Electrospun Fiber with Microfluidic Device as a New Template to Study Endothelial Cell Alignment

Ravikanth Konjeti, Yi Liang, Ian Baird, Lucas H. Hofmeister, and Hak-Joon Sung

KEYWORDS. Microfluidics, cell alignment, microenvironment, Endothelial cells

BRIEF. The development of a microfluidic device to study endothelial cell alignment in the presence of competing stimuli.

ABSTRACT. This study developed a novel microfluidic platform for studying the effect of electrospun poly(ε-caprolactone) (PCL) fibers on endothelial cells. Electrospinning parameters were optimized to reliably produce aligned PCL fibers. Polymer fibers were then incorporated into microfluidic devices to simultaneously subject cells to fibers and fluid shear. We determined that endothelial cells prefer to align with a given fiber alignment. Work is still being done to incorporate fluid flow as a competing signal in this microfluidic model.

INTRODUCTION.

Cardiovascular disease (CVD) is the leading cause of mortality in developed countries. CVD causes over 25% of deaths in the United States while an estimated 17.3 million people died worldwide in 2008 from cardiovascular diseases [1]. Vascular mechanotransduction, the conversion of mechanical motion into chemical signaling, is a major contributor to CVD development. The endothelium, an inner monolayer of cells that line all blood vessels, is a major mediator in control of mechanotransduction. Hence, study of endothelial cell (ECs) response to mechanical stimuli can provide ground-breaking strides in CVD treatments.

Endothelial cells within the body are highly responsive to many different stimuli, especially fluid shear from blood flow and membrane signals from the cellular matrix. These signals influence cell morphology and alignment. Studies have shown that the presence of a disease, such as aneurism and arteriosclerosis, alters the alignment of the endothelial cells [2,3]. On the contrary, stimuli given to the cell from the microenvironment, such as polymer fibers and fluid shear, have shown to affect and change the cell alignment as ECs react to their surroundings [4,5]. By understanding the correlation between cells and stimuli in regards to cell alignment, the effects of disease mechanisms and infiltrations can be better understood.

This study examined the effects of one of the two competing microenvironmental cues, fiber matrix, on Human Umbilical Vein Endothelial Cells (HUVECs). As they are easily cultured, the HUVECs were subjected to poly(ε-caprolactone) (PCL) nanofibers to control matrix morphology. A microfluidic device was adopted to study the effects of the signals on HUVEC alignment. We hypothesize that cells will align parallel to fiber direction. By better understanding the basic levels of cellular communication and interaction within its given environment, further strides can be made in the development of cures and treatments for cardiovascular diseases.

MATERIALS AND METHODS.

Electrospinning.

Electrospinning was used to fabricate polymer fiber mats for control of matrix morphology during endothelial cell alignment tests. Electrospinning is a method that uses high voltages to eject polymer solution in fibrous form evenly onto a spinning substrate. A 10% PCL solution in 70:30 chloroform:dimethylformamide was spun onto Polydimethylsiloxane (PDMS) with and without flanking aluminum strips. PCL was incorporated due to its predominant usage in bioengineering and regenerative medicine.

Fiber Optimization.

The electrospinning parameters we first characterized to produce polymer fibers with different alignments. Six different parameters were systematically altered: voltage, spin rate, flow rate, distance, drum diameter, and duration. Each of these parameters affects the different aspects of the fiber alignment.

Microfluidic Device Design.

A microfluidic device was created to examine endothelial cells while providing controlled fluid flow. The design consisted of an input, through which the cells and media would enter the device; the channels, where cells would adhere and proliferate while being subjected to the fibers and flow; and the output, through which dead cells and old media are removed. A schematic, created in AutoCad (Autodesk, San Francisco, California), shown in Figure 1 is the template of the actual device. The devices were produced through photolithography-soft lithography using a photoepoxy (MicroChem SU-8 2100, Newton, Massachusetts) and PDMS (Dow Corning Sylgard 184, Midland Michigan).

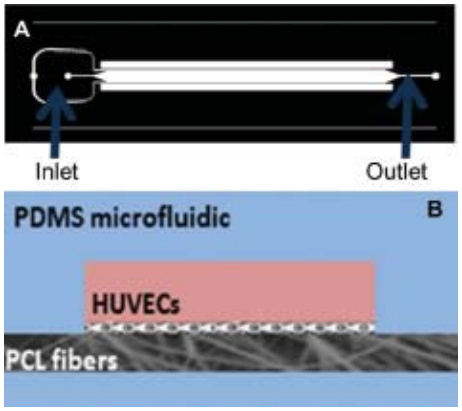

Figure 1 (A) Top view of microfluidic design. (B) Section view showing HUVECs cultured on PCL fibers.

Cell Alignment on Fiber Mats.

Cell orientation was first studied through a single isolated physical signal provided by the aligned electrospun PCL fibers. Samples obtained from the electrospinner were sterilized under ultraviolet light in a closed tissue culture hood for 30 minutes. Samples were then placed in a well plate and washed twice with PBS. The wells were then filled with M200 with Low Serum Growth Supplement kit culture media (Invitrogen, Carlsbad, California) and HUVECs were then trypsinized from a plate culture and seeded onto the center of the substrates. After 24 hours, the substates were imaged on a Nikon Ti inverted microscope and analyzed through ImageJ (NIH, Bethesda, MD). Cell and fiber angles were then compared using a two sided students T-test.

RESULTS.

Fiber Optimization.

Electrospinning parameter optimization was used to identify electrospinning parameters which produced aligned fibers. Images of the parameter optimization are represented below (Figure 2) while quantitative data is shown in Table 1. Fiber alignment is presented as ±standard deviation of fiber angle. The most highly aligned fiber parameters are highlighted in yellow.

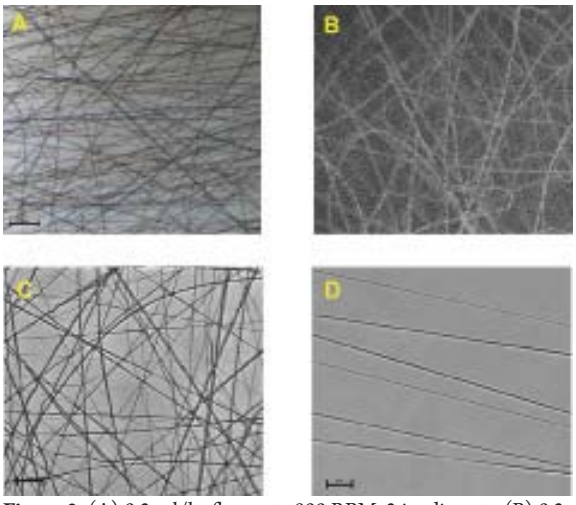

Figure 2. (A) 0.2 ml/hr flow rate, 800 RPM, 2 in. diameter(B) 0.2 ml/hr flow rate, 1000 RPM, 2 in. diameter (C) 0.05 ml/hr flow rate, 900 RPM, 2 in. diameter (D) 0.02 ml/hr flow rate, 1350 RPM, 3.33 in. diameter (Supported Materials). Images are of the electrospun PDMS substrates.

Table 1. A table of the parameters and alignment results.

Voltage (kV)	Flow Rate (mL/h)	Distance (mm)	Spin Rate (RPM)	Time (min)	Drum Diameter (in)	Fiber Alignment
10	0.60	100	900	10	2	±42.8˚
15	1.20	100	900	10	2	±47.4 ˚
10	0.40	100	800	15	2	±54.5 ˚
10	0.20	100	800	10	2	±46.7 ˚
10	0.20	100	1000	25	2	±40.6 ˚
15	0.40	70	600	15	2	±52.5 ˚
15	0.10	50	800	20	2	±41.2 ˚
15	0.20	50	900	15	2	±52.5 ˚
15	0.05	75	900	15	2	±40.5 ˚
15	0.01	75	900	10	2	±49.0 ˚
10	0.05	50	900	20	2	±44.4 ˚
10	0.01	50	900	20	2	±36.1 ˚
15	0.01	50	1300	20	3.33	±53.7 ˚
10	0.02	150	1350	20	3.33	±4.1 ˚

Fibers were imaged and the alignment of the PCL fibers was analyzed using ImageJ. The fiber distribution is determined by creating a horizontal and vertical axis on the image. The fibers are then identified and the angles are derived through the arbitrary axes on the image. The frequency of the fiber angles is shown in Figure 3.

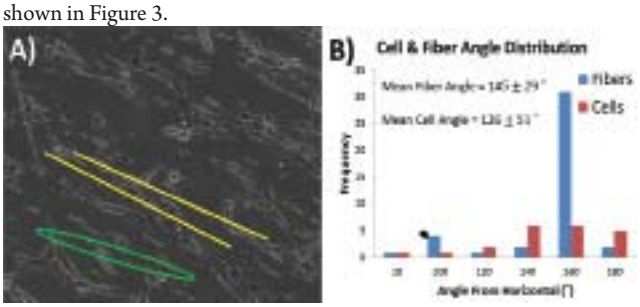

Figure 3. (A) An image of seeded cells on electrospun aligned fiber mats. The yellow lines show an example of the aligned fibers. The green region highlights the area where cell are aligned (B) A histogram showing the angle distribution of the cells seeded and the PCL fiber distribution

The highest frequency of the fiber alignment at the 160 degree mark strongly correlates with the high peaks of the cell alignment. Mean cell angle is not significantly different from the fiber angle ($p<0.05$) hence, indicating that cells aligned on PCL fibers. However, the variance presently shows only a preferential alignment between the two. The median shows that the center of the data set falls within the most frequent fiber angle. These statistics show that cell do in fact align with fibers.

DISCUSSION.

First and foremost, the electrospinning optimization proved to be beneficial. The polymer deposition drastically changed as the flow rate, spin rate, and drum diameter changed. Drum diameter and flow rate had the largest effect on polymer fiber morphology of all of the parameters. Drum diameter changes the linear velocity of the surface of the rotating mandrel according to the equation below.

$$\textbf{Linear Velocity} = \frac{\textbf{Drum Diameter}}{\textbf{2}} \times \textbf{Spin Rate}$$

Using the equation, we see that increasing the diameter and the spin rate can increase the linear velocity, which affects the speed at the surface of the substrate. By increasing the linear velocity, the fibers are then theoretically stretched across the PDMS substrate. Previous research has shown that high spin rates and low flow rates can produce aligned fiber through electrospinning [6]. The spin duration determined the density of the fiber deposition upon the PDMS; longer time intervals allots for more fiber deposition. Comparing the parameter optimization and aluminum placement, the parameters seemed to have had a greater influence on alignment. The methods of ensuring fiber alignment through modifications with the electrospinning apparatus have caused a significant difference with the fiber alignment. Using aluminum strips and a parameter set up at 1350 RPM at a 100 mm distance at 10 kV with a range of 0.1 and 0.2 ml/hr flow rate on a 3.33 diameter drum, aligned PCL fibers can be produced for future experiments. This method can be used in future tests to produce not only aligned PCL fibers, but also numerous other polymer compositions upon different substrates.

After optimizing electrospinning parameters to produce aligned fibers, these parameters were used to produce aligned fiber mats for cell experiments. On experimental mats, fibers were aligned with an angle distribution of 145±29 degrees ($p<0.05$). The aligned electrospun fibers were seeded with cells to see their effect on cell alignment. The difference in means, a 126 degree value for the fibers and a 145 degree mean for the cells, shows a relative alignment among the two. The HUVECs were found to be significantly aligned with fibers ($p<0.025$), indicating that HUVECs alignment is parallel to that of its surrounding environment. More testing can provide more conclusive results. By increasing fiber density and cell incubation time, cell alignment could be further improved.

The microfluidic experiment results are lacking due to difficulties with culturing HUVECs in microfluidic devices. Long-term cell viability within the devices has been difficult to achieve despite the ease of HUVEC plate culturing. There has been evidence of cell adhesion to the microfluidics but cell death has caused the nullification of the microfluidic tests. Finding an optimum flow rate could possibly solve this issue. With slow flow rates, cells death is triggered by the lack of nutrients whereas fast flow rates cause cells to detach from the microfluidic channel. Hence, a flow rate must be resolute for cell culturing within the device.

The cell alignment elucidates the effect of microenvironmental factors. The effects of the PCL fibers shows that the cells do tend to align along the direction of the fibers. This supports previous work, and provides a novel method of studying the alignment of human umbilical vein endothelial cells in a controlled environment.

CONCLUSION.

The study overall has provided insight into the effects of microenvironmental factors on a major cell function, in this case, endothelial cell alignment. Through the usage of electrospinning, fibrous polycaprolactone on a PDMS substrate provides a physical signal for HUVEC alignment. The seeded cells upon these fibers were able to show that cells to interact with these physical signals and preferentially align parallel to the direction of the fibers.

The novelty of the microfluidics application in studying morphological indicators of cell responses adds to an already flexible branch of microfluidics. Despite the continual efforts in troubleshooting the microfluidics, the study has shown that proper parameters can provide an apparatus that allows for the microenvironmental study of cells.

First and foremost, cell viability in microfluidics must be achieved to complete the study. By finding a flow rate that can provide continuous nutrients to the cells without detachment, proper cell viability can be maintained for the competitive signals to be tested. This can generate a better image of the microenvironmental cues on cell alignment. Dealing with the electrospinning method, finding an optimized setting can provide consistency in the production of fibrous PCL as well as production of different densities, which can affect the amount of physical signaling the cells receive. Although we found significant alignment, the high variance is an indication that improvements must be made for future tests. By increasing the density of the fiber mats, we could potentially eliminate the high range in variance. Higher densities can provide a stronger barrier between the cells, forcing a certain alignment. Also, repeated tests of the cell and fiber alignment can further validate the results found within this study and provide a stronger correlation between cell and fiber alignment.

The apparatus developed here can act as a model for future tests in studying microenvironmental cues. The application of this model can similarly be used to study other functions of cells outside of morphology and alignment.

By understanding the effects of individual signals on cell alignment, cardiovascular diseases could be better understood as these diseases manipulate the endothelial alignment. This study provides a model for future tests in studying microenvironmental signals on cell alignment.

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SUPPORTING INFORMATION.

S1. Table of Parameters
S2. Cell Seed in Devices
S3. Aluminum Strips Placement
S4. Fiber Images

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Ravikanth Konjeti is a student at Martin Luther King Jr. Magnet High School and enrolled in the School for Science and Math at Vanderbilt.