LOCAL OXYGEN LEVEL IS DENSITY DEPENDENT IN MICROCHANNEL CULTURE

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ABSTRACT

We present a measurement method of oxygen levels around cells cultured in a microchannel. Micro-scale silicate beads immobilised with Pt(II) octaethylporphine were used to measure the oxygen levels. Measurement of oxygen level was achieved by seeding beads near cells and observating the fluorescence emitted from the beads with a standard microscope. We demonstrated that the oxygen level approached physiologic levels as cell density increased to 400 cells/mm^2.

KEYWORDS: oxygen level, cell density, micro-scale beads

INTRODUCTION

Oxygen is an important physiologic parameter and changing the oxygen level around cells affects their behavior. (e.g. proliferation[1] and hypoxia[2]). The oxygen level around cells *in vivo* is 2-5 %[3] which is much lower than the level used in standard incubators. MEF (Mouse Embryonic Fiblobrasts) cultured in 3 % oxygen grew faster than those cultured in 20 %, and showed no sign of senescence [1]. Therefore, to study cell biology using cultured cells, the oxygen level in a media should be known and controlled. Optical measurement using oxygen sensitive dyes is suitable for oxygen sensing in a microchannel culture because it does not consume oxygen in contrast to a conventional oxygen electrode. While other optical methods have been reported including the use of fluorscense lifetime imaging (FLIM) which requires more specialized imaging instruments and solution-based dyes[4], our approach is compatible with standard epifluorescent microscopy found in most labs.

Pt(II) octaethylporphine (PtOEP) is one of the oxygen sensing dyes and it was reported that silicate micro-scale beads immobilized with the PtOEP showed linier response in wide oxygen range[5]. In this study, we measured oxygen levels around cells cultured in microchannes by using PtOEP labeled beads.

EXPERIMENTAL

We used NIH-3T3 fibroblast cells in this study. Dulbecco's Modified Eagle Medium (DMEM) with 10 % fetal bovine serum (FBS) was used as a culture media. The cells were seeded with 200 and 400 cells/mm^2 in PDMS microchannels of width 1 mm, height 250 μm, and length 10 mm. These were cultured for 24 hours at 37 degree C, and 20 % oxygen. We seeded the oxygen sensing beads in the microchannel and the fluorescence was imaged at three hours post seeding the beads to ensure steady state behavior. The cells and beads were seeded by using a passive pumping method[6]. To obtain calibration curves, nitrogen gas was used to vary the oxygen level in the microchannel.

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RESULTS AND DISCUSSION

We calibrated the beads at two oxygen levels, 0% and 20 %, which is a saturated oxygen level. We seeded the beads in the microchannel filled with the culture media, and observed fluorescence emitted from the beads when oxygen levels in the microchannels were at 20 %, an atomosphric oxygen level, and at 0 %. Figure 1 shows fluorescence images of the beads in the microchannel at 20 % (A) and 0 % (B). Intensity of fluorescence emitted from the beads increases as oxygen level decreases (shown in Fig. 1). Figure 2 shows the intensity of the fluorescence at different oxygen levels. We determined a calibration curve from this result and confirmed it matched previous results[4].

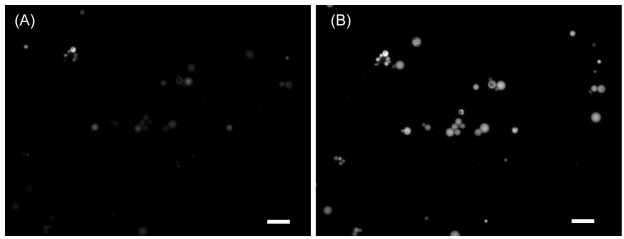

Figure 1 Fluorescence images emitted from the oxygen sensing beads in the culture microchannel, oxygen 20% (A), 0 % (B). Scale bar: 100 μm

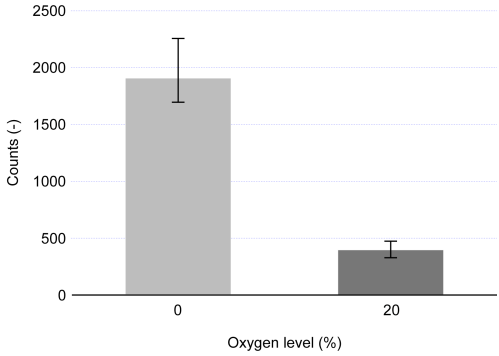

Figure 2 Intensity of fluorescence emitted from the oxygen sensing beads when oxygen level in the media was 0 % and 20 %.

Figure 3 shows bright field and fluorescence images of the oxygen sensing beads with the cells cultured in microchannels when cell density was 200 and 400 cells/mm^2. Figure 4 shows oxygen levels estimated by the intensity of fluorescence emitted from the beads. The oxygen levels at 200 and 400 cells were about 70 (7 %) and 90 μM (5%), respectively, the oxygen level around the cells decreased to almost physiological level as cell density increased to 400 cells/mm^2. These results indicate that the oxygen level around cells is relatively low even in PDMS -based microchannels.

CONCLUSIONS

We applied the oxygen sensing beads to measure the local oxygen level in the microchannel culture and found that the local oxygen level was dependant on the cell density, and it was similar to physiologic levels when cell density was high. Advantages of the bead-based imaging approach is compatibility with standard instruments and simplicity which should enable widespread use.

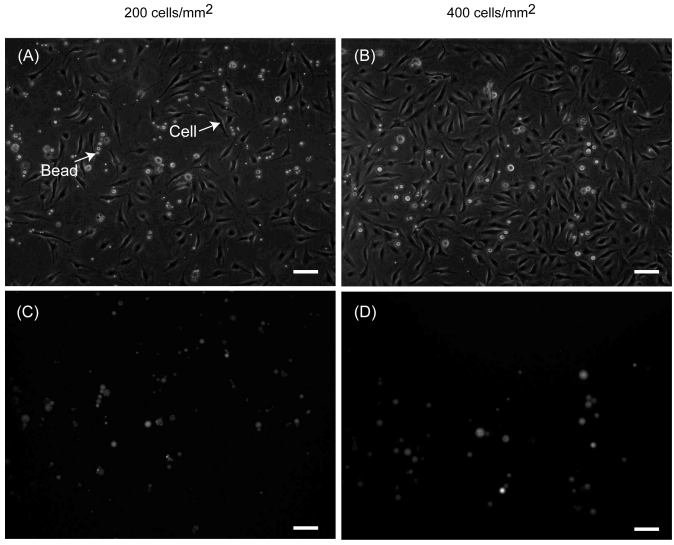

Figure 3 Bright field and fluorescence images of the oxygen sensing beads with the NIH-3T3 cells cultured in microchannels. A, C: 200 cells/mm², B, D: 400 cells/mm² The cells are fusiform and look like black. Most of beads are smaller than the cells and look like white. Scale bar: 100 μm

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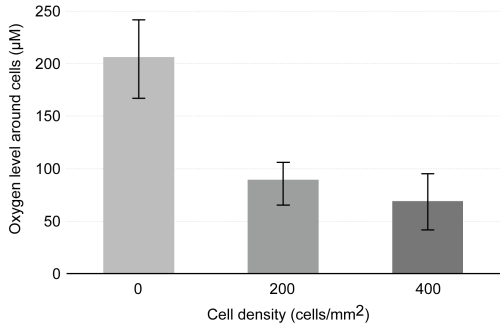

Figure 4 Oxygen level around the NIH-3T3 cells when cell density was 0, 200, 400 cells/mm².