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TEER measurements of C2Bbe1 cells in a standalone device

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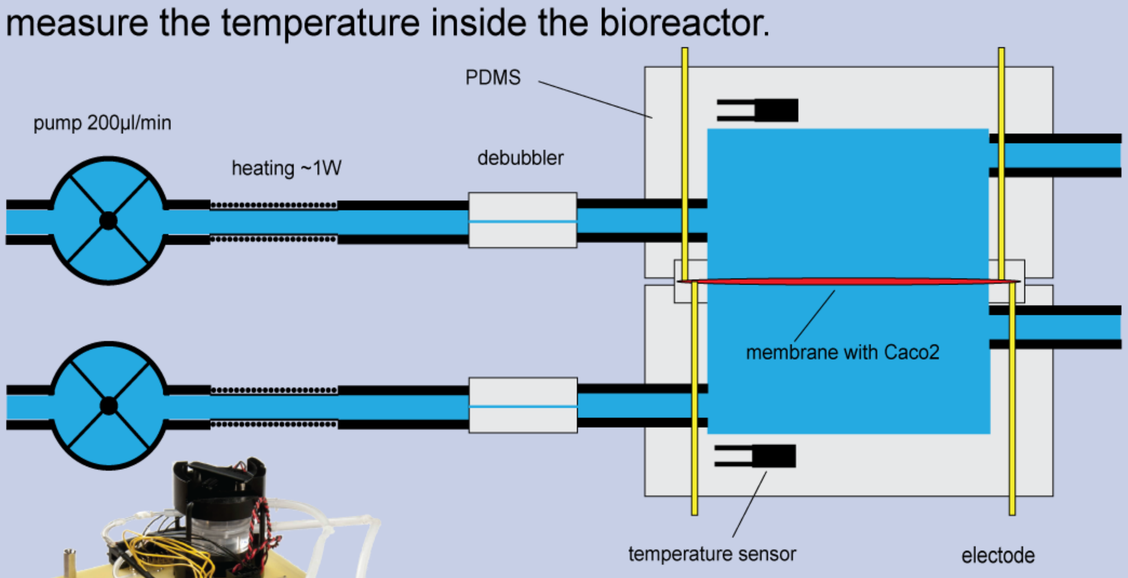
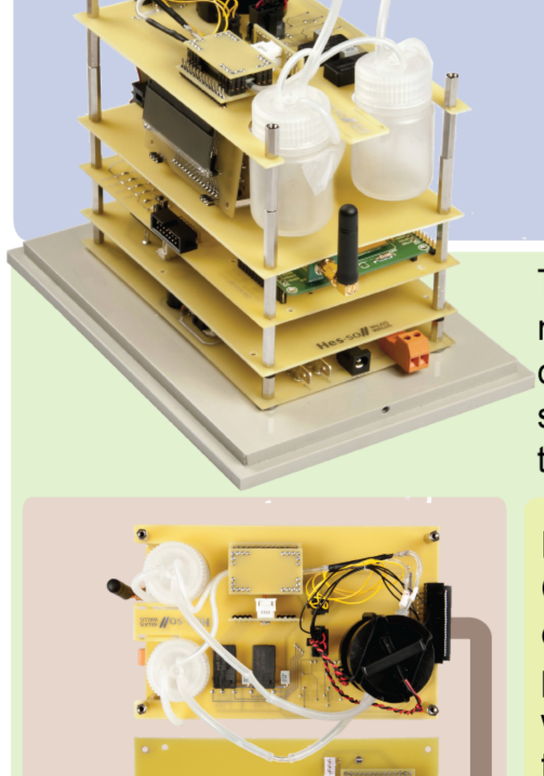
Hes·so VALAIS WALLIS

Objectives
To build a compact autonomous fluidic system that measures the Trans Epithelial Electrical Resistance (TEER) of C2Bbe1 cells. This value provides information about the water quality. The system consists of an fluidic setup with pumps, heating to maintain the temperature in the bioreactor, a debubbler to take out disturbing bubbles, an electrical sensing unit, a processor and communicating unit.

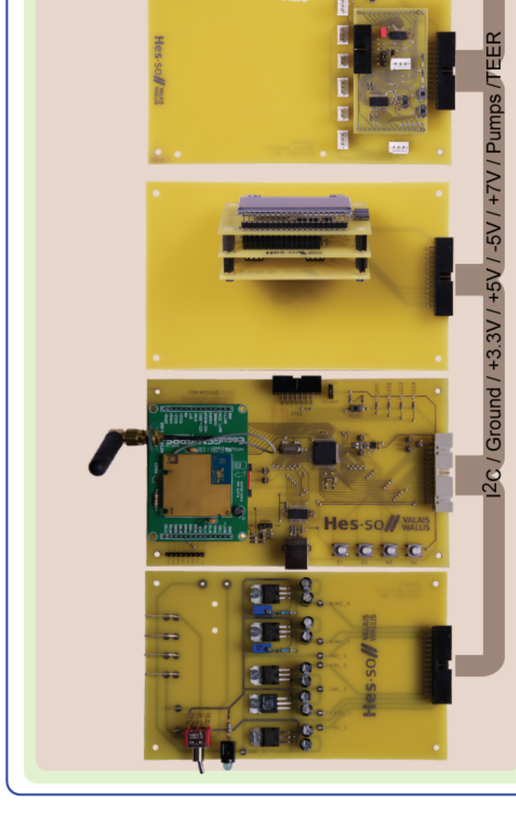
Design of the fluidics: pumps, debubbler, bioreactor and temperature control

The bioreactor with all the tubing including the heating, the reservoir the silicon membrane and even the pumps are sterilized by autoclaving. The debubbler is made of a PTFE filter membrane [1] but was not yet autoclaved and to prevent bio fouling and contamination between different experiments it should be changed every time. The bioreactor is made of two PDMS blocks that holds the membrane in place. Two sensors are placed in the PDMS close to the liquid to measure the temperature inside the bioreactor.





The overall system is 11x18x19 cm^3. It needs a 12V power supply and communicates via GSM. This device sends the results in predefined periods to a mobile phone.



Fluidic board
C2Bbe1 cells in a temperature controlled bioreactor. 2 Bartels mp6 pumps control the flow (200 µl/min) with debubblers, heating elements and temperature sensors.

pump and temp. control board
Processor to control temperature and pumps.

TEER board
Current source for TEER measurement and small display.

Micro-processor board
MSP430 16 bit based micro-controller, GSM module and USB connectivity for LabView access.

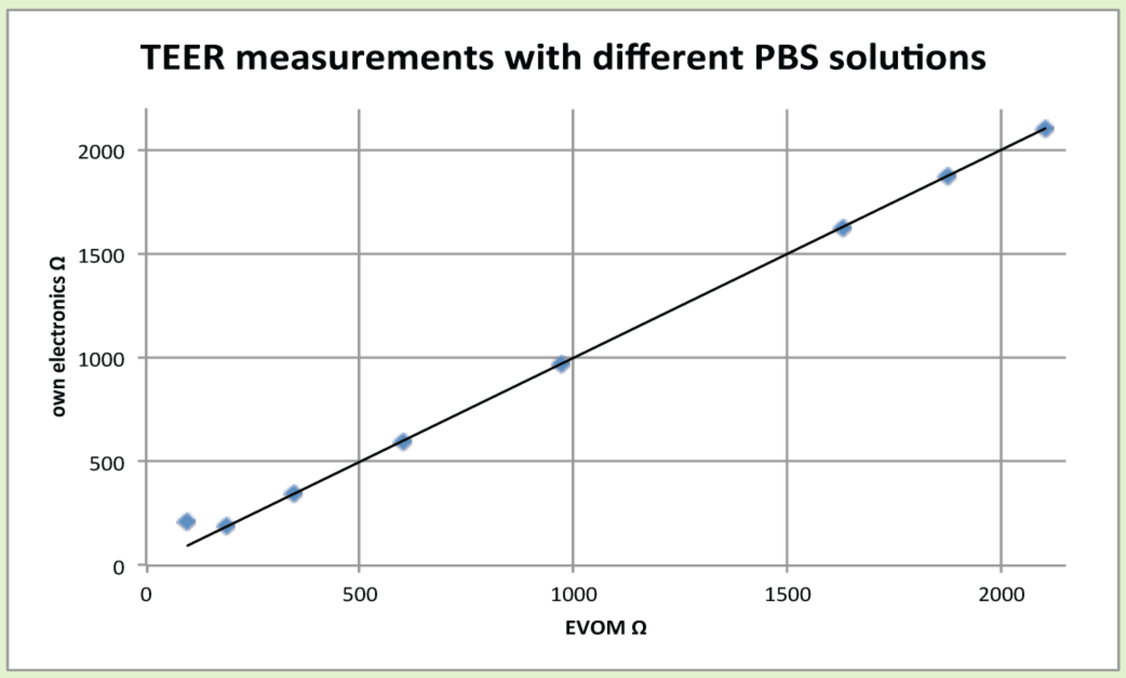
Power supply board
Accept 12V
Supply -5V, 5V, 7V and 3.3V

TEER measurement

An alternative current with a square wave 20µA @ 12.5Hz is generated with a microprocessor and some dedicated analog electronics and connected to two gold electrodes. Another pair of identical electrodes is used to measure the voltage across the C2Bbe1 cells. The signal is amplified, rectified and the root mean square value calculated.

Test of electronics

In order to test the electronics for the TEER measurement, the developed system and a commercialized Epithelial Voltohmmeters EVOM2 from World Precision Instruments (WPI) were compared. Resistance measurements were taking on 24mm ø polystyrene membranes with 3µm pore diameters were taken using various concentrations of Phosphate buffered saline solutions (PBS) STX2 electrodes (WPI) made of Ag/AgCl were used for taking these measurements.



The graph above shows the linear relation of different PBS solutions between the developed electronics and the commercialized Epithelial Voltohmmeters EVOM2 from World Precision Instruments (WPI).

TEER measurement of C2Bbe1

The C2Bbe1 were 21 days cultured directly on the silicon nitrude membrane. During this time the cells are growing and the resistance increases steadily. After the 21 days the value stays stable. If there is a toxicant in the water that is destroying the C2Bbe1 layer, the resistance value is dropping enormous. The TEER value obtained with both instruments after 21 days is around 460Ωcm^2 and can drop to around 50Ωcm^2 when the water is contaminated. The challenge is to keep the C2Bbe1 in good condition over the measurement time. Therefore a bioreactor with better insulation to save electricity and to allow for longer measurement times in the field has to be developed.

References

1.High-throughput micro-debubblers for bubble removal with sub-microliter dead volume. Harald van Lintel, Guillaume Mernier and Philippe Renaud Micromachines 2012, 3