Automated Monitoring of Micro-bioreactors

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The use of in-vitro micro-tissue models for toxicological assays is an ever-growing field. Spheroids are an example of a tissue model gaining popularity for this type of in-vitro testing. These tests are in general performed in multi-well plates, and biomarkers for toxicity assessment are often measured using well-plate-based assays. This approach can be used for the determination of a large panel of biomarkers, but is difficult when performing (semi)-continuous monitoring.

Within the EU project Hemibio[1] (Hepatic Microfluidic Bioreactor), a hepatic tissue model system is being developed for in-vitro repeated dose toxicity up to 30 days. A set of sensors is integrated downstream from the micro-bioreactor where the tissue model is cultivated in order to maintain optimal culture conditions for the cells, monitor their metabolism and potential cell death and detect and quantify their response to exposure to potentially toxic compounds.

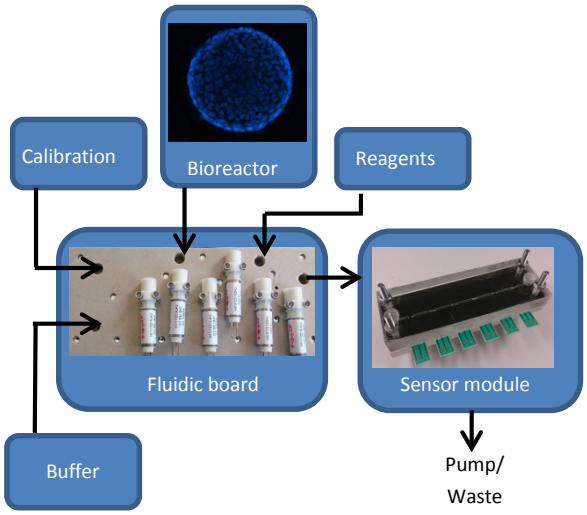

Figure 1: Schematic of the automated sampling and monitoring system. The custom-made sensing module designed for Hemibio, is capable of accommodating up to 6 sensors in a series and has a total volume in the channel of less than 50 µl.

The automated sampling, handling and sensing platform was designed by CSEM to be interfaced with the Hemibio bioreactor in order to have an in-line detection system, as illustrated in Figure 1. The sensor module is decoupled from the bioreactor, and placed after its outlet. In this way the cell culture is completely independent from the presence of sensors, which will not interfere with the biological material (and vice-versa). The modular concept allows for an easy exchange of the sensor in case of failing sensors, an easy re-calibration of sensors and as well the modularity in the choice of the sensors to be used for a specific experiment.

Given the unified dimensional footprint for all the electrochemical-based sensors, this panel of markers can be modified or expanded in the future, to specific metabolites related to diseases of interest, or even to different tissue models. The developed monitoring setup can be adapted to future new bioreactor designs.

Within the Hemibio project the selected parameters to be monitored with the sensor module are glucose, lactate, ALT (enzyme marker specific for hepatocyte cell death). Commercial fluorescence-based oxygen sensor beads were placed directly in the bioreactor.

The detection of ALT is indirect: the marker measured is a product of the enzymatic reaction catalyzed by ALT. The rate of production of this product provides the indication for the concentration of the enzyme present in the sample. The measurement of the product from the enzymatic reaction takes place over time, and requires the implementation of a reaction chamber, to allow time for the reaction to take place, and the implementation of multiple injection times.

Validation of the automated setup has been made by using cell culture supernatant withdrawn from the Hemibio bioreactor with HepG2 cells exposed to a toxic insult, by adding 200 µM rotenone and 0.4% DMSO to the perfusion medium. A clear increase in the signal for both oxygen and ALT is visible, (Figure 2[2]), which indicates that these two parameters can monitor cellular damages after a toxic insult.

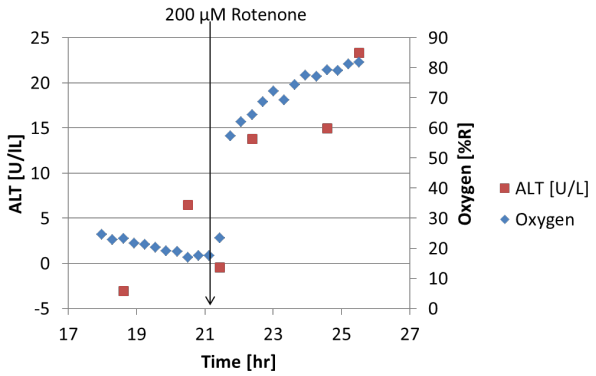

Figure 2: Monitoring of ALT and O_2 levels of a HepG2 culture before and after exposure to 200 µM rotenone[2].

[1] www.hemibio.eu, project funded by the Cosmetics Europe association and the European Union FP7, grant agreement Nr. 266777

[2] T. Gocht, M. Schwartz (Ed.), Towards the Replacement of in-vivo Repeated Dose Systemic Toxicity Testing, SEURAT-1 Annual Report Nr. 5, submitted for publication