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A New Non-Invasive Technique for Measuring 3D-Oxygen Gradients in Wells During Mammalian Cell Culture

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Abstract:

Oxygen tension plays an important role in overall cell function and fate, regulating gene expression, and cell differentiation. Although there is extensive literature available that supports the previous statement, little information is to be found about accurate O_2 measurements during culture. In fact, O_2 concentration at the cell layer during culture is commonly assumed to be equal to that of the incubator atmosphere. This assumption does not consider oxygen diffusion properties, cell type, cell density, media composition, time in culture nor height of the cell culture medium column. In this study, we developed a non-invasive, optical sensor foil-based technique suitable for measuring the 3D oxygen gradient that is formed during cell culture as a result of normal cell respiration. For this propose, we created a 3D printed ramp to which surface an oxygen optode sensor foil was attached. The ramps were positioned inside the culture wells of 24 well plate prior cell seeding. This set up in conjunction with the VisiSens TD camera system allows to investigate the oxygen gradient formation during culture. Cultivation was performed with three different initial cell densities of the cell line A549 that were seeded on the plate containing the ramps with the oxygen sensors. The O_2 gradient obtained after 96 h of culture showed significantly lower O_2 concentrations closer to the bottom of the well in high cell density cultures compared to that of lower cell density cultures. Furthermore, it was very interesting to observe that even with low cell density culture, oxygen concentration near the cell layer was lower than that of the incubator atmosphere. The obtained oxygen gradient after 96 h was used to calculate the oxygen consumption rate (OCR) of the A549 cells, and the obtained value of ~100 fmol/h/cell matches the OCR value already reported in the literature for this cell line. Moreover, we found our set up to be unique in its ability to measure oxygen gradient formation in several wells of a cell culture plate simultaneously and in a non-invasive manner.

Keywords: oxygen, gradient, respiration rate, non-invasive, ramp, optical-based, sensor foil