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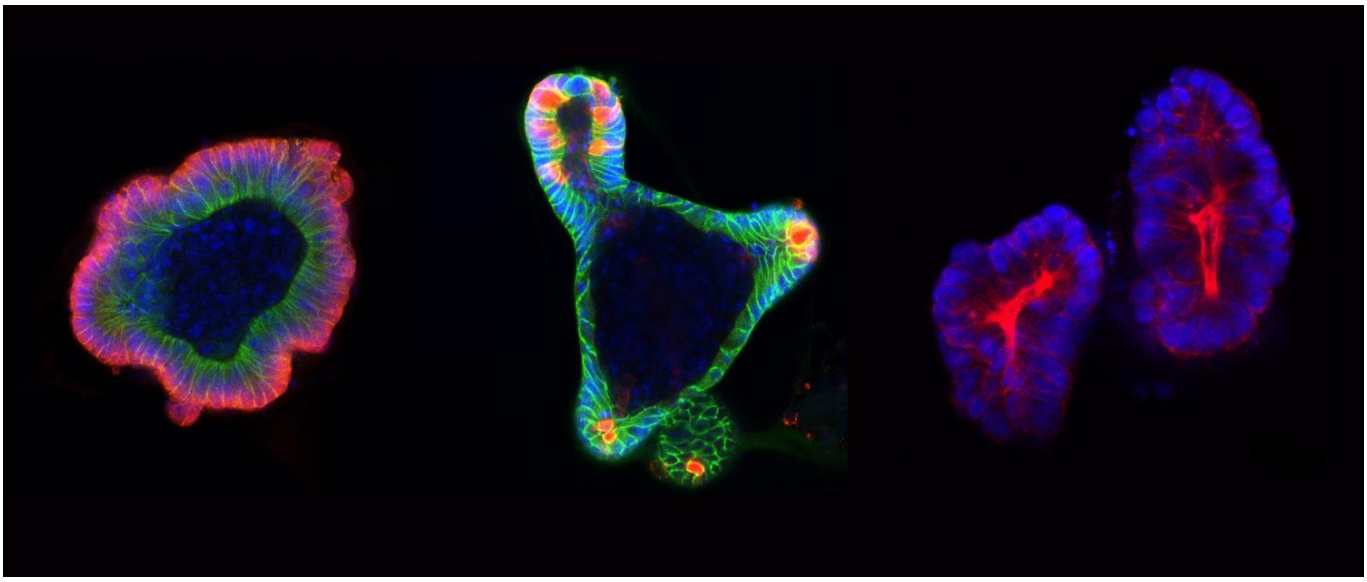

Nutrient distribution in an organoid bioreactor

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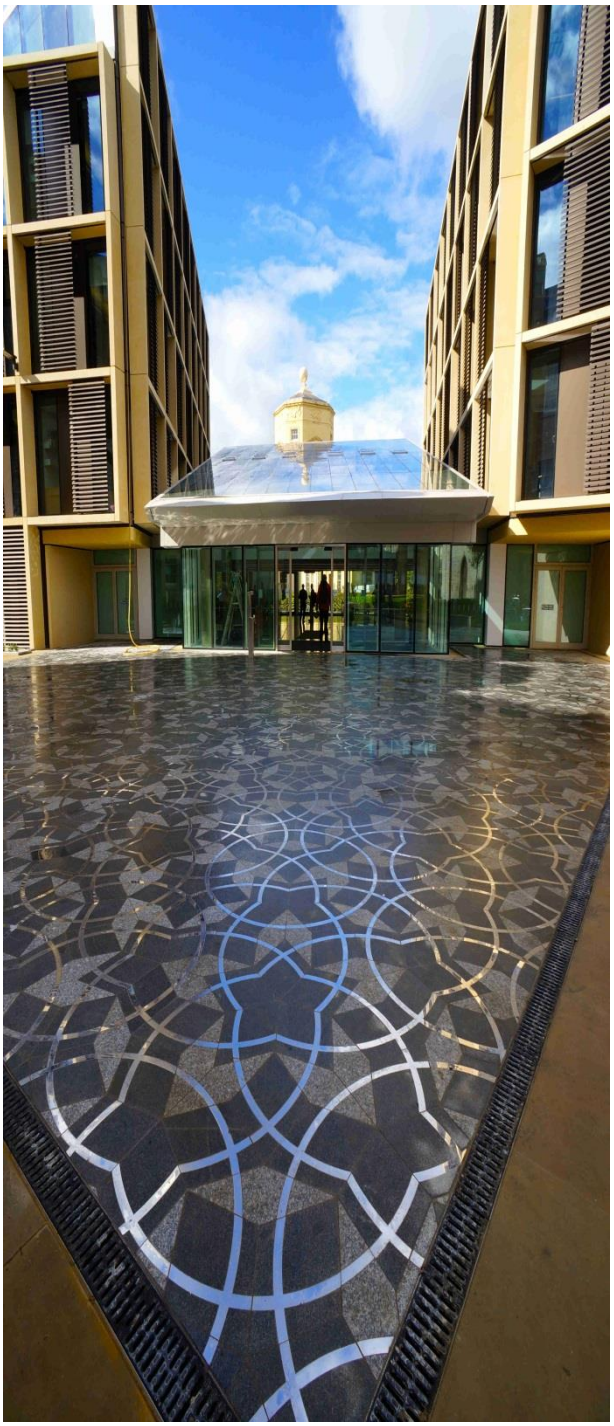
Cellesce



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1. Introduction

Background

Organoids are clusters of specialized cells that provide more representative behaviour than 2-D cell lines

Organoids, clusters of artificially cultured specialised cells with organ-like properties, have long been used as physical models for biological processes, such as tissue growth and disease progression. The Extra Cellular Matrix (ECM) in which they are grown provides the structure needed to trigger specialisation and to exhibit native cell growth behaviour, meaning they are a representative proxy for *in vivo* cells. This makes them preferable to traditional cultures grown on flat surfaces, known as 2-D cell lines.

In the case of colorectal cancer research, intestinal stem cells (more specifically crypt-based columnar cells) are of interest, as they can be used for screening of drugs or monitoring the mutational evolution of the disease. Cellesce cultivate organoids from tumour cells particularly for use in high-throughput drug screening since they provide a more realistic picture of how human cells will respond to treatment than 2-D cultures, and can also be used for comparison against healthy cells.

Bioreactor set up

The method followed by Cellesce for growing organoids involves seeding single cells from patient tumours in a layer of hydrogel (which acts as the ECM) residing below a culture media, which in turn sits below air that is maintained at ambient levels of oxygen (Figure 1, top). The hydrogel provides a structure for cells to grow in, mimicking the human environment. In the static system, the media is changed daily when discoloration in the media is noticed. The dynamic system involves generating a flow across the top of the hydrogel to replenish nutrient and carry away waste products. Organoids are removed from the bioreactor after 2-6 days, by which point they have grown to up to 6 cells in size.

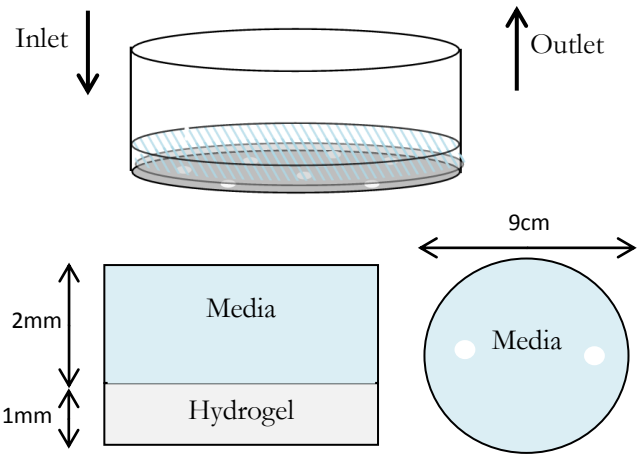

Figure 1: Bioreactor setup. Organoids are suspended in layer of hydrogel below a layer of culture media. Media is replenished via flow across the dish.

A key priority for Cellesce is assuring uniformity and reproducibility in the size of organoids. This depends on optimising the nutrient concentration through the dish. The key nutrients are oxygen and glucose. Growth is also impaired by the build-up of lactate, a waste product from the metabolism of glucose. Cellesce would like to ensure high, uniform nutrient concentration everywhere in the hydrogel, which is why it is necessary to change the culture media regularly. One method for replenishing the culture media is via a flow across the top of the dish. The typical sizes of the dish are shown in Figure 1.

Our aim is to build a mathematical model to describe the change in concentration of the nutrients in a bioreactor in order to determine how to configure the experimental set-up (eg, the seeding density and when to change the culture media) in order to grow high quality organoids.

2. A mathematical model for the case of no nutrient flow.

We restrict our attention initially to modelling the distribution of oxygen in the experimental system (a similar approach can be applied to uptake of glucose and production of lactate), focusing on the situation where there is no flow. We use a 1-dimensional diffusion-reaction model to describe the evolution of the oxygen concentration in the hydrogel, where the reaction accounts for consumption of oxygen by the cells.

We assume that the cells are distributed uniformly throughout the hydrogel and that we are operating in a regime where cells are not dividing so the oxygen consumption rate does not explicitly depend on time. Whilst this is not strictly true, it is a reasonable approximation for modelling purposes as the timescale for cell growth is much longer than the rate at which nutrient is being taken up (it takes roughly 1 day for a cell to divide). We model the rate of oxygen consumption according to the Michaelis-Menten model for enzymatic reactions, where uptake is given by

$$R = \lambda_{max} \frac{c}{\kappa + c}$$

In the limit of high concentration, the Michaelis-Menten model for oxygen uptake behaves as though constant, whilst in the limit of low concentration, uptake is proportional to surrounding concentration

where c is the surrounding concentration of nutrient in the hydrogel (measured in mol/l) and κ is the concentration level at which uptake is half of λ_{max} (the maximum rate of uptake for the dish, calculated as the rate of uptake per cell times the number of cells in the dish). In the limit of high concentrations, consumption is almost constant, and in the limit of low concentrations, consumption is proportional to the surrounding concentration. In both of these cases, we can find analytical solutions for the concentration. We assume an initial concentration of zero oxygen in the hydrogel, a fixed concentration c_0 at the top of the hydrogel (ie. the base of the culture media) and no concentration flux out the base of the bioreactor.

Parameter values

The values used in the model are outlined in table 2. These were provided by Cellesce where available or else taken from the relevant literature. Discrepancies in values, particularly as regards the *critical concentration level* (the level at which growth of cells is impaired), suggests experimental data may be needed to corroborate the predictions of the model Significantly, cancer cells demonstrate atypical behaviour when compared to healthy cells, with higher consumption and also an ability to survive better at low nutrient and or high lactate concentrations.

	Initial concentration in media (mol/l)	Rate of uptake (mol/cell/s)	Critical concentration level (mol/l)	Michaelis-Menten constant, κ, (mol/l)	Diffusivity (mm^2/s)
Oxygen	2.78×10^{-4}	8×10^{-17}	4×10^{-5}	8×10^{-6}	5×10^{-4}
Glucose	1.75×10^{-2}	8×10^{-16}	3×10^{-4}	3×10^{-3}	3×10^{-4}
Lactate	-	4×10^{-16}	5×10^{-3}	-	10^{-4}

Table 2: Parameter values. These were obtained provided by Cellesce where available and otherwise estimated from the available literature.

Number of viable organoids

First, we suppose that the concentration along the top of the hydrogel is maintained at a constant concentration. Using values from literature and Cellesce experimental data (outlined in table 1), the ratio between the timescale for diffusion through the hydrogel and the timescale for oxygen uptake in the dish is small, suggesting that there is ample nutrient in the bioreactor to support cell growth. In the limits of large and small concentrations, we can obtain analytical expressions for the concentration of nutrient in the bioreactor. These provide upper and lower bounds for the Michaelis-Menten model. The solution in each case is composed of a term that decays exponentially in time and a steady state term. This steady state term can be used to determine the number of cells that can be sustained for a given height of hydrogel. For example, in the limit of large concentration, we found that

$$N < \frac{2\,D(c_0 - c_{critical})}{\lambda h^2}$$

where D is the diffusivity, c_0 the initial concentration, $c_{critical}$ the concentration at which cell growth is impaired, λ the consumption rate per cell (ie, $\lambda N = \lambda_{max}$), and h the height of the dish. Rearranging this equation, we can establish a critical height, corresponding to the depth to which cells can be seeded (at the same density) before the steady state concentration reaches a critical level. Our solution suggests that the current experimental set up is enough to sustain 50 million cells, equivalent to roughly 9 million organoids at the size at which they are removed from the bioreactor. However, Cellesce uses a seeding of 3 million cells, and they observe cell death, and a discoloration in the culture media. This suggests that we need to revise our assumption that the nutrient concentration across the top of the dish is constantly maintained. We consider instead a model where nutrient is depleted over time – most relevant for nutrients other than oxygen such as glucose. We also want to consider how to model the production of waste products, particularly lactate.

The high rate of diffusion in comparison to uptake leads to very small spatial variations in concentration throughout the dish

Nutrient depletion over time

Our model assumes a fixed concentration of oxygen along the top of the hydrogel. This is applicable in the case where the oxygen concentration in the ambient air layer is sufficient to replenish the concentration in the media, or if the media is being constantly replenished. However, this is not applicable for insufficient flow. In addition, the concentration of lactate and glucose are not affected by the ambient air region. For these reasons, we also consider a model where the concentration of nutrient or waste product is finite and depletes over time.

We solve this model numerically to determine the concentration of oxygen, glucose and lactate over time. In addition, the rate of diffusivity relative to uptake meant the spatial variations throughout the dish were small over a long timescale. Therefore, we calculated the depletion for the whole system using a spatially averaged concentration and consumption rate across both the hydrogel and culture media regions. The depletion of oxygen over time is shown in Figure 2, where we compare the numerical solution for the concentration of oxygen at the base of the hydrogel and the averaged concentration throughout the hydrogel-media region of the bioreactor.

Crucially, the spatially averaged model leads to explicit formulas for identifying the time at which overall concentration in the bioreactor will reach a critical level, below which growth is impaired. We find that, using the values for oxygen consumption, we predict a depletion on the order of hours, much shorter that the observed time for media discoloration. Using the values for glucose consumption, we predict that the critical level is reached after 1.5 days. However, we also predict that lactate concentration levels become critical after roughly one day, which coincides with the timeline at which discoloration of the culture media is observed.

Using a spatially averaged model, we can provide estimates for the time until the concentration of nutrient in the bioreactor becomes critical

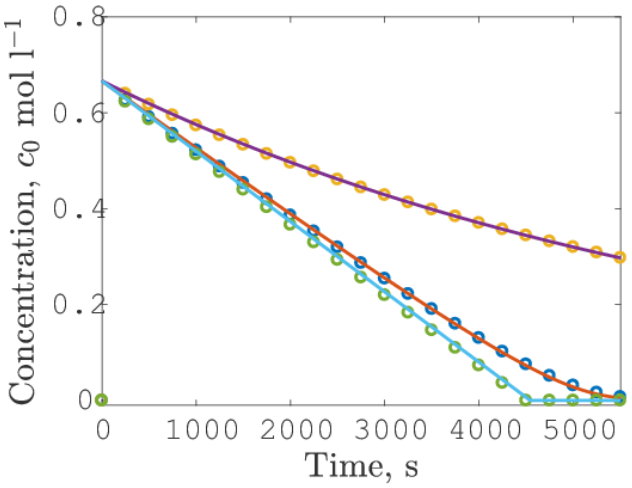

Figure 2: Depletion of oxygen over time according to the three models for consumption: Michaelis-Menten (red/blue), constant (turquoise/green) and proportional (purple/orange). Small spatial variation in concentration means average concentration for the combined hydrogel-media region (straight line) is a good approximation of the concentration at the based of the dish (circles).

A summary our predictions of the critical heights and times is presented in Table 2. The variations in reported values for uptake, critical levels and the Michaelis-Menten reaction constant, are likely to affect the exact values predicted. In particular, there is a wide discrepancy in reported 'critical concentration' levels, ie. the level at which cell growth is negatively affected.

	Critical height (mm)	Critical Time: constant consumption (days)	Critical Time: proportional consumption (days)	Critical Time: Michaelis-Menten (days)
Oxygen	4	0.06	0.08	0.06
Glucose	10	1.2	3.6	3.06
Lactate	-	1.0	-	-

Table 2: In the model where concentration is maintained we calculate the critical height, corresponding to the depth to which cells can be seeded (at the same density) before the concentration reaches a critical level that impairs cell growth. Where concentration is not maintained, we use a spatially averaged model we calculate the critical time at which the base of the dish reaches a critical level. The time at which average lactate concentration reaches a critical value coincides with the time at which media discoloration is observed experimentally.

3. Dynamic system

The results from our static model indicate the need for replenishment of the culture media, particularly to maintain glucose concentrations and carry away waste product. One of the ways that Cellesce hope to achieve this is by generating a flow across the top of the hydrogel via an inlet and an outlet port (as shown in Figure 1).

The Peclet number for the system (the ratio between the horizontal rate of advection and the horizontal rate of diffusion) is O(10), indicating that advection plays a prominent role

in the transport of nutrient, despite the slow flow. One potential issue is the possibility of stagnant regions, and so we simulate the flow across the top of the dish to determine whether this is the case. We model the system using Stokes equations for slow flow and substitute the resulting fluid velocity profile into an advection-diffusion equation for the concentration of oxygen in the culture media (assuming negligible amount is removed by the cells).

In Figure 2, we present the velocity profile across the dish. We see that, apart from regions of high speed at the inlet and outlet, the flow across the dish was relatively uniform, if slightly higher along the central channel. Hence, provided that the flow rate is sufficient to ensure that the media leaving the system is not below the relevant critical level (or above in the case of waste product), our results suggest this set-up is sufficient for maintaining a sufficient concentration of nutrient across the top of the hydrogel. The flow required to achieve this can be estimated from using the values from table 1, or measured experimentally.

To estimate the required volumetric flow rate of v ($m^3\ s^{-1}$), we consider the estimated concentration of nutrient or waste product leaving the system relative to the critical concentration to be $\lambda V\ c_0/v\ c_{crit}$, where V is the volume of culture media. We note that, as demonstrated in the previous section, replacing the media daily is not sufficient to maintain the required oxygen level, and therefore this demonstrates that the ambient air above the hydrogel plays a key role in maintaining the concentration of oxygen in the media. However, this estimation suggests that, for both glucose and lactate, the flow rate used by Cellesce is sufficient to maintain the concentration above a critical level. Hence, the flow system is important for maintaining a fresh supply of glucose and carrying away lactate.

Simulating the flow across the dish suggests higher flow down the central channel, but discrepancies across the dish are slight

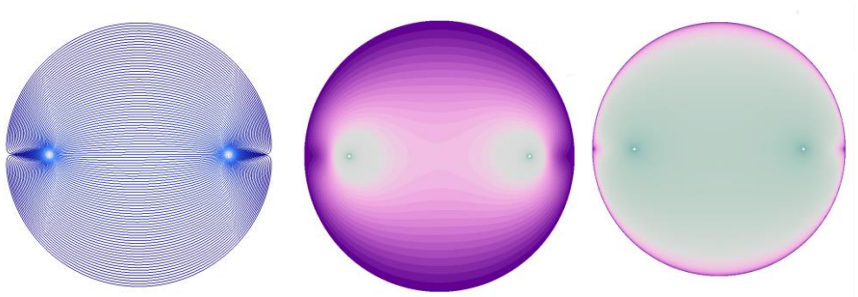

Figure 2: Flow streamlines indicate all regions of the dish are reached. Wee see that velocity is slightly higher through the middle channel. However, on a log scale, we see that only the edges form stagnant regions

4. Discussion, conclusions & recommendations

We have built a mathematical model to describe the concentration of nutrients in an organoid bioreactor. Using mathematical modelling we were able to provide estimates for critical numbers of organoids that could be sustained in a bioreactor, and a critical time after which the concentration of nutrient would drop to a level which may impact on organoid growth. A key outcome was identifying lactate build up as a critical factor in limiting growth, and illustrating the need for an ambient air region above the culture media to maintain the oxygen concentration in the media.

We analysed the flow in the Cellesce bioreactor set-up and found no notable stagnant regions, except at the very edges of the dish, suggesting their method is suitable for providing a fresh supply of nutrients everywhere across the dish. This is dependent on ensuring a flow rate sufficient that the media leaving the system is not below the critical value. To improve the evenness of flow, extra inlet and outlet ports might create a more even flow across the dish, however our simulation suggests discrepancies are slight.

A key factor to bear in mind is the wide discrepancy in parameter values, particularly the ambiguity regarding the critical concentrations below which the cells stop operating; hypoxia is particularly difficult to classify in tumour cells which can thrive in much lower conditions than healthy cells.

To take this work further, experimental data to corroborate values (for example rate of consumption) could be collected by the Cellesce team. In addition, it would be beneficial to consider a model where uptake of nutrient and production of waste products are modelled together, as these factors are co-dependent; for example low oxygen levels lead to increased levels of lactate production. Future models that incorporate both flow and uptake should also consider cell growth, as this occurs on a similar timescale to the flow.

5. Potential impact

Using mathematical modelling techniques, we can determine operating conditions for the bioreactor, including seeding density, the optimum timings for changing culture media, and how to set up media concentrations. Understanding variations in nutrient across the bioreactor will also be useful for ensuring quality in the growth of organoids. These techniques are quicker than conducting experiments and also more easily adapted to see the effects of changing parameter values.

Dr Marianne Ellis, Chief Technology Officer and Founding Member, said *"Combining mathematical modelling with experimental modelling allows faster and better optimisation. Being able to get spatial and temporal data from the mathematical models provides another level of information we cannot get from taking positional readings alone. An iterative design approach has allowed us to assess our current settings and predict future operating conditions, which would otherwise take months and be expensive if done solely in the lab."*