STUDY OF CELL MORPHOLOGY ON SU-8 MICROFIBERS

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中文摘要– 本論文主題為研究應用電紡技術所製作出的 SU-8 微纖維絲來作為一種控制細胞型態之一種表面處理技術。我們分別將電紡出的 SU-8 微纖維絲收集在玻璃及聚丙烯薄膜表面，以研究細胞與微纖維絲的交互作用。實驗結果顯示，乳癌細胞及其內部肌動蛋白皆會沿著 SU-8 微纖維絲排列。此技術將可應用來作為基材的表面處理，作為引導細胞排列之應用。

Abstract–This research aimed to study the correlations between cell morphology and substrate topology. In particular, electrospun SU-8 microfibers on glass and polypropylene substrates were used to study how cancer cells would behave morphologically when cultured in direct contact on fibrous surfaces. Experimental findings suggested that breast adenocarcinoma MDA-MB-231 cells exhibited morphological changes that corresponded to the fibrous topology. Specifically, the morphologies of the cultured cells seemed to preferentially align and conform directionally to the SU-8 microfibers. This preliminary results showed the potential of using SU-8 microfibers on cell culture surfaces as a geometrical factor to direct morphological changes in active cells such as MDA-MB-231.

Introduction

Cells are exquisitely sensitive and responsive to their surrounding microenvironment, and their changes in morphology and polarization in response to environmental signals influence their functions. The three main categories of signals are: soluble signals, signals transmitted from cell to cell, and insoluble signals transmitted from environment to cell, such as substrate geometries and concentration profiles of adherent molecules. For example, previous research showed that when substrate area available for cell to anchor and adhere was controlled and restricted in the form of circular islands, cell morphology and proliferation could be modulated by the island size [1]. Further investigations showed that there existed a critical dimension of these islands that triggered profound cell behavior and cell shape changes, where focal contacts and actin microfilament bundles accumulated in a circle at the margin [2]. Later, microcontact printing method was used to create specific patterns of extracellular matrix protein on substrate surfaces, and thereby making it possible to place cells in predetermined locations and arrays, separated by defined distances, and to dictate their shape [3]. More recently, controlled total cell anchoring areas on suspended surfaces submerged in media have also been demonstrated. It was based on SiO2 microbridges coated with fibronectin as controlled micro-structures specifically for studying the biomechanical behaviors of cells [4]. The research reported in this paper is motivated by the need to further understand the influences of microscale features on surface topologies on cell morphological behaviors. The engineering approaches with electrospinning method to create biocompatible SU-8 microfibers [5] coated with fibronectin on the culture substrate and the experimental findings on how such substrate influenced cell morphology are presented in this paper.

Materials and Method

1. SU-8 fiber preparation

The solution used for electrospinning was SU-8 2010 diluted with methyl ethyl ketone (MEK) in 90% (w/w) (SU-8 2010/MEK) concentration. The solution was stirred for one hour to ensure homogenous mixing. Then the resulting solution was drawn into a 10ml syringe, which was fixed onto an NE-1000 syringe pump to achieve a controlled and steady total volume flow rate during the electrospinning process. The optimized flow rate used in this study was found to be 50 μl/min. A stainless steel sheet covered with PVC tape was used as ground plate. A 22 mm × 22 mm cover glass for collecting the SU-8 fibers was placed on top of the stainless steel sheet. A 14 kV negatively biased voltage was applied to the stainless steel syringe needle, and the distance between the tip of the needle and the collector substrate was varied between 3 and 5 cm. The needle tip was polished to ensure the resulting Taylor cone during electrospinning was symmetric and oriented downward towards the ground collector, as shown in Figure 1. After the electrospinning process was initiated and stabilized, SU-8 microfibers were collected at the end of the electrospinning duration, which was varied between 30 s and 3 min. The collected SU-8 fibers on the cover glass or the 0.1 mm-thick polypropylene (PP) sheet were then exposed to UV light from a halogen lamp for 15 min

followed by a 175°C hard bake.

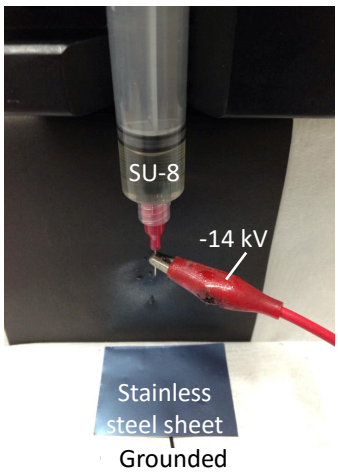

Figure 1. Experimental setup of the electrospinning process.

2. Cell culture and morphological studies

The cells chosen for this study were breast adenocarcinoma MDA-MB-231 cells. They were first cultured in a flask with Dulbecco's Modified Eagle Medium (DMEM) mixed with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Before loading the cells onto the glass coverslides or PP sheets with collected SU-8 microfibers, 5 µg/ml concentrated fibronectin (Merck) in sterile 1X phosphate-buffered saline (PBS) solution was used to coat fibronectin onto the surfaces for 15 minutes. After flushing the excess fibronectin with PBS solution, MDA-MB-231 cells were loaded on the substrates and allowed time to form adhesion with the fibronectin-coated surfaces. Cells that did not adhere on the substrates were flushed away after 2 hours. The remaining cells were cultured for 13 to 14 hours inside an incubator with 5% CO2 ambient. Afterwards, the cells were fixed and 4',6-diamidino-2-phenylindole (DAPI) was used to stain the nuclei, and the F-actin were stained with rhodamine phalloidin. The cells were then studied under an Olympus X71 inverted microscope with fluorescent imaging.

Results and Discussions

During the initial optimization studies of the electrospinning process, it was found that SU-8 microfibers of any desirable lengths were not formed correctly on the exposed stainless steel collector. Instead, short segments of SU-8 fibers were deposited onto the stainless steel surface. Subsequently, a glass coverslide was placed on top of the stainless steel plate as the collector. The electrospun SU-8 collected on the coverslide included both microfibers and variously-sized beads. We postulated that the formation of beads might be due to the cohesion force or surface tension (or both) of the SU-8 solution. Since SU-8 behaves hydrophobically on glass surfaces and hydrophilically on PP, we further postulated that if beads were formed on glass collector because of its hydrophobicity, then the use of hydrophilic PP should minimize the formation of beads. Figure 2 shows the images comparing electrospun SU-8 microfibers on glass (2A-2C) and PP (2D).

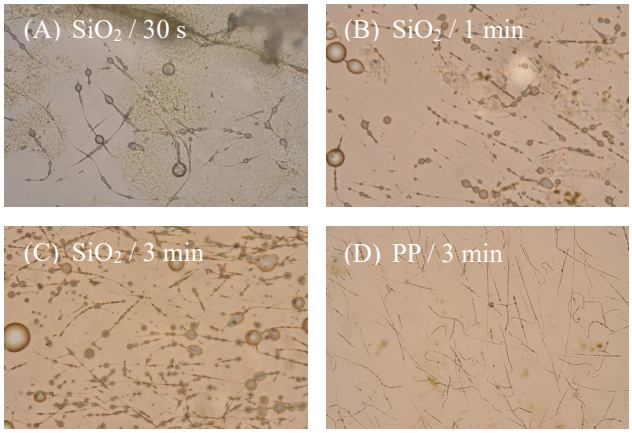

Figure 2. Electrospun SU-8 fibers on glass (A-C) and PP (D) collectors under different processing time (200X magnifications).

The experimental results suggested that larger SU-8 beads together with microfibers were formed on glass substrate when processing time was extended. On the other hand, electrospun SU-8 microfibers were quite stable at considerable length once they were formed on PP substrates (Figure 2D). These observations were consistent with the hypothesis that substrate hydrophobicity could play an important role in stable formation of electrspun SU-8 microfibers. The diameters of the SU-8 microfibers under these processing conditions were observed to be around 1 µm.

Both SU-8 microfiber coated glass and PP substrates were used for studying the influence of microfibers on the morphology of MDA-MB-231 cells. The influences of the microstructures provided by SU-8 microfibers was studied after 14-hour culture. Cells were fixed before first cell division. Figure 3 shows the cell morphologies of two of the cells interaced with SU-8 microfibers. It shows that cells did follow the orientation of SU-8 microfibers. It is also verified by the distribution of F-actin (red). It shows that the cytoskeleton network of cells react to the microstructures of SU-8 microfibers and coordinate its structure to the substrate topology. However, due to the SU-8 microfibers were relatively short. The morphology of cells were not fully corellated to the orientations of microfibers.

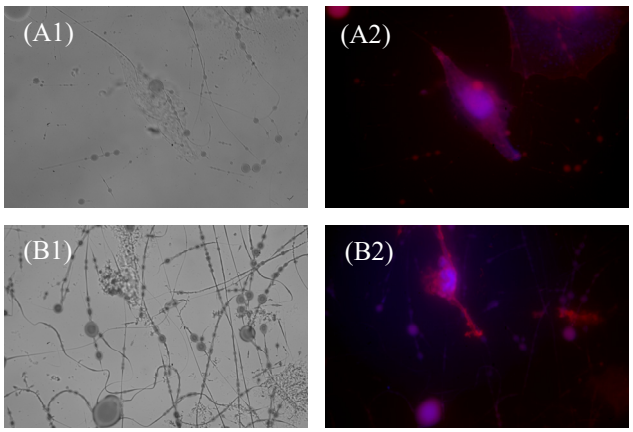

Figure 3 (A&B). MDA-MB-231 cells on a SU-8 microfiber coated glass slides. Left: bright-field images; Right: fluorescent images at 400X magnifications (blue nucleus; red F-actin).

To further understand the influence of SU-8 microfibers on cell morphologies, we use electrospun SU-8 microfibers on PP substrates. In this case, the SU-8 microfibers were more uniform and considerably longer than one cell length. Figure 4 shows the experimental results of cells cultured after 13 hours on PP. The correlations between cell morphology and SU-8 microfibers were more pronounced than those on glass substrates. In Figure 4A, two cells lined on both sides of one SU-8 microfiber. Their F-actin fibers (red) were also oriented in the same direction. Figure 4B shows one cell anchored on top of one SU-8 microfiber with the cell body spanning across the entire length. These results suggested that cells could directly interact with substrate topological features in the micro scale. By patterning the surface topologies with a SU-8 microfibers, we could control the orientation and morphology of cells. Potential applications in tissue engineering could include cardiomyocyte maturations [6] and axonal growth of neurons [7].

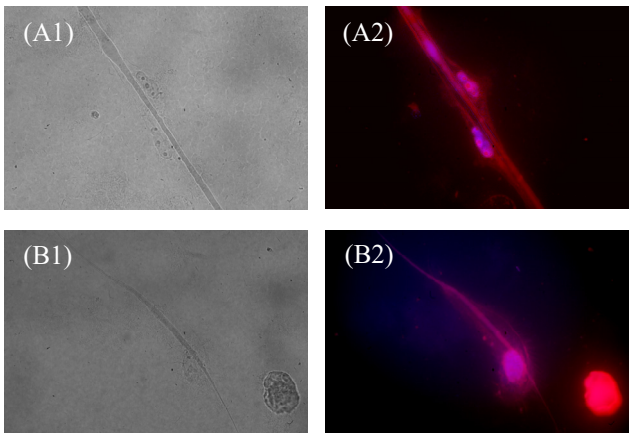

Figure 4(A&B) . MDA-MB-231 cells on a SU-8 microfiber coated PP sheets. Left: bright-field images; Right: fluorescent images at 1000X magnifications (blue nucleus; red actin).

Conclusion

We have demonstrated the techniques to generate SU-8 microfibers with electrospinning on glass and PP with varying degrees of uniformity and lengths. It was found that the hydrophobicity of the collector could be an important factor in minimizing bead formation based on comparing hydrophobic glass substrates and hydrophilic polypropylene sheets with respect to the wettability of SU-8 solution. We have shown that long SU-8 microfibers could be formed on PP sheets while SU-8 beads would be generated on glass substrates. Our experimental findings suggested that microfibers with width on the order of 1 μm could influence cell morphology and cytoskeleton orientations, demonstrating that SU-8 microfibers could serve as a means to control the orientations and polarizations of cells, and further to promote cardiomyocyte maturation or axonal growth in tissue engineering research.

Acknowledments

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