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“All-in-one” cell-based biosensor for on-site monitoring of cell response

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Introduction

Biosensors play an important role in many areas and become part of our everyday life. They have rapidly been evolving and expanding due to the recent advances in material science, nano- and biotechnology, and microfluidics. The importance of biosensors has once again been demonstrated, particularly as a consequence of the coronavirus disease 2019 (COVID-19) [1–3].

Biosensors, which utilize living cells, are called “cell-based biosensors”. This kind of biosensors can either detect the reaction of cells specific to analytes (for example, molecules or biomarkers) directly or monitor their physiologically relevant functions [4]. The light-addressable potentiometric sensor (LAPS) can be used as a biosensor, for which different regions (measurement sites) on the sensor surface can be addressed by light illumination [5–8]. This feature represents a remarkable advantage in achieving spatial resolution in biosensing applications [9,10]. For example, using a LAPS chip that has a pH-sensitive transducer layer, a desired sensing point on the sensor surface can be addressed to measure the extracellular acidification (EA) of the cells above that region [11]. Moreover, a larger area can be addressed point-by-point in a scanning manner. In this way, the distribution of the concentration of the EA (extracellular pH (pH_e)) can be visualized in form of a chemical image [12]. Fig. 1 shows a representative schematic of the LAPS chip for cell investigations and its set-up.

Despite an increased number of publications and research focusing on electrochemical cell-based biosensors to detect substances of interest such as pollutants [13], toxins [14], or viruses like SARS-CoV-2 [15], there are only a few cell-based biosensors currently in use on the market. One important reason for this may be found in the biosensor principle, which contains a “living” component such as mammalian cells: their efficient preservation and transportation to the end-user of the sensor system in a ready-to-use concept is still a major challenge. This issue often hinders the practical applicability and commercialization [13–15]. Therefore, there is a growing need for preservation methods and tools to enable ready-to-use, on-site and on-demand concepts.

In this study, a strategy (that is called on-sensor cryopreservation) has been developed, based on a

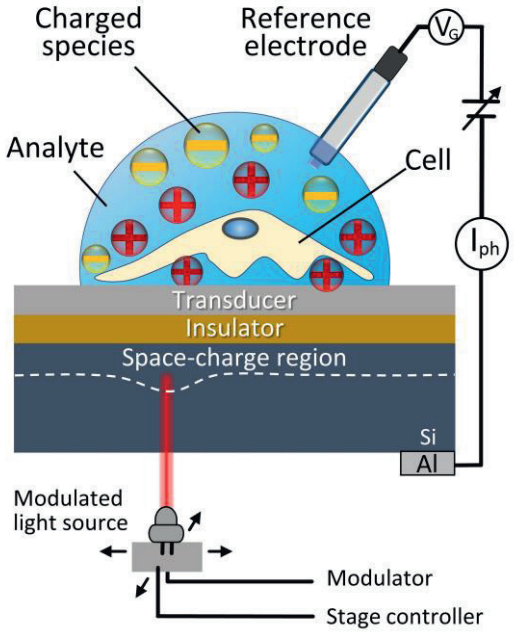

Fig. 1: Schematic representation of the light-addressable potentiometric sensor (LAPS) for cell investigations and measurement set-up.

method for preserving living components directly on a biosensor surface by freezing (-80 °C). To achieve a high cell recovery after the cryopreservation process, the rigid sensor surface (Ta_2O_5) was modified with elastic electrospun polymer fibers consisting of polyethylene vinyl acetate (PEVA), which has a low glass-transition temperature and a high thermal expansion coefficient. The biosensor chip is consequently integrated into a microfluidic system to obtain a cryo-chip, which is important for precise control of the microenvironment. This miniaturization ensures a fast thawing process that has a vital impact on cell survival.

Methods and Materials

Fabrication of LAPS chips

The LAPS chip consisting of an Al/p-Si/SiO_2/Ta_2O_5 structure was fabricated based on a p-doped silicon wafer (thickness: 400 µm). An insulating layer (30 nm SiO_2) was grown by thermal dry oxidation procedure (1000 °C). Thereafter, electron-beam evaporation

was used to deposit a tantalum layer on the SiO_2 layer. A thermal dry oxidation step (520 °C) was performed to obtain a 60 nm Ta_2O_5 layer as a pH-sensitive transducer. For an ohmic contact, an aluminum layer (300 nm) was deposited at the rear side of the sensor chip by electron-beam evaporation. The wafer was divided into single chips by dicing them with a size of 2 cm x 2 cm. An illumination window for the rear side of the sensor chip was defined by partially removing the aluminum layer using 5% hydrofluoric acid. After fabrication, the sensor functionality was characterized using an electrochemical spectrum analyzer (Zahner-Elektrik GmbH) [16,17].

Electrospinning of PEVA on the LAPS and surface characterization

To obtain high cell (monolayer) recovery after cryopreservation the sensor surface was functionalized with PEVA (vinyl acetate 40 wt%) fibers. They were prepared by dissolving PEVA beads in chloroform and *N,N*-Dimethylformamide (3:2, v/v), followed up by an electrospinning technique to deposit them to the sensor layer. Morphological examinations of the fibers were performed using scanning electron microscopy (SEM) and digital microscopy.

O_2 plasma treatment

Good wettability of the surface is critical for cell culturing. O_2 plasma treatment (PT) was used to increase the wettability of the modified sensor surface. PT was performed as described in ref. [9]. Afterwards, the wettability changes were observed by water contact angle measurements.

Microfluidic integration

A self-adhesive microfluidic slide (sticky-Slide VI 0.4; ibidi GmbH) with a height of 400 µm and a width of 3.8 mm was used for creating two identical but separated channels on the LAPS chip. One of the channels served as a reference channel. As this chip system is used for cryopreservation of the cells, this chip system is named “cryo-chip”. The cryo-chip system is shown in Fig. 2.

Cell culturing and cryopreservation

To test the feasibility of the cryo-chip, a model cell line, Chinese hamster ovary (CHO-K1) cells (DSMZ, Germany), was selected to perform cell culturing and analysis. Before seeding the cells, the microfluidic channels were sterilized by flushing them with 70% ethanol for one hour. Cell culturing was performed as mentioned in the literature [11]. 40000 cells/cm^2 were seeded into each channel. As control samples, elastic coverslips (uncoated, ibidi GmbH) and unmodified LAPS chip, were also used as substrate material. After 48 h of incubation, the cell viability was determined with a Cell Counting Kit (CCK)-8 assay (Dojindo Laboratories, Kumamoto). The cells inside the channels were incubated in an incubator for 1 h with CCK-8 test solution at a 1:10 dilution in the medium. After the incubation, the solution was transferred into well plates, and the absorbance value was read at 450 nm on a microplate reader.

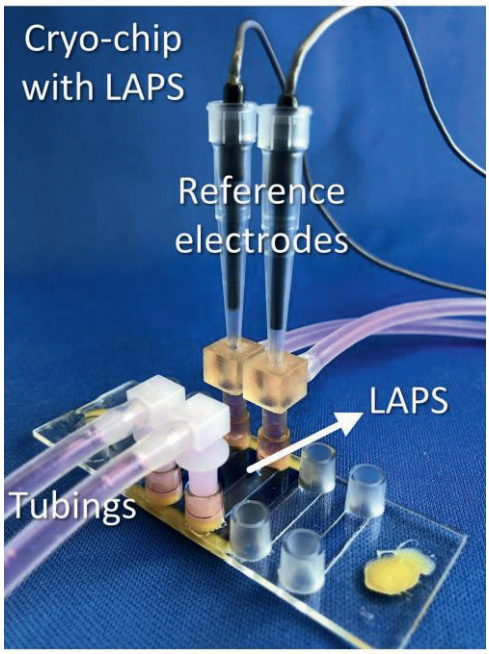

Fig. 2: A cryo-chip consisting of a modified LAPS, which is integrated into a microfluidic system. Two identical and separated microfluidic channels are placed on the same LAPS chip having a differential measurement set-up.

For cryopreservation, a cryoprotectant solution was prepared from 90% fetal calf serum and 10% dimethyl sulfoxide. The channels of the cryo-chip were filled with this solution and placed into a freezing container at −80 °C. After keeping the cryo-chips in the freezer overnight, they were quickly thawed in an incubator (37 °C) and the cryoprotectant solution in the channels was replaced with a fresh culture medium. The cell viability and recovery after thawing was analyzed with the CCK-8 assay as mentioned above.

Sensor signal measurement after cryopreservation

To enable differential measurements, one of the two channels of the cryo-chip was labeled as a “reference channel”, whereas the other was defined as “active culture channel”. After thawing, a micro-agar salt-bridge including Ag/AgCl reference electrode, tubes, connectors and a 10 mL glass syringe containing the measurement medium were connected to the chip under aseptic conditions (see Fig. 2). A 3D-printed adapter with four spring-loaded pins was assembled to create an ohmic rear side connection of the LAPS.

This way, the chip was connected to the self-developed LAPS measurement set-up. Extracellular acidification of the cells was monitored as previously reported in the literature [11,18].

Results and Discussion

Electrospun PEVA fibers were successfully attached to the sensor surface after optimization of the electrospinning parameters. An optimum fiber structure was obtained with the parameters, in which polymer solution concentration, flow rate, spinning duration, tip-to-connector distance, and power supply were 13% (w/v), 1.5 mL/h, 60 seconds, 10 cm, and 18 kV, respectively. Fig. 3 demonstrates the bare sensor surface (top) and the modified sensor surface (bottom) after electrospinning of the PEVA fibers.

Tab. 1 shows the results regarding the cell recovery on different substrate surfaces before and after on-sensor cryopreservation. The LAPS surface with PEVA and PT showed the highest recovery rate among the tested surfaces. In addition, the results did not statistically differ between the LAPS surface and the polymer coverslip (p=0.053). The results showed that on-sensor cryopreservation using a cryo-chip containing a modified LAPS is effective for keeping cells viable during cryopreservation. More details about the advantages of elastic polymers on monolayer cell cryopreservation were reported elsewhere [19,20].

Tab. 1: Cell recovery on different surfaces with regard to the on-sensor cryopreservation (−80 °C, overnight) using the newly developed cryo-chips.

Surfaces	Cell recovery (%)
polymer coverslip	76.3 ± 5.9
LAPS	36.3 ± 0.6
LAPS with PEVA fibers	49.5 ± 3
LAPS with PEVA fibers and plasma treatment	66.2 ± 2.6

The sensing performance of the cryo-chip was validated before and after the on-sensor cryopreservation using adherent CHO-K1 cells as a model organism. Tab. 2 depicts the average photocurrent alterations in response to the cell activity before and after cryopreservation. As can be seen from the table, the photocurrent in the active culture channel (with cells) starts to increase just after the thawing process, compared to the reference channel (without cells). This increase was even more pronounced after further cell culturing for 6 hours. The results indicate extracellular acidification in the active culture channel and support the results regarding the cell recovery using the new cryo-chip system.

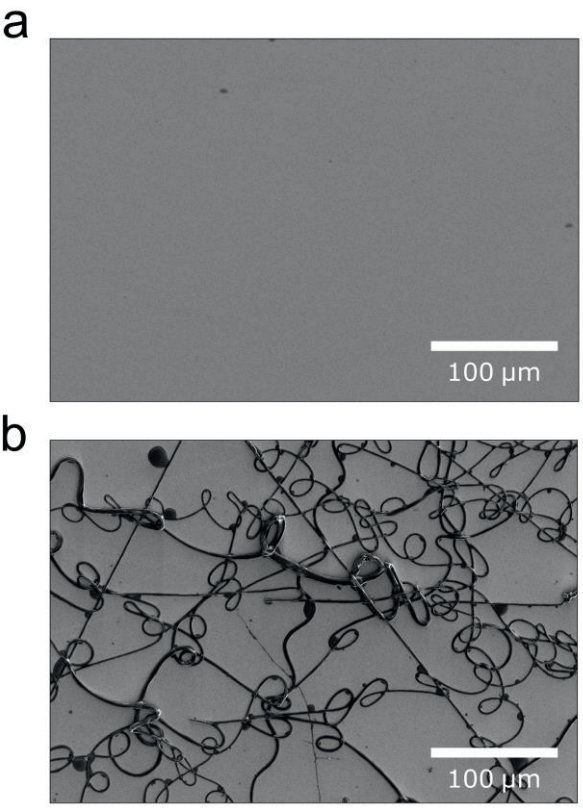

Fig. 3: SEM images representing a) a bare LAPS chip surface and b) a LAPS surface modified with electrospun PEVA fibers.

Tab. 2: Average photocurrent alterations measured by the cryo-chip (two channels) indicating the cell activity, and the extracellular acidification before and after on-sensor cryopreservation (−80 °C, overnight).

Measurement	Average photocurrent values (nA)	
	Reference channel	Active culture channel
Before cryo-preservation	124.6 ± 18.1	124.3 ± 20.5
After thawing	122.8 ± 17.6	131.5 ± 20.4
6 h after thawing	118.6 ± 16.7	160.9 ± 23.1

In conclusion, this on-sensor cryopreservation is effective not only for keeping cells viable during cryopreservation but also for detection of the extracellular acidification of adherent CHO-K1 cells directly after thawing. Being able to perform on-chip cryopreservation at the manufacturing stage and transporting this ready-to-use system via cold-chain transport could therefore pave a way for an “off-the-shelf” and “all-in-one” system applicable to on-site applications.

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