Micro-arrayed pH and Oxygen Sensor for Cell Chips

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INTRODUCTION

Biochip technologies such as DNA chips, protein chips and cell chips are now attracting a great deal of attention. We are developing a micro-well array chip system for parallel monitoring of single cell function. This micro-well array chip has a quarter million wells on one chip (ca. 20mm x 20mm), and diameter of each well is 10 μm and only one cell can be occupied for each well.

Monitoring of cell activity is necessary for the drug screening, toxicity assessment, and so on. Since most of cells incorporate sugars and oxygen, and produce organic acids (such as lactic acid), pH and oxygen concentration are good indicators for cell activity. In this study, we prepared optical pH and oxygen sensors in each well for monitoring of cell activity (Fig.1).

EXPERIMENTAL

Fluorescein isothiocyanate (FITC) was used as a pH sensing indicator. FITC was covalently bound to the surface of the amine group introduced slide glass. Then polydimethylsiloxane(PDMS) film which has 0.01 to 2 mm diameter holes was put onto the slide glass. Fluorescence intensity of each well was measured by using a high resolution micro-array scanner.

As an oxygen sensing indicator, Tris(1,10-phenanthroline)ruthenium(II) chloride (Ru complex) was used. Polysulfone membrane containing Ru complex was formed onto a PMMA plate. Further procedures were the same as pH sensor array.

RESULTS AND DISCUSSION

The pH responses of the micro optical pH sensor array was investigated by using a 2 mm diameter well array and phosphate buffers. As shown in Fig.2, fluorescence intensity obtained by the micro array scanner was increased by the increase of pH between 6.5 and 8.0. Good correlation was observed between pH and the total fluorescence intensity of each well image, as shown in Fig.3.

In the same way, fluorescence image was observed by using a 10 μm diameter well array. Higher fluorescence intensity was obtained with pH8 buffer compared with pH6.5 buffer.

The oxygen response of a micro optical oxygen sensor array was investigated by using an O_2-aerated water and a N_2-aerated water. Fluorescence intensity was increased in a N_2-aerated water.

Finally, cell activity was detected by using a micro optical pH sensor array and mouse lymphocytes. Cellulose acetate membrane was formed onto the FITC layer in order to escape the direct attachment of cells to the sensor layer. Fluorescence intensity was compared after 30 min incubation with live cells and dead cells (treated with ethanol). The wells with live cells showed lower fluorescence intensity (lower pH), and cell activity could be monitored with this micro optical pH sensor array.

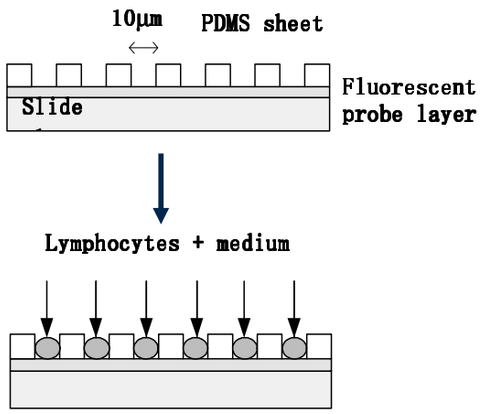

Fig.1 Micro-arrayed chemical sensor for cell chips

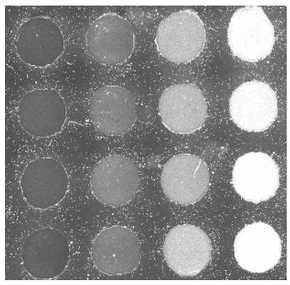

Fig.2 Fluorescent image of pH sensor array for pH6.5, 7.0, 7.5 and 8.0 phosphate buffers (From the left)

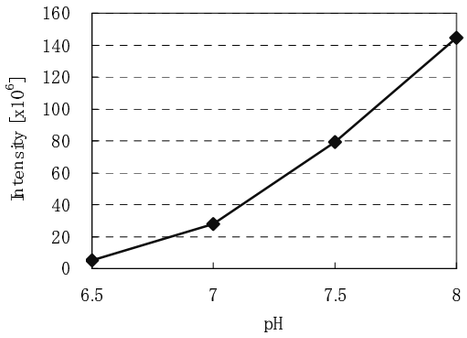

Fig.3 Calibration curve of the pH sensor array