X JORNADA DE JÓVENES INVESTIGADORES DEL I3A

Adhesion Study of a Monolayer of Endothelial Cells

Itziar Ríos Ruiz[1], Sara Oliván García[1,2], Miguel Ángel Martínez Barca[1,2] , Estefanía Peña Baquedano[1,2]

1.Aragon Institute of Engineering Research (I3A). University of Zaragoza, Spain
2.Centro de Investigación Biomédica en Red de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Spain

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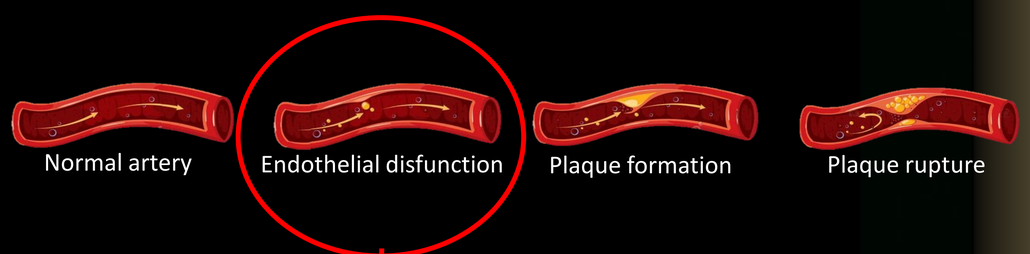
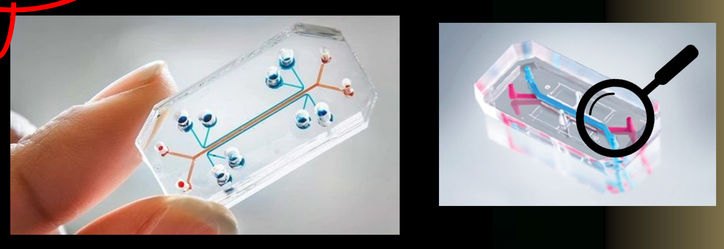
MOTIVATION

THE PROCESS OF ATHEROSCLEROSIS

Atherosclerosis is a complex disease and, despite its incidence, the mechanisms of its initiation and development are still under study. It is known to start with a disfunction in the endothelium, the most inner layer of the vessels, which consequently affects their permeability.

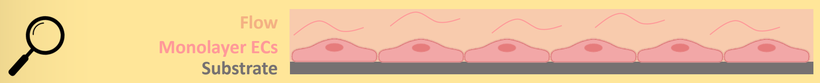


In order to study the role of the endothelium in the disease, microfluidics has emerged as a powerful tool to obtain information of the response of endothelial cells (ECs) to flow.

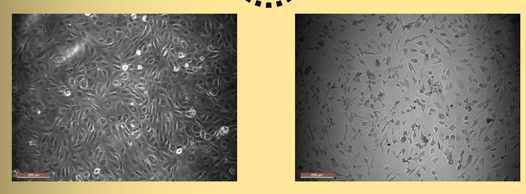


MICROFLUIDIC TESTS

In these microfluidic experiments, ECs are subjected to different flow conditions, in order to obtain their response, mainly in terms of shape and orientation.



Flow tests tend to last several hours as the stationary response of the cells is pursued. These experiments can be aggresive to ECs and make them detach from the substrate.



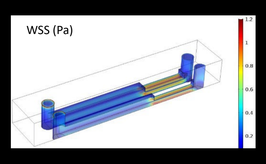
AIM

The aim of this work is to study different adhesion treatments and check their performance in a monolayer of ECs subjected to flow. Moreover, the effect of the substrate in which the ECs are seeded is also studied, as well as the use of a plasma treatment prior to the addition of the adhesion protein. The efficacy of these conditions is measured by counting the number of cells that remain after the flow tests.

METHODS

MICROFLUIDIC DEVICE

The microfluidic device used for these experiments has three channels and is designed to reach two different levels of wall shear stress (WSS) in each. It is fabricated by pouring PDMS in a mould and leaving it overnight at 60°C to cure. Afterwards, once removed from the mould, the device is mounted in the substrate. In this study, two different substrates have been used, a glass slide and PDMS. Finally, the device is washed with PBS to remove any posible depositions from the PDMS during fabrication.



SURFACE TREATMENTS

PDMS and glass are highly hydrophobic materials, thus surface treatments to enhance their hydrophilicity are desirable. In this study, the devices are initially subjected to oxygen plasma for 60 seconds. Afterwards, the adhesion protein is seeded in the device and left to incubate for 1h. When the incubation time ends, the devices are gently cleansed three times with PBS in order to remove any residual traces of the proteins. Cells are subsequently seeded and incubated for a monolayer to form.

Coating	Collagen	Matrigel	Fibronectin
Concentration	100 µg/ml	50 µl/cm2	5 µg/cm2

EXPERIMENTAL SETUP

Peristaltic pump

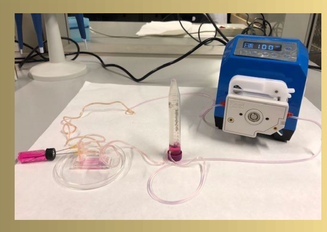
HUVEC (Human Umbilical Vein Endothelial Cells)

Fluid damper

Dextran (↑ fluid viscosity)

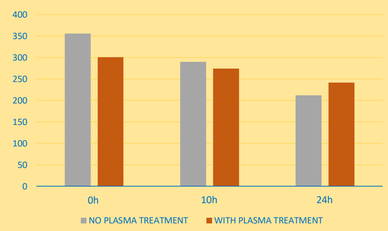
4,5 ml/min

24 hours

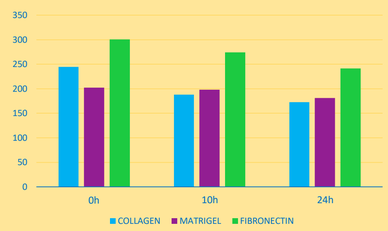


RESULTS

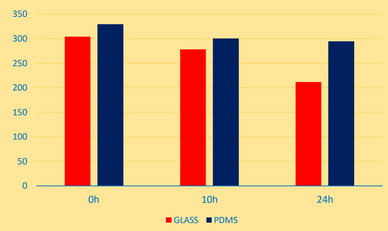
PLASMA TREATMENT



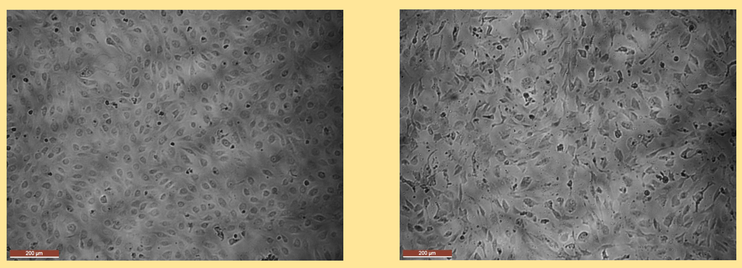
ADHESION PROTEIN



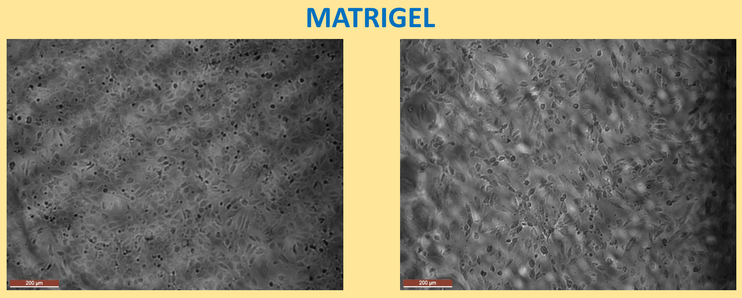
SUBSTRATE



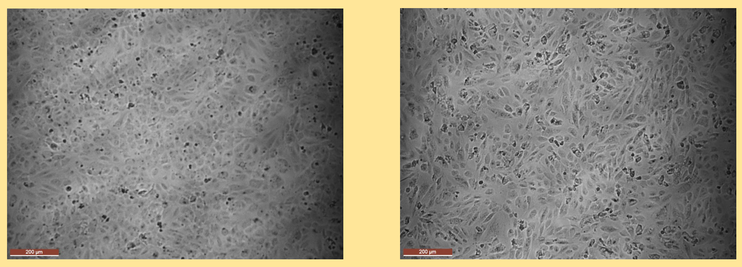
COLLAGEN



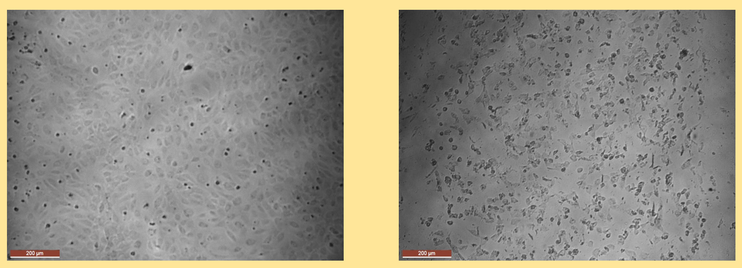
MATRIGEL



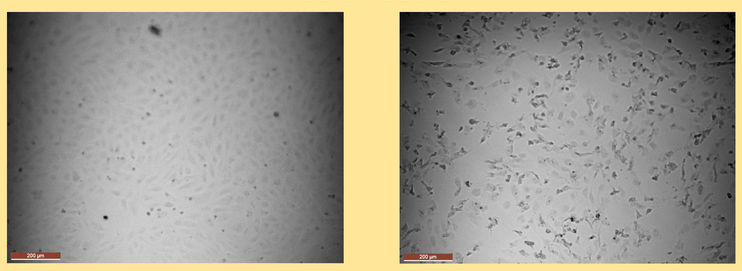
FIBRONECTIN



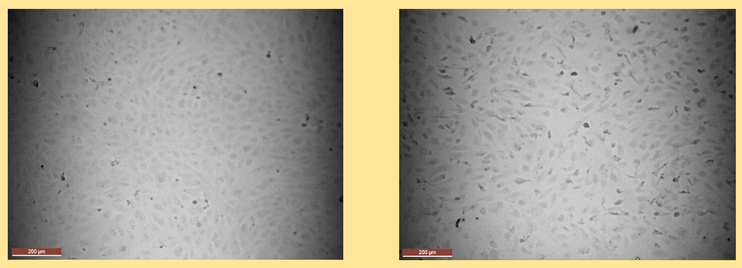
WITHOUT PLASMA TREATMENT



GLASS SLIDE



PDMS



CONCLUSIONS

- Plasma treatment is necessary before treating the surface with any adhesion protein
- Fibronectin proved to be the best adhesion protein for HUVECs in 24h flow tests
- A PDMS substrate was better for cell adhesion than a glass slide
- The use of a fluid damper reduces ECs detachment as peristaltic pulses are softened
- The type of adhesion protein did not affect cell response to flow

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