Integration of Customized TEER Electrodes in a Microphysiological System Fabricated with an Innovative Low-Cost Production Technique

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***Abstract*—Biological barriers (BB) are structures within our bodies that are crucial for tissue homeostasis and drug absorption. Dysfunction of these barriers is associated with various diseases, underscoring the need for physiologically relevant in vitro models. Microphysiological systems (MPS), or Organ-on-Chip (OoC) systems, are cutting-edge models explored as alternatives for drug screening. A limitation of current OoC systems is the absence of sample parameter monitoring capabilities, leading to active research in MPS sensorization. Trans Epithelial Electric Resistance (TEER) measurements are essential for quantifying barrier permeability non-invasively. Although commercial TEER systems are widely used, they may not be suitable for MPS applications. Consequently, there is growing interest in developing customized TEER electrodes for specific MPS devices. The integration of electrodes into MPS often lacks standardized guidelines, impacting the performance and scalability of TEER monitoring systems. To address these challenges, this work proposes five key features for a rational prototyping process of MPS-specific TEER electrodes. In line with these features, the study focuses on a specific modular MPS : the True Tissue on Platform (TToP). This work propose an innovative and versatile approach for electrodes fabrication and implements it in the TToP static module for TEER measurements. This approach involves the use of gold in form of edible sheets as economic source of the metal for electrodes production. TToP static module compatible electrodes are designed to emulate commercial ones while addressing their limitations. This approach enable a reliable comparison with the standard throughout the project. An assessment of the production process parameters is done to evaluate the coupling of materials and fabrication techniques used. This is followed by the validation of electrodes produced, assessing their biocompatibility, functionality and reliability in measurements.**

***Keywords*—TEER, biological barriers, sensors design, microphysiological systems**

I. INTRODUCTION

Biological barriers allow the separation between different compartments of the human body or between the body and the external environment. They have a fundamental role in controlling the absorption of exogenous substances, as well as in the maintenance of homeostasis in different body compartments. Given the significance of this physiological structure the generation of physiologically relevant in-vitro models of biological barriers can play a key role in understanding human diseases and in the development of more predictive methods for assessing toxicity and drug or nutrient absorption [1]. These in vitro models span from traditional ones, such as transwell inserts, to more advanced models called microphysiological systems (MPS), that provide physiological stimuli to the sample in order to better reproduce the in-vivo environment. To assess the proper functioning of the biological barrier in the in vitro model, the Trans Epithelial Electrical Resistance (TEER) can be measured. This method allows, through the placement of electrodes across the sample, the monitoring of the tissue permeability and therefore its state of health. This technique guarantees the collection of real-time data, in a non-invasive and quantitative way [3]. For this reason many studies are now focusing on the production and integration of TEER biosensors inside in vitro models. TEER measurements are typically conducted using commercial measurements system, like EVOM3 Voltohmmeter coupled with STX electrodes. However, these widely used systems, come with several limitations, related to materials biocompatibility, costs, and complex MPS suitability [2]. In the current literature, customized TEER electrodes production processes often lack specific guidelines, leading to reduced performance and limited scalability. To address this issue, this work proposes five key features for a rational prototyping process for customized TEER electrodes: easy and low-cost manufacturing, materials biocompatibility, simulation guided design, stable positioning, comparison with the standard. With this rational view, this work aims to prototype customized electrodes for TEER measurements in a specific MPS: the True Tissue on Platform (TToP) [5] . TToP device present two bicompartmental modules: an open-well static module compatible with standard 12-well plates, and a perfusion module that enables continuous recirculation of cell culture medium. With the main focus on the static module, customized gold electrodes are designed and validated for TEER measurements on the device

II. MATERIALS AND METHODS

A. Design and Fabrication process

The electrode structure was designed to fit TToP static module and to facilitate a reliable comparison with standard TEER measurement systems. To achieve this, the geometry of the custom electrodes closely mimics that of the STX2-PLUS electrodes, hereinafter referred to as “commercial electrodes” . These electrodes are among the most commonly used "chopstick" style electrodes in literature. Therefore electrodes present a slim elongated structure with two terminals, one for

connection with the electronics and one for sensing purposes. Some dimensions adaptation are introduces to fit TToP device and electrodes materials constraints. Inspired by the work of Santos and co workers [4], an innovative method for electrodes manufacturing was introduced. Gold in form of edible sheets was used as a cheap and versatile source of the metal. Hence, TToP custom electrodes feature gold sensing and connecting components integrated with PMMA and tape for support and insulating layers. Material processing is accomplished using CO2 laser cutting techniques and manual assembling.

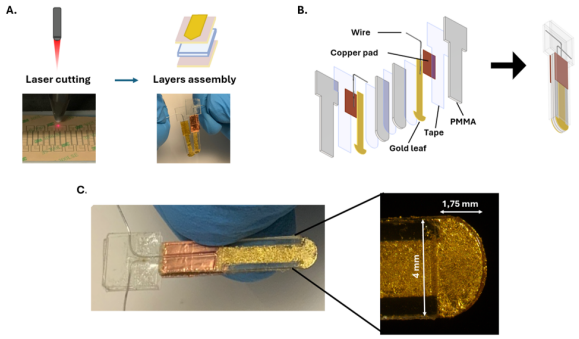

Fig. 1: (A) Schematic view and photos of the production process main phases: laser shaping of materials and layers assembling; (B) Exploded view of a single electrode; (C) Image of the gold customized electrode with sensitive part dimensions

B. In silico validation

Due to technical constraints related to materials and machinery, the design of the customized electrodes slightly changes from that of the commercial ones. Consequently, computational Finite Element Method (FEM) simulations were performed to validate and compare the customized electrodes design and material with the standard. These simulations were conducted using COMSOL Multiphysics v.6.0 software (COMSOL Inc., Burlington, MA, USA) in AC/DC module, with sensitivity (S) selected as the guiding parameter. Sensitivity accounts how much each portion of the sample volume contribute to the overall measured resistance. Simulation of both commercial and custom gold electrodes under their working conditions aimed to assess the distribution of Sensitivity across the sample area. Ideally, sensitivity would exhibit a completely uniform distribution over the sample area. However, minor deviations of customized electrodes from the standard are acceptable as long as significant differences are avoided.

C. Functionality validation

Following the validation of the design and the optimization of the production process, electrodes are tested and compared to commercial ones on biological samples. For this purpose twelve static TToP modules were seeded with EA.hy926 cells and cultured until confluent layer formation. Cells were cultured in sterile conditions using Dulbecco's Modified Eagle Medium (DMEM) with 4.5 g/L D-Glucose, L-glutamine and sodium pyruvate, with 10% FBS, 1% Pen-Strep, 1X HAT (hypoxanthine- aminopterin- thymidine) in a 37°C, 5% CO2 standard incubator.
To enable an efficient measurement setup, an electrode housing was designed and 3D printed. Customized electrodes are connected to EVOM3 VoltOhmmeter through an adapted in order to perform TEER measurments.

D. Materials biocompatibility

The next step for a complete validation involves verifying the biocompatibility of the customized electrodes. The validation experiments involved the use of custom-designed holders, which were 3D printed and fixed to electrode blades. These holders allowed the electrodes to be immersed in the wells of a 12-well plate at a specific distance from the cell layer. In this way materials could remained immersed in the cell culture medium allowing the assessment of toxic compounds releases. Both gold and commercial electrodes underwent this testing for 5 days, initially on endothelial cells cultured in standard wells, followed by the same cells cultured in TToP static modules. Possible toxic effects on the cell layer was evaluated through bright field images, fluorescence images and cell density computation. Fluorescence images were taken at the end point, after day 5 of culture. Cells were fixed in Formalin 10% and stained with DAPI and Acridine Orange (AO).

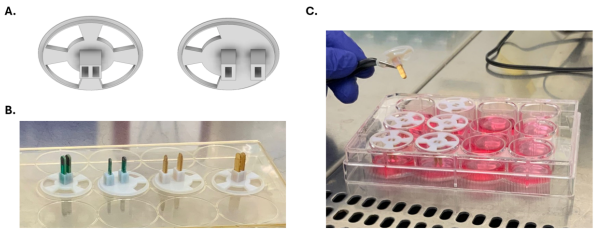

Fig. 2: (A) CAD model of the holder for standard wells and TToP static module; (B) Image of the two types of holders with gold and commercial electrodes fixed; (C) Image of the electrodes blades put in contact with cells in a multiwell plate through the 3D printed holders

III. Results

A. Production parameters

1) Production time: The production process,utilizing stratification and laser cutting, enables simultaneous manufacturing of multiple pieces, significantly reducing time. The choice of the number of pieces to produce in parallel is flexible. Increasing the number of pieces produced in parallel, increase production time, but not in a linear fashion. Doubling the number of pieces does not double the fabrication time since certain steps are more time-efficient with increased parallel production. With this technique the time required for production of ten electrodes amount to only fifty minutes,which is an impressive result considering the simplicity of the process.

2) Production costs: The production process described differs from typical electrode production methods as it doesn't require the use of clean rooms. Clean rooms offer significant benefits in terms of product quality and regulatory compliance, however they also come with challenges related to complexity, operational limitations, and costs related to maintenance and energy consumption. Therefore the presented fabrication process guarantees economic savings in terms of material used and machinery needed. Including both material costs and expenses for operators (60€ per hour) and energy, the production of a single piece remains below 5 €, which is largely under prices of commercially available electrodes for TEER measurements especially gold ones [6].

3) Production rate of success: After refinement of the design and production technique, the rate of defective electrodes decreased significantly to just 7.69% of the total produced, despite the delicate nature of gold sheets and the manual assembly process, which increases the risk of damage.

B. Sensitivity distribution

For the in silico validation, sensitivity distribution across the sample area was examined using sensitivity heatmaps. The simulations compared the performance of customized and commercial electrodes under varying sample conditions, including scenarios with lower or higher resistance (corresponding to lower or higher TEER values). Output data demonstrated two important concepts: (i) there's no significant difference in measurement reliability between customized and commercial electrodes, (ii) Sensitivity is homogeneously distributed, ranging from 0.95 to 1.6 in the worst case.

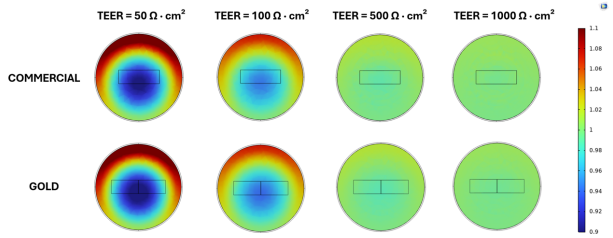

Fig. 3: Commercial (up) vs Gold customized electrodes (down) sensitivity heatmaps for different values of TEER

C. Measurements on biological samples

Twelve static TToP modules were seeded with EA.hy926 cells and cultured until confluent layer formation. Resistance measurements with a gold couple and with commercial electrodes were performed in triplicated and compared to each other as shown in Fig. 4. No statistical differences are present in measurements between commercial and gold electrode pairs for all twelve samples (Ordinary one-way ANOVA, GraphPad 8.0.2).

D. Materials biological effects

The biological validation of the customized gold electrodes aimed to demonstrate on one hand limitations related to standard electrodes, attributed to their material toxicity, and on the other hand compatibility of gold custom electrodes with long-term exposure to the sample environment.

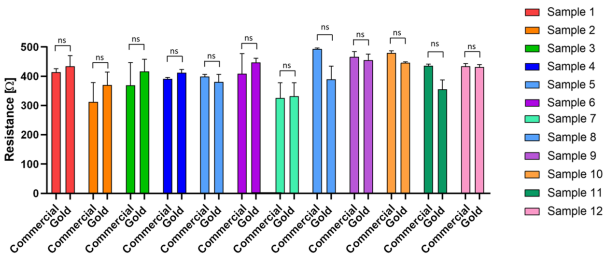

Fig. 4: Resistance measurements performed on the twelve TToP samples with gold electrodes and commercial electrodes

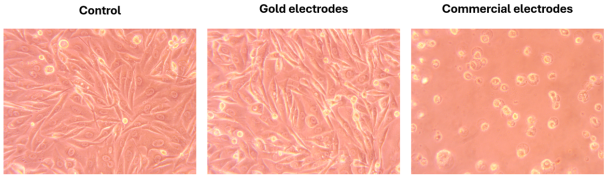

Fig. 5: Bright field images of samples being in contact with the two types of electrodes and compared to the control

Qualitative examination of bright field images assess changes of cells healthy morphology after prolonged exposure to commercial electrodes material. On the contrary this effect is not encountered in samples being in contact to gold electrodes that show no differences from the control. Same conclusion can be drawn by visual inspection of fluorescence images of samples stained with acridine and DAPI.

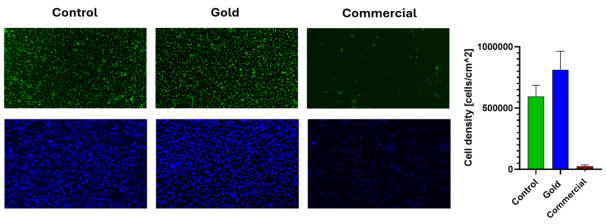

Fig. 6: Fluorescence images of samples exposed for five days to the two different types of electrodes. Cells were labeled with AO and DAPI (left). On the right: cell density in TToP samples in contact with gold and commercial electrodes blades. Symbol "ns" stands for no statistical difference, while the asterisk indicate a P value <0,05. Time point: day five of continuous interaction with materials. For the density computation, three images of the sample area were used for cell counting.

Cell density computations and analysis (using Ordinary one-way ANOVA, GraphPad 8.0.2) confirm the observed trends. No statistical differences are present between gold electrodes and the control, while commercial electrodes show significant difference when compared to the control and gold electrodes. The increased cell density associated with the gold sample is

not indicative of enhanced cell viability by gold, but rather reflects general variability within the samples.

IV. CONCLUSION

This work focused on prototyping and validating customized gold electrodes for TEER measurements in TToP static module. A novel fabrication process was developed, utilizing low-cost materials and techniques, leading to an high-quality product. Various aspects of electrodes performance were examined throughout the project, including design validation through sensitivity-guided in silico simulations, which confirmed the reliability of the electrode design. Measures on biological samples demonstrated gold electrodes functionality and closeness to the standard ones (commercial electrodes). Nevertheless, further experiments are necessary to demonstrate the robustness of the measurement system. Biological experiments revealed the long-term compatibility of the customized gold electrodes with the cells environment, highlighting their potential for permanent integration into MPS without risk of sample damage. In contrast, commercial electrodes were found to be inadequate for prolonged contact with the cellular environment.

The use of gold as an electrode material in MPS sensorization is widespread in literature. However, edible gold sheet as a source of the material is still never used in TEER measurements applications, making this work highly innovative. Furthermore, the versatility of the production process enables the design of electrodes for closed-well sandwich-like MPS which is a topic of great interest in current research. It can be concluded that TToP gold customized electrodes efficiently enhanced the five key parameters discussed in the introduction for a rational electrodes prototyping. From a future perspective, the integration of electrodes into a high-throughput device is expected to overcome the current limitations of TEER measurement systems.

V. ACKNOWLEDGMENTS

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