INTEGRATED MICROFLUIDIC TISSUE BARRIER SENSOR MODULE FOR A STANDARDIZED AND MODULAR ORGAN-ON-CHIP PLATFORM

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ABSTRACT

We present the fluidic and electrical packaging of a novel silicon-based trans-epithelial electrical resistance (TEER) sensor chip designed for a modular and standardized organ-on-chip (OoC) platform. The package comprises three key components: the housing of the TEER chip, microfluidic routing for seamless integration with the platform, and electrical connections to a platform-integrated potentiostat. This modular solution enables continuous impedance measurements while maintaining unobstructed optical access to the tissue culture region. Experiments confirmed leak-free fluid flow across the stacked microfluidic channels and stable sensitivity of TiN electrodes to PBS. The TEER module retains optical transparency, biocompatibility, and industrial scalability, supporting advanced *in situ* tissue barrier assessments in standardized OoC systems.

KEYWORDS

Barrier-on-chip, microfluidic platform technologies, organ-on-chip, standardization, trans-epithelial electrical resistance

INTRODUCTION

Organ-on-chip (OoC) technology has emerged as an innovative approach in biomedical research, offering dynamic and physiologically relevant models of human organs and diseases. By replicating the microarchitecture and biochemical and mechanical cues of living tissues, OoCs provide significant advantages over traditional static two-dimensional (2D) and three-dimensional (3D) cell cultures, which often fail to capture the complex interactions present *in vivo*. The improved recreation of the microtissue environment in OoCs by the incorporation of liquid flow related mechanical cues enhances the human relevance of human cell based new approach methods [1].

A critical aspect within these microfluidic systems is to evaluate the functionality of tissue barriers, which can be done by measuring trans-epithelial electrical resistance (TEER). TEER provides a quantitative assessment of cell layer integrity and serves as a non-invasive, real-time indicator of barrier function. Integrating TEER measurements into OoC platforms has been demonstrated in various studies, highlighting its utility in assessing human epithelial barrier function [2].

While conventional TEER measurements typically rely on two-electrode configurations, a four-electrode system offers significant advantages, especially for detecting low resistance values. By eliminating the influence of electrode-medium contact resistance and mitigating the effects of wire and contact resistances, the four-electrode approach enhances measurement precision and reliability, ensuring more accurate impedance readings [3]. This enhancement is particularly valuable for applications requiring high-sensitivity assessments of barrier integrity in microfluidic environments.

In our previous work, we developed an advanced TEER chip that demonstrated biocompatibility, optical transparency, and seamless integration of a four-electrode TEER measurement geometry [4]. This silicon-based OoC device incorporates two pairs of titanium nitride (TiN) microelectrodes patterned along two vertically stacked microchannels separated by an ultra-thin, microporous, optically transparent silicon nitride membrane (Fig. 1A).

Building upon this foundation, the current study focuses on the fluidic and electrical integration of the TEER sensor chip into the standardized Translational OoC platform (TOP) [5]. We present the comprehensive packaging of the TEER chip into a standardized fluidic and electrical module, enabling the study of single or multi-organ interactions within the standardized OoC Platform (Fig. 1B). This packaging solution enhances the reproducibility and versatility of OoC systems, contributing to more predictive and physiologically relevant *in vitro* models for biomedical research and drug development.

FABRICATION PROCESS

The full process flow for the packaging of the TEER chip into a TEER module compatible with the TOP is illustrated in Fig. 1C-E, outlining the sequential steps involved in manufacturing and assembling the device. The overall packaging of the OoC system consists of multiple elements, including sealing of the microfluidic channels, integration of fluidic routing components, and electrical interfacing using wire-bonding from chip to custom-designed printed circuit board (PCB (Fig. 1C). Each of these aspects is carefully engineered to ensure robust operation, minimal leakage, and reliable electrical connectivity for impedance measurements.

The silicon-based TEER chip is fabricated through a scalable, wafer-level batch process, and features two vertically stacked microchannels separated by an optically transparent, submicron-thin, microporous silicon nitride membrane (Fig. 1A) [4]. The TEER chip is sealed with exposed ports for liquid routing. Laser-cut polyethylene terephthalate (PET) layers are bonded to the top and bottom

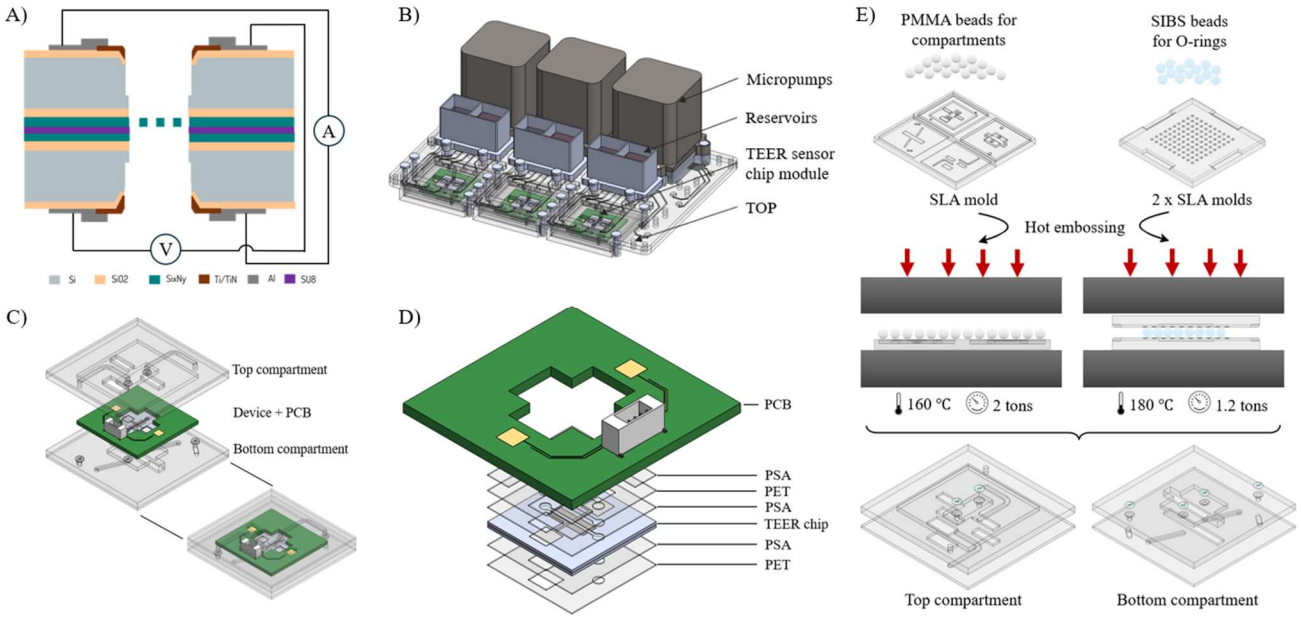

Figure 1: A) Sketch of the TEER sensor chip cross-section, highlighting the four slanted-wall electrodes and the optically-transparent membrane. B) Integration of TEER modules into the TOP. C) Exploded view of the TEER module. D) Exploded view of the TEER chip packaging. The chip is bonded to a custom-designed PCB. E) Design and assembly of top and bottom compartments with O-rings for sensor module packaging.

surfaces of the chip using pressure-sensitive adhesive (PSA), ensuring controlled fluid flow while maintaining open access to microchannels for external systems. Fluidic routing follows the open-sourced, ISO 22916-compliant TOP design rules (TDRs), which provide standardized port layouts for seamless module-to-platform integration [6]. The fluidic package consists of two distinct compartments, as depicted in Fig. 1C. The upper compartment is used to guide fluids through the top microchannel, while the lower compartment facilitates fluid flows to the bottom microchannel and provides a pathway between the TEER chip and the platform. To prevent leakage and maintain isolation between the compartments, soft O-rings are utilized at critical interface points.

The fabrication of the compartments and sealing components involves a combination of additive manufacturing and polymer processing techniques. Specifically, high-temperature V2 resin molds are 3D-printed using stereolithography (SLA). The polymer compartments are then manufactured via hot embossing. Poly(methyl methacrylate) (PMMA) and poly(styrene-block-isobutylene-block-styrene) (SIBS) are selected for the compartment material and the O-rings, respectively. A temperature of 160 °C is used to melt the PMMA which is pressed into the cavities of the mold at a pressure of 2.5 MPa for 15 minutes. The demolding takes place after the hot embossing had cooled down to below 100 °C.

By employing these optimized fabrication techniques and material selections, the TEER chip module achieves a high level of integration with its electrical and fluidic components, ensuring both functional reliability and ease of use in experimental applications. The modular and standardized design approach supports reproducibility and versatility, making the TEER chip adaptable for various OoC research and development applications.

EXPERIMENTAL RESULTS

The fabricated TEER module was evaluated for its fluidic and electrical performance before *in vitro* biocompatibility and preliminary cell culture assessments. To verify leak-free integration, the module was interfaced with the TOP and tested under flow conditions. Electrical functionality of the fabricated chips, including the integrated electrodes, was assessed prior to biological experiments. Electrical characterization was performed using an electrochemical setup, where the TEER chip electrodes served as working electrodes in a common electrolyte solution, with reference and counter electrodes completing the circuit. Biocompatibility was evaluated through *in vitro* experiments using Caco-2 cells, assessing the suitability of the sensor chip for cell-based studies.

Fluidic interface test

The fluidic integration of the TEER module with the TOP was assessed to verify sealing efficacy and operational reliability. To evaluate interface integrity, the microfluidic channels were perfused with a dyed aqueous solution, enabling visual detection of any leakage at critical junctions. As depicted in Fig. 2, fluid flow remained restricted to the designated pathways, demonstrating effective sealing achieved through the bonding of the PET layers with PSA and the incorporation of soft O-rings. The leak-free operation of the TEER module within the TOP platform highlights the robustness of the sealing and assembly process, ensuring stable integration for future applications.

Electrical characterization

Electrochemical impedance spectroscopy (EIS) was performed to estimate the impedance of the microelectrodes. EIS characterizes the resistance and reactance of

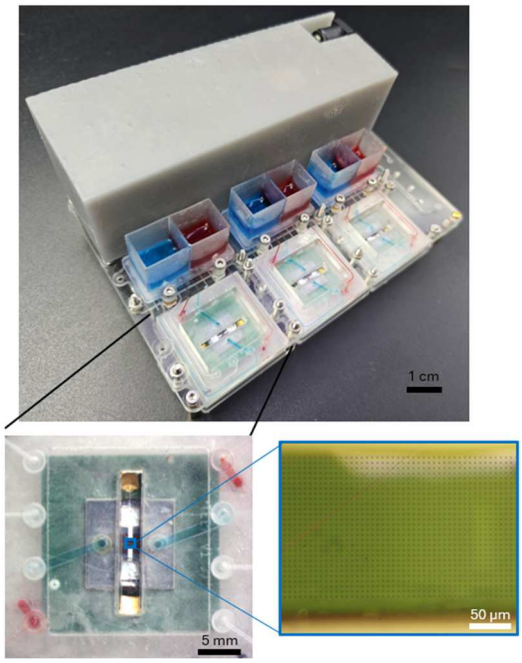

Figure 2: Leak-free flow across three TEER modules by means of the TOP (top). Microscope images on the bottom show the fluidic interface between a single TEER module and the TOP (left), and a close-up of the microfluidic channel and microporous membrane (right).

microelectrodes by applying an alternating current or voltage across a range of frequencies and measuring the system's response [7].

To evaluate the electrical performance of the TEER module, impedance measurements were conducted using PBS solutions at concentrations of 1x and 10x. The TEER chip incorporates TiN electrodes over slanted microchannel sidewalls. They were arranged in a four-terminal configuration within a two-electrode setup to minimize the influence of wire resistance (Fig. 3). The four TiN microelectrodes were designated as the working electrode, working sense, counter, and reference electrodes. For signal acquisition, the electrodes were connected to a portable potentiostat (PalmSens4) using wired connections, secured with crocodile clips to ensure stable electrical contact. A sinusoidal voltage signal with a root-mean-square (RMS) amplitude of 20 mV was applied across a frequency range from 1 Hz to 0.1 MHz. Each PBS concentration was tested in three independent measurements to ensure consistency, as depicted in Fig. 4.

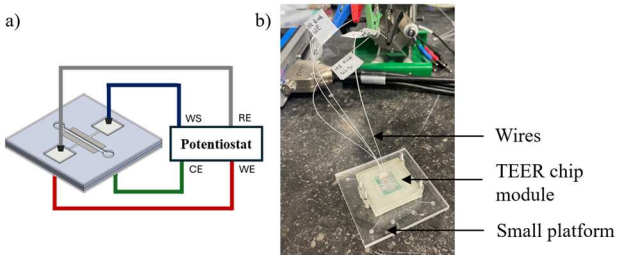

Figure 3: a) Setup of EIS measurements using a 4-electrode 4-terminal connection mode with the working electrode (WE), working sense (WS), counter (CE), and reference (RE) electrodes. b) The TEER module connected to a small platform and a portable potentiostat.

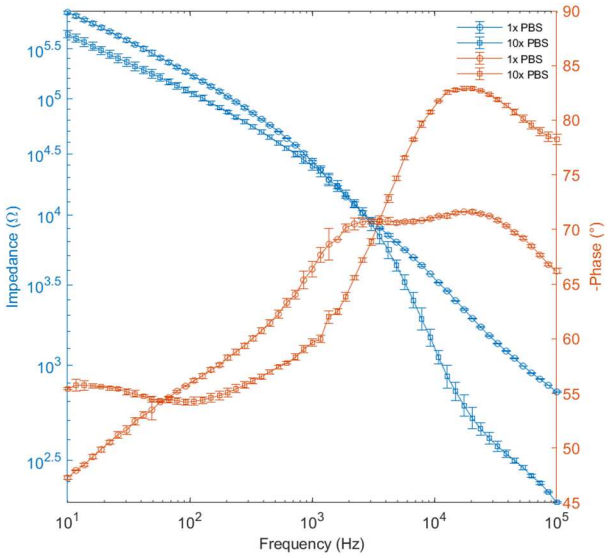

Figure 4: Electric impedance data recorded from the four TiN electrodes of the sensor chip using the TEER module and TOP-integrated potentiostat for 1x and 10x PBS solutions. Error bars represent the standard deviation of three independent measurements for each concentration.

EIS measurements reveal a frequency-dependent response influenced by PBS concentration. The impedance magnitude shows an S-shaped profile, high at low frequencies and decreasing with increasing frequency, reflecting the transition from interfacial polarization to bulk electrolyte behavior.

Unlike typical TEER systems, our chip does not exhibit a distinct high-frequency plateau representing bulk electrolyte resistance. We hypothesize this is due to the device's 3D geometry: vertically-stacked channels separated by a microporous SiN membrane, and slanted sidewall electrodes. This configuration likely introduces spatially-distributed fields and overlapping impedance contributions, which convolve with the resistive regime.

As PBS concentration increases from 1x to 10x, impedance decreases particularly at high frequencies due to improved ionic conductivity. However, in the mid-frequency range (~10^3 Hz), impedance values converge, reducing contrast between conditions. Despite this, phase values remain consistent, 10x PBS showing a sharper, higher peak, while 1x PBS exhibits a broader, flatter response, suggesting overlapping relaxation processes.

These features may arise from distributed double-layer capacitance, membrane resistance, and ion mobility within the chip's confined geometry. The absence of multiple distinct phase peaks indicates that the broadening may be due to overlapping effects rather than distinct RC elements.

While the chip effectively senses conductivity differences, the lack of plateaus complicates biological interpretation. An equivalent circuit model, capturing solution resistance, membrane impedance, and distributed capacitance, will help deconvoluting these contributions.

These results confirm that the chip is able to determine differences in electrolyte conductivity, supporting its suitability for impedance-based sensing applications within the TEER module.

Biological characterization

Biocompatibility of the TEER chip was demonstrated through *in vitro* culture of human intestinal epithelial Caco-2 cells (ATCC, HTB-37). Caco-2 cells were seeded at 200.000 cells/cm^2 on the chip membrane. Cells were cultured in MEM (Gibco, 21090-022), supplemented with 20% fetal bovine serum (Capricorn, FBS-11A), 1% Penicillin and Streptomycin (Fisher Scientific, 15070063) and 2 mM L-Glutamine (Fisher Scientific, 25030024) for 3 days and subsequently fixed for 15 min with 3.7% paraformaldehyde. Barrier formation was assessed through the staining of tight-junction marker ZO-1. Fixed cells were permeabilized with 0.3% Triton X-100 for 10 min and stained with rabbit-anti-ZO-1 polyclonal antibody (Invitrogen™, PA585256, 1:100) for 90 minutes, followed by a secondary staining with Alexa Fluor™ 647 (Invitrogen™, A31573, 1:250) and cellular nucleus staining Hoechst 33342 (Invitrogen, H3570, 1:500) for 30 minutes in the dark. Images were obtained with a Re-scan confocal microscope.

Caco-2 cells grown on the chip membrane appeared to have a healthy morphology. ZO-1 staining showed that the Caco-2 cells formed a confluent epithelial monolayer with an uninterrupted arrangement of cells (Fig. 5).

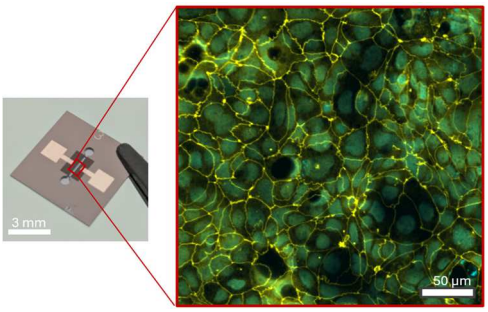

Figure 5: Biocompatibility of the TEER chip (left) was demonstrated using Caco-2 cells (right). On the 3rd day of culture, Caco-2 cells were stained for the nucleus (cyan) and tight junction marker ZO-1 (yellow), showing that the Caco-2 cells formed an uninterrupted, confluent epithelial barrier on the chip membrane.

The evidence provided in this analysis suggests that TEER sensor chips are compatible with the attachment and proliferation of Caco-2 cells, which allowed the formation of a confluent monolayer within a period of 3 days. This observation indicates that the silicon-based TEER sensor chip supports cellular growth and monolayer formation for TEER measurements.

CONCLUSION

We have demonstrated a fluidic and electrical packaging solution for a novel silicon-based TEER sensor chip, developed for integration into a modular and standardized OoC platform. The packaging design encompasses three primary components: a protective housing for the TEER chip, a microfluidic routing system for efficient interfacing with the platform, and electrical connections to an integrated potentiostat. This approach facilitates continuous impedance measurements and preserves optical transparency for real-time monitoring of the sensor 's membrane.

Experimental validation of the system confirmed effective leak-free fluid flow through the TEER module and stable PBS sensitivity of the TiN electrodes, demonstrating reliable performance under various testing conditions. The packaging solution maintains the optical transparency, and does not influence the properties of the sensor chip. This development provides an essential tool for enhancing the reproducibility and versatility of OoC-based studies, advancing the field of *in vitro* tissue models for drug development and disease research.

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