Adhesion Study of a Monolayer of Endothelial Cells

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Abstract

Cell adhesion is crucial for flow microfluidic tests, as cell detachment can imply an invalidation of the results of an experiment. Here, we studied the effect of different coating treatments and substrates in the adhesion and preservation of a monolayer of endothelial cells.

Introduction

The mechanism of atherosclerosis involves many complex micro- and macroscale processes. The disease is known to start with a disfunctional endothelium, which implies an increased permeability of the vessel wall. Several experimental tests, especially in the field of microfluidics, have been developed in order to study endothelial cell (EC) response to flow [1], which would help relate the permeability of the endothelium to different blood flow conditions [2]. ECs in general respond to laminar and unidirectional flow by aligning and elongating [3]. And although this response to flow is reported to happen significantly in the first hours of the experiment [4], microfluidic tests with longer durations have shown that ECs keep on elongating and alingning throughout more than 24 hours [5].

One of the main challenges of these longer microfluidic tests is the adhesion of ECs to the substrate. ECs are delicate and flow tests can be harsh. If ECs detach during a flow experiment, the results are invalid as cells will modify their behaviour if they are not forming a homogeneous monolayer.

In this study, we have performed and adhesion study on human umbilical vein endothelial cells (HUVECs), considering the substrate material as well as the adhesion treatment for the surfaces.

Materials and methods

The device and experimental tests are described in this section.

Microfluidic device

For this study, a microfluidic device previously developed was used to apply the flow conditions in the monolayer of HUVECs. These conditions were computed with fluid-structure interaction simulations, see Fig. 1.a. The device is made of PDMS. It is prepared by pouring PDMS in a resin mould and, once polimerised, it is attached to the substrate. In this study, this substrate is either a glass slide, see Fig. 1.b, or a PDMS base of around 1.6 mm in thickness.

Coatings and flow tests

The adhesion treatment of the microfluidic devices was performed as follows. The hydrophilicity of the substrate was enhanced by a O_2 plasma treatment for 60 seconds. Immediately after, a coating suspension was introduced in the device and kept in the incubator for one hour. Afterwards, the suspension was carefully removed and the device was cleansed three times with PBS, in order to remove all traces of the suspension. Afterwards, HUVECs were seeded in the microfluidic device and left to attach and grow in the incubator overnight. Table 1 gathers the information of the adhesion treatments.

Before the tests, the formation of a homogeneous monolayer was checked and phase contrast images were taken as initiation of the experiments. Flow was applied with a peristaltic pump and its inherent pulsatility was reduced by the use of a damper. Images were taken at 16 and 24 hours of the experiment. These images were processed afterwards with a self-developed algorithm and cell counting was performed automatically.

Results

The best adhesión treatment for HUVECs was found to be fibronectin, see Fig. 2. Moreover, PDMS as substrate showed better behaviour for cell adhesion than glass slides.

Conclusions

This is the first step needed to be developed before a flow microfluidic test that is to be performed for more than a few hours. Cell adhesion was notably different for the coating treatments we used, highlighting the importance to optimise the adhesion procedures in the experiments. Once the adhesion of ECs is assured, flow tests with different conditions can be performed to obtain cell response in terms of these parameters.

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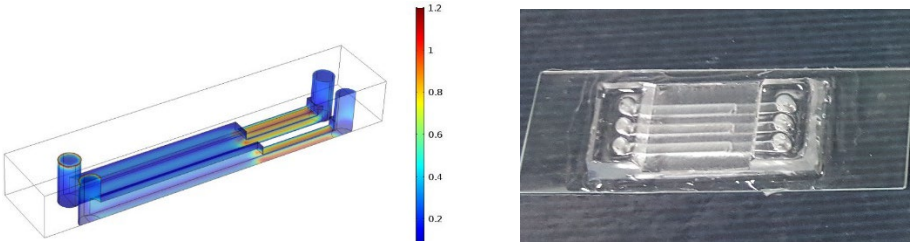
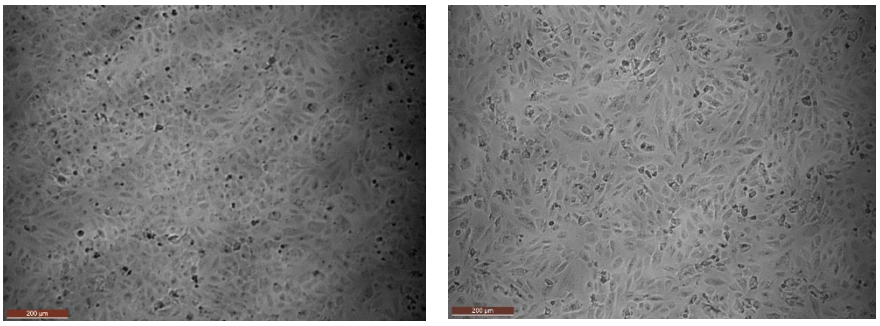
Table 1. Concentration of the adhesión treatments used with HUVEC.

Treatment	Collagen	Fibronectin	Matrigel
Concentration	100 µg/ml	5 µg/cm^2	50 µl/cm^2



(a) **(b)**

Figure 1: (a) Symmetrical cut of the geometry of the device and values of wall shear stress (WSS). (b) Microfluidic device mounted on a glass slide.



(a) **(b)**

Figure 2: Monolayers of HUVEC before (a) and after (b) the adhesion study with fibronectin coating.