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.

Epithelial and endothelial cells form intercellular tight junctions when cultured to a monolayer. 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 enterocyte cell line after 21 days. Caco-2 cells can produce TEER values of up to 600 Ohm cm².

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.

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.

Membrane, electrode and fluids 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.

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.

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 CO2 concentration and temperature.