XI JORNADA DE JÓVENES INVESTIGADORES DEL I3A

SURFACE MODIFICATIONS OF COP-BASED MICROFLUIDIC DEVICES FOR IMPROVED IMMOBILIZATION OF HYDROGEL PROTEINS: LONG TERM 3D CULTURE WITH CONTRACTILE CELL TYPES AND ISCHEMIA MODEL.

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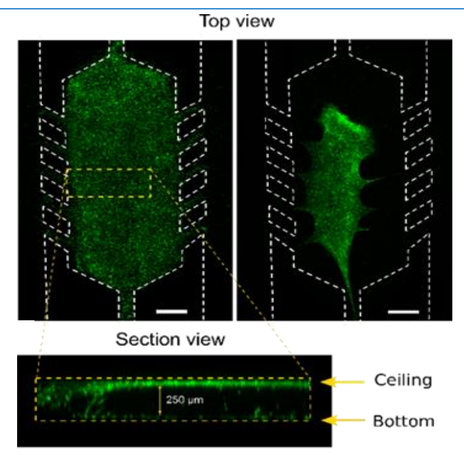
INTRODUCTION

Microfluidic devices require supporting matrices or scaffolds to support 3D cell cultures in order to mimic the spatial complexity and mechanical properties of in vivo microenvironment. In collagen hydrogel based 3D cultures, fibroblast-like cells elongate and adhere to fibers via integrins, exerting forces that release fibers. If hydrogel fibers have insufficient resistance to the applied force, cells deform the fibers, and might eventually lead to a collapse. Although contraction and remodeling process is a natural outcome from cellular expansion and interaction with the matrix, this phenomenon may ruin the hydrogel architecture within the microfluidic device, disrupting the compartmentalization and creating an obstacle to long time or high cell concentration assays.

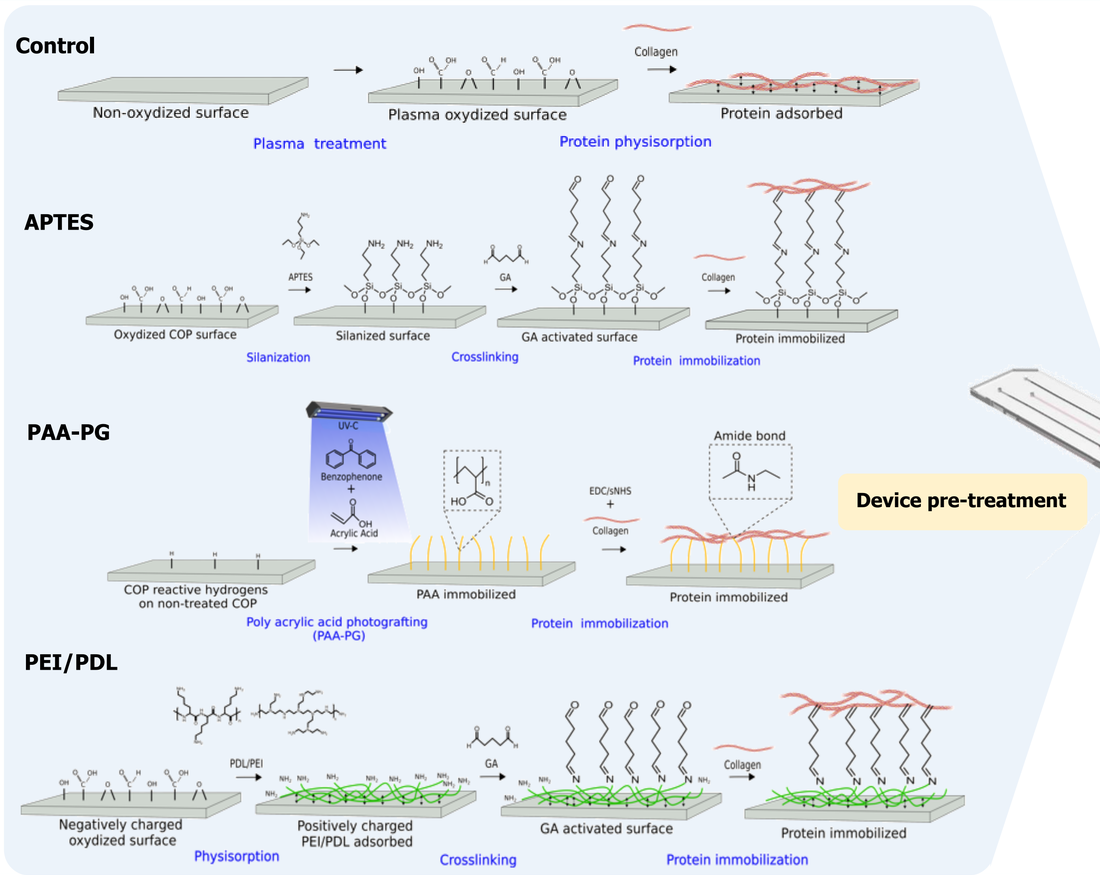
Therefore, we encouraged to test described surface treatments for PDMS on COP-based microfluidic devices (COP-MD), to form stronger bonds with collagen as ECM protein model, avoiding hydrogels cell collapse and detachment from COP surfaces, in order to closely mimic natural microenvironments in microfluidic devices.

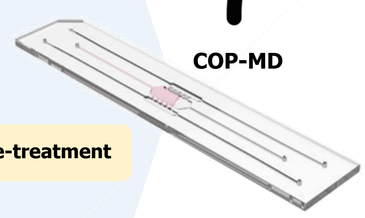


METHODS

COP surface was previously plasma treated to bind either **APTES**, **PEI** or **PDL** through C=O and C-O, finally crosslinking with bis-aldehyde **GA** between amino groups from APTES, PEI or PDL and **collagen I**.

We covalently coupled collagen I to **poly(acrylic acid) photografted** on COP surface via carbodiimide-mediated amide formation before hydrogel polymerization (**PAA-PG**).

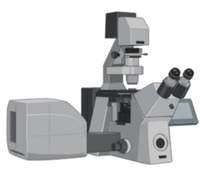




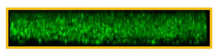
Device pre-treatment

COP-MD

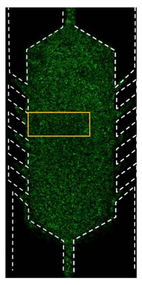
Microscopy monitoring and data analysis



Confocal microscopy



Transversal/section view



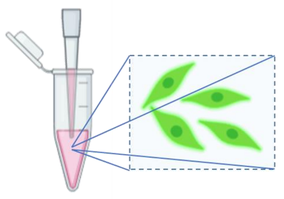
Top view

3D cell culture

Human Cardiac Fibroblasts

10^7 cells/ml

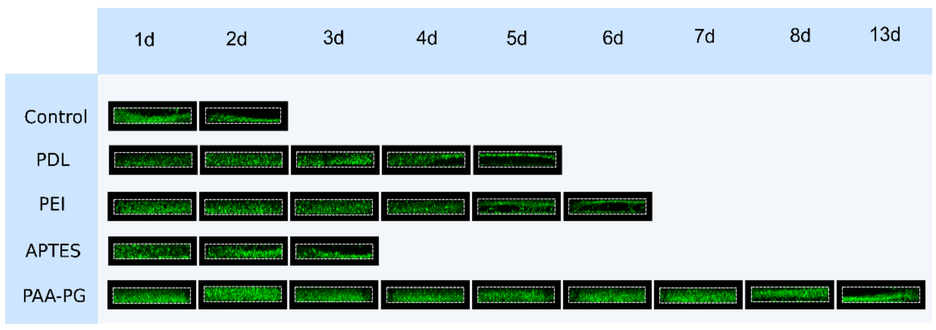
Collagen hydrogel 1.2 mg/ml



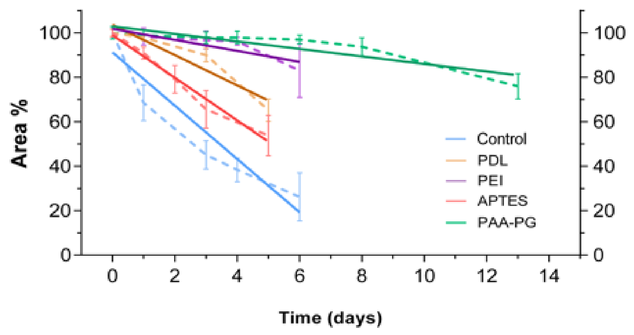
RESULTS

Immobilization efficiency of collagen hydrogel was defined as the hydrogel **resistance to collapse** at different time points after incubation of human cardiac fibroblasts (HCF) embedded collagen hydrogels within the devices.

It was determined by quantification of the **hydrogel transversal area within the devices at the studied time points**, since a top view of 3D cultures may lead usually to wrong conclusions.



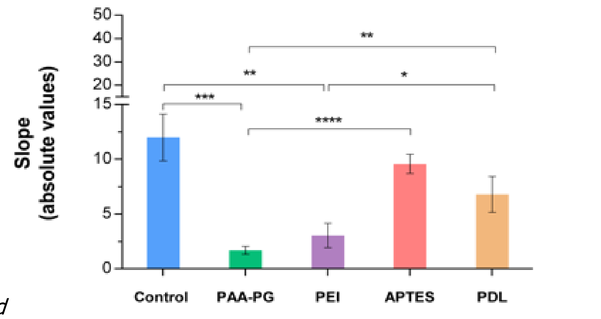
Transversal area evolution overtime of HCF labelled with green-fluorescent DiO Vybrant™ dye.



PAA-PG surface treatment of COP-MD prevents 1.2 mg/ml collagen hydrogels collapse longer time than other treatments.

Area percentage of HCF embedded collagen hydrogels within the device chamber over time (dashed line) and trend lines (continuous line).

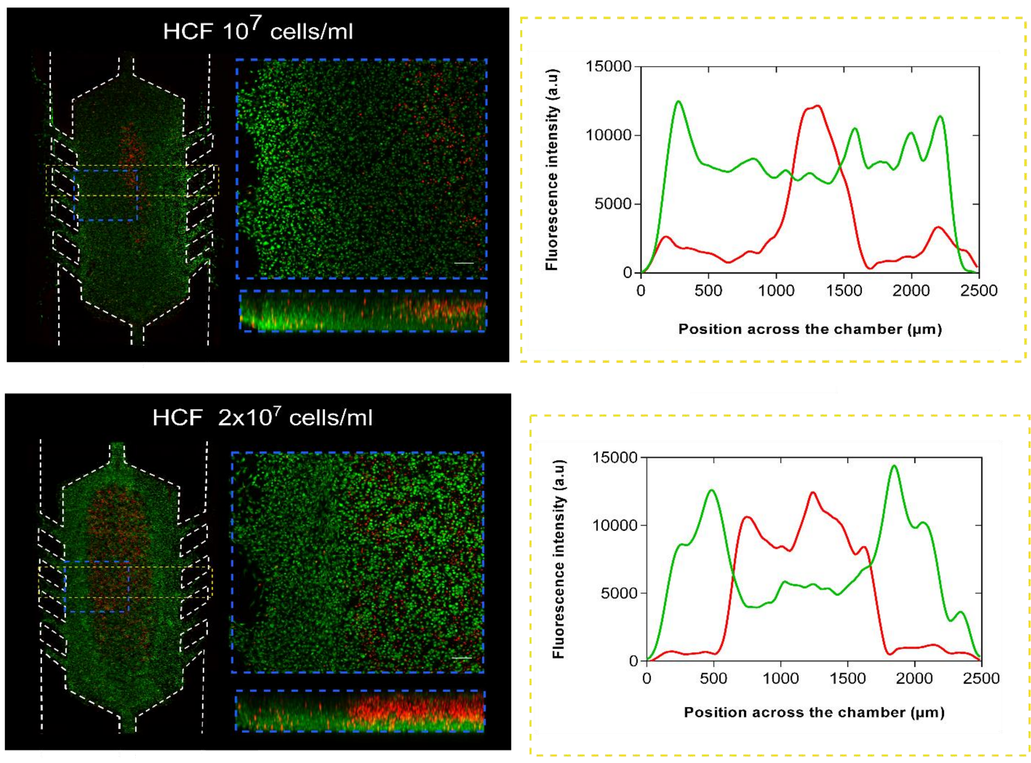
Real time micrograph acquisition of control and PAA-PG treated devices with 2x10⁷cells/ml.



Statistical comparison among the slopes of trend lines.

Evaluation of PAA-PG immobilization technique for the *in vitro* simulation of the necrotic core formation.

Due to COP gas impermeability, we studied the application of PAA-PG pre-treatment to generate a **necrotic core model** since it allows long term 3D culture, preserving collagen hydrogel structure. We were able to generate oxygen and nutrient gradients that lead to formation of necrotic cores. In fact, different necrotic core sizes were developed depending on initial HCF density seeding.



*Top and transversal view of **alive**/**dead** fibroblasts of 10^7 and $2x10^7$ HCF/ml at 48 hours after calcein (CAM)/propidium iodide (PI) staining (green/red). The graphs show quantification of CAM (green) and PI (red) fluorescence intensity profile along the chamber device (yellow bordered regions). Scale bar 100 µm.*

CONCLUSIONS

We conclude that PAA-PG is the most effective method that allows long term culture at high cell densities of contractile cell types such as fibroblasts. The implementation of PAA-PG treatment to COP-MD opens a new field on *in vitro* model generation where collagen hydrogel structure preservation is required. This constitutes a basis for the development of more patho-physiologically and relevant co-culture models of wound healing, tumor microenvironment and ischemia within microfluidic devices.

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