EFFECT OF TOPOGRAPHIC CUES ON IN VITRO CULTURED TRABECULAR MESHWORK ENDOTHELIAL CELLS

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ABSTRACT

This paper reports the response of trabecular meshwork (TM) endothelial cells to various topographical cues at microscale and nanoscale. Cells are cultured on substrates with aligned and random features fabricated using electrospinning and microfabrication. The expression of myocilin, a characteristic protein of TM cells, is examined. The results show that all the topographic surfaces up-regulate myocilin than the planar surface. At microscale, the myocilin expression of cells on random features are significantly greater than those on linearly aligned features. This trend diminishes when the feature size decreases to the nanoscale. It is also found that the microstructured nanofibrous matrix combining the topographic characteristics of both aligned and random features not only exhibits significantly up-regulated myocilin, but also demonstrates cell alignment. The result indicates that topographic cue is a potent regulator of TM function. Its effect on the outflow resistance can be used to design new therapeutics for regulation of intraocular pressure.

KEYWORDS: Electrospinning, Microfabrication, Topographic cue; Trabecular meshwork endothelial cells

INTRODUCTION

Aqueous humor is a clear, colorless fluid that delivers metabolites and removes catabolites in the eye. It is produced by the ciliary body, flows radially inward, baths the lens, and drains out of the eye through specialized tissues known as outflow facility. If the flow resistance of the outflow facility increases, the intraocular pressure (IOP) increases. This is called primary open angle glaucoma, a leading cause of blindness. Figure 1 illustrates the outflow facility of the aqueous humor, which consists of a three-dimensional matrix of trabecular meshwork (TM) and the inner wall endothelium of Schlemm's canal. Juxtacanalicular tissue is the outmost region of the TM that has relatively dense matrix structures. This tissue is believed has a significant contribution to the IOP increase [1]. In the JCT, TM cells reside on the nanoscale extracellular matrix (ECM). The cells are also in direct contact with the inner wall surface of the Schlemm's canal which has various topographic features [2, 3]. Therefore, the effect of topographic cues on TM and hence the IOP needs to be understood.

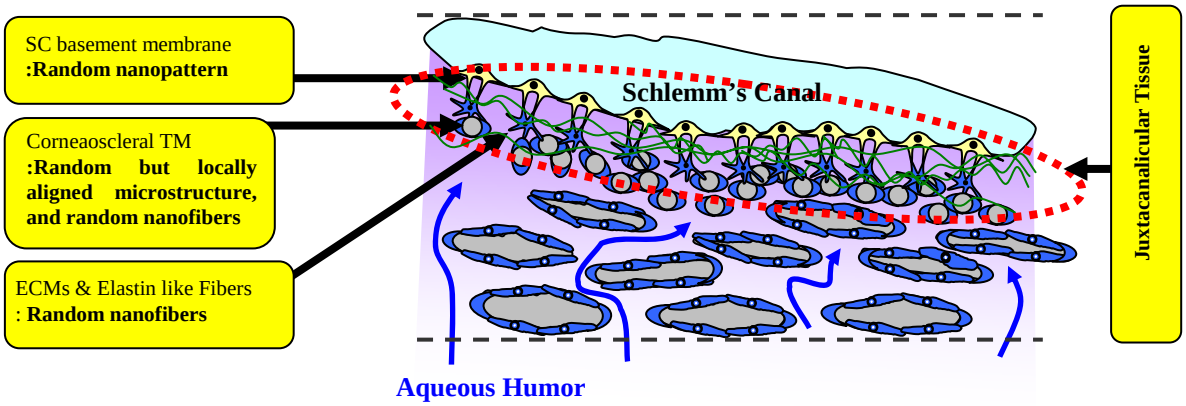

Figure 1: Schematic view of the outflow facility of aqueous humor.

Most *in vitro* studies of TM tissues, however, are over-simplified. These studies overlook the local topographic cues and culture TM cells on planar surfaces [4]. The propound topographic difference between the planar surfaces and the complicated *in vivo* topographic environment may cause vastly different cell responses, making it challenging to interpret the data with biological relevance. It has found that TM cells cultured on linear nanostructures exhibit a significantly greater level of myocilin, a characteristic protein that can be used to distinguish TM cells, than those cultured on the planar surface [5-6]. Despite the exciting finding, a systematic examination of the effect of topographic cues on TM is yet to come.

In this study, a systematic examination of the effect of microscale and nanoscale topographic cues on TM behavior is reported. Linearly aligned and random aligned features are fabricated using solithography and electrospinning process. These features either mimic the natural topography in the *in vivo* environment, or represent engineered topographies that may be potent for regulating the TM behavior. The effect of the characteristic dimensions of these topographies and the alignment states on TM cells are investigated.

EXPERIMENTAL

Figure 2 illustrates the experimental groups of different topographic cues. The random features include microbubble surfaces with the characteristic dimensions at microscale and randomly aligned electrospun nanofibers with the characteristic dimensions at nanoscale. The aligned features include linear microstructures with the characteristic dimensions at microscale and aligned electrospun nanofibers with the characteristic dimensions at nanoscale. In addition, a porous matrix with microstructured electrospun nanofibers is used, where the linear microstructures is made up of randomly aligned nanofibers. A planar substrate is used as the control group. To eliminate the effect of materials, all the specimens are made of poly(etherurethane)urea (PEUU) polymer. In particular, the microbubble patterns is generated by dissolving KCl crystals into PEUU prepolymer. After the polymer is cured and the KCl is crystallized, the salt is dissolved by immersing the entire specimen into aqueous solution. Linearly aligned PEUU microstructures are fabricated using a standard softlithographic process. Randomly aligned PEUU nanofibers are fabricated using a custom-designed electrospinning setup, where the polymer is pumped through a syringe that is connected to a high positive voltage. The electrostatic force whips the polymer on a planar collecting substrate that is set as the ground. Linearly aligned PEUU nanofibers are fabricated by electrospinning the polymer on a rotating collector spinning at 2500rpm. Microstructured nanofibers are fabricated by electrospinning the polymer on a micropatterned collecting surface.

Human TM endothelial cells are obtained from three donor eyes and cultured on the specimens. After two-week culturing, the TM cells are fixed. Actin filaments, nuclei, and myocilin are immunostained. Western blotting is performed to assess myocilin expression. There are three specimens in each group. T-tests with Bonferroni correction are used for multiple comparisons.

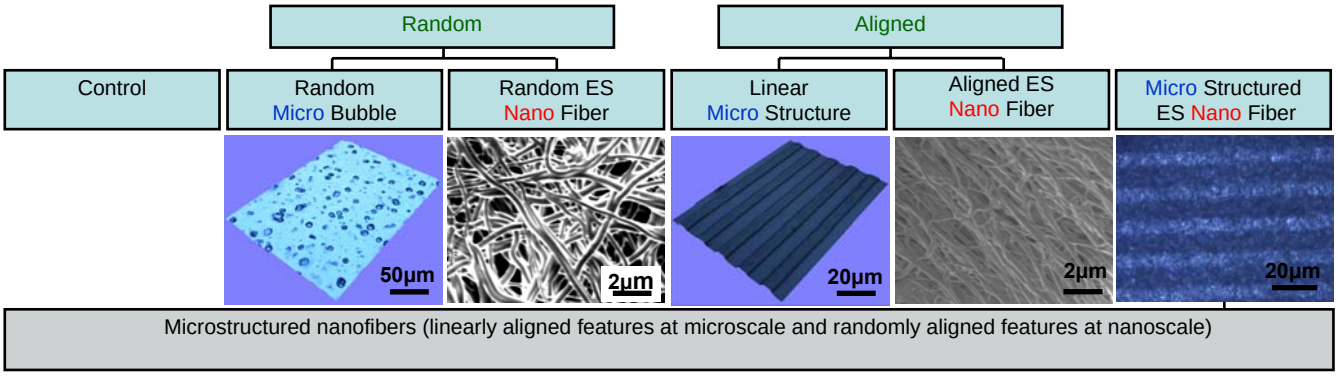

Figure 2: Experimental groups for topographic study of TM cells.

RESULTS AND DISCUSSION

The immunofluorescence images show that microscale and nanoscale topographies can both align TM cells along the longitudinal axis of the linear structures. The morphology is in contrast to the randomly oriented TM structures in the control group (Figure 3). It is also found that myocilin expression in the topographic groups are greater than that in the control group, as indicated by the enhanced intensities of myocilin in cytosol.

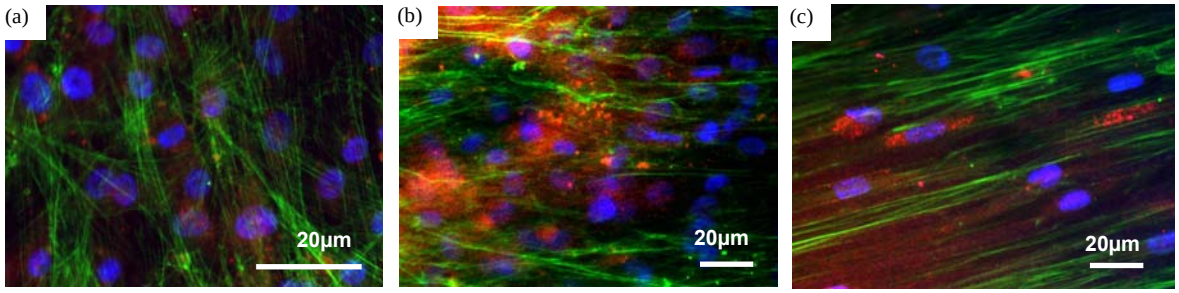

Figure 3: Immunofluorescence micrographs of TM cells cultured on substrates with different topographies; (a) flat substrate (control); (b) linearly aligned electrospun nanofibers,; and (c) linearly aligned microstructures. Myocilin (red), actin filament (green), and nuclei (blue).

Statistical analysis shows that myocilin expression in the group of random microbubbles is significantly greater than that in the group of aligned microstructures with $P<0.005$ (Figure 4a). When the characteristic dimension of the topographies decreases to nanoscale, myocilin expression seems to increase, while the difference of myocilin expressions between the linearly aligned and randomly aligned groups diminishes (Figure 4c). It is also found that the myocilin expression in the micro-

structured nanofibers group is significantly greater than that in the aligned microstructures group (Figure 4c), which indicates that the randomly aligned nanotopography in the microstructured nanofibrous matrix plays a significant role in up-regulating myocilin expression. Since these random nanofibers share similar topography with natural ECMs, the result may suggest that the fibrous structures in ECMs are important in maintaining the *in vivo* function of TM tissues. Finally, comparison between the groups of random microbubbles and the microstructured nanofibrous matrix shows that cell alignment and myocilin expression can be controlled independently.

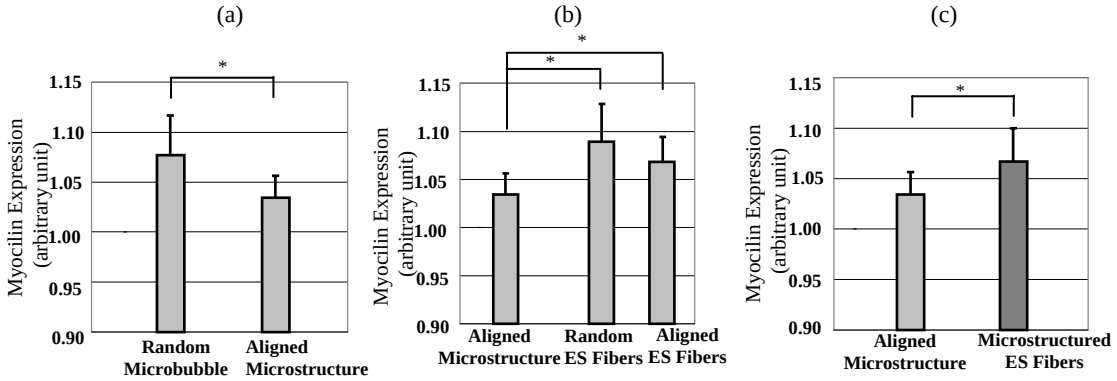

Figure 4: Statistical analysis of myocilin expressions in different specimens. (a) microscale topography (b) nanoscale topography and a comparison with the linearly aligned microscale topography; and (c) comparison of the linearly aligned microscale topography and the microstructured nanofibrous matrix. All data are normalized by the control value. Statistically significant values are marked by asterisks.

CONCLUSION

Topographic cues are important factors that can lead to more *in vivo* like characteristics of human TM endothelial cells. Nanoscale topographies seem to be more effective in up-regulating myocilin than microscale counterparts. At microscale, randomly aligned features are more effective in up-regulating myocilin than linearly aligned features. Linearly aligned TM cells with enhanced myocilin expression can be obtained by culturing cells on microstructured nanofibrous matrix. This is useful for studying the layer of TM cells that is immediately adjacent to the linearly aligned Schlemm's canal endothelial cells. It is also demonstrated that the alignment and the myocilin expression can be independently controlled, which offers a way for investigating their individual and coupled impacts on the outflow resistance.

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