A systematically reduced model for organoid expansion

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1. Introduction

Organoids are:

- three-dimensional multi–cellular structures;
- grown *in vitro* to **recapitulate** *in vivo* micro–anatomy;
- **derived from patient tissue** and retain many of their features (e.g. structure, pathology, heterogeneous cellular composition [1]);
- a **more realistic model** of *in vivo* cells than flat 2D cell line cultures;
- suitable for use in large–throughput drug screens;
- labour–intensive and time–consuming to grow.

Cellesce utilise bioreactor technology to grow organoids at scale. Key priorities are ensuring **reproducibility** of organoid output and **uniformity** of organoid size, through bioreactor design and identifying suitable operating conditions.

Achieving these goals is time–consuming via experimental work alone. Through the development of a mechanistic mathematical model, we are able to provide quantitative predictions of fluid flow and metabolite concentrations throughout the bioreactor.

Key Question: *How do the operating conditions affect the nutrient delivery to and waste removal from the organoids?*

The key experimental control is the **flow rate**. We consider the effect of the flow velocity on the metabolite concentrations within the bioreactor and the extent to which the flow facilitates nutrient delivery and waste removal.

Organoids are cultured within the 'CXP1' bioreactor (see Figure 1) using the following protocol:

- Organoids are seeded as single cells in a thin layer of hydrogel, which acts as a porous scaffold;
- Nutrient–rich culture media is pumped across the top of the hydrogel;
- The organoids are grown for approximately 7 days, until they are around $40-80\mu$m in diameter and contain about 50 cells;
- Organoids are extracted from the hydrogel and tested for quantity, size, and viability.

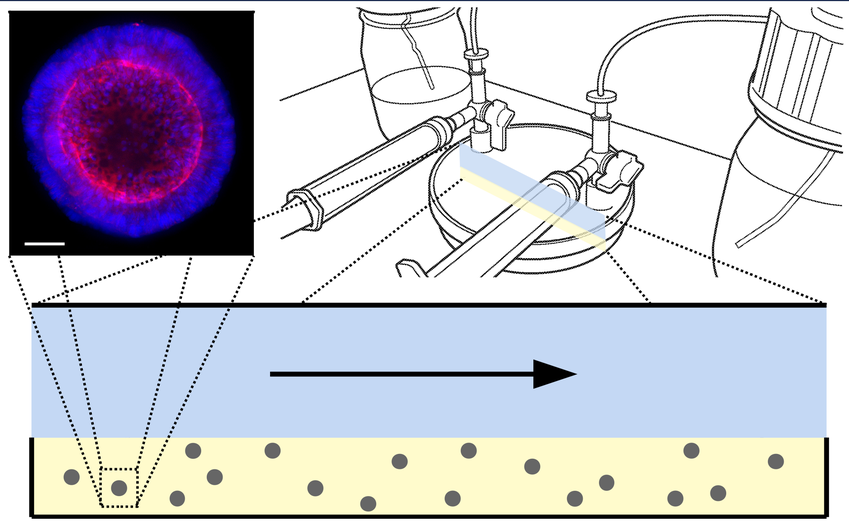

Figure 1 – **(Top left)** Example of colorectal organoid[4], stained for nuclear (blue) and cytoskeletal (red) markers for imaging. Scale 50μm. **(Top right)** Schematic of 'CXP1' bioreactor [2]. **(Bottom)** Two–dimensional reduction of bioreactor, with arrow showing direction of media flow. Blue is media, yellow is hydrogel, grey is organoid biomass.

2. Model development

We mathematically model the glucose and lactate concentrations in two distinct regions, representing the media and hydrogel layers [3]. We consider the following governing equations for glucose, c_i, and lactate, w_i, in the hydrogel, $i = H$, and in the media, $i = M$, layers:

$$\frac{\partial c_H}{\partial t} = \underbrace{D_{CH}\nabla^2 c_H}_{\text{diffusion of glucose}} - \underbrace{r(c_H)}_{\text{glucose consumption per cell}} \underbrace{n(t)}_{\text{cell density}}, \quad (1)$$

$$\frac{\partial w_H}{\partial t} = \underbrace{D_{WH}\nabla^2 w_H}_{\text{diffusion of lactate}} + \underbrace{s(c_H)}_{\text{lactate production per cell}} \underbrace{n(t)}_{\text{cell density}}, \quad (2)$$

$$\frac{\partial c_M}{\partial t} + \underbrace{u(z)\frac{\partial c_M}{\partial x}}_{\text{advection of glucose by media flow}} = \underbrace{D_{CM}\nabla^2 c_M}_{\text{diffusion of glucose}}, \quad (3)$$

$$\frac{\partial c_M}{\partial t} + \underbrace{u(z)\frac{\partial w_M}{\partial x}}_{\text{advection of lactate by media flow}} = \underbrace{D_{WM}\nabla^2 w_M}_{\text{diffusion of lactate}}, \quad (4)$$

$$n(t) = N_0 e^{pt}, \quad r = \nu c_H, \quad s = 2\nu c_H. \quad (5)$$

Slow viscous flow implies half–Poiseuille flow profile:

$$u(z) = [u]\frac{(z-h_H)^2}{(h_M-h_H)^2}, \quad (6)$$

where $[u]$ is the peak flow velocity.

Definition	
D_{Ci}	glucose diffusivity
D_{Wi}	lactate diffusivity
$c_{-\infty}$	inlet glucose concentration
N_0	cell seeding density
ν	glucose consumption rate

Hydrogel: $(x, z) \in [0,\, L] \times [0,\, h_H]$.
Media: $(x, z) \in [0,\, L] \times [h_H,\, h_M]$.
The aspect ratio of the domain is denoted by $\epsilon = h_M/L \ll 1$.

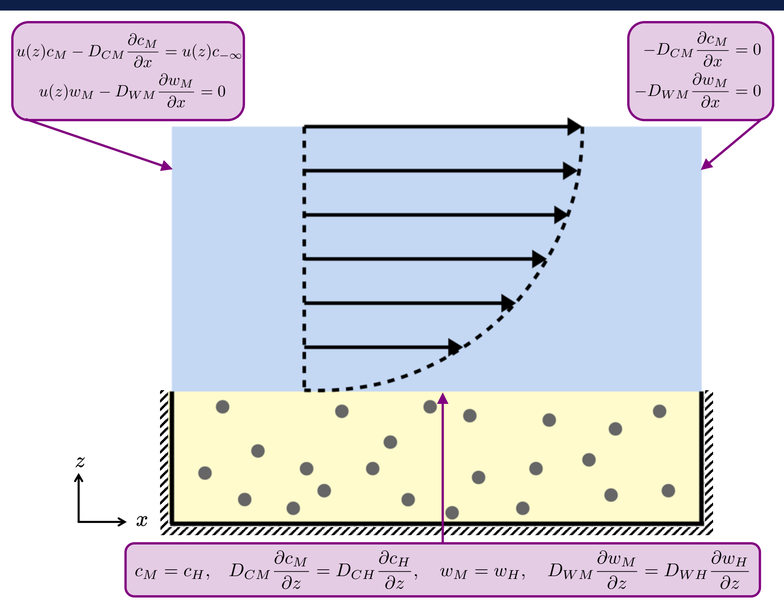

Figure 2 – Boundary conditions for the media (blue) and hydrogel (yellow) for Eqs. (1)–(4). At the media–hydrogel interface, we impose continuity of concentration and flux. At the impermeable hashed boundaries, we impose no flux.

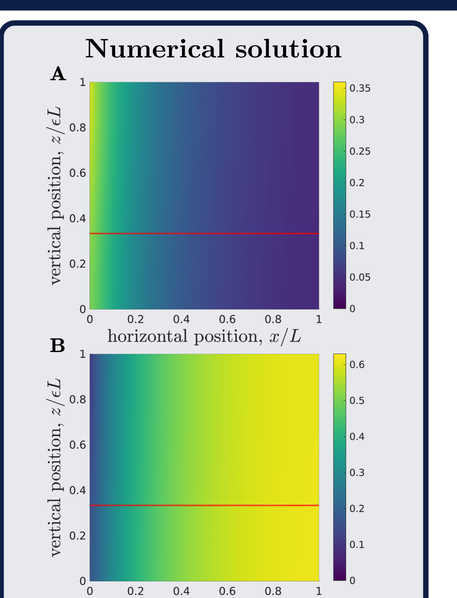

Figure 3 – Glucose (**A**) and lactate (**B**) concentration at 7 days. Parameters: Eq. (16).

3. Model reduction

We derive reduced models of the full system, Eqs. (1)–(4), for metabolite concentrations $c(t, x) = c_M = c_H$ and $w(t, x) = w_M = w_H$ by considering the system in the limit $\epsilon \to 0$ and obtaining the leading–order behaviour of the system by averaging in z, where $\bar{u}$ is the depth–averaged flow velocity and we define the parameters $\theta = h_H/(h_M - h_H)$, and $D_C = (D_{CM} + \theta D_{CH})$ and $D_W = (D_{WM} + \theta D_{WH})$:

Longwave approximation: $\frac{[u]}{L} \sim \nu N_0 \sim \frac{D_{ij}}{L^2}$

$$(1+\theta)\frac{\partial c}{\partial t} + \bar{u}\frac{\partial c}{\partial x} = D_C\frac{\partial^2 c}{\partial x^2} - \theta\nu N_0 c e^{pt}, \quad (7)$$

$$(1+\theta)\frac{\partial w}{\partial t} + \bar{u}\frac{\partial w}{\partial x} = D_W\frac{\partial^2 w}{\partial x^2} + 2\theta\nu N_0 c e^{pt}, \quad (8)$$

with boundary and initial conditions:

$$\bar{u}c - D_C\frac{\partial c}{\partial x} = \bar{u}, \ \bar{u}w - D_W\frac{\partial w}{\partial x} = 0 \ \text{ at } x = 0, \quad (9)$$

$$\frac{\partial c}{\partial x} = 0, \quad \frac{\partial w}{\partial x} = 0 \text{ at } x = L, \quad (10)$$

$$c = \frac{c_{-\infty}}{1+\theta}, \quad w = 0 \text{ at } t = 0. \quad (11)$$

Sublimit model: $\frac{[u]}{L} \sim \nu N_0 \gg \frac{D_{ij}}{L^2}$

$$(1+\theta)\frac{\partial c}{\partial t} + \bar{u}\frac{\partial c}{\partial x} = -\theta\nu N_0 c e^{pt}, \quad (12)$$

$$(1+\theta)\frac{\partial w}{\partial t} + \bar{u}\frac{\partial w}{\partial x} = 2\theta\nu N_0 c e^{pt}, \quad (13)$$

with boundary and initial conditions:

$$c = c_{-\infty}, \ w = 0 \ \text{ at } x = 0, \quad (14)$$

$$c = \frac{c_{-\infty}}{1+\theta}, \quad w = 0 \text{ at } t = 0. \quad (15)$$

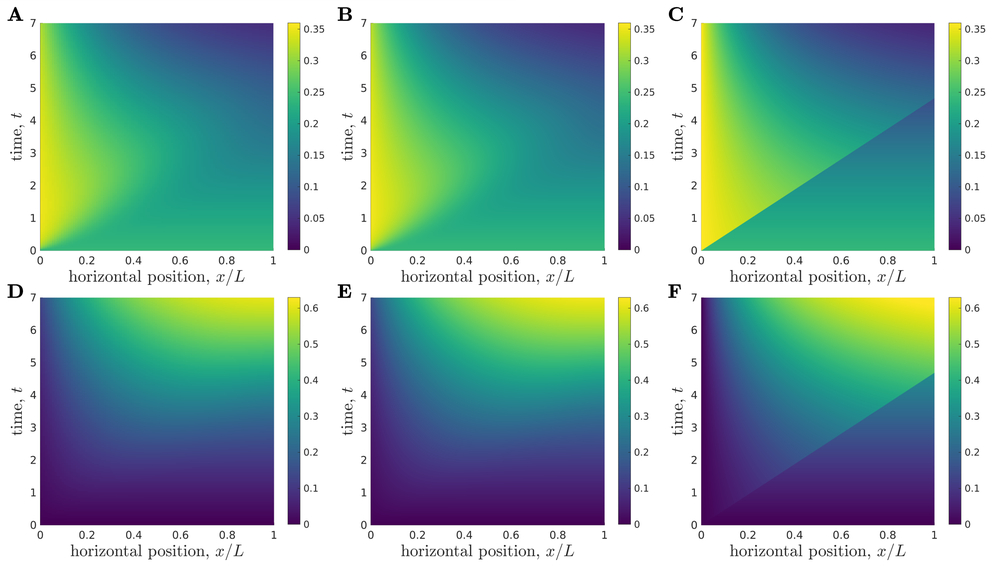

Figure 4 – Results showing how glucose (**A, B, C**) and lactate (**D, E, F**) concentrations change over time during a typical experiment. (**A, D**) Results from z-averaged full model; (**B, E**) longwave approximation; (**C, F**) sublimit of longwave approximation. Parameter values given in Eq. (16).

$$\begin{aligned} &c_{-\infty} = 0.36\text{mol}\,\text{m}^{-2}, && p = 3.9\times10^{-6}\text{s}^{-1}, && [u] = 10^{-6}\text{m}\,\text{s}^{-1}, && N_0 = 2.7-4\times10^{10}\text{cell}\,\text{m}^{-2}, \\ &L = 9\times10^{-2}\text{m}, && h_H = 1\times10^{-3}\text{m}, && h_M = 3\times10^{-3}\text{m}, && \nu = 9.4\times10^{-17}\text{m}^2\,\text{cell}^{-1}\,\text{s}^{-1}, \\ &D_{CH} = D_{CM} = 6\times10^{-10}\text{m}^2\,\text{s}^{-1}, && D_{WH} = 1.2\times10^{-9}\text{m}^2\,\text{s}^{-1}, && D_{WM} = 1.4\times10^{-9}\text{m}^2\,\text{s}^{-1}. \end{aligned} \quad (16)$$

4. Effect of flow rate for a specified organoid line

We characterise a cell line via the cell proliferation and glucose uptake rates. We consider the following metrics to optimise the CXP1 operating parameters, e.g. **peak media velocity**, for a specified cell line.

Minimise resource wastage

Maximise **glucose conversion**, the ratio of glucose consumed to glucose supplied:

$$Q(t) = \frac{\theta\nu N_0 \int_0^t \int_0^L c e^{pt}\ \mathrm{d}x\mathrm{d}t}{(h_M - h_H)c_{-\infty}\int_0^t \bar{u}\ \mathrm{d}t}$$

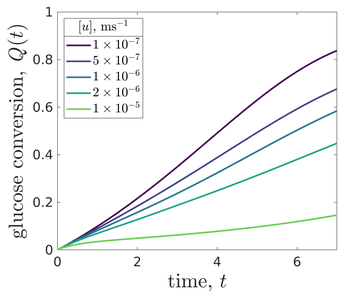

Figure 5 – Glucose conversion evolving in time for five different flow rates. Parameters: Eq. (16).

Cells are negatively affected by high lactate concentrations

Minimise the **maximum lactate concentration** in the bioreactor:

$$w_{\max}(t) = \max_x\left(w(t,x)\right),$$

with maximum tolerated lactate concentration w_{tol}.

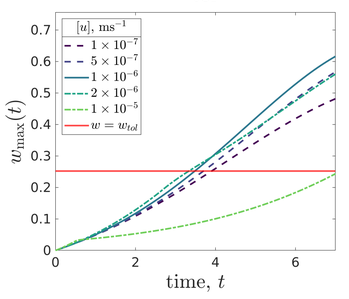

Figure 6 – Maximum lactate concentration evolving in time for five different flow rates. Parameters: Eq. (16).

Minimise the **fraction of the domain with lactate concentrations above the maximum tolerated level**:

$$P_U(t) = \frac{1}{L}\int_0^L H(w - w_{\text{tol}})\mathrm{d}x$$

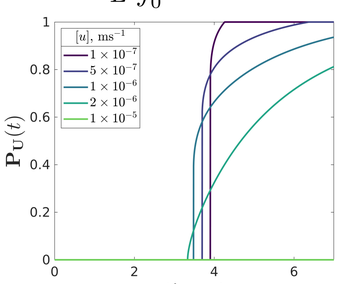

Figure 7 – Dynamic fraction of uninhabitable domain for five different flow rates. Parameters: Eq. (16).

The **turn–off time** is the time at which intolerable lactate levels are first experienced, $t_{\text{off}} = \min(t)$ for $t \in \{t : w \geq w_{\text{tol}}\}$.

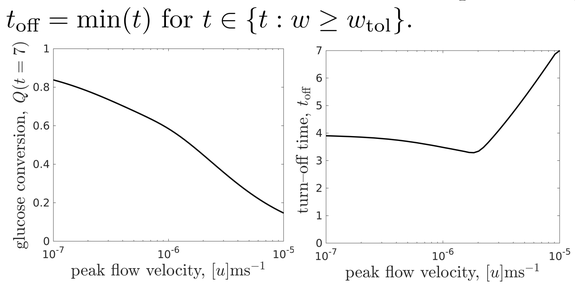

Figure 8 – Glucose conversion at day 7 (**left**) and turn–off time (**right**) against peak flow velocity. Parameters given in Eq. (16).

The "optimal" operating parameters will determine metabolite concentrations which:

(1) yield a specified value for glucose conversion;
(2) predict a turn–off time which is greater than the run time of the experiment;
(3) maintain a glucose consumption rate per cell which is sufficient for cellular proliferation.

The specific values and relative importance of each requirement is dependent on the user.
Our model reduction facilitates rapid calculation of each metric.

5. Key points

- Presented an unsteady, two–dimensional model for metabolite transport within the CXP1 bioreactor.
- Derived two reduced models by exploiting extreme spatial and temporal parameter ratios in system.
- Provided framework for improving organoid viability through varying bioreactor operating conditions.

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Cellesce® organoids expanded

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References

[1] Drost, J. & Clevers, H. (2018). Organoids in cancer research. *Nature Reviews Cancer* 18, 407-418
[2] Ellis, M. J., Chaudhuri, J., & Dale, T. C. (2019) Methods for culturing organoids, U.S. Patent Application No. 16/316,573
[3] Ellis, M. A. et al. (2021). *A systematically reduced mathematical model for organoid expansion.* Manuscript submitted for publication.