USING OXYGEN-CONSUMING THERMOSET PLASTICS TO GENERATE HYPOXIC CONDITIONS IN MICROFLUIDIC DEVICES FOR POTENTIAL CELL CULTURE APPLICATIONS

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ABSTRACT

The precise control of the oxygen concentration in a cellular environment allows the study of cells under physiologically relevant conditions. This work reports on a novel method for the generation of reduced dissolved oxygen concentrations in microfluidic chambers for cell- and organ-on-chip applications. Using a thermoset polymeric material (OSTEMER^{TM}), which effectively scavenges dissolved oxygen (DO), microfluidic devices have been fabricated where oxygen was rapidly depleted from the microfluidic chamber. It is shown that hypoxic and anaerobic conditions can be generated through the inherent scavenging property of the material itself, without any additional chemical additives.

KEYWORDS: Oxygen scavenging, thiol-ene, OSTE+

INTRODUCTION

Oxygen plays a key role in mammalian cellular functions in normal human physiology as well as disease states. Its concentration varies tremendously in human body, from nearly anoxic in cartilage to fully oxygenated in arterial blood 13.2%. Moreover, in the human body a sudden loss of oxygen supply can cause irreversible impairment like ischemic brain damage. Oxygen control in perfused and non-perfused microfluidic devices was previously accomplished by diffusion across a gas-permeable material (PDMS) [1], or by introduction of an oxygen scavenger directly into the growth medium [2]. In contrast, the method reported here uses an oxygen scavenging material and therefore differs from that of other groups in the specific method of on-chip integration. We have previously shown that this material is compatible with different cell lines [3] and is highly suitable for the rapid fabrication of complex microfluidic devices [3, 4, 5].

EXPERIMENTAL

Master moulds were fabricated using standard lithography (DFR on Si wafer) with an 0.5% Teflon AF spin coated anti-adhesion layer. Microfluidic devices were replica moulded using OSTEMER 322 (Mercene Labs). Oxygen concentrations were measured in UV- (700 mJ/cm^2, Bio-link BLX Crosslinker) and heat cured chambers using an optical sensor (Piccolo 2, Pyro Science) in combination with a platinum(II) indicator (PtTPTBPF). The oxygen indicator dye was incorporated into amine-functionalized polystyrene beads. Since reactive epoxy groups are present at the OSTEMER surface after the first curing step, the beads were covalently liked via the NH-group. Electrochemical measurements we performed using a needle sensor (OX-NP, Unisense).

RESULTS AND DISCUSSION

To investigate the particle-to-polymer bonding strength, an open microchamber was immersed into an ultrasonic bath for 10 min and microscopic investigation revealed no visible loss of beads (see Fig. 1A). To validate that the oxygen scavenging is not an artefact of this measurement technique (e.g., catalysed by Pt), we performed measurements using an Clark type electrode sensor in an OSTEMER test chamber (~15 µL). As shown in Fig. 1B, the data clearly demonstrates that DO is scavenged irrespective of the measurement method used. On the other hand, using the same setup, we observed that the DO concentration does not decrease in a chip made from PMMA (data not shown).

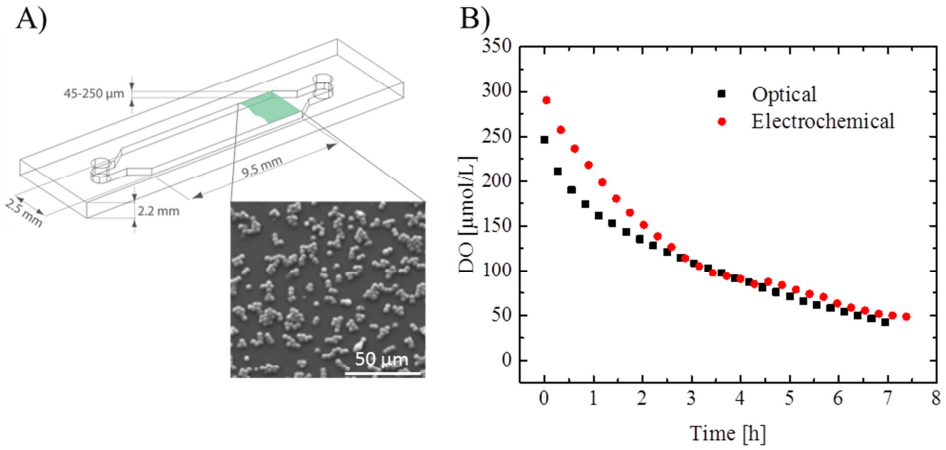

Figure 1: A) Microdevices with integrated oxygen indicator. Schematic of a microchamber with indicated dimensions (left) and a SEM image of the indicator beads attached at the surface of OSTEMER (bottom). Schematic not to scale. B) Oxygen scavenging of aerated ddH2O in an OSTEMER chamber (~15 µL) measured using optical and electrochemical sensors. The disturbance in the electrochemical sensor signal is due to physical movements of the sensor.

CONCLUSION

In the work presented here, we specifically used a thermoset plastic based on thiol-ene chemistry (OSTEMER^{TM} 322) to demonstrate that hypoxic conditions can be achieved in a microfluidic chamber. The presented method neither requires the addition of oxygen scavenging compounds to the growth medium nor bulky pressurized gas cylinders with tedious gas interconnections, thus provides an ideal platform for anaerobic cultivation of cells. In general, microfluidic chambers with low-oxygen or even anaerobic conditions have a broad applicability for a number of cell studies where control of oxygen concentration and especially oxygen-free environments are essential.

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REFERENCES

[1] Skolimowski, M., et al., *Microfluidic dissolved oxygen gradient generator biochip as a useful tool in bacterial biofilm studies*, Lab Chip, **10**(16): p. 2162-2169, 2010.

[2] Wang, L., et al., *Construction of oxygen and chemical concentration gradients in a single microfluidic device for studying tumor cell-drug interactions in a dynamic hypoxia microenvironment*, Lab Chip, **13**(4): p. 695-705, 2013.

[3] Sticker, D., et al., *Multi-layered, membrane-integrated microfluidics based on replica molding of a thiol-ene epoxy thermoset for organ-on-a-chip applications*, Lab Chip, **15**(24): p. 4542-54, 2015.

[4] Sticker, D., et al., *Microfluidic Migration and Wound Healing Assay Based on Mechanically Induced Injuries of Defined and Highly Reproducible Areas*, Anal Chem, **89**(4): p. 2326-2333, 2017.

[5] Carlborg, C. F., et al., *Beyond PDMS: off-stoichiometry thiol–ene (OSTE) based soft lithography for rapid prototyping of microfluidic devices*, Lab Chip, **11**, 3136-3147, 2011.

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