

# CHARACTERIZATION OF ALTERATIONS IN ELASTIC MODULUS OF CULTURED HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS SUBJECTED TO CYCLIC TENSION

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## INTRODUCTION

Mechanical environment of arterial endothelial cells (ECs) is an important factor affecting endothelial functionality. ECs respond to the mechanical stimuli by adaptation and remodeling [1]. ECs are constantly subjected to pressure induced cyclic stretch *in vivo*. The aim of this study is to characterize effects of cyclic loading on the Young's modulus of ECs subjected to cyclic stretch.

## METHODS

Human Umbilical Vein Endothelial Cells (HUVECs) were cultured using DMEM with 10% fetal bovine serum plus other supplements. Cells were transferred on the central region of silicon membrane, pre-coated with collagen type I. After one night incubation for a proper attachment, cells were subjected to uniaxial cyclic stretch using a custom built apparatus. Test conditions include load frequency of 1 Hz, strain amplitudes of 10% and 20%, and test durations of 2, 4, 6 and 8 hours. After detaching from substrate with Trypsine, mechanical properties of ECs were studied using Micropipette aspiration method according to published protocols [2]. During application of pressure, cell images were recorded through invert microscope.

Cells are assumed to be homogenous and incompressible [2]. After applying negative pressure, endothelial cells deform and aspirate in, according to the elastic model described by the following equation [2].

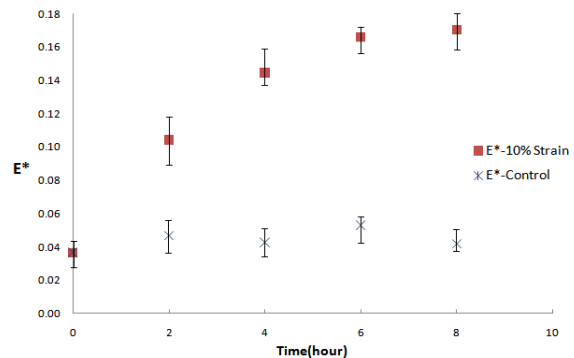
$$E = \frac{3a \Delta P \varphi(n)}{2\pi L}$$

Where E is Young's modulus of Endothelial cells, a is micropipette inner radius, L is aspirated length,  $\Delta P$  is aspiration pressure, and  $\varphi(n)$  is a function describing geometry of micropipette.  $E^*$  (the ratio of E to aspiration pressure) was determined according to above equation.

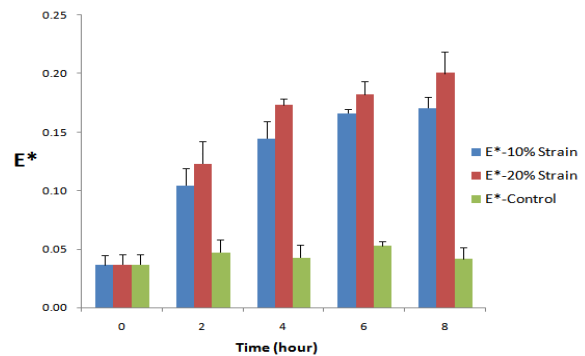
## RESULTS AND DISCUSSION

Morphological alterations of ECs due to cyclic tension have been shown in previous research [3]. In addition to morphological changes, mechanical properties of ECs are influenced by their mechanical environment. Figure 1 describes stiffening of ECs by increase of number of load cycles. By elevation of strain amplitude, the degree of stiffening is intensified (Fig 2). Stiffening of ECs with loading time and amplitude was statistically significant using ANOVA analysis ( $P < 0.03$ ). Results indicate that ECs respond to pulsatile stretch by structural adaptation determined by increased stiffness. Previous studies showed that Actin filaments are major determinants of mechanical properties of ECs. It has been shown that actin microfilaments of ECs form stress fibers due to cyclic stretch [4]. The formation of such stress fibers might be the

main source of increased Young's modulus in the stretched ECs.



**Figure 1:** Alteration of cell stiffness with test duration (hours) for strain amplitude of 10%.



**Figure 2:** Alteration of cell stiffness with test duration (hours) for strain amplitudes of 10% and 20%.

## CONCLUSIONS

In this study we examined effect of cyclic stretch on the young's modulus of ECs using micropipette aspiration method. We concluded that cyclic stretch causes stiffening of ECs. This is one of adaptive responses of cells to their physico-mechanical environment. Such phenomenon is of importance in reconstruction of damaged tissues using engineered cells.

## REFERENCES

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