MONITORING OF THE RESPIRATORY ACTIVITY OF MULTICELLULAR TUMOR SPHEROIDS IN AN INTEGRATED MICROFLUIDIC DEVICE

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ABSTRACT

We report an integrated sensing device consisting of an ultra-microelectrode array (UMEA) sensor placed in a nanoliter-volume chamber for monitoring the respiratory activity of multicellular tumor spheroids (MCTS). The UMEA sensor is made of platinum, and consists of 16 UMEs of 2 µm diameter spaced by 20 µm. This sensor is integrated in a microfluidic device equipped with trapping structures for capture of one MCTS. The dissolved oxygen concentration is measured using chronoamperometry at a reduction potential of -0.6 V by applying voltage pulses for 5 ms. The measurement near MCTS shows a decrease in DO concentration from 7.8 mg/L to 6.7 mg/L within 2 hours, which is caused by the respiration of MCTS.

KEYWORDS: Oxygen sensing, Multicellular tumor spheroid, Ultra-microelectrode, chronoamperometry

INTRODUCTION

A cell respiratory activity is an overall indicator of its metabolism, and a non-invasive marker for cell growth, differentiation or viability in drug testing assays [1]. In the field of assisted reproductive technologies (ART), the embryo oxygen consumption has been identified as a marker for its viability and quality [2]. Typically, the oxygen consumption of a biological sample is determined by measuring variations in the dissolved oxygen concentration in its vicinity, using optical or electrochemical sensors. In previous work, we developed an electrochemical sensor based on an array of ultra-microelectrodes (UME) together with a novel sensing protocol using very short measurement times (t < 5 ms) to minimize the oxygen consumed by the sensor itself [3]. Here, we apply this sensor and sensing principle for measurements on multi-cellular tumor spheroids, and we integrate the sensor in a microfluidic device to monitor the oxygen consumption of individual multicellular tumor spheroids.

EXPERIMENTAL

The sensor device (Fig. 1a&b), which consists of an array of 16 UME with a 2-µm diameter, is fabricated as reported previously [3]. The fluidic part includes a 630-nL chamber equipped with a trapping site for the capture of a single MCTS on the sensor. This fluidic layer is fabricated from PDMS using soft-lithography, and manually aligned on the sensing substrate. The device is placed in a custom-made chip-holder (Fig.1c) to facilitate its connection to the potentiostat. For O_2 measurements, a reduction potential of -0.6 V is applied as a 5-ms pulse, while continuously recording the current. For all biological measurements, the device is first coated with Pluronic to prevent undesired adsorption of proteins on the sensing area and biofouling.

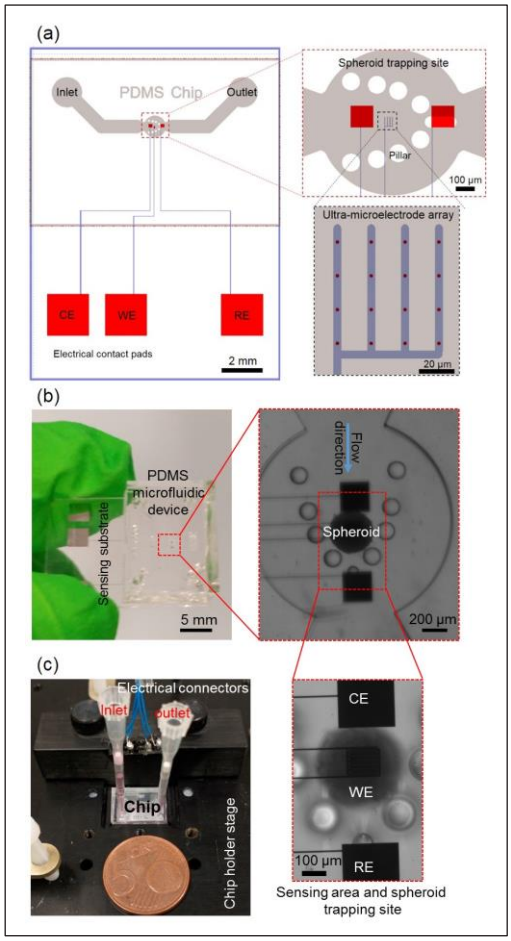

Figure 1: (a) Schematic representation of the entire sensing device. (b) Assembled microfluidic device with a zoom view on the chamber, in which a 300-µm diameter MCTS is trapped. (c) Custom-made chip-holder.

RESULTS AND DISCUSSION

First, the performance of the sensor is characterized and calibrated in DMEM cell culture medium To that end, nitrogen is bubbled in the solution on the sensor to create oxygen-deprived conditions, and chronoamperometric (CA) measurements are

continuously performed to monitor variations in the dissolved oxygen concentration using both the UMEA and an external sensor (Unisense, Denmark). From these measurements, a calibration curve is established and the device sensitivity is determined (Fig. 2a). The sensor sensitivity in DMEM media is -0.24 $nA.s^{0.5}.L.mg^{-1}$, which is comparable to the sensitivity found in phosphate buffer (-0.49 $nA.s^{0.5}.L.mg^{-1}$) [3]. Next, the sensor is integrated in a microfluidic device, in which individual MCTS prepared from co-culture of breast tumor cells and fibroblasts, are introduced (Fig. 1b). The dissolved oxygen concentration near the MCTS concentration is measured over 2 hours on two different days. Fig. 2b present the results of these single MCTS measurements. After about one hour, the DO concentration decreases, which is due to the depletion of oxygen near the UMEA caused by the oxygen consumption by the MCTS. Typically, a decrease to *ca*. 6.7 mg/L was found, together with an oxygen depletion rate of 0.03 $mg.L^{-1}.min^{-1}$. Furthermore, we observe an offset of about 0.3 mg/L in the sensor response between the two days of measurement. This offset can be due, for instance, to a slight displacement of the MCTS in the device, further away from the UMEA.

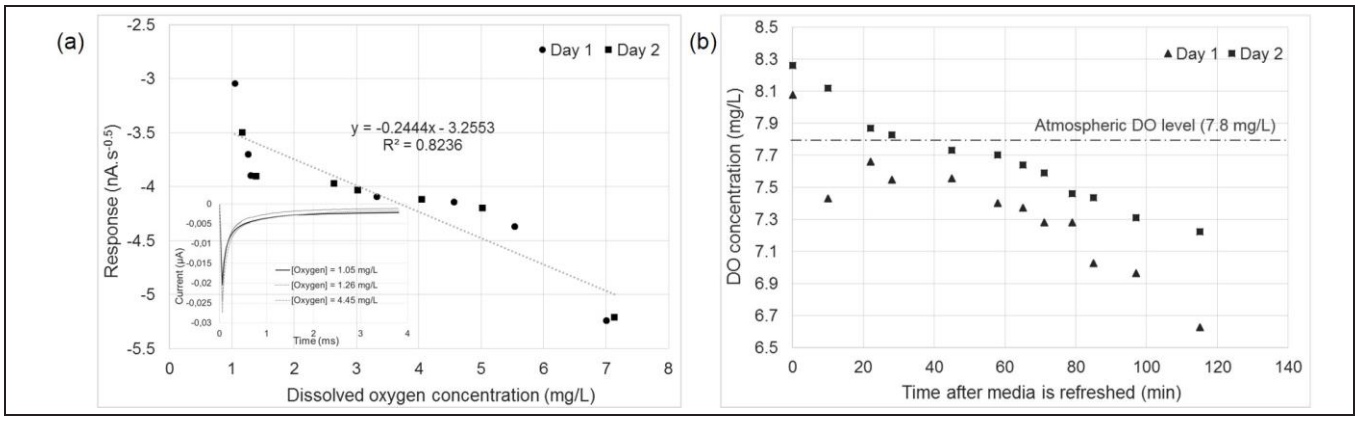

Figure 2: (a) Calibration of the UMEA sensor in DMEM medium against a commercially available sensor. Inset: Recorded chronoamperometric response of the UMEA sensor for various measured concentrations in dissolved oxygen (DO) in DMEM cell culture medium. (b) Dissolved oxygen concentration for two days near the MCTS. Time zero is defined as the moment where medium was refreshed in the device.

CONCLUSION

In this paper, we demonstrate the applicability of a UMEA sensor used with a short measurement protocol to monitor the respiratory activity of multi-cellular tumor spheroids. Calibration of the UMEA sensor using a short measurement time approach in the microfluidic device shows good linear correlation between the sensor response and the dissolved oxygen concentration in the device. Furthermore, a sensitivity of -0.24 $nA.s^{0.5}.L.mg^{-1}$ is derived from this calibration curve. Finally, continuous monitoring of the respiratory activity of individual MCTS indicates a decrease in the dissolved oxygen concentration to *ca*. 6.7 mg/L in about 2 h. In future studies, this platform can be employed for drug testing on MCTS samples, and to monitor the quality of mammalian embryos.

ACKNOWLEDGEMENTS

We would like to thank Jean-Baptiste Blondé for providing MCTS for our experiments. The authors thank the MIRA institute for financial support for this work.

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