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**Effects of Aligned Fiber Substrates and P12 Peptide on Cell Migration**

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**Kao Li**

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## Abstract

Cell migration is the critical process in the development and maintenance of multicellular organisms. It's a highly integrated multistep process that orchestrates a series of cellular behavior, such as embryonic development, wound healing and immune responses. A lot of effort has been dedicated to studying the basic principle and mechanism of cell movement since decades ago[1-3]. Cells can migrate in single cell or en masse fashion. We have already known that En masse cell migration is more related with physiological processes of tissue formation. And previous study has shown that cells would respond to different substrates, with various morphology or stiffness [4]. Also, we believe there should be an evident and reasonable difference of migration fashions between young and aged cells as it should be. In this paper, we studied and demonstrated the migration of fluorescent human dermal fibroblast on PMMA flat thin film and fiber substrates on day 1,2,3,4 with en masse technique. We also applied P12, a fibronectin-derived cell survival peptide, on cells to see whether it could work in a positive way to promote cell migration as it did in enhancing cell survival in culture and improving wound healing in rat

skin[5]. The results showed that cells move faster on aligned fibrous substrates than those on the planar thin film and the velocity on fiber would speed up as days went by. What's more exciting was that P12 accelerated cell migration on both kinds of substrates, which holds the potential to achieving fast wound healing.

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**Figure 4.** The modular structure of FN. (A) FN consists 12 type I modules (rectangles), 2 type II modules (violet ovals) and 15 – 17 type III modules (ovals). The alternatively spliced domains, IIIB, IIIA and the V region, are in yellow. Binding domains for fibrin, collagen, cells and heparin are indicated; dimer forms via cysteine pair at the C-terminus (SS). The cell-binding domain (blue ovals) includes III10 with the RGD cell binding sequence and III9 which contains the synergy site. Sites of FN – FN interaction include the assembly domain in I1 – 5 as well as III1 – 2 and III12 – 14 (red). The 70 kDa N-terminal fragment is underlined (blue). The ribbon structures of FN type I1, type II1 and type III10 modules were drawn using PyMOL molecular graphic system. (B) Type I and type II modules were drawn with coordinates from residues 63 – 107, PDB ID1QGB and residues 10 – 60, PDB 2FN2, respectively. The cysteine residues and disulfide bonds are shown in purple. (C) The ribbon structure of human FN type III10 module

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## List of Abbreviations

HFB: human dermal fibroblast

FN: fibronectin

PMMA: poly(methyl 2-methylpropenoate)

ECM: extracellular matrix

Tg: glass transition temperature

PS: Penicillin-Streptomycin

FBS: fetal bovine serum

OM: optical microscope

DMEM: Dulbecco's Modified Eagle Medium

**Acknowledgment**

I would like to sincerely thank my advisor Professor Miriam Rafailovich for giving me a chance to work with her. She really gave me a lot of help and guidance in my research. Since I was a beginner when I joined the project, she always encouraged me that I have done a good work. Her encouragement and suggestion made me always feel confident in my research.

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Finally, I thank my mom and my dad, for their love and concern for me at all times.

# Chapter 1 Introduction

Cell migration is a highly integrated and orchestrated process that regulates embryonic morphogenesis; contributes repair and regeneration of impaired tissue organization; and drives disease progression of cancer cells, atherosclerosis, arthritis and mental retardation. Researchers have been dedicated to studying the mechanism of cell migration for a long time due to the belief that a better understanding of the principle by which cells migrate may contribute to the development of novel therapeutic strategies for controlling cellular activities, for example, the contagion of invasive tumor cells and would healing. Previously, most papers are focused on cell signaling pathways[6-8] involved in the process of cell movement, variation of substrates morphology or special structure[9,10].

## 1.1 Basic mechanisms for cell migration

Cells often migrate in response to specific external signals, including chemical signals and mechanical signals. Many previous researches have shown that cells can sense stiffness of the substrates they are seeded through their adhesions to the extracellular matrix (ECM) and automatically respond by altering their cytoskeletal organization and tension accordingly[11]. People has even tested the feasibility of various kinds of physical forces to obtain controlled cell migration[12]. Also, it has been demonstrated that cell movement can also be guided by purely physical interactions at the cell-substrate interface[13].

Cells fulfill the mission of migration by very different mechanisms. Many prokaryotic organisms and sperm cells which are less intricate use their flagella to propel themselves. While eukaryotic cell migration typically is far more complex and can comprise complicated and combinations of cooperating different migration mechanisms. It’s generally related to drastic deformation of cellular shape which are driven by the cytoskeleton. Two very distinct migration

scenarios are crawling motion (most commonly studied) and blebbing motility[14], while under the microscope we can clearly see the fluorescent fibroblasts move in the former fashion. In addition, there are two main theories for how the cells protrudes their front edges: the cytoskeletal model and membrane flow model. It is suggested that both of these two underlying processes are effective in the process of cell extension. The cytoskeletal model theory is based on the observation that there is rapid formation of a mass of actin, a globular multi-functional protein that forms microfilaments found in all eukaryotic cells. Such fact brought out a hypothesis that actin filaments lead to the protrusion of the leading edge and is the main driving force of its advancing[15]. The second theory arouse from the research results that the front part of cell is the recruitment site where membrane of the internal cellular membrane pools are restored to the cell surface at the end of endocytic circle[16]. This led to the hypothesis that the protrusion of the leading edge was due to the additional cell membrane added to the front part. In this theory, the newly formed actin works to stabilize the added membrane in order that a highly structured extension, or lamella, is formed instead of a bubble-like structure (or bleb) at its front[17]. It's also required to possess a fresh supply of integrins, transmembrane receptors that are the bridges for cell-cell and cell-extracellular matrix (ECM) interactions and attach the cells onto the surface. It is thus possible that these integrins are endocytosed toward the rear of the cell and carried back to the cell's front by exocytosis process, in order to be recruited to form new attachments to the substrate.

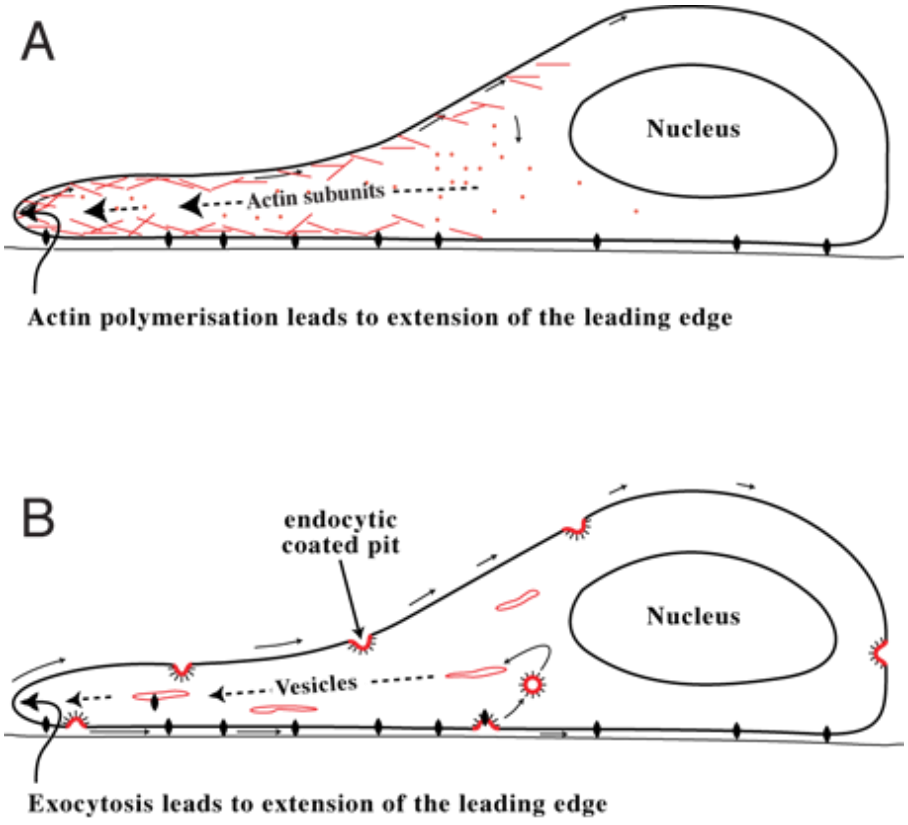


**Figure 1. Two Schemes for Particle Migration on Motile Fibroblasts.**

**In both schemes, the cells are moving to the left.**

**(A) Polarized actin cycle in which actin filaments polymerize at the leading edge, pushing forward the cell's front part and causes protrusion. Actin depolymerization process in front of the cell's nucleus generates actin subunits (g-actin) which diffuse to the cell's front for reuse. Filamentous actin, moving rearward, pulls tines (small arrows) and associated particles on the cell surface towards the cell's backside. Particles or patches often migrate with actin, although this is not always the case.**

**(B) Polarized endocytic cycle in which exocytosis at the leading edge extends the cell surface, bringing a fresh source of substratum attachments with it. Endocytosis by coated**

**pits occurs randomly on the cell surface, generating a lipid flow in the plasma membrane which decreases with increasing distance from the leading edge: this is indicated by the decreasing size of the arrows along the plasmalemma. This flow pushes on substratum-attached feet, providing a fluid drive to advance the cell. Particles or patches on the cell surface are swept towards the back of the cell, where rearward movement due to flow is balanced by random movement due to Brownian motion.[17]**

What's more, migrating cells also possess a certain polarity, a front and a rear, without which cells would spread in all directions randomly. When a cell moves randomly, its temporary front would easily become passive and yield right to other part and region to form a new front. In contrast, a directionally moved cell forced by chemotaxis represents an enhanced stabilized front as the cell advances toward a higher concentration of the stimulating chemical. This specific polarization is reflected in a molecular level: certain molecules are restricted to stay in particular area of the cells' inner surface. Researches have proved that the phospholipid PIP3, activated Rac and CDC42 are found at the front of the cell, whereas Rho GTPase and PTEN are found near the rear part[18,19]. One more thing is that it's been widely believed that microtubules also work to influence cell migration for many years, the mechanism by which they do so still remained controversial. Microtubules are not required for random movement on planer surface, but have been proven to be necessary for directional migration and efficient protrusion of the front edge[20]. Even on micropatterned aligned fibrous structures, disassemble of microtubules would change the cells' migration fashion from highly persistent to a striking oscillation[21]. Therefore, there are some underlying and intrinsic differences both in cell and molecule level when cells spread randomly or are forced to crawl directionally. In the circular process of cell shape transformation, cells overall shape undergoes a protrusion and contraction. Additionally, it

has already been proven that detachment treatment of the cell's front and trailing edge lead to significantly different phenomenon for traction force loss, which showed a distinct nature of mechanical interactions at the leading versus trailing edges[22].

In this paper, we applied two kinds of substrates with planar thin film and uniaxially fiber structure, both made by PMMA to make sure they possessed the same chemical component. The result showed that cells migrated on one layer aligned parallel fiber were highly polarized as they could only move toward two directions, in which case the migration velocity was faster than on the flat film. The velocity curve also showed that speed on the fiber accelerated as days went by and reached maximum on day 4.

## 1.2 Electrospinning

To obtain the fiber structured substrates, we used modified electrospinning caster with a rotating drum. Eclectrospinning is one of the most effective and efficient ways to obtain one-dimensional and also layer-by-layer 3-dimensional nanostructures[23]. During electrospinning procedure, a high voltage is applied to a metallic tubule which is connected with a container holding the polymer solution with proper concentration, viscosity, conductivity and surface tension. With the help of a high enough electric field, the electrostatic force generated is sufficient to overcome the surface tension of the polymer solution and therefore leads to the ejection of subtle jet out from the tubule. The polymer jet with electric charges undergoes a stretching and whipping process, giving rise to a number of continuous fibers, while at the same time the solvent is evaporated. The sprayed fibers whose diameters can be as small as tens of nanometers are then forced by electric field to fly forward until get collected by a grounded conductor. The fibers often represent as a randomly arranged and non-woven mat.

At last, migration distance within 1hour of cells without P12 and with P12 were measured with the help of day MetaMorph®-operated modified optical fluorescent microscope. And the images and data were analyzed and compared to investigate the effects of fiber structured substrates and P12 on cell migration.

### 1.3 Fibronectin

We have proven that cells don't grow well on pure PMMA, so we coated the substrates with fibronectin, a high-molecular weight (~440kDa) glycoprotein of the extracellular matrix. As we all know, the extracellular matrix (ECM) could work as a base and framework for cell adhesion, supports and enable cell migration and compartmentalize tissues separately to fulfill different missions [24] [25]. Fibronectin is one of the most components of ECM which regulates various cellular activities via direct combination with cell surface integrin receptors and it was the first to be intensively investigated. The subunits of FN are composed of 3 types of repeating modules, type Ⅰ, type Ⅱ and type Ⅲ. These modules comprise functional domains that mediate interactions with other ECM components, with cell surface receptors and with FN itself [26]. In FN, there are also various functional domains which work as binding sites for bacteria, fibrin, heparin and collagen. For example, an important cell-binding site (RGD) exists in the FNⅢ10 repeat, which interacts with the main FN receptor α5β1. Type I and II modules contain two intrachain disulfide bonds while type III modules do not have disulfide bridges (Fig. 1B). Each type I repeat has stacked β -sheets enclosing a hydrophobic core that contains highly conserved aromatic amino acids and the pair of disulfide bonds [27].Type II modules consist of two anti-parallel β -sheets that are perpendicular to each other and linked by disulfide bonds[28]. There are

17 type III repeats encoded in the FN gene; 15 are constitutively included and 2 are alternatively spliced[29]. High-resolution structures of type III modules have been determined by crystallography and NMR. These repeats are composed of seven β -sheets arranged into two anti-parallel β -sheets connected by flexible loops[30]. Strands A, B and E form one β -sheet and strands C, CV, F and G form the other to enclose a hydrophobic core (Fig. 1C)[31]. Therefore, FN molecules have the ability to work as a substratum and scaffold for cell growth by interacting with other extracellular molecules, including themselves, for various biological functions. Since we have proven that cells don't favor exposed PMMA, we coated fibronectin onto the thin film and fiber substrates to make sure cells could grow normally and healthily during culturing.

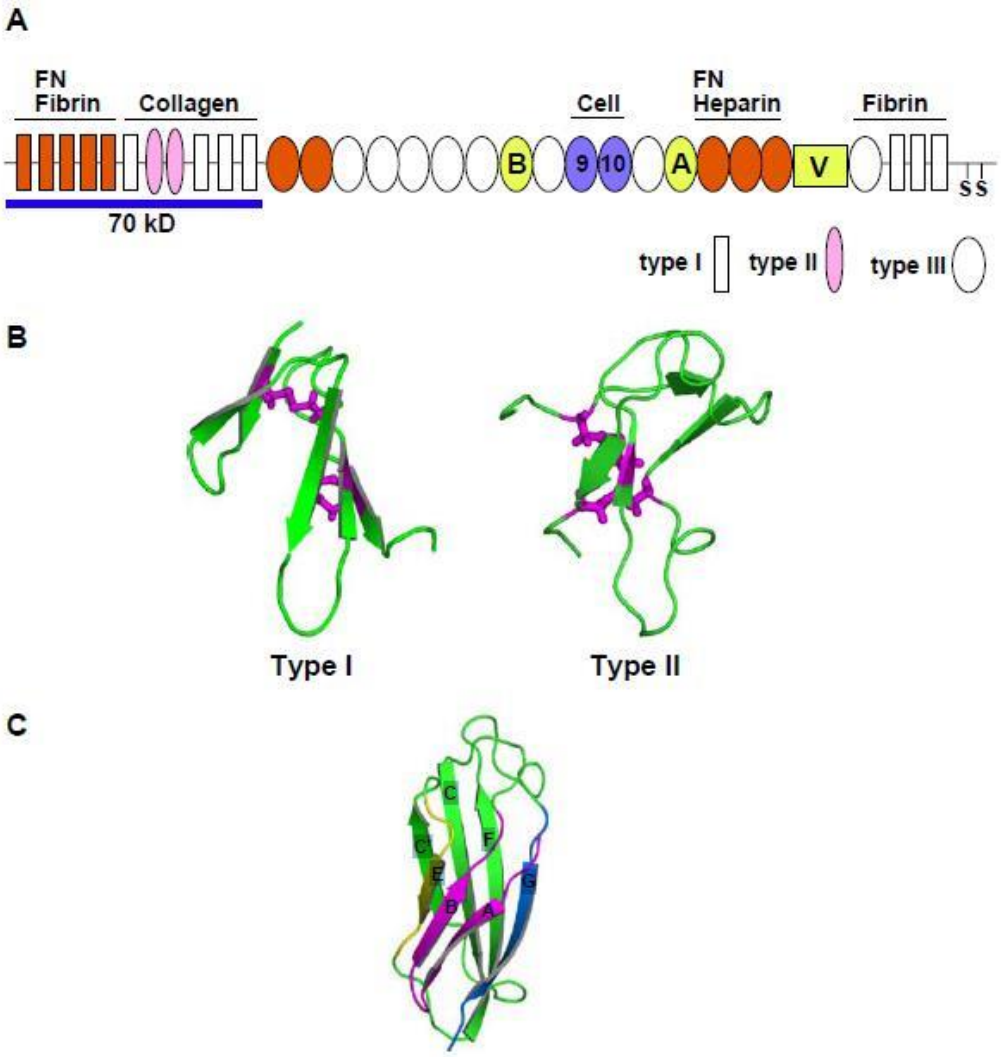


**Figure 2. The modular structure of FN. (A) FN consists 12 type I modules (rectangles), 2 type II modules (violet ovals) and 15– 17 type III modules (ovals). The alternatively spliced domains, IIIB, IIIA and the V region, are in yellow. Binding domains for fibrin, collagen, cells and heparin are indicated; dimer forms via cysteine pair at the C-terminus (SS). The cell-binding domain (blue ovals) includes III10 with the RGD cell binding sequence and III9 which contains the synergy site. Sites of FN–FN interaction include the assembly domain in I1–5 as well as III1– 2 and III12 – 14 (red). The 70 kDa N-terminal fragment is underlined (blue). The ribbon structures of FN type I1, type II1 and type III10 modules**

**were drawn using PyMOL molecular graphic system. (B) Type I and type II modules were drawn with coordinates from residues 63– 107, PDB ID1QGB and residues 10– 60, PDB 2FN2, respectively. The cysteine residues and disulfide bonds are shown in purple. (C) The ribbon structure of human FN type III10 module was drawn with coordinates from residues 6 – 94, PDB ID 1FNA.The strands A, B, C, CV, E, F, G are indicated.[26]**

While during the process of inflammatory phase of wound healing, blood leukocytes sense the chemical gradients generating from the wound area by directed migration in single cells fashion. In more cases, tissue cells move in en masse way instead of single cells. For instance, epidermal cells migrate across the sound space as a sheet in a tractor-tread manner related with dynamic formation and dissolution of cell junctions [32]. It is also generally accepted that epithelial cells migrate en masse as a continuous coherent sheet, with most cells retaining their relative positions to each other [33]. So we applied en masse method to plate the cells to mimic a collective cell migration fashion which usually occurred during wound healing process.

### 1.4 P12 peptide

After cells are plated on the substrates, we added P12 solution of concentration of 10mM in full cell culture medium 1 hour before microscopic pictures were taken on day 1,2,3,4. We believe 1h is enough for P12 to react. P12 is a recently found peptide comprising 14 amino acids which is derived from the first fibronectin type III domain of fibronectin. P12 belongs to a new class of bioactive peptides that the discoverers call epiviosamines, a name that combines the Greek word, epivios, which means “to survive in the face of adversity”, with amine for peptideP12 has been proven to possess the capability of enhancing cell survival in culture and promoting the process of wound healing in mice skin. We tested the velocity of cells on both kinds of substrates with and without the presence of P12 in order to verify any possible positive influence that P12 could

exert on cell migration. If P12 could contribute to an improving and enhanced cell migration without any unwanted and undesirable effects, it holds highly promising therapeutic benefits that P12 could work as a promoting agent for fast wound healing.

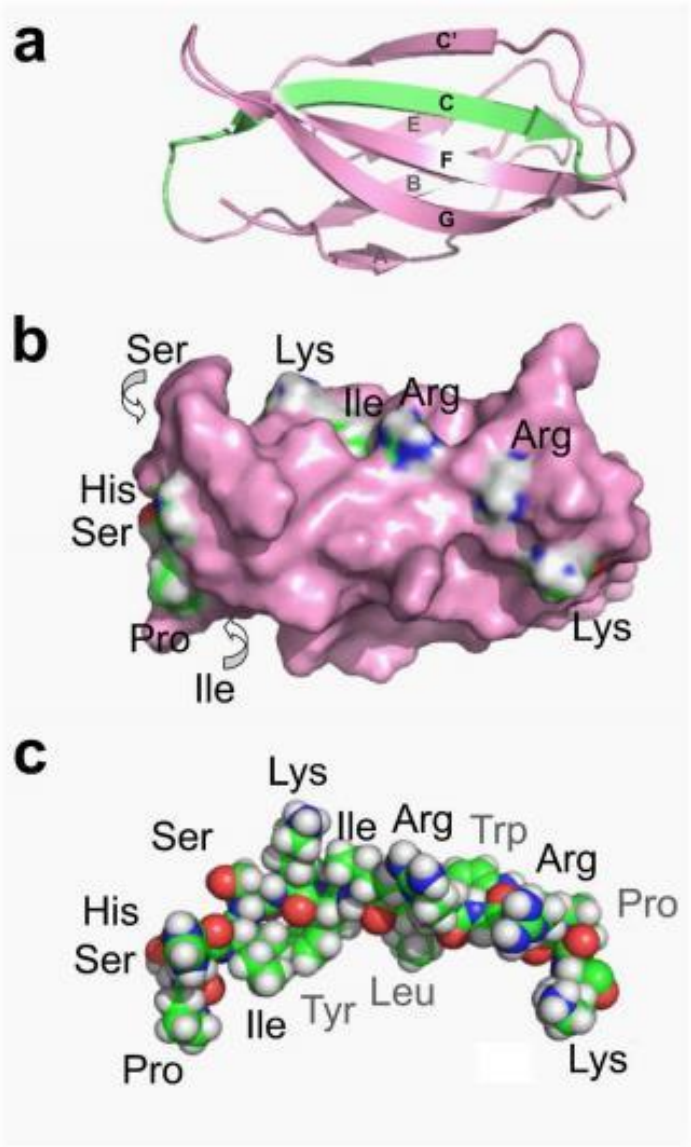


**Figure 3. Structural model of P12. (a) The parent domain, FNIII1, consists of a b-sandwich structure with A, B, and E strands on one side and C, C0, F, and G strands on the other. The region corresponding to P12 is shown in green. (b) A surface-model shows that the**

**majority of the P12 residues are exposed on the surface (labeled in black). Ile-4 and Ser-5 are hidden on the back. (c) The structure of P12 extracted from III1 is shown with a space-filling model. Surface-exposed residues are labeled in black; other residues are in gray.** [34]

# Chapter 2. Materials and Methods

## 2.1 Preparation of the substrates:

### 2.1.1 Materials

We purchase poly(methyl methacrylate) from Sigma-Aldrich company in Milwaukee, WI, USA with the molecular weight of 120,000 and use it without any further modification or purification. poly(methyl methacrylate) is a transparent thermostatic polymer with Tg (glass transition temperature) of 99 ℃ and melting temperature of 160℃.

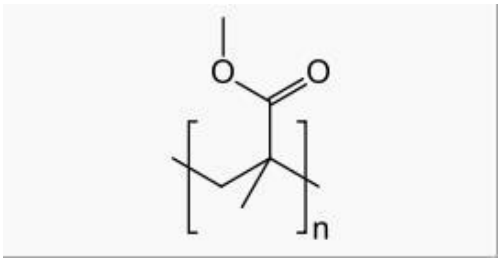


**Figure 4. Poly(methyl 2-methylpropenoate)**

The substrates we use is cover glass coated with 1)PMMA flat thin film and 2) PMMA flat thin film with PMMA microfiber aligned on it. To form satisfying substrates, it requires the polymer to be perfectly adherent to the surface of the glass sheet, yet there must not be any further reactions between them under the incubation condition. Suitable cover glasses with 12mm diameter were purchased from Electron Microscopy Sciences, PA.

### 2.1.2 Preparation Methods of the substrates:

In order to make the cover glasses ideal bases for the polymer to attach, we must completely remove any interferent on the surface, such as organic and inorganic substance and particles. The glass sheets are washed in methanol with sonicator for 15 minutes and afterwards in de-ionized water for 3 times, during which we believe the residual impurities attached could be eliminated.

Spin –Casting, Electro-Spin and Annealing of Thin Film and Fiber Substrates

PMMA solution for spin-casting was made with concentration of 20mg/ml in Tetrahydrofuran. It was then spun cast on washed cover glass after completely dissolution at 2,500 rpm for 30 seconds on a Headway Spin Coater. Every cover glass needed to be spun for 30 seconds first to get rid of the water attached on the surface before PMMA solution drops were added.

Microfiber structure was coated on thin film substrates afterwards by electrospinning process, which were used as comparison with simple flat film. Conventional electrospinning method was

modified in our lab to obtain microfibers in uniaxially aligned arrays deposited on PMMA flat film. The key to the success was the application of a rotating drum with a speed controller to collect the fiber jet.

Here, we used it to generate microfibers with uniaxially aligned arrays. We used a syringe as the reservoir and a rotating drum to collect the fibers. The PMMA solution for such process was made in chloroform with weight ratio of 1:4. Cover glass slices coated with flat thin film were attached on aluminum foil by double sided tape in columns and wrapped up onto a cylinder rotator which would spin at a wanted rate as the fibers were spun upon the surface of the substrates. In this way, we can get neatly arranged parallel fiber structured substrates.

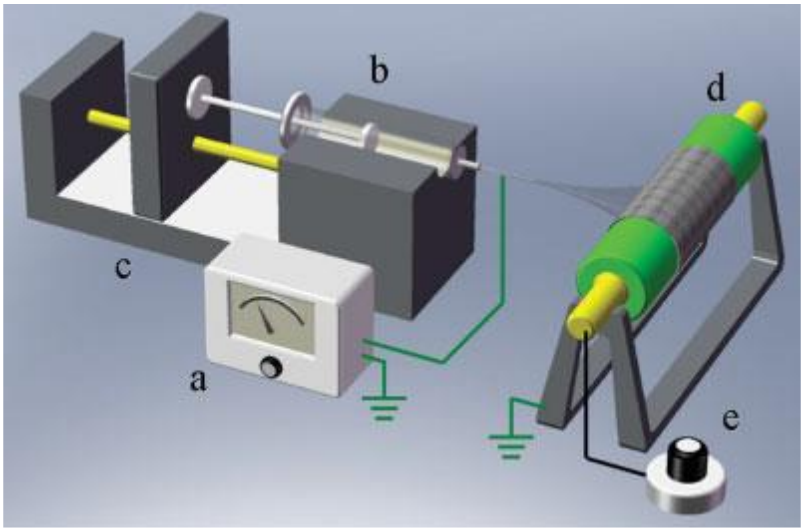


**Figure 5. Schematic of the modified electrospinning apparatus in our lab. The polymer solution was loaded in the syringe and 5–8 kV electricity was provided by (a) a high-voltage power supply between (b) the needle and the ground. The flow rate of solution, 50 μL /min, was controlled by the (c) syringe pump. Two fiber collectors were used, a stationary**

**aluminum disc or (d) a rotating drum wrapped with aluminum foil. The alignment was controlled by (e) the motor rotation speed controller and the collection time.** [35]

Both the thin film and fiber substrates were annealed in an oil trapped vacuum oven in which the pressure could drop below $10^3$ Torr for overnight at 120-130℃, which is high enough to kill most bacteria. Annealing process under the temperature above the Tg (glass transition temperature) of the polymer is also essential to get a uniform and stable polymer layer, during which the polymer chains are free to move so that the remaining inner stress of could be released. In addition, annealing could also help to eliminate any trace of solvent left on the surface, which otherwise would do harm to the cell growth on the substrates.

## 2.2 Cell culture and measurement

### 2.2.1 Cell plating method

To study the mechanism of cell migration on different substrates, we used fluorescent human dermal fibroblasts (mT EGFP 6 passage). The cells were cultured in full cell culture medium, which is Dulbecco's Modified Eagle Medium (DMEM) (standard media for fibroblast culture, including 4.5f/L D-Glucose, L-Glutamine and no sodium pyruvate) plus with 10% Fetal Bovine Serum (FBS)(HyClone company, filtered with triple 0.1 μm pore size filtration system ) and 1% Penicillin-Streptomycin (PS). Cells are cultured in an incubator produced by Cryostar Industries Inc. in which the temperature was 37℃ and $CO_2$ concentration was 5%.

Before cells are plated, all substrates are coated with fibronectin in advance because based on results of former experiments conducted by our group, most cells would die one day after plating. Fibronectin solution was made with concentration of 30ug/ml in 1ml SF-DMEM. And substrates were kept in the incubator in such solution for 2h.

All these types of cells are plated on flat thin film and fiber substrates with en mass method. Then they were observed under a Leica microscope and migration is pictured every 10 minutes within an hour on day 1, 2, 3, 4.

In our experiment, cells are plated on to the substrates following such steps: After the cells cultured in tissue culture flasks reached 80-95% confluence, the culture medium was completely removed and flaks were washed with Dulbecco's Phosphate Buffered Saline (DPBS), with no calcium chloride or magnesium chloride, for 3 times. 2 ml of 0.05% trypsin w/ EDTA was added and cells were detached from the surface after 4 minutes' incubation, since 37℃ is the optimum enzymatic reaction temperature. Rap the flasks gently after taken out of incubator to make sure the cells are completely detached. Then, we add 4 ml full DMEM to neutralize the trypsin and prevent the surface proteins to be over damaged. Collect all the cell suspension in 15ml tube and centrifuge them at 1x1000 rpm for 4mins. After one rinse in 2% (w/v) BSA DMEM, cells were resuspended with SF-DMEM and counted. After number counting, we suspended the cells in 0.2% agarose SF-DMEM solution to obtain a concentration of $1.5x10^7$. Then agarose droplets with cell concentration (1.25 μL) was placed on the surface of substrates in 24-well plates. Samples were put into refrigerator at 4℃ for 10 mins to make sure the agarose gels became solidification state. At last, 1 ml full-media was added to each well to ensure normal growth of fibroblasts.[25]

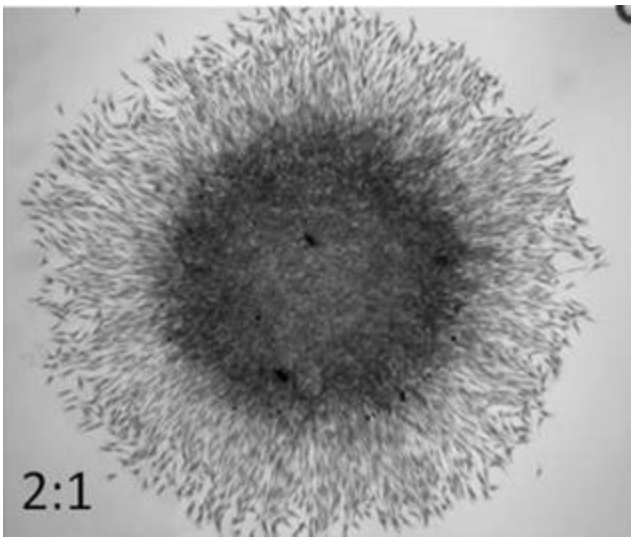


**Figure 6. Fluorescent microscopy image of en masse cell droplet in agarose gel.[4]**

### 2.2.2 Microscopic imaging and measurement of cell migration

In our experiment, time-lapse pictures were taken with the interval of 10 minutes within 1 hour every day MetaMorph®-operated CoolSNAP™ HQ camera (Universal Imaging Corporation, Downingtown, PA) attached to a Nikon Diaphot-TMD inverted microscope fitted with a 37℃ stage incubator and a 10x objective lens. Each group of these 6 pictures are edited into short movies by sequential play to show the location change and details of cell shape deformation. With the help of "XYZ Distance Measure" apps provided by MetaMorph software, we measured the distance of migration of 15 randomly picked cells. And we triplicated the measurement for each cell and took the mean value. At last, velocity values of cells on different structured substrates and with/without P12 were compared. The detailed information about the way cells migrated provided by the images were also gathered and investigated.

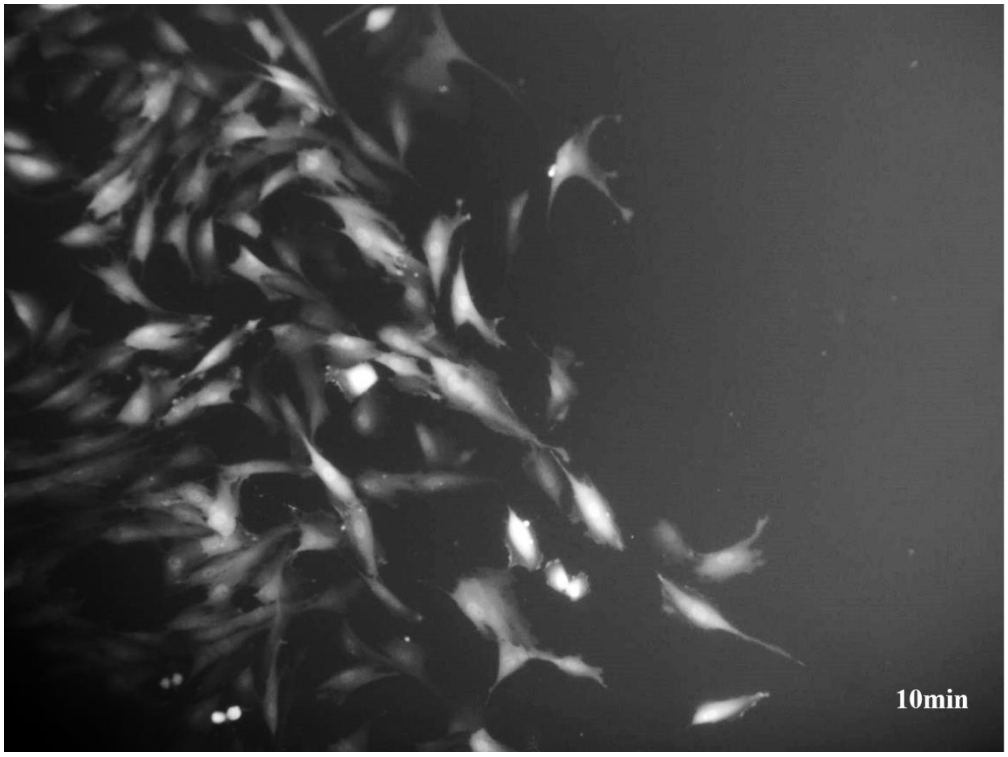


**Figure 7. Optical fluorescent microscopic image of fluorescent HFBs on thin film on day 3.**

## Chapter 3 Data Analysis and Discussion

### 3.1 Characterization of substrates

Highly aligned PMMA fibrous scaffold were engineered by modified electrospinning method with solution of 20% PMMA in our lab. The drum rotation speed was set at 6750r/min to get the ideal structure because we found that such speed had a critical influence on the alignment fashion of the fibers deposited on the cover glasses, including the diameter an parallelization of fibers as well as the spacing size. When the rotation was set at 6750 r/min, the approximate distance between two neighbouring fibers could be controlled based on the deposition time[35].

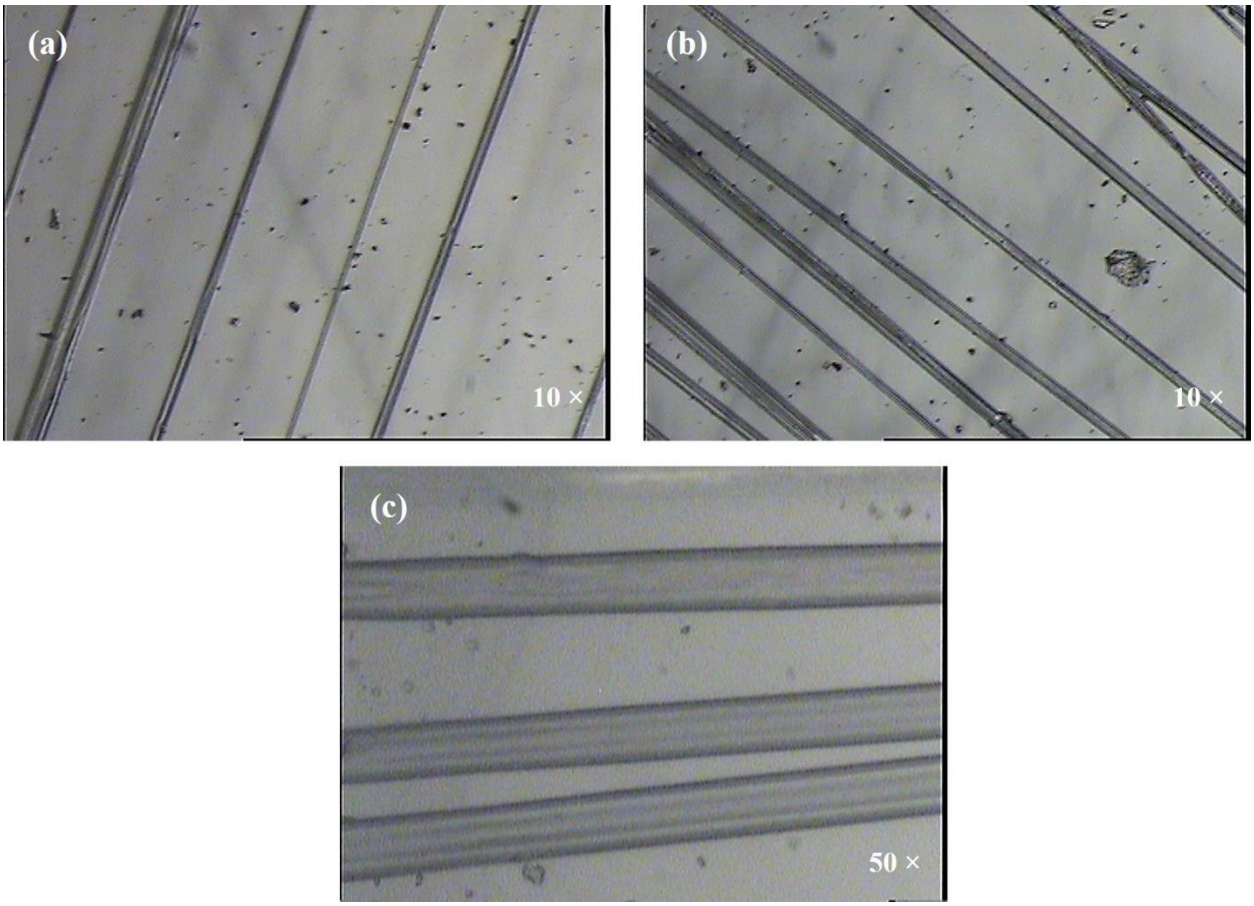


**Figure 8. Optical microscopic images of PMMA electrospinning fibers: (a) and (b) with 10-time lens; (c) with 50-time lens.**

### 3.2 Time-lapse measurement of cell migration

Agarose gel droplets containing fluorescent HFB cell solution with concentration of $1.5x10^7$ cell/ml were seeded onto thin film and aligned fibrous PMMA substrates in 24-well cell culture dishes. They were put in a 37 ℃, 5% CO2, 95% humidity incubator (Napco Scientific Company, Tualatin, OR) for 24h, 48h, 72h, 94h in full medium standard for HFB culture. Time-lapse images of the en mass cell migration were recorded every 10 minutes within 60min with the help of MetaMorph®-operated CoolSNAP™ HQ camera (Universal Imaging Corporation, Downingtown, PA) attached to a Nikon Diaphot-TMD inverted microscope fitted with a 37℃ stage incubator and a 10x objective lens. In addition, P12 contained medium was added to a group of samples with both thin film and fiber substrates 1 hour before the measurement. After finishing of the record work of 6 pictures, they were played in stack as a short movie. We randomly pick out 15 cells from each record and measured the distance between each cell's original and final position. Measurement for each cell was operated with 3 replicates and averaging number was taken and used as the final value.

#### 3.2.1 Thin film versus fiber

Dermal wound healing is a complex process which comprises a series of synergistic and collaborative efforts of many different tissues and cell lineages, such as cell proliferation, formation and organization of extracellular matrix (ECM) and rapid cell coverage on would area[36]. It has been widely accepted that cell migration is one of the critical factors in almost all these processes and therefore it has been intensely investigated for a long time. Due to lack of deep understanding and advanced equipment, most of the previous studies were conducted on flat substrates and hence the research conclusions about cell migration mechanism and principle have limitations. While migration on three dimensional structured substrates may be more

relevant to practical situation because extracellular matrix on which cell locomotion happens is fibrillar[37]. What's more, compared to single cell, en masse migration has been proven to be more related with wound healing and many other cellular procedures. Based on these two considerations, we plated cells in agarose gel droplets on both planar and fiber substrates and investigated the underlying mechanism through velocity values and observation of the transformation of cell shapes.

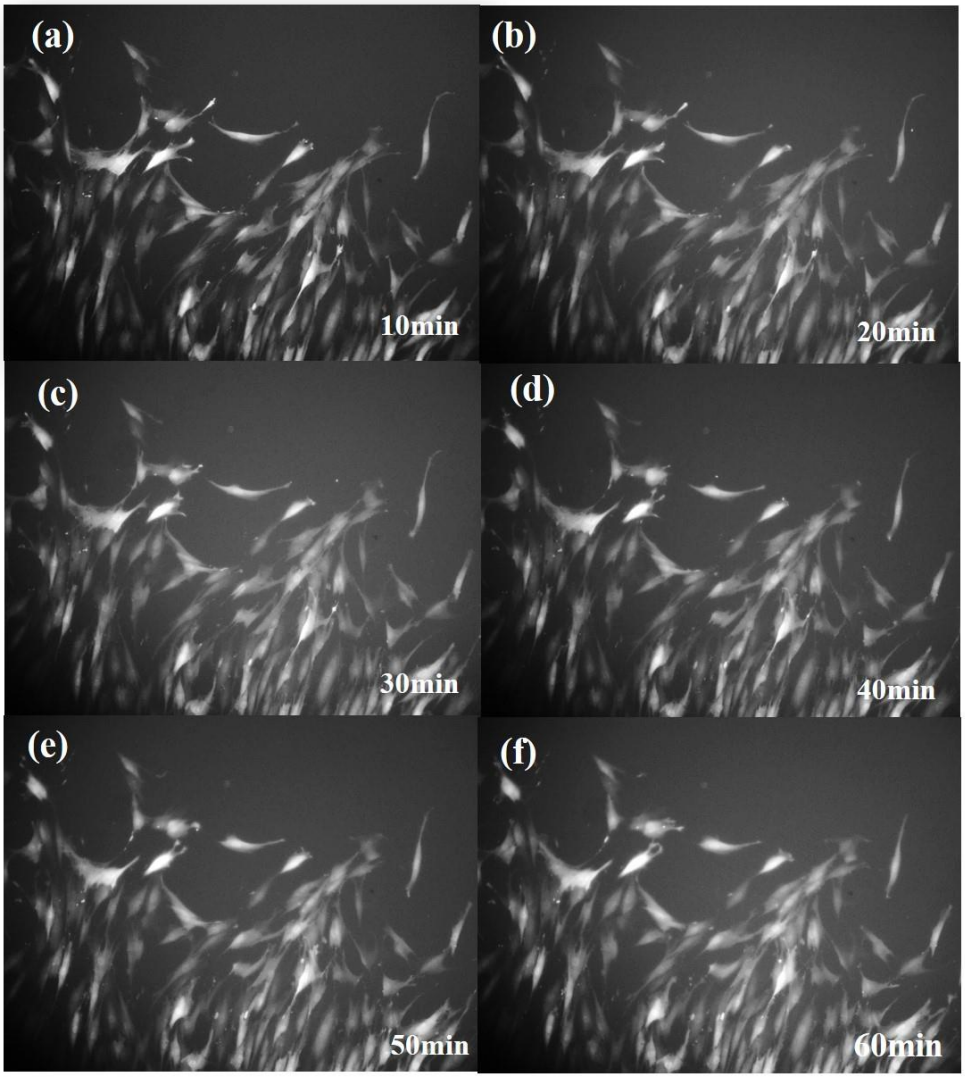


**Figure 9. Time-lapse fluorescence microscopy images of control fluorescent HFBs on thin film of D1. From (a)-(f) are images continuously taken every 10min within 1 hour.**

When played in short movie, we can see most of the cells crawled more or less. And there was part of the cell protrude first (lead edge) and an afterward contraction of the rest regions (trailing edge). However, cells spread out by random trajectories without any rules.

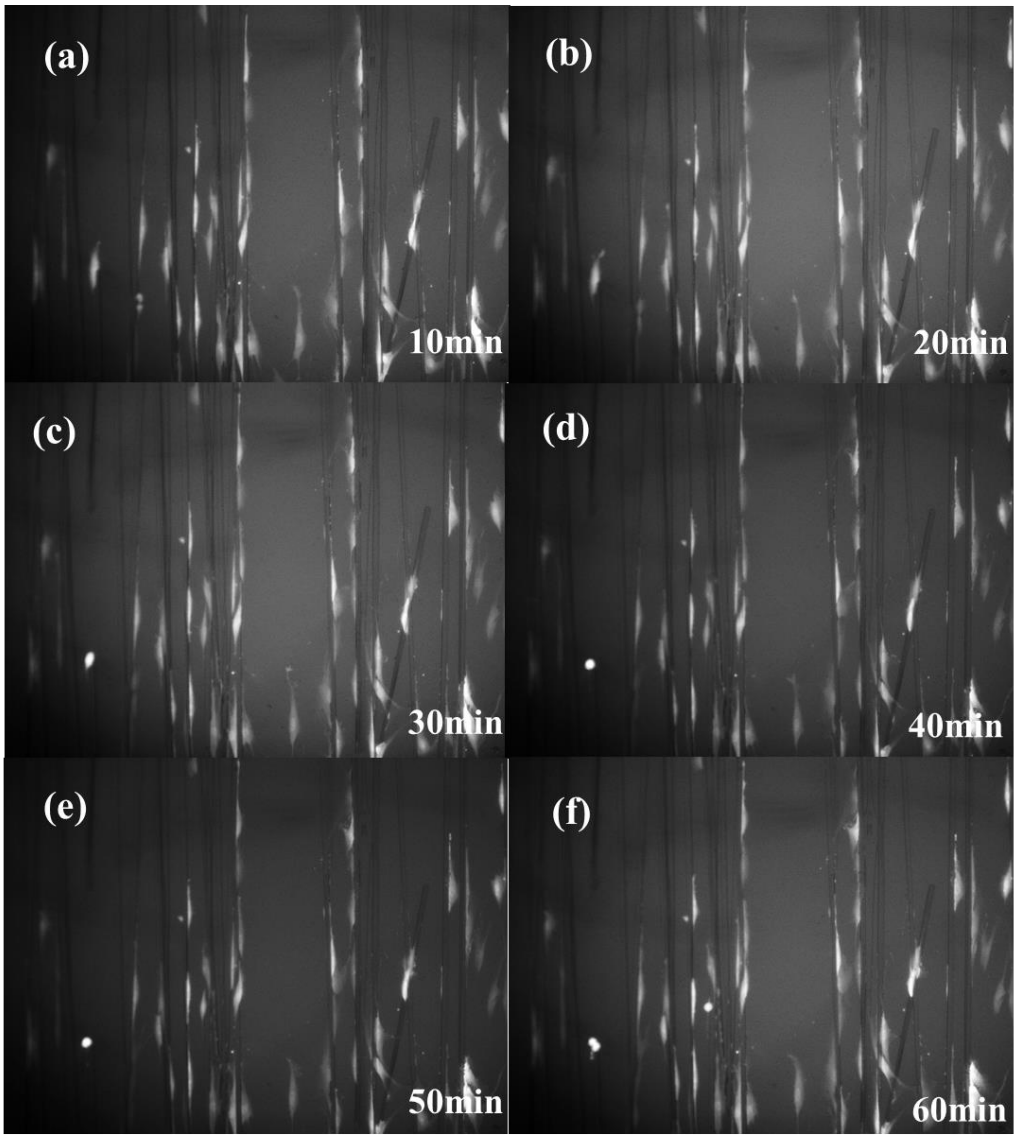


**Figure 10. Time-lapse fluorescence microscopy images of control fluorescent HFB on fiber of D1. From a-f are images continuously taken every 10min within 1 hour.**

When cells were plated onto aligned fibers, they would migrate along the fiber tracks and migration directions are limited to only two directions. Cell migration became very persistent because there were specific leading and trailing edges and they were traveling aiming at one distance consistently.

We measured the distance of randomly picked 15 cell's movement within 1h.

| | Control TF | Control Fiber | P12 TF | P12 Fiber |
|---|---|---|---|---|
| Day 1 | 36.56 | 37.3 | 45.27 | 51.04 |
| Day 2 | 32.54 | 48.28 | 40.86 | 48.28 |
| Day 3 | 42.07 | 45.92 | 31.13 | 53.64 |
| Day 4 | 43.56 | 56.28 | 53.13 | 64.6 |

**Table 1. Migration distance of control cells with and without P12 on flat thin film vs on fiber on day1, day2, day3 and day4 ( μ m).**

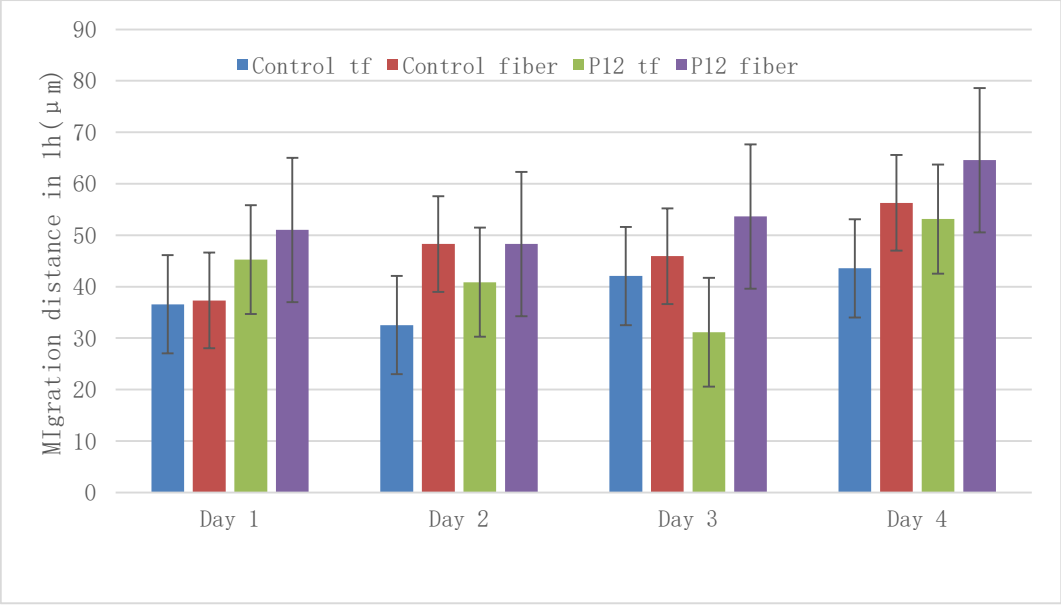


**Figure 11. Comparison of migration distance of control cells with and without P12 on flat thin film vs on fiber on day1, day2, day3 and day4 ( μ m).**

Comparison of the average values of cells on planar and on fiber showed that migration speed on fiber were faster than those on flat thin film on all 4 days. Therefore, directional migration is more efficient on velocity than random migration and fiber structured substrates could contribute to an enhanced cell mobility by 3%-60%. Inaccuracy of data may be due to intercellular obstruction when cells were confluent in one area. And there was a theory that en masse cell migration of fibroblasts on the planar surface has a spatially dependent velocity distribution which decreases exponentially in time towards the single cell value[9].

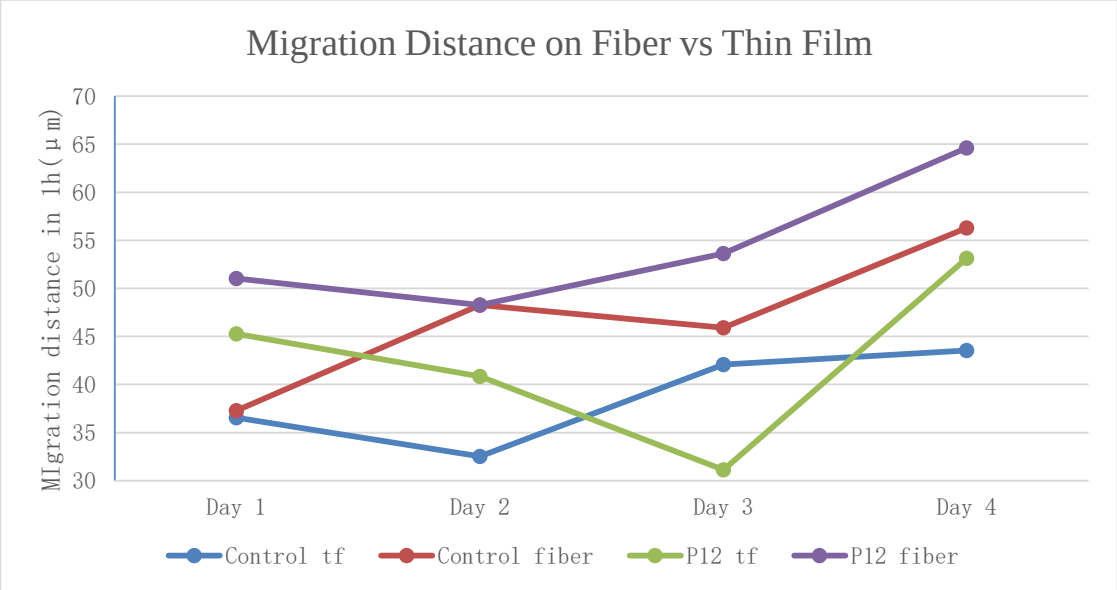


**Figure 12. Tendency of the velocity of cell movement versus time**

The curve above shows the trend of how the velocity ( μ m/h) of cells on both kinds of substrates changed over time. Velocity values on the 4$^{th}$ day are all higher than those on the 1$^{st}$ day. However, speed increasement of the velocity on fiber was more significant than that on the planar thin film. The cells on fiber accelerated by 25% and 50% without and with P12 respectively, while there were only slight enhancements for cells on thin film.

Thus, judging from the above two points, particularly aligned fiber substrate holds the promising potential to work as an efficient scaffold for fast wound healing not only because it could regulate and lead to a directional migration but also has the capacity to promote the cell migration. We plan to build layer-by-layer 3D scaffolds and test the cell migration under such relatively more complicated situation in the future.

As talked above, cell migration process in single-cell level is based on a cyclic mechanism: (1) the formation of a lamellipodium, on which attachment of cells to the substrate, and the adhesion of cells to each other are based; (2) translocation of the nucleus in the direction of motion, and (3) detachment and subsequent contraction of the trailing edge[38]. After analyzing the short videos recorded by the microscope about the cyclic process of cell locomotion, we found that the contraction behavior of the cells on fiber seemed to be less than that of cells on thin film, which needs to be further verified. We assume that there's a relationship between polarity and contraction rate of the cell shape, which we plan to testify in the future.

### 3.2.2 The effect of P12 peptide on cell migration

As mentioned before, P12, a 14 amino acid peptide derived from the first fibronectin (FN) type III domain of fibronectin, holds the potential to promote cell migration the same way as it works to enhance cell survival[39]. We compared the movement distance of cells with and without P12, on both flat and fiber substrates.

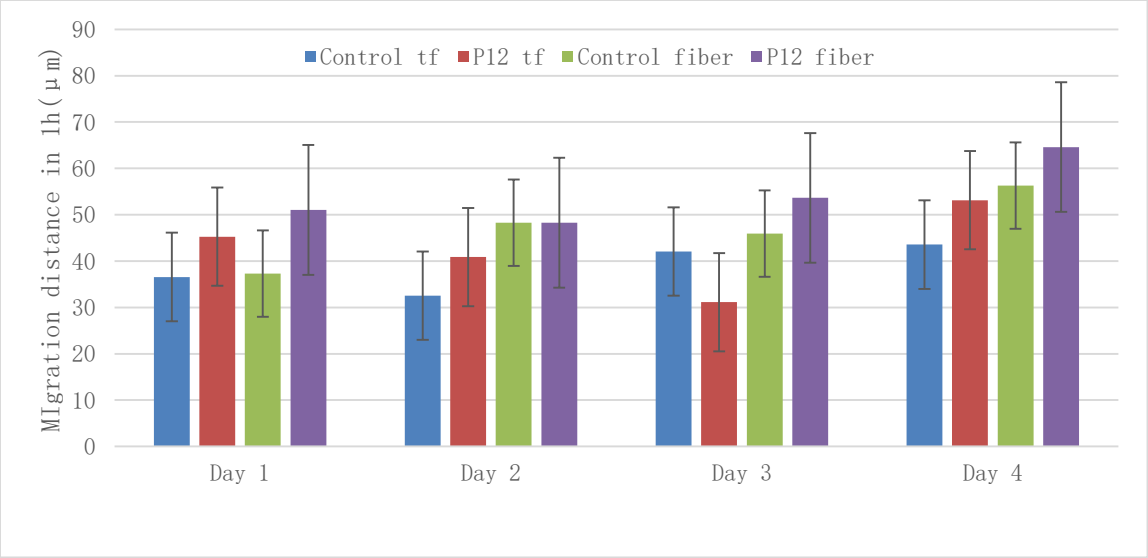


**Figure 13. Comparison of migration distance of cells with P12 and without P12 on day1-4.**

As can be seen from the histogram above, P12 accelerated the velocity of cell migration on all 4 days and on both kinds of substrates (only exception occurred on data of thin film on day3).

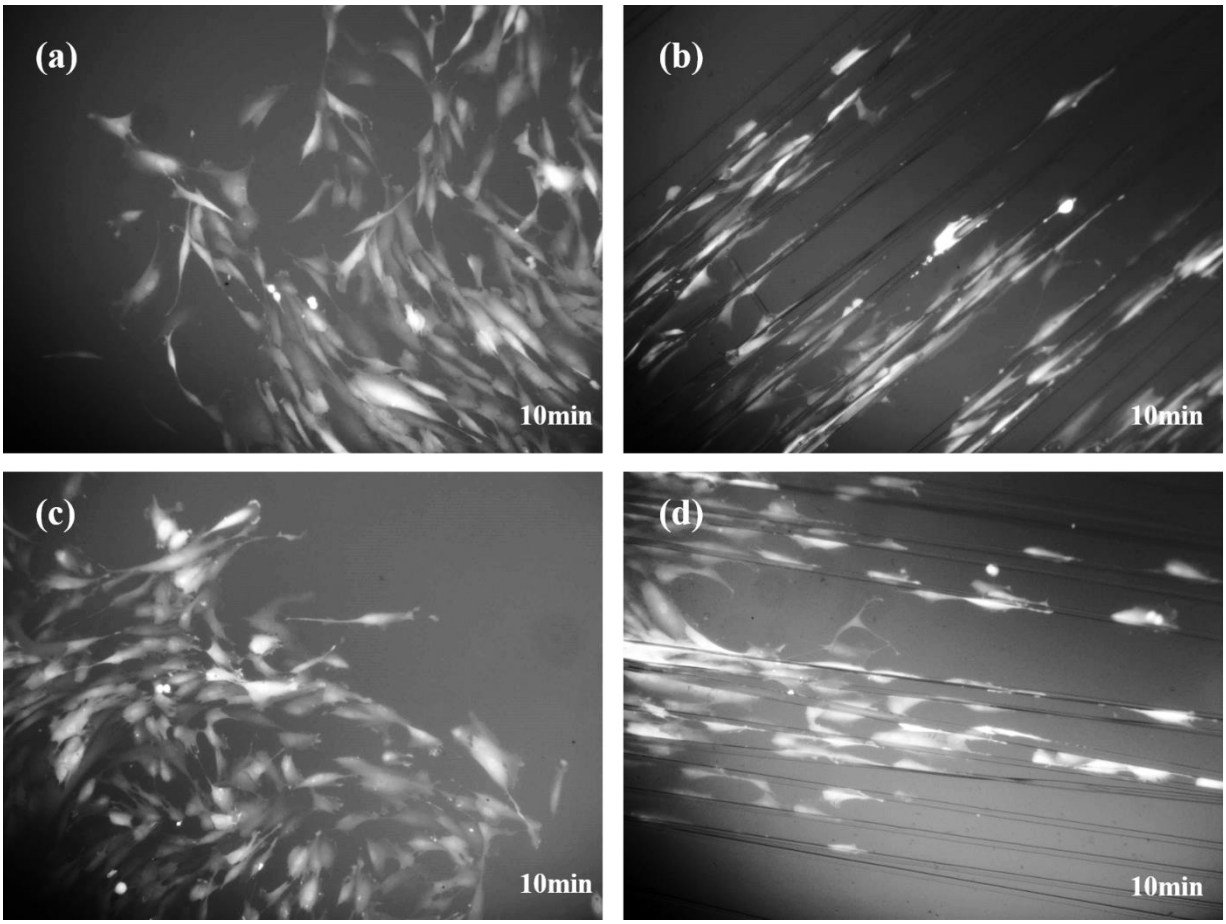


**Figure 14. Optical fluorescent microscopy images of cells without P12 on (a) planar thin film; (b) cells without P12 on fiber; (c) cells with P12 on planar thin film; cells with P12 on fiber on day4.**

As can be seen from the images above, when added with P12, cells still remain their normal morphology and no pathologic changes occurred. Additional proliferation test will be conducted to further prove the reliability and applicability of such peptide as an enhancement agent for cells.

## Conclusion

Cell migration plays a critical role in a wide range of physiological and pathological phenomena as well as in scaffold-based tissue engineering. Cell migration behavior is known to be regulated by biochemistry from extracellular and intracellular signaling and physical interactions with surrounding ECM and substrates, which means it's influenced by a wide variety of factors. In this paper, by testing locomotion distance in 1h on fibronectin coated PMMA substrates, we provided evidence that substrates of aligned fiber structure could lead to faster cell migration than planar thin film structured ones. Thus, fiber structured scaffold is applicable to direct cell locomotion and contribute to a controlled and desirable cell migration. We also tested a newly discovered FN derived peptide, P12, on HFBs on both kinds of substrates. The results show that P12 turned to be an effective enhancement agent since it lead to a faster cell migration in both conditions and meanwhile, didn’t cause any undesirable and detrimental changes to cell morphology and survival.

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