A System for High-Reproducibility Vessel-on-Chip Fabrication

V. Guadagno[1], C. Raineri[1], T. Mencarini[1], S. Bozzi[1], E. Raschi[2], C. Bodio[2], P.L. Meroni[2], A. Redaelli[1]

[1]Dept. of Electronics, Information and Bioengineering, Politecnico di Milano, Milan, Italy

[2]Istituto Auxologico Italiano

Understanding endothelial interactions with pathological elements and blood cells is crucial for advancing vascular disease research. The endothelium plays a central role in maintaining vascular homeostasis, regulating blood flow, and preventing thrombosis. However, in pathological conditions specific interactions can disrupt endothelial function, promoting a prothrombotic state and increasing the risk of vascular complications [1]. To accurately study these processes, developing advanced 3D vessel models that replicate the extracellular environment and physiological culture conditions of endothelial cells is essential, offering greater reliability than traditional 2D *in vitro* systems.

Vessel-on-Chip (VoC) constitute a promising technology for culturing endothelial cells in 3D biologically relevant extracellular environments while allowing controlled perfusion [2,3]. These lumens can be created by inserting a template into a hydrogel precursor and removing it after gelation. However, manual extraction introduces high intra- and inter-operator variability, affecting lumen reproducibility and endothelialisation outcomes. Factors such as extraction speed, movement consistency, and vibrations can significantly impact lumen geometry.

In the perspective of developing high-fidelity 3D VoC models, we present a novel fabrication method enhancing reproducibility by minimizing operator-dependent variability. We designed a motorized system that automates needle extraction, the most critical step for reproducibility. The system consists of a linear actuator that extracts the needle, with guides ensuring optimal alignment along x and y, while the vertical structure of the clamping clip ensures versatility along the z direction, accommodating slight variations between devices. Three operational modes, controlled by buttons, allow for clamping, extraction, and emergency stop in case of setbacks.

We validated the system on our designed VoC, where a 160 µm lumen is obtained within a Collagen I hydrogel using an acupuncture needle as a template. The lumens are characterized via confocal microscopy with FITC-labeled collagen coating. Comparative analysis of manually and automatically extracted lumens demonstrated improved structural consistency and reproducibility using our motorized system, highlighting its potential for standardizing VoC fabrication and advancing vascular research.

[1] D.J. Medina-Leyte et al., *International Journal of International Sciences* (2021)

[2] A.M.A.O. Pollet, J.M.J der Toonder., *Bioengineering* (2020)

[3] Y. Juste-Lanas et al., *APL Bioengineering* (2023)