DESIGN AND VALIDATION OF AN *IN VITRO* PLATFORM FOR LYMPHOCYTES RECIRCULATION IN CANCER IMMUNOTHERAPY APPLICATIONS

Paolo Ritter (1,2), Stefania Oliveto (2,3), Claudio Conci (1), Emanuela Jacchetti (1), Stefano Biffo (2,3), Manuela Teresa Raimondi (1)

1. Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano – piazza Leonardo da Vinci 32, Milan, Italy; 2. National Institute of Molecular Genetics "Romeo ed Enrica Invernizzi" - INGM – via Francesco Sforza 35, Milan, Italy; 3. Department of Biosciences, University of Milan – via Giovanni Celoria 26, Milan, Italy

Introduction

In recent years, the development and validation of immunotherapy models played an increasingly relevant role in biological research. As compared to traditional therapies such as surgery, chemotherapy, and radiotherapy, immunotherapy is expected to be one of the most promising approaches to tumor treatment. [1]. In addition, the introduction of three-dimensional (3D) cellular models is assisting in lowering high failure rates in research. The primary causes of these failures include partial translation and a failure to confirm experimental conclusions gained from two-dimensional (2D) in vitro models during *in vivo* animal research. In this regard, our research seeks to develop a predictive and experimental *in vitro* model capable of providing a controlled environment for the interaction of cancer and immune cells. To that purpose, we developed an ad-hoc millifluidicdevice (MOAB-Nichoid) that combines the primary features of two well-established culturing supports: the Nichoid, a nanostructured polymeric scaffold [2], and the MOAB, a miniaturized optically accessible bioreactor [3].

Methods

The MOAB-Nichoid was realized by two-photon polymerizing (2PP) the Nichoid scaffolds. These were then embedded into the chambers of the MOAB with a gluing procedure.

The design and realization of the MOAB-Nichoid was accompanied by the development of a hydraulic circuit that, coupled with a syringe pump, would allow the recirculation of suspended cells through the bioreactor. This circuit required the insertion of a dual check valve and a custom-made reservoir to collect the cells suspension and allowing its recirculation.

Finite element modelling was setup by means of COMSOL Multiphysics software suing the laminar flow and particle tracing physics to assess the behavior of the circulating cells inside bioreactor chamber. First we retrieved parameters such as flow velocity, pressure, and shear stress in the bioreactor chamber; then these were used to simulate the flow of immune cells by means of particle tracing.

Results

We realized 14 samples of the MOAB-Nichoid platform consisting in three independent chambers each (Fig.1e-f). These allowed to perform both a functional and a biological validation, confirming the feasibility and reproducibility of immune cell recirculation experiments through the developed bioreactor.

The first step of the validation process focused on the assessment of the adequate functioning of the hydraulic circuit. Further *in vitro* analyses evaluated the immune cells recirculation capability of the setup, confirming and completing the predictions made on the basis of the *in silico* model.

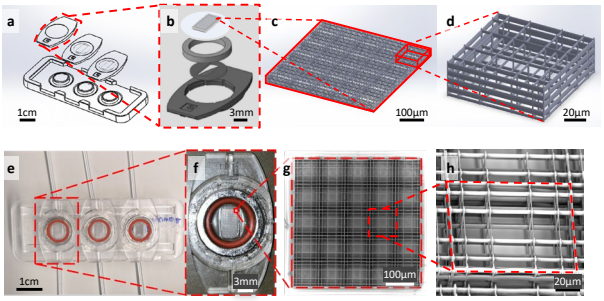

Figure 1: a. CAD of the bioreactor; b. CAD of the lid of the single chamber with the array of Nichoids; c. CAD of Nichoid block; d. CAD of the single niche; e. Picture of the MOAB Nichoid; f. Picture of the lid of the single chamber with the polymerized scaffold; g. SEM image of the Nichoid block; h. SEM image of the single niche.

Discussion

The *in vitro* model called MOAB-Nichoid was demonstrated to be suitable for immune cells recirculation. Furthermore, it will allow for the culture of cells derived by solid cancers, standing as an optimal candidate for an animal-free patient-specific platform for testing the efficacy of cancer immunotherapies, increasing the effectiveness of cancer treatment.

References

1. De Ponte Conti et al, eLife, 10, 2021.
2. Testa et al, Frontiers in Bioeng and Biotech, 10, 2023.
3. Perottoni et al, Lab on a Chip, 21, 2021.

Acknowledgements

This work was supported by: European Research Council (ERC, projects BEACONSANDEGG, G.A. 101053122 and NICHILD, G.A. 101068512); European Commission (EU, FET-OPEN project IN2SIGHT, G.A. 964481 and flIMAGIN3D, G.A. 101073507); NC3Rs (project MOAB, G.A. NC/C019201/1); AIRC (IC 2022 27021 to S.B.).