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Oxygen Monitoring in Cell Cultures

Real-Time, Noninvasive Measurement in Multi well Plates Is Now Possible During Incubation

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Cell culture has historically been performed at near ambient atmospheric (18-21%) partial pressure of oxygen (pO_2). Special instrumentation is needed to maintain physiological levels of oxygen tension. In addition hypoxia, defined as a lower than normal pO_2 at the cell or tissue level, has long been known to be detrimental to cell metabolism and viability.

Culturing cells in ambient atmosphere (21% pO_2) insures the cells have "enough" oxygen but most cells experience an *in vivo* pO_2 between 0.7 and 7%. Recent studies have shown that cells grown at 21% pO_2 have altered phenotypes and gene expression levels as compared to cells grown at a more physiologically "normoxic" level.

Incubators and glove boxes can be set for a relevant physiologic pO_2 atmosphere, but because cellular respiration removes O_2 from the liquid media (rather than the gaseous atmosphere), the gaseous pO_2 level for the incubation is not a good estimation of the pO_2 the cells are actually experiencing. Instead, there is a need to measure the pO_2 at the level of the cellular monolayer, the pericellular pO_2, to know the precise oxygen conditions to which the cells are being exposed.

Noninvasive oxygen detection

The SensorDish® Reader is a small apparatus which can optically detect pericellular oxygen or pH levels in a 24-or 6-well plate during incubation (*Figure 1*). The plates (OxoDish® for O_2) contain an integrated sensor spot at the bottom of each transparent well. The spots are illuminated by the Reader, which interprets the resulting phase angle shift in relation to O_2 levels enabling noninvasive continuous monitoring of pericellular pO_2 levels and can be a representation of cellular oxygen consumption.

Real-time measurements of oxygen and pH in mammalian cell cultures are important to understanding the metabolic dynamics in cell cultures. The SensorDish Reader PLUS (SDR), available from Coy Laboratory Products, includes the tools for measuring temperature and pressure that are needed for the SensorDish Reader to measure pericellular pO_2.

The SDR allows the time course of cellular oxygen consumption to be followed in response to environmental conditions or toxicological insults. This type of treatment-effect monitoring could also be valuable for other applications such as tissue engineering and stem cell research.

In the present work, the SensorDish Reader was utilized to monitor pericellular pO_2 for cells plated at three different cell densities and varying gaseous oxygen tensions [19% pO_2 (*Figure 2A*) and 7% pO_2 (*Figure 2B*)].

In addition, the oxygen consumption rates in response to mitochondrial modulators (*Figure 3*) were also assessed. Mouse embryonic fibroblasts (MEFs) were used as a representative mammalian cell type, carbonyl cyanide m-chlorophenylhydrazone (CCCP) was used as a mitochondrial uncoupler, antimycin as an inhibitor of Complex III of the electron transport chain, and dimethyl sulfoxide (DMSO) as the solvent control.

Effects of cell density

The first experiment used the Sensor-Dish Reader to investigate the effects of cell density on the cellular oxygen consumption rate and subsequent pericellular pO_2 levels. The oxygen profile (*Figure 2A*) clearly shows an effect of cell density on the oxygen consumption level and suggests that diffusion of O_2 from the atmosphere into the media is a limiting step. There is a proportional deviation of pericellular pO_2 from the media-only control based on the number of cells initially plated during the course of the incubation, implying that individual cellular respiration is equivalent at all the cell densities tested.

The second experiment tested the Reader at lower physiological ambient oxygen levels on the cell density induced observations of the first experiment. The oxygen profiles confirm the effect of cell density on oxygen consumption and the resulting pericellular O_2 level. (*Figure 2B*).

In the 7% pO_2 media only treatment it takes seven hours or more to reach equilibrium with the glove-box oxygen concentration. Pre-equilibrating media for experimental use may be critical to obtain and maintain the desired liquid pO_2. The pericellular pO_2 in the highest cell densities dropped below the gaseous incubation pO_2 (7%) after only 1½ hours and began to level off at ~2% pO_2. Both of these experiments imply that pericellular pO_2 levels may be much lower than anticipated after a few hours of incubation, which could compromise experimental results.

Effects of mitochondrial modulators

The third experiment was designed to investigate the effects of specific mitochondrial modulators on cellular oxygen consumption and subsequent pO_2 levels in the media. The oxygen profile shows a pronounced effect on cellular oxygen consumption (*Figure 3*).

Measurements at the three- to four- hour time points indicate that there was little variation in oxygen consumption prior to receiving the dosed media. Within 30 minutes of dosing, observable differences emerge in the oxygen consumption curves between the treatments. DMSO, the vehicle control, caused little variation in the average pericellular pO_2 level in the MEF cells. Adding antimycin produced an observable decrease in oxygen consumption while treatment with CCCP resulted in an observable increase in cellular oxygen consumption.

Conclusions

Using the SensorDish Reader, we monitored pericellular pO_2 levels in mammalian cell cultures and showed cell density dependent oxygen consumption and variation in cellular oxygen demand in response to toxic compounds. The density dependent differences observed at 19% pO_2 were also observed at 7% pO_2, a more physiologically relevant oxygen level. At higher cell densities, in both pO_2 levels, diffusion of O_2 from the atmosphere into the media was insufficient to compensate for O_2

utilization by the cells. This is important for cellular studies as numerous examples show cellular response varies with localized O_2 levels.

In addition, cell culture media without any cells took at least 7 hours to reach equilibrium when exposed to a 7% pO_2 atmosphere. Therefore, pre-incubation of the media at the desired oxygen level is recommended and utilization of the Coy O_2 Control Glove Box enables control and maintenance of the desired oxygen level during pre-incubation, set-up, incubation, and treatment.

Also, gaseous pO_2 during incubation is a poor measure of the pericellular pO_2 to which the cells are actually exposed. The SDR allows for constant and rapid quantification of pericellular pO_2 levels which are a more realistic measure of the oxygen concentration which cells are actually experiencing. This in turn can be used as a measure of cellular oxygen demand and metabolic function.

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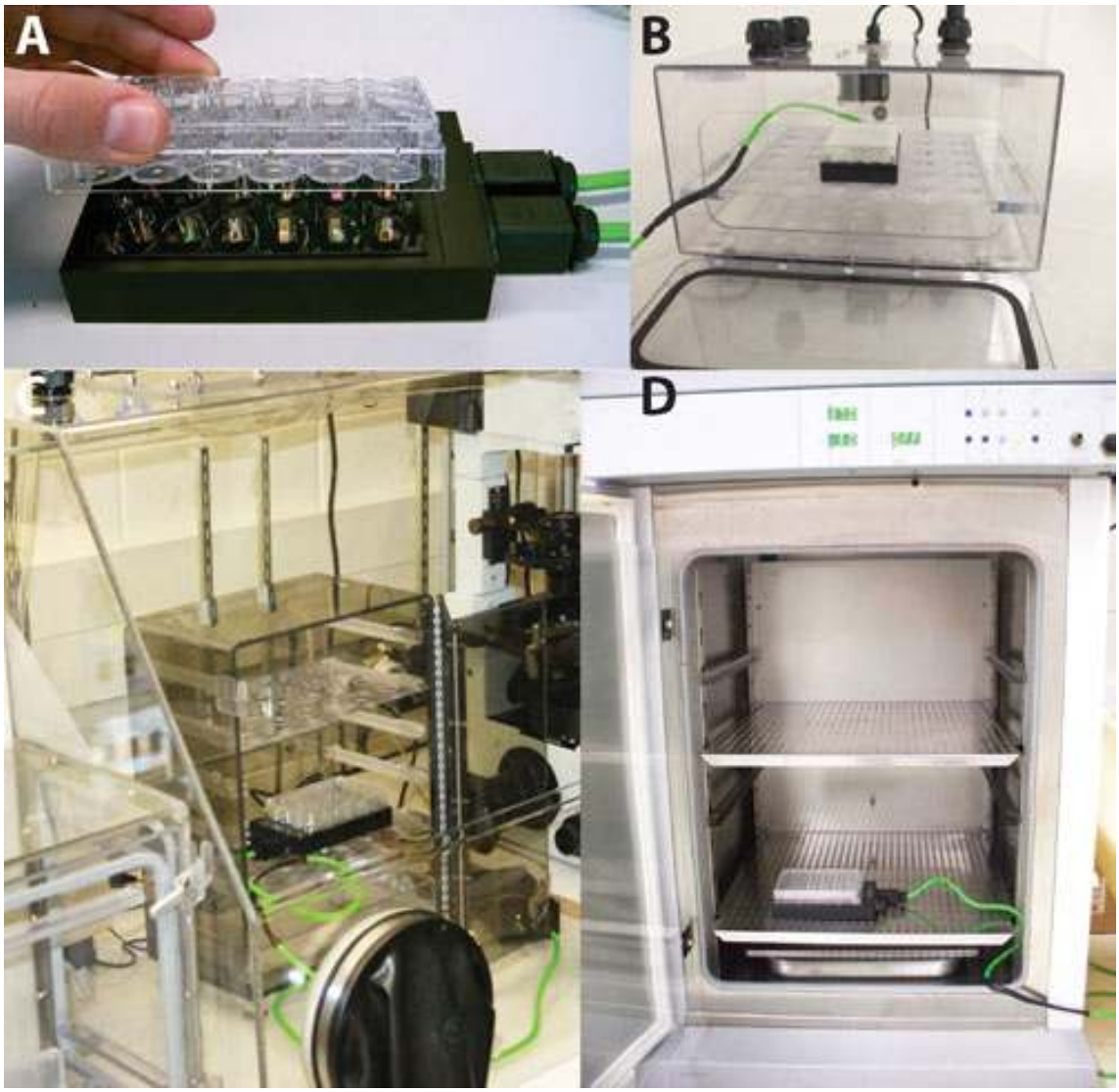

Figure 1. (A) SensorDish Reader with a 24-well multidish (OxoDish) for real-time monitoring of dissolved oxygen in a (B) cabinet, (C) glove box, and (D) incubator

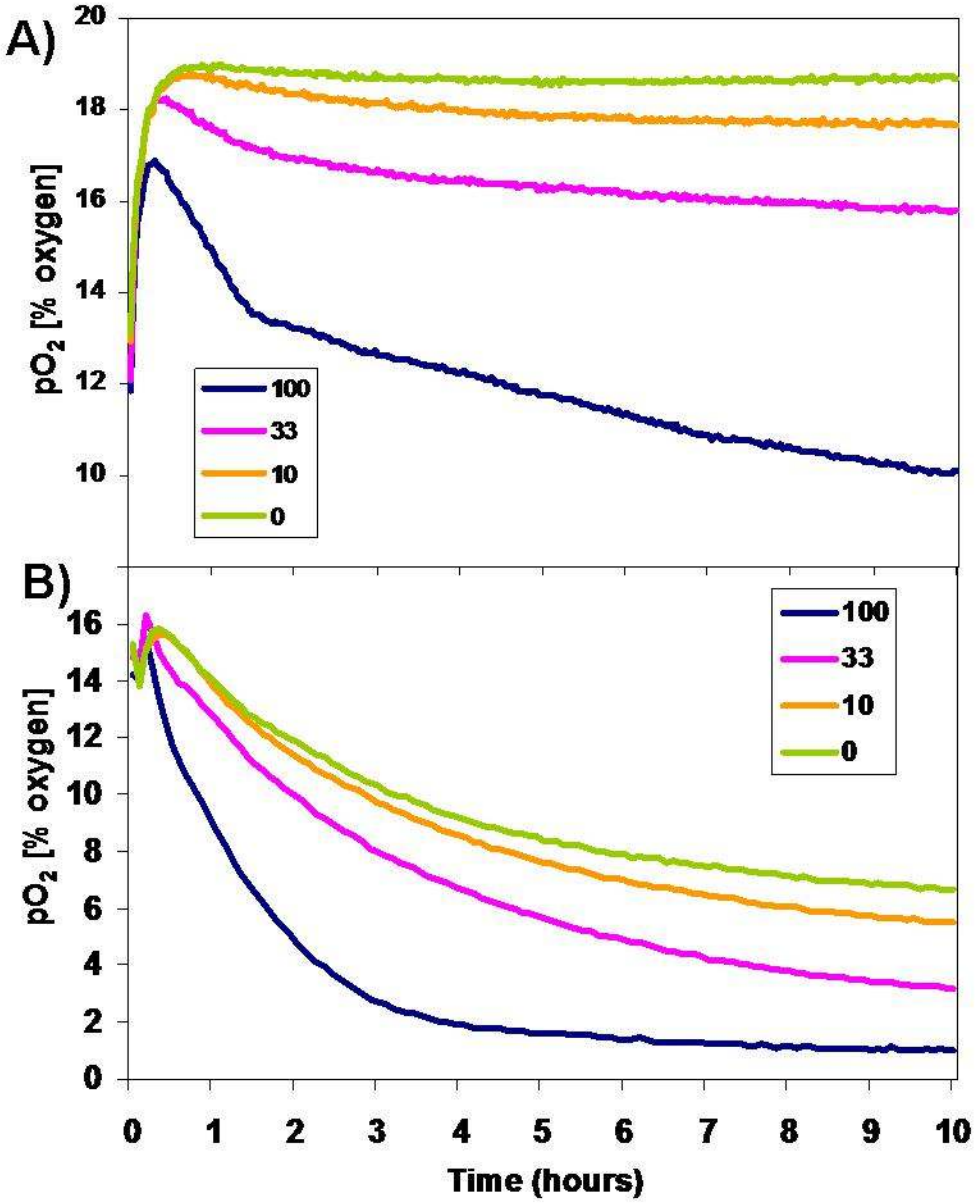

Figure 2. Average pericellular pO_2 levels of MEF cells plated at differing densities (100,000, 33,000, 10,000 and 0 cells/cm^2) and exposed to (A) ~19%, and (B) 7% ambient pO_2 as controlled by a Coy O_2 Controlled Glove Box. n=3 for each treatment. 1 ml media/well. At both 19% and 7% O_2, diffusion is unable to keep up with the O_2 loss in media due to respiration. Note the need to pre-equilibrate media to obtain the desired O_2 level in the liquid.

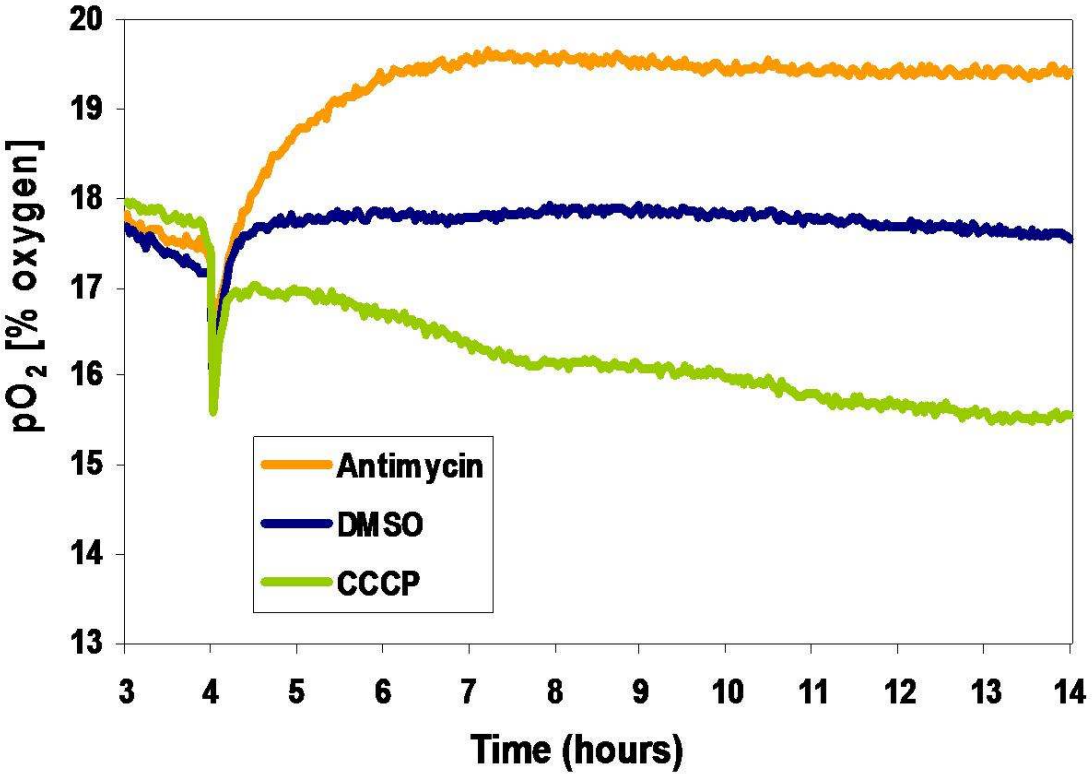

Figure 3. Average pO_2 levels in the culture media of MEF cells exposed to antimycin, DMSO, and CCCP at four hours. n=4 for each treatment. 1 ml media/well with 1 ml/well of mineral oil to limit gaseous diffusion. Together the SDR and O_2 Controlled Glove Box enable in-culture comparative studies of O_2 utilization.