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FNSNF

Transepithelial Electrical Resistance as an Evironmental sensor.

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Current methods for testing water pollution are expensive and does not test for a wide variety of toxicants. This study aims to develop a cell-based autonomous biosensing microsystem for testing water quality. Transepithelial electrical resistance (TEER) measurements will be used to monitor the integrity of a monolayer of epithelial cells in an automated fluidics system. The system will be installed at a river or stream and will sample the water. A drop in TEER will be taken as indicative of the presence of toxicants in the water sample and will be automatically relayed to an operator notifying them of a problem. Work has also been carried out comparing the sensitivity of TEER measurements with the lactate dehydrogenase assay and an assay used to detect the production reactive oxygen species when samples are exposed to heavy metals.

Cell Model

Cell which form intercellular tight junctions are utilized to give high TEER values. Tight junctions are found in several places such as; Kidneys, Intestine, BBB, Lung epithelia.

Tight junctions prevent the free passage of ions and molecules through the intercellular space, allowing active transport.

Currently we are using the C2Bbe1 (caco-2 clone) cell line which are a human colon cell line which differentiate into an entrocytic cell line after 21 days

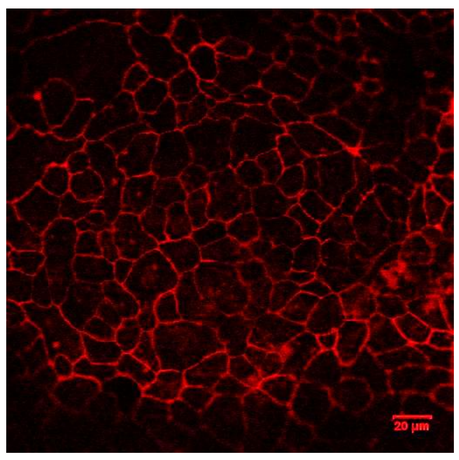


Fig1: Caco-2 cells on silicon nitride membrane stained for ZO-1 (red)

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.

TEER Measurements

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

Cells such as caco-2 cells produce TEER values of about 600Ω.cm^2.

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

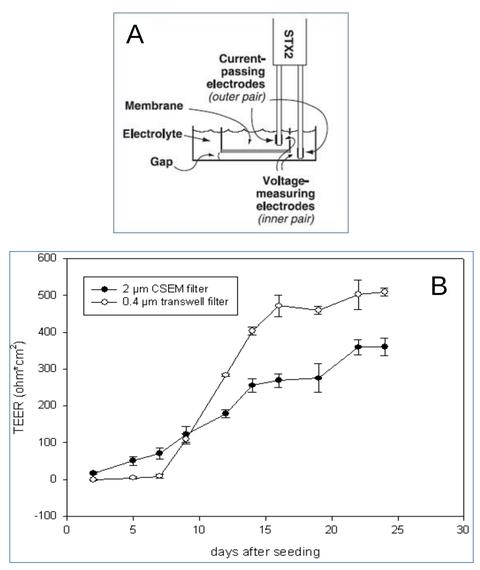


*Fig2: **A** Shows the tradition set up with STX2 electrodes. **B** shows TEER measurements on Transwell filters and CSEM permeable supports*

SEM and TEM Analysis of Monolayers

Cell were grown on silicon nitride supports for 21 days until full differentiation and then imaged by Scanning Electron Microscopy (SEM) and Tranmission Electron Mircroscopy (TEM).

For SEM cells were fixed in glutaraldehyde and then deydrated using critical point drying to give the excellent detail.

TEM images were obtained using an EPOM embedding method. Following 4 days of EPOM embedding the samples are sectioned using a diamond cutter.

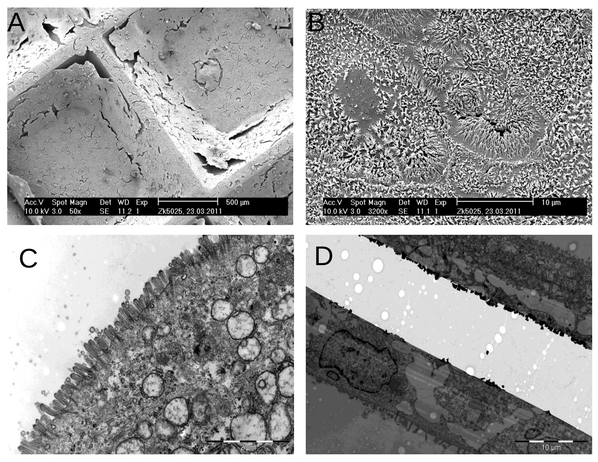


*Fig3: **A + B** show SEM images of a monolayer of C2Bbe1 cells on a silicon nitride support. Image B shows villi which are indicative of a well differentiated monolayer of cells. **C+D** are TEM images. Cell organelles can be seen here which indicate a well differentiated monolayer of cells. Tight junctions can also be viewed.*

Silicon Nitride Supports

Ultra thin porous silicon nitride membranes (500nm thick) are fabricated in house by CSEM with integrated platinum electrodes. 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 1 and 3 microns.

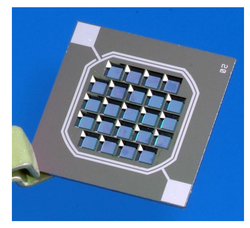
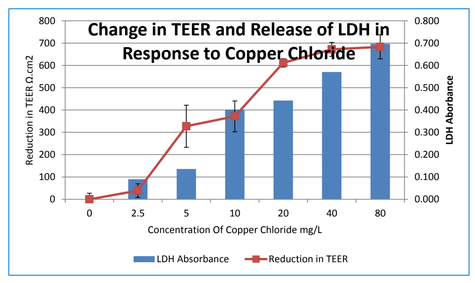


Fig4: Silicon Nitride permeable insert with 23 silicon nitride pads and integrated platinum electrodes

TEER Comparison with LDH and ROS Production

A comparison in sensitivity between TEER measurements and the production of reactive oxygen species (ROS) and LDH in response to heavy metal salts was carried out. It was found that at about 5mg/L concentration, TEER measurements were more sensitive than both assays, however at higher concentrations TEER measurements would plateau sooner than LDH release would.

Other heavy metals to be used include nickel chloride. Pesticides atrazine and fenitrothion will be used as a different kind of environmental toxicant.



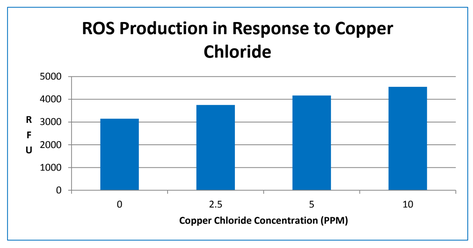


Fig6 A shows the reduction in TEER (line) and the production of of LDH (Bar) in repsonse to various concentrations of copper chloride. B shows the production of reactive ocygen species in response to copper chloride.

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.

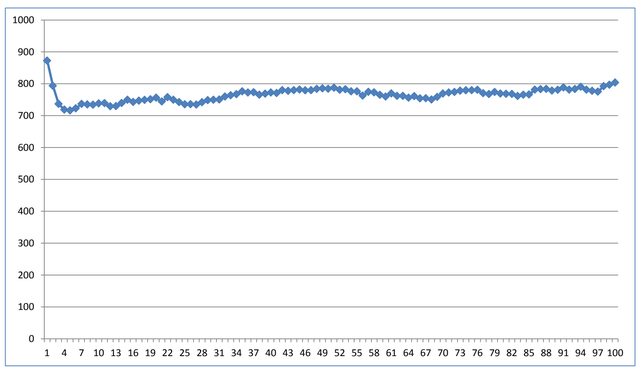


Fig6: Shows continuous TEER measurements taken over a period of 16 hours on fully differentiated C2Bbe1 cells.

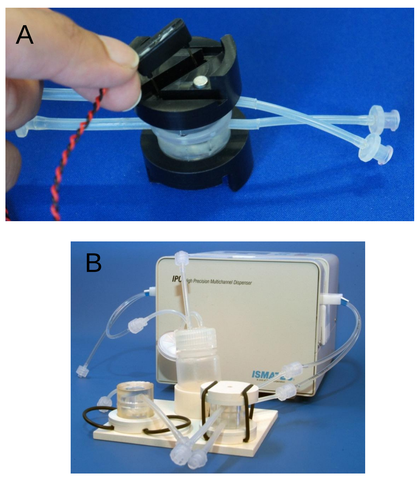


*Fig7: **A** shows the PDMS chamber which holds the silicon nitride support. The electrical contacts for taking the TEER measurements are also seen. **B** shows the full pump system for continuous flow conditions.*

Outlook

Stable TEER measurements over relatively long periods of time have been achieved. This paves the way to integrate the system into an autonomous system whereby all the attributes that are required for the cells to be maintained are present