รายงานวิจัยฉบับสมบูรณ์

โครงการผลกระทบของเส้นใยไหมนาโนต่อการเคลื่อนที่ของเซลล์เพื่อการสมานแผล

The Effect of Electrospun SF Nanofibers on Cell Migration for Wound Healing Application

โดย นางสาวพิมพ์พร อุทยารัตน์ และคณะ

พฤษภาคม 2555

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สนับสนุนโดยสำนักกองทุนสนับสนุนการวิจัยและสถาบันเทคโนโลยีนิวเคลียร์แห่งชาติ (องค์การมหาชน)

Abstract

The ability of tissue-engineered scaffolds to be interactive with cells is desirable in directing certain cellular behaviors. To investigate the role of substrate topography in wound healing, we developed electrospun silk nanofibers into nonwoven and aligned patterns and evaluated their influence on the cell morphology and cell migration. Using real-time imaging, we have demonstrated that most cells from suspension adopted an elongated morphology immediately upon cell attachment on the aligned pattern, whereas a few cells on the nonwoven pattern showed the same response on isolated, individual nanofibers. To simulate the migration of skin cells from the wound appendage, microcarrier beads were used to deliver cell monolayers on both patterns. The cells showed to migrate along tracks of individual nanofibers with relatively similar migration velocities. However, only the cells on aligned pattern exhibited the persistence in directional migration until confluence. Concomitant to the orientation of cell shape, intracellular actin filaments of individual cells within the monolayers also aligned parallel to nanofibers. Taken together, the incorporation of such directional guidance on the surface of wound dressings and skin grafts may be useful in facilitating cell migration and re-epithelialization process.

Keyword: silk nanofibers, cell migration, microcarrier beads, actin filaments

บทคัดย่อ

ความสามารถของวัสดุทดแทนเนื้อเยื่อชีวภาพในการทำปฏิกิริยาหรือสื่อสารกับเซลล์ที่ยึดเกาะบนพื้นผิวเป็นสิ่งสำคัญที่ช่วยเหนี่ยวนำให้เซลล์มีการตอบสนองต่อวัสดุตามที่ต้องการ งานวิจัยนี้มีวัตถุประสงค์ที่จะศึกษาผลของโครงสร้างบนพื้นผิววัสดุที่อยู่ในรูปเส้นใยไหมขนาดนาโนเมตรที่มีลวดลายแบบซ้อนทับกันไปมาและแบบขนานต่อการแสดงออกด้านรูปร่าง และการเคลื่อนที่ของเซลล์โดยใช้เทคนิคการถ่ายภาพฟลูออเรสเซนต์ตามเวลาจริง สำหรับการแสดงออกด้านรูปร่างของเซลล์พบว่าบนพื้นผิวที่เส้นใยมีลวดลายแบบขนาน เซลล์ส่วนใหญ่จะยืดตัวตามเส้นใยทันทีหลังการยึดเกาะก่อนจะปรับเปลี่ยนรูปร่างให้มีลักษณะเรียวคล้ายวงรีในขณะที่เซลล์บางส่วนบนพื้นผิวที่เส้นใยมีลวดลายแบบซ้อนทับกันไปมามีการตอบสนองดังกล่าวเฉพาะบนเส้นใยเดี่ยวที่ไม่เกาะกลุ่มกับเส้นใยอื่น ๆ ในส่วนของการศึกษาด้านการเคลื่อนที่ของเซลล์ได้ใช้ลูกปัดขนาดไมโครเมตรเพื่อปล่อยเซลล์ลงบนพื้นผิวเส้นใยไหม เพื่อเป็นการจำลองการเคลื่อนที่ของเซลล์จากผิวหนังโดยรอบบริเวณบาดแผลระหว่างการสร้างเนื่อเยื่อผิวหนังใหม่ ผลการทดลองพบว่าเซลล์โดยส่วนใหญ่เลือกที่จะเคลื่อนที่ตามเค้าโครงเส้นใยบนพื้นผิวทั้งสองแบบด้วยความเร็วที่ใกล้เคียงกัน เนื่องจากบนพื้นผิวที่เส้นใยมีลวดลายแบบขนาน การเรียงขนานของเส้นใยสามารถเหนี่ยวนำให้เซลล์ทั้งหมดเคลื่อนที่ตามเค้าโครงเส้นใยไปในทิศทางเดียวกัน ซึ่งแตกต่างจากการเคลื่อนที่ของเซลล์อย่างไม่มีทิศทางโดยรวมที่แน่นอนบนพื้นผิวที่เส้นใยมีลวดลายแบบซ้อนทับกันไปมา ทั้งนี้ไม่พบการเคลื่อนที่ออกนอกทิศทางเค้าโครงของเส้นใยบนพื้นผิวที่เส้นใยมีลวดลายแบบเรียงตัวจนกระทั่งเซลล์เจริญเป็นแผ่นเซลล์ครอบคลุมพื้นผิวทั้งหมด นอกจากนี้เซลล์เดี่ยวแต่ละเซลล์บนแผ่นเซลล์ยังสามารถคงรูปร่างเรียวตามเส้นใยไหมได้ โดยมีการจัดเรียงเส้นใยโปรตีนแอกทินภายในเซลล์ไปในทิศทางขนานกับเส้นใยไหมบนพื้นผิว ผลการทดลองที่ได้จากงานวิจัยนี้สามารถนำไปประยุกต์ใช้กับแผ่นปิดแผลและผิวหนังเทียมเพื่อเหนี่ยวนำการเคลื่อนที่ของเซลล์ให้ไปในทิศทางเดียวกัน ซึ่งอาจเป็นประโยชน์ต่อการสร้างชั้นผิวหนังใหม่ในกระบวนการสมานแผล

คำสำคัญ: เส้นใยไหมขนาดนาโนเมตร การเคลื่อนที่ของเซลล์ ลูกปัดขนาดไมโครเมตร เส้นใยโปรตีนแอกทิน

Executive Summary

Cell migration is a complex biological process and one of the critical factors that facilitate re-epithelialization of damaged skin during wound healing. The aim of this research was to develop and evaluate patterned nanofibers as a potential source of topographic stimulants on wound dressings for cell migration. In the fabrication of nanofibers by electrospinning, silk fibroin (SF) protein was chosen as a material due to its long track record in biomedical applications. We have established in this study the electrospinning parameters to generate smooth and continuous SF nanofibers into nonwoven and aligned patterns. To gain a better understanding of how nanofibers as well as their organization affect cell morphology and cell migration, the time-lapse imaging technique was used to observe the cells' responses to nanofibers in real-time. In terms of cell morphology, our results have demonstrated that changes in the cell shape immediately followed cell adhesion on nanofibers. Interestingly, isolated nanofibers within the nonwoven pattern were also capable of guiding cell elongation parallel to the fiber direction although the uniform alignment of the elongated cell shape was obtained only on the aligned pattern composed of parallel nanofibers.

As the polarization of cell shape is the first step in the migration process, the ability of cells to become elongated along nanofibers may further facilitate cell migration. To test this guidance provided by nanofibers on cell migration, microcarrier beads coated with cell monolayers were used to deliver cells onto both patterns. This microcarrier bead setup allowed for the simulation of skin cell migration from the wound appendage into the wound as the cells migrated outward from the beads into the surrounding area. The real-time imaging showed that upon leaving microcarrier beads, the movement of cells followed along tracks of nanofibers on both patterns. Similar to changes in the cell morphology, the migration of cells in a uniform direction was obtained on aligned pattern and persisted until

the cells formed a confluent monolayer. Based on these results, aligned nanofibers can serve as stimulants for directional cell migration, and also potentially serve as coating on wound dressings and skin grafts. Future study in animal model is needed for the complete evaluation of patterned nanofibers before their clinical use.

During the two years of this study, we have published our data on the characterization of silk protein and the fabrication of nanofibers in Fibers and Polymers journal and also in a poster presentation at the 8th Asian Pacific Burn Congress Conference. Results from the study of cell migration were presented in an oral presentation at the 1st annual international meeting of the Society of Molecular Imaging of Thailand. Details of all the publications and also a short article for public distribution are given in the appendix.

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1. Introduction

The loss of skin tissue from burn injuries requires coverage by wound dressings or skin grafts to prevent the body from dehydration and infection. In Thailand most of the commercial wound dressings used in the treatment of burn wounds is imported from abroad. As the expense of treatment scales with the extent of wounds, patients from the low-income background may not have access to suitable wound dressings throughout the course of their treatment. Thus, it is necessary to develop wound healing materials based on resources available in the country to provide a sufficient and affordable treatment for all patients.

Natural polymers such as silk fibroin (SF) and chitosan have been extensively investigated in recent years as a potential candidate for wound healing application [1-7]. In the biomedical area, SF in particular has a long establishment as suture for surgical operation due to its excellent biocompatibility with minimal adverse effect [5,6,8]. The SF protein can be easily extracted from cocoons of silkworms and regenerated into silk fibers. Due to the well-established silk textile industry in the country, there is an abundant source of cocoons available for the production of wound dressings. As these cocoons are usually disregarded from the textile industry, the alternative application of cocoons in the biomedical area can significantly increase their efficacy and reduce the amount of cocoons being wasted.

In this research we regenerated SF nanofibers from fibroin protein derived from cocoons of silkworms and evaluated their effect on skin cells. Responses in the cell morphology and cell migration, which is one of the critical factors that facilitate wound healing, were observed in real-time. Our data have demonstrated that these nanofibers,

depending on their organization, were capable of controlling the direction of cell migration, which may be useful in wound healing application.

2. Literature Review

In wound healing cell migration is one of the critical factors that facilitate re-epithelialization of the wounded skin [9]. During wound healing *in vivo*, the cells receive chemical and physical signals from the surrounding extracellular matrix (ECM) to orchestrate their coordinated movement. These signals, however, may lack in two-dimensional (2D) cell culture on flat surfaces [10,11], especially the physical signals such as compliance, porosity and topography [10,12] that constitute the ECM structure. Since most proteins of the ECM are assembled into fibers with a size scale from nanometers to micrometers [10,12,13], the ability to simulate fibrous structure can recapitulate part of the ECM onto a flat, synthetic surface.

The first evidence of cell-fibril interaction is described in a 'contact guidance' phenomenon [14] in which the oriented synthetic matrix provides topographic cues to guide cell orientation and cell migration upon cell attachment on the matrix. This appearance of aligned cell shape and directional cell migration is supported by the alignment of intracellular cytoskeleton, actin microfilaments and microtubules [15-18]. Previous studies of fibroblasts in three-dimensional (3D) matrices have shown that the cells adopted different morphology and migration behavior when surrounded by collagen fibers compared to flat surfaces [19-21]. In terms of wound healing, Xie *et al*. [22] has reported that dural substitutes composed of radially aligned poly(ε-caprolactone) (PCL) nanofibers significantly enhanced the *ex vivo* wound closure on dura mater compared to flat collagen membranes. This radial alignment of PCL nanofibers induced the cells to migrate directionally into the wounded area, which in turn facilitating the formation of a new cell sheet. This ability of fibrous substrates to control cell migration may potentially be applied to various wound healing applications such as skin, tendon and nerve.

The electrospinning technique offers a method to construct biomimetic scaffolds composed of intricate fibrous structure that emulates the natural assembly of ECM fibers in soft tissue [8,23-25]. In the electrospinning process a power supply creates an electric field between a solution reservoir and a target. A jet of solution is dispensed from the reservoir and drawn across the electric field to deposit as fibers on the collecting target [26]. The size of fibers can be varied from nanometer to micrometer, depending on the polymer concentration, the power supply voltage and the distance between the collecting target and the jet dispenser [24,26-28]. Patterning of these fibers into various arrangements such as radial alignment and uniaxial alignment can be achieved by modifying the targets that collect fibers [22,29], or applying mechanical straining to fibers after the electrospinning process [13,30].

Besides the use of synthetic polymer in commercially available wound dressings, recent studies worldwide have focused on the development of SF as the new generation of wound dressings due to its satisfactory results in animal model and abundant availability in nature [1,4,6]. The SF protein is the main component located in the core of silk fibers [8,27] and can be easily extracted from cocoons of silkworms. Previous studies have shown that SF can be regenerated into fibers by electrospinning technique to fabricate various types of scaffold such as tendon [8,26], cartilage [31], and bone [32,33]. For wound dressings, SF protein has been processed into 2D membranes [2,3,6] and 3D sponges [1]. However, the fabrication of SF mats composed of electrospun SF fibers has not yet been investigated as dressing materials.

During the regeneration of damaged skin tissue, dermal fibroblasts and keratinocytes play an important role in the re-epithelialization process. While fibroblasts migrate to the wound site to lay down a collagen-rich matrix that replaces a blot clot [9] and to contract the

wound, the migration of keratinocytes creates a new epidermal layer. This *in vivo* cell migration involves the collective movement of many rather than individual cells. *In vitro* various assays such as agarose droplet [11,34] and tissue explant [13,22] are used to provide a cell source for *en mass* migration on the substrates tested as dressing materials. In addition, the microcarrier bead assay conventionally used in the study of angiogenesis [35] can also be applied to deliver cell monolayers on the substrate. Similar to the agarose droplet and tissue explant assays, the continuous outward migration of cells from the monolayer-coated beads onto the surrounding substrate mimics the cell migration from the wound margin into the wound. Therefore, the electrospinning technique and the microcarrier bead assay can be combined to develop and evaluate an interactive SF dressing for its potential use in skin wound healing.

3. Research Objective

The main goal of this research is to develop electrospun silk membrane as an interactive wound dressing. To achieve the goal, the following three specific objectives have to be met:

3.1 To fabricate silk fibroin membrane composed of smooth and continuous nanofibers that are organized into nonwoven and aligned patterns

3.2 To investigate the effect nonwoven and aligned patterns on the migration of dermal fibroblasts

3.3 To investigate the effect of aligned nanofibers on the arrangement of intracellular actin filaments

4. Summary of Research Activities

All of the research activities accomplished in this project are summarized in Table 1. In the first year the fabrication process of SF nanofibers by electrospinning technique was established, and in the second year the cell migration experiments were conducted on nanofibers. The time-lapse experiments of cell migration were in collaboration with Dr. Peter Lelkes at Drexel University, Philadelphia, US.

Table 1. Summary of Research Activities

Research Activities	**Year 1**		**Year 2**	
	Months 1-6	**Months 7-12**	**Months 1-6**	**Months 7-12**
1. Preparation of reagents and equipments	→			
2. Extraction of SF and characterization of molecular weight by gel eletrophoresis	→			
3. Electrospinning SF nanofibers and characterization by SEM	→	→		
4. Fabrication of SF membranes composed of nonwoven and aligned nanofibers, conversion to β-pleated sheets in methanol and characterization of membrane structure by SEM		→		
5. Optimization of fibroblasts/beads ratio and characterization of monolayer-coated beads		→	→	
6. 1) Use the conditions obtained in (5) to set up microcarrier bead assay at the Lelkes Lab 2) Time-lapse observation of fibroblast migration			→	
7. Visualization of actin filaments			→	
8. Data analysis and report preparation			→	→

5. Materials and Methods

5.1 Extraction and Characterization of SF Protein

SF protein was extracted from cocoons of Thai silkworms *Bombyx mori* (variant Nangnoi Si Sa Ket). The as-received cocoons from Chiang Mai Sericulture Center (Chiang Mai, Thailand) were first degummed by boiling in 0.5% (w/v) sodium carbonate (Na_2CO_3) solution twice, 30 min each, to remove the sericin coating. After rinsing in warm double-distilled water, the cocoons were let dry at room temperature. To extract SF protein, the dry, degummed cocoons were then dissolved in 9.6 M LiBr to yield a 10% (w/v) solution and heated at 70 ºC for 2 h. The extracted SF solution was then filtered through Whatman filter paper to remove debris and dialyzed in a CelluSep tubular membrane (cut-off MW 12,000–14,000) against double-distilled water for 96 h at 4 ºC. The dialysate was then lyophilized and stored at -20 ºC prior to electrospinning.

5.2 Patterning SF Fibers by Electrospinning

Prior to the electrospinning process, lyophilized protein was dissolved in hexafluoroisopropanol (HFP, Sigma) to yield a 15% (w/v) SF solution and stirred overnight. During the electrospinning process, an infusion pump fed SF solution at a constant rate of 1.0 mL/h through a blunt 22-gauge stainless steel needle while a high voltage of 10 kV was applied between the needle and the fiber collector placed at a distance of 10 cm away from the needle. Circular glass cover slips with diameter of 22 mm on a flat Al sheet were used to collect electrospun fibers into a nonwoven pattern. To organize fibers into an aligned pattern, the SF solution was electrospun onto parallel, stainless steel rods to span the fibers across the rods. These aligned fibers were then transferred onto glass cover slips. About 0.06 – 0.08 mL of SF solution was used in the electrospinning of individual aligned or nonwoven patterns. The fiber morphology was inspected by light microscopy and scanning

electron microscopy (SEM) on JOEL JSM 6380 LV. Prior to cell migration study, all samples were immersed in 90% methanol for 1 h to convert the random coils of SF molecules to β-sheets to prevent rapid fiber dissolution in aqueous environment.

5.3 Cell Culture

Primary human dermal fibroblasts were purchased from Cascade Biologics (Life Technologies, Grand Island, NY). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Mediatech, Manassas, VA) supplemented with 10% fetal bovine serum, 4.5 g/L glucose, sodium pyruvate, 1% L-glutamine and 1% penicillin and streptomycin (complete DMEM) in T75 tissue culture flasks pre-coated with 10 μg/ml fibronectin (Sigma, St. Louise, MO) in a 37ºC, 5% CO_2 and relatively 95% humidity chamber. The cells were sub-cultured at 90% confluence and cell passages 6-10 were used in all experiments.

5.4 Setup of Microcarrier Beads

Dextran beads (cytodex, Sigma) with diameters in the range of 150 – 200 μm were used as microcarrier beads to deliver fibroblasts monolayers on electrospun nanofibers for cell migration study. Two hundred-mg beads were first hydrated in 1X PBS overnight, resuspended in fresh PBS to a final concentration of 10 mg/mL before sterilized by autoclave. To promote cell attachment on the bead surface, stock of microcarrier beads were coated with 40 μg/ml gelatin and kept refrigerated. For quality control purpose, the number of beads in 2 mg/ml solution was counted from three independent samples and estimated to be 6,000.

For the preparation of fibroblast monolayer on microcarrier beads, we adapted a static coating method [35] to culture fibroblasts on the bead surface. Approximately 1.5×10^3

beads were mixed with 3×10^6 cells in 4 mL of complete DMEM during gentle re-suspension in 15-mL centrifuge tube before transferred onto P35 petri dish, and incubated for 48 h. To inspect the formation of fibroblast monolayers, the cells's nuclei were stained with bisBenzamide (H 33258, Sigma) before examined by light and fluorescence microscopy techniques.

5.5 Time-lapse Fluorescence Microscopy of Cell Morphology and Cell Migration

To understand how individual cells from suspension respond to nanofibers upon cell attachment, the cell spreading was first observed in real-time. All nonwoven and aligned nanofiber samples were sterilized by immersion in 70% ethanol and UV irradiated before secured on P35 petri dishes by a thin layer of petroleum jelly. The samples were then coated with 10 µg/mL bovine fibronectin (Sigma) to promote initial cell adhesion. The confluent cell monolayers in tissue culture flask were labeled with fluorescence dye, DiI (V-22885, Life Technologies), before seeding on the samples. Immediately after seeding, snap shots of cell spreading on the surface were taken every 6 min for the entire duration of 1 h using Jenoptik imaging software. Data were collected from about 3-4 samples from each of the nonwoven and aligned patterns. The cells were then allowed to proliferate and form a confluent monolayer on nanofibers before fixed and stained for actin filaments.

For the study of cell migration, after the cells formed monolayers on microcarrier beads, the cell membrane was labeled with DiI and seeded on the samples. After incubation for at least 16 h to allow the beads to fully attached on nanofibers, snap shots of the cell movement were captured every 15 min for the duration of 2.5 - 4 h. Data were collected from about 4-5 samples from each of the nonwoven and aligned patterns. Bare glass cover slips without nanofibers were used as a control. The setup for all time-lapse experiments is shown in Figure 1. Selected samples were fixed and stained immediately for actin filaments

after time-lapse observation. The rest of the samples were further incubated until the cells reach confluence before staining for actin filament and cell nuclei.

5.6 Visualization of Actin Filaments

To visualize the intracellular actin filaments, the samples were fixed in 3.7% paraformaldehyde for 15 min. After three washes in 1X PBS, the cells were stained in a 1:50 solution of Alexa Fluor 488-conjugated phalloidin (Life Technologies) for 30 min. The cells were then washed with 1X PBS three times, 10 min per wash, and mounted with 10 µL of Prolong gold containing DAPI (Life Technologies). The samples were examined by either fluorescence microscopy or confocal microscopy.

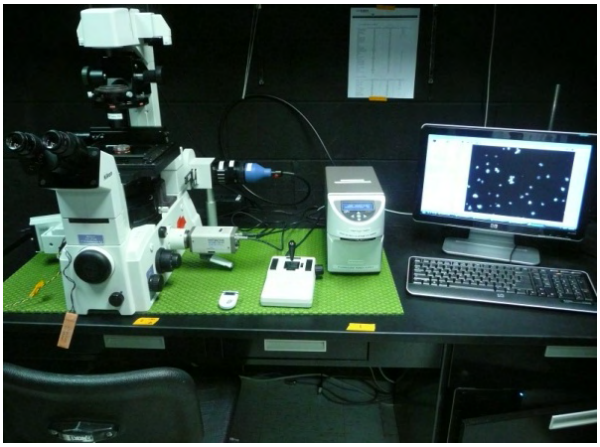


Figure 1. Experimental setup for time-lapse fluorescence microscopy

5.7 Data Analysis

The diameter of electrospun fibers were measured from SEM images of 100 – 150 fibers taken from 5-10 different samples. The periodicity of nanofibers within the nonwoven and aligned patterns was analyzed by fast Fourier transform (Image-Pro Plus version 7, Media Cybernetics, Inc., Bethesda, MD). To quantify changes in cell shape within 1 h of

attachment on nanofibers, the cells were categorized into 2 groups; polygonal and elongated morphology. The polygonal morphology was used to define cells that extended their membrane randomly in all directions or assumed a round shape, whereas the elongated morphology referred to cells that extend the cell shape in a bipolar manner. Data were taken from about 100-200 cells on each of the nonwoven and aligned patterns. In addition, a shape factor, which is defined as $(4\times\pi\times\text{area})/(\text{perimeter}^2)$, was also used to quantify the cell shape on the scale of 0 (line, elongated) to 1 (round). In the study of cell migration, after the microcarrier beads were coated with dermal fibroblasts, the number of cells per bead was estimated from 34 beads taken from 4 different sets of experiments. To quantify the velocity of cell migration, the distances traveled by the cells were traced from the translocation of cell nuclei using track measurement function of the Image-Pro Plus software. The migration velocity was estimated from about 30 cells from each of the nonwoven and aligned patterns. An unpaired t-test with $p \leq 0.05$ (GraphPad Prism, GraphPad Software, Inc, CA, USA) considered statistically significant was used in the statistical comparisons between samples.

6. Results

6.1 Characterization of SF Protein

The yellow cocoons of Thai silkworms *B. mori* (variant Nangnoi Si Sa Ket) were composed of sericin and fibroin proteins (Fig. 2A). Using SDS-PAGE technique, the extracted SF protein from the core of fibers was found to consist of at least two major components; one with molecular weight above 238 kDA and the other with molecular weight between 20-25 kDa (Figs. 2B and 2C). These two components of SF protein correspond to heavy-chain fibroin (ca. 220 -500 kDa) [3,8] and light-chain fibroin (ca. 25 kDa) [36,37], respectively, as reported in previous studies. Therefore, the SF protein obtained from variant Nangnoi Si Sa Ket contains similar protein components to other variants within the family of *B. mori*.

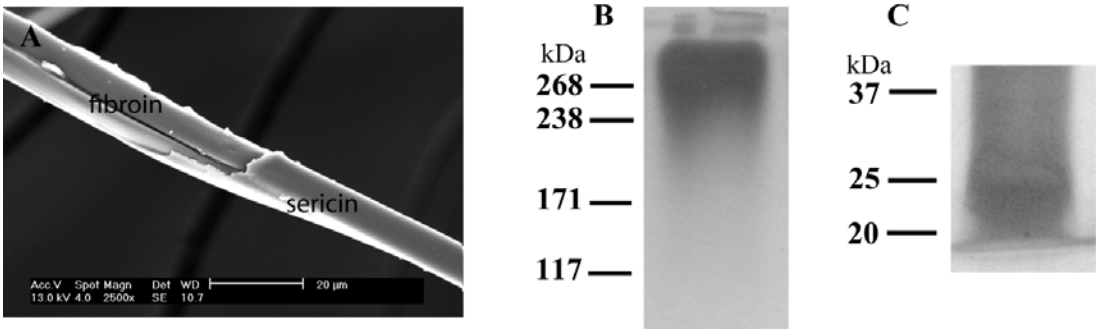


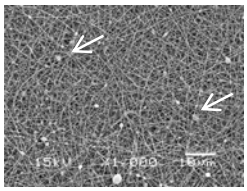
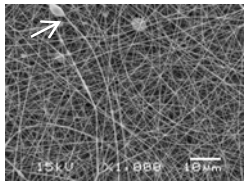
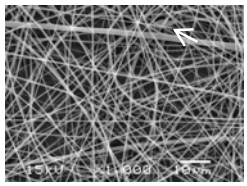
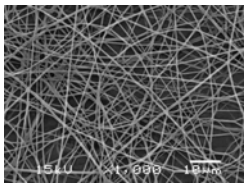
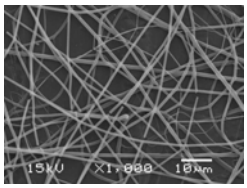
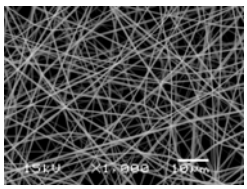
Figure 2. Characterization of SF protein. (A) SEM image of natural silk fiber taken from cocoons of Thai silkworms *B. mori* (variant Nangnoi Si Sa Ket). The sericin protein appears as coating on the outer surface and the fibroin protein makes up the core of silk fiber. (B) High molecular weight and (C) low molecular weight protein bands of fibroin separated by SDS-PAGE on 6% and 8% gel, respectively.

6.2 Electrospun SF Nanofibers into Nonwoven and Aligned Patterns

To generate smooth and continuous silk nanofibers, the electrospinning parameters including SF concentration in HFP solution, voltage and flow rate were optimized. Fibroin concentrations ranged from 5 - 15% (w/v), the electrospinning voltages of 8, 10 and 12 kV, and the flow rates of 0.8 and 1.0 mL/h were tested during the electrospinning process. The formation of nanofibers formed at each condition was summarized in Table 2.

At fibroin concentration lower than 10% (w/v), the electrospun fibers appeared with beads and varicosities, whereas the fibers became smooth and continuous when the concentration was raised to 15% (w/v). In addition, the size of electrospun SF nanofibers also increased with increasing fibroin concentration. With variation in electrospinning voltage, the decrease in voltages from 12 to 8 kV caused nanofibers to merge together into junctions (Fig. 3). In this study, the minimum voltage of 10 kV was found to be necessary for the formation of straight and continuous nanofibers (Fig. 3B). Further adjustment of electrospinning flow rate did not affect the morphology of fibers, although the combination of lower voltage and higher flow rate (Table 2: 12 kV and 0.8 mL/h vs. 10 kV and 1.0 mL/h) slightly caused the decrease in fiber diameter by ~ 20%. Thus, among these electrospinning parameters, the concentration of fibroin solution provided the strongest effect on the formation of smooth and continuous silk nanofibers. In this experiment, the 15% (w/v) SF solution fed through the spinneret at flow rate of 1 mL/h through a 10 kV electric field was found to be the optimal condition to generate SF nanofibers without any irregularities.

Table 2. Effect of electrospinning parameters on the formation of SF nanofibers

concentration (%)	voltage (kV)	flow rate (mL/h)	fiber diameter (nm)	fiber morphology
5	12	0.8	154 ± 4.3	
8	12	0.8	244 ± 6.2	
10	12	0.8	451 ± 10.8	
15	12	0.8	944 ± 47.2	
15	10	0.8	959 ± 31.6	
15	10	1.0	775 ± 26.9	

Data are mean ± standard error of the mean collected from 100 - 150 fibers for each condition. Arrows indicate beads and varicosities on the fibers.

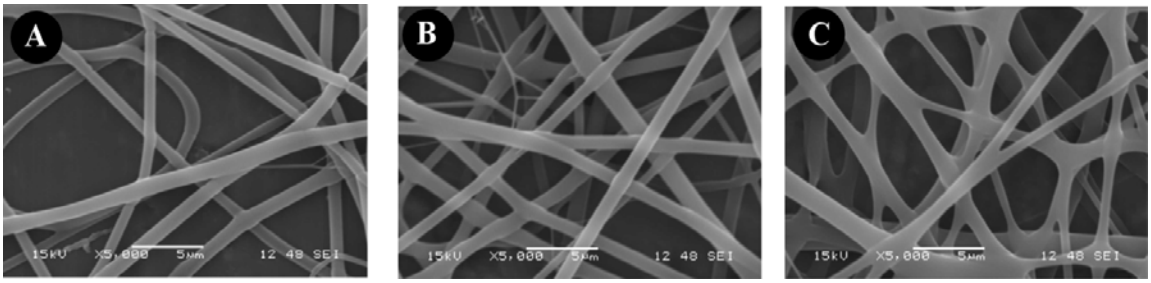


Figure 3. Effect of electrospinning voltages (A) 12, (B) 10 and (C) 8 kV on the morphology of SF nanofibers. Scale bar is 5 μm on all images.

This optimal electrospinning condition was applied to generate nanfibers into the nonwoven and aligned patterns on glass coverslips for cell migration study. Using Fourier transform analysis, the fast Fourier transform (FFT) of nonwoven pattern appeared in a cloud of dots, whereas the FFT of aligned pattern showed a line of dots (Figs. 4A and 4B, insets). Thus, the Fourier transform analysis confirmed that the aligned pattern indeed composed of nanofibers arranged in periodicity. In addition, these methods used to organize nanofibers into different patterns did not alter the fiber diameter as it appeared to be similar, about 600 - 700 nm, for both patterns (Figs. 4C and 4D). Therefore, we conclude that silk fibroin protein extracted from the cocoons of silkworms can be regenerated into smooth and continuous SF nanofibers that organized into nonwoven and aligned patterns with similar fiber diameter by electrospinning. These nanofibers will serve as patterned substrates for the study of cell morphology and cell migration.

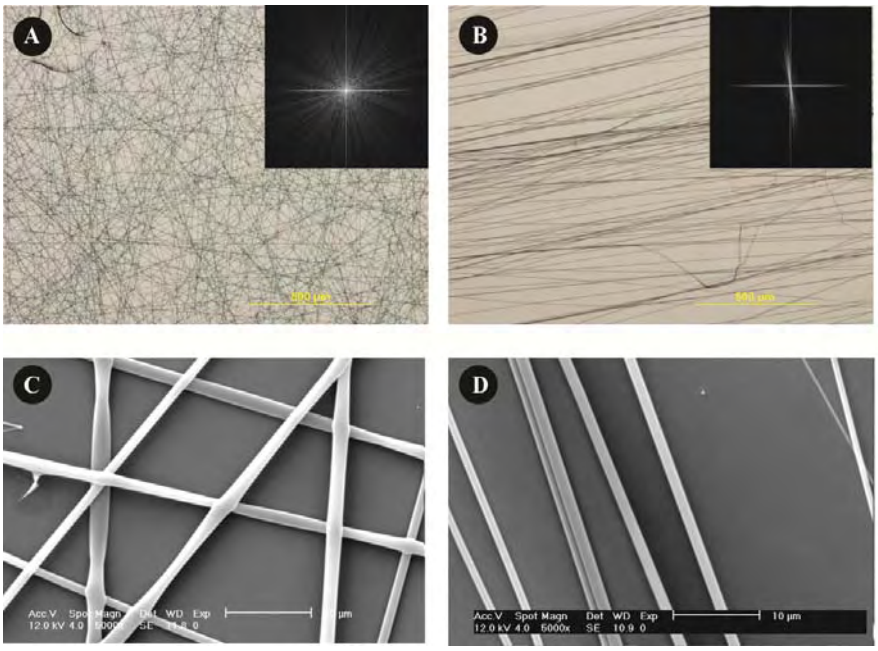


Figure 4. Visualization of electrospun SF nanofibers. Bright field images of SF nanofibers arranged into (A) nonwoven and (B) aligned patterns with FFT images shown in insets. SEM images in (C) and (D) show the corresponding nanofibers organized into nonwoven and aligned patterns, respectively, at 5000X magnification.

6.3 Chemical Treatment of SF Nanofibers

The secondary structure of SF can be converted from random coils to ordered β-sheet by immersing in 90% methanol for 1 h, preventing the electrospun nanofibers from rapid degradation in aqueous environment. Changes in the structure of SF protein were characterized by ATR-FTIR (Fig. 5). The FTIR spectra showed shifts in N-H bending vibration intensity from 1652 to 1628 cm^{-1} (amide I) and 1543 to 1530 cm^{-1} (amide II), with an additional shoulder at 1266 cm^{-1} (amide III). These shifts in N-H bending vibration correspond to the presence of β-sheet in the methanol-treated fibroin protein reported in previous studies [38-40].

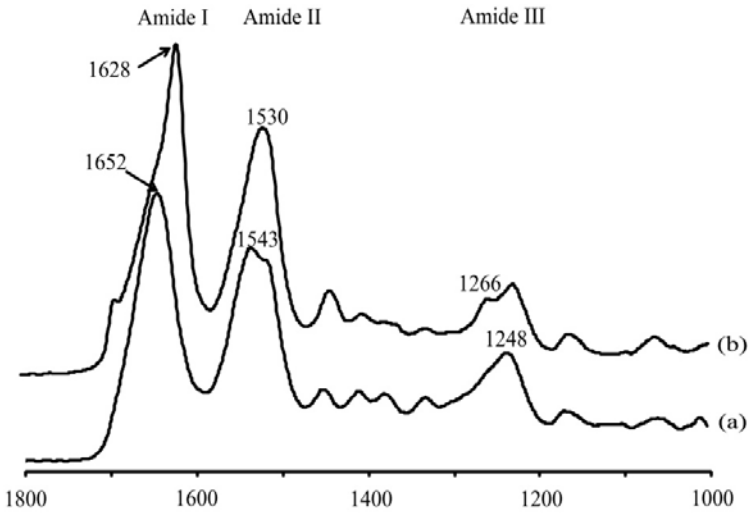


Figure 5. FTIR spectra of SF nanofibers (a) before and (b) after methanol treatment

Figure 6 shows SEM images of nanofibers within the nonwoven and aligned patterns after immersion in methanol for 1 h. Although the methanol treatment induced changes in the secondary structure of SF protein, the fiber morphology remained the same in both patterns. Subsequently, these nanofibers maintained their structural integrity throughout the duration of cell migration study.

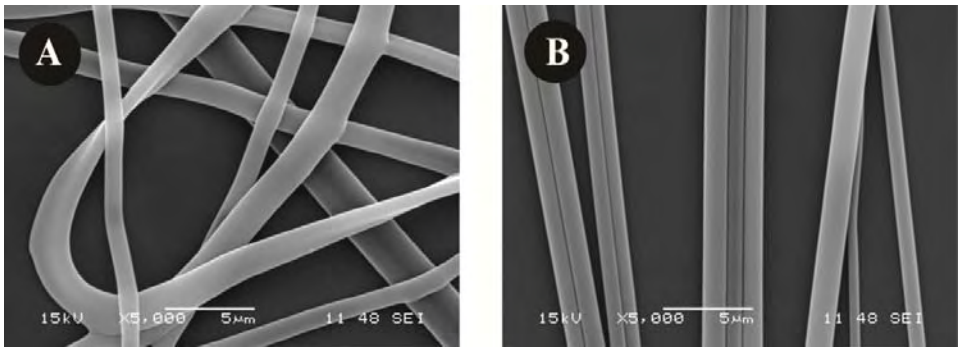


Figure 6. SEM images of methanol-treated (A) nonwoven and (B) aligned nanofibers

6.4 Microcarrier Beads Coated with Fibroblast Monolayers

Using the static coating method, fibroblasts formed monolayers on microcarrier beads within 48 h after the cells were incubated with beads. Figure 7 shows the uniform distribution of cells over the bead surface. The number of cells on monolayer-coated beads was quantified from fluorescence images of nuclei staining and estimated to be 40 ± 1.9 cells per bead. These monolayer-coated beads will be used in the time-lapse observation of cell migration.

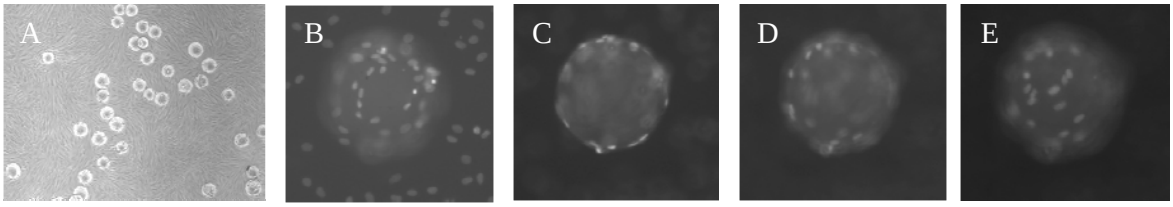


Figure 7. Fibroblast attachment on microcarrier beads using static coating method. (A) Phase contrast image taken at objective 4X at 48 h post incubation. Staining of cell nuclei (B) – (E) shows the distribution of cells over the bead surface from the bottom most (B) layer up to the top most (E) layer. Images (B) – (E) are taken at objective 20X.

6.5 Responses in Cell Morphology to Nonwoven and Aligned Patterns

Changes in the cell shape as guided by nanofibers could be observed within minutes after cell attachment. On nonwoven nanofibers, most cells extended the cell shape randomly in all direction (Fig. 8, solid arrow) as they adhered on junctions where nanofibers cross with others. However, a few cells stretched and elongated the cell shape on isolated, individual nanofibers (dashed arrow). On the aligned pattern, the majority of cells stretched and elongated parallel to the fiber direction. Interestingly, we observed that during cell spreading the cell membrane could form many pods (Figs. 8F – 8H, solid arrows) to navigate on adjacent nanofibers simultaneously before eventually extend the entire membrane along the fiber direction.

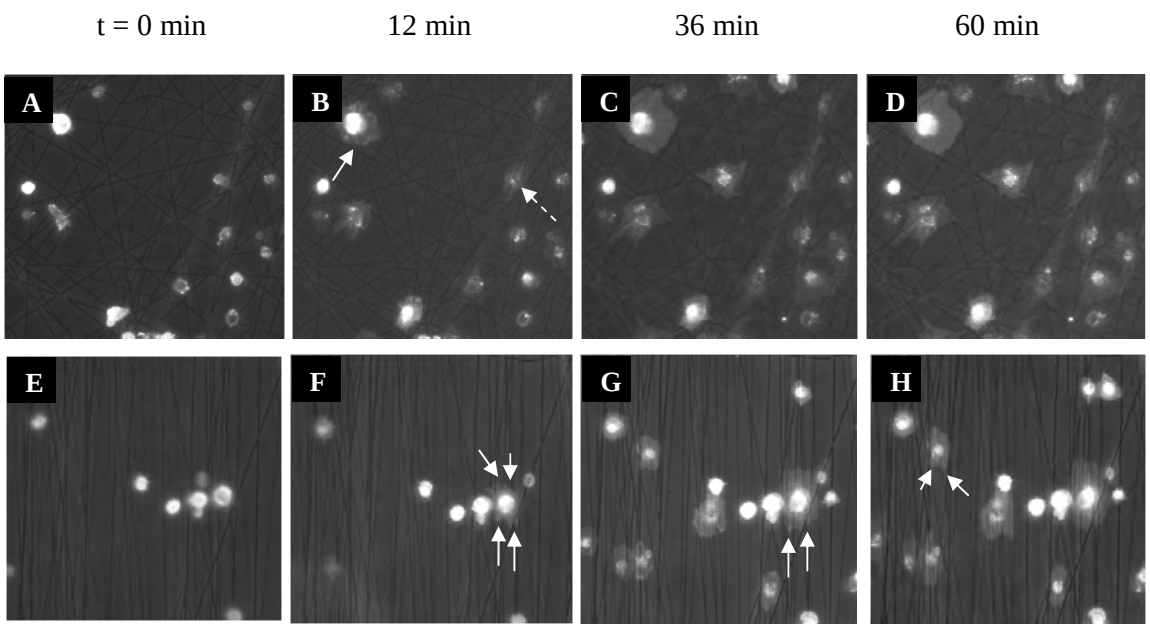


Figure 8. Responses in the cell morphology on (A) - (D) nonwoven and (F) - (H) aligned nanofibers observed in real-time. All images are taken at objective 10X.

To quantify changes in cell shape within 1 h of cell attachment, the cells were grouped into polygonal or elongated morphology and also measured by a shape factor. Figure 9 shows that the elongated morphology is dominated on aligned pattern in contrast to the polygonal morphology observed in the majority of cells on nonwoven pattern. Similarly, the shape factors of cells on nonwoven and aligned patterns were 0.60 ± 0.02 and 0.33 ± 0.02, respectively, which present another indicative of the round-like and the line-like cell shape. After the cells formed a confluent monolayer on aligned patterns, we observed that individual cells within the monolayer could maintain an elongated morphology and aligned the cell shape parallel to nanofibers (Fig. 10). From this real-time imaging of cell morphology, we conclude that isolated SF nanofibers could guide cell elongation but the uniform alignment of cell shape was obtained only on nanofibers of the aligned pattern.

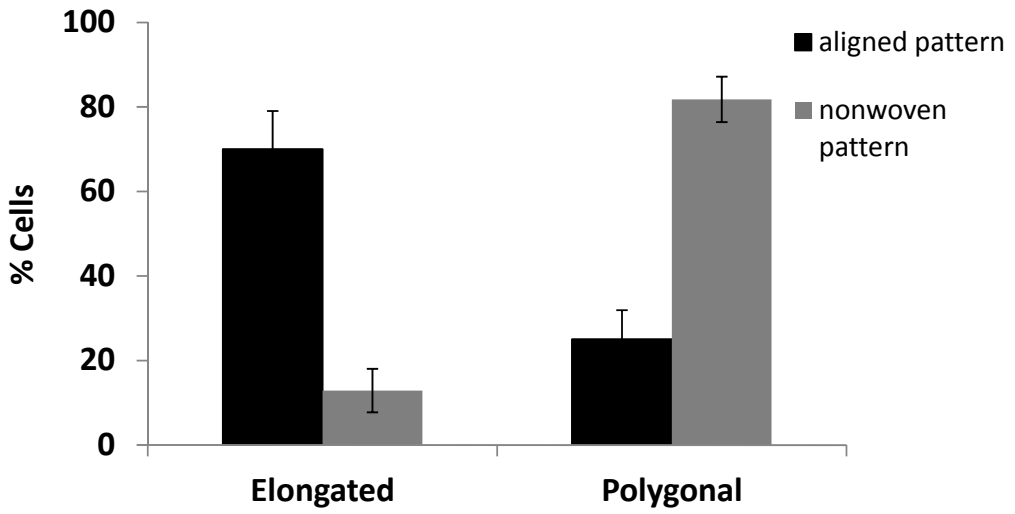


Figure 9. Quantification of cell morphology on nonwoven and aligned patterns

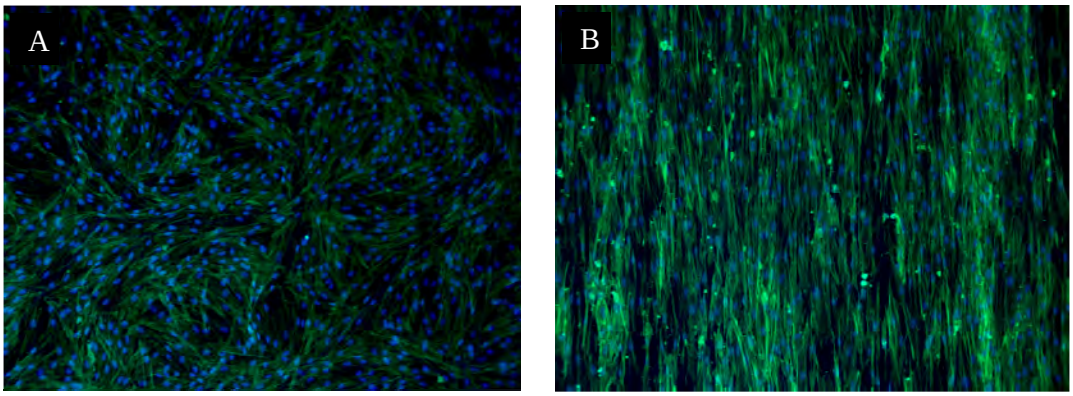


Figure 10. Fluorescence images of intracellular actin filament staining (green) of the cell monolayers on (A) nonwoven and (B) aligned patterns taken at objective lens 4X. The cell nuclei are shown in blue.

6.6 Cell Migration on Nonwoven and Aligned Patterns

In this experiment, dermal fibroblast migration from microcarrier beads was used to simulate the migration of cells from wound appendage into the wounded area. Figure 11 shows the time-lapse imaging of dermal fibroblasts migrating away from beads onto

patterned nanofibers. We found that the maximum duration of 4 h was sufficient to observe the cells move the entire cell body out of the beads. On both patterns, the cells followed individual track of nanofibers as they migrated away from the beads as pointed by solid arrows. Interestingly, the cells that have already left the bead also continued to migrate along the direction of nanofibers as pointed by arrow heads. Thus, despite different fiber organizations, the cells could recognize and respond the contact guidance provided by individual nanofiber.

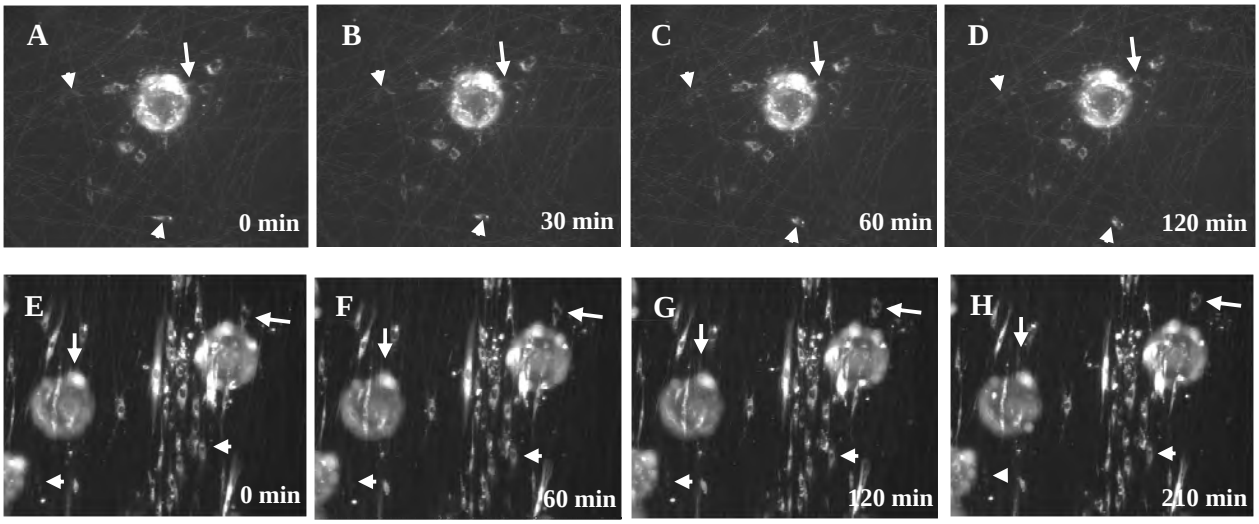


Figure 11. Time-lapse observation of fibroblast migration on (A) - (D) nonwoven and (F) - (H) aligned patterns. All images are taken at objective 10X. Solid arrows indicate the cells that are leaving the beads onto nanofibers. Arrow heads points at cells migrating along the fiber contours.

Following real-time imaging, the direction of cell movement on nonwoven, aligned and bare glass cover slip (control) substrates was monitored at 24, 48 and 72 h (Fig. 12). On aligned pattern, the alignment of nanofibers guided the uniform direction of cell migration which was absent on the nonwoven pattern as well as on control substrate. This further indicates the ability of aligned nanofibers in maintaining the persistent direction of cell

migration, which results in the different *en mass* migration behavior as shown in Figure 13. On the aligned pattern, the area occupied by cells migrated out of the beads resembles a

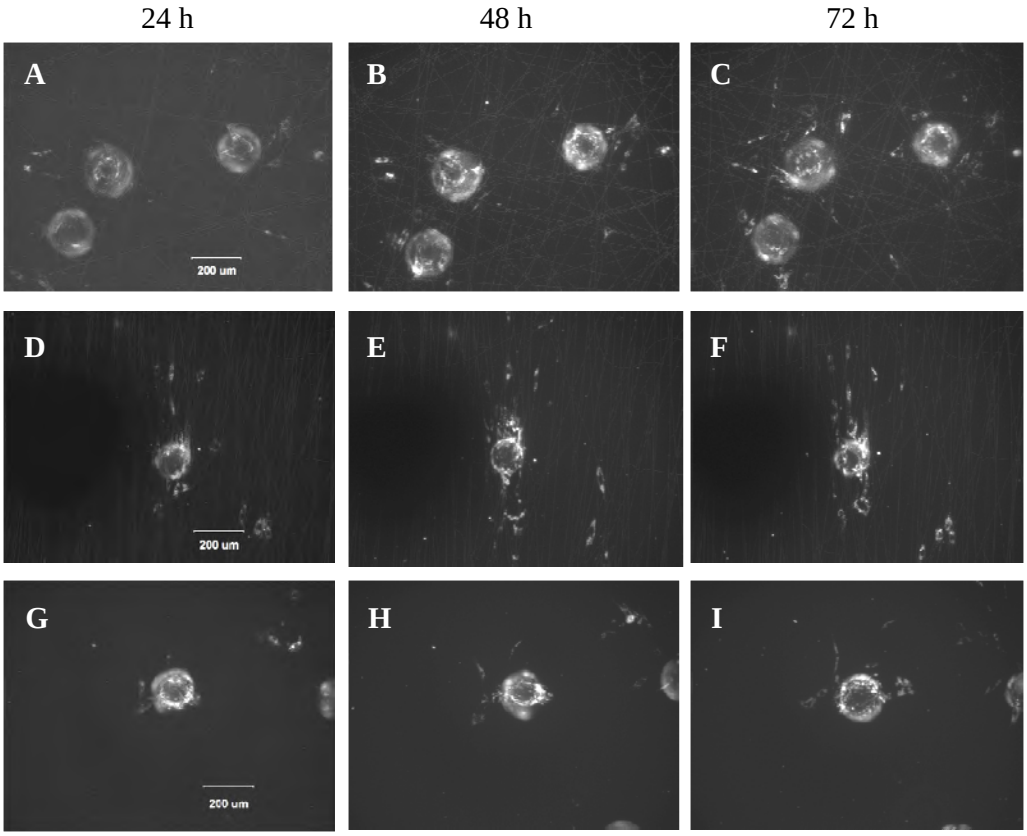


Figure 12. Snap shots of fibroblast migration on (A) - (C) nonwoven, (D) - (F) aligned and (G) - (I) coverslip substrates at 24, 48 and 72 h post time-lapse imaging. Scale bars are 200 μm for all images.

rectangular shape, whereas on the nonwoven pattern the area appears in a circular shape. This directional migration was maintained until the cells reached confluence on the aligned pattern. Therefore, the persistent cell migration in a predetermined direction can be controlled by the uniform alignment of nanofibers.

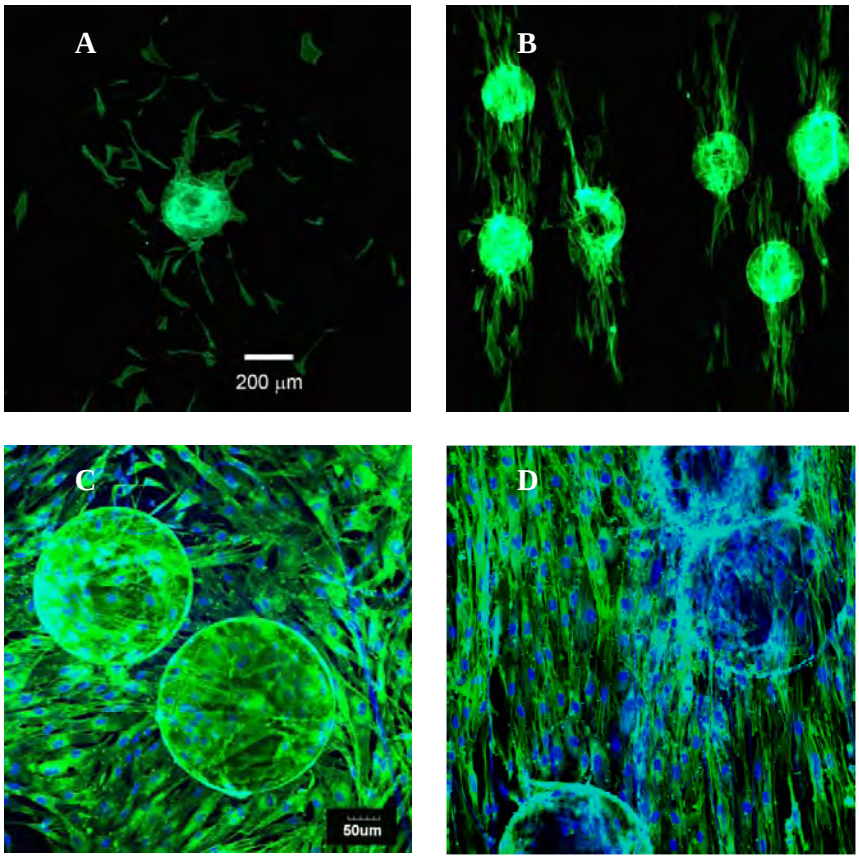


Figure 13. Fluorescence images of actin filament staining (green) of cells on (A) nonwoven and (B) aligned patterns after 72 h of cell migration out of microcarrier beads. (C) and (D) are confocal images of cell monolayers at confluence on nonwoven and aligned patterns, respectively. Cell nuclei are shown in blue. Scale bars are 200 µm for (A) and (B), and 500 µm for (C) and (D).

7. Discussion

As demonstrated in this research, cell morphology and cell migration on a synthetic biomaterial can be controlled by the well-define topographic structure presenting on the material's surface. With the use of electrospinning technique, the electrospun fibers with size in the nanometer scale can serve as an effective topographic coating on a flat surface. Our real-time imaging showed that the responses in cell shape and cell migration were instantaneous after the cells adhered on the fibrous patterns.

The use of microcarrier beads to deliver cell monolayers on the surface offers a method to evaluate the migration of cells from different type of substrates similar to the droplet assay [11]. Unlike the scratch wound assay [13] in which the cells were first allowed to attach and adapt their morphology on the tested surface, cells migrating out of the beads have to make new contacts on the tested surface they attach to. This prevents any previous establishment of the cells to affect their migration behavior.

In our system, aligned SF nanofibers can recapitulate the fibrillar structure of natural ECM. Previous study of cell migration on a 1D micropatterned line has shown that the micrometer-scale lines, 1-5 μm wide, could promote the rapid uniaxial migration of fibroblasts, a phenomenon that was absent in a regular 2D culture, similar to the cell-derived, oriented 3D matrix composed of fibronectin fibers [41]. The elongation as well as migration velocity on wider lines, however, became less pronounced as the cell spreading was no longer conformed to a uniaxial path and started to navigate in other directions. Because SF nanofibers of the aligned pattern in our system are in close proximity with their neighbors, this organization of nanofibers can closely mimic the structure of the cell-derived, oriented 3D matrix that contains dense fibronectin fibers. As demonstrated by our real-time imaging, different parts of the cell membrane that adhered to adjacent, parallel nanofibers were shown to coordinately extend down the fibers during cell attachment and

cell migration. Therefore, the regulation of directional cell responses on aligned SF nanofibers is based primarily on cell-nanofiber interaction without any confinement effect.

The process of cell migration consists of 3 coordinated, sequential steps: (1) membrane protrusion and focal adhesion formation at the cell front, (2) forward movement of the cell body, and (3) detachment of the trailing edge at the cell rear [18]. The ability of the cell protrusion to proceed in a uniaxial manner can in turn guide the subsequent migration in a predetermined path. The persistence in both the cell elongated morphology and cell migration direction obtained on aligned SF nanofibers in our study is similar to the observation reported previously on aligned PMMA fibers [11]. Despite the difference in fiber diameters (~ 700 nm for SF and 8 μm for PMMA [11]), both SF and PMMA fibers can guide the directional migration of dermal fibroblasts along the fibers. The directional migration of the cells is also supported by the elongation and alignment of cell shape parallel to the fibers. Thus, this adaptation of the cell shape to aligned nanofibers may help the cells keep their migration path along a predetermined direction. The mechanism underlying this interaction has yet to be investigated in future study.

8. Conclusion

In this research we reported the fabrication of patterned SF nanofibers by electrospinning and the evaluation of these patterns on the morphology and migration of dermal fibroblasts. The electrospinning parameters were established to generate smooth and continuous SF nanofibers into nonwoven and aligned patterns. Both the cell elongation and directional cell migration could be guided by individual nanofibers although the uniform, elongated cell morphology was obtained only on the aligned pattern, which persisted until the cells reach confluence. Similar to the alignment of cell shape, the directional migration of the cells was also uniform on the aligned pattern as the cells maintained their migration path along nanofibers. Based on these results, nanofibers organized into the aligned pattern can be used to control both the cell morphology and the cell migration.

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10. Outputs and Publication

In terms of the fundamental knowledge, we have established the method to fabricate silk nanofibers from fibroin protein with different patterns, nonwoven and aligned, by electrospinning. In addition, we also demonstrated that isolated, individual nanofibers can guide elongated cell morphology and directional cell migration although the uniform response of both behaviors was obtained only on nanofibers of the aligned pattern. As for the innovation aspect, patterned nanofibers can potentially be used as an interactive dressing material or skin graft in wound healing application.

Results from this research have been presented in a seminar, two conferences and a journal paper. The fabrication of nanofibers was presented in the seminar for the Research and Development group at the Thailand Institute of Nuclear Technology on November 26, 2553 (appendix 1). A poster presentation entitled “Electrospun Silk Fibroin Mats for Wound Healing Application” was given at the 8th Asian Pacific Burn Congress Conference during September 11 – September 14, 2554 (appendix 2). The real-time study of cell responses to nanofibers was presented in an oral presentation entitled “Manipulation of dermal fibroblast orientation and migration by electrospun silk nanofibers” at the 1st annual international meeting of the Society of Molecular Imaging of Thailand during April 9 – April 11, 2555 (appendix 3). The characterization of fibroin protein and the fabrication of electrospun nanofibers are currently in press in Fibers and Polymers Journal (appendix 4). Lastly, a short article about this research for public distribution is given in appendix 5.

11. Appendix

1. Seminar for the Research and Development group at Thailand Institute of Nuclear Technology (Public Organization)

รายชื่อผู้เข้ารับฟังการบรรยายทางวิชาการเรื่อง

Fabrication of Silk Fibroin Nanofibers by Electrospinning

วันที่ 26 พฤศจิกายน 2553 ณ ห้องประชุม อาคาร 8 สถาบันเทคโนโลยีนิวเคลียร์แห่งชาติ (องค์การมหาชน)

1. วรรณี [illegible]
2. [illegible]
3. ศิราณี ศรีเวียง
4. พิริยา แก้วพุก[illegible]
5. [illegible]
6. [illegible]
7. [illegible]
8. [illegible]
9. [illegible]
10. [illegible]
11. [illegible]
12. [illegible]
13. วันชัย [illegible]
14. [illegible]
15. [illegible]
16. [illegible]
17. [illegible]
18. [illegible]
19. [illegible]
20. [illegible]
21. ศิริลักษณ์ ชูแก้ว
22. [illegible] คำหวาน
23. [illegible] เอี่ยมศิริ

2. Abstract for the 8th Asian Pacific Burn Congress conference

ELECTROSPUN SILK FIBROIN MATS FOR WOUND HEALING APPLICATION

Pimpon Uttyarat[1], Suwimol Jetawattana[1],Wannee Angkhasirisap[2], Thanee Sukglin[2], and Nantana Choomchuay[3]
[1]Research and Development Division, Thailand Institute of Nuclear Technology (Public Organization)
[2]Office of Academic Services, National Laboratory Animal Center, Mahidol University
[3]Pathology Department, Faculty of Medicine, Srinakharinwirot University

Objective: The aim of this study was to develop a prototypic wound dressing from silk fibroin by electrospinning to promote cellular response and wound healing in rat model.

Materials & Methods: Silk fibroin protein was extracted from cocoons of silkworms *Bombyx mori* (variant Nangnoi Si Sa Ket) and fabricated into mats consisting of fibroin nanofibers by electrospinning. Fibroblasts were used as a cell model to investigate the response of cells to the organization of nanofibers, nonwoven vs. aligned patterns. The cells were pre-labeled with fluorescence dye for microscopic observation on nanofibers at 2 h post incubation. For wound healing study, male Sprague-Dawley rats weighing 270 ± 20 g were anesthetized before full thickness wounds with circular area of 28.3 mm^2 were excised on the dorsal area and covered with nonwoven fibroin mats. The wounds were cleaned, recorded and replaced with new mats every 3 days. Biopsy specimens were collected on day 7, 14 and 21 for histological examination of inflammatory response, collagen synthesis and epithelial formation.

Result: On aligned nanofibers, most cells uniformly oriented their shape parallel to fibers, whereas the cells on nonwoven nanofibers did not show a unidirectional orientation. For wound healing, nonwoven fibroin mats promoted the complete wound closure by day 17 without severe inflammatory response. Histological examination showed that within 7 days post surgery moderate collagen synthesis and epithelial regeneration in wounds covered with fibroin mats were comparable to those treated with commercial wound dressings.

Conclusions: Fibroin mats composed of electrospun nanofibers were shown to induce cellular response and promote wound healing.

3. Abstract for the 1st annual international meeting of the Society of Molecular Imaging of Thailand

Manipulation of dermal fibroblast orientation and migration by electrospun silk nanofibers

Pimpon Uttayarat[1], Suwimol Jetawattana[1], Peter I. Lelkes[2]

[1]Thailand Institute of Nuclear Technology, Nakon Nayok, Thailand

[2]School of Bioengineering, Science and Health Systems, Drexel University, Philadelphia, USA

The ability of tissue-engineered scaffolds to interact with cells is important in directing many cellular responses such as cell morphology, migration and proliferation. The cell migration in particular plays an important role in determining the fate and structural organization of cells and tissue *in vivo*. In this research, we aimed to investigate the effect of silk fibroin (SF) nanofibers with different fiber orientations on the migration of dermal fibroblast for wound healing application. SF protein was extracted from cocoons of Thai silkworms *Bombyx mori* (variant Nangnoi Si Sa Ket) and fabricated into nonwoven and aligned nanofibers by electrospinning. Microcarrier beads coated with monolayers of dermal fibroblasts were used to deliver cells onto electrospun SF substrates as a way to mimic the cell migration from wound appendage during re-epithelialization. The migration of cells on SF nanofibers was recorded by time-lapse fluorescence microscopy. Results demonstrated that on substrates with aligned SF nanofibers, the cells uniformly migrated away from microcarrier beads in the direction parallel to fibers. By contrast, the majority of cells on substrates with nonwoven SF nanofibers migrated in random directions with some cells migrated directionally along the fibers. At confluence the cells on aligned SF nanofibers maintained the elongated cell shape in the direction parallel to nanofibers. This nanotopographic guidance on the substrate conferred by aligned nanofibers may be used to effectively guide cell alignment and directional cell migration during wound healing.

4. Abstract for Fibers and Polymers Journal

Antimicrobial Electrospun Silk Fibroin Mats with Silver Nanoparticles for Wound Dressing Application

Pimpon Uttayarat*, Suwimol Jetawattana, Phiriyatorn Suwanmala, Jarurattana Eamsiri, Theeranan Tangthong and Suchada Pongpat

Thailand Institute of Nuclear Technology (Public Organization), 16 Vibhavadee Rangsit Rd, Chatuchak, Bangkok, 10900 Thailand

In this report, silk fibroin (SF) mats coated with silver nanoparticles (AgNPs) were manufactured as a prototypic wound dressing and evaluated for antimicrobial properties. SF was extracted from cocoons of Thai silkworms *Bombyx mori* (variant Nangnoi Si Sa Ket) and fabricated into nonwoven mats by electrospinning. In a one-step synthesis method, colloidal AgNPs were prepared from silver nitrate by gamma irradiation and inspected by transmission electron microscopy. Using the *in vitro* disc diffusion and growth-inhibition assays, AgNP-coated SF mats effectively inhibited the growth of *Staphyllococus aureus* and *Pseudomonas aeruginosa* when the coating solution containing colloidal AgNPs was $\geq$ 4 mM, or equivalent to 50.4 ng/cm^2 of adsorbed AgNPs. Based on these results, the AgNP-coated SF mats can potentially be used as antimicrobial wound dressings.

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Fibers and Polymers

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Date: 20-03-2012
To: "Pimpon Uttayarat" puttayar@alumni.upenn.edu,puttayar@gmail.com
From: "Ji Ho Youk" youk@inha.ac.kr
Subject: FIPO: Your manuscript entitled Antimicrobial Electrospun Silk Fibroin Mats with Silver Nanoparticles for Wound Dressing Application

Ref.: Ms. No. FIPO-D-11-00422R1
Antimicrobial Electrospun Silk Fibroin Mats with Silver Nanoparticles for Wound Dressing Application
Fibers and Polymers

Dear Dr. Uttayarat,

I am pleased to accept your paper for publication in Fibers and Polymers.

It was accepted on 20-03-2012.

Thank you for submitting your work to this journal.

With kind regards

Ji Ho Youk
Associate Editor
Fibers and Polymers

5. Article for public distribution

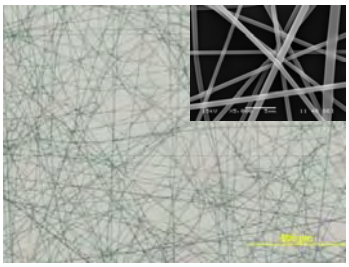
เส้นไหมในธรรมชาติเป็นที่รู้จักอย่างแพร่หลายในอุตสาหกรรมทอผ้า และมีประวัติอันยาวนานเป็นระยะเวลากว่าร้อยปีในการใช้เป็นไหมเย็บแผลทางการแพทย์ เส้นไหมประกอบไปด้วยโปรตีนหลักสองชนิด คือ โปรตีนไหมไฟโบรอินซึ่งเป็นแกนหลักของเส้นไหม และโปรตีนไหมเซริซินที่เคลือบเป็นกาวไหมอยู่บนผิวนอกสุด นอกจากเส้นไหมแล้วรังไหมที่ห่อหุ้มหนอนไหมในระยะดักแด้ก็มีโปรตีนทั้งสองชนิดนี้เป็นส่วนประกอบด้วยเช่นกัน เนื่องจากรังไหมไม่ได้ถูกนำมาใช้ประโยชน์ในอุตสาหกรรมทอผ้า จึงกลายเป็นวัสดุเหลือทิ้งจากการเลี้ยงไหม ดังนั้นการสกัดเอาโปรตีนไหมจากรังไหมเพื่อพัฒนาต่อยอดให้เป็นผลิตภัณฑ์จะเป็นการช่วยเพิ่มมูลค่าของรังไหมเหลือทิ้งให้เกิดประโยชน์สูงสุด

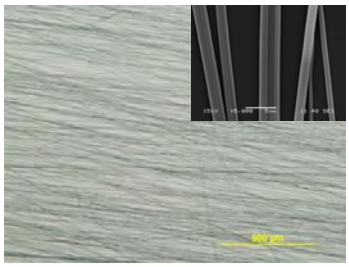
ปัจจุบันงานวิจัยหลายชิ้นทางการแพทย์ได้ให้ความสำคัญกับการพัฒนาวัสดุปิดแผลจากโปรตีนไหมไฟโบรอิน เนื่องจากเป็นวัตถุดิบที่หาได้ในธรรมชาติและสามารถเข้ากันกับร่างกายได้ดีโดยไม่ก่อให้เกิดอาการแพ้อย่างรุนแรง โครงการวิจัยนี้จึงมีวัตถุประสงค์ที่จะขึ้นรูปเส้นใยไหมที่มีขนาดระดับนาโนเมตรจากโปรตีนไหมไฟโบรอินและศึกษาการตอบสนองด้านรูปร่างและการเคลื่อนที่ของเซลล์ผิวหนังบนเส้นใยเพื่อพัฒนาเป็นวัสดุปิดแผล ในขั้นตอนการขึ้นรูปเส้นใยได้ใช้เทคนิคการปั่นเส้นใยด้วยไฟฟ้าสถิต (electrospinning) โดยฉีดสารละลายโปรตีนไหมไฟโบรอินผ่านสนามไฟฟ้าเพื่อให้เกิดเป็นเส้นใยบนฉากรับ เส้นใยที่เตรียมได้มีลวดลายการจัดเรียงแตกต่างกันสองแบบ คือ แบบซ้อนทับกันไปมา (nonwoven) และแบบขนาน (aligned) จากการวิเคราะห์ด้วยกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราดพบว่าเส้นใยไหมที่ขึ้นรูปได้มีขนาดประมาณ 600-700 นาโนเมตรซึ่งเล็กกว่าเส้นไหมธรรมชาติเกือบหนึ่งพันเท่า เมื่อนำเซลล์ผิวหนังมาเลี้ยงบนพื้นผิวที่มีเส้นใยทั้งสองแบบครอบคลุมอยู่พบว่าพื้นผิวที่เส้นใยไหมมีการจัดเรียงแบบขนานสามารถเหนี่ยวนำให้เซลล์ยืดตัวและมีรูปร่างเรียวคล้ายวงรีไปในทิศทางเดียวกันกับเส้นใยได้ทั้งหมด ซึ่งแตกต่างจากพื้นผิวที่เส้นใยไหมมีลวดลายแบบซ้อนทับกันไปมาที่เซลล์ไม่มีการจัดเรียงรูปร่างไปในทิศทางเดียวกัน นอกจากนี้เส้นใยไหมนาโนที่มีลวดลายแบบขนานยังช่วยกระตุ้นให้เซลล์ทั้งหมดเคลื่อนที่ไปในทิศทางเดียวกันตามเค้าโครงเส้นใยจนกระทั่งเซลล์เจริญเป็นแผ่นเซลล์ครอบคลุมพื้นผิว ถึงแม้ว่าเซลล์บนพื้นผิวที่เส้นใยมีลวดลายแบบซ้อนทับกันไปมา

จะสามารถเคลื่อนที่ตามเค้าโครงของเส้นใยเดี่ยวได้เช่นเดียวกัน แต่การเคลื่อนที่ของเซลล์โดยรวมไม่ได้เป็นไปในทิศทางเดียวกันเหมือนกับเซลล์บนพื้นผิวที่เส้นใยมีลวดลายแบบขนาน ดังนั้นรูปร่างและการเคลื่อนที่ของเซลล์สามารถถูกกำหนดให้ไปในทิศทางที่ต้องการได้โดยด้วยการเหนี่ยวนำจากโครงสร้างพื้นผิวบนวัสดุ และเส้นใยไหมนาโนที่มีลวดลายแบบขนานนี้อาจนำไปประยุกต์ใช้ให้เป็นส่วนหนึ่งของโครงสร้างพื้นผิวของวัสดุปิดแผลเพื่อช่วยในการสมานแผล

รังไหมเริ่มต้น

เส้นใยไหมนาโน
(ซ้าย) แบบซ้อนทับกันไปมา
(ขวา) แบบขนาน





แผ่นเซลล์ผิวหนังบนพื้นเส้นใยไหมนาโน
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