Effects of varying degrees of surface strain anisotropies on endothelial cells

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\textbf{Introduction:} Cyclic strain is well known to affect cell behavior. It is also known that isotropic and anisotropic strain can affect cells differently\textsuperscript{(1)}. While in-vivo cells experience varying degrees of anisotropy (d.o.a.), in-vitro anisotropic strain studies have mostly focused on uniaxial strains. In order to create a better understanding of cellular behaviour under physiological strain conditions, the response of cells to strains with varying d.o.a. should be investigated.

In this study, we determined the effects of varying d.o.a. on human umbilical vein endothelial cells (HUVECs) using a newly developed device. Studies like this can be used to determine and optimize the mechanical stimulation needed to elicit physiological cellular responses, and are beneficial for applications such as tissue engineering and organs-on-chip systems.

\textbf{Materials and Methods:} The device, which is a modified version of our previously developed device to apply surface strains in combination with flow induced shear stresses to cells\textsuperscript{(2)}, has 100 units producing various anisotropic strains (1a). This is achieved by stretching a polydimethylsiloxane (PDMS) membrane over circular pillars into surrounding ellipse trenches. The dimensions of the ellipse determine the d.o.a., which is defined as the ratio of maximum to minimum principal surface strains. Each condition contains four replicates. The presence of fluid flow channels allows for the determination of combined effects of anisotropic strains and flow induced shear stresses, which is currently under investigation.

Computational models were used to aid in the device design (1b) and the strains were empirically characterized by tracking beads embedded in the membrane (1c).
HUVECs were seeded on the device, allowed to attach for 18 hours and then stained with CellTracker Green CMFDA. The cells were mechanically stimulated (maximum principal strain up to 10% at 1 Hz, sine strain profile) and imaged after 0, 6 and 19 hours of stimulation. The cells were then fixed and stained with Alexa 488 Phalloidin (actin stain) and DAPI (nuclear stain) and imaged again. Images were analyzed using CellProfiler to detect the effects of the mechanical stimuli on the cells.

**Results:** The models and empirical measurements showed that strains with varying d.o.a. could be generated on the device. Maximum principal strains up to ~20% could be achieved.

HUVECs were found to become elongated and align along the minimum principal strain direction. Alignment was visible after 6 hours of stimulation and increased with longer stimulation times. Apart from that, an increase in d.o.a. resulted in increased cell alignment (2a-c).

**Conclusions:** HUVECs respond to various d.o.a. With an increasing d.o.a., the cells show an increasing alignment response. The variations in response of cells highlight the need to study the effects of strains of varying d.o.a. on cells. Our device permits such
experiments with an increased throughput, which makes it an important tool to better understand these mechanobiological principles.

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References: