Cell seeding and flow-induced stimulation in automated culture platform for physiologically relevant 3D vascular co-culture modelling

E. Pederzani[1], A. S. Bolandrina[1], C. E. Campiglio[2], E. Carrara[3], M. Ripamonti[3], S. Garbellini[1], E. Gorlini[1], A. Caldiroli[4], S. A. Riboldi[4], A. Remuzzi[2], G. B. Fiore[1], M. Soncini[1]

[1]*Department of Electronics, Information and Bioengineering, Politecnico di Milano, via Giuseppe Ponzio 34, Milano, IT*
[2]*Department of Management, Information and Production Engineering, University of Bergamo, via Pasubio 7, Dalmine, IT*
[3]*Department of Biomedical Engineering, Istituto di Ricerche Farmacologiche Mario Negri-IRCCS, Bergamo, IT*
[4]*Dialybrid S.r.l., R&D, Via Vivaldi 32/A, Cantù, IT*

First author: Elia Pederzani (elia.pederzani@polimi.it)
Presenting author: Alessia Sofia Bolandrina (alessiasofia.bolandrina@polimi.it)

Abstract

Tissue-engineered vascular grafts aim to address the shortage of vascular substitutes by harnessing endogenous regeneration. However, their success remains limited due to the complex interplay between neo-tissue formation, scaffold degradation, and hemodynamics. Although preclinical animal testing remains a standard, but species- and model-specific differences which hinder clinical translation. To better investigate these complex phenomena, 3D *in vitro* models, providing more physiologically relevant environment than conventional approaches, have become essential.

In this context, we developed an advanced culture system capable of replicating *in vivo*-like flow-induced stimuli and performing automated cell seeding. The system enables the housing of tubular constructs, allowing precise control and monitoring of fluid dynamic and mechano-chemical stimuli. The platform was designed with a focus on versatility and automation, with closed-loop control functions ensuring consistent and reproducible operation while minimizing operator-dependent variability.

Through a multidisciplinary approach, combining *in silico* modelling and biological experiments, we define an automated cell seeding procedure to obtain a 3D luminal co-culture. Human umbilical vein endothelial cells (HUVECs) and human aortic smooth muscle cells (HASMCs) were automatically seeded. Co-culture was maintained for five days, with flow-induced stimuli applied during the last three. Immunostaining confirmed a well-organized and layered structure, with HUVECs above densely packed HASMCs. This 3D model advances the study of vascular mechanobiology, cellular and cell-scaffold interactions, enhancing research in vascular engineering field.

The developed system represents an effective platform to investigate these phenomena, serving as a reliable alternative to animal models while significantly reducing the cost and ethical concerns of preclinical and clinical research.

Acknowledgments: This work was funded by the National Plan for NRRP Complementary Investments (PNC, established with the decree-law 6 May 2021, n. 59, converted by law n. 101 of 2021) in the call for the funding of research initiatives for technologies and innovative trajectories in

the health and care sectors (Directorial Decree n. 931 of 06-06-2022) - project n. PNC0000003 - AdvaNced Technologies for Human-centEred Medicine (project acronym: ANTHEM). This work reflects only the authors' views and opinions, neither the Ministry for University and Research nor the European Commission can be considered responsible for them.

Disclosure of conflicts of interest: Yes, Alice Caldiroli and Stefania Adele Riboldi are employees of Dialybrid S.r.l., manufacturer of the vascular grafts used in this study

Ethical Statements: No