

ENGINEERING OF VASCULARIZED 3D TISSUES ON CHIP



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The vascular system, also called circulatory system, is an organ system that allows the circulation of blood and the transport of nutrients, oxygen, carbon dioxide, hormones, and blood cells in human body to provide nourishment, stabilize temperature and pH, and maintain homeostasis. Most of the tissues and organs constituting human body depend on vascularization for their sustenance and to correctly fulfil their functions.

Since tissue engineering has been developed, it represents one of the main strategies that bioengineering has to obtain *in vitro* substitutes for wounded tissues in human body, replicating the physiological environment with low risk of rejection after *in vivo* implantation. One of the main issues regarding tissue engineering is represented by nutrients diffusion during biohybrid formation, which is insufficient in preventing necrosis of tissues in the scale of millimeters before *in vivo* application.

My PhD project relates to microfluidics research field and will focus on the design and the production of a microfluidic device, whose function will be the investigation of the effects of fluidodynamics, mechanical stimuli such as shear stress, geometric cues and time-space control over biochemical gradients on the development of an accessible vascular network inside an *in vitro* 3D engineered tissue. Mechanical and physical parameters such as shear stress, flow rate, pressure and geometrical cues have been demonstrated to influence the behaviour of cells in living tissues both *in vivo* and *in vitro*. The scientific question I want to address concerns how the control of such engineering parameters with a microfluidics approach can induce biological phenomena such as angiogenesis and anastomosis of blood vessels in a viable 3D engineered tissue – physiological or pathological - and build an accessible vessel network that can be investigated in several aspects.

The easiest way to increase the exchange of nutrients and metabolites in *in vitro* 3D cultures is providing a dynamic culture, which can be obtained in bioreactors or with a microfluidic approach. The custom design of a microfluidic device and the controlled use of cell media flows – with syringe pumps or by hydrostatic pressure - can reduce the negative effects of scarce diffusion inside the extracellular matrix (ECM).

Another solution to the problem may be vascularization, which consists in the process of formation of blood vessels. The presence of a perfusable network may switch the mechanism of molecule transport, from diffusive to convective, so that nutrients and oxygen can be conveyed through vessels *lumen* and can be exchanged through the endothelial walls, reducing the impact of diffusion coefficient. The formation of a mature capillary network inside a biohybrid may also better represent the biological environment, and can provide a deeper understanding of physiological phenomena in a 3D model *in vitro*.

Microfluidics can be used for inducing capillary formation inside a biohybrid, a phenomenon which needs fine control over parameters such as flow rate, pressure and gradients for biochemical and mechanical stimulation of different cells, that microfluidic devices can guarantee. Once vascularization is obtained, the biohybrid may be implanted *in vivo* with a reduced time of adaptation to human body, or the vascular network may be kept perfusable by using the microfluidic device. Basically, an organ-on-chip can be obtained as a platform to investigate and analyze a circulatory system *in vitro*.

Usually, *in vitro* vascular networks are generated by two distinct approaches: by endothelial-lined channels, or by self-assembled networks. The approach in this project will be the combination of the two described strategies: the designed microfluidic device will present a part – *i.e.* the channels – which will be lined with ECs, but the channels will present a communication pore on the side wall that directly connect to the tissue chamber. Vasculogenesis will be induced inside the engineered 3D tissue. There will be an interface connection between channels and chamber constituted by the lateral surface of the tissue itself, which will undergo endothelial lining as the channel walls of the chip. Controlling transendothelial flow at the communication pores, shear stress on the lining ECs, VEGF gradients – from the inlet or directly secreted by fibroblasts in the tissue – and flow rates, sprouts will depart from the channel-chamber interface and anastomose to the pre-vascular network inside the biohybrid.

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Francesco Del Giudice, PhD student XXXV cycle, May 2021