Ecole Nationale Supérieure des Mines SAINT-ETIENNE

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Department of Bioelectronics

INSPIRING INNOVATION | INNOVANTE PAR TRADITION

Novel *in vitro* models for pathogen detection based on organic transistors integrated with living cells

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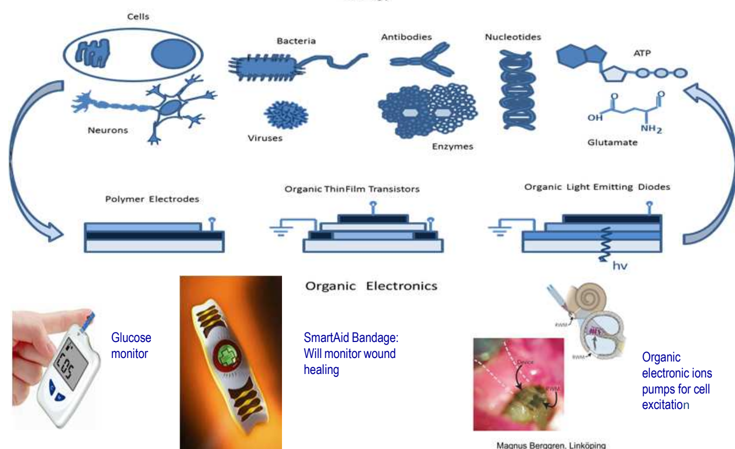
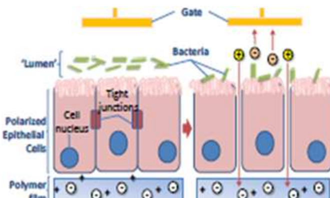
Introduction

Bioelectronics research seeks to understand the interface between biological systems and electronic materials. In particular, an understanding of how cells interact with organic semiconductors and how that interaction can be controlled, would aid the design and implementation of numerous biomedical devices.



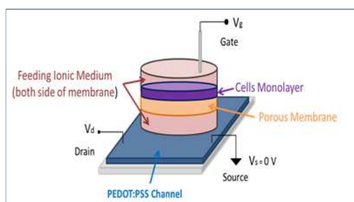
The advent of organic electronics has created a unique opportunity to integrate the worlds of electronics and biology, using devices such as the organic electrochemical transistor (OECT), which provide a very sensitive way to detect minute ionic currents. These devices have unprecedented sensitivity, in a format that can be mass produced at low-cost. The aim of this study was to integrate barrier tissue layers with OECTs to yield devices that can detect minute disruptions in barrier function, as seen by a decrease in transepithelial resistance (TER). In this study, OECTs were integrated with gastro-intestinal cell monolayers that form a barrier tissue layer. The device was shown to be able to sensitively detect decreases in TER upon exposure to a model toxin.



The epithelium is represented with tight junctions between the cells (left panel). The polymer film below contains holes (+) and counter ions (-). Upon assault by pathogens (e.g. bacteria) the tight junctions are disrupted (right panel). Ions from the medium can then enter the conducting polymer film when a gate voltage (Vg) is applied, and de-dope it. This results in a decrease in the drain current.

Materials & Methods

OECT device



Upon assault by a pathogen, the tight junctions are disrupted and ions from the electrolyte can migrate into the conducting polymer film and de-dope it.
Ethanol was used at 10% v/v to breach the tight junctions. Using LabView software and Keithley 2400 for data correction and analysis, the gate voltage was pulsed continuously and the modulation in the drain current monitored as a function of time.

Cell Line

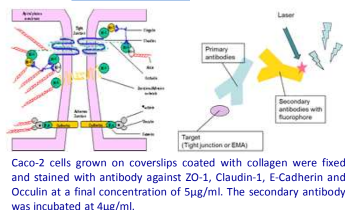
For these studies we use the Caco-2 cell line as representative of the intestinal epithelium because they are known to form a polarized monolayer with an apical brush border comparable to that of the human colon.

Characterization of barrier tissue layers

CellZscope

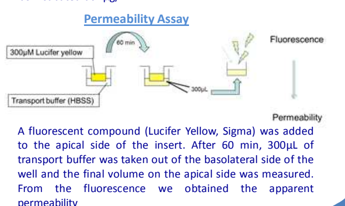
Mesure of TransEpithelial Resistance
Measure of intactness of the tissue layer

Immunofluorescence



Caco-2 cells grown on coverslips coated with collagen were fixed and stained with antibody against ZO-1, Claudin-1, E-Cadherin and Occulin at a final concentration of 5µg/ml. The secondary antibody was incubated at 4µg/ml.

Permeability Assay

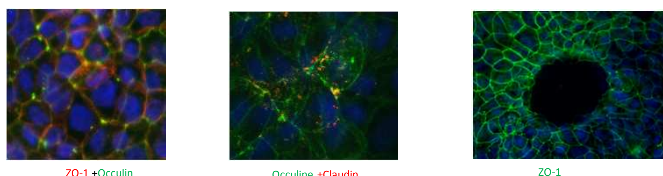


A fluorescent compound (Lucifer Yellow, Sigma) was added to the apical side of the insert. After 60 min, 300µL of transport buffer was taken out of the basolateral side of the well and the final volume on the apical side was measured. From the fluorescence we obtained the apparent permeability

Results

Differentiation of Caco-2 monolayers

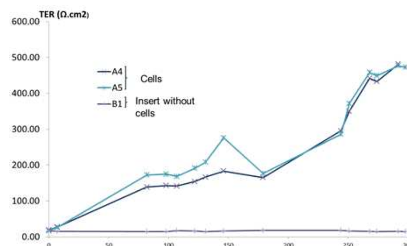
Immunofluorescence

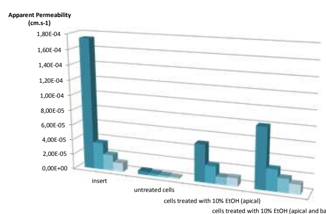


Immunofluorescence was carried out 21 days after cell seeding to characterize tight junction proteins on the differentiated cell monolayers. Antibodies against tight junction proteins showed distinct localization of these proteins on the cell periphery with co-localization also observed.

CellZscope

TER increased over a period of 14 days and then reached a plateau at approximately 400 Ω.cm².

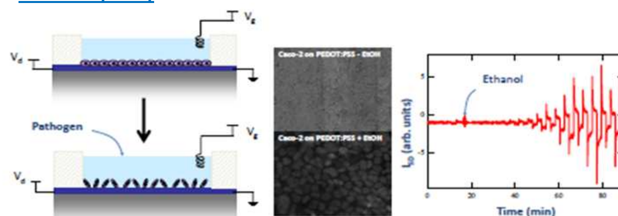




Permeability Assay

The apparent permeability is related to the human absorption of drug. For a differentiated monolayer, the permeability of Lucifer Yellow is $1x10^{-6}$cm.s^{-1}.

Preliminary assay



Modulation of the drain current of an OECT approx. 20 min after the overlaying Caco-2 cell monolayer was assaulted with ethanol (a model toxin). The gate voltage was pulsed between 0 and 0.3 V (not shown). This data, obtained with unoptimized devices and monolayers, supports the hypothesis that OECTs can detect breaches in tight-junctions.

Conclusion

Results show the successful integration of OECTs with Caco-2 cells, and the subsequent detection of disruption in TER, which is consistent with a breach of barrier tissue integrity. Future work will include testing with pathogens and toxins, and the integration of other cell types, to create additional in vitro cell models for pathogen and toxin detection, thus generating a system which is inexpensive, rapid and reduces animal experimentation.

Acknowledgements