PMMA fibrous scaffold, the sample was removed and rotated to 90$^o$ and the procedure was repeated for the next layer. The substrates used for measuring the two dimensional (2D) migration consisted of a single layer of parallel fibers. The substrate used for measuring three dimensional (3D) migration consisted of two layers of fibers, oriented at 90$^o$ to each other and

The fiber diameter distribution of PMMA scaffolds was calculated by analyzing scanning electron microscope (SEM) images with Image Tool. For cell seeding, fiber-

carrying coverslips were sterilized with ultraviolet (UV) light for 20 min. Serum free Dulbecco's Modified Eagle medium (DMEM) containing 30 μg/ml intact human plasma fibronectin (Fn) (Calbiochem, San Diego, CA) was added into each sample and incubated at 37°C for 2 h.

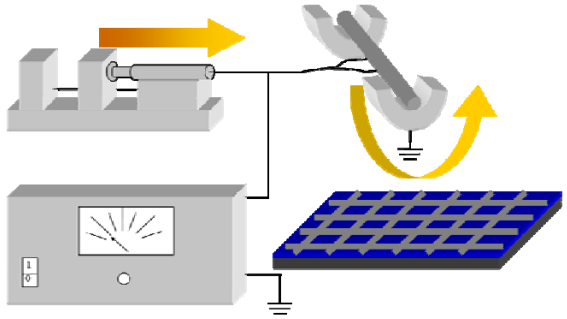

Figure 1: Schematic illustration of the procedure of fabricating PMMA cross-aligned fibrous scaffold. PMMA solution was loaded in the syringe and pushed out by syringe pump. When a high voltage (5-8kV) is applied between the needle and the collector, the fibers will be deposited on the rotating drum.

## 2.2 Cell Culture and Membrane Staining

Primary human dermal fibroblasts were obtained from Clonetics (San Diego, CA) and used between passages 11 and 12. The cells were cultured in DMEM supplemented with 10% fetal bovine serum (HyClone, Logan, UT) and an antibiotic mix of penicillin strepmycin, and L-Glutamine (full-DMEM), in a 37°C, 5% $CO_2$, 95% humidity incubator (Napco Scientific Company, Tualatin, OR).

The standard phase contrast microscopy limited the visualization of the cells on the fibers. To visualize the cells under fluorescent microscopy in real time, cell membrane were stained with DiD (Invitrogen, Carlsbad, CA). The DiD lipid-labeling procedure was obtained by a modification of the manufacture's instruction. Briefly, cells were washed well with phosphate buffer saline (PBS) and re-suspended at density of $1 \times 10^6$ cells/ml in serum free DMEM containing DiD at a concentration of 3.5 μg/ml, incubated for 30 min at 37°C. The labeled suspension tubes were centrifuged and the supernatant was removed.

## 2.3 Cellular F-actin Cytoskeleton and Nucleus Organization

To observe cells under fluorescent microscope, cells were fixed, permeabilized, followed by staining with Alexa Fluor 488 Phalloidin (Invitrogen, Carlsbad, CA) and propidium iodide (PI, Sigma Chemical Co., St. Louis, MO) for actin cytoskeleton and nucleus, respectively. The morphology of the cells was visualized with Nikon Diaphot-TMD inverted microscope or Leica TCS SP2 laser scanning confocal microscope (LSCM, Leica microsystem Inc., Bannock burn, IL).

## 2.4 Assessment of Cell Migration Velocity on Different PMMA Substrates

The agarose drop assay is a method that is frequently used to analyze cell mobility [5]. This method is performed by packing cells at high density in a small drop of agarose placed on the substrate to be studied. Migratory properties of cells are evaluated by measuring the ability of the cells to migrate out of the agarose drop. Fibroblasts to be tested were stained with DiD as described above, and then re-suspended in a volume of 0.2% (w/v) agarose solution to obtain the final cell density of $1.5 \times 10^7$ cells/ml. Droplets of 1.25 μl the cells suspension were delivered with a sterile micropipette into the wells with different substrates. The wells were then placed at 4°C for 15 min to allow the agarose to solidify. After cooling, the agarose droplets were covered with full-DMEM gently.

After incubation for a predetermined time, time-lapse images of the cells were recorded every 15 min for up to 60 min with a MetaMorph-operated CoolSNAP$^{TM}$HQ camera attached to a Nikon Diaphot-TMD inverted microscope fitted with a 37°C incubator stage and a 10× objective lens. For each sample, the migration speed was determined from the time-lapse images using MetaMorph, which tracked the distance covered by the center of a nucleus very 15 min over a period of 1 h. The same measurement was done after 6, 16 and 24 h of cell culture. Each measurement represents the average and standard deviation for 20 cells, with 3 duplicates and the entire curve was repeated at least 4 times.

# 3 RESULTS AND DISCUSSION

## 3.1 Cell Migrating Track on PMMA Spun Cast Film and Aligned Fibrous Scaffold

Previously, we showed that the appearance of the cells on an electrospun fiber mat, whose diameter was smaller than one micron, was similar to that of the cells cultured on a flat film. In addition to fiber diameter, orientation is another crucial parameter which can determine cell migration. To detect the effect of fiber orientation, the agarose droplet was placed on a PMMA thin film and scaffold composed of aligned fibers with diameter of 8 μm. From figure 2, we can clearly see that cells appeared to migrate radially on PMMA thin film. However, cells migrated along the direction of the electrospun fibers.

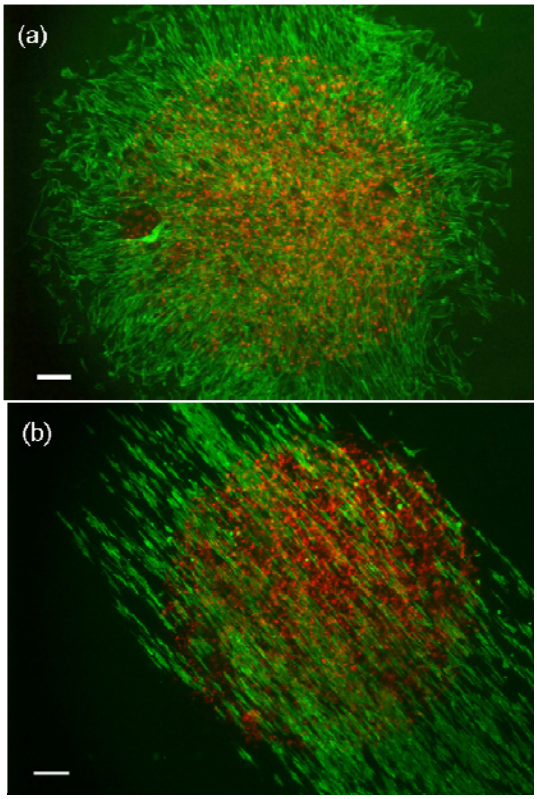


Figure 2: Fluorescence microscope overlap images of the live cells, stained with DiD (red), onto the image of the cells incubated for 24 h, fixed and stained for F-actin with Alexa Fluor (green), where the edge of the droplet is clearly marked: the agarose droplet deposited on Fn-coated PMMA (a) film and (b) aligned fiber. Scale bar=0.5 mm.

## 3.2 Cell Migration Velocity on PMMA Spun Cast Film and Aligned Fibrous Scaffold

Average speed of en masse cell migration was measured using time-lapse imaging and MetaMorph software. In order to measure cell migration velocity reproducibly, we chose to focus on cells at the leading edge of the corona, since it was easier to determine their position. The average cell migration speed, of the fibroblasts at the leading edge of the corona formed on different substrates, is plotted as a function of incubation time in figure 3. From the figure, we can see that on the flat film surface (figure 3, black line), the migration velocity decreased exponentially with incubation time. On the aligned fibrous scaffold, cell migration velocity remained constant in time and of the same magnitude as that of the single cell, or the asymptotic value reached by the cells on the Fn-coated flat surface.

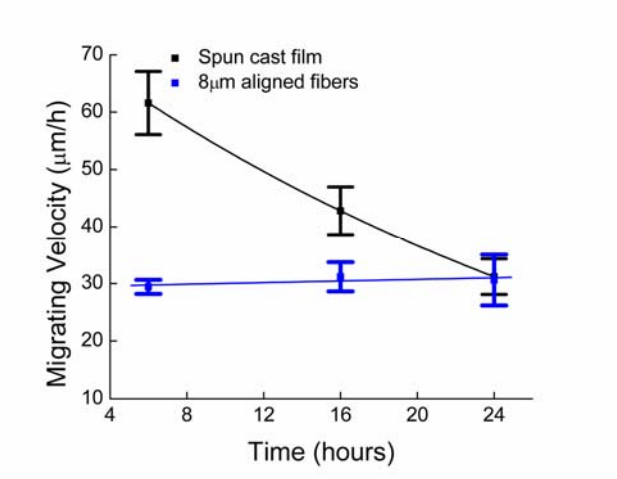


Figure 3: Cell migration velocity as a function of incubation time. Each measurement represents the average and standard deviation for 20 cells, with 3 duplicates and the entire curve was repeated at least 4 times.

## 3.2 Cell Migrating on PMMA Cross-aligned Fibrous Scaffold

A true 3D scaffold consists of alternating layers of fibers. The cell migration results on cross-aligned scaffold are shown in figure 4. From the figure, we could see that most of the cells were oriented in the cross hatched pattern of the matrix.

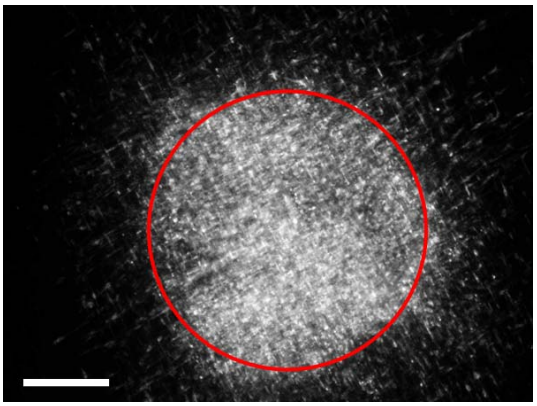

Figure 4: Fluorescent microscope image of live cells, stained with DiD, migrating from an agarose droplet onto Fn-adsorbed PMMA cross-aligned fibrous scaffold after 24 hours incubation. The ring surrounds the periphery of the original droplets. Scale bar=100 μm.

In figure 5 we show a SEM image of the cells that were fixed and dried on the fibers. From the figure we can see that even though most of the cytoplasm of the cells was centered on one fiber, a large number of filopodia extended out to the neighboring fiber. In this manner, the cells could sense the cell density in their environment.

We could also see that when the cells migrated on the 3D cross-aligned fibers, cells will not "flow" and migrate through the spaces between the fibers. Rather, cells will adhere to the fibers and either remain on a straight course or follow the fiber on the layer above or below. In figure 5 we also show two cells on a cross hatched fiber mat. The arrows point to one cell, which is oriented entirely on one fiber and migrating in a straight course. The second set of arrows point to another cell, whose cytoplasm is bent at 90° as the cell began to migrate to a lower layer and follows the fiber oriented at 90°. Since the two cells are in close proximity, they are trying to move away from each other, which become possible only through the 90° rotation of one of the cells.

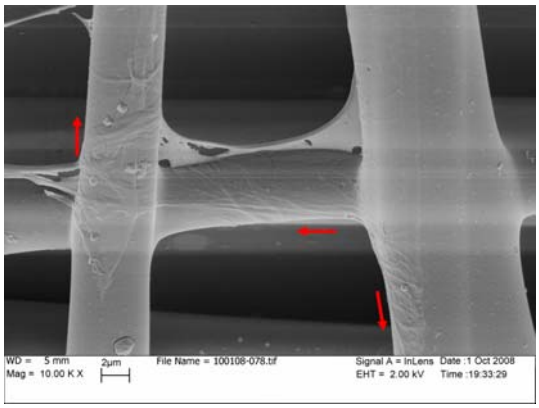


Figure 5: SEM image of cells migrating on the cross-aligned PMMA scaffold. Note that the cells extended between adjacent fibers.

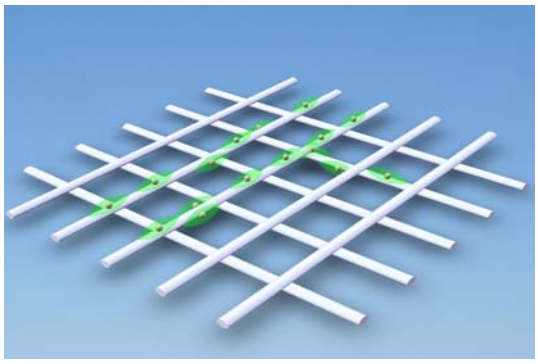

Figure 6: Cartoon of cells plated on a scaffold composted of two layers of aligned fibers oriented at 90° to each other. The cells migrated by following the contours of the fibers. At a junction, the cells can either continue straight on one fiber or follow the fiber oriented at 90° into the next layer.

## 4 CONCLUSION

The migration of cells on 3D electrospun scaffolds is summarized in figure 6. Cells prefer fibers to the flat surface of identical chemical composition [6]. As a result, when an agarose droplet containing dermal fibroblast cells are plated on the surface of the aligned electrospun fibers fiber mat, where the diameter of the fibers is larger than 1 micrometer, the cells migrate along the fibers. In contrast to cells migrating from a droplet placed on a flat film, the cells migrate with constant velocity, similar to that observed for single cell migration. On a flat surface, the cell migration is radial, as cells try to reduce the cell density. The velocity is initially fast and eventually reduces to that of the single cell velocity as the cell density decreases. On fibers, the cell density is constant, as is the cell migration velocity. When placed on a three dimensional mat, such as the one shown in figure 6, the cells will migrate from one level to another following the contour of the fibers. Hence the migration is unaffected the by size of the "holes" formed by the fibers.

## 5 ACKNOWLEDGMENTS

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