DESIGN AND OPTIMIZATION OF MICROFLUIDIC AND ORGAN-ON-A-CHIP DEVICES - MODELLING

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ABSTRACT

Organ-on-a-chip technology has been in development for in vitro simulation of microenvironments found within living structures, enlarging the study of organ behaviour, disease progression and drug delivery. The present project aims to employ a finite element approach, to design and optimize a device for characterization and prediction of chemical concentration gradients. Through a developed numerical model, the optimization process has been enhanced, and testing conditions have been simulated and defined to be as close to those found in vivo.

Keywords: organ-on-a-chip, microfluidic devices, chemical gradient, finite element analysis, modelling.

INTRODUCTION

Tissue Engineering emerged as a powerful tool for translational biomedical applications, as well as to understand and study disease mechanisms. However, it presents considerable limitations, as currently, it cannot recapitulate complex circulation systems, its macroscale requires a large amount of cells, and typically, these tools have tissue to fluid volume ratios which are not physiological. Organ-on-a-chip devices have evolved to overcome these limitations, by integrating engineered tissue within physiologically relevant microfluidic systems, developing in vitro models capable of simulating the microenvironments found in vivo, within organs present in the human body (Healy et al. 2017).

The human body operates under a broad range of chemical concentrations gradients, known to play influential roles on specific physiological functions. The functional unit of the liver, the hepatic acinus, works under a highly defined oxygen concentration gradients, which impact multiple cellular functions, such as metabolism, protein synthesis and antioxidative mechanisms (Kietzmann et al. 2017). Optimally developed organ-on-a-chip devices, designed to simulate chemical concentration gradients, emerge as powerful tools for in vitro studying of disease progression and drug testing (Lee-Montiel et al. 2017).

The present paper aims to design and optimize an organ-on-a-chip device, focused on simulation of chemical concentration gradients, by employing a finite element approach and develop a numerical model that contemplates the relevant physics, boundary-conditions and microenvironments known in vivo. Through this numerical approach, it is intended to achieve optimal experimental conditions, further validation through real world data.
CONCENTRATION GRADIENT DEVICE

The device presented by this paper, was based on an initial design and concept – the Concentration Gradient Device - conceived and provided by the Biomedical Interfaces at Glasgow (BIG) Research Group, with whom this project was carried on.

This device has two inlets [1], through which flow is admitted into the system, and one outlet [5], whereby flow exits the same system (Figure 1). A species concentration differential is established by admitting the respective species solely on one of either inlets. Between inlet and outlet ports, this device presents three major features: a gradient generator [2], defined by branching of the two inlets, into a multiple array of serpentine-like channels; an array of intermediate channels [3], connecting features [2] and [4]; and a gradient displayer [4], embodied by a main chamber, where organoids are placed in, through the same inlets, and their reaction to the established gradient studied. The concentration gradient is generated by continuous laminar flow perfusion, and controlled diffusive mixing of the species introduced into the system, enabling long-term cell investigation.

The Concentration Gradient Device intends to simulate a broad range of concentration gradients, regardless its species, expanding its application to a wider field of study. The optimization process will be carried accordingly, having, however, a more significant focus towards simulation of oxygen tension, as present in the liver (Sharma et al. 2019).

![Fig. 1 - The first design and respective sections of the Oxygen Gradient Device](image)

A FINITE ELEMENT APPROACH

A numerical model was developed based on the initial design for the Concentration Gradient Device, as provided by Biomedical Interfaces at Glasgow (BIG) Research Group. Modelling was carried on COMSOL Multiphysics v4.3 software. Through finite element analysis, it was intended to model the intrinsic flow, chemical and geometric characteristics of the device, such as to perform studies, focused on obtaining the ideal respective features, that better define the concentration gradient displayed at the main chamber, whilst providing a safe microenvironment for cellular growth.
Flow, within the device, was simulated using the “Laminar Flow” Module, from COMSOL. Reynolds Number was regarded as a marker to assess the relative influence of both inertial and viscous forces, and ultimately determine the nature of the flow within the device. A low Reynolds Number ($Re < 2000$) indicates that inertial forces govern flow, and therefore, it should be described as Laminar (Gadegaard et al. 2019). However, for a very small Reynolds Number ($Re \ll 1$), viscous forces become dominant, and flow is defined as Creeping. The Reynolds Number can be expressed as it follows:

$$Re = \frac{\rho u Dh}{\mu}$$  \hspace{1cm} (1)

where: $\rho \ [kg/m^3]$ is the water density at 37 [$^\circ$C]; $u \ [m/s]$ is the flow velocity; $Dh \ [m]$ is the hydraulic diameter; and $\mu \ [m^2/s]$ the dynamic viscosity of water at 37 [$^\circ$C]; and it was calculated to be comprehended within the following values, for serpentine channels (SC) and main chamber (MC) respectively:

$$Re_{SC} = 1.33$$ \hspace{1cm} (2)

$$Re_{MC} = 2.33$$ \hspace{1cm} (3)

indicating that flow should be modelled as Laminar. This approach is further strengthened by literature (Nguyen et al. 2013).

Thereby, fluid motion within the device is solved by the Navier-Stokes Equation, applied for an incompressible fluid, expressed as follows:

$$\rho(u \cdot \nabla u) = \nabla \cdot \left[ -pI + \mu(\nabla u + (\nabla u)^T) - 12 \frac{\mu u}{Dh^2} + F \right]$$  \hspace{1cm} (4)

where $p \ [Pa]$ is the fluid pressure and $F \ [N]$ the external forces applied, while the term $12 \frac{\mu u}{Dh^2}$ accounts for the shallow nature of the device’s channels. The Navier-Stokes Equation is solved together with the Continuity Equation:

$$\rho \cdot \nabla u = 0$$  \hspace{1cm} (5)

ensuring both the conservation of momentum and conservation of mass, respectively.

To simulate the transport mechanisms of the dissolved species, COMSOL’s “Transport of Diluted Species” Module was employed, engaging the Convection-Diffusion Equation, to solve for the combined effect of both mechanisms. This Equation can be expressed as follows:

$$\nabla \cdot (-D_i \cdot \nabla c_i) + u \cdot \nabla c_i = Ri$$  \hspace{1cm} (6)
where $D_i \ [m^2/\text{s}]$ is the diffusion coefficient of species $i$; $c_i \ [\text{mol/m}^3]$ is the concentration of the same species; and $R_i$ accounts for a sink, or source, of species $i$.

Studies were performed as stationary, as the study of cell culture response to concentration gradients is done long-term, and characterized by a steady state solution for both engaged physics, without any time dependent feature.

**PROPERTIES AND BOUNDARY CONDITIONS**

Media is modelled to be Water, with features as shown in Table 1. Media is admitted into the system, with equal magnitude, through both inlets, to simulate continuous flow perfusion into the device. Walls are modelled as a no-slip boundary condition. Fluid exits the system through the outlet, where the boundary condition is defined to exhibit no viscous stress and a magnitude pressure of 0 $[\text{Pa}]$.

The Diluted Species is modelled to be Oxygen, with characteristics as shown in Table 1. Oxygen is admitted into the system at the left inlet by a continuous supply of magnitude 0.17075 $[\text{mol/m}^3]$. The right inlet, however, is defined to provide no supply of Oxygen. Walls are modelled to show no flux, in or out, of the mentioned species, in order to account for Polystyrene’s impermeability. Oxygen will leave the system through the outlet. The initial concentration of Oxygen within the device was defined as being null.

The relevant definitions regarding media, dissolved species and testing conditions used for modelling, are expressed in Table 1.

<table>
<thead>
<tr>
<th>Testing Temperature</th>
<th>37 $[^\circ \text{C}]$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density of Water at 37$[^\circ \text{C}]$</td>
<td>993.30 $[\text{kg/m}^3]$</td>
</tr>
<tr>
<td>Dynamic Viscosity of Water at 37$[^\circ \text{C}]$</td>
<td>0.6913 $[\text{mPa}\cdot\text{s}]$</td>
</tr>
<tr>
<td>Diffusion Coefficient of Oxygen in Water at 37$[^\circ \text{C}]$</td>
<td>$3.35 \times 10^{-9} \ [\text{m}^2/\text{s}]$</td>
</tr>
<tr>
<td>Concentration of Oxygen in Water at 37$[^\circ \text{C}]$</td>
<td>0.17075 $[\text{mol/m}^3]$</td>
</tr>
</tbody>
</table>

**MODEL PARAMETERIZATION**

The device was initially modelled as designed by Biomedical Interfaces at Glasgow (BIG) Research Group. However, to enhance the optimization process, the model was further described by a set of design parameters, fully defining the entire geometry of the device. Therefore, the model created is dynamic, and enables a wide range of geometric possibilities and combinations (Figure 2), so that optimal design features to enhance the gradient may be easily found and implemented.

Fig. 2 - Design capabilities allowed by model parameterization
In Figure 2, geometry (a) represents the first design; geometry (b) changes basic features, such as width of channels, length of serpentes, width of main chamber and length of main chamber; geometry (c) exhibits more complex variations, such as difference in width between serpentine and intermediate channels, shape of main chamber outlet, and decreased number of serpentine lines; and geometry (d) presents further possible modifications, regarding number of serpentine loops, increased number of serpentine lines and increased number of inlets.

**GRADIENT ANALYSIS**

The optimization process has the target of generating a deterministic and well-defined gradient, uniformly spread across the Main Chamber’s width and length, thereby providing a controlled microenvironment for cell culture, where concentration levels can be maintained long-term and for specific and pre-determined areas of interest. Such feature is intended to allow for a more accurate study of cellular response to concentration gradients.

To allow for a clear and quantifiable way to analyse the gradient displayed, a 7x5 probe grid was implemented within the Main Chamber, so that uniform studies maybe performed throughout the entire length of the optimization process. From this grid, values for concentration levels, flow, shear stress and diffusion can be taken. Vertical lines are designated as Concentration Lines – a geometric place where concentration levels should remain steady and uniform – and horizontal lines, as Gradient Lines – a geometric place where a smooth and well-defined gradient should be displayed (Figure 3a).

Playing a key role to generate the displayed concentration levels at the gradient, Serpentes spread across the Generator Area should also be analysed with care. Throughout these channels, diffusive mixing of species occurs, due to a concentration differential at the start of each Serpentine. By the end of this channel, mixing should reach a steady-state, and present a uniform concentration level. To control such variations, probes were also implemented, transversal to flow direction, across the length of Serpentes (Figure 3b).

In Figure 3, picture (a) exhibits the 7x5 probe grid, where Concentration Lines are represented by the red vertical dashed lines, and Gradient Lines by the blue horizontal dotted lines; picture (b) shows in green continuous lines the probes set on serpentine channels.

**MESH REFINEMENT**

Establishing a correct mesh refinement is crucial to ensure result accuracy, whilst keeping simulations time efficient. Therefore, a finite element model demands that a study about mesh refinement is completed, by assessing and characterizing its influence on the derived results, whilst defining the optimal mesh for the intended simulations.
To evaluate the liability of results generated by the developed model, for a given mesh, concentration of oxygen throughout the device was taken into consideration, as it should not exceed the concentration supply at the inlet – 0.17075 [mol/m³] – which accounts for media containing 18% of dissolved oxygen, as referenced for oxygen in water at 37[ºC] (Ochs et al. 2014).

A first study was performed, by increasing the total number of elements, uniformly, within the mesh. The initial solution was computed for a “Coarser” mesh, with a total number of 28572 meshing elements. The number of elements was increased by steps, ultimately being ran for a total number of 1522023 meshing elements, equivalent to an “Extremely Refined” mesh, further scaled by a factor of 10. Time costs for simulation ranged between 19[sec] and 5594[sec] respectively. As shown in Figure 4, it is clear that with increasing number of elements, thus creating a finer mesh, oxygen concentration levels decrease and eventually stabilize at the percentage referenced (18%). From the performed study, it was also possible to understand that in the Main Chamber and in the first length of the Serpentine Channels, critical domains for simulations, the optimal number of elements for each was respectively 239837 (Figure 4b) and 166972 (Figure 4c), where the error was determined as null. Therefore, mesh refinement should be focused on the respective domains, and relaxed throughout the rest of the device, attempting for a more time-efficient model, whilst providing the same result accuracy.

The previous conclusion lead to a second study, executed by implementing a domain customizable mesh, initially refined to the element size obtained by the previous study, and followed by a continuous decrease of the total number of elements within the less critical domains of simulation. Ultimately, an optimized mesh was obtained, contemplating the optimal number of elements for both Main Chamber and the first length of the Serpentine Channels, as previously determined, whilst keeping a coarser mesh for the rest of the device. Therefore, the optimal mesh presents a decreased total number of meshing elements of 553091 (Figure 4a) with a decreased computational time of 291[sec] while maintaining the same accuracy of results, where oxygen concentration values remain below the referenced percentage of 18% dissolved oxygen.

Fig. 4 - Percentage of Oxygen Concentration plotted as function of the number of meshing elements, for the whole device (in blue), main chamber (in red) and initial length of Serpentine Channels (in yellow)

OVERVIEW OF PARAMETERS

The present model is described by a high number of variables that, before being optimized, need to have their own individual influence ranked and characterized within the entire spectrum of the optimizable parameters. This spectrum includes every design feature, so that the entire geometry of the device is assessed, as well as flow magnitude determined to both inlets, to evaluate the optimal flow conditions.
Therefore, an initial study was carried to determine the order of influence each parameter has in gradient development at the main chamber. From this approach, it was possible to organize every parameters within a hierarchical scale of influence on the gradient displayed. Inlet Flow was understood to be the main variable for gradient development, while design features were only interpreted as means for refinement of the same gradient. Thereby, simulations carried afterwards, were performed as a function of Inlet Flow, and lead to further development on characterizing the effect of each individual parameter on gradient refinement. The major parameters considered for optimization are further specified, ranked and characterized in Table 2.

Table 2 - List of parameters considered for optimization

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rank</th>
<th>Effect</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inlet Flow</td>
<td>+++</td>
<td>Gradient development</td>
<td>High Flows may cause harmful environments for cell culture</td>
</tr>
<tr>
<td>Width of Serpentine Channels</td>
<td>++</td>
<td>Control over mixing of species at Serpentines</td>
<td>Width should be defined in regard to the size of Organoids</td>
</tr>
<tr>
<td>Number of Serpentine Loops</td>
<td>++</td>
<td>Regulate the length for mixing of species at Serpentines</td>
<td></td>
</tr>
<tr>
<td>Length of Serpentines</td>
<td>++</td>
<td>Regulate the length for mixing of species at Serpentines</td>
<td></td>
</tr>
<tr>
<td>Width of Intermediate Channels</td>
<td>++</td>
<td>Control over stability of the gradient and concentration lines</td>
<td>Wider channels may cause highly unstable gradients</td>
</tr>
<tr>
<td>Distance between Intermediate Channels</td>
<td>++</td>
<td>Control over stability of the gradient</td>
<td>Distance should be defined considering the resolution limits of the printer</td>
</tr>
<tr>
<td>Width of Main Chamber</td>
<td>++</td>
<td>Define the width of the gradient</td>
<td>A wider chamber may cause highly unstable concentration lines</td>
</tr>
<tr>
<td>Shape of Main Chamber Outlet</td>
<td>++</td>
<td>Stability control of gradient and concentration lines, at the final length of the Main Chamber</td>
<td></td>
</tr>
<tr>
<td>Height of Channels</td>
<td>+*</td>
<td>Regulate flow induced Shear Stress</td>
<td>High Shear Stress may cause harmful conditions for cell culture</td>
</tr>
<tr>
<td>Length of Main Chamber</td>
<td>+</td>
<td>Section concentration lines</td>
<td></td>
</tr>
<tr>
<td>Length of Inlet/Outlet Channels</td>
<td>+</td>
<td>Regulate overall length of device</td>
<td></td>
</tr>
<tr>
<td>Distance between Main Chamber and Serpentines</td>
<td>+</td>
<td>Regulate overall length of device</td>
<td></td>
</tr>
<tr>
<td>Distance between Serpentines</td>
<td>+</td>
<td>Regulate overall width of device</td>
<td></td>
</tr>
</tbody>
</table>

"+++": Gradient Developer – Parameters in which gradient generation is dependent on;
"++": Gradient Refiner – Parameters presenting very distinct features regarding gradient enhancement;
"+": Gradient Neutral – Parameters that, while being important for other purposes, exhibit a very small contribution towards gradient modelling.

*: Defining the optimal Height of Channels, while showing very little effect on gradient modelling, was recognized to be highly important on controlling flow induced shear stress, and avoid values for which cellular culture is compromised.
RESULTS

The optimization process was executed in a top-down approach, starting at the inlet, by defining flow rate, and building from there downwards, until the outlet is reached, following flow direction. This method was employed due to the dependency shown by a given point within the device, towards the features presented by another point positioned before.

The coming analysis will present the features, and respective arguments, that define the optimal solution, following the referred top-down approach. Geometric parameters will be further characterized by comparison between the initial and the optimized design.

**Flow Rate: $1.5 \times 10^3 \, [ul/h]$**—An initial study was performed to define an interval containing the optimal flow rate, by considering steps of order 10 between each value. The flow rate should be ranging between $1 \times 10^3$ and $1 \times 10^4 \, [ul/h]$, as this was the interval where a more uniform and steady gradient would be exhibited across the entire length of the main chamber. The difference in gradient spreading between different inlet flows may be seen in Figure 5a. This approach was followed by a finer study of the obtained interval, defined by steps of $0.5 \times 10^3 \, [ul/h]$, from which the optimal flow rate was defined to be $1.5 \times 10^3 \, [ul/h]$, as it secured the best balance between steady concentration lines (Figure 5b) and a smooth gradient throughout Figure 5c. The value obtained for flow rate was further validated by comparison with a similar sized chamber, for concentration gradients, found in literature, and recalling a flow rate of $44 \, [ul/min] = 2.64 \, [ul/h]$ (Folch et al. 2008).

![Diffusion profile of Oxygen for inlet flows of 1 [ul/h] (on the left) and the optimal flow rate of 1.5 × 10³ [ul/h] (on the right); Steady oxygen concentration lines across the entire length of the device; Smooth gradient line across the main chamber’s width](image)

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(Folch et al. 2008)
Width of Serpentine Channels: 0.3 [mm]; Number of Serpentine Loops: 4; Length of Serpentine Channels: 42.66 [mm] – Due to the defined flow rate, the number of serpentine loops and their respective length was increased to enable full mixing of oxygen at each serpentine unit, by providing a longer path for diffusion to occur. The width of the respective channels was kept the same, so that the Organoids considered for experimental testing, and characterized by a diameter of 0.25 [mm], are not blocked by the channels, and may arrive to the main chamber. Through Figure 6, it is possible to observe the differences caused at each serpentine by changing the mentioned design parameters.

![Fig. 6 - Oxygen concentration levels across the serpentine’s width, measured at the outlet of the respective channel. The gradient displayed was obtained from: (a) the initial design, where full mixing did not occur; (b) the optimized design, with enhanced number of serpentine loops and overall length, enabling the obtention of a steady state for the oxygen concentration levels](image)

Width of Intermediate Channels: 0.5 [mm]; Distance Between Intermediate Channels: 0.15 [mm]; Width of Main Chamber: 3.1 [mm]; Length of Main Chamber: 8 [mm] – Both width of intermediate channels and main chamber were increased, allowing the gradient to spread and flatten across a wider line - by 47.6% - and ultimately, make it more readable for future experimental testing. The distance between intermediate channels was kept the same, while the length of the main chamber was decreased, sectioning concentration lines to an interval where the difference in oxygen concentration remains under 0.1% (Figure 7) The reduced length of the main chamber also contributed to make the device 31.7% smaller, with an overall dimension of 44.1 × 22.2 [mm].

![Fig. 7 - Sectioning the main chamber to prevent a non-desirable variation in the oxygen concentration lines](image)
Shape of the Main Chamber Outlet: Triangular Outlet – Changing from a rectangular shaped outlet to a triangular one, meant that concentration lines became steadier, especially towards the end, without exhibiting an abrupt change as in the previous design. Differences can be observed in Figure 8.

![Oxygen concentration line in: a) initial design; b) optimized design, where the slope is less abrupt](image)

**Fig. 8 - Oxygen concentration line in: a) initial design; b) optimized design, where the slope is less abrupt**

Height of Channels: 0.3 [mm] – Maintaining height of channels at 0.3 [mm] will result in a free flow path, between the main chamber’s upper wall and Organoid interfaces, of only 0.05 [mm] high, further inducing shear stress with values as high as 0.39 [Pa] (Figure 9), close to those found in vivo – between 0.5 [Pa] and 2 [Pa] (Takayama et al. 2005) – and reported to have a positive effect in cellular function (Tanaka et al. 2006).

![Section area of the main chamber, showing the shear stress profile over the area left for free flow, comprised by both Organoid and main chamber’s upper wall interfaces (Organoid interface was approximated as a continuous wall)](image)

**Fig. 9 - Section area of the main chamber, showing the shear stress profile over the area left for free flow, comprised by both Organoid and main chamber’s upper wall interfaces (Organoid interface was approximated as a continuous wall)**

CONCLUSIONS AND FUTURE WORK

By employing a finite element framework for optimization, it was possible to better define testing parameters, improve gradient modelling features, as well as to closely simulating conditions found in vivo, overall developing a model and design, capable of creating a more deterministic, controllable and accurate microenvironment for Organoid culture. For future work, it is intended to explore more complex design features, that may enrich and diversify the gradient displayed, and establish a new study regarding different diluted species and their respective influence. And to further validate the developed finite element model, an experimental prototype is also impending, to establish comparisons between experimental and numerical solutions.
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