BIOMIMETIC BIOREACTOR FOR AIR-LIQUID INTERFACE CULTURE - SKIN TISSUE ENGINEERING APPLICATION

Beatrice Masante (1,2), Stefano Gabetti (1), Simona Villata (3), Désirée Baruffaldi (3), Alessandro Sanginario (4), Francesca Frascella (3), Alberto L. Audenino (1), Diana Massai (1)

1. PolitoBIOMed Lab and Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Italy; 2. Department of Surgical Sciences, University of Torino, Italy; 3. Department of Applied Science and Technology, Politecnico di Torino, Italy; 4. Department of Electronics and Telecommunications, Politecnico di Torino, Italy

Introduction

In vivo, epithelial tissues are continuously in contact with the external environment [1]. For mimicking such condition *in vitro*, the air-liquid interface (ALI) approach is widely adopted [2]. Traditionally, ALI is obtained by using a transwell (with a semipermeable membrane) inserted in a well, in order to separate the liquid and the air compartments [3]. However, such static culture condition does not mimic the nutrient and gas transport provided by the native microvasculature and can lead to gradient formation. To overcome this limitation, culture medium recirculation can be adopted, knowing that it is crucial to avoid bubble formation and stagnation under the ALI, to prevent sample drying. Here, we developed a biomimetic bioreactor for ALI culture with recirculating culture medium, to be used for skin tissue engineering investigations.

Materials and methods

In detail, the bioreactor was designed for: 1) guaranteeing ALI culture; 2) allowing culture medium recirculation; 3) ensuring a continuous nutrient and oxygen transport across the ALI while avoiding air bubble stagnation under it; 4) being easy-to-use with conventional lab equipment. To fulfill these requirements, the bioreactor is composed of: 1) a culture chamber (CC) for housing a commercial transwell, used for separating the liquid and the air compartments; 2) a recirculation circuit for continuous culture medium flow. In detail, the CC (diameter = 37 mm, height = 24 mm) was designed (Solidworks) and manufactured by multijet printing (VisiJet M2S-HT250, ProJet MJP 2500 Plus, 3D Systems), and it consists of a lid and a cylindrical base with barbed inlet and outlet ports for tubing (inner diameter = 1 mm) connection (Fig.1A). The CC is part of a closed-loop recirculation circuit based on a multi-channel peristaltic pump (Ismatec), a medium reservoir, oxygen-permeable tubing (platinum-cured silicone tubing, Darwin microfluidics) and luer lock connectors (Fig. 1B). With the inlet and the outlet tubing both mounted on a pump cassette, the recirculation circuit ensures a constant volume of culture medium inside the CC with a controlled free liquid surface. Stationary computational fluid dynamic (CFD) simulations (COMSOL Multiphysics) were performed for supporting the optimization of the CC geometry. The CC was modeled with 3 domains (Fig. 1C) imposing inlet and outlet flow rates in the range 0.1-0.5 mL/min, for guaranteeing capillary-like flow velocity values within the CC [4]. Finally, performance tests were carried out in-house using demineralized water and testing different flow rates (0.1-0.5 mL/min) for 2 days for assessing the reliability of the bioreactor and possible air bubble stagnation.

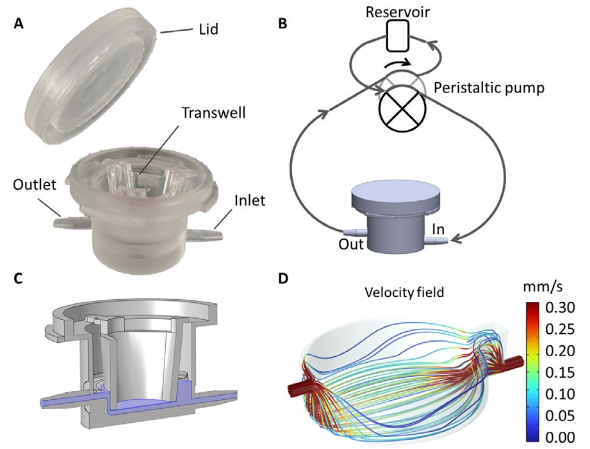

Figure 1: A) Picture of the bioreactor CC housing a transwell; B) Circulation circuit scheme; C) Front section of the modelled CC domains (fluid domain in blue); D) Flow streamlines developing within CC imposing flow rate = 0.1 mL/min.

Results

The CFD results showed that the flow streamlines follow the internal geometry of the culture chamber and flow tangential to the transwell membrane, avoiding recirculation regions (Fig. 1D). Preliminary in-house tests confirmed the bioreactor ease of assembling and use and its reliability. In particular, it was assessed that a constant volume of culture medium was maintained and, in case of air bubble presence, these were easily expelled within the CC at the free liquid surface before reaching the transwell membrane.

Conclusion

A biomimetic bioreactor providing ALI culture with recirculating culture medium was developed. Performance tests confirmed its ease of use, reliability, and the absence of air bubble stagnation under the transwell membrane. Biological tests with 3D models of skin, based on cell-seeded gelatin-methacryloyl (GelMA) hydrogels are ongoing. The constructs will be mechanically characterized by nanoindentation and compared with human skin tissue samples.

References

1. Rimal, et al, Appl. Mater. Today 25, 101213, 2021.
2. Chen, et al, Reprod Domest Anim, 3:38-45, 2019.
3. Bluhmki, et al, Sci. Rep, 10:13022, 2020.
4. Fagrell, et al, Am J Physiol, 233(2):H318-H321, 1977