Response of Corneal Epithelial Cells to Mechanical Properties of their Substrate

Molladavoodi S[1]; Medley JB[1]; Kwon HJ[1]; Gorbet MB[2]
[1] Department of Mechanical and Mechatronics Engineering
[2] Department of Systems Design Engineering
University of Waterloo, Waterloo, ON, Canada

Introduction:

In the physiological environment of the human body, chemical signals such as signaling molecules, growth factors or chemoattractants can induce specific behaviour. Nowadays, it is well-recognized that cells not only respond to chemical signals, but also respond to mechanical signals [1]. These mechanical stimuli can be either external mechanical stresses being applied to the cells [2] or they can be derived from the mechanical properties of the material substrate that cells are interacting with [1].

The mechanical properties of the cornea have been reported to change with age (increased elastic modulus [3]) as well as with diseases such as keratoconus, whereby a significant thinning of the cornea and a reduced elastic modulus have been observed [4,5]. Although morphological changes have been observed in corneal epithelial cells [6,7], it is not known whether variations in the elastic modulus of the cornea affects corneal epithelial cell behavior. In the current study, human corneal epithelial cells (HCEC) were cultured on substrates with different mechanical properties and the expression of adhesion molecules (integrin α_3 and β_1) was studied. These cell membrane receptors influence cell adhesion (cell spreading and hemidesmosome stability), wound healing and also contribute to the control of the epithelial cell cycle [8].

Materials and Methods:

Polyacrylamide (PAA) membranes with different elastic moduli were prepared. First, coverslips were activated by soaking in (3-aminopropyl) trimethoxysilane (APTMS) for 10 minutes and then in glutaraldehyde for 30 minutes. Then, polyacrylamide solutions with different concentrations of acrylamide and bis-acrylamide were prepared. Polymerization was started by adding 10% ammonium persulfate solution (APS) and tetramethylethylenediamine (TEMED) to the solutions. A small amount of this solution (~15 µl) was put on a microscope slide and the activated coverslip was placed on top thus spreading it. This setup was left for 15-30 minutes for the polymer solution to gel. Then the coverslip was peeled from microscope slide and the surface of PAA gel was treated with Sulfo-SANPAH for 10 minutes using a UV light source. Surfaces of PAA gels were coated with collagen type I and left at 37 ^{o}C for 45 minutes. Cells were then seeded on different gels and samples were incubated overnight. Cells were also seeded on tissue culture treated polystyrene (TCPS) and they acted as control cells.

Mechanical properties of substrates were measured by a simple compression test using a universal material testing machine (Texture analyzer.xt Plus, Stable Micro Systems, New Jersey) with a 49 N (5 kgf) load cell. In order to study cell behavior, adhesion molecules and specifically integrin-$\alpha_3\beta_1$ was studied using flow cytometry. Following overnight incubation, cells were detached from membranes and were incubated with fluorescently-labeled antibodies against integrin-β_1 (CD29) and integrin-α_3 (CD49c). Cells were then fixed and analyzed using flow cytometry.

For statistical analysis, samples were compared using the Student t-test. A p value of less than 0.05 was required for statistical significance. The number of experiments was equal to three with different cell passages. For each experiment, all materials were tested at the same time.

Results and Discussion:

As expected (Table 1), increasing Bis-crylamide concentration resulted in changes in the mechanical properties of the PAA substrates [9]. Change in expression of Integrins α3 and β1 are shown in Figure 1.

Table 1: PAA substrates used in this study

Sample name	Acrylamide concentration (%)	Bis-acrylamide concentration (%)	Elastic modulus (kPa)
Compliant	10	0.01	4
Medium	10	0.10	26
Stiff	10	0.30	69

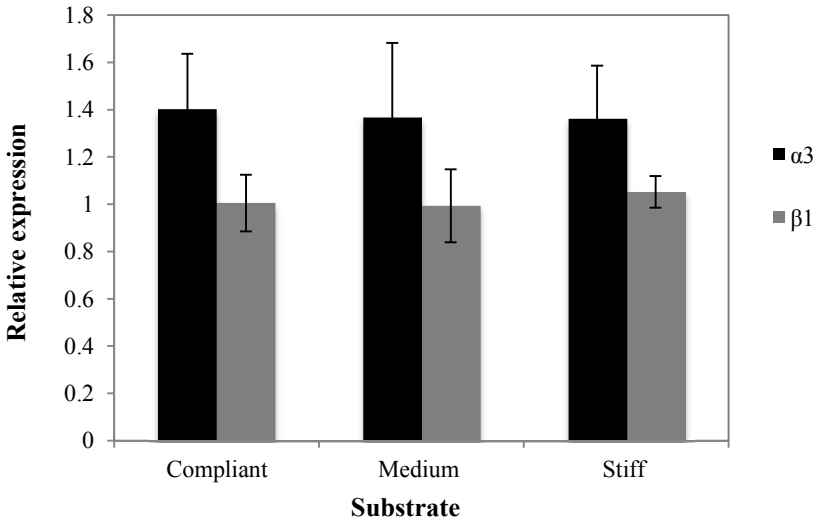

Figure 1: Relative expression of markers after overnight incubation on PAA substrates. Integrin expression was measured by flow cytometry and given as a percentage of cells grown on TCPS.

To determine the elastic modulus of the substrates, compression tests were also performed on samples after UV treatment. While UV treatment increased stiffness slightly, the difference in elastic modulus between samples remained and samples could still be categorized as compliant, medium and stiff.

When compared to control cells (cells seeded on TCPS), slight upregulation in integrin-α_3 was observed, with no change in integrin-β_1. Contrary to previous studies with fibroblasts which showed increase in cell attachment with increasing mechanical properties in collagen scaffolds [10], in our current study, the difference in stiffness of the substrate did not show statistically significant changes in corneal epithelial cell expression of α_3 and β_1. Since α_3 and β_1 are important receptors in cell adhesion and wound healing, the lack of response to variations in stiffness warrants further investigation. To gain a better understanding of the corneal epithelial cell behavior on materials with different stiffness, future work will include characterizing cell morphology (cytoskeleton) as well as cell activation.

References: [1] Discher DE, Janmey P, Wang Y. Science 2005;310:1139. [2] Zaidel-Bar R, Kam Z, Geiger B. J Cell Sci 2005;118:3997. [3] Last JA, Liliensiek SJ, Nealey PF, Murphy CJ. Struct Biol 2009;167:19. [4] Piñero DP, Nieto JC, Lopez-Miguel A. J Cataract Refract Surg 2012;38:2167. [5] Romero-Jiménez M, Santodomingo-Rubido J, Wolffsohn JS. Contact Lens and Anterior Eye 2010;33:15. [6] Efron N, Hollingsworth JG. CLIN EXP OPTOM. 2008;91:34. [7] Tsubota K, Mashima Y, Murata H, Sato N, Ogata T. Cornea 1995;14:77. [8] Stepp MA. Exp Eye Res 2006;83:3. [9] Pelham RJ, Wang Y (1997) Cell locomotion and focal adhesions are regulated by substrate flexibility. Proc Natl Acad Sci USA, 94, 13661. [10] Haugh MG, Murphy CM, McKiernan RC, Altenbuchner C, O'Brien FJ (2011). Tissue Eng Pt A, 17,1201.