A Decoupled Multi-Stimulus Bioreactor for Studying Complex Chemo-Mechanical Microenvironments *In Vitro*

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Statement of Purpose: In the body, cells exist in a complex chemo-mechanical microenvironment, which works to regulate the cell's functional behavior[1]. In recent years, many novel bioreactor systems have been developed to expose cells to individual stimuli such as fluid wall shear stress (WSS), cyclic stretching, hydrostatic pressure, substrate stiffness, substrate topography, and extracellular matrix proteins. These systems have led to the growing appreciation for the role mechanical forces have in regulating cellular behavior; however, few approaches are material independent and allow for the systematic variation of multiple combinations of forces in a single device[2]. We have developed the Universal Mechano-Tester™, a unique, cost-efficient, bioreactor system for independently and dynamically varying WSS, flow regime, cyclic stretch, hydrostatic pressure, and cell culture substrate including the substrate's stiffness, topography, and extracellular matrix (ECM) components.

Methods: A novel polydimethylsiloxane (PDMS) chamber was designed to decouple mechanical forces for their independent control during culture. It was fabricated by casting Sylgard 184 PDMS elastomer in custom 3D printed molds as two halves. The bottom half featured a specific defined inset region, termed the cell culture region (CCR), which was filled with either PDMS or polyacrylamide (PA). Benzophenone was used to covalently bond PA to PDMS[3]. Surface topography was transferred to the CCR using molds placed over the region during the curing process of the filler material. The two halves of the chamber were then sealed together using PDMS as an adhesive and sterilized. The CCR was treated with sulfo-SANPAH to conjugate RGD peptide or a DNA aptamer with an amine end group to the substrate surface. Human umbilical vein endothelial cells (HUVECs) were either seeded onto the CCR by filling the inside of the chamber with a cell suspension or flowed through the chamber and for capture on the CCR. The chamber was fitted with custom 3D printed Luer connectors to facilitate connection to a flow circuit. Extensive finite element (FEM) and computational fluid dynamics (CFD) simulations were conducted using the ANSYS 19.1 engineering simulation software to understand the fluid-structure interaction that arose from the simultaneous stretching of the chamber and flowing of fluid through the chamber flow channel.

Results: The chamber featured a rectangular flow channel (100 x 10 x 2 mm) and a pair of perpendicularly oriented protruding struts for stretching (**Figure 1**).

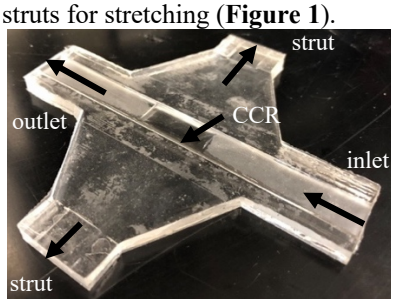

Figure 1: Bioreactor chamber

The location of the CCR was determined from FEM simulations of the chamber defined as the region in which a near-uniform strain field developed; specifically, a centrally located 20 x 10 mm rectangle in the flow channel wall. The struts when stretched a distance of 3.5 mm resulted in 10% strain of the CCR, which varied linearly with distance. In stretching the chamber, the flow channel geometry dynamically changes resulting in a varying WSS over the CCR. By dynamically adjusting the inlet flow rate, we hypothesized we could compensate for this variation to realize a constant WSS during stretching. We simulated this using a 1 Hz, 10% strain sinusoidal stretching profile and an inlet velocity of the form, $V_{in} = V_{ave} - A\sin(2\pi t + \phi)$, where V_{ave} is the average inlet velocity, A is the velocity amplitude, and ϕ is the phase angle. The variation in the WSS over the center of the CCR was minimized using an A of 19.5 mm/s and a ϕ of 100º. The chamber was stretched using a bidirectional linear actuator; the hydrostatic pressure was varied by changing the relative height between a media reservoir and the chamber; the flow was varied using a peristaltic pump. The electronics were controlled using a low-cost, Arduino-based microcontroller system. Conjugation of the RGD peptide and the DNA aptamer was confirmed using ATR-FTIR by the presence of broad N-H stretching band centered at ~3500 cm^{-1}, which was not present in neat PDMS or sulfo-SANPAH treated PDMS. PA successfully filled the CCR and bonded with the PDMS chamber to support strain transfer during stretching. The chamber was shown suitable for cell culture by the attachment and capture of HUVECs on the CCR when conjugated with RGD peptide and the DNA aptamer.

Conclusions: The Universal MechanoTester™ is able to decouple mechanical forces for their independent control. The CCR allows for material independence from the chamber construction. Well-established conjugation chemistries enable this system to be used with various materials and ECM proteins. Moreover, it supports the testing of new approaches for material design, such as aptamer surface functionalization for cell capture under dynamic conditions. The Universal MechanoTester™ will advance the study of cellular behavior in complex chemo-mechanical microenvironments.

References:

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