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Electrospinning of PVA/sericin nanofiber and the effect on epithelial-mesenchymal transition of A549 cells

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ABSTRACT

This research aims to investigate the cell-nanomaterial interaction between epithelial-mesenchymal transition of A549 cell and electrospinning nanofibers composed of polyvinyl alcohol (PVA)/silk sericin (SS). The electrospinning of regenerated nanofiber was performed with water as a spinning solvent and glutaraldehyde as a chemical cross-linker. Solution concentration, applied voltage and spin distances as well as other parameters were optimized to generate fine nanofibers with smooth surface in good homogeneity. From the scanning electron microscopy (SEM) analysis, the nanofibers had an average diameter of 200 nm. Epithelial-mesenchymal transition (EMT) is a process by which epithelial cells lose their cell polarity to become mesenchymal stem cells. This transition is affected by multiple biochemical and physical factors in cell metabolism cascade. Herein, we investigate the biophysical effect on A549 EMT by culturing cells on nanofibrous mats with different topography and composition. The cell viability was evaluated by biochemical assay and its morphology was observed with SEM. The results demonstrate that cells appropriately attached to the surface of the nanofibrous mats with extended morphology by their filopodia. Gene expression analysis was conducted by real-time PCR using multiple markers for detecting EMT: *N-cadherin* (*NCad*), Vimentin (*Vim*), Fibronectin (*Fib*) and Matrix metallopeptidase (*MMP9*). An increasing expression pattern was observed on *NCad, Vim, Fib*, with respect to a negative control as cell cultured on polystyrene dish. This result indicates the 200 nm PVA/SS nanofibers may induce A549 cells to process epithelial–mesenchymal transition during the culturing.

1. Introduction

The extracellular matrix (ECM) affects the fate and activity of cells and tissues by both biochemical and biophysical factors. The interaction between resident cell and ECM is critical in determining resultant cell activity and fate, such as proliferation, differentiation and migration [1–7]. Surface topography of culturing material acts as the "first-step" factor affecting cell seeding and adhesion. Cells sense and transduce the physical and mechanical properties of their microenvironment through the direct interaction between the cell membrane and surface on ECM [8]. With respect to physical properties, such as topography, elasticity, gradients and geometry, cells can sense underlying topographic change and modified its physiological morphology. Thus cell activity can be regulated as consequent of sensing different extracellular physical factors [9]. Therefore, to understand the cascades of topographic sensing process and its influence on cell fate is critical in biomedical engineering design and healthcare research.

To investigate the interaction between the nanomaterial topology and cell fate, nanofabrication is the key step as controlling the topographic surface of culturing material as ECM, which regulates cell activity. Nanofabrication method such as soft lithography, electron beam lithography, and photolithography etc. are wildly used in surface etching to generate nanoscale patterns such as nanogroove, nanopillar, and nanoarray [8–18]. Besides surface lithography technique as mentioned above, polymer by electrospinning is another conventional and efficient method to fabricate the designed nanostructure, especially for nanofibers. By controlling different parameters such as solution concentration, applied voltage, and spin distance etc., nanofibers can be equipped with unique properties such as high surface to volume ratio, high porosity and designed alignment [3–5,19–25].Many polymers dissolved in different solvent can be fabricated into functional mat for

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Table 1
Primer sequences used for qRT-PCR procedures.

Gene	Forward (5′-3′)	Reverse (5′-3′)
N-cad	TGCGTTCTCTATCCAGAGGCT	TGCGTTCTCTATCCAGAGGCT
Vim	GACGCCATCAACACCGAGTT	CTTTGTCGTTGGTTAGCTGGT
Fib	GAGAATAAGCTGTACCATCGCAA	CGACCACATAGGAAGTCCCAG
Mmp9	CGACCACATAGGAAGTCCCAG	CGACCACATAGGAAGTCCCAG

biomedical engineering such as cell culture, tissue regeneration and drug delivery by regulating cell activity on the nanofibrous mat. For example, chitosan/silk composited nanofiber can modulate the osteogenic differentiation and proliferation of human mesenchymal stem cells; nanofibrous mat is able to mitigate hypertrophic scar contraction; gold coated collagen nanofiber enhances stem cells differentiation; [26,26,26]and carboxymethyl cellulose enables nanofibrous scaffold with enhanced biomimetic potential for bone tissue generation [1,23, 26–28].

Lung cancer is one of the leading causes of death among cancers worldwide, the metastasis of lung cancer cell is a major obstacle to cancer treatment [29]. However, the metastasis is a multiple process requires the interaction between cell and ECM as the cell attach to the surface to form focal adhesion by its receptors [29,30]. Surface topography on culturing material is critical on determining cell adhesion followed by multiple-steps locomotion. Since EMT has a key role on the early process of metastasis of cancer cells, to understand the interaction between cell and material topography is critical to understanding the process of the cancer development [29–32]. A549 lung epithelial cell is a good candidate to determine the topographic effect on EMT. Because A549 is cancerous so that the lung cell is responsive to the surface topographic stimuli. This is able to provide insight into malignancy triggered by topographic factors. But there is limited research on the interaction between nanofibrous niche and A549 EMT.

For nanofibrous mat fabrication, the natural proteins are the right candidates for electrospinning due to their unique properties. In comparison with synthetic polymers, natural polymers usually exhibit better biocompatibility but lower spinnability. Therefore, a rational combination of natural protein and synthetic polymer is good solution for healthcare engineering. Silks from silkworms (*Bombyx mori*) are mainly composed of two different macromolecular proteins, i.e., fibroin (the inner brins) and sericin (outer coating) [33]. Silk sericin (SS) protein is a raw material emerging into biomedical applications due to its outstanding biological properties, which consists of 18 kinds of amino acids and most of which have strong polar side groups such as hydroxyl, carboxyl, and amino groups. In this case, this work is designed to investigate the interaction between A549 EMT and PVA/SS nanofibrous mat with different topographic patterns and chemical composition.

2. Experimental

2.1. Materials

Polyvinyl alcohol (PVA, degree of polymerization, 1788; Mw = 72,600–81,400; hydrolysis 87.0–89.0%) and glutaraldehyde (GA, 50% aqueous solution) were purchased from Aladdin, China. Hydrochloric acid (HCl, 37% reagent grade), one of cross-linking agents and sodium carbonate anhydride (Na_2CO_3), the main chemicals for degumming were supplied by HUSHI. Glycine, ethyl alcohol and phosphate buffer solution (PBS) were bought from Sangon Biotech, China.

2.2. Preparation of silk sericin protein

Bombyx mori cocoons bought from Jiaxing, China, were cut into dime-sized pieces and disposed of silkworms with approximately 5 g. Cocoons were exposed to the ultraviolet irradiation for 5 min, immersed in 75% ethanol for 15 min, then dried in the clean hood to maintain aseptic. The pieces of cocoons were boiled at 100 °Cfor 30 min in 0.02 M Na_2CO_3 solution. The remaining soap was filtered with 0.45 μm and 0.22 μm sterile filters twice. Silk sericin (SS) solution was collected by centrifuge at 4500 rpm for 20 min with ultra-centrifugal filters (15 mL, 10 K), sealed in box and stored at 4 °C before cell experiments.

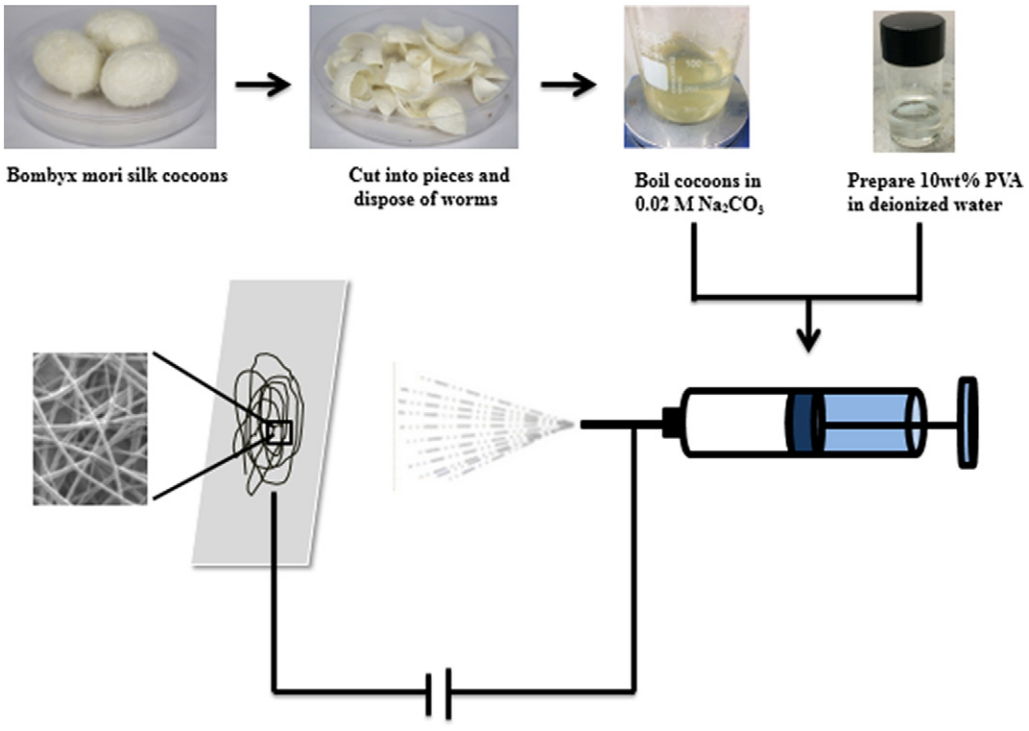

Fig. 1. Schematic of the silk sericin extraction procedure and electrospinning system.