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MICRO- AND MACRO-VASCULAR ENDOTHELIAL CELLS BEHAVE DIFFERENTLY ON POLYCAPROLACTONE MEMBRANES WITH SMOOTH AND ROUGH SURPHACE TOPOGRAPHY

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KEYWORDS

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INTROCDUCTION

Endothelial cell (EC) monolayer formation and its integrity is a crucial factor for the eventual success when implanting blood contacting devices or designing artificial blood vessels. It is of great interest to develop a scaffold for tissue engineering purposes that helps cells to sustain their biological functionality. Surface topography of the cell surrounding environment is one of the features that determine the cell behaviour and its function.

In the field of tissue engineering and regenerative medicine, when it comes to the development of a scaffold, apart from choosing an appropriate material that should be biocompatible and non-toxic for the cells, it is also necessary to design and fabricate the substrate with an appropriate technique. These various processing methods would allow the material to obtain, among other desirable features, distinct surface properties. These surface properties are to be tailored for specific biological responses. One of the major goals is therefore to create a scaffold that can mimic extracellular matrix (ECM) in order to provide cells with an environment similar to their own in a biological system.

In our work we proposed a novel bi-layer model for construct an artificial blood vessel composed by a biodegradable polyester-polycaprolactone processed by means of solvent casting and electrospinning.

The aim this particular study was to evaluate the effect of surface topography, rougher electrospinning and smoother solvent casting surfaces, over macro (HUVECs) and microvascular (HPMEC-ST1.6R) EC adhesion, proliferation and gene expression profile.

MATHERIALS AND METHODS

I. Bi-layer scaffold fabrication

Solvent cast polycaprolactone (SC PCL) layer was obtained by polymer dissolution in chloroform (20% (w/v) and casting on glass Petri dishes. The nanofibre mesh PCL (NF PCL) layer was fabricated by electrospinning of a 17% PCL (w/v) solution in chloroform and dimethylformamide (in ratio 7:3). Both layers were merged down by layering the NF PCL over the SC PCL using chloroform.

II. Cell source and culture conditions

Macrovascular human umbilical cord vein endothelial cells (HUVECs) were obtained by 0.1% collagenase type I digestion. Cell phenotype was positively confirmed by immunocytochemistry for surface specific antigen CD31 (PECAM-1) and intracellular marker, von Willebrand Factor (vWF). Human pulmonary microvascular endothelial cell line (HPMEC-ST1.6R) was generated by transfection and displayed the major constitutively expressed and inducible endothelial phenotypic markers. Cells were cultured in M199 supplemented with 20% FBS, Glutamax I, Endothelial Cell Growth Supplement and heparin at 37°C and 5% CO_2. HPMEC-ST1.6R were additionally treated with geneticin 418 for selection of the transfected cells.

Both cell types were seeded SC PCL and NF PCL at $8x10^4$ cell/well in 24 well plates. The DNA of the samples was collected to infer about cell proliferation. The level of expression of EC markers (vWF, PDGF-B, PECAM-1, VE-Cadherin) was determined by real time Q-PCR. The expression of vWF and PECAM-1 at the protein level was confirmed by immunocytochemistry and visualized in a Confocal Microscope.

RESULTS AND DISCUSSION

SEM analysis revealed that obtained SC PCL had smooth but not even surface (fig. 1A), while electrospun NF PCL showed random distribution of nanofibres in the diameter range from 180 nm to 2.30 µm (fig. 1B).

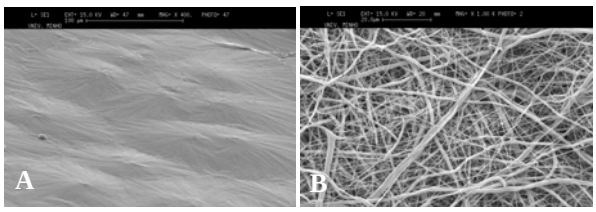

Figure 1. SEM surface images of SC PCL (A) and NF PCL (B).

The DNA quantification results (fig. 2) showed that HUVECs did not proliferate on the NF contrarily to HPMEC-ST1.6R. In addition, both cell types proliferated at normal rates on the smooth SC membranes.

The level of expression of vWF (fig. 3), like for the other markers, was more affected on the HUVECs than on the HPMEC-ST1.6R cultures, which depicted a significantly lower expression than HUVECs. While the expression of vWF was not affected during culture,

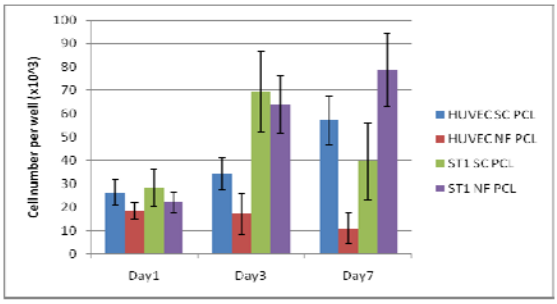

Figure 2. Cell number obtained by dsDNA quantification.

HUVECs vWF expression on SC decreased from day 1 to day 3 and stabilised until day 7. Contrarily, on the NF the HUVECs vWF expression seemed to be constant until day 3 then increasing until day 7.

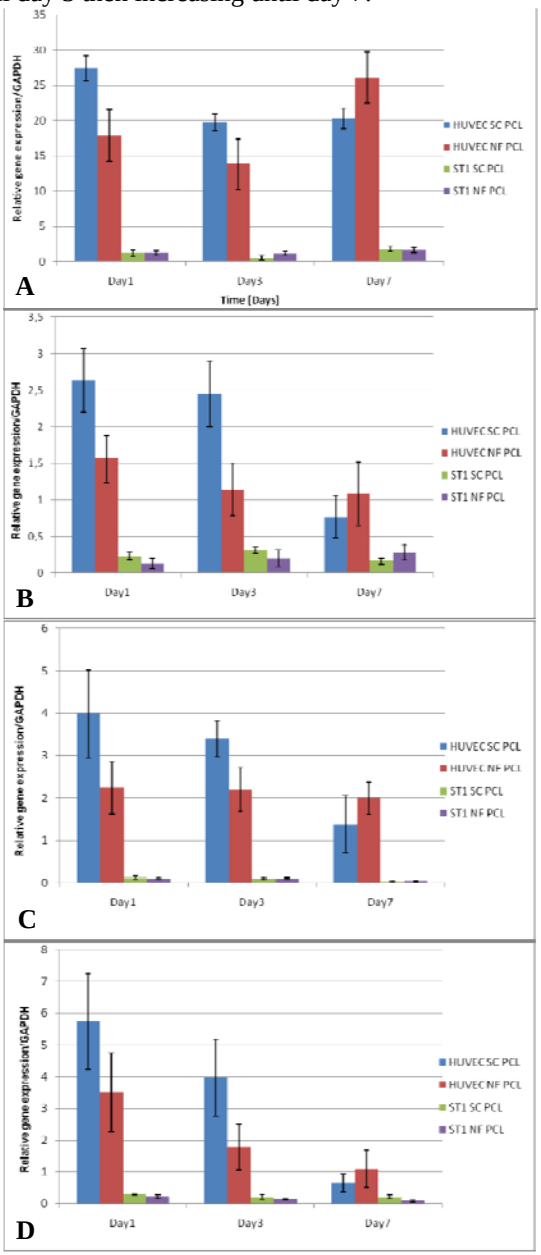

Figure 3. Gene expression level of vWF (A), VE-cadherin (B), PECAM-1 (C) and PDGF-BB (D).

The gene expression results were confirmed at the protein level (fig. 4)

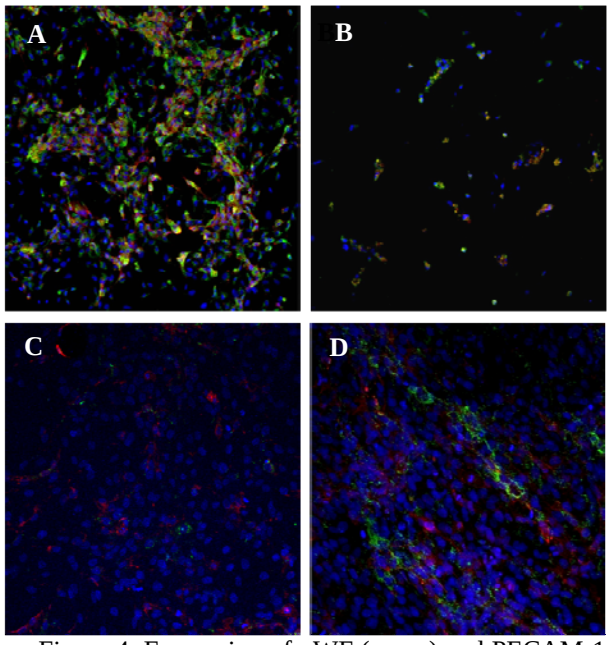

Figure 4. Expression of vWF (green) and PECAM-1 (red) in HUVEC (A,B) and HPMEC-ST1.6R (C,D) on SC (A,C) and NF (B,D) surfaces counterstained with DAPI (blue) after 7 days of culture.

CONCLUSIONS

The proliferation level and gene expression for HUVECs was better on SC PCL and it can be clearly stated that these ECs of macrovascular origin preferred smooth (SC) over rough (NF) surfaces.
Contrary to HUVECs, microvascular HPMEC-ST1.6R proliferated better on NF PCL, holding the gene expression level on the same.
Nanofibre meshes seem to be more appropriate mimicking the natural environment for microvascular ECs while smooth solvent cast membranes are preferable for the ECs when aiming at engineering larger diameter blood vessels.
HUVECs were shown to be more responsive to surface topography than HPMEC-ST1.

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