Real-time cellular impedance monitoring and imaging in a dual-flow bioreactor

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Abstract

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. Here, we present an advanced cell culture system able to mimic the dynamic environment of biological barriers, while monitoring cell behaviour through real-time impedance measurements and imaging. Caco-2 cells were cultured in the Trans Epithelial Electric Impedance (TEEI) bioreactor under both flow and static conditions. The cells in dynamic conditions developed higher impedance values at low frequencies and showed a typical RC behaviour, while the controls showed minimal capacitive behaviour. These results highlighted the differences between flow and static conditions and the ability of the TEEI measurements to provide a more precise indication of monolayer formation.

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Key words: Cellular impedance; bioreactors; TEER monitoring; Caco-2 cells.

Conference presentation: this paper was presented at the Second Centro 3R Annual Meeting - 3Rs in Italian Universities, 2019, June 20-21, University of Genoa, Italy.

Received for publication: 28 October 2019.
Accepted for publication: 11 November 2019.

Biomedical Science and Engineering 2019; 3(s3):104
doi:10.4081/bse.2019.104

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 such as nutrients and xenobiotics, as well as in the maintenance of homeostasis in different body compartments. The integrity of the barrier is usually characterised by measuring the Trans Epithelial Electric Resistance (TEER). However, Impedance spectroscopy, *i.e.* the application of a frequency sweep of current, can provide additional information on the capacitive component of the cellular barrier (Figure 1A).[1] Here, we present a new millifluidic double-flow bioreactor, which integrates a TEEI measuring system.

Materials and Methods

The TEEI bioreactor is an adaptation of the modular, dual flow commercial Live Box 2 bioreactor (LB2, IVTech S.r.l. - Massarosa, Italy). Silver circular electrodes were integrated on the internal surface of the glass slides placed at the top and bottom of the bioreactor. Spring contacts were inte-

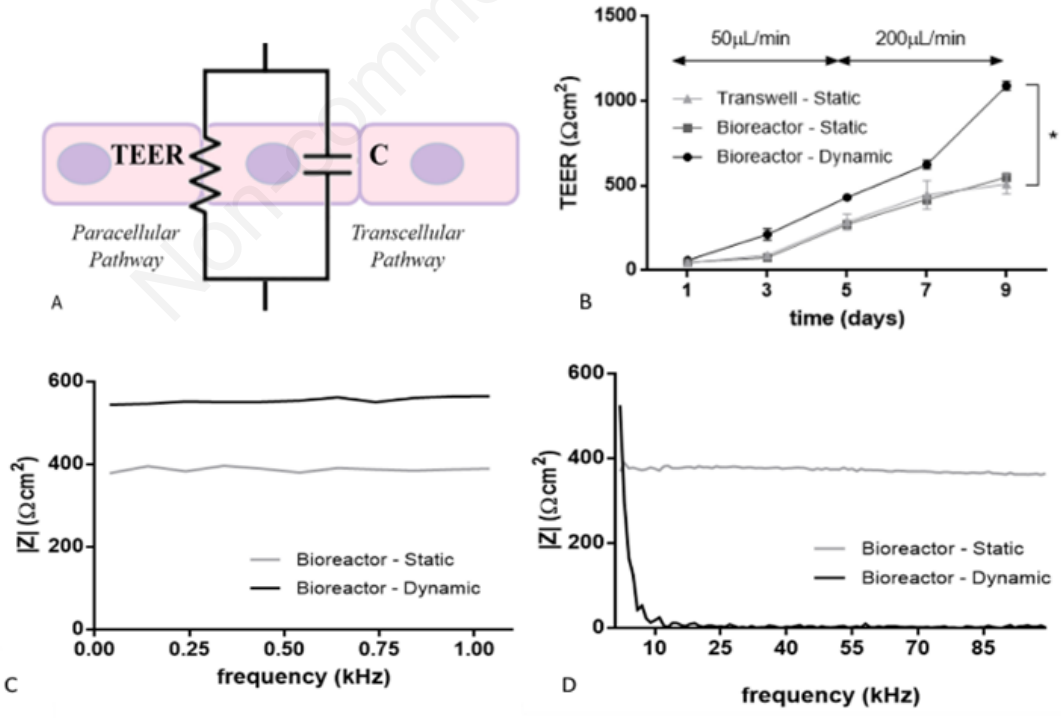

Figure 1. A) Electric equivalent of an epithelial or endothelial cell layer, B) TEER measurements during cell culture performed with the impedance-meter (f<1 kHz) in the bioreactors and with the EVOM in the transwells (*=P<0.05 between static and dynamic conditions). TEEI measurement 5 days after seeding in the bioreactor in static and dynamic conditions: C) low frequencies (0.40- 1 kHz) and D) high frequencies (2-100 kHz).

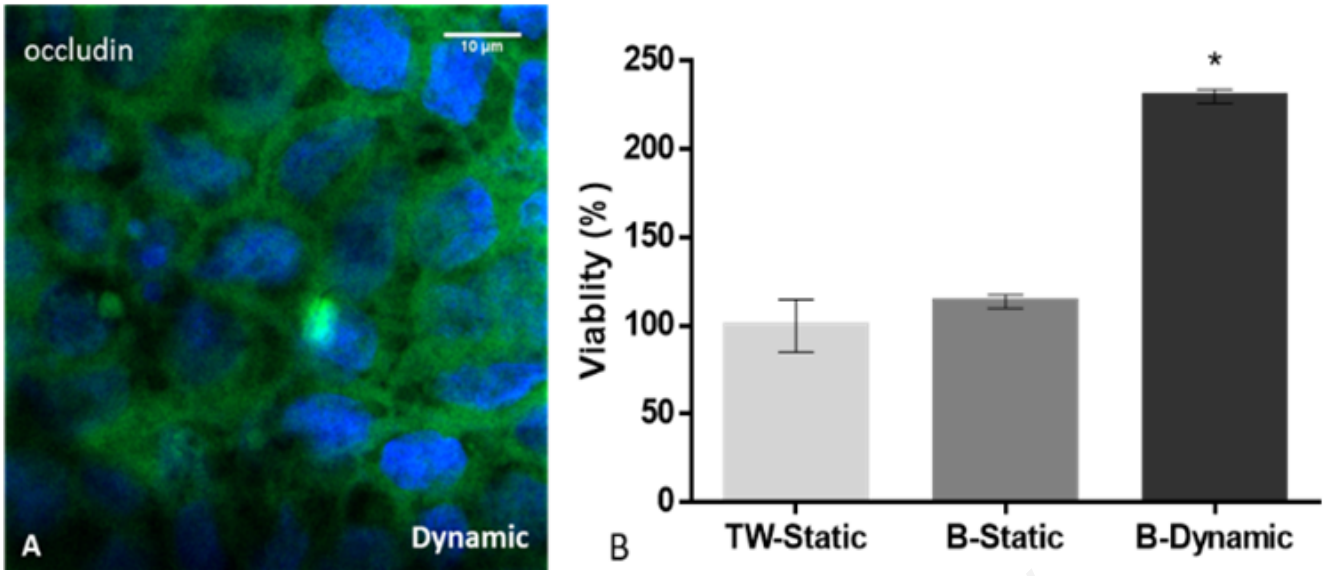

Figure 2. A) Confocal Imaging at day 9 in dynamic conditions (40X magnification); B) Viability at day 9 in the transwell and in the bioreactors (data are normalised with respect to static controls in transwells) *=P<0.05.

grated in the bioreactor clamp system allowing to interface the bioreactor with the measuring circuit, based on the AD5933 and an analog front-end that adapt the chip to biological measurements.[2] Caco 2 cells ($1x10^5$ cells/cm^2) were seeded both in the tranwell and cultured for 9 days in static and dynamic conditions. At the end of the culture, cell viability was assessed with a resazurin based in metabolic assay (Sigma Aldrich). Then, the cells were fixed and stained with DAPI and occludin monoclonal antibody (OC-3F10) directly conjugated with Alexa Flour 488 (Thermo-Fisher, Massachusetts, USA) and images were acquired with a confocal microscope (Nikon A1, Tokyo, Japan).

Results

Dynamic conditions were able not only to improve barrier tightness, as shown by the higher TEER values (Figure 1B), but also cell viability (Figure 2B). Moreover, as it possible to observe in Figure 1 C-D, impedance measurements provided a more precise indication regarding monolayer formation. FInally, the presence of tight junctions was assessed with immunostaining for occluding (Figure 2A).

Conclusions

In this work a new dual-flow bioreactor with integrated TEEI monitoring was developed and tested with an intestinal *in vitro* model, demonstrating the importance of dynamic conditions for barrier-forming cells. Thus, this sensorized system can be used to improve the relevance of further *in vitro* studies.

References

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