Biomimetic air-liquid interface milli-bioreactor for skin tissue engineering applications

B. Masante,[1,2,3], S. Gabetti,[1,3] D. Baruffaldi,[4] S. Villata,[4], A. Sanginario,[5] A.L. Audenino,[1] F. Frascella,[3,4] D. Massai[1,3]

[1]Department of Mechanical and Aerospace Engineering and PolitoBIOMed Lab, Politecnico di Torino; [2]Department of Surgical Sciences, University of Torino; [3]Interuniversity Center for the Promotion of the 3Rs Principles in Teaching and Research, Pisa; [4]Department of Applied Science and Technology, Politecnico di Torino; [5]Department of Electronics and Telecommunications, Politecnico di Torino, Italy

Conventional approaches for testing drugs and cosmetics are based on *in vitro* monolayer culture or animal models, with clear limitations related to low biomimicry and ethical issues, respectively. Here, we developed a biomimetic milli-bioreactor for modelling *in vitro* the Air-Liquid Interface (ALI), to be used for culturing Three-Dimensional (3D) skin tissue models for drug testing and Skin Tissue Engineering (STE) applications.

The milli-bioreactor Culture Chamber (CC), made of a citocompatible and autoclavable resin (VisiJet M2S-HT250; 3D Systems, Rock Hill, USA), was designed for housing standard transwell inserts and is part of a closed-loop recirculation circuit (priming volume <1.5 mL), based on a multichannel peristaltic pump (Longer, Futian, China). The optimization of the CC geometry was guided by Computational Fluid Dynamics (CFD) simulations (COMSOL Multiphysics), performed imposing different inlet and outlet flow rates (0.1-0.5 mL/min). Performance tests for assessing the bioreactor reliability were carried out. Finally, for sterility maintenance assessment, Gelatin-Methacryloyl (GelMa) 10% w/v hydrogels laden with A540 GFP+ cells were poured in transwell inserts and cultured for 10 days in DMEM (gibco; Thermo Fisher, Waltham, USA) within the milli-bioreactor in static conditions. Cellular viability was qualitatively assessed by fluorescence analysis.

CFD results demonstrated that the flow streamlines within the CC run tangentially to the transwell membrane, preventing recirculation regions. Performance tests confirmed the ease of use and reliability of the milli-bioreactor, without air bubble stagnation. Fluorescence analysis showed high cell viability, confirming the bioreactor sterility maintenance.

The proposed milli-bioreactor is a powerful parallelizable tool for investigating the complexity of skin tissue, overcoming the limitations of conventional cell culture methods and representing a viable alternative to animal models. Biological tests with 3D skin tissue models are ongoing.

Correspondence: B. Masante
E-mail: beatrice.masante@polito.it

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