Toward scalable in vitro models: a novel experimental and computational pipeline for the identification of cellular metabolic parameters

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Abstract

Oxygen utilization by cells has a crucial role in the design of advanced in vitro models. The aim of this study is to develop an experimental and computational pipeline for identifying oxygen metabolism parameters. We applied the approach to HepG2 cell monolayer cultures, demonstrating that such parameters depend on cell density.

Introduction

Size-dependent scaling of oxygen metabolic rate has been recently introduced as a reliable testbed for the translational power of in vitro cell aggregates.1 In fact, oxygen is a key player in cellular respiration and has a major influence on cell growth and fate. However, its low solubility in culture media may result in an inadequate supply, limiting the perspective of 3D cell cultures.2 Generally, oxygen consumption is described by the Michaelis-Menten’s (MM) equation:

\[ R(x,y,z,t) = -\rho_c OC R \left( \frac{C(x,y,z,t)}{k_M + C(x,y,z,t)} \right) \]  

where \( C \) [mol m\(^{-3}\)] is the oxygen concentration in the culture medium, \( \rho_c \) [cell m\(^{-3}\)] is the cell density, OCR [mol cell\(^{-1}\) s\(^{-1}\)] is the average oxygen consumption rate, and \( k_M \) [mol m\(^{-3}\)] is the MM constant. OCR and \( k_M \) are kinetic parameters assuming values specific for the cell type and have been usually intended as constants. Although, Magliaro et al. have recently shown their dependence on cell density in 3D in vitro constructs, estimating consumption parameters by optimizing the MM model fitting on experimental measurements of surface oxygen concentrations at the bottom of the constructs. To determine if the same behaviour is also manifested in monolayers, we developed an experimental and computational pipeline for identifying OCR and \( k_M \) values in 2D cultures varying cell density, as summarized in Figure 1.

Materials and Methods

Experimental measurements. Experiments were performed using an oxygen sensor spot (OXSP5, Pyroscience GmbH) attached to the bottom of a well of a 96-well plate, placed in a temperature-controlled chamber. Before HepG2 cell seeding (Table 1), the sensors were coated with gelatin to promote cell adhesion. At the beginning of each experiment, a two-point calibration was set for establishing the level of 100% oxygen saturation and the totally hypoxic condition. Furthermore, the initial condition was set at 100% oxygen saturation by dispensing fresh culture medium to the monolayer, while the upper boundary was rendered oxygen impermeable by combining the use of a layer of mineral oil and an oxygen consuming chemical filter (ThermoFisher Scientific). In this configuration, we acquired the average oxygen concentration at the cell surface until steady state was reached, repeating the experiment at least in triplicate for each cell density.

Parameter identification. The in vitro experiments were “replicated” in silico, simulating the dynamics of the system in Matlab (R2020b, The MathWorks, Inc.). Given the cylindrical geometry of the system and assuming that cells are homogeneously distributed on the bottom of the well, oxygen dynamics can be assumed as dependent on the axial direction only. The system is physically governed by oxygen transport and consumption described by the second Fick’s law (Eq. 2):

\[ \frac{\partial C(x,t)}{\partial t} = D \frac{\partial^2 C(x,t)}{\partial x^2} \]  

and by the following initial (Eq. 3) and boundary (Eq. 4, 5) conditions:

\[ C(x,0) = C_0 \]  

\[ J_c(H,t) = 0 \]  

\[ J_c(0,t) = -\rho_c OC R \left( \frac{C(0,t)}{k_M + C(0,t)} \right) \]  

where \( H \) [m] is the culture medium height, \( C_0 \) [mol m\(^{-3}\)] is the oxygen concentration in saturated culture medium and \( J_c \) [mol m\(^{-2}\) s\(^{-1}\)] is the oxygen flux modelling the cell layer consumption. OCR values were identified for each \( \rho_c \) by iteratively minimizing the mean square error between \( C(0,t) \) predicted in silico and the corresponding experimental concentration profile (Figure 1). Statistical analyses were performed using the non-parametric Kruskal-Wallis test and non-linear correlation with respect to cell density (GraphPad Prism 7).

Results

The Kruskal-Wallis test highlighted a statistically significant difference between OCRs at different \( \rho_c \) (\( p < 0.0001 \)). Median values are reported in Table 1.

| \( \rho_c \) [cell m\(^{-3}\)] | Median OCR [mol cell\(^{-1}\) s\(^{-1}\)] |
|-------------------------------|-------------------------------------|
| 1.0×10\(^3\)                  | 0.6409                              |
| 2.0×10\(^3\)                  | 0.6109                              |
| 3.0×10\(^3\)                  | 0.5809                              |
| 4.0×10\(^3\)                  | 0.5509                              |

Discussion and Conclusions

This study defines an experimental and
computational pipeline allowing the estimation of metabolic parameters and cell density in monolayer cultures. In particular, the approach has been applied to HepG2 cells. The results show that OCR depends on cell density, suggesting cooperative metabolic behaviour when oxygen availability is limited. The same analysis is ongoing for kM.

References
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