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A microfluidic cell culture device with integrated microelectrodes for barrier studies

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INTRODUCTION

Trans-epithelial electrical resistance (TEER) is one of the widely used and conceivably the most straightforward technique for understanding the integrity of an epithelial or endothelial cell layer (1,2). This paper describes a simple and straightforward fabrication process of microelectrodes in a multi-layer and multi-chamber lab-on-a-chip device for measuring TEER. We proposed using a combination of two different metals for fabricating the microelectrodes to acquire TEER measurements in the microdevice: a low melting temperature indium alloy (InBiSn) on the one hand, and platinum (Pt) on the other hand.

FABRICATION OF MICROELECTRODES

The microfluidic device was fabricated using thiol-ene 'click' chemistry (3). The design and fabrication of the microfluidic chip have been reported earlier (4). Two different metals were used to fabricate the microelectrodes:
Top electrodes = Platinum wire, Bottom electrodes = Indium-based alloy (InBiSn, In 51%, Bi 32.5%, Sn 16.5% by weight)

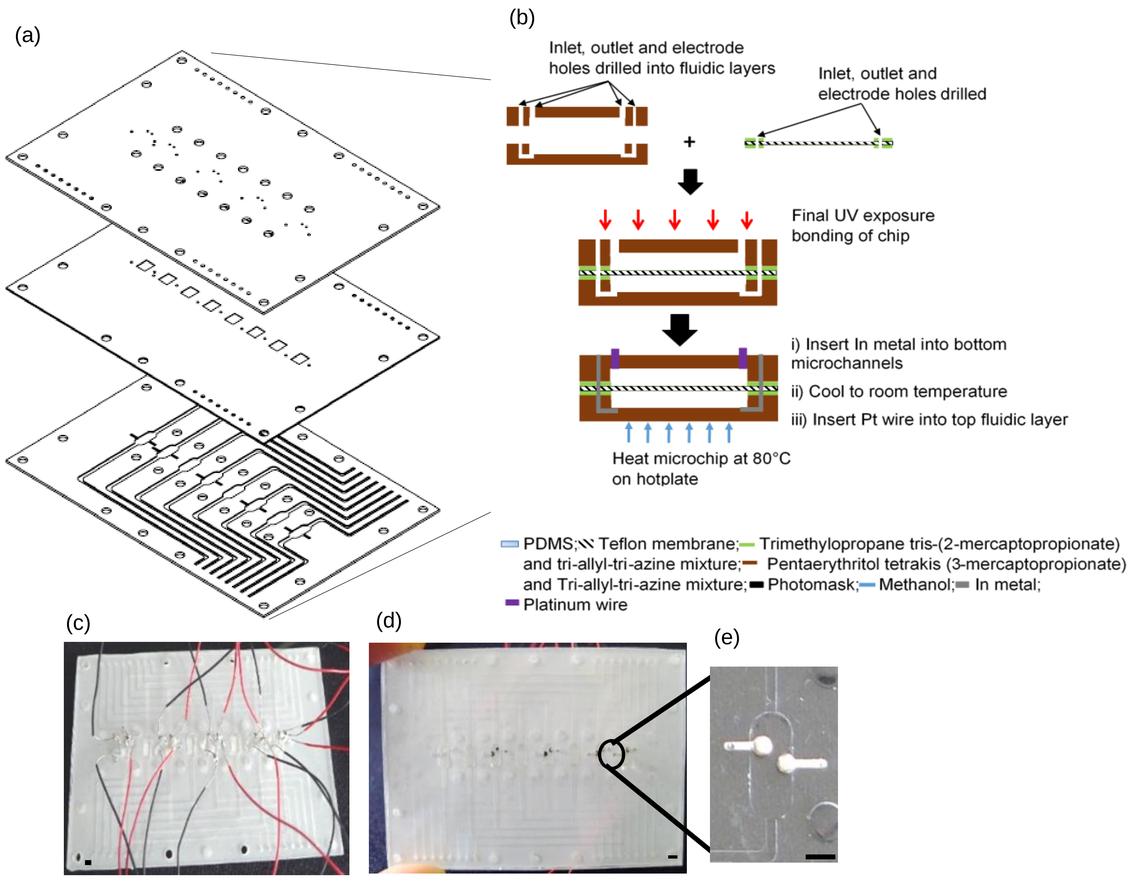


Figure 1. Design and development overview of microelectrodes embedded in the multi-layer and multi chamber thiol-ene microchip for Caco-2 cell culture: (a) Schematic drawing of the 3 layers present in the thiol-ene microchip. (b) Schematic drawing of the process to embed the microelectrodes on the microchip. (c) Top view of the completed microchip with embedded electrodes and connecting wires. (d) Underside of thiol-ene microfluidic chip with the InBiSn electrode embedded in the microchamber. (e) Expanded view of the InBiSn electrode. (Scale bar = 2mm)..

RESULTS AND DISCUSSION

BIOCOMPATIBILITY STUDIES WITH INDIUM ALLOY

The biocompatibility of InBiSn was evaluated by culturing Caco-2 cells in the presence of small pieces of the alloy. Phase contrast microscopic images confirmed that the Caco-2 cells cultured in the microwells containing the InBiSn metal have multiplied. The viability of the Caco-2 cells was further determined with live/dead cell stains. The fluorescent images of the cells showed that the mean cell viability was > 95% in all the microwells containing the metal (n = 3) (Fig. 2d). The results were comparable to the control microwells (≈ 100% cell viability). As Pt is biocompatible and is widely used in medical devices (5) we conclude that the Pt and InBiSn electrode material are biocompatible.

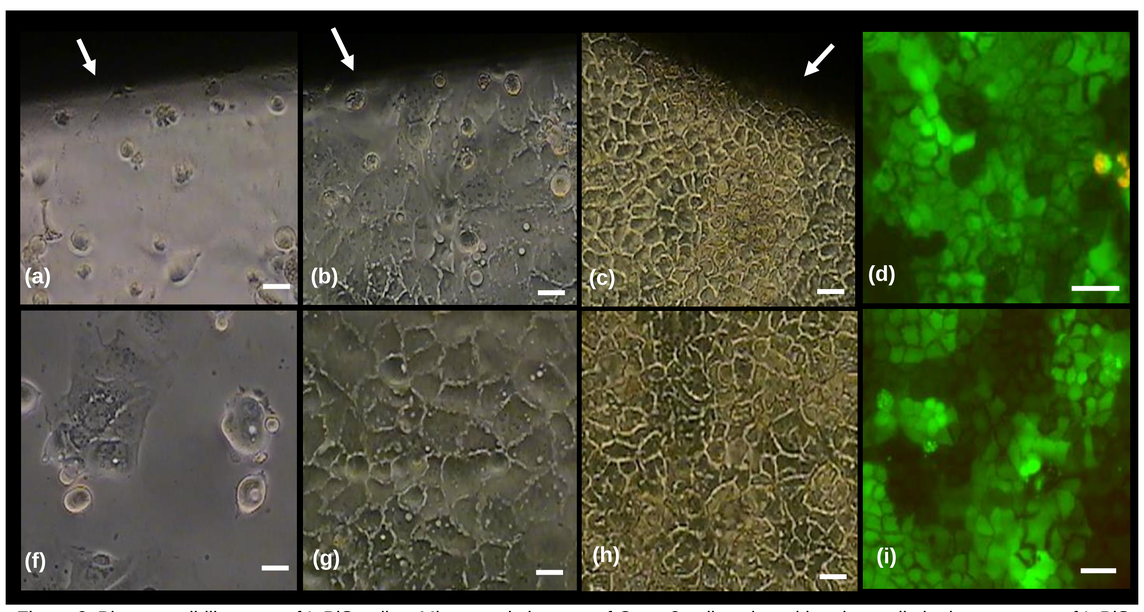


Figure 2. Biocompatibility tests of InBiSn alloy. Microscopic images of Caco-2 cells cultured in microwells in the presence of InBiSn metal (a) – (d) and without the metal (f) – (i). Microscopic images were taken on day 1, day 3 and day 6 of Caco-2 cell culture. White arrows in (a) – (c) indicate the InBiSn metal. Live/dead fluorescent images were taken on day 5 of cell culture. Live cells were fluorescently stained with calcein, shown in green, and dead cells are stained with ethidium homodimer-1 shown in red. Scale bar = 50 µm. Magnification at 10x.

RESULTS AND DISCUSSIONS

REAL-TIME TEER MEASUREMENTS

The microfluidic chip with microelectrodes was assembled onto a cell culture platform with MAINSTREAM components (6) and seeded with Caco-2 cells or CT26 cells, respectively. The TEER measurements recorded for the microchambers seeded with Caco-2 cells showed significant increase with time. In contrast, chambers seeded with CT26 cells resulted in an overall low TEER value (Fig. 3).

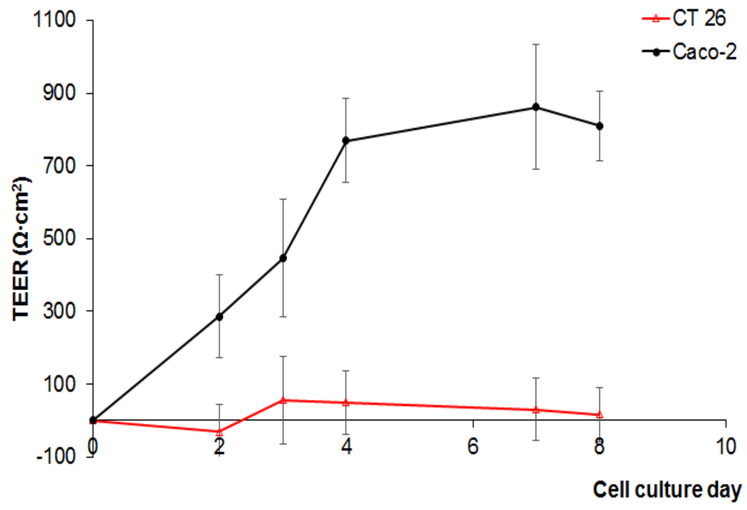
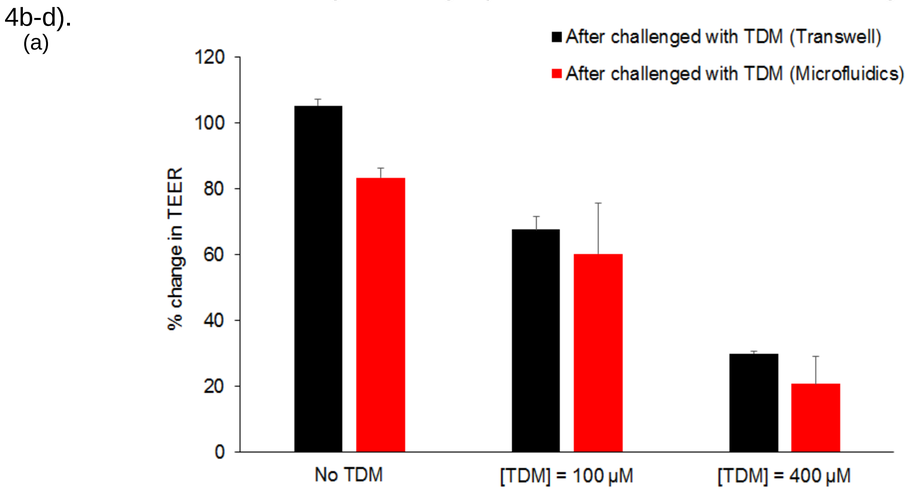


Figure 3. TEER measurements of Caco-2 cells and CT 26 cells. (n = 6, mean ± SD)

MICROELECTRODES FOR SENSING DYNAMIC BARRIER CHANGES

Day 8 Caco-2 cell layers were challenged by the membrane enhancer tetradecyl-β-D-Maltoside (TDM) (7). This resulted in a decrease in TEER values for both Transwell and microfluidic systems (Fig 4a). Further analysis of the disrupted Caco-2 barrier was conducted by immunofluorescence staining of the tight junctions and fluorescence staining of the nucleus (Fig 4b-d).



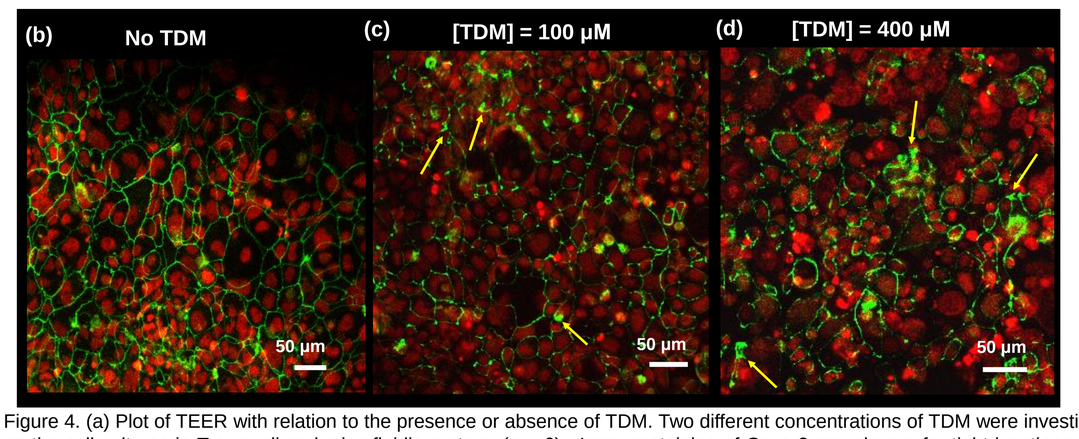


Figure 4. (a) Plot of TEER with relation to the presence or absence of TDM. Two different concentrations of TDM were investigated on the cell cultures in Transwell and microfluidic system. (n = 6). Immunostaining of Caco-2 monolayers for tight junctions, ZO-1 occludens (green fluorescence) and nucleus (red fluorescence) when Caco-2 cells were subjected to: (b) no TDM; (c) [TDM] = 100 µM; (d) [TDM] = 400 µM. Cells were stained on day 8 of cell culture. Magnification was 10x.

CONCLUSION

Here, a simple and straightforward procedure for using two different metals to fabricate the microelectrodes in a compact, multi-chamber microfluidic cell culture device for measuring cell barrier function is presented. The metals used for fabricating the microelectrodes were biocompatible and showed capability in measuring TEER across the cells layers. Additionally, the electrodes were capable in sensing dynamic changes to the barrier property when the cells were challenged with a membrane enhancer. Immunofluorescence staining towards the tight junctions of the Caco-2 monolayers was also conducted to further confirm the validity of the TEER measurements. Such a set-up potentially provides a solution to the limited existing equipment for acquiring TEER measurements in compact microfluidic devices for cell culture.

ACKNOWLEDGEMENTS

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