

Design of a Flow Chamber to Study Shear Stress Induced Endothelial Cell Orientation On/Within Different Modified 3D- Fibrin Matrices

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INTRODUCTION: All tissues require sufficient blood perfusion in order to function properly, as deficient perfusion often lead to loss of function. In hopes of returning sufficient perfusion to ischemic tissues, therapeutic angiogenesis attempts to stimulate the body's endogenous ability to develop new blood vessels. One approach is to stimulate wound healing and tissue regeneration with 3D-fibrin hydrogel matrices that present specific adhesion sequences for integrin attachment. Here we explore the integration of the sixth Ig-like domain of L1 (L1Ig6) into a fibrin matrix, which interacts with the $\alpha_v\beta_3$ integrin present on endothelial cells. It has previously been shown that *in vivo* interaction between L1Ig6 and $\alpha_v\beta_3$ stimulates angiogenesis [1].

Under physiological conditions, endothelial cells are exposed to a wide range of mechanical shear stresses from blood flow, resulting in gene regulation and cellular rearrangements [2]. To date, however, the effects of shear stress on endothelial cells in/on L1Ig6-modified 3D-fibrin hydrogel matrices have not been investigated.

The aim of this project was to develop a flow chamber to study HUVECs cultured on/within 3D-3D-fibrin hydrogel matrices and characterize different flow characteristics.

METHODS: Parallel plate flow channels have become the standard in researching cellular responses to shear flow. Therefore, an adapted version was designed that allowed cell culturing in 3D-fibrin matrices. PDMS was used as chamber material as it is gas permeable. For optimal analysis of the cells in the flow device, the overall dimensions of the chamber are limited to that of a standard microscope slide (76 x 26 mm).

Important in the development of parallel plate flow chambers are the dimensions. For laminar flow, the Reynolds numbers must lie below 2000. The ratio height/width for most flow chambers lies below 0.015 [3]. Given the assigned range for these two parameters, flow can be assumed to be within an infinite parallel plate. This gives the following shear stress (τ) and entrance length (l_e) formulas:

$$\tau = (6\mu Q) / (h^2w) \quad (1)$$

$$l_e = Re(0.08 h) \quad (2)$$

RESULTS: The newly designed flow chamber is seen in Figure 1. The reservoir for 3D-matrices and cell culture is located in the middle of the channel to ensure a homogeneous shear stress over it that is neither influenced by entrance or wall effects. Both at the entrance and at the exit fluid reservoirs have been placed, which both facilitate tube attachment and even fluid flow.

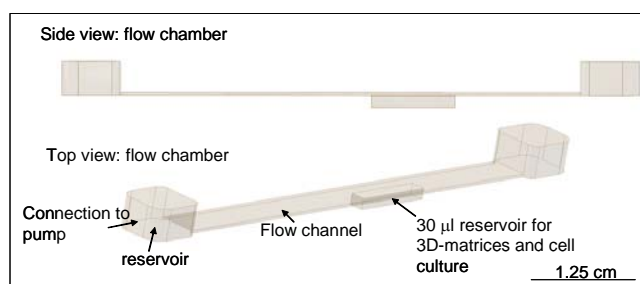


Fig. 1: Schematic of the designed flow chamber.

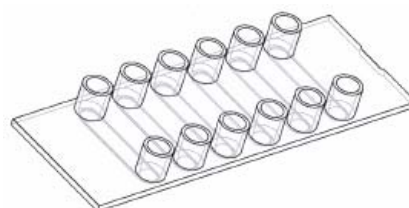


Fig. 2: Commercial μ -Slide flow chamber (Ibidi).

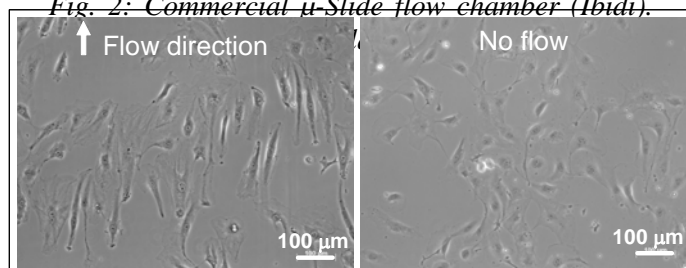


Fig. 3: HUVECs cultured on commercial μ -Slide flow chambers orient along the direction of flow.

DISCUSSION & CONCLUSIONS: The 3D-fibrin matrices will be inserted into the novel designed flow chamber and cellular behavior over characteristic physiological shear stresses will be analyzed. Cellular behavior will be compared between cells cultured in 3D-matrices and on 2D-surfaces.

REFERENCES: ¹Hall, et al. (2004) *Angiogenesis* 7:213-223. ² Risau (1997) *Nature* **386**:671-674. ³Chung (2003) *Computers and Structures* **81**:535-546.