Surface Modifications of COP-Based Microfluidic Devices for Improved Immobilization of Hydrogel Proteins: Long Term 3D Culture with Contractile Cell Types and Ischemia Model.

Sandra González-Lana [1,3], Teodora Randelovic [1,2], Jesús Ciriza [1,2,4], María López-Valdeolivas [5], Rosa Monge [1,3], Carlos Sánchez-Somolinos [5], Ignacio Ochoa [1,2,4]

[1] Tissue Microenvironment Lab (TME). Aragón Institute of Engineering Research (I3A). University of Zaragoza, C/ Mariano Esquillor s/n, 500018 Zaragoza, Spain.

[2] Institute for Health Research Aragón (IIS Aragón)

[3] BEONCHIP S.L.

[3] CIBER in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN).

[4] Aragón Institute of Nanoscience and Materials (INMA)

Tel. +34-976762707 e-mail: sangolan@unizar.es

Resume

Hydrogel confinement is critical in microfluidics to generate gradients, as cell remodelling process boundaries the development of biological models such as ischemia or fibrosis. Here, we test the immobilization efficiency of collagen hydrogels cells embedded to treated COP-based microfluidic devices to preserve 3D structure and avoid gel contraction.

Introduction

The tissue microenvironment plays a key role in tissue homeostasis and disease progression, nonetheless the traditional *in vitro* cell culture platforms have been limited by the lack of adequate biomimetic stimuli. Thanks to the microfluidic technology applied to cell culture, these complex microenvironments can be nowadays recreated combining hydrogels, cells and microfluidic devices. However, from microfluidic platforms emerge other limitations. Therefore, when cultured 3D hydrogels within microfluidic devices, contractile cells elongate and adhere to the gel fibres, applying forces that can eventually alter the structure [1][2]. Though contraction and remodelling process is a natural outcome from cellular expansion and interaction with the matrix [3], this phenomenon may ruin the hydrogel architecture within the microfluidic device, disrupting the compartmentalization and creating an obstacle to long time or high cell concentrated assays.

Several approaches have been made in the past to overcome this limitation [4][5], most of them focused on the microfluidic devices based on PDMS. However, other materials such as COP/COC are gaining popularity for microfluidic applications due to their multiple advantages over PDMS.

Thus, in this work we 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.

Matherial and methods

We compared four surface treatments in COP-MD, prior to immobilization of fibroblast embedded collagen hydrogels. We covalently coupled collagen I to poly (acrylic acid) photografted on COP surface via carbodiimide-mediated amide formation before hydrogel polymerization (PAA-PG). Except for PAA-PG treatment, 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.

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 (figure 1).

Results

Collagen I was immobilized to PAA-PG, APTES, PEI and PDL pre-treated COP-MD surfaces, taking as control a plasma treated surface. PAA-PG treatment showed the longest hydrogel structure preservation, keeping over 90% area after 8 days of cell culture, when the first fissures between the device surface and the hydrogel began to be

displayed, and showing statistically significant differences compared to control ($p < 0.001$) (Figure 2).

The presence of fibroblasts in co-culture of different cell types is often needed to create more biomimetic *in vitro* models. Some assays require high cell densities within the 3D matrix as the self-induced ischemia models, where the fibroblast role is particularly important. Hence, we tested if PAA-PG pre-treatment could overcome this methodological limitation, focusing on a necrotic core model with HCF in collagen hydrogels (10^7 and $2x10^7$ cells/ml). Cell viability staining after 48h showed a necrotic core in the innermost hydrogel region indicating that pre-treatment with PAA-PG allows the generation of oxygen and nutrient gradients that lead to formation of necrotic cores, unreproducible without collagen hydrogel immobilization (figure 3).

Conclusions

Altogether our results indicate that chemical surface modification of COP devices avoids the quick collagen collapse in 3D hydrogels, with poly-acrylic acid photografting (PAA-PG) results as the most effective surface treatment for covalent bonding to collagen hydrogels embedding 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 isquemia within microfluidic devices.

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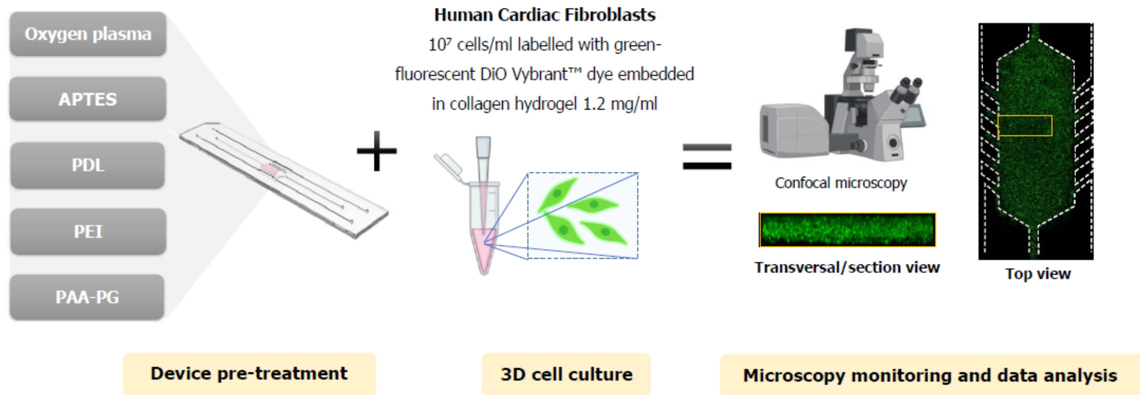


Figure 1. Scheme of workflow.

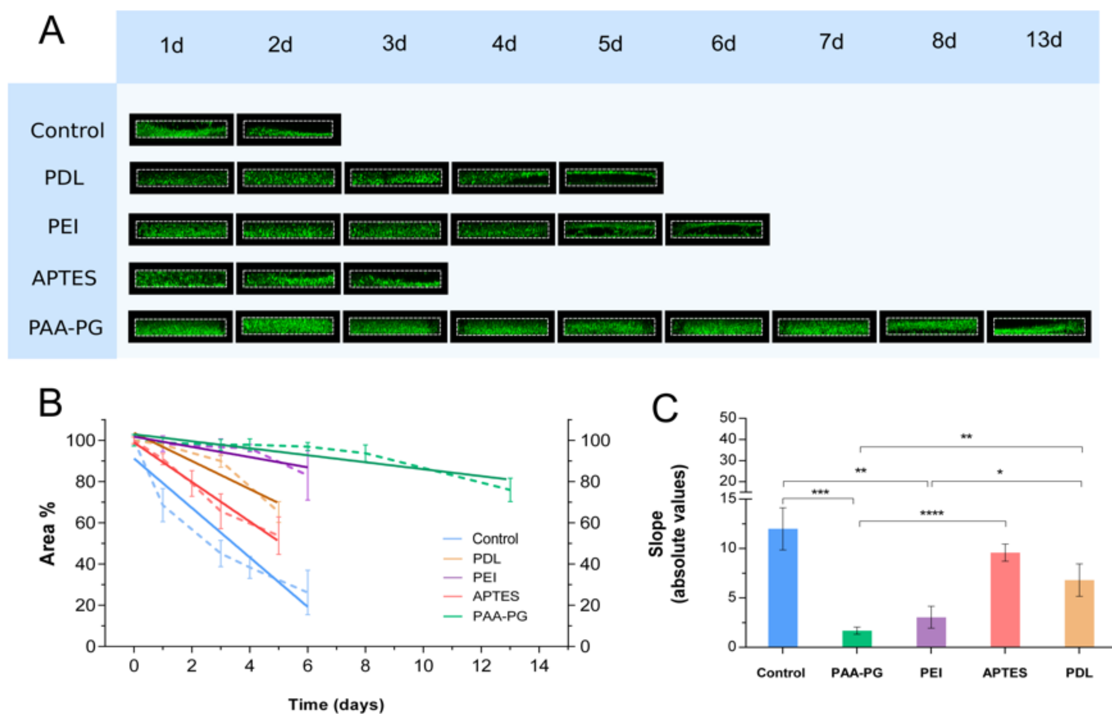
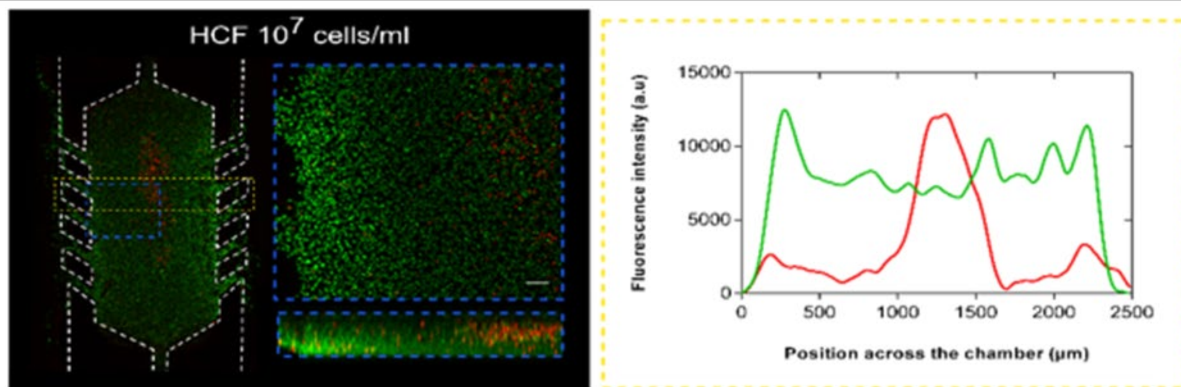


Figure 2. Culture of fibroblasts (10^7 cells/ml) embedded in collagen after device pre-treatments. A) Transversal area evolution overtime of fibroblasts labelled with green-fluorescent dye. B) Area percentage of fibroblasts embedded collagen hydrogels within the device chamber over time (dashed line) after treatment with different conditions and trend lines (continuous line). Error bars SEM. Note: ****$p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

A



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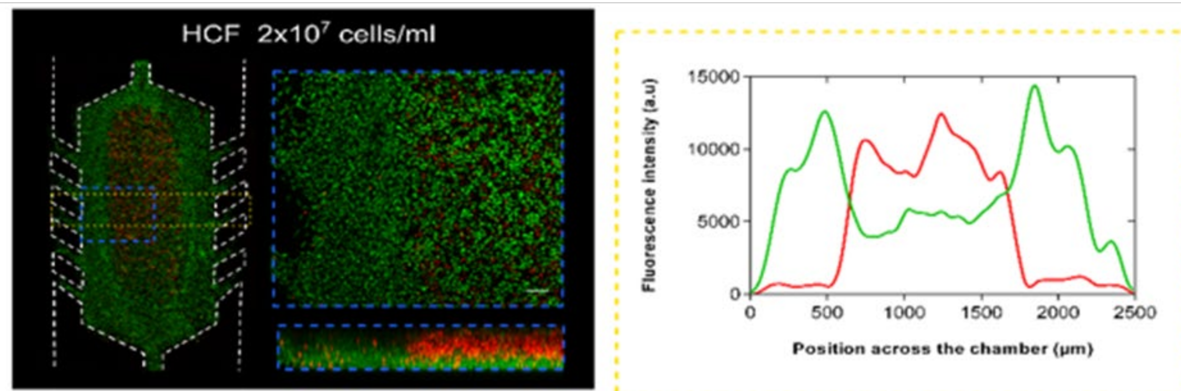

Figure 3. Top and transversal view of alive/dead fibroblasts of 10^7 (A) and $2x10^7$ (B) cells/ml at 48 hours after calcein (CAM)/propidium iodide (PI) staining (green/red). The graphs show quantification of CAM (green) and PI (red) fluorescence intensity profile along the chamber device (yellow bordered regions). Scale bar 100 µm.