

In vitro study of shear stress over endothelial cells by Micro Particle Image Velocimetry (μ PIV)

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The endothelium is a layer of thin cells that lines the interior surface of blood vessels, forming an interface between circulating blood in the lumen and the rest of the vessel wall. Studies on endothelial cells have revealed that they are involved in many aspect of vascular biology like the control of blood pressure, blood clotting, atherosclerosis, formation of new blood vessels, inflammation and swelling.

Blood flow in vessels causes the onset of mechanical forces acting on the inner walls. These fluid-vessel interactions lead to pressure, strain and shear forces. The endothelial cells experience most of these flow induced shear stresses. Shear stress over endothelial cells has been proven to play a crucial role in cardiovascular development, being involved in the mechano-transduction processes leading to atherogenesis, atherosclerosis, as well as other cardiovascular diseases. Moreover, *in vitro* studies of flow on endothelium have shown that flow-induced shear stress modulates gene expression.

Object of the present study is to investigate in detail, *in vitro*, the isolated mechanical effect of shear stress on an endothelial cells culture in a flow chamber under controlled fluid dynamical conditions. In this work a procedure was set to determine height variations and shear stress distribution over the cultured endothelial cell layer. To do this, Micro Particle Image Velocimetry (μ PIV) was used to measure the velocity fields for the shear stress evaluation, and an ad hoc flow chamber, suitable for culturing endothelial cells and equipped with an optical access for fluidic investigations was designed.

The setup consists in a modular flow chamber composed by a holder in which a microscope slide can be inserted as well as a rubber gasket that defines the dimensions of the micro-channel. The height of the channel is 120 μ m while the width can vary from 1.5 mm to 4 mm depending on the shape of the gasket used so that small liquid volume is involved (the fluid of culture used is a nutrition medium at 37°C). The endothelial cells layer grows on the glass plate separately and it is put in the chamber afterwards.

As for the μ PIV system, two identical Nd:YAG lasers were used, with beam combining optics delivering light pulses ($\lambda=532$ nm) along the same optical path; an inverted microscope was applied and a combination of green and red filter, together with a beam splitter allowed to obtain an epi-fluorescent imaging system; a high performance cooled CCD double frame camera was used, with a 1376 \times 1040 pixels resolution (6.45 \times 6.45 μ m pixel size). Red fluorescent PEG coated polymer microspheres with a 560 nm diameter were used as tracer particles.

The μ PIV measurements allowed obtaining the velocity fields of several planes parallel to the cell layer. Assuming a no-slip condition at the cell surface exposed to flow, the topography of the layer and the velocity gradient, hence the wall shear stress (assuming the fluid of culture Newtonian) can be determined.

An optimization of the measurement procedure was performed using Monte-Carlo simulations and an evaluation of the uncertainty of the final measurement is also presented. This procedure allows to obtain the shear stress distribution on the endothelial cells layer with good accuracy (6% of full range) and high spatial resolution (2.58 μ m \times 2.58 μ m).

Furthermore results are presented from measurements taken on human endothelial cells subjected to a flow induced shear stress of 0.6 Pa. In figure 1 is shown a 3D reconstruction of the endothelial layer topography where a colormap of the wall shear stress is superimposed. Bumps corresponding to the cells nuclei which experience the highest shear stresses can be noticed.

We plan to use this set-up to compare the wall shear stress and the gene expression of the cells themselves. That can give useful information to better understand the relationship between shear stress and endothelial cells development.

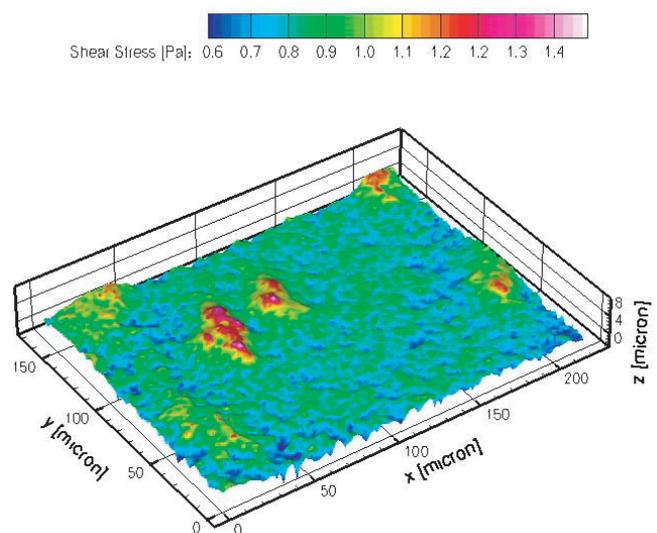


Fig. 1. Measured wall shear stress on endothelium layer