

Non-Invasive Monitoring of Oxygen Consumption by 3-Dimensional Tissue Constructs

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Abstract

1000 cells per well of either WI-38s (a lung fibroblast cell line) or primary rat hepatocytes were seeded onto two different 3-dimensional scaffolds, and cultured in 96-well BD™ Oxygen Biosensor Systems (BD OBS) for two weeks. Oxygen concentration in the wells was monitored daily using the BD™ OBS and was used to determine total oxygen consumption. Both cell lines showed clear differences in their oxygen utilization behavior between the two scaffolds, and these differences correlated with differences measured destructively by other assays. Oxygen data was supplemented by sacrificing replicates at several stages along the way to assess total cell count. For the hepatocytes, cytochrome P450 (CYP) 3A4 activity, a marker of differentiation, was also measured. Hepatocytes cultured on open-pore PLA (OPLA) scaffold showed an exponential increase in both oxygen consumption and cell number through day 10, followed by a plateauing of these measurements. Total CYP450 activity remained fairly constant throughout the study, but when normalized to the number of cells present showed a trend opposite to that of cell number. By contrast, hepatocytes seeded onto a composite collagen scaffold showed minimal increase in either oxygen consumption or cell number, but showed a significantly greater specific CYP3A4 activity, consistent with these cells retaining a more differentiated and less proliferative phenotype than the hepatocytes on the OPLA.

Introduction

As tissue engineering pushes past the boundaries of traditional 2-dimensional cell culture, new challenges emerge for assessing the state of the cells. Among the challenges associated with the culture of cells in 3D are the ability to nondestructively monitor cell viability and the phenotypic or differentiation state of the cells. The BD™ Oxygen Biosensor System (BD OBS) enables repeated and non-invasive monitoring of the rate of oxygen consumption by cells, making it ideally suited for use with engineered tissue constructs, where it is frequently difficult to assess viability visually. By pairing information about oxygen consumption with complementary assay information about cell number and cell differentiation, it is possible to obtain significant insight into the state of the tissue as a function of its culture conditions.

Materials and Methods

WI-38s and primary rat hepatocytes (Cedra, Inc.) were cultured in Hepato-STIM media (BD Biosciences, Cat. No. 355056). Experiments were commenced by adding one each of either BD™ 3D Collagen Composite Scaffold (Cat. No. 354613) or BD 3D OPLA Scaffold (Cat. No. 354614) to the wells of a 96-well BD™ Oxygen Biosensor System (Cat. No. 353830). Scaffolds (see **Figure 1**) were seeded by adding 100 µL of cells (at 10⁴ per mL), and incubating for one hour, and then adding an additional 150 µL of media. Oxygen consumption was measured in a BMG FluoSTAR plate reader at 485 nm excitation, 595 nm emission. Fluorescence values for each well were normalized to their initial value and then to the values of no-cell controls at each time point. Fluorescence values so-normalized were used to compute equilibrium oxygen concentrations, per **Equation 1** (see below). These values, in turn, were used to compute an equilibrium rate of oxygen consumption, per **Equation 2**. At several stages throughout the experiment, replicate samples were sacrificed to assay cytochrome P450 activity by monitoring the increase in fluorescence due to the cleavage of the CYP3A4 substrate 7-benzylxyquinoline (BD Biosciences Cat. No. 451725), or total cell count via the Pico Green Assay Kit (Molecular Probes).

Dissolved oxygen concentration ($[O_2]$, dO_2) at the well bottom is inversely proportional to BD OBS signal, as governed by Stern-Volmer theory (see reference 1 for a derivation)¹:

$$[O_2] = \frac{[O_2]_a}{N} \times \frac{N_{max} - N}{N_{max} - 1} \quad (1)$$

N is the normalized fluorescence intensity (as defined above) of the well of interest, N_{max} is the maximum such ratio (which occurs when dO_2 is zero, and is typically ~6), and $[O_2]_a$ is the ambient oxygen concentration (taken as 195 µM at 37°C in 5% CO₂).

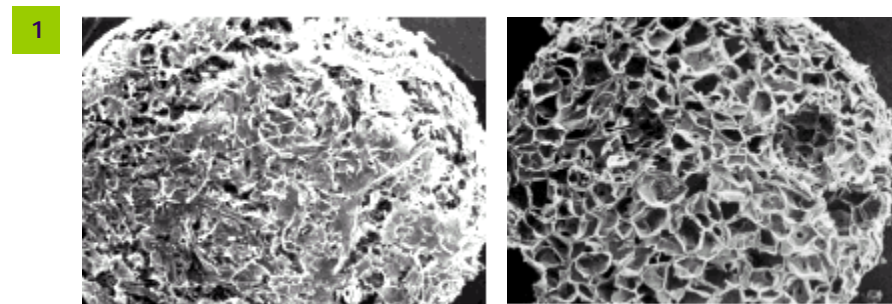
Oxygen consumption rate (OCR) was computed using a model derived by Mamchaoui & Saumon² from the steady-state solution to Fick's Law.

$$OCR = D S I \Delta p / h \quad (2)$$

D is the diffusion constant of oxygen in the media (3.3 x 10⁻⁵ cm²/s), S is the surface area (0.31 cm² for a 96-well plate) of the well, h is the diffusion path length in cm (the distance between the atmosphere and the cells - volume divided by surface area), I is a units conversion factor, and Δp is the difference in oxygen concentration (or partial pressure) between the air/media interface and the media/cell interface. **Figure 2** schematically depicts the geometry involved.

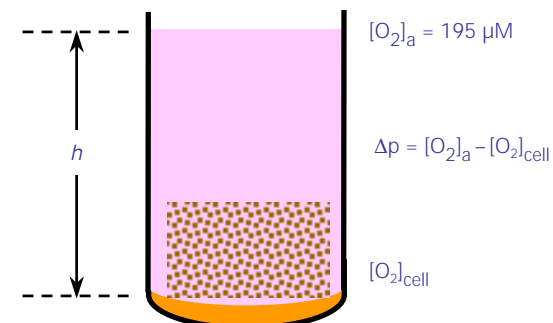
References

1. Timmins, Mark, and Guarino, Richard, "Monitoring the Oxygen Consumption Rates of Cells in Culture," *The CellLine* 12:1 (2002).
2. Mamchaoui, K., and Saumon, G., *Am. J. Physiol. Lung Cell Mol. Physiol.* 278 (2000) L858.



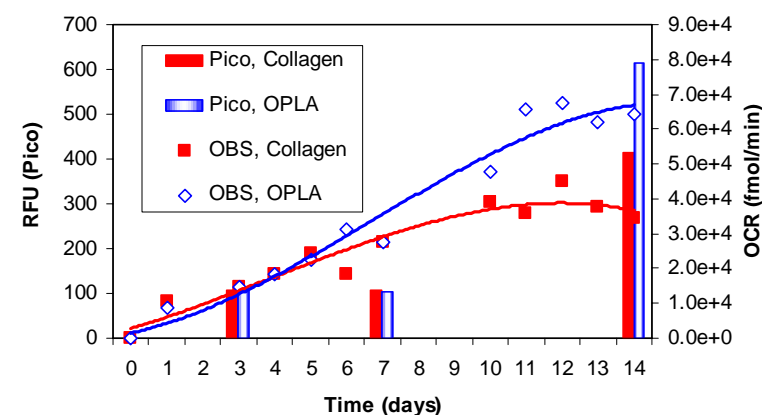
SEMs of the BD™ 3D Collagen Composite Scaffold (left) and the BD 3D OPLA scaffold (right). Both scaffolds are 5 mm in diameter and 3 mm tall.

2 Schematic (approximately to scale) of the geometry relevant to the oxygen diffusion model.



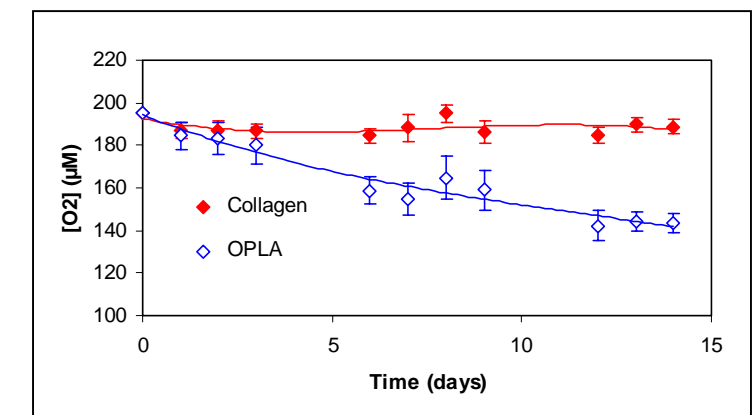
The pink area represents the 250 µL of media (depth h), the speckled rectangle represents the scaffold, and the orange represents the oxygen-sensing film which affords a reading of the oxygen concentration at the well bottom. For simplicity, this analysis of oxygen consumption explicitly ignores the fact that the well bottoms are round and that the cells on the scaffolds are not all precisely at the well bottom. For 250 µL in a 96-well plate, h is 0.80 cm.

3 WI-38's on OPLA and Composite Collagen Scaffolds Normalized BD™ OBS and Raw Pico Green Data



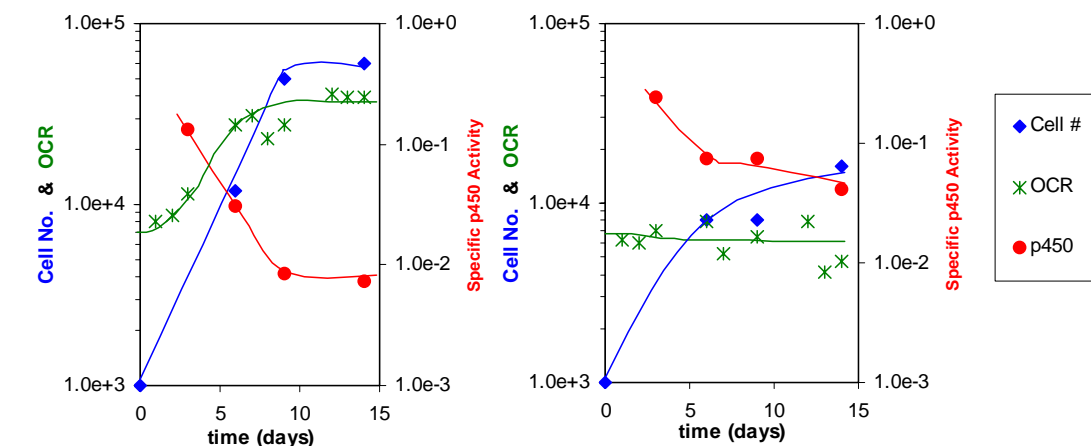
Normalized BD™ OBS signal (dots) and raw Pico Green fluorescence (bars) as a function of time for WI-38 cells on OPLA and Composite Collagen scaffolds. Both scaffolds supported growth of this fibroblastic cell line, although it can be seen that cell number and oxygen utilization were both greater on the OPLA scaffold. Oxygen consumption is clearly predictive of cell number.

4 Dissolved Oxygen Concentration over Time for Hepatocytes on Scaffolds in BD™ Oxygen Biosensor System



Oxygen concentration computed from the BD™ OBS fluorescence for rat hepatocytes on OPLA and Composite Collagen scaffolds. Proliferation on the collagen scaffold was so limited, oxygen concentration at the well bottom barely decreased from ambient. Media was changed on day 8.

5 Hepatocytes on OPLA Scaffold and Hepatocytes on Collagen Scaffold



Oxygen Consumption Rate (OCR) computed from the oxygen concentrations shown in Figure 4 (*), cell number computed from Pico Green fluorescence (◆), and specific P450 activity reported as fluorescence divided by cell number (●) as a function of time for rat hepatocytes on OPLA and Composite Collagen scaffolds. Cells proliferated exponentially on OPLA for 10 days, but to a significantly less degree on collagen, where the absolute P450 activity was ~50% greater. When normalized to cell number, however, specific P450 activity was nearly 10-fold greater.

Conclusions

- WI-38's proliferated on both scaffolds, but to a greater degree on OPLA, as evidenced by both oxygen consumption and cell number
- Hepatocytes proliferated and de-differentiated to a much greater degree on OPLA than on composite collagen scaffold, and retained much greater specific P450 activity on collagen.
- Oxygen consumption computed from nondestructive BD OBS data correlated positively with cell number ascertained by Pico Green and inversely with specific P450 activity toward 7-BQ.