DEVELOPMENT OF AN ADVANCED CULTURE SYSTEM TO INVESTIGATE VASCULAR TISSUE ENGINEERING BIOMECHANISMS

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Introduction

Vascular tissue engineering aims to regenerate vessels "at the target site" using cell-free scaffolds supporting endogenous regeneration. Ideally, the regenerated tissue is remodelled, guided by physiological hemodynamic loads, to resemble a vessel with native-like structural and functional properties [1]. Despite encouraging *in vivo* proof-of-concept studies [2], intimal hyperplasia and early stenosis remain prevalent complications in graft implantation. With the aim of investigating these phenomena, we succeeded in developing an advanced and versatile culture system able to perform cell seeding and mimic *in vivo*-like stimuli (pre-tensioning, wall shear stress (WSS) and cyclic pressure) for long-term experiments. Currently, experiments with different seeding and culture conditions and related biological analyses are ongoing to verify the suitability of the system to be used in a cell culture lab and its performances under different experimental protocols.

Methods

The system consists of a culture chamber, a rotating mixer for semi-automatic cell seeding, a pinch-valve to generate a pulsatile stress, and two fluid dynamic circuits (luminal and extraluminal compartments) each one composed by a roller pump, a reservoir and an air chamber (Figure 1). A custom graphic user interface allows to act on each peripheral device in manual control or to set an experiment by specifying the duration, the pumps' flow rate, the pressure regime, and the culture chamber's rotation speed for the seeding.

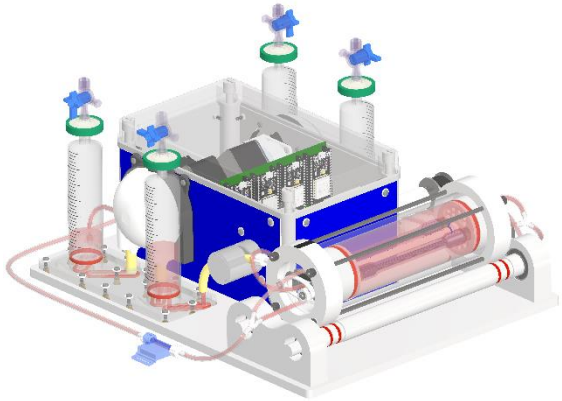

Figure 1: Culture system assembly.

Three-layered electrospun grafts ($Ø_{in}$=6 mm, l=60 mm), composed of a nanometric mesh of silk fibroin (SF) and polyurethane (PU) enclosed within SF layers, were manufactured and used for these tests [3].

Human umbilical vein endothelial cells (HUVECs) [4] seeding was achieved using discrete rotations (4 angular position, each kept for 30 minutes). We evaluated flow-induced HUVECs morphology, cell adhesion to the substrate, cell orientation (SEM, immunofluorescence staining of e.g. F-actin, VE-Cadherin, pFAK, paxillin, ZO-1), and gene expression (e.g. KLF-2, vWF).

Results and discussion

The system was successfully subjected to bench tests to verify the no cytotoxicity, the compatibility with the assembly procedures under laminar flow hood, the hydraulic sealing (up to 400 mmHg), and the maintenance of sterility inside an incubator up to 21 days. We believe that the versatility and easiness of use are the features that most distinguishes the culture system here developed and presented from those commercially available or present in the state-of-the-art. Indeed, the system is robust and reliable in setting and exploit different seeding and stimuli protocols, allowing to perform a fine tuning of the experimental procedures. After cell seeding, we observed that HUVECs almost completely covered the graft and establish a complex cell-graft and cell-cell interaction network (Figure 2). All these observations endorse the possibility to obtain a functional cell-populated graft to subject to physio-pathological environments of fluid flow.

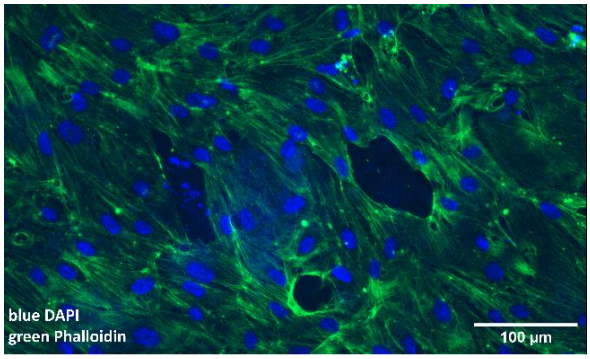

Figure 2: Immunostaining image.

The developed culture system and the drafting of *ad hoc* experimental protocols will allow to establish an accurate *in vitro* model to investigate complex biological interactions in vascular tissue engineering.

References

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