Real-time multi-analyte online monitoring of 3d cell cultures by integrated enzyme-based biosensors in hanging drop networks

Conference Paper**Author(s):**

Misun, Patrick ; Rothe, Jörg; Hierlemann, Andreas ; Frey, Olivier

Publication date:

2015

Permanent link:

<https://doi.org/10.3929/ethz-b-000111658>

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Originally published in:

<https://doi.org/10.1109/TRANSDUCERS.2015.7181252>

Funding acknowledgement:

296257 - The Body-on-a-Chip (BoC) (EC)

142440 - The Body on a Chip - Human 3-D tissue analogues in microfluidic systems (SNF)

REAL-TIME MULTI-ANALYTE ONLINE MONITORING OF 3D CELL CULTURES BY INTEGRATED ENZYME-BASED BIOSENSORS IN HANGING DROP NETWORKS

Novelty/ Progress Claims

We present the integration of enzyme-based lactate and glucose biosensors into hanging drop networks. Hanging-drop networks are an ideal microfluidic platform for formation, cultivation, and continuous and longterm observation of 3D microtissues. The implementation of biosensors enables continuous in-situ online monitoring of effects of different culturing conditions and compound dosages on the microtissues. A hybrid approach including glass sensor modules embedded into a microfluidic PDMS chip facilitated system integration. The biosensors enabled real-time recording of lactate production and glucose consumption of human colon carcinoma spheroids.

Background/ State of the Art

3D spheroids become important tissue models in pharmaceutical industry and basic research for drug development and toxicity studies, as they represent simple organ models that are more representative than conventional 2D cell cultures.

Currently, two factors limit their broad application. Suitable culturing systems that can host multiple spheroids in and can be combined with fluidic networks that enable continuous medium supply and compound exposure are missing. Further, most of the readout methods are optimized for 2D cell cultures, which now have to be adapted and modified for the requirements of 3D tissue formats.

Description of the New Method or System

The hanging drop network is based on an inverted PDMS substrate that comprises hydrophilic regions surrounded by hydrophobic rims that guide the liquid and form a completely open microfluidic hanging-drop and channel system [1].

The hanging drops form below the 8 circular regions and are interconnected through narrow channels (Figure 1a). PDMS as substrate material is biocompatible and gives the possibility to locally change its wetting properties through O₂-plasma treatment.

A small glass plug-in with integrated electrodes constitutes the biosensor read out unit, which is inserted in a small rectangular recess. Additional rim structures on the plug-in complete the patterns on the PDMS to obtain a leakage-free microfluidic network

(Figure 1b). The modular setup offers the advantage of performing the biosensor functionalization and calibration before plugging it into the microfluidic PDMS substrate.

The biosensor is functionalized through coating the platinum working electrodes (WE) with a membrane containing lactate oxidase (LOx) [2], or glucose oxidase (GOx). The enzymes in both cases catalyze the reaction of the analyte, i.e., glucose or lactate, to produce peroxide that can be amperometrically detected (Figure 2a). Figure 2b shows the hanging drop chip containing a 3D-spheroid at a sensing site. Calibration curves of the biosensors are shown in Figure 3a for the glucose sensor and in Figure 3b for the lactate sensor. The calibration was performed in PBS in a beaker at 37°C. The functionality of the biosensors in the microfluidic hanging-drop network has been tested with a flow protocol (Figure 4) switching between PBS, 0.5-mM lactate, and 0.5-mM glucose solutions.

Experimental Results

Cancerous tissue typically shows an enhanced glucose uptake and an increased glycolysis rate, followed by the anaerobic fermentation of pyruvate into lactate. In-situ detection of glucose uptake and lactate production by a human colon carcinoma microtissue (HCT116) is shown in Figure 4a. Two spheroids were introduced in each drop, where WE 1&2 and WE 3&4 were located. After complete liquid exchange with 0.5-mM glucose solution, the flow was stopped, and glucose consumption and lactate accumulation in the hanging drop could be detected and monitored by the biosensors over 60min. Control WEs show a slight cross-talk due to peroxide diffusion. The microtissues were then removed and, as expected, no lactate and glucose concentration changes were detected. A subsequent flow-through calibration confirmed the functionality of the sensors and that the spheroid metabolism is the origin of the detected transients.

The online readout enables the acquisition of information on the metabolic activity of 3D-microtissues in real-time and allows for precise control of the cell culture conditions in the microfluidic networks.

Word Count: 600

Figure 1: (a) Top view of the microfluidic 2x4-hanging drop chip showing hydrophobic PDMS rims. The biosensor glass plug-in is placed into a recess and completes the microfluidic network. (b) Close-up of the electrodes. 4 working electrodes (WE), one counter electrode (CE), and an Ag/AgCl pseudo-reference electrode (RE) are integrated.

Figure 2: (a) Cross-sectional view along "A" in Figure 1b. The drop forms between PDMS rim and SU-8 rim on the glass insert. The close-up shows the working principle of the enzyme electrode. (b) Photograph of the chip including a 3D microtissue located in one of the two sensing drop sites.

Figure 3: (a) Glucose calibration curve and (b) lactate calibration curve performed in PBS at 37 °C ($n=4$). Linearity up to 1 mM analyte and a good reproducibility of sensitivity was observed in several trials.

Figure 4: (a) (1) Detection of glucose consumption and lactate accumulation in the drop, in which the HCT116 cancer spheroids were located. Blank WEs show a small current increase due to peroxide cross-talk from the functionalized electrode in the same drop. After spheroid removal a calibration was performed in which first (2) 0.5 mM glucose and then (3) 0.5 mM lactate were added. The two-step signal increase during the lactate calibration is due to peroxide accumulation near the electrode at no flow. (b) Flow protocol used for liquid renewal in the hanging drops.

References:

- [1] Frey, O. *et al.* *Nat. Commun.* 2014. 5:4250.
- [2] Frey, O. *et al.* *EuroSensors 2014 Conference.* Brescia, Italy.