

# A MICROFLUIDIC ARRAY PLATFORM FOR SIMULTANEOUS CELL CULTURE UNDER VARIOUS OXYGEN TENSIONS

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## ABSTRACT

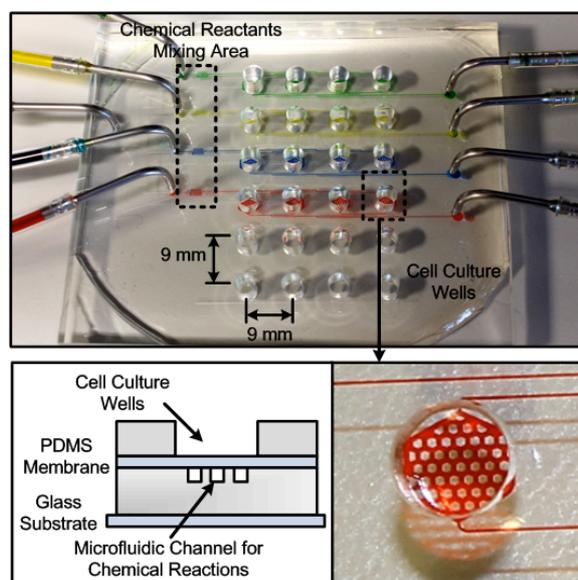
Oxygen is capable of affecting cellular response in various ways. In this research, we developed a microfluidic array platform capable of simultaneously testing multiple drug conditions, and performing cell culture under various oxygen tensions. We performed tests using a hypoxia-activated anti-cancer drug, triapazamine (TPZ), on human alveolar epithelial cell (A549) using the platform. The experimental results indicate the cell viabilities of the drug treated A549 cells decreased more than 20% under low oxygen tensions compared to normoxia. The developed platform is promising to provide a more meaningful *in vitro* cell model for pharmaceutical development with the desired high throughput capabilities.

## KEYWORDS

Microfluidics, Cell Culture, Oxygen Tension, Polydimethylsiloxane (PDMS).

## INTRODUCTION

Oxygen tension plays an important role in regulating various cellular functions in both normal physiology and disease states [1]. Therefore, conventional *in vitro* cell culture models under normoxia often possess limited predictive capabilities. A traditional method of setting an oxygen tension in a liquid medium is by saturating it with a gas mixture at the desired level of oxygen, which requires bulky gas cylinders, sophisticated control, and tedious interconnections. Moreover, only single oxygen tension can be tested at the same time. In this research, we have developed a microfluidic array platform capable of simultaneously performing cell culture under various oxygen tensions.

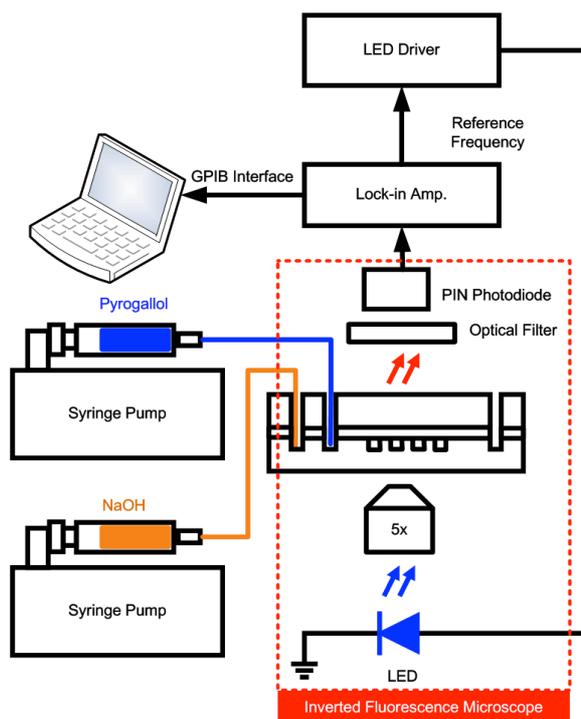


**Figure 1.** Photo of the fabricated microfluidic cell culture array platform for simultaneous cell culture under various oxygen tensions. The device is constructed by two PDMS layers separated by a 200  $\mu\text{m}$ -thick PDMS membrane, and a glass substrate. The oxygen tensions in the cell culture wells are controlled using the spatially confined chemical reactions (pyrogallol+NaOH) in the underneath microfluidic channels. The cell culture wells are arranged in the same dimensions of the conventional 96-well plate.

## EXPERIMENT

In the developed microfluidic cell culture array platform, the oxygen tensions are controlled by the spatially confined chemical reaction method that previously developed in our lab [2]. The method has been shown to be efficient and cell incubator compatible. The developed microfluidic cell culture array platform consists of a glass substrate and two PDMS layers: a top layer for cell culture and a bottom layer for oxygen tension control. The two

layers are separated by a PDMS membrane with a thickness of 200  $\mu\text{m}$  as shown in Fig. 1. The top cell culture wells (4 mm in diameter) are arranged in the same dimension as a standard 96-well plate (9 mm apart in both directions) commonly used in the biological lab. The microfluidic channels constructed in the bottom layer are exploited for the oxygen scavenging chemical reactions to control the oxygen tensions in the cell culture wells. In order to efficiently scavenge the oxygen, the chemical reactants (pyrogallol and NaOH) are introduced into the device from two separated inlets. The chemicals start to mix and react with each other when flowing through the meander-shape channels. The widths of the microfluidic channels underneath the cell culture wells are increased to enlarge the gas exchange areas for efficient oxygen tension control. The membrane sandwiched between the PDMS layers prevents the cell culture medium directly contacting the chemical reactants, while maintains excellent oxygen permeability. The oxygen tensions in the cell culture wells are calibrated by measuring the relative fluorescence lifetime of an oxygen sensitive fluorescence dye, tris(2,2'-bipyridyl) ruthenium (III) chloride [3], while flowing chemicals in the underneath channels with various concentrations using the experimental setup as shown in Fig 2.



**Figure 2.** Schematic of the experimental setup of the relative fluorescence lifetime measurement for oxygen tension estimation. The setup is based on an inverted fluorescence microscope with a LED and a PIN photodiode as a light source and a photo detector, respectively.

## RESULTS

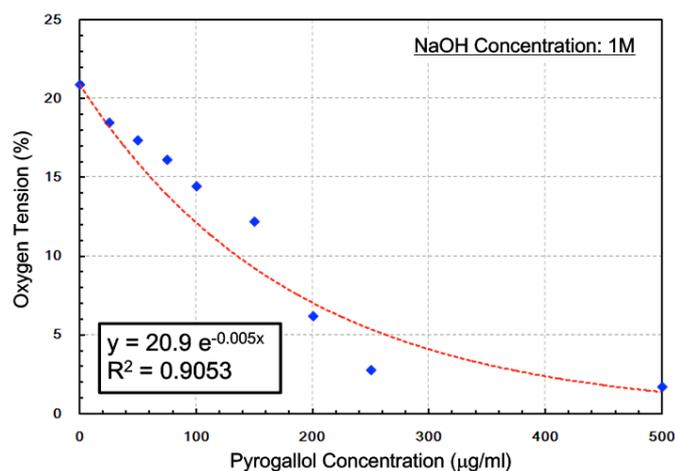
Figure 3 shows the calibration results that demonstrate the oxygen tension controllability of the platform. The platform can achieve oxygen tensions from less than 2% to normoxia in a single device without interfering gas contents in a cell incubator. Furthermore, we performed tests using a hypoxia-activated anti-cancer drug, triapazamine (TPZ), on human alveolar epithelial cell (A549) using the platform. Figure 4 shows the experimental results that indicate the cell viabilities of the drug treated A549 cells decreased more than 20% under low oxygen tensions compared to normoxia.

## CONCLUSION

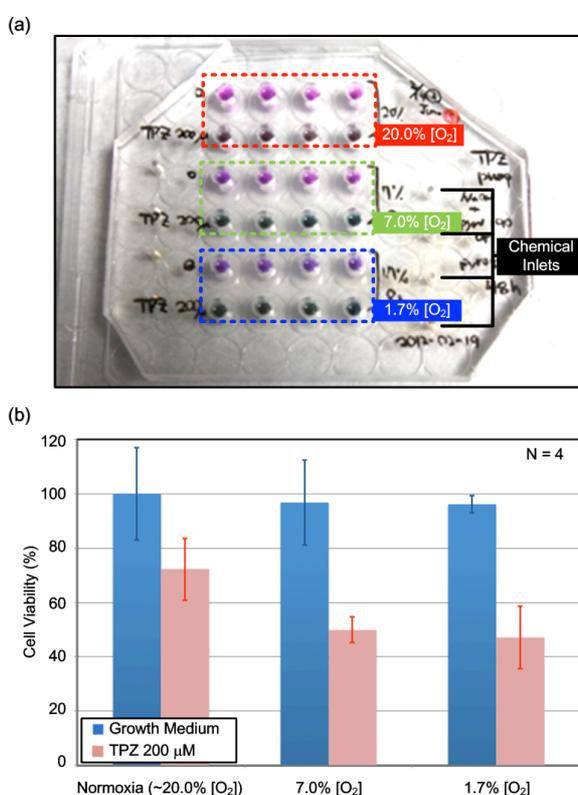
In conclusion, the platform takes advantage of microfluidic phenomena while exhibiting the combinatorial diversities achieved by microarrays. Importantly, the platform is compatible with existing cell incubators and high-throughput instruments for cost-effective setup and straightforward operation.

## ACKNOWLEDGEMENTS

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**Figure 3.** Experimentally measured oxygen tensions in the cell culture wells while introducing chemical reactants with various concentrations (NaOH: 1M, and pyrogallol 0 to 500 µg/ml) for oxygen scavenging into the microfluidic channels at the bottom layer.



**Figure 4.** (a) Photo of the device after cell culture with drug treatments and PrestoBlue cell viability assay under various oxygen tensions on a single chip. (b) The relative A549 cell viabilities (normalized to the cell cultured in the growth medium under normoxia for 12 hrs) after 12 hr hypoxia-activated anti-cancer drug (TPZ) treatments under various oxygen tensions using the developed microfluidic cell culture array platform. Data are expressed as the mean +/- SD.

## REFERENCES

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