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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 (In85Sn in the one hand, and platinum (Pt) on the other hand.

FABRICATION OF MICROELECTRODES

The microfluidic device was fabricated using thin-foil ‘tick’chemistry (3). The design and fabrication of the microfluidic chip were reported earlier (4). Two different metals were used to fabricate the microelectrodes. Top electrodes = Platinum wire, Bottom electrodes = Indium-based alloy (In85Sn in 51%, Bi 32.5%, Sn 16.5% by weight).

RESULTS AND DISCUSSION

BIOMICROTABILITY STUDIES WITH INDIUM ALLOY

The biocompatibility of In85Sn 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 microfluidics containing the In85Sn metal have multiplied. The viability of the Caco-2 cells was further determined with live/dead cell staining. The fluorescent images of the cells showed that the mean cell viability was >95% in all the microchambers containing the metal (n = 3) (Fig. 2d). The results were comparable to the control microchambers (100% cell viability). As Pt is biocompatible and is widely used in medical devices (6) we conclude that the Pt and In85Sn electrode material are biocompatible.

MICROELECTRODES FOR SENSING DYNAMIC BARRIER CHANGES

Day 8 Caco-2 cell layers were challenged by the membrane enhancer tetradeacyl-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).

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.

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REFERENCES


Figure 1. Design and development overview of microelectrodes embedded in the multi-layer and multi-chamber thin-foil microchip for Caco-2 cell culture. (a) Schematic drawing of the 3 layers present in the thin-foil 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 thin-foil microfluidic chip with the In85Sn electrode embedded in the microchannel. (e) Expanded view of the In85Sn electrode. (Scale bar = 3mm)

Figure 2. Biocompatibility test of In85Sn alloy. Microscopic images of Caco-2 cells cultured in microfluidics in the presence of In85Sn metal ([TDM] = 400 μM) and without the metal ([TDM] = 0 μM). Microscopic images were taken on day 1, day 3 and day 6 of Caco-2 cell culture. White arrows in (i) - (iii) indicate the In85Sn 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 of 10x.

Figure 3. TEER measurements of Caco-2 cells and CT26 cells. (a) Linear scale of CT26 and Caco-2 cell culture. (b) Graph showing the TEER measurements of Caco-2 cells and CT26 cells. The y-axis represents the resistance of the barrier and the x-axis represents the time in days. (c) Bar graph showing the TEER measurements of Caco-2 cells and CT26 cells. The y-axis represents the resistance of the barrier and the x-axis represents the time in days. (d) Line graph showing the TEER measurements of Caco-2 cells and CT26 cells. The y-axis represents the resistance of the barrier and the x-axis represents the time in days.

Figure 4. (a) Plot of TEER with respect to the presence or absence of TDM. Two different concentrations of TDM were investigated on the cell cultures in Transwell and microfluidic system. (b - e): Immunofluorescence staining of Caco-2 monolayer for tight junctions, ZO-1 antibody (green fluorescence) and nucleus (red fluorescence) when Caco-2 cells were subjected to (a) No TDM; (b) [TDM] = 100 μM; (c) [TDM] = 400 μM. Cells were observed on day 8 of cell culture. Magnification was 10x.