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

Piera Mancini,[1] Ermes Botte,[1,2] Chiara Magliaro,[1] Arti Ahluwalia[1,2]

[1]Research Center "E. Piaggio", University of Pisa, Pisa; [2]Department of Information Engineering, University of Pisa, Pisa, Italy

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:

$$R(x,y,z,t) = -\rho_c OCR \frac{C(x,y,z,t)}{k_M + C(x,y,z,t)} \quad (1)$$

where C [mol m^{-3}] is the oxygen concentration, ρ_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,[3] 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 carried out 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):

$$\frac{\partial C(z,t)}{\partial t} = D \frac{\partial^2 C(z,t)}{\partial z^2} \quad (2)$$

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

$$C(z,0) = C_0 \quad (3)$$

$$J_c(H,t) = 0 \quad (4)$$

$$J_c(0,t) = -\rho_c OCR \frac{C(0,t)}{k_M + C(0,t)} \quad (5)$$

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 ρ_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 (Graph Pad Prism 7).

Results

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

Also, a significant correlation ($R^2 = 0.6409$) between OCR and ρ_c emerges through the non-linear fitting, as shown in Figure 2.

Discussion and Conclusions

This study defines an experimental and

Correspondence: Piera Mancini, Research Center "E. Piaggio", University of Pisa, Pisa, Italy.
E-mail: p.mancini1@studenti.unipi.it

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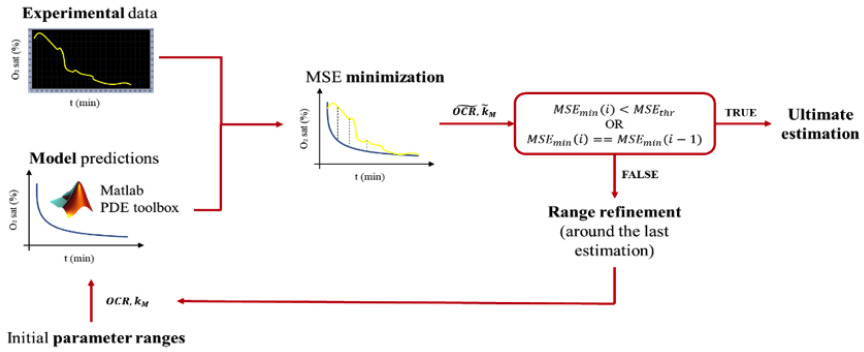

Figure 1. Block diagram illustrating the experimental and computational pipeline developed for identifying kinetic parameters characterizing oxygen metabolism in cell monolayers.

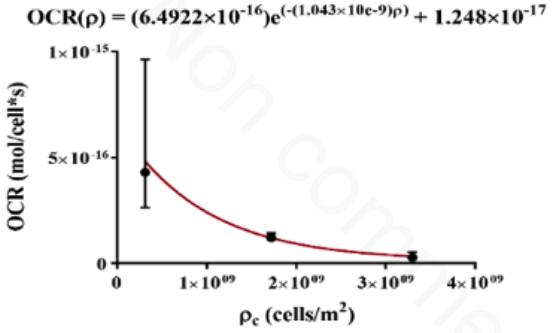

Figure 2. The non-linear dependence of OCR on ρc and its analytical formulation as a single-phase decay function. proteome

Table 1. OCR identified for each ρc. The number of experimental measurements N is also reported.

ρ_c [cell m^{-2}]	OCR [mol cell^{-1} s^{-1}]	N
3.10×10^8	4.30×10^{-16}	5
1.72×10^9	1.20×10^{-16}	6
3.30×10^9	2.79×10^{-17}	5

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 k_M.

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