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Transepithelial Electrical Resistance on Cell Cultures for *in vitro* Toxicity Testing of Water Samples

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The OECD has set guidelines for tests of new toxicants, which involve exposing live fish to analytes for up to 96 hours and testing how many fish have died at intervals of 24, 48, 72 and then finally 96 hours. Recently there has been a huge drive to refine and to develop new *in-vitro* methods that could be used to reduce animal testing. We have developed a device which will continuously monitor the integrity of a monolayer of epithelial cells using Transepithelial electrical resistance (TEER) in a cell-based autonomous device with the aim to improve on traditional cytotoxicity assays and to reduce animal testing.

Cell Model

Epithelial and endothelial cells form intercellular tight junctions when cultured to a monolayer. Tight junctions are found in several places in the body such as; Kidneys, Intestine, BBB, Lung epithelia.

Tight junctions prevent the free passage of ions and molecules across the cell monolayer through the intercellular space (paracellular transport). Tight junctions can be used as a measure of cell health.

We are using the C2Bbe1 (caco-2 clone) cell line which is a human colon cell line which differentiates into an entrocytic cell line after 21 days. Caco-2 cells can produce TEER values of up to 600Ω.cm^2.

In the future we are looking to use a fish cell line. Advantages of this would include, culture at a lower temperature, no CO2 dependence, can withstand varying osmotic pressures.

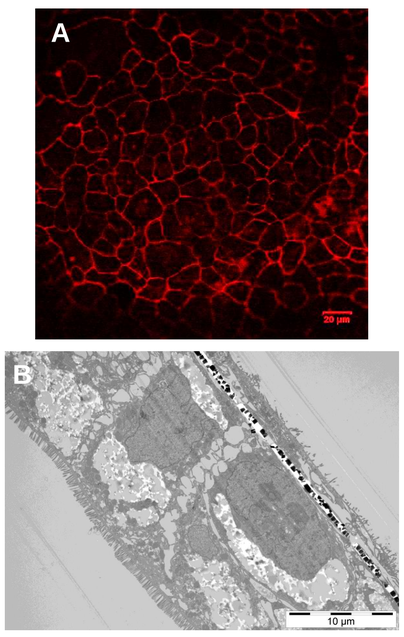


*Fig1: **A** Caco-2 cells on silicon nitride membrane stained for ZO-1 (red) **B** Shows a TEM cross section of caco-2 cells on silicon nitride membranes*

TEER Measurements

TEER measurements as a measurement of tight junctions can be used to determine the health of a monolayer of cells in an easily quantifiable method.

The traditional method for measuring TEER values is to use STX2 electrodes using a 4 point measurement system on Transwell permeable inserts. One set of electrodes is placed in the basolateral compartment and the other in the apical compartment.

Measurements which are currently carried out can be unreliable and lead to high standard deviations

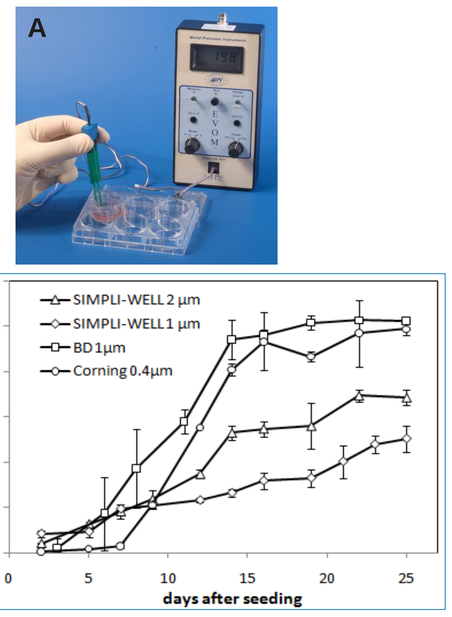


*Fig2: **A** Shows the tradition set up with STX2 electrodes. **B** shows TEER measurements on Transwell filters and CSEM permeable supports*

Silicon Nitride Supports

Caco-2 cells are grown on ultra thin porous silicon nitride membranes (500nm thick) which are fabricated in house by CSEM with integrated platinum electrodes on-chip. By integrating the electrodes on the cell supports, measurements can be made more easily and reproducibly. The membranes have excellent transport properties and good growth of epithelial cells is observed

Pore sizes can be between 0.5µm and 3µm.

A custom holder for the support was developed so that cells can be grown for 21 days in well plate in a normal incubator before being transferred into the fluidics device for measurements

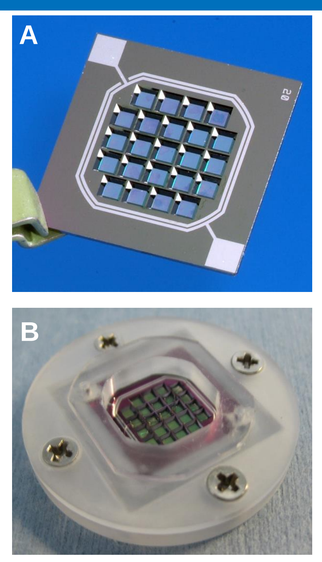


*Fig3:**A** Silicon Nitride permeable insert with 23 silicon nitride pads and integrated platinum electrodes **B** Shows the custom holder for the membrane*

Fluidics Device

Membrane, electrode and fluidics can be integrated into a single device. The system is divided into an apical and a basolateral compartment as it is with the traditional Transwell inserts. Without this separation TEER measurements would be impossible.

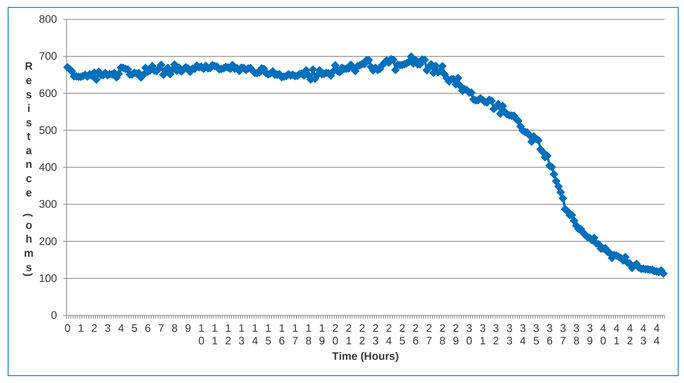


Fig6: Shows continuous TEER measurements taken over a period of 44 hours on fully differentiated C2Bbe1 cells and a drop in TEER in response to a toxicant

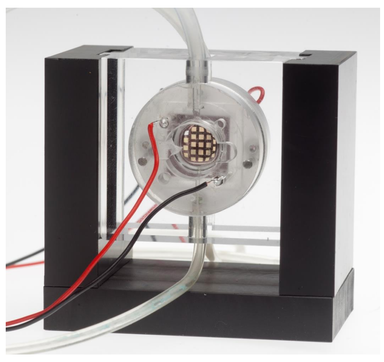


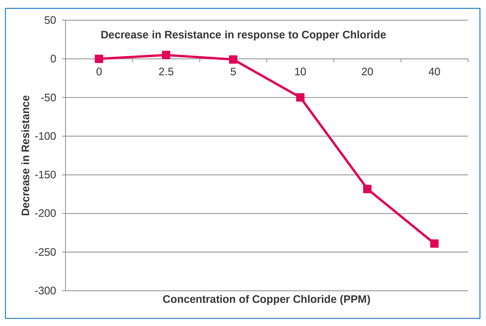
Fig4: The system is integrated into a fluidics device and clamped together to ensure water tightness

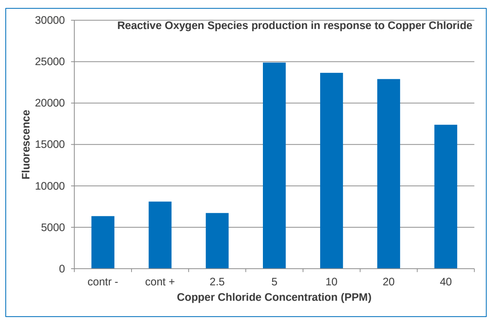
Cell Response to Copper Chloride

Cell were grown for 21 days and treated with between 2.5-10ppm of Copper Chloride.

The cell response to copper chloride was measured as a decrease in TEER or the production of lactate dehydrogenase (LDH) or reactive oxygen species (ROS).

A significant effect was seen earlier using TEER measurements than for the LDH assay. The ROS assay was unreliable due to cells detaching at higher concentrations.





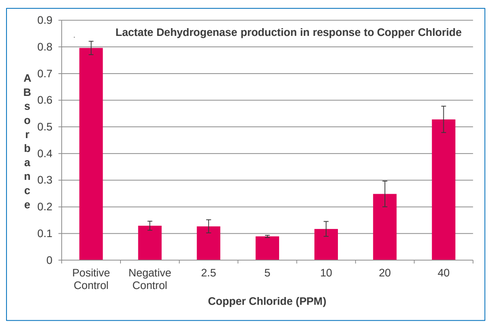


*Fig7: **A** shows the decrease in TEER in response to Copper Chloride. **B** shows the production of LDH in response to copper chloride. **C** Shows the production of ROS in response to copper chloride.*

Outlook

Good initial results have been obtained on the novel silicon nitride supports. Good cell growth has been observed with a fully differentiated monolayer of cells with mature tight junction formation. TEER measurements have also been taken under flow and static conditions on the silicon nitride supports.

The next step involves integrating the fluidics system into an autonomous system which will control CO_2 concentration and temperature.

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