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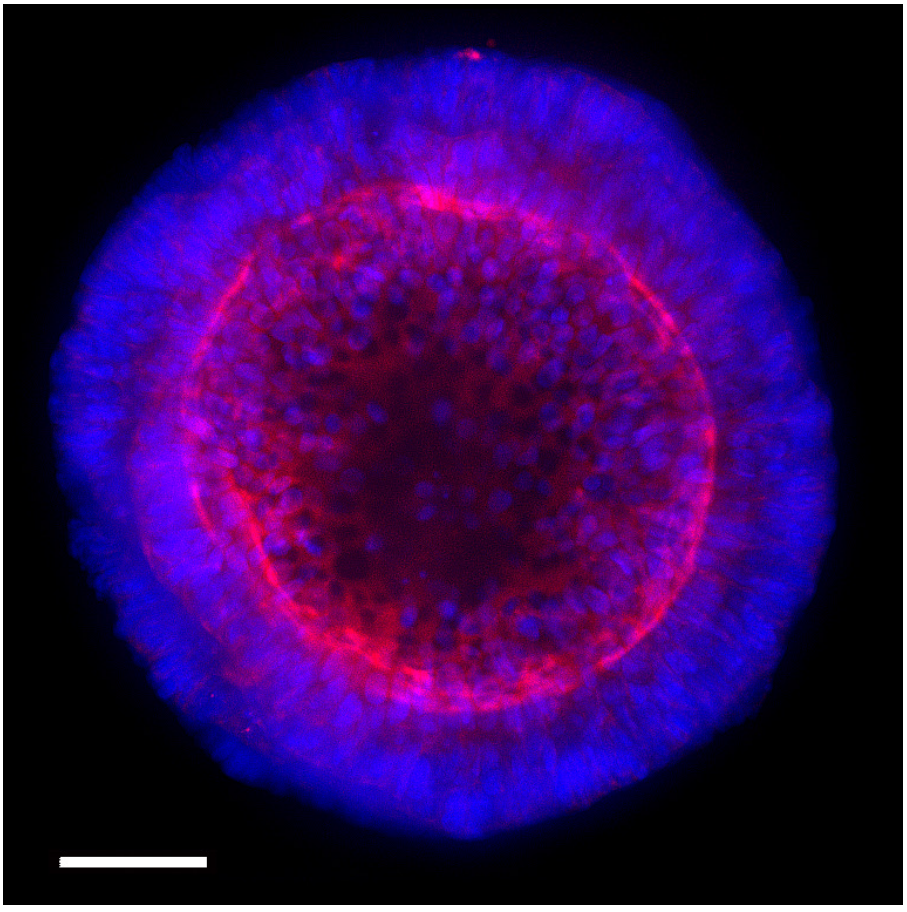

Oxygen concentration in the CXP1 bioreactor

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Cellesce®
organoids expanded

Contents

1 Introduction

Background

Organoids are three-dimensional, multi-cellular structures which are grown *in vitro* and successfully recapitulate realistic tissue-specific structure and function [1]. Organoids can recreate a realistic *in vivo* anatomy as they contain the key cell types of their tissue of origin, *i.e.* stem cells, which allow extended culture studies, and differentiated cells, which retain physiologically relevant functions. For example, organoids grown from breast cells produce milk proteins, while organoids grown from cancer cells are invasive. The Extra Cellular Matrix (ECM) in which organoids are grown provides the structure and signalling interactions needed to trigger cellular specialisation and for the organoids to exhibit native cell growth behaviour. Because organoids retain many features of the human tissues from which they are originally derived, such as the tissue structure, pathology, and heterogeneous cellular composition, this makes them a more representative proxy for *in vivo* cells than flat 2D cell line cultures grown at the bottom of a dish. Organoid technology improves efficiency and predictability of efficacy and toxicity assays, reducing the need for animal testing. Cellesce aim to feed organoid technology into the next generation of drug discovery, since it has the potential to save time and resources early in the drug discovery pipeline.

Organoids are three-dimensional, multi-cellular structures, which are grown *in vitro* but successfully recreate a realistic *in vivo* micro-anatomy.

Only in the past 15 years have suitable conditions for long-term growth of organoids been developed. Previously, organoids had only been grown at small scale in specialist research laboratories. The culturing of organoids is a highly labour intensive and time-consuming process. The small quantities and variability between batches makes the manual growth process unsuitable for large-throughput drug screens. However, the development of suitable culture conditions has allowed Cellesce to utilise bioreactor technology to grow organoids at scale.

Cellesce is currently using their 'Cellesce Expansion 1 (CXP1)' technology to expand colorectal cancer organoids for use by pharmaceutical companies and academic researchers. Part of the technology includes a bioreactor, which utilises flow of nutrient-rich media to enhance oxygen delivery to the organoids and facilities the removal of waste products. A key priority is ensuring uniformity in organoid size and reproducibility; this depends on the bioreactor design and operating conditions. Full optimisation of the bioreactor conditions requires understanding the spatial and temporal information regarding flow and the resulting oxygen and metabolite concentrations throughout the bioreactor [2]. This information is impractical, inefficient, and expensive to collect empirically, and thus our aim is to build and solve mathematical models for the oxygen distribution within a hydrogel layer in the CXP1 bioreactor, in order to optimise oxygen delivery to ensure high-quality organoids are produced.

A bioreactor is a device or system for culturing cells or tissues in a closed, controllable environment which supports biological growth.

CXP1 bioreactor set-up

We show a schematic of the CXP1 bioreactor in Figure 1. Organoids are seeded as single cells in a thin layer of hydrogel which acts as a porous scaffold, providing the structure needed for cell growth. Media is pumped across the top of the hydrogel through an inlet pipe, flowing across the bioreactor. The media is then pumped out of the bioreactor through the outlet pipe. The organoids are grown in the bioreactor for roughly 7 days, with the precise duration dependent on the cell line. Once the organoids have grown from single cells (roughly $\sim 10\mu$m) to organoids of roughly 50 cells ($\sim 40-80\mu$m), they are extracted from the hydrogel and tested for size, viability, and number. The organoids are then frozen and drug assays are later performed to check organoid viability.

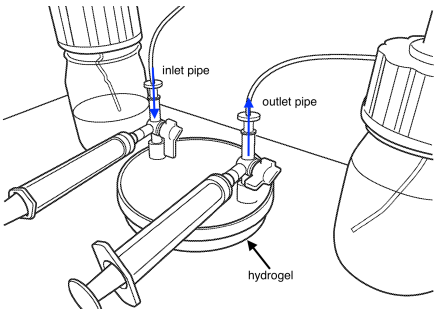


Figure 1 – CXP1 bioreactor. Patent WO 2018/011558 A1.

2 Model for one organoid

We begin by modelling the oxygen concentration using a reaction-diffusion model through the hydrogel. We simplify the geometry of the CXP1 bioreactor into layers as shown in Figure 2.

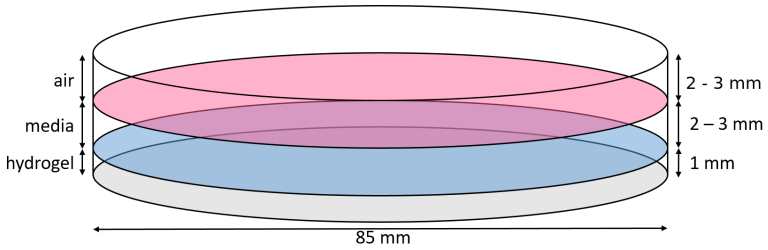


Figure 2 – Simplified CXP1 bioreactor set-up.

We assume that the organoids remain fixed in the hydrogel. For simplicity, we neglect organoid development by assuming we are in a regime where the cells are not dividing. We assume there is no radial variation across the dish and are interested in the variation in oxygen concentration with depth. We also assume that there is no oxygen leaking out of the bottom of the bioreactor. We neglect any fluid flow and focus solely on the diffusive transport of oxygen within the hydrogel. Thus, we consider a one-dimensional domain (Figure 3) and begin by modelling one organoid acting as a local oxygen sink somewhere within the hydrogel layer.

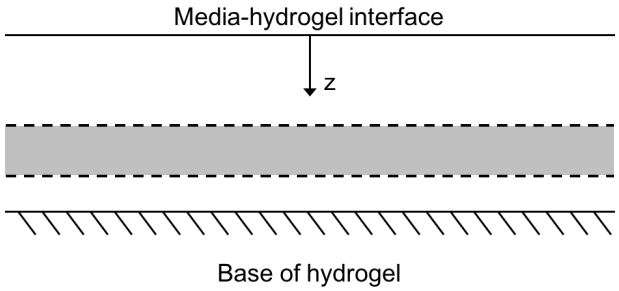
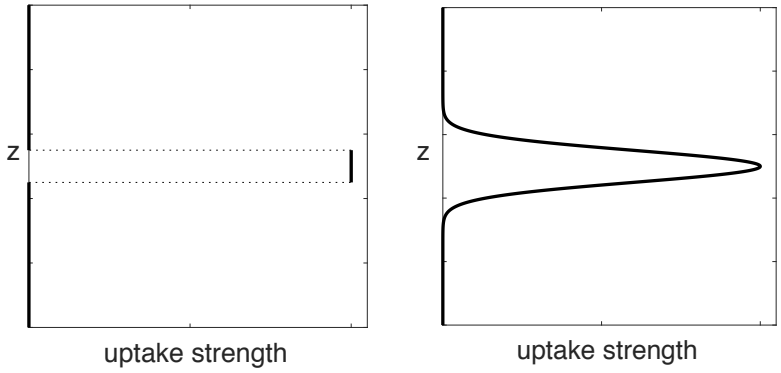


Figure 3 – One-dimensional problem domain, where the shaded region represents the organoid.

Forms of oxygen uptake by organoids

We start by considering how to model the uptake of oxygen by the organoid. There are many existing reaction-kinetics models which we could use and we choose to assume that the organoid oxygen uptake is proportional to the local oxygen concentration, sometimes referred to as first-order kinetics. Since the organoid has finite width, we also need to model how the various internal components of the organoid take up the oxygen. We choose the simplest possible model, in which all parts of the organoid have the same uptake, resulting in the uptake shown in Figure 4a, which we call the *top-hat* case. However, we also consider the smoothed version of the uptake given in Figure 4b in order to remove the discontinuities when we solve our model numerically, called the *Gaussian* case.

When the organoid is very small in comparison to the depth of the hydrogel layer, we can consider the organoid as a point sink. This allows us to perform a mathematical technique called a *boundary layer analysis* and obtain a formula for the concentration of oxygen in the bioreactor, based on the form of the uptake close to the organoid. If we assume that the media maintains a constant concentration of oxygen at the media-hydrogel interface and that there is no flux of oxygen through the base of the hydrogel, we obtain a formula for the minimum concentration in the system, which varies as the position of the organoid varies and is smallest when the organoid is located at the base of the hydrogel layer.



(a) Top-hat. **(b) Gaussian.**

Figure 4 – The two different forms of sink we consider to model the oxygen uptake by organoids.

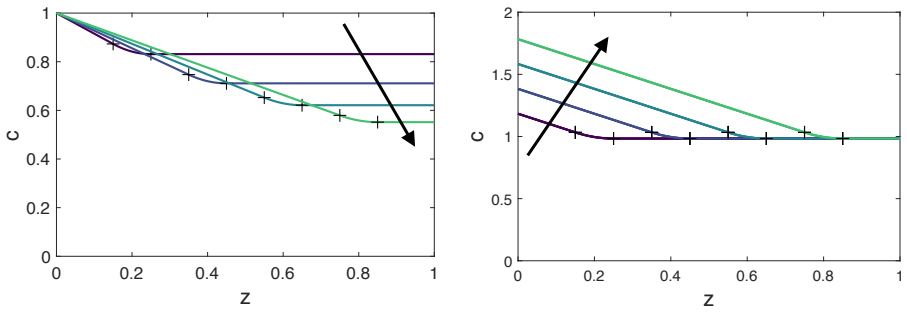
Importance of media-hydrogel interface

It is of great importance to ensure that the correct physical model is used for the coupling between the hydrogel and media regions. We relate the concentration in the media to the concentration in the hydrogel via appropriate boundary conditions.

Different physical models for the interaction between the media and the hydrogel changes the qualitative behaviour of the oxygen concentration in the bioreactor.

One model would be to assume that the media acts as a well-mixed bath of oxygen, ensuring there is a constant oxygen concentration at the top of the hydrogel. Using this assumption, we find that the position of the organoid affects the oxygen concentration profile in the dish, as shown in Figure 5a. When the organoid is positioned closer to the bottom of the bioreactor, both the volumetrically averaged and minimum oxygen concentrations in the bioreactor decrease. Our results suggest that seeding the hydrogel with organoids nearer the top of the bioreactor would be advantageous, since then the organoids would be exposed to a higher oxygen concentration. Cellesce have already implemented a protocol to reduce the number of organoids positioned towards the base of the bioreactor, since the organoids do not grow as they should once settled on the bottom of the dish.

A different assumption at the media-hydrogel interface would be to consider the media to supply a constant flux of oxygen to the hydrogel. Although the flow of fresh media is likely ensure a nearly uniform oxygen concentration in the media, the media does not contain an overwhelmingly greater amount of oxygen in comparison to the hydrogel. We find that using a prescribed influx boundary condition at the media-hydrogel interface removes the dependence of the minimum concentration on the position of the organoid, as shown in Figure 5b. Both of these simple models provide insight into the true situation in which the flux and chemical potentials are constant across the media-hydrogel interface.

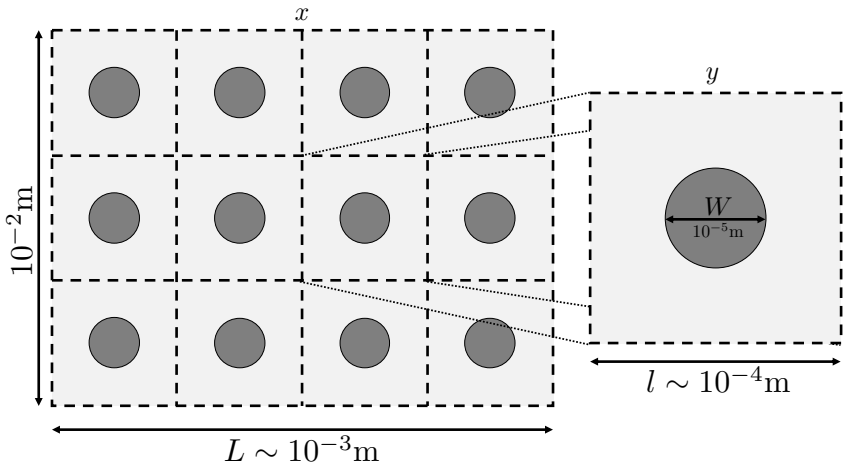


(a) Constant oxygen source. **(b) Constant oxygen influx.**

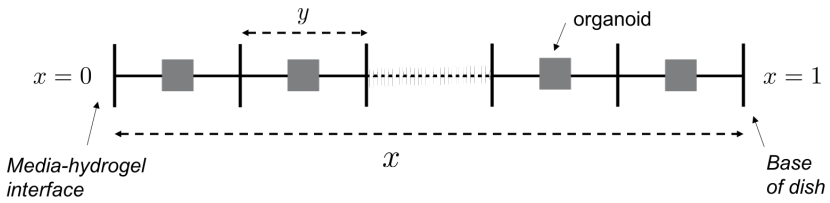
Figure 5 – Concentration profiles as the midpoint of the uptake region changes (representing change in position of the organoid). The arrows indicate increasing the distance between the organoid and the top surface in the *top-hat* case.

3 Model for many organoids

Our model for a single organoid could become prohibitively computationally expensive in the case of many sinks and in the extension to higher dimensions. To overcome this issue, we wish to model the numerous organoids as an effective oxygen sink across the whole domain. The bioreactor is initially seeded with single cells, which have diameter of approximately 10^{-5}m. The CXP1 bioreactor set-up is such that, if seeded uniformly, each organoid would be contained within a box with sides of length approximately 10^{-4}m. This separation of scales, shown in Figure 6, suggests that upscaling using mathematical homogenization is a suitable way forward.



(a) A two-dimensional projection of the three-dimensional problem set-up.



(b) The one-dimensional simplified geometry.

Figure 6 – Diagrams of the possible domains.

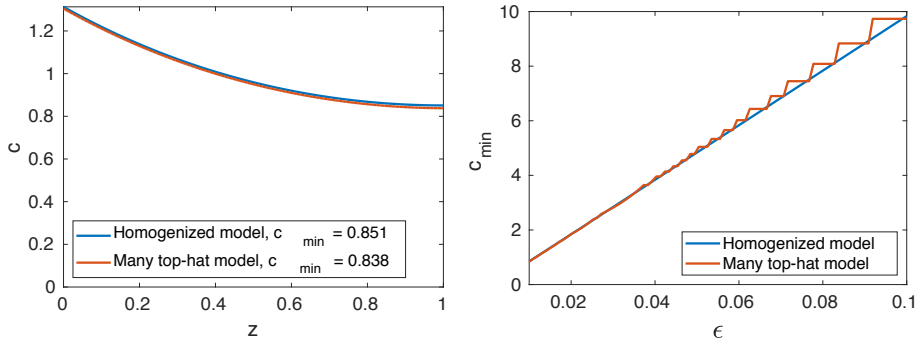
Mathematical homogenization is a technique to upscale a slowly varying microscale problem to find an averaged model for the macroscale behaviour.

Homogenization is a mathematical technique to systematically upscale a microscale problem (the problem on the length scale of an organoid) to obtain a model for the governing macroscale behaviour (the problem on the length scale of the bioreactor). We consider the diffusion and uptake of oxygen through hydrogel seeded with organoids. We model the organoids as regions where there is oxygen uptake, which we assume to be proportional to the concentration. We assume that oxygen transport occurs via diffusion with diffusivity, D_o, in the organoid, which is different to the diffusivity, D_h, in the hydrogel, and we couple the equations in each region using continutity of flux and continuity of concentration. A similar problem is considered in [3]. For simplicity, we consider organoids of a fixed size. We define ϵ to be the ratio of the periodic cell length, l to the length of the bioreactor L, and for simplicity consider the reduced one-dimensional domain in Figure 6b. We also apply a constant flux boundary condition at the media-hydrogel interface.

We perform the homogenization and the resulting governing equation is a reaction-diffusion system, in which the reaction term is an *effective uptake* term and the diffusion coefficient is an *effective diffusivity*, which both capture the microscale behaviour of the system. We solved the resulting homogenized model analytically.

Comparison to previous model

We want to determine how well the analytical solution to the homogenized model agrees with the full system in which there are many uniformly-spaced organoids of the same uptake-strength and same width at periodic intervals. The number of organoids in the domain is inversely proportional to ϵ, i.e. $\epsilon = 0.1$ corresponds to 10 organoids regions. A comparison of both models with 100 organoids is given in Figure 7a. We can see that the behaviour is qualitatively the same throughout the domain, with an error of size (ϵ). Although we expect the solution to agree in the limit of many organoids ($\epsilon \to 0$), it is of interest to understand the error between solution for non-small values of ϵ. In Figure 7b, we compare the minimum concentration in the bioreactor for the averaged and full models as the number of organoids varies. We see that, as the number of organoids becomes large, the solutions converge. However, even in the extreme case of only 10 uptake regions, the solutions are not hugely different. This comparison is highly promising, as it shows that homogenization is a sensible avenue for further development of physically relevant but mathematically tractable models.



(a) Concentration profiles for the homogenized and the many top-hat models with 100 uniformly-spaced uptake regions ($\epsilon = 0.01$). **(b) Minimum concentration for the homogenized and many top-hat models as the number of sinks changes.**

Figure 7 – Comparison of the homogenized model to the many top-hat model.

4 Discussion, conclusions, & recommendations

We have presented a number of models for oxygen distribution in the hydrogel layer of the bioreactor. We initially considered the case of one organoid in the bioreactor and we found that choosing the appropriate boundary condition for the media-hydrogel interface is important, as different boundary conditions can produce significantly different behaviour. We considered the limit of the organoid acting as a point sink and computed the minimum oxygen concentration in the bioreactor.

The homogenized model and many top-hat model agree well.

We then examined the case of many organoids and developed an averaged model, which treats the organoids as sinks over the entire domain. We solved the model to find a formula for the concentration and compared this to a numerical solution of the corresponding full system in the case where we assume a *top-hat* function for the oxygen consumption and a constant oxygen influx, and we see good agreement when the number of organoids is large.

There are many avenues for further development of our mathematical models. For example, we will model the interface between the media and hydrogel layers more accurately. We could also consider different types of reaction kinetics for the uptake of oxygen, such as the Michaelis-Menten model. This will be of particular interest when we consider the distribution of other nutrients and waste products as well as oxygen. Also of interest is an investigation of the effect of media flow, since we have seen the significance of the coupling of the media and hydrogel layers through imposing appropriate boundary conditions. It would be advantageous to compare the oxygen concentration predicted by the model to experimental values for the average oxygen concentration in the in-flow and out-flow pipes collected by the team at Cellesce. In the CXP1 system, Cellesce do not have direct control of the organoid seeding process. They put a

well-mixed organoid-hydrogel suspension in the bioreactor but they cannot guarantee perfect uniformity in organoid distribution. Thus, we will compare our homogenized model to the many top-hat model with stochasticity in the placement and strength of the oxygen uptake regions. We could also extend the homogenized model to higher dimensions. Lastly, we could include more cell biology into the models. Thus far we have neglected the growth of the organoids and the mechanics of organoid-organoid and organoid-hydrogel interactions. However, experiments at Cellesce have shown that these are important the production of high quality, viable organoids and will be a key ingredient of future models.

5 Potential impact

Mathematical modelling has the potential to improve bioreactor operating conditions by reducing the effort required for experimental optimisation, which is both time consuming and expensive. Understanding the variations in oxygen concentration within the bioreactor will provide insight into ensuring high quality and uniformity in organoid growth. Our early-stage models have a lot of potential for further extension, enabling understanding of the distribution of nutrients and assisting in the development of the next generation of bioreactor.

Dr Kim Luetchford, Senior Scientist at Cellesce, said: "*Mathematical modelling allows us to gain insights that we would otherwise be unable to achieve. Measuring oxygen levels spatially and temporally is impossible at the scale at which we work, but modelling can provide us with that data. This demonstrates to us the importance of mathematicians working with experimentalists to optimise and better understand our cell culture conditions, and refine them efficiently in the future. Working efficiently saves us both time and money, and refining our conditions will help us minimise waste while still generating a high quality, viable cellular product.*"

References

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