SINGLE CELL METABOLIC PROFILING USING MULTIPLEXED, PHOTO-PATTERNED FLUORESCENCE SENSOR ARRAYS

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ABSTRACT

We present a multi-step photo-polymerization process for patterning triple fluorescence sensor arrays on fused silica chips. The sensor arrays can hermetically seal with microwell arrays containing single live cells for simultaneous measurements of metabolic parameters, such as oxygen consumption rate (OCR) and extracellular acidification rate (ECAR).

KEYWORDS: single cell, multiparameter, metabolic analysis, multi-step photo-polymerization

INTRODUCTION

Single-cell metabolic analysis is of particular interest for understanding cellular heterogeneity, disease initiation, progression, and drug resistance [1-2]. Earlier we reported OCR measurements using a variety of microfluidic device configurations [3]. We further demonstrated the preparation of a dual pH and oxygen sensor with the capacity for simultaneous measurements of OCR and ECAR by patterning an array of oxygen and pH sensors either using a plasma removal method [4] or a multiple micro-pocket method [5]. However, performance of fluorescence sensors was compromised either due to the harsh plasma treatment or large microwell volume. In this paper, we report a multi-step photo-polymerization method for patterning multiplexed fluorescence sensor arrays for single cell metabolic profiling.

EXPERIMENTAL

Four-inch double-sided polished fused silica wafers (University wafer, Boston, MA) were used as substrate material. AZ4330 positive photoresists and AZ300 MIF developer were purchased from Capital scientific, CA. A mixture of 1 part ammonium hydroxide (27 wt%), 1 part hydrogen peroxide (30 wt%) and 5 parts DI water, and a mixture of 1 part hydrochloric acid (35 wt%), 1 part hydrogen peroxide (30 wt%) and 5 parts DI water were used for RCA 1 clean (base clean) and RCA 2 clean (acid clean), respectively. Photomasks were procured from Photoscience Inc. for patterning chrome L-marker. Trimethylsilylpropyl acrylate (TMSPA) and Perfluorooctyltriethoxysilane was acquired from Sigma–Aldrich. Edwards Auto 306 E-beam Evaporator (Edwards, NY) was used for chrome deposition on fused silica wafer. Automatic Dicing Saw (DAD 3220, DISCO, CA) was operated to dice four inch wafer to 13 mm square. Maskless Photolithography System (SF-100, Intelligent Micro Patterning LLC, FL) was used to UV expose the photo-polymerizable sensors at 435 nm wavelength.

RESULTS AND DISCUSSION

The schematic representation for the multi-step photo-patterning of triple fluorescence sensor arrays for single cell metabolic profiling is shown in Fig. 1. The 13 x 13 mm fused silica dies with chrome L-marker at the edge were fabricated using standard lithography process. We activated the surface by 5 min oxygen plasma treatment (Harrick PDC-32G, Harrick Plasma, NY), followed by overnight vapor salinization using over 3-acryloxypropyl trimethoxysilane. We used three AutoCAD visual masks to sequentially photo-polymerize a reference sensor (non-responsive to pH and oxygen), fluorescein-derived pH sensor and platinum prophyrin-derived oxygen sensor. Each of the visual masks has the same L-marker as that on the fused silica die, which we used for alignment during the multi-step exposure process. We photo-patterned 3 x 3 arrays of triple sensors with 300 μm pitch. Three different sensors, each with a 20 μm diameter, were confined in a 90 μm diameter circle. The fluorescence images collected using three

sets of fluorescence filters, the bright field image and the overlaid pseudo color image of polymerized sensors are shown in Figure 1. The oxygen and pH responses are shown in Figure 2. The oxygen sensor responds linearly from deoxygenated solution to oxygenated solution. We fabricated microwell arrays containing lips for confinement of single cells using an HF wet etching process [3]. The pitch of the microwell array is 300 μm while the inner diameters of lips are 110 μm which can form enclosed microchamber with the multiplexed sensor arrays for "draw-down" metabolic profiling (Figure 3a). We loaded microwell arrays with single cells (CPA, Barrett's Esophagus cell line) and incubated for 24 hours (Figure 3b) before "draw-down". One microwell containing no cell was used as control. The "draw-down" was performed by aligning sensor arrays to the microwell arrays on an inverted microscope. The fluorescence images from triple sensor arrays were automatically collected for 120 minutes at 1 minute intervals. OCR, ECAR and reference were plotted in Figure 4.

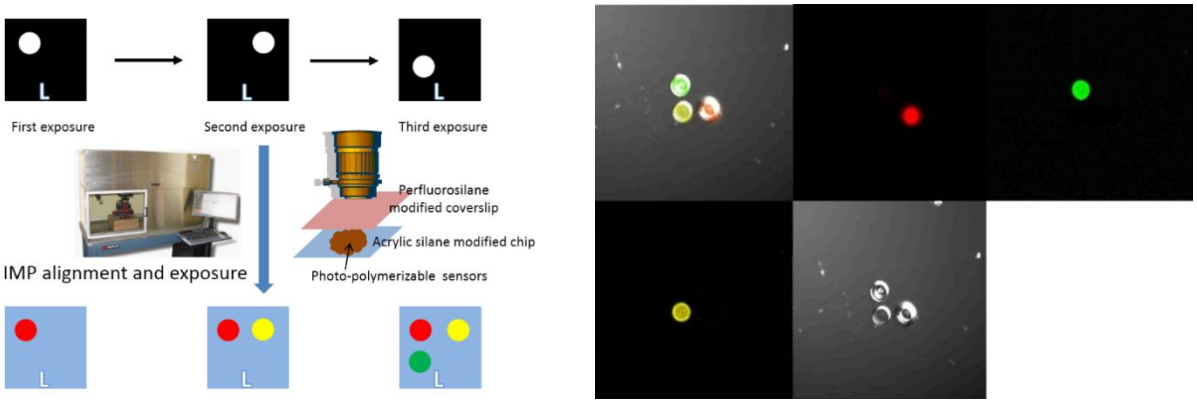

Figure 1: Schematic representation of multi-step photopatterning of triple sensor array for single cell metabolic profiling and the fluorescence images, bright field image and overlaid image of sensor array.

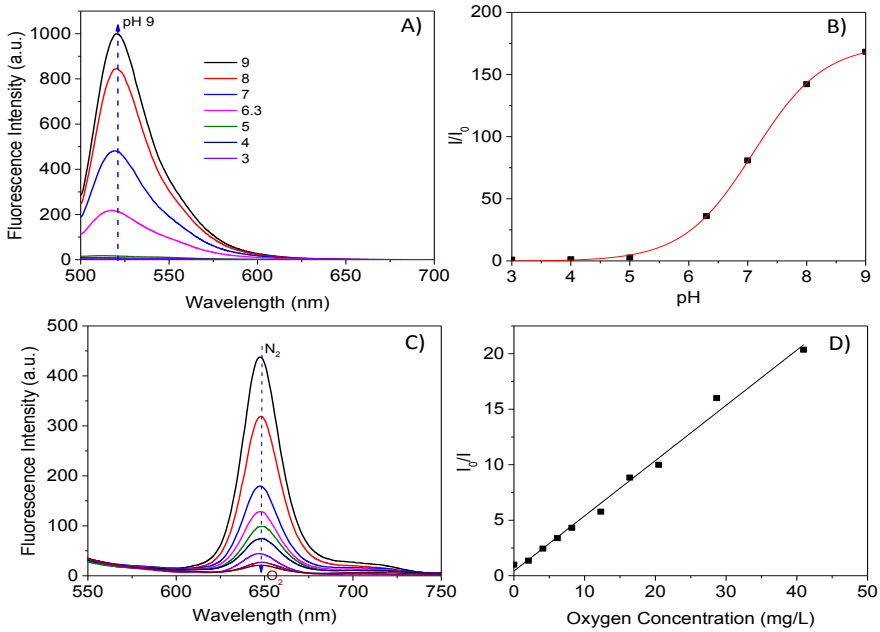

Figure 2: (A) pH sensor responses (488 nm excitation). (B) pH responses (515 nm emission). I_0 measured at pH=3. (C) Oxygen sensor responses (405 nm excitation). (D) Stern-Volmer plot of the oxygen responses (650 nm emission). I_0 measured at deoxygenated condition.

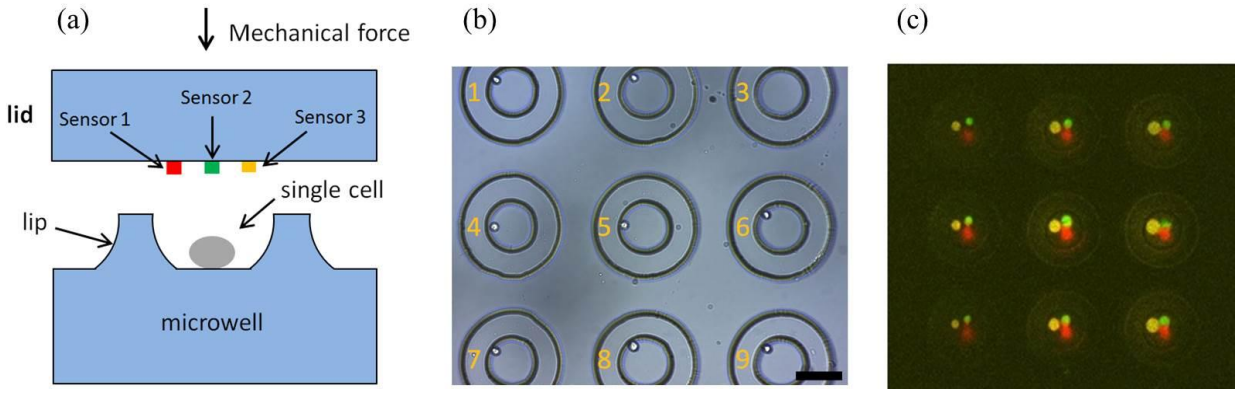

Figure 3: (a) Schematic representation of metabolic profiling "draw-down" method. (b) Microwell loaded with single cells. Scale bar: 100µm. (c) Pseudo color images of a triple sensor array sealed with cell loaded microwells.

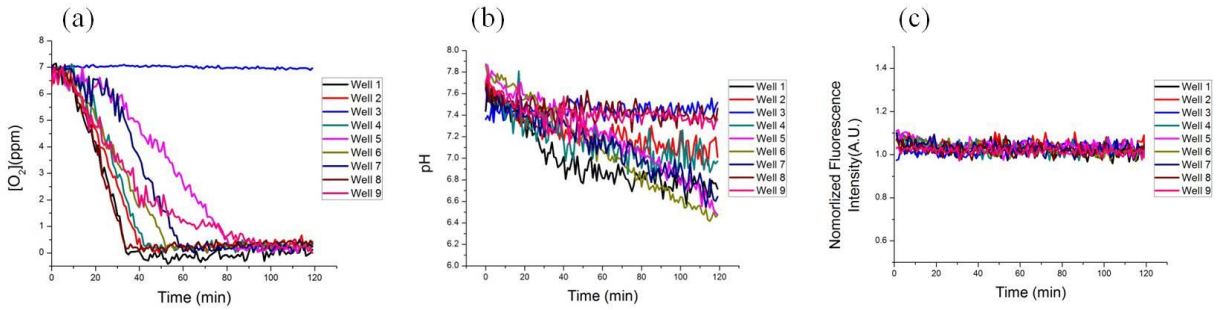

Figure 4: Single cell metabolic profiling (a) OCR; (b) ECAR and (c) reference.

CONCLUSION

We successfully presented a three-step photo-polymerization process for patterning triple sensor arrays and measured OCR and ECAR of live single cells. The demonstration of multiple spatially resolved sensors provides a foundation for high-throughput, multiparameter analysis of live cell respiration and other metabolic parameters at the single-cell, multiple-cell and tissue level.

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