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d-LIVER

ICT-enabled, cellular artificial liver system incorporating personalized patient management and support

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D5.4: Report on the evaluation of concepts for closed-loop control of toxin concentration in cell environment.

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RE	Restricted to a group specified by the consortium (including Commission Services)	
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1. Executive Summary

Closed loop control of toxin concentration inside the bio-artificial liver (BAL) requires a sensor based device to control and regulate e. g. perfusion, pH and other parameters inside the BAL. Different toxin concentrations were investigated in 8 ml bioreactors to evaluate the response of the cells and to define measures for regulation of cell culture. The results showed that changes in cell behaviour can be graded into different states dependent on the toxin concentration. Measures and procedures for cell recovery or culture termination can be undertaken dependent on the actual state of the bioreactor culture. The recovery of cells inside the BAL can be used to setup a system with two reactors: one in recovery mode and one in active mode. Switching from one reactor to the other defines a closed loop system to eliminate dangerous toxin concentrations.

2. Introduction

Deliverable D5.4 reports the design of a system which incorporates many different sensors (some of which have been developed in the project and some commercially available) for control of perfusion conditions and monitoring cell quality (Figure 1). Sensors were integrated at different sites of the perfusion circuit, depending on functional requirements.

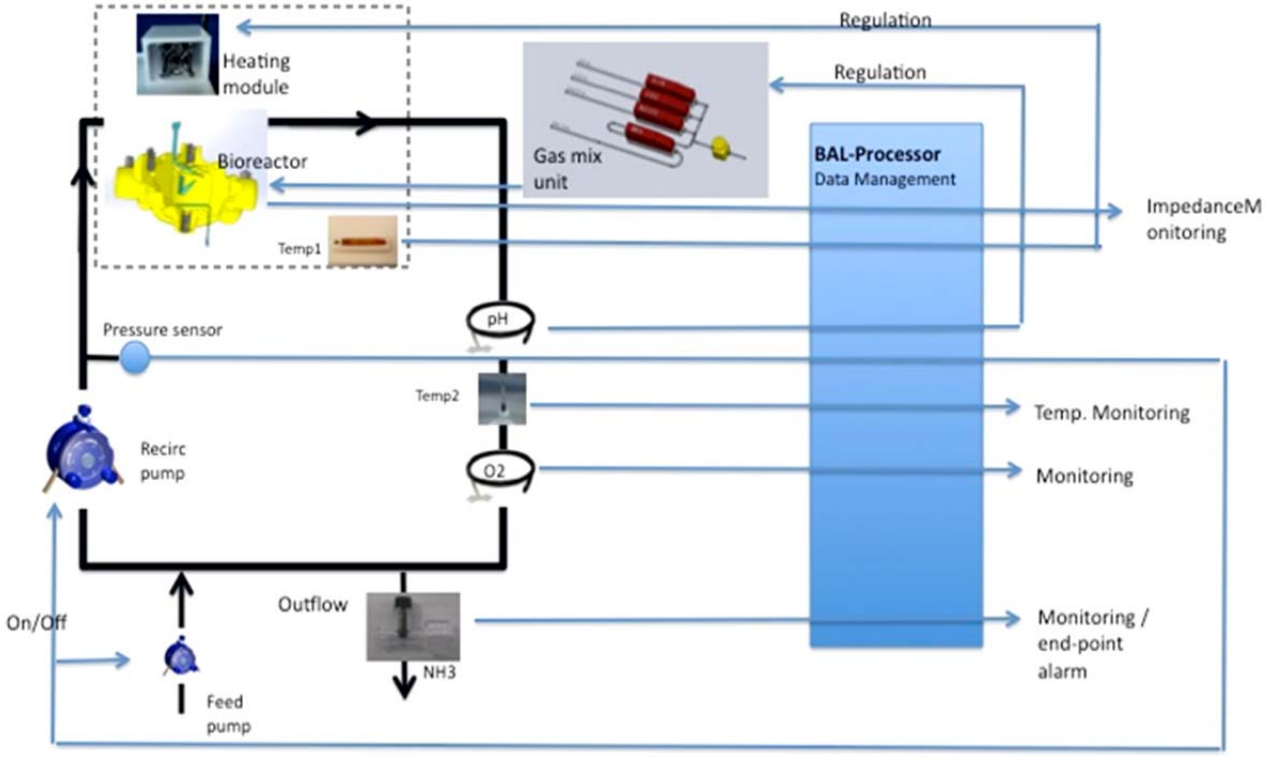

Figure 1: Schematic of system incorporating different sensors for control of system functions and cell culture monitoring.

Closed-loop controlled parameters:

- Temperature: Maintenance of a constant level within the bioreactor chamber by measurement via electronic temperature sensors and closed-loop control of the heating system.
- pH-value: Maintenance of a constant level in the perfusion liquid (culture medium or plasma) by continuous sensor-based measurement within the recirculation line and closed-loop control via CO_2 adjustment in the gas mix.

Preset value adjusted parameters:

- Flow rate of gas mixture: Four mass flow controllers regulate the supply of a gas mixture and the proportion of gases (compressed air, carbon dioxide and oxygen) in gas mixture.
- Culture medium/plasma recirculation rate: Maintaining of recirculation pump flow rate at a preset value via automatic readjustment of the pump motor speed.
- Culture medium/plasma feed rate: Maintaining of feed pump at a preset value via automatic readjustment of the pump motor speed.
- System pressures: Measurement in the recirculation circuit before (pre-pressure) and behind (post-pressure) the bioreactor and also within the cell compartment (intra-pressure) and maintenance of pre-, intra- and post-pressure within a predefined range (by exceeding the range, recirculation and feed pump will stop).

Parameters measured on-line:

- Oxygen saturation of culture medium: Continuous measurement via disposable O_2 sensors within the recirculation line.
- Impedance sensor: Continuous measurement with newly developed sensor strips integrated into the BAL.
- Ammonium sensor: Continuous measurement with newly developed sensors positioned at the BAL inlet and outlet.

3. Impedance measurement and cell recovery after toxin exposure in the BAL

Evaluation of the impedance foil based sensor with primary porcine liver cells in petri dishes revealed that attachment of the cells on the sensor resulted in an increased impedance value. Additional characterization of these cells on the sensor showed PAS (periodic acid-Schiff) positive staining for the detection of hepatocyte characteristic intracellular glycogen.

Experiments on cell recovery after toxin exposure in the BAL were performed in 8 ml bioreactors, using B-13 cells as model cell source in the BAL unit. The cells were trans-differentiated over 10 days into B-13/H cells by continuous application of 10 µM dexamethasone. Methapyrilene, which has been reported to exert a toxic effect on B-13/H cells (Probert *et al*., 2014), was used as a test substance to analyse the effect of toxin exposure and to evaluate potential recovery of cells from toxic stress in the BAL.

Bioreactor experiments were performed at a recirculation rate of 20 ml/min, a feed rate of 6 ml/h and a gas perfusion rate of 40 ml/min (95% air, 5% CO_2). In order to induce different grades of injury, methapyrilene was applied at increasing concentrations into the BAL. After continuous toxin perfusion the bioreactors were rinsed with culture medium to remove remaining toxic substance from the bioreactor system. Thereafter medium perfusion was continued to regenerate the system and enable cell recovery from possible cell stress/cell damage.

Daily medium samples were taken from the bioreactor perfusates and analysed for enzyme release (aspartate transaminase [AST], lactate dehydrogenase [LDH], alanine transaminase [ALT], glutamate dehydrogenase [GLDH]), glucose, lactate, ammonium and urea concentrations.

Methapyrilene application resulted in a dose-dependent increase in enzyme leakage in the bioreactors. Glucose consumption and lactate production rates showed a temporary decrease after exposure to low concentrations of the substance, which was followed by an increase to levels comparable with the control bioreactor. Further increase of the methapyrilene dose led to a drastic decline of glucose consumption and lactate production rates, and values remained nearly zero until culture termination. The time-courses of ammonia and urea production confirmed the finding of dose-dependent enhancement of methapyrilene toxicity.

The results from the experiments on methapyrilene toxicity showed that the response of the cell behaviour can be graded into different states dependent on the toxin concentration (Figure 2).

- "Normal" → no significant stress observed, continue culture
- "Subcritical" → moderate stress detected, recovery possible, e.g. rinse BAL
- "Critical" → irreversible cell damage, no recovery possible, terminate BAL

Decisions for cell recovery or culture termination can therefore be taken dependent on the actual state of the bioreactor culture.

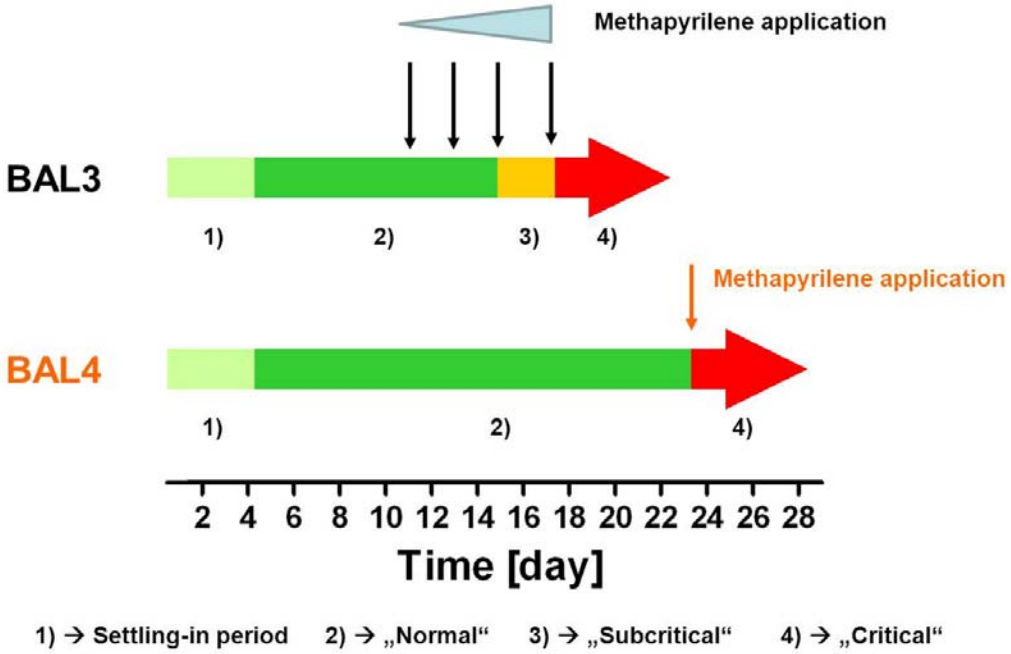

Figure 2: Schematic illustration of cell behaviour under application of toxic stress. The figure shows the response of trans-differentiated B-13 cells in two in two bioreactors (BAL3 and BAL4) to increasing concentrations of methapyrilene as an example of the application of toxic stress in the BAL.

4. Conclusions

Different toxin concentrations were investigated in 8 ml bioreactors to evaluate the response of the cells and to define measures for the regulation of cell culture. The exposure to increasing toxin concentrations in the BAL resulted in increasing stress levels from slight to severe cell damage. Thus, cell behaviour can be graded into different states dependent upon the toxin concentration: “Normal”, “Subcritical” and “Critical” (see D5.3). Measures and procedures for cell recovery or culture termination can be performed dependent on the actual state of the bioreactor culture. In the “Normal” state the BAL culture requires no specific measures and may be continued. In the “Subcritical” state measures for cell recovery in the BAL can be instigated. In the case of irreversible injury (“Critical state”) the culture must be terminated. Further, in the “Subcritical” state, one option for possible recovery of cells inside the BAL is to rinse the system to flush out the toxic compounds. The recovery period can be bridged by setting up a system with two reactors: one in recovery mode and one in active mode. Switching from one reactor to the other defines a closed loop system to eliminate dangerous toxin concentrations in the BAL. In some cases, albumin dialysis could be used as an additional tool to detoxify the patient plasma prior to entering the bioreactor.

5. References

Probert, P.M.E., Chung, G.W., Cockell, S.J., Agius, L., Mosesso, P., White, S.A., Oakley, F., Brown, C.D.A., Wright, M.C. Utility of B-13 progenitor-derived hepatocytes in hepatotoxicity and genotoxicity studies. *Toxicol. Sci.* 137(2), 350, 2014.