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# Electrochemical microsensors for monitoring the functions of liver cells in a modular based microfluidic system

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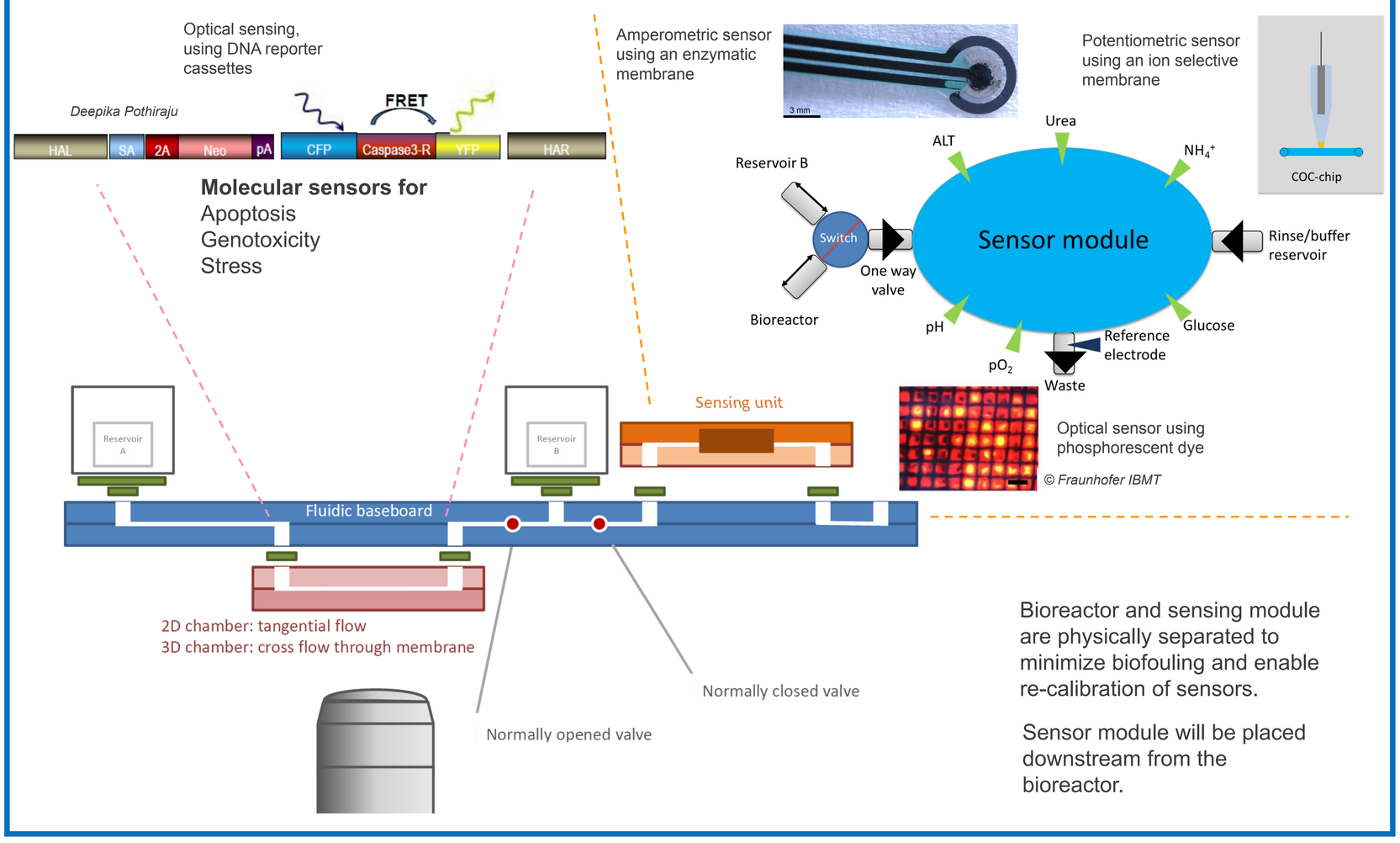
**European legislation is restricting the use of animals for toxicological studies, which increases the need for alternative testing methods. The HEMIBIO project aims at developing a hepatic 3D micro bioreactor reproducing the complexity of the liver for preclinical toxicity testing. One of the major challenges in building a 3D-liver bioreactor is the lack of data on the complex environment present inside the bioreactor where the cells live, aggregate and differentiate. Microsensors located in the direct vicinity of the cells can play a vital role, by monitoring cell culture conditions and thus help mimic the natural microenvironment.**

## Modular approach and non invasive sensing




Bioreactor and sensing module are physically separated to minimize biofouling and enable re-calibration of sensors.

Sensor module will be placed downstream from the bioreactor.

## Cell death and cell function

| Sensor | Typical concentration in vitro (Human cells) |
|---|---|
| ALT | 5 U/mio cells for healthy culture and 12.6 U/mio cells for culture exposed to 10 mM CCl4 for 6h[1] |
| Urea | 478 nmol/h/mio cells[2] |
| $NH_4^+$ | Detoxification rate: 1 µmol/h/mio cells[3] |

1, Ou et al., Chemical-Biological Interactions vol. 185, 94-100, 2010
2, Bartolo et al., Biomaterials vol. 30, 2531-2543, 2009
3, Enosawa et al., Cell transplantation vol. 5, 39-40, 1996

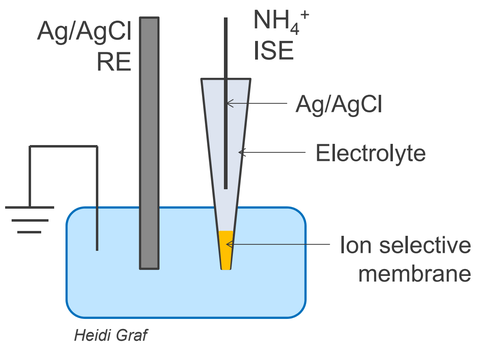
Alanine transaminase (ALT) is an intracellular enzyme specific to hepatocytes. ALT will only be present in cell culture medium when the cell membrane ruptures, either by injury or cell death.

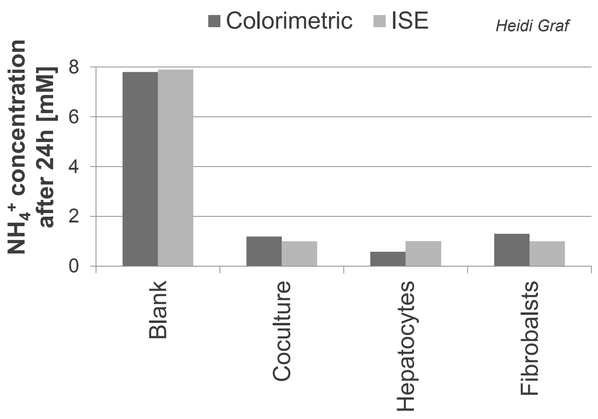
Urea production has been chosen as cell function marker for hepatocytes. Healthy cells will produce urea at a constant rate, while the rate will decrease if the cells are not thriving or a link in the urea cycle is disturbed.

Ammonium detoxification is not specific to hepatocytes. Detoxification rate is indicative for the performance of the entire cell culture.

## $NH_4^+$ microfluidic ion selective electrode

The ion selective electrode in a pipette-tip has been validated against a commercial colorimetric assay kit

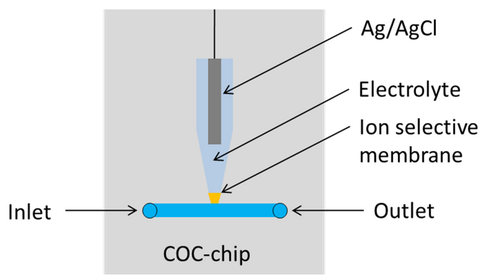


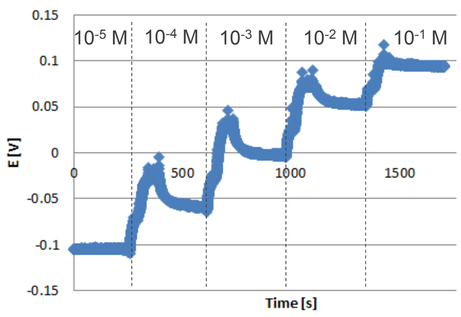




### Microfluidic integration

Miniaturization of previous piptte-tip ISE combined with direct integration into a microfluidic channel in a Cyclic Olefin Copolymer (COC) chip.

- Transparent to visible light and UV
- Robust
- Adapted for medical and pharmaceutical industry (FDA approved)
- Negligible liquid absorption
- Resistant to acids, alkalis and polar organics



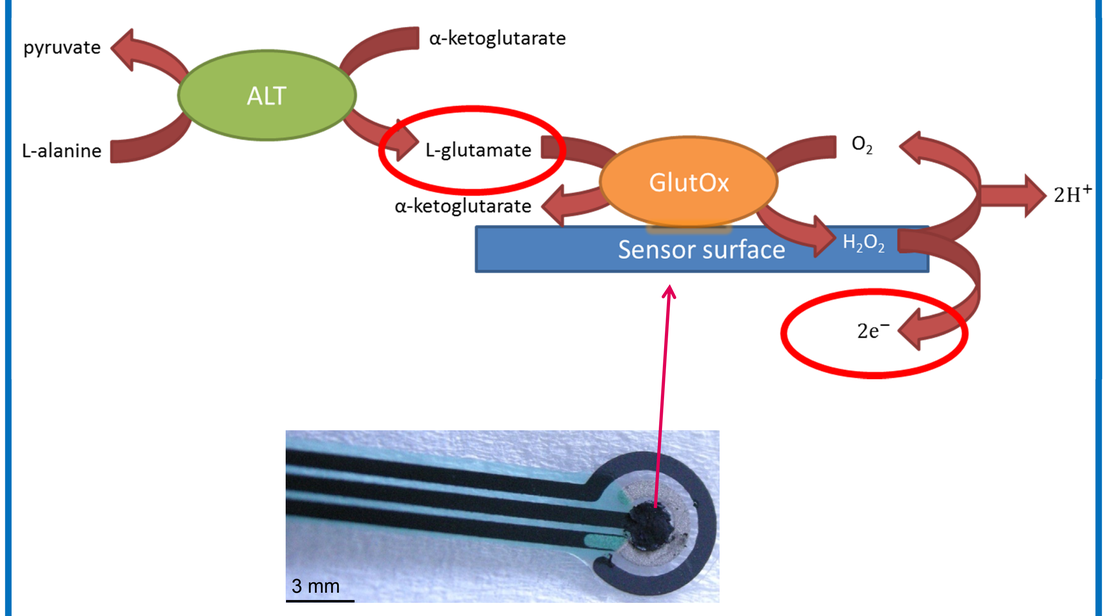

Dynamic response of $K^+$-sensitive microfluidic ISE to sequential injections of KCl calibration solutions of concentrations $10^{-5}$ M to $10^{-1}$ M. A near-nernstian response is observed.

## Alanine Transaminase (ALT) sensing

Indirect detection of ALT is possible by sensing its reaction product L-glutamate. The glutamate is converted into hydrogen peroxide by the enzyme glutamate oxidase (GlutOx), immobilized at the sensor surface. Hydrogen peroxide is finally oxidized at the electrode surface and the electrons released, are registrered by an external electronic circuit.

$$\text{L-alanine} + \alpha\text{-ketoglutarate} \xrightarrow{ALT} \text{L-glutamate} + \text{pyruvate}$$

$$\text{L-glutamate} + O_2 \xrightarrow{GlutOx} H_2O_2 \rightarrow O_2 + 2H^+ + 2e^-$$




The enzyme glutamate oxidase is immobilized on the sensor surface.

## Conclusion

Ion-selective electrode miniaturization and microfluidic integration has been initiated, and its feasibility proven.

ALT will be indirectly detected by amperometric measurement of glutamate.

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