

# DEVELOPMENT OF A COMPUTATIONAL/EXPERIMENTAL MODEL OF 3D VASCULARIZED TISSUES

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## Introduction

Before undergoing clinical translation, it is essential evaluating the physiological reactions caused by biomaterials implantation. Integration of tridimensional (3D) cell culture systems and computational studies can help modelling the behavior of living tissues [1]. Computational fluid dynamics (CFD) analyses together with the application of a millifluidic optically accessible bioreactor (MOAB), suitable for dynamic culturing of cells in a 3D microscopic environment, have revealed to be a quick and reliable method for modelling vascularized tissue regeneration *in vitro*.

## Methods

The cell culture platform consists of two rows of nine 3D microgrids, each of 500x500x40  $\mu\text{m}$ , fabricated by 2-photon polymerization of the SZ2080 biocompatible photosensitive resin [2]. Each microgrid is characterized by pores of 50x50x20  $\mu\text{m}^3$  (Figure 1). Cell proliferation was evaluated both on a flat substrate and inside the microgrids in static and perfused conditions (inside the MOAB). CFD analyses allowed to set up the optimal parameters and the shear stress values acting on 3D cells cultures. Co-cultures of endothelial cells and fibroblasts were performed and proliferation, together with tridimensional organization, were evaluated by confocal laser scanning microscopy (CLSM) (Figure 3). Specific growth factors (VEGF and TGF- $\beta$ 1) were administered for stimulating vessels formation.

## Results

CFD analyses were performed for determining the optimal flow rate (10  $\mu\text{l}/\text{min}$ ) for achieving a maximum shear stress value of 0.1 mPa inside the microgrids (Figure 2). Co-cultures actively proliferated both in static and dynamic conditions and cell viability was not affected as shown by CLSM imaging. Upon administration of growth factors, co-cultures reorganized starting from day 4.

## Discussion

CFD analyses allowed to evaluate optimal parameters for cell culture in dynamic conditions. The micro-scaffold and the bioreactor's setup efficiently supported endothelial cells and fibroblasts growth. Experiments showed that this cell culture system is a quick and reliable tool for modelling vascularized tissue regeneration *in vitro*. Evaluation of vessels and

connective tissue formation is ongoing in co-cultures. Administration of VEGF and TGF- $\beta$ 1 will be performed for estimating their cellular uptake/release kinetics.

## Figures

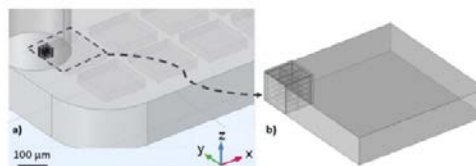


Figure 1: a) Design of the bioreactor constituted by a chamber filled with micro-scaffolds (inset, b)).

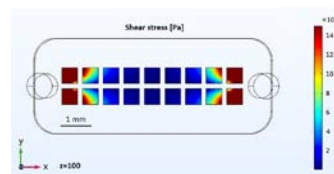


Figure 2: Graphical representation of the estimated shear stress acting on the upper boundary surface of each micro-scaffold. The color map allows to identify the most stressed micro-scaffolds (in red).

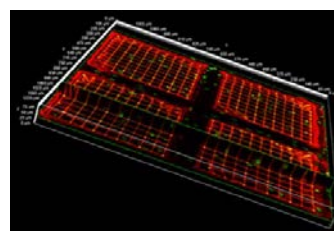


Figure 3: Endothelial cells cultured in the 3D micro-scaffolds imaged by confocal laser scanning microscopy upon live (green)/dead (red) staining.

## References

1. A. Marturano-Kruik et al., PNAS, 115(6), 1256-1261, 2018.
2. E. Jacchetti et al., Sci Rep. 11(1):3021, 2021.

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