VARIATION OF CELLS IN CONTROLLED OXYGEN TENSION BY MICRO-FLUIDIC DEVICE

Sanghun Ji, Dami An, Eunjin Lee, Kyam Lee and Jeongyun Kim

1Department of Physics, College of Natural Science, Dankook University,
2Department of Nanobiomedical science, Graduate School, Dankook University and
3Department of Horticultural Science and Biotechnology, Seoul National University, S.KOREA

ABSTRACT

In this research, we report the microfluidic based oxygen tension controllable high throughput hypoxia system for cell biology research. This device has thin PDMS membrane between gas gradient generation layer and cell culture layer to transfer the gas using higher gas permeable property of the thin PDMS membrane. This system can be used for researching a fundamental understanding the effect of hypoxia environment on to stem cells and cancer cells.

KEYWORDS: Microfluidic, Oxygen tension, Hypoxia

INTRODUCTION

In the stem cell biology research, oxygen tension is one of important parameters [1]. Cell variation dependent on oxygen concentration was researched by other groups but these spaces in which the cells are located under the different oxygen tension dose not isolated each environment [2, 3]. These hypoxia chamber does not perfect to control the oxygen tension in none isolated chamber, because these cells are exposed the same media with diffusion in single chamber. Therefore, we controlled oxygen tension in each isolated cell culture chamber using micro fluidic device through serpentine diffusion channel and observed cells variation under different gas compositions.

OXYGEN CONTROL SYSTEM AND MICROFLUIDIC CHIP

Oxygen tension control system is operated automatically by PC and LabVIEW program(Fig.1). Microfluidic device that fabricated with poly dimethylsiloxane (PDMS) by soft lithography consists of cell culture layer and gas channel layer with oxygen gradient generator and pneumatic control(Fig.2). To generate diverse oxygen tension from 0% to 20% in microfluidic, pure nitrogen gas and 5% Oxygen mixture gas in nitrogen as normoxia are delivered into microfluidic channel through mass flow controller (MFC) with LabVIEW control. To prevent dry out in cell culture chamber, every gases were passed through each humidity chamber before introducing into device. The delivered mixture gases form the oxygen tension gradient through serpentine channel called as ‘Christmas tree diffusion channel’ and passes out below the cell culture chambers. Then, the oxygen gas mixtures are diffused into the cell culture chamber through gas permeable thin PDMS membrane with 50 μm thickness(Fig.3, b). The thickness of PDMS membrane is important parameter to control the gas permeability.

Figure 1: Schematic of oxygen control system
Therefore, their thickness was exactly controlled using spin-coater. Each cell culture chamber supplied difference oxygen tension without any interference between neighbor culture chambers by microvalve after injecting cells and refreshing media every 3 hours. Color dyes were used to show the devices operation scheme(Fig.3, a). The blue and yellow dyes mean pure nitrogen gas and 5% oxygen mixture gas in nitrogen respectively. The gradient color dyes from blue to yellow filled each gas chambers. The violet color dye as cell suspension was introduced into cell culture chambers and separated by closed microvalves.

**Figure 2. Schematic of oxygen gradient generation device**

**EXPERIMENTAL RESULTS**

We confirmed the gas permeability and cell culture condition of our microfluidic device. It is very difficult to detect the oxygen tension in nano scale volume liquid, especially in microfluidic channel. So, bromothymol blue (BTB) indicator solution was used to detect carbon dioxide tension instead of oxygen. BTB indicator solution has yellow color in acid condition and blue one in base. After filling the cell culture chamber with BTB solution, nitrogen and carbon dioxide gases were introduced below cell culture chamber through serpentine gradient generator. BTB indicator solution has blue color initially in pH7 at fresh air environment, which the blue color shows inside tubing connected into device.

**Figure 3. Gradient generator performance and schematic of chamber cross section. (a) Blue dye means nitrogen and yellow dye means 5% oxygen. Formed gas gradient from 0 to 5% by oxygen gradient generator is injected each gas chamber. (b) Injected gases are diffused into cell culture chamber.**
The color of BTB indicator solution in higher carbon dioxide tension area was changed from blue to yellow as increasing carbon dioxide tension in each chambers(Figure 4).

![Image of BTB solution](image1.jpg)

*Figure 4. The color change of BTB solution depends on CO₂ tension through gas permeable membrane.*

To evaluate cell culture environment in the device, PC3-TR (a TRAIL resistant subpopulation of PC3) was incubated in culture chambers for 1 day with flowing 5% oxygen gas after treatment the surface of channel with fibronectin. Figure 5 shows PC3-TR cells well spread on the cell culture chamber. Current work focuses on optimizing the differentiation condition of dental pulp stem cells controlled by oxygen tension and cancer cell therapy under hypoxia environment.

![Image of PC3-TR cells](image2.jpg)

*Figure 5. After 3hour microscope image of injected PC3-TR to fibronectin treated cell culture chamber.*

**CONCLUSION**

We have developed a microfluidic based oxygen tension controllable high throughput hypoxia system using gas permeable property of PDMS. This system may be a promising approach for stem cell research and cancer research under hypoxia environment.

**REFERENCES**


**CONTACT**

*Jeongyun Kim, Ph.D., tel: +82-41-550-1255; jeongyunkim@dankook.ac.kr*