Application of Computational Fluid Dynamics as a Simulation-based Design Guide in a Microfluidic 3D Living Cell Array (3D LCA) Device

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Application of Computational Fluid Dynamics as a Simulation-based Design Guide in a Microfluidic 3D Living Cell Array (3D LCA) Device

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Submitted in partial fulfillment of the requirement for the degree

Master of Engineering (Biomedical)
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Abstract

The use of computational fluid dynamics (CFD) as a tool in the aerodynamics and oil industry provides a reinforcement to efficiency in the design of aircrafts or for understanding the flow through pipes. A similar approach can be taken towards microfluidics, where traditionally devices have been designed based on experience or physiological replication, and a CFD simulation is shown post-manufacture to show the flow and diffusion patterns present. In this study, a reverse approach is suggested, by designing the device first in CFD and perform simulations on variations of alterable parameters to get insight on how the changing parameters affect the conditions within the device. A 3D Microfluidic Living Cell Array (3D LCA) wherein cells are embedded in a hydrogel is used as the model to perform the simulations on. It is shown that alterations of certain parameters (such as pore size) can have drastic effects on the nutrient supply and waste removal mechanics of the 3D cell microchamber, while other factors such as the permeability of the medium of cell culture (hydrogel) does not affect the glucose concentration but does affect the O₂ and CO₂ concentrations generated within the microchamber.
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1. Introduction

Computational Fluid Dynamics (CFD) has taken tremendous strides since its advent in the earlier half of the twentieth century. Although the principles were there, the execution of those theories were restrained until the computer industry, driven partly (but strongly) by the necessity of computing power to solve computational fluid dynamics problems, took great leaps in producing more powerful processors and efficient algorithms since the 1970's[1]. The field of CFD has come far enough such that the ownership of CFD software is congruent to owning a wind virtual tunnel. Although computational techniques will never replace true experimental manifestations - since mathematical equations are only models of what we perceive and not a holistic representation of every component a system contains - most of the equations used in the field are derived from physically or experimentally accurate and observed principles. Amongst these principles are the Navier-Stokes' equations, the diffusion (or heat) equation, Stokes-Einstein equation (which represents the diffusivity of molecules based on their size and affinity), the Darcy and Brinkman equations that define porous flow, and many more. Simultaneous solutions of these equations via numerical approximation are what gives CFD its convenience and power.

Dynamic Macro-Systems

The design of dynamic flow systems already exist in the form of various larger devices and in microfluidic devices. Amongst the larger devices are systems that perform on a scale that would not be feasible on a simple microscopic scale - such as bioreactors seeded with hepatocytes, renal dialysis systems, or even artificial hearts. In the case of a bioreactor, the supply of nutrients to the hepatocytes and the corresponding removal of wastes from the bioreactor is of quintessential importance to keep the cells in the natural state (and healthy) for as long as possible. [2] In devices such as the artificial heart, the diffusion factors of nutrients are not pertinent, rather, the flow pattern within the device such as to provide the body with a proper blood supply (approx. 4-5 L/min) and to prevent the damage of the device (such as from cavitation) is to be ensured. Thus, the dynamic demands of various devices may vary from simple flow requirements to the maintenance of a complex balance of nutrient supply and demand.
Devices could be designed based on experience or physiologically relevance. One example is to design a bioreactor (or microfluidic device) in which the distance between the perfusing media and the hepatocytes (or cultured cells) are no more than 100-200 µm away from the perfusing channel. This distance comes from Warburg's calculations for the diffusion limitation of a small molecule (such as oxygen or glucose), and has been experimentally or physiologically confirmed by others.[3]

*The Tumor Microenvironment*

The tumor microenvironment is comprised of tumor cells (parenchymal cells) and the associated stromal structure and cells, including carcinoma-associated fibroblasts, leukocytes, bone-marrow derived cells, and blood and lymphatic vascular endothelial cells. The microenvironment is also characterized by irregular vasculature, high interstitial fluid pressure, high pH levels, abnormal inflammatory response and metabolic chaos.[4-7] Although it is argued as to whether this chaotic environment is the cause or effect of the tumor microenvironment, it is certain that the carcinogenic disposition of cells and such an aberrant microenvironment are correlated.[8] The chaotic microenvironment can promote tumor progression, as various studies have shown.[9-11] These studies also suggest that the normalization (return to normal function) of blood vessels by rendering them less leaky through administration of chemicals that reduce vascular permeability (e.g. VEGF inhibitors) can improve the prognosis of the patient and work synergistically with chemo- or radio-therapy.[4, 6, 7] One of the hallmarks of the tumor microenvironment is hypoxia, which in turn promotes the accumulation of the constitutively produced hypoxia-inducing factor-α (HIF-α), further stimulating tumor progression by promoting various other inducible factors in the cells.[10, 12, 13] Some of the inducible enzymes or factors by HIF-α include vasoactive proteins (endothelin-1, NO synthase-2, plasminogen activator inhibitor-1, vascular endothelial growth factor(VEGF), VEGF receptor), hormones and receptors (such as α1B-adrenergic receptors, transforming growth factor β5), and enzymes that promote energy and iron metabolism. All these factors help the cell to adapt to the low-oxygen environment.[14]
Developing a 3D microfluidic living cell array (3D LCA) device

One of the goals in trying to design a microfluidic device is to mimic the tumor microenvironment, including the blood vessel which supplies the nutrients and a scaffold (gel) into which the perfusing nutrients can diffuse into while housing the cells. Designing a device for investigating physiologically accurate cellular activity will by necessity be a 3D culture with a continuous perfusion, as opposed to simple, static 2D (or 3D) culture. Static 2D/3D cultures or animal models are poor (or inaccurate) imitations of the true in vivo dynamic representation of human physiological conditions. Mimicking the in vivo conditions by 3D in vitro dynamic models will provide a more accurate drug responses of cancer cells (cell lines or biopsy samples). A 3D microfluidic LCA mimicking such microenvironment enables accurate, high-throughput drug screening on an efficient scale. Previous work from our lab produced a device consisting of multiple wells arranged in an array format with each well being an individual cell culture chamber.[15] There are three layers in the device: (1) the top rectangular channels with a width of 790 µm (corresponding to the width of pulmonary veins, >500 µm) and height of 130 µm; (2) a middle and gas permeable membrane with 40 µm clustered pores, which provides a frame over which endothelial cells can be cultured and still allow perfusion and diffusion between the top channel and the bottom chamber; and (3) the bottom chambers right underneath the clustered pores with a diameter of 770 µm, and a depth of approximately 100 µm, where cells can be are seeded and encapsulated in hydrogel (e.g. PuraMatrix). The measured viscous resistance \( \frac{1}{\text{permeability}} \) of PuraMatrix is \( 5 \times 10^{14} \text{ m}^2 \), which is a first step in emulating the stiffness of the tumor microenvironment in the tumor microenvironment, depending on the tumor site, the permeability can increase by up to two orders of magnitude (to the order of \( 5 \times 10^{12} \text{ m}^2 \) visc. resistance)[16]. The lack of functional lymphatic systems within a solid tumor is captured in the device by not having an outlet at the bottom of the chamber.

The importance of design
Designing by experiment does not always meet the conditions we need to mimic in a cell culture model. Instead of taking a trial-and-error approach by intuition, or using a post-manufacture CFD analysis to “show” what is going on in the device, it would be more economical and efficient to use CFD to guide the understanding of how different device parameters would provide a different set of conditions in the cell culture. The current 3D LCA device is hardly a perfect representation of the tumor microenvironment, but is a step towards that goal. A CFD model can provide an insight into the effects on flow and diffusive profiles induced by changing the porosity and thickness of simulated basement membrane (the middle layer) or by changing the permeability of the microenvironment (the hydrogel). By understanding the physiological effects that are being recreated, such as low $pO_2$ and pH, or high interstitial fluid pressure, it is possible to grasp the scope of the environment that the seeded tumor cells in the device are exposed to, and how these parameters can be changed to provide a device with the optimal (or desired) conditions. For instance, if the pleiotropic effects of hypoxia needs to be studied (such as testing drugs that only work in hypoxic conditions [8]), there would be a necessity to induce a relatively hypoxic state in the extracellular environment.

The computational power of CFD is avidly used in the aerodynamics and oil industry to simulate models of flow over vehicles or of oil flow in pipes prior to the construction of the actual objects[1]. A similar approach can be taken towards the design of biological devices such as 3D microfluidic devices. In this study, only one well of the entire device is considered to keep the computational time and resources within reason. By altering certain physical parameters of the device itself, and by providing the correct inputs to the system, a generalized model is obtained which provides the optimal conditions that are sought from the device to be built. Although there will be practical limitations of the accuracy of the device itself (for instance, the actual permeability of the constructed hydrogel in the device may not be fully isotropic and slightly different from the parameter input into the software) as well as manufacturing errors that cannot be pre-determined, the simulated scenarios of the environment produced in the device itself will provide a starting point on the selections to use that will optimize the parameters (e.g. fluid flow...
through the device, pO₂ and pH in the cell culture chamber, etc.) by showing effects on such parameters that are induced from alterable parameters in the device simulation (e.g. pore size of the membrane within the device, permeability of the hydrogel, concentration of glucose or other nutrients, etc). The discrepancy of the simulation from the actual observed result can be noted later, after the device has been manufactured and experimental tests are run.

2. Methods & Materials

Equations and Assumptions of Importance to Fluid Mechanics Models in Biological Systems

Computational Fluid Dynamics obeys the principles of fluid dynamics, which are derived from observed physical principles. The equations that are most important and used in the computation are provided in this section. These concepts are inherently included in the software package used, ANSYS Fluent, part of the ANSYS®v14 Workbench. ANSYS Fluent is a powerful CFD tool with customizable geometries, material properties, flow profiles, and reaction rates, which are all essential to the definition of the model being simulated. It is important to understand the equations that are being implemented or worked with as a mathematical model with incorrectly formalized parameters will provide an incorrect and most likely irrelevant solution that may evade verification due to the lack of realistic experimental implementation.

Preliminary Mass, Momentum, and Energy conservation Equations

Non-uniform flow patterns can lead to poor nutrient supply and non-uniform shear stress distribution. Although in this paper the focus is not placed on shear stresses, it may be pertinent to those who wish to culture cells that are sensitive to shear stresses (such as endothelial cells). Typically in a μ-fluidic (microfluidic) device, the flow profiles are well defined if the parameters are chosen accordingly.

The continuity equation which essentially comes from the reasoning that mass can neither be gained nor lost but only transferred, is represented as:
\[
\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \mathbf{V}) = 0 \quad (1a) \quad \text{or} \quad \frac{\partial \rho}{\partial t} + \rho \nabla \cdot \mathbf{V} = 0 \quad (1b)
\]

Where \( \mathbf{V} \) is the velocity vector of the fluid flow, \( \rho \) is the fluid density, \( \frac{\partial \rho}{\partial t} \) is the total differential change in \( \rho \cdot \frac{\partial \rho}{\partial t} \) is the partial change of density with respect to time (local change), and \( \nabla \) is the del operator as defined in standard vector calculus books.

The momentum conservation (Navier-Stokes) equations are as follows:

\[
\rho \frac{D u}{D t} = -\frac{\partial p}{\partial x} + \frac{\partial \tau_{xx}}{\partial x} + \frac{\partial \tau_{yx}}{\partial y} + \frac{\partial \tau_{zx}}{\partial z} + \rho f_x \quad (2a)
\]

\[
\rho \frac{D v}{D t} = -\frac{\partial p}{\partial y} + \frac{\partial \tau_{xy}}{\partial x} + \frac{\partial \tau_{yy}}{\partial y} + \frac{\partial \tau_{zy}}{\partial z} + \rho f_y \quad (2b)
\]

\[
\rho \frac{D w}{D t} = -\frac{\partial p}{\partial z} + \frac{\partial \tau_{xz}}{\partial x} + \frac{\partial \tau_{yz}}{\partial y} + \frac{\partial \tau_{zz}}{\partial z} + \rho f_z \quad (2c)
\]

Where \( p \) is the fluid pressure, \( \rho \) is the fluid density, \( u, v, \) and \( w \) are the velocity components in the Cartesian \( x,y, \) and \( z \) directions, \( \tau_{ij} \) are elements from the shear stress matrix in the respective direction \( (i) \) along the plane defined by the \( j^{th} \) normal vector, and \( f_i \) is the body force per unit volume in the \( i^{th} \) direction.

The Navier-Stokes’ Equations for viscous Newtonian fluids is given by the set of equations in 2a-2c, with the added constitutive information for the stress-viscosity relationship (\( \tau \) is proportional to velocity gradients):

\[
\tau_{xx} = \lambda (\nabla \cdot \mathbf{V}) + 2\mu \frac{\partial u}{\partial x}, \quad (3a) \quad \tau_{yy} = \lambda (\nabla \cdot \mathbf{V}) + 2\mu \frac{\partial v}{\partial y}, \quad (3b) \quad \tau_{zz} = \lambda (\nabla \cdot \mathbf{V}) + 2\mu \frac{\partial w}{\partial z}, \quad (3c)
\]

\[
\tau_{xy} = \mu \left[ \frac{\partial v}{\partial x} + \frac{\partial u}{\partial y} \right], \quad (3d) \quad \tau_{yz} = \mu \left[ \frac{\partial w}{\partial y} + \frac{\partial v}{\partial z} \right], \quad (3e) \quad \tau_{xz} = \mu \left[ \frac{\partial u}{\partial z} + \frac{\partial w}{\partial x} \right]. \quad (3f)
\]

Where \( \lambda \) is the second viscosity coefficient, while \( \mu \) is the molecular viscosity. As per Stokes’s hypothesis,
\[ \lambda = -\frac{2}{3} \mu \quad (4) \]

This is frequently used but the validity has not been confirmed. When defining the viscosity of a problem, we typically define \( \mu \).

Energy must also be conserved in the system, and the derived energy conservation equations become:

\[ \frac{\partial}{\partial t} \left[ \rho \left( e + \frac{v^2}{2} \right) \right] + \nabla \cdot \left[ \rho \left( e + \frac{v^2}{2} \right) \mathbf{V} \right] = \rho q + \frac{\partial}{\partial x} \left( k \frac{\partial T}{\partial x} \right) + \frac{\partial}{\partial y} \left( k \frac{\partial T}{\partial y} \right) + \frac{\partial}{\partial z} \left( k \frac{\partial T}{\partial z} \right) - \frac{\partial (\mu p)}{\partial x} - \frac{\partial (\mu p)}{\partial y} - \frac{\partial (\mu p)}{\partial z} \]

\[ \frac{\partial (\omega p)}{\partial x} + \frac{\partial (\omega u_{xy})}{\partial y} + \frac{\partial (\omega u_{xz})}{\partial z} + \frac{\partial (\omega v_{xy})}{\partial x} + \frac{\partial (\omega v_{yz})}{\partial z} + \frac{\partial (\omega w_{xz})}{\partial y} + \frac{\partial (\omega w_{yz})}{\partial z} + \frac{\partial (\omega w_{xz})}{\partial y} + \frac{\partial (\omega w_{yz})}{\partial z} + \rho f \cdot \mathbf{V} \]

\[ (5) \]

Note the difference between the scalar velocity magnitude \( V \) and the vector velocity \( \mathbf{V} \).

An important number that characterizes flow is the Reynolds number, given as:

\[ Re = \frac{\rho V d}{\mu} \quad (6) \]

Where \( \mu \) is the dynamic viscosity, \( d \) is the characteristic length of the problem, \( V \) is the characteristic velocity of the problem, and \( \rho \) is the fluid density. A Reynolds number less than 1400 corresponds to laminar flow, and above it we transition into intermittent or fully developed turbulent flow. Note that in a \( \mu \)-fluidic device, doing a simple dimensional analysis will reveal that the Reynolds number is much less than 1:

\[ \rho \sim \frac{10^3 kg}{m^3}, \quad V = \frac{10^{-4} m}{s}, \quad d \sim 10^{-3} m, \quad \mu \sim 10^{-3} Pa \cdot s, \] giving the value of \( Re \sim 0.1 \). Thus we can use a laminar solver for the CFD model (CFD softwares have different solving methods for laminar and turbulent flow).

**Flow through porous media**
Newtonian fluid flow through a porous medium at low Reynolds number could be modeled using Darcy’s flow, given as:

$$\frac{-\mu q}{\kappa} = \nabla p \quad (7a), \quad v_s = \frac{q}{n} \quad (7b),$$

where 'q' is the fluid velocity (calculated), n is the porosity, and $v_s$ is the true fluid velocity to account for the limited volume introduced by having a porous material.

The scale of µ-fluidic devices are so small that the Reynolds number is small (<1) by the very implementation of the device. However, Darcy’s flow does not capture the viscous effects, and inviscid flow typically cannot account for the no-slip boundary conditions. For modeling flow through thin slices or small sections, the gradient of velocity becomes an important factor and cannot be neglected by using simply Darcy’s flow model. In such a case, the Brinkman model is used, which accounts for the viscous effects of the fluid. The equation is given as:

$$\mu \nabla^2 u_s - \frac{\mu}{\kappa} u_s = \nabla p \quad (8)$$

Where $u_s$ is the superficial velocity vector, $\mu$ is the viscosity, and $\kappa$ the permeability of the porous medium.

Note that the equation reduces to the Navier-Stokes equation or Darcy’s equation depending on whether the viscous forces or drag forces become dominant. Thus, in the transition between these two flows – such as flow in the µ-fluidic device where the flow goes from free-flow into a porous media, the full Brinkman implementation should be used. This allows the continuity of velocity and shear stresses, especially at the interface. Note as an example that flow in open channels (permeability being almost infinite) will reduce the Brinkman equation to the Navier-Stokes equation for incompressible flow. [17]

*Diffusivity and Convection*
The primary purpose to having a constant flow is to mimic, to a certain degree, the physiological condition of having a continuous supply of fresh nutrients as well as a continuous “exhaust” or flushing out of waste products. A well balanced system should match the supply of nutrients to the reaction rate of the cells such that there is neither an accumulation nor a deficit. Thus, in a well balanced system, at dynamic equilibrium, the local increase in concentration \( \frac{\partial c}{\partial t} \) should be 0. The convection-diffusion equation is as follows:

\[
\frac{dc}{dt} + \nabla \cdot N - R = 0, \quad (9a)
\]

\[
N = -D \nabla C + v \cdot C \quad (9b)
\]

Where the variables are:

- \( C \): concentration of the species (mol/m\(^3\)),
- \( N \): molar flux (mol/m\(^2\).s),
- \( R \): consumption rate of the species (mol/m\(^3\).s),
- \( v \): velocity as calculated from Brinkman’s equation (m/s), and
- \( D \): diffusivity of the solute (m\(^2\)/s)

At equilibrium, \( \frac{dc}{dt} = 0 \), reducing the equation to:

\[
\nabla \cdot (-D \nabla C + v \cdot C) = R \quad (9c)
\]

Bulk diffusivity varies from the actual effective diffusivity since the entire area normal to the diffusive flux is not available in the porous medium. The effective diffusivity of a species through a porous structure thus differs from the bulk diffusivity due to the porosity and tortuosity of the material. Tortuosity is defined as the ratio between the actual distance traveled by a species particle between two
points and the smallest distance between those very two points, and it is commonly represented by the variable $\tau$, and this often takes values ranging from 2 to 10.[18] Representing the porosity as $\varphi$, there is an equation that relates the bulk diffusivity to diffusivity throughout a porous medium (using oxygen as an example):

$$D_e = \frac{D_{O_2}\varphi}{\tau}, \quad (10)$$

Where $D_{O_2}$ is the bulk diffusivity of the species (in this case oxygen) in the media perfusing through the porous structure, $\varphi$ and $\tau$ are, as aforementioned, the porosity and tortuosity, respectively.

A system may or may not be diffusion limited, and the quantitative way to acknowledge this is to calculate the Peclet (Pe) number. The $Pe$ number is a ratio of the convective transport and the dispersion (or diffusive) coefficient, and is given as:

$$Pe = \frac{vd}{D} \quad (11)$$

Where $D$ is the diffusion coefficient as before, $v$ is the characteristic fluid velocity, and $d$ is the characteristic length of the model in use. A $Pe$ number between 0 and 1 indicates that the transport of the species to the site of interest is primarily diffusion limited, at larger $Pe$ the transport of the species is mainly convection-driven.

A model equation by Warburg predicts the possible thickness of a region of healthy cells possible by diffusion alone using blood as the non-circulating fluid would be 1mm for a cylinder-shaped tissue or complete developing organism, calculated by:

$$r_{\text{max}} = \sqrt[4]{\frac{4D_{O_2}B^p}{V_0}} \quad (12)$$

Where $r_{\text{max}}$ is the maximum distance, $D_{O_2}B$ is the bulk diffusivity of oxygen in blood, $P$ is the partial pressure in the culture medium (or blood), and $V_{O_2}$ is the rate of oxygen consumption in the tissue. In
physiological conditions, especially around capillaries, the distance limit set by diffusive transport of nutrients is typically 100-200 µm, cells beyond this region enter a state of hypoxia (such as the necrotic cells in the center of a solid tumor mass deprived of oxygen due to their distance from active capillaries at the tumor periphery). This phenomenon is also commonly noticed in bioreactor design, where, for a hepatocyte culture, it was shown that a reactor design that relies solely on diffusion for mass transfer requires that the cells be within 150-200 µm of an oxygen source to survive and proliferate.

**Reaction Mechanisms and Rates:**

Reaction kinetics can be implemented by user-defined methods into a CFD application. The bulk volumetric reaction can be defined that occurs at given regions or zones in a model (such as the bottom chamber, the top channel, or the middle membrane) – or can be discretized based on location within the model. It lies upon the user to select the model most suitable for the reaction model.

Reactions that define the glucose consumption or oxygen consumption rate are typically modeled using Michaelis-Menten kinetics:

\[-r = \frac{MC}{K+C} \quad (13)\]

Where \( r \) is the reaction rate (positive indicates creation of the species), \( M \) is the maximum reaction rate possible (such as by increasing the species), \( K \) is the Michaelis constant (the concentration of the species at which the reaction rate is half of \( V \)), and \( C \) is the current concentration of the species itself.

In a culture where there is cell growth, the reaction rate is modeled as:

\[-r = \frac{1}{Y_{X/n}} \frac{dX}{dt} + mX \quad (14)\]

Where \( X \) is the cell concentration, and \( Y_{X/n} \) is the yield coefficient of cell concentration on component concentration (how the growth rate affects the consumption of the species), and \( m \) is the maintainence
rate for the cells are currently in existence. Under limiting nutrient and space considerations (and cell-cell contact inhibition), the growth of cells can typically be modeled as:

\[
\frac{dx}{dt} = \mu X \left(1 - \frac{x}{X_{\text{max}}}\right) \tag{15}
\]

where \(\mu\) is the growth rate constant, and \(X_{\text{max}}\) is the maximum cell density permissible. The nutrient limitation is inherently considered in the growth rate by modeling it as another equation:

\[
\mu = \frac{\mu_{\text{max}} C}{K + C} \tag{16}
\]

Where \(C\) is the concentration of the nutrient as in the Michaelis-Menten equation.

The typical values for the consumption rate used in a 3D model are derived from 2D cultures done on a plastic culture surface. Cells respond differently when cultured on different substrates or when grown in different configurations (2D or 3D), and experimentally determined kinetic parameters that are truer to 3D culture should be collected in order to make the CFD model closer to a realistic realization.

However, for the purposes of this paper, the cultures were done in a 2D culture plate using PC9 non-small-cell lung cancer cells. The procedure and data are discussed later, in the “Parameters defined in the model” sub-section and the Results section.

Consider the quasi-static case in which the residence time of the nutrients is much smaller than the cell growth rate, in which case we can ignore the cell growth rate and model the system at a fixed, known, cell density. In this case, eqn. 14 reduces to \(-r = mX\). An important assumption here is that \(K\) is much smaller than \(C\), since otherwise if \(K \gg C\), the \(\mu\) term becomes large enough such that the \(\frac{dx}{dt}\) term cannot be ignored. A small \(K\) value also denotes that the nutrient uptake rate is almost independent in sensitivity to the local concentration of the nutrient itself. Such examples are seen in chondrocytes and hepatocytes where the uptake rate is reduced by only 2-10% with a 50% reduction in oxygen
concentration. On the other hand, there was a 35% reduction in smooth muscle cells for the same 50% reduction in oxygen tension.[17]

**Parameters defined in or by the model**

The shear stress in a rectangular channel is given as:

\[
\tau = 6\mu Q / wh^2, 
\]

where \(Q\) is the flow rate, \(w\) and \(h\) are the channel width and height respectively, and \(\mu\) is the dynamic viscosity of the fluid in question. Using a flow rate of 100 \(\mu\)m/sec, the calculated shear stress value is = 0.05dyne/cm\(^2\), which is within the ideal range for a wide variety of mammalian cell lines. (<0.1 dyne/cm\(^2\) and >0.01 dyne/cm\(^2\))[3] As described before, the simulated device comprises of one well in the device, which is made up of three layers: (1) a top layer with a width of 790 \(\mu\)m, a height of 100 \(\mu\)m, and a length of 1600 \(\mu\)m, (2) a gas-permeable middle membrane layer with pore sizes varying from 10-40 \(\mu\)m, and the thickness of this layer varying from 10-40 \(\mu\)m as well, and (3) the bottom cell culture chamber which is 200 \(\mu\)m in depth with a diameter of 770 \(\mu\)m, containing a 99% isotropic porous material (to simulate Puramatrix hydrogel) with a viscous resistance (1/permeability) ranging from 5x10\(^{14}\) m\(^{-2}\) down to 5x10\(^{12}\) m\(^{-2}\). This cell culture chamber is assumed to be comprised of 50% of PC9 non-small cell lung cancer cells (as in physiological conditions), which in turn affects the reaction rate.[8]

**Peclet Number:**

The Peclet number on a dimensional analysis basis for the models used in this paper is:

\[
Pe = \frac{vd}{D}, \\text{where } v \sim \frac{10^{-11} \text{to} 10^{-8} m}{s}, \quad d \sim 10^{-4} m, \quad D \sim \frac{10^{-7} m^2}{s},
\]

Which gives \(Pe \sim 10^{-6} \text{ to } 10^{-3} \left(< < 1\right). The velocity chosen is the velocity in the porous media (as obtained after simulations), indicating that in the porous media, the transport of nutrients is mainly
diffusion driven. However, it is shown by simulation results that depending on the magnitude and direction of velocity, the effects of diffusion can be countered.

**Diffusivities used:**

The diffusion coefficients employed in this model[15], at a reference temperature of 298.15 K, are: (Note: all numbers are in units of $10^{-9}$ m$^2$/s)

In water:

$\text{O}_2$: 2.1, $\text{CO}_2$: 1.6, Glucose: 0.71, Water: 2.2

In PDMS:

$\text{O}_2$: 1.6, $\text{CO}_2$: 1.1, Glucose: 0, Water: 0

**Input Concentrations:**

Culture systems typically allow, at atmospheric oxygen tension, up to 0.2 mmol/L of $\text{O}_2$, which, at a molar mass of 15.99 (or 16) g/mol, equates to approximately $3.2 \times 10^{-6}$ g/cm$^3$ in solution. Assuming water as the primary solvent and with a density of 1 g/cm$^3$, this gives a mass fraction of dissolved $\text{O}_2$ of $3.2 \times 10^{-6}$ g/g.

Similarly, under physiological conditions, there is approximately 1 g/L of glucose in blood. This formulation is also used in several common media. This concentration equates to 1 g/1000 cm$^3$, or, using water as the base solvent, 1 g/1000 g = 0.001 g/g when expressed as a mass fraction.

Although typical cell culture incubators are run at 5% $\text{CO}_2$, for the purposes of this simulation, there was no input $\text{CO}_2$ so as to see the progressive build up within the chamber based only on the cells' reaction rate as well as convective and diffusive effects.

**Reaction Rate:**
The reaction rate was measured for cells cultured in 8 wells of two 6-well culture plates, seeded at a density of approximately 400,000 cells/ml. The cells were given 12 hours to attach to the plates. At this time, 2 additional control samples with no cells (only media) was loaded onto two empty wells of the 6 well-plates, and over the course of the next 8 hours, media from the plates were collected for each sample every hour. At the end of the 8 hours, the measured average cell density was 418,000 cells/ml. The 2 additional controls having no cells (which were averaged) were used to normalize the data from the 8 samples. The amount of glucose in the medium was measured using a GAGO Glucose Assay Kit (Sigma Aldrich). Over the duration of the 8 hours of sample collection, 20 µl of the medium from each sample was collected every hour after lightly shaking the culture plate to help make the media well-mixed. Each collected sample was stored in each well of a 48-well culture plate which was refrigerated and sealed after every collection. 1ml of deionized water was added to each of the samples to bring the concentration to the recommended amount in the kit's protocol (20-80 µg/ml). From these diluted samples, 30 µl from each sample was loaded onto each well of a 96-well plate. To each of these 30 µl samples, 60 µl of the GAGO glucose assay reagent was added and incubated for 45 minutes at 37°C. After the incubation period, another 60 µl of sulphuric acid (at 12 N molality) was added to stop the reaction. The absorbance from each sample was then read and used as a measure to determine the glucose concentration, with the 4.5g/L concentration in the media being the standard to calibrate against. The results collected over every hour for 8 hours are tabulated in the Results section, and from the table, and a calculated average consumption rate of 0.00195 mg/ml-hr, or, 5.272x10⁻⁸ kmol/m³-s sec was used. Typically, under the 0.2mmol O₂/L described before, the rate of oxygen uptake by human skin fibroblasts is about 0.064mmol O₂/L-h, while it was 0.3mmol O₂/L-h for the same seeding density of hepatocytes. This equates to a range of 1.78x10⁻⁸ – 8.33x10⁻⁸ kmol/m³-s sec. The basic equation for aerobic respiration is:

\[ C_6H_{12}O_6 (glucose) + 6O_2 \xrightarrow{\text{yields}} 6H_2O + 6CO_2 \]
Thus, the rate of glucose consumption should be $\frac{1}{6}$th of the $O_2$ consumption rate by stoichiometric evaluation. Therefore, the rate of glucose consumption matched with the rate of $O_2$ consumption is $2.96 \times 10^{-9} - 1.39 \times 10^8$ kmol/m$^3$-sec. The measured rate in PC9 cells of $5.272 \times 10^8$ kmol/m$^3$-sec is within an order of magnitude of this range.

Chamber parameters used:

Various parameters were altered in the simulation for the intended device. These parameters include:

1. Pore sizes in the middle membrane: 40 µm, 30 µm, 20 µm, and 10 µm
2. Thickness of the middle membrane: 40 µm, 30 µm, 20 µm, and 10 µm
3. Viscous resistances $\left(\frac{1}{\text{permeability}}\right)$ of the porous bottom chamber: $5 \times 10^{14}$, $5 \times 10^{13}$, and $5 \times 10^{12}$ m$^{-2}$

Fixed dimensions in the model include:

1. 200 µm depth and 770 µm diameter for the bottom chamber
2. Top channel width of 790 µm, height of 100 µm, and length of 1600 µm
3. 770 µm diameter for the middle chamber to sit on top of the bottom chamber perfectly

Not all parameters were altered, for instance, to collect the data for the change of velocity profiles (or in the change of nutrient concentration) based on the change in permeability, one particular case was picked (such as the 20 µm pore size and a membrane thickness of 30 µm) and the permeability altered in the bottom chamber for those physical conditions. The Results section elucidates on the models picked to display the corresponding data.

The Model: ANSYS FLuent v. 14

The structure of the model and its meshed counterpart is shown in Fig 1. Depending on ANSYS Fluent's own adaptive meshing (and through some mesh inflation when necessary), a typical mesh element will
have 100,000 - 500,000 nodes. Smaller pore sizes introduce more discontinuities, creating a mesh with more elements than the same structure with a larger pore size.

**Figure 1:** The model designed with the pores is shown in (A). The generated mesh is shown in (B).
3. Results and Discussion

I. Measurement of Glucose consumption rate:

Glucose consumption rate was measured using the Glucose Assay-GO(GAGO) Kit from Sigma Aldrich.

The averaged absorption values from the controls are tabulated in the last column of Table 1, while the absorbance values are tabulated with each sample corresponding to each column. ( (x) - represents unavailable data). A higher absorbance value corresponds to a higher glucose level, with the reference value signifying a concentration of glucose at 4.5g/L when the absorbance is 150. At certain time points it may appear that the glucose concentration goes up instead of dropping, such as in sample # 2 during the 2-3 hour period. The increase is actually due to the medium evaporating over time, thereby concentrating the glucose levels in the medium, and this effect is noted in the reference well(s) as their absorbance (and hence concentration of glucose) steadily increase over time. It is important to normalize the measured value of the absorbance from the medium in the cell cultures with the medium without cells. This correctly captures the drop in glucose levels over time in the cell cultures. [Table 2]

<table>
<thead>
<tr>
<th>Time(hours)</th>
<th>Sample #</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>84</td>
<td>87</td>
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<tr>
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<td>72</td>
<td>83</td>
</tr>
<tr>
<td>8</td>
<td>75</td>
<td>75</td>
</tr>
</tbody>
</table>

Table 1: The measured absorbance values are tabulated above for 8 samples, over a period of 8 hours. The reference sample is the medium, at a concentration of 4.5g/L. The reference values are an average of two samples.

The absorbance values are then normalized to glucose concentrations based on the calibration that the media was originally constituted of 4.5g/L (a value of 1 is equivalent to this concentration).
Table 2: The measured absorbance values are expressed as a fraction of the corresponding reference value at the given time. Normalization is important to account for the effect of the medium evaporating over time in the incubator and increasing the concentration of glucose within it.

The third table below simply sums up the consumption rate observed in each sample, following from the normalized amount of glucose measured in the media.

Table 3: Measuring the consumption rate as a difference of the normalized absorbance values. Since the values are normalized, the change noted here is already expressed as the fractional change from the original 4.5g/L reference concentration.

The average consumption amongst all samples comes out be about 2.6%(±0.7%). This equates to 0.0855-0.1485mg/ml-hr for each well in the cell culture.

The graphical representation of the tabulated data is shown below:
Figure 2: The data shown in TABLE is shown here in compact form, representing the averaged normalized absorbance value over time.

Cell density as counted using the Hemacytometer (Hausser Scientific, USA) was 418,000 cells/ml.

*Calculating Cell consumption rate to use in the simulation:*

Using the average consumption of 0.117 mg/ml (2.6% of 4.5g/L), and a cell count of 418,000 cells/ml, the consumption per cell is quantified as 4.0625x10⁻⁹ mg/ml-hr per cell. With chamber dimensions in the bottom chamber of height 200 µm, a diameter of 770 µm, and an assumed spherical cell shape in 3D culture with a radius of 10 µm, the number of cells that can be accommodated in the bottom chamber at a seeding density of 50% is 8,420. *The consumption rate for this number of cells in the 0.00705mm³ chamber volume is 9.50x10⁻⁹ g/L-s or 5.27210x10⁻⁸ Kmol/m³-s using the molar mass of glucose as 180.0634g/mol. The reaction rates in ANSYS Fluent v.14 have to be implemented in units of Kmol/m³-s.*

The effects that are expected from the alteration of different parameters within the modeled device fit the expectations approached from a qualitative intuition (with a few exceptions); however, the quantitative values allows the placement of a scale to this intuitive disposition.
Figure 3: (A) shows the isometric representation of the chamber, with the direction of flow of the medium as shown. (B) The location of the plane on which the contours or streamlines are displayed is shown here in isometric view.

Figure 4: The plane shown in Fig (B) is shown here from the side, as is used to represent various contours and streamlines. Any plotted data for particular variables (velocity, O$_2$, CO$_2$, and glucose concentrations) are plotted along one of the lines (Line 1 - Line 5) as shown on the plane, in the direction away from the middle membrane as shown ("Distance from the middle membrane"). Since the bottom chamber is 200µm thick, any plotted data will extend up to 200 µm.
Representation of Data/Results

Note that the results shown in the figures below are either shown from an isometric viewpoint (Fig. 3) or at the cross-section through the longitudinal axis of the device (with flow going from the left to the right direction) as in Fig. 4. The graphs are plotted with data acquired along 5 lines through this cross-section, spaced 150 µm apart between each pair, as also shown in Fig 4. The lines are labeled 1 through 5 progressively going from left to right (in the direction of flow). The data is plotted along the lines as we move away from the middle membrane (shown in the figure as "Distance from middle membrane").
Changes in pore size

Figure 5: The streamlines along the plane is shown for dimensions: Pore Diameter of 40µm, membrane thickness of 40µm, and a viscous resistance(hydrogel) of $5 \times 10^{14} \text{ m}^{-2}$.

Figure 6: The contour along the plane is shown for dimensions: Pore Diameter of 40µm, membrane thickness of 40µm, and a viscous resistance(hydrogel) of $5 \times 10^{14} \text{ m}^{-2}$.

Figure 7: The streamlines along the plane is shown for dimensions: Pore Diameter of 20µm, membrane thickness of 40µm, and a viscous resistance(hydrogel) of $5 \times 10^{14} \text{ m}^{-2}$.
Figure 8: The streamlines along the plane is shown for dimensions: Pore Diameter of 20µm, membrane thickness of 40µm, and a viscous resistance (hydrogel) of $5 \times 10^{14} \, \text{m}^{-2}$. 

Figure 9: The streamlines along the plane is shown for dimensions: Pore Diameter of 10µm, membrane thickness of 40µm, and a viscous resistance (hydrogel) of $5 \times 10^{14} \, \text{m}^{-2}$. 

Figure 10: The streamlines along the plane is shown for dimensions: Pore Diameter of 10µm, membrane thickness of 40µm, and a viscous resistance (hydrogel) of $5 \times 10^{14} \, \text{m}^{-2}$. 
Figure 11: The streamlines along the isometric view is shown for dimensions: Pore Diameter of 10µm, membrane thickness of 40µm, and a viscous resistance(hydrogel) of $5 \times 10^{14}$ m$^{-2}$.

Effect on streamlines: As the pore size is decreased from 40 µm to 10 µm, the flow becomes smoother – almost a similar effect to that seen in lasers or optical fibers – the larger the length to width ratio of the tube through with the laser or light passes, the more the likelihood of a collimated light beam coming out at the output end. This effect is noticed by looking at the streamlines from Figs. 5, 7, and 9.
Figure 12: The velocity along Line 1 is shown for different pore sizes, membrane thickness of 40µm, and a viscous resistance(hydrogel) of 5x10^{14} m^2.

**Effect on velocity profile**

Reducing the pore size typically constrains the flow of fluid tangential to the main flow. If the flow was directly fluxing through the middle layer only, then by the principles of momentum and mass conservation (or even by Bernoulli's principle), the flow speed should increase with a decrease in pore size. However, in the case where the fluid is taking a secondary flow path through the membrane pores and hydrogel, the size of the pores positively correlate to the flow - the larger the pore size, the faster the perfusion speed through the gel. The change is most significant between the 40 µm and 20 µm pore sizes or between the 40 µm and 10 µm pore sizes. The range of velocities as seen from the data along Line 1 (Fig. 12) is as follows: 5.1x10^{-9} to 5.12x10^{-12} m/s for the 40 µm pore, 5.73x10^{-9} to 1.36x10^{-12} m/s for the 20 µm pore, and 3.67x10^{-9} to 2.10x10^{-12} m/s for the 10 µm pore. When the velocity is plotted on a log
scale, the velocity gradients along the chamber depth for the different pore sizes show a similar profile between 20 and 40 µm, but in terms of actual velocity magnitude, the 10 and 20 µm are closer on the log scale. One noticeable point is that less than a depth of ~60 µm, the flow from the 20 µm pore is higher than in the 10 µm pores, but after that this role switches with the 10 µm pore providing a slightly faster flow.

**Effect on oxygen concentration**

The conditions created by using a 10 µm pore shows drastic results over the use of a 40 µm pore. With a 10 µm pore size, the chamber is seen to receive more oxygen than in the case of the 40 µm pore, by about 0.8% (Fig. 13) This is possibly due to the gas permeable membrane which is open to diffusion of oxygen, and the comparatively “turbulent” flow in the 40 µm pore size device may drive against the diffusive gradient of oxygen.

**Figure 13:** The O<sub>2</sub> concentration along Line 1 is shown for 40 µm and 10 µm pore sizes, membrane thickness of 40 µm, and a viscous resistance(hydrogel) of 5x10<sup>14</sup> m<sup>-2</sup>

**Effect on Glucose Concentration**
The levels of glucose drop slightly in the 10 µm pore size compared to the 40 µm pore size structure. This is expected as the flow is reduced in the smaller pore and glucose cannot diffuse through the gas permeable middle membrane like O$_2$ or CO$_2$. The difference is about 0.5%.

![Glucose Concentration Graph](image)

**Figure 14:** The glucose concentration along Line 1 is shown for 40 µm and 10 µm pore sizes, membrane thickness of 40µm, and a viscous resistance(hydrogel) of $5 \times 10^{14}$ m$^{-2}$

**Effect on Carbon Dioxide Concentration:**

The carbon dioxide profile shows a slightly higher concentration for the 10 µm pores compared to the 40 µm pores (by about 1.7%). This is most likely attributable to the slow flow through the medium, allowing only diffusion to move the CO$_2$ from the bottom of the chamber to the top. For the rather mixed, non-streamed flow through the 40 µm pores, the CO$_2$ can be delivered faster to the middle membrane from where it can be convected and diffused out.
Figure 15: The CO$_2$ concentration along Line 1 is shown for 40 µm and 10 µm pore sizes, membrane thickness of 40µm, and a viscous resistance(hydrogel) of $5 \times 10^{14}$ m$^{-2}$. 
Changes in middle membrane thickness (change in the pore depth)

**Figure 16:** The contour along the plane is shown for dimensions: Pore Diameter of 20µm, membrane thickness of 40µm, and a viscous resistance(hydrogel) of $5 \times 10^{14}$ m$^{-2}$.

**Figure 17:** The contour along the plane is shown for dimensions: Pore Diameter of 20µm, membrane thickness of 30µm, and a viscous resistance(hydrogel) of $5 \times 10^{14}$ m$^{-2}$.
Section: Changes in Membrane Thickness

**Figure 18:** The contour along the plane is shown for dimensions: Pore Diameter of 20µm, membrane thickness of 10µm, and a viscous resistance(hydrogel) of $5 \times 10^{14}$ m$^{-2}$.

**Figure 19:** The streamlines along the plane is shown for dimensions: Pore Diameter of 40µm, membrane thickness of 40µm, and a viscous resistance(hydrogel) of $5 \times 10^{14}$ m$^{-2}$.
Figure 20: The streamlines along the plane is shown for dimensions: Pore Diameter of 40µm, membrane thickness of 30µm, and a viscous resistance(hydrogel) of 5x10^{14} m^{-2}.

Figure 21: The streamlines along the plane is shown for dimensions: Pore Diameter of 40µm, membrane thickness of 20µm, and a viscous resistance(hydrogel) of 5x10^{14} m^{-2}.
Effect on streamlines

The reduction of membrane thickness creates less divergence of the flow at the pore region, creating a more streamlined flow through the bottom chamber (Fig. 19-22). This may not be clearly evident in the case of 20 µm pores, but is clear in the 40 µm pores as the flow in the 40 µm thick middle membrane shows a seemingly divergent pattern, as if there were 3 different regions of flow in the bottom chamber (Fig. 19), which becomes more convergent as the membrane size is reduced as visualized in the model with a 10 µm thick middle layer. (Fig. 22)
Effect on velocity profile

The velocity profile depicts that the flow conditions with the thicker middle membranes (30 µm and 40 µm) are almost just a shifted version of the flow through the 20µm thick membrane (using data from line 2, Fig. 23), shifted by the same amount that the membrane size is reduced by with each case (10 µm). This is true in flows that are relatively similar in profile (in this case, the 20 µm pore size creates a nice streamed flow, as opposed to the 40 µm pores.) It is observed that any given location will have a higher net velocity if the membrane thickness is reduced.

**Figure 24:** The contour along the plane is shown for dimensions: Pore diameter of 20µm, membrane thickness of 40µm, and a viscous resistance(hydrogel) of $5 \times 10^{14}$ m$^{-2}$.

**Figure 25:** The contour along the plane is shown for dimensions: Pore diameter of 20µm, membrane thickness of 30µm, and a viscous resistance(hydrogel) of $5 \times 10^{14}$ m$^{-2}$.
Figure 26: The contour along the plane is shown for dimensions: Pore diameter of 20µm, membrane thickness of 20µm, and a viscous resistance(hydrogel) of 5x10^{14} m^{-2}.

Figure 27: The oxygen concentration along Line 1 is shown for different membrane thicknesses, with dimensions: Pore diameter of 20µm and a viscous resistance(hydrogel) of 5x10^{14} m^{-2}. The graph is not entirely smooth, perhaps due to discretization of elements within the model.

**Effect on oxygen concentration**

O₂ concentration overall increases while the gradient decreases when the membrane thickness is reduced. This is intuitive as the bottom chamber is brought closer to the source of the flow in the top
Section: Changes in Membrane Thickness

chamber. $O_2$ concentrations in the 40, 30, and 20 µm thick membranes fluctuate in the ranges of 2.44-2.86 µg/g, 2.44-2.92 µg/g, and 2.61-2.97 µg/g respectively, as we move from the bottom to the top of the hydrogel chamber along line 1. The gradients produced in the 40, 30, and 20 µm thick membranes fluctuate (going up along line 1) from 1.14 - 2.14ng/g-µm, 1.14 - 2.08 ng/g-µm, and 1.14 -1.73 ng/g-µm respectively, showing that a decrease in membrane thickness reduces the gradients introduced in the bottom chamber. On a log scale, the $O_2$ concentrations show a similar profile, with a small amount of shift up the graph with reduced membrane thickness (Fig. 27). A look at the contour map reveals this slight shift along with an decreasing oxygen concentration as the membrane thickness is increased. (Fig. 24-26)

![Figure 28](image)

**Figure 28**: The glucose concentration along Line 1 is shown for different membrane thicknesses, with dimensions: Pore diameter of 20µm and a viscous resistance(hydrogel) of $5 \times 10^{14}$ m$^{-2}$.

**Effect on Glucose Concentration:**

The gradient in glucose concentration is not altered significantly as all the curves follow a similar trend for the different membrane thicknesses. However, due to the proximity to the source (the top chamber), the 20 µm thick membrane model permits a higher concentration of glucose into the bottom
Section: Changes in Membrane Thickness

chamber, with a lower concentration in the 30 µm thick membrane, and the lowest being in the 40 µm thick membrane. The ranges for each of these models, respectively, are: 606-831 µg/g, 574-802 µg/g and 542-772 µg/g along the data on Line 1 (Fig. 28).

Figure 29: The CO₂ concentration along Line 1 is shown for different membrane thicknesses, with dimensions: Pore diameter of 20µm and a viscous resistance(hydrogel) of 5x10⁻¹⁴ m².

Effect on Carbon Dioxide Concentration

The gradient in the CO₂ concentration drops slightly with decreasing membrane thickness (<0.1%), but is otherwise considerably unaltered within the different membrane thickness samples. The actual concentration profiles are simply shifted versions of similar curves (Fig. 29). The concentration of CO₂ goes up with membrane thickness, using data from FIGURE (line 3), the concentration range for CO₂ in the 40, 30, and 20 µm pore sizes are 185-302 ng/g, 176-293 ng/g, 166-284 ng/g respectively. The ranges depict that reduced membrane thickness also reduces the range of CO₂ produced in the bottom chamber, thus creating a reduced gradient (although the change is <0.1%).
Changes in hydrogel permeability

Figure 30: The streamlines along the plane is shown for dimensions: Pore diameter of 40µm, membrane thickness of 40µm, and a viscous resistance(hydrogel) of $5 \times 10^{14}$ m$^{-2}$.

Figure 31: The streamlines along the plane is shown for dimensions: Pore diameter of 40µm, membrane thickness of 40µm, and a viscous resistance(hydrogel) of $5 \times 10^{12}$ m$^{-2}$.

Effect on streamlines

In the example shown with membranes of 40 µm thickness and 40 µm pore sizes(Fig. 30) it is noticed that the stiffest hydrogel (viscous resistance of $5 \times 10^{14}$ m$^{-2}$) provides the least compliance to the fluid flow through the gel - the flow is almost divided into separate regions or apparent vortices as viewed
from the cross-section. As the gel is made more permeable towards a viscous resistance of $5 \times 10^{12}$ m$^{-2}$, the flow becomes less diverged. (Fig. 31)

![Figure 32](image)

**Figure 32:** The velocity along Line 1 is shown for different gel stiffnesses, with dimensions: Pore diameter of 20µm, membrane thickness of 40µm, and a viscous resistance(hydrogel) of $5 \times 10^{14}$ m$^{-2}$.

**Effect on velocity profile**

As the permeability increases, the net velocity magnitude fluxing through the chamber (with hydrogel) also increases. This should generally be the case, as in the extreme case of no resistance (infinite permeability, i.e., no hydrogel but only the original liquid phase of the perfusing medium), there would be no retardation to the flow through the bottom chamber. The increase in permeability by one order of magnitude typically raises the velocity by about one order of magnitude as well. Looking specifically at the data from (Fig. 32), at line 1, the velocity in the stiffest gel is on the order of $10^{-5}$ to $10^{-4}$ µm/sec at about 15 - 190 µm away from the middle membrane. In the intermediate gel, this range speeds up to $10^{-4}$ to $10^{-3}$ µm/sec, and to $10^{-3}$ to $10^{-2}$ µm/sec in the softest gel. A similar shift in the order of magnitude can be seen at the data along all locations (lines). At the middle of the bottom chamber (along
line 3), the average speed is on the order of $10^4$, $10^3$, $10^2$ µm/sec respectively for the stiffest, intermediate, and softest gel respectively.

**Figure 33:** The contours along the plane is shown for dimensions: Pore Diameter of 20µm, membrane thickness of 30µm, and a viscous resistance(hydrogel) of $5 \times 10^{14}$ m$^{-2}$.

**Figure 34:** The contours along the plane is shown for dimensions: Pore Diameter of 20µm, membrane thickness of 30µm, and a viscous resistance(hydrogel) of $5 \times 10^{13}$ m$^{-2}$.
**Figure 35**: The streamlines along the plane is shown for dimensions: Pore Diameter of 20µm, membrane thickness of 30µm, and a viscous resistance(hydrogel) of $5 \times 10^{12}$ m$^{-2}$.

**Figure 36**: The velocity along Line 1 is shown for different gel stiffnesses, with dimensions: Pore diameter of 20µm, membrane thickness of 30µm.

**Effect on oxygen concentration**

The results for the oxygen profile is rather counterintuitive when compared across gels of different stiffness Fig. 33-35. Following along the data on line 1,(Fig. 36)the gradient follows a similar trend across all locations - for the first 40 µm from the surface of the middle membrane, the gradient in O$_2$ concentration (as a mass fraction, µg/g) gradient is about 0.003 µg/g-µm. This gradient drops to 0.0025 µg/g-µm over the next 40 µm(40 - 80 µm) , to 0.002 µg/g-µm over the next 40 µm (80 - 120 µm), to 0.0017 µg/g-µm over the next 10 µm (120-130 µm) and to about 0.0013 µg/g-µm from 130 µm on downwards. This trend is seen in all three gels, however, the trend for the softest gel slightly varies after 130 µm in depth, where the gradient drops to about 0.001 µg/g-µm from 130 -150 µm depth, to 0.0007 from 150 - 170 µm depth, and about 0.0004 µg/g-µm in the remainder of the chamber. Although the gradients are more similar in the stiffer and intermediate gels, the actual concentration of O$_2$ in the simulated gels show a closer resemblance between the stiffest and softest gels than the intermediate gel,
with the intermediate gel having the highest O\textsubscript{2} concentration. Looking along the data on line 1 (Fig. 36), the range of O\textsubscript{2} concentration for the stiffest and intermediate are 2.53 - 2.92 µg/g and 2.70 - 2.99 µg/g respectively, seeming to convey the idea that the better perfusion (higher velocities) in the intermediate gel allows better transport of oxygen into the chamber. In the softest gel, however, the velocity profiles are the highest compared to the other two gels, but the O\textsubscript{2} concentration drops back to 2.53 - 2.91 µg/g, very similar to that in the stiffest gel.

![Figure 37: The glucose concentration along Line 1 is shown for different gel stiffnesses, with dimensions: Pore diameter of 20µm, membrane thickness of 30µm.](image)

**Effect on Glucose Concentration**

The glucose concentration shows a very replicated similarity between the gels, unlike the oxygen and carbon dioxide profiles. Along line 3, for instance, the range for the stiffest and intermediate gels are 0.574 - 0.802 mg/g and 0.573 - 0.801 respectively, while a higher range of 0. 583-0.803 mg/g is seen in the softest gel. This relatively identical profile in all three different gels suggest that due to the abundance of glucose, its concentration is not affected by the flow rate, it is a reaction that is diffusion-dominated, where the diffusion is sufficient to overcome its consumption rate.
Figure 38: The CO\(_2\) concentration along Line 1 is shown for different gel stiffnesses, with dimensions: Pore diameter of 20\(\mu\)m, membrane thickness of 30\(\mu\)m.

**Effect on Carbon Dioxide Concentration**

The carbon dioxide generated in the chamber again shows a similarity between the stiffest and softest gels. Comparing along the data in line 3 (Fig. 3), the range in these two gels are 176 -293 ng/g and 176 - 294 ng/g respectively. In the intermediate gel, this range decreases (presumably due to better perfusion) to 92 - 174 ng/g, which is outside of the range of either the stiffest or softest gels.

**The unexpected case**

The unusual O\(_2\) and CO\(_2\) pattern noticed with the change in permeability warrants some discussion. The velocity magnitude by itself shows an increase as the permeability increases, as better perfusion by virtue of increased velocity is seen in the glucose concentration being the highest in the softest gel. Glucose, however, is an abundant resource in the media (1mg/g) and is not a limiting factor for cell metabolic rates. O\(_2\), due to its low solubility, defines the metabolic limitations more so than glucose (0.2mmol/L or approximately 3.2mg/g). Having the media perfusing at a faster velocity through the gel may lead to the thought that the O\(_2\) will be better delivered by convection, which supplements the
already present diffusion of $O_2$ into the bottom chamber. Although this seems to hold true when comparing the stiffest and intermediate gels, the same does not hold true for the softest gel.

To explain this unexpected phenomenon, a contour of the net velocity magnitude (Figs. 39,40) and the positive (upwards) y-direction velocity component (Figs. 41,42) may provide a better insight into the problem. The y-component of the stiffest gel is not shown because only the velocity range between $7 \times 10^{-9}$ and $10^{-7}$ m/s is sought, but from the net magnitude contour of the stiffest gel in Fig. 40, it is easy to see that those values will be out of range.

**Figure 39:** Velocity magnitude profiles in the range of $10^{-8}$ m/s to $5.4 \times 10^{-7}$ m/s in the bottom chamber with viscous resistances of (A) $5 \times 10^{12}$ m$^{-2}$, and (B) $5 \times 10^{12}$ m$^{-2}$. Both cases have same chamber dimensions: 20 µm pore size, 30 µm membrane thickness.
Figure 40: Velocity magnitude profiles in the range of $10^{-12}$ m/s to $5.4 \times 10^{-9}$ m/s in the bottom chamber with a viscous resistance of $5 \times 10^{14}$ m$^2$. The dimensions are the same as the chambers in Fig. 39 (20 µm pore size, 30 µm membrane thickness). Note that the velocity magnitude in this system is well out of an order of magnitude of the systems in Fig. 39 (the intermediate and softer gel).

Figure 41: Another look at velocity in the chamber with the softest gel. This time, only the positive y-direction (upwards) velocity is shown, in the range of $10^{-9}$ m/s to $5.4 \times 10^{-7}$ m/s. The chamber dimensions are still at a 20 µm pore size and a 30 µm membrane thickness.
Figure 42: Positive y-velocity components in the chamber with the intermediate gel, in the range of $10^{-9}$ m/s to $5.4 \times 10^{-7}$ m/s. The chamber dimensions are still at a 20 µm pore size and a 30 µm membrane thickness.

The range is shown only on the order of 0.01-0.1 µm/sec. As is clear from the contour, this velocity regime is only existent in close to the middle membrane for the softest gel, while such a relatively high velocity profile is absent from the other two gels. One way to explain this is the lack of diffusive support in the softest gel by employing Fick’s Law of Diffusion. Using a typical fluid element to analyze the factors affecting diffusion and flow (Fig. 43), the following assumptions and conventions for the analysis of the element are made:

1. The problem is one-dimensional: the velocity components are only in one direction (in the x-direction), and diffusion can happen only along this direction as well.

2. The velocity entering the fluid element is the same as the velocity exiting the element (mass conservation principle with no mass accumulation within the fluid element).

3. The concentration at the input side is $C_i$ and at the output side it is $C_o$. 
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(4) $\Delta C = (C_o - C_i)$

(5) The area through which the fluid is fluxing in or out is called $A$, while the thickness of the fluid element is $d$.

(6) The diffusivity of the species is $D$.

(7) $J$ is the diffusive flux of the species (in units of $g/m^2\cdot s$)

Using the above conventions, Fick's Law of Diffusion can be written as:

$$J = -D \nabla C,$$

where we can approximate to the first order that $\nabla C \approx \frac{\Delta C}{d}$.

The net flux in mass is: $JA = -\frac{D\Delta C}{d}A$. But the net flux can also be defined by a superficial velocity of the species itself. The species (in this case, $O_2$) has a certain concentration immediately outside of the fluid element (at $C_o$), and the flux into the element cannot be higher than the number of species particles that $C_o$ can provide. Thus, if we assume there is a superficial species "velocity" - which is the velocity $v_s$ required at which, given a concentration of $C_o$, the flux will match the value of $J$.

In other words: $JA = -\frac{D\Delta C}{d}A = v_s A C_o$,

which simplifies to: $v_s = -\frac{D\Delta C}{dC_o}$. Since $\Delta C < 0$, we can just consider the magnitude of the velocity:

$$v_s = \left| \frac{D\Delta C}{dC_o} \right|$$

An order of magnitude analysis on this equation uses the following assumptions for the fluid element:

$$D \sim \frac{10^{-9}m^2}{s}, \quad \Delta C \sim \frac{10^{-3}g}{g}, \quad C_o \sim \frac{2g}{g}, \quad d \sim 10^{-6} - 10^{-5}m (or 1 - 10\mu m)$$

These values give: $v_s \sim 5 \times 10^{-8} \frac{m}{s} = 5 \times 10^{-7} \frac{m}{s}$ (or $5 \times 10^{-2} \frac{\mu m}{s} = 5 \times 10^{-3} \frac{\mu m}{s}$).
Figure 43: Visualizing a fluid element which has a one-dimensional flow through it, an input concentration of $C_i$, an output concentration of $C_o$, and area on A perpendicular to the bulk flow velocity $v$, a distance of $d$ from one end to the other and a diffusive flux $J$ (at a superficial speed $v_s$). See text for further description of the model.

This value of $v_s$ is on the same order as that of the bulk fluid flow seen near the middle membrane of the softest gel (Fig. 41). Thus, in theory it can be surmised that although there is a an effort for diffusive influx to occur from the top channel towards the bottom, the bulk flow velocity opposing that velocity is of similar magnitude, thereby negating the diffusive influx. In the other two gels, the diffusive influx (as calculated here) is more than two orders of magnitude higher than the bulk flow speed, thus the cells are provided with abundant $O_2$, at least in the intermediate gels. In the case of the stiffest gel, although there is diffusive influx, the convective supply of $O_2$ is very slow (one to two orders of magnitude lower).
magnitude less than that in the intermediate gel). This means that in this gel, it can take about 10-100 fold of the time that it takes in the intermediate gel to supply O₂ to the cells in the hydrogel convectively, making this situation heavily diffusion-driven. In the softest gel, the convective component is there, but the diffusive component is missing. It would appear that the intermediate gel provides a more adequate balance between convective and diffusive supply of O₂.
4. Conclusion

This study was conducted in order to understand the way that altering parameters will change flow and diffusive behavior in the microchamber. There are many parameters that can be tuned, and whether the optimum conditions specific to the requirements of the user can be chosen can determine what parameters to specify the device manufacture to. The validity of the simulation should be verified in a few models by use of various markers (for pO$_2$, pH, glucose) [14, 21, 22] and techniques such as fluorescence recovery after photobleaching (FRAP). [23]

The data obtained can be advantageous in terms of design considerations. For instance, changing the permeability in the bottom chamber by change of the viscous resistance from $5 \times 10^{14}$ m$^{-2}$ to $5 \times 10^{13}$ m$^{-2}$ gives a faster flow and better delivery of oxygen as expected, but in making the gel even softer to a viscous resistance of $5 \times 10^{12}$ m$^{-2}$, the oxygen content drops back to the same conditions as that in the stiffest gel. Thus, the intermediate gel shows the best perfusion and delivery of oxygen out of the three permeabilities. This allows us to use this as a tool to provide the same oxygenated conditions just by alteration of the hydrogel's permeability to the correct degree. For our case, we can change the peptide percentage of PuraMatrix to tune the gel permeability. Tumor cells can respond to flow, and has competing mechanisms to go against or with the flow. [24] To be able to generate two diverse flow conditions while keeping nutrient amounts the same (such as glucose), it is possible to study the isolated effect of flow on the cultured cells without the need to regulate the amount of oxygen supplied in the perfusing medium to ensure the same oxygenated conditions.[25, 26]

The typical interstitial fluid flow is on the order of 0.1-2 µm/sec. One such case in which this flow speed is achieved is when the viscous resistance is at $5 \times 10^{12}$ m$^{-2}$. In the other permeabilities, the fluid convection speed is very low, on the order of $10^{-3}$ to $10^{-6}$ µm/sec. That does not invalidate these models for use as cell culture. Cancer cells typically harbor a microenvironment marked by high interstitial fluid pressure and very low fluid flow due to the lack of functional lymphatics. [8] Studies show that
normalizing the capillary structure and repairing the lymphatic architecture allows drugs to perfuse better into the cancer cells as well as a slightly better prognosis (about 6 months). The microvessels surrounding a solid tumor, however, is very permeable. This situation can be studied in the microchamber by using a large pore size (40 µm) and high viscous resistance of the hydrogel (5×10^{14} m^{-2}). The same study can be performed on a smaller pore size (20 µm) with the same or different permeability to see the effect of a reduced permeability only in the middle membrane (not in the solid tumor, which is represented by the hydrogel). In terms of clinical application, using biopsy samples instead of cell cultures, one could test whether altering the different parameters will affect the tumor cells and aid in drug screening and give an insight into whether altering those parameters in the physiology of the patient will truly have an effect on the cancer cells, including conditions not mentioned in this study (such as hyperthermia) that can be used in conjunction with the microfluidic device to test for the synergistic effects of multiple stimuli.[26-28]

By having a chart representative of the conditions induced in the microchamber through the use of CFD, the observed experimental results can be explained in conjunction with them.
5. Summary

In this study, a few characteristics in the CFD model of the original 3D LCA device has been altered to note the changes introduced in the microchamber due to these alterations. In general, the trend found was that: (1) velocities correlate positively with changes in pore size and permeability, and inversely correlates with membrane thickness; (2) the glucose profile remains unchanged based on permeability, but does increase with an increase in pore size or a decrease in membrane thickness. For instance, if a stiff microenvironment needs to be simulated in the real device, based on the simulation results, it would be optimal to use 20 µm pore sizes for smooth, streamlined flow but with a membrane thickness of only 20 µm so that velocities on the order similar to the interstitial fluid flow can be reached (~ 0.1 µm/sec). The profile for CO\textsubscript{2} has a very high inverse correlation to the O\textsubscript{2} profile, thus, based on the simulation results, knowing one may help estimate the other. This is not the case for glucose as there is a situation where there is an almost identical glucose profile (for different permeabilities) but very different O\textsubscript{2} and CO\textsubscript{2} profiles. Using only experience and physiological revelations to guide the design of the device may not necessarily give the expected profile within the microchamber, and CFD can step in to provide the insight that can also motivate the design towards an optimal direction without relying on trial-and-error methods of optimization.
6. References:


7. Supplemental Figures:

This section is intended only to supplement the data in this study, and the study can be read independently of this section. It showcases some of the other profiles from the same conditions that were not shown in the study (from varying locations).
Figure S-1: The streamlines along the plane is shown for dimensions: Pore Diameter of 20µm, membrane thickness of 30µm, and a viscous resistance(hydrogel) of $5 \times 10^{14}$ m$^{-2}$.

Figure S-2: The streamlines along the plane is shown for dimensions: Pore Diameter of 20µm, membrane thickness of 30µm, and a viscous resistance(hydrogel) of $5 \times 10^{13}$ m$^{-2}$.
Figure S-3: The streamlines along the plane is shown for dimensions: Pore Diameter of 20µm, membrane thickness of 30µm, and a viscous resistance(hydrogel) of $5 \times 10^{13}$ m$^{-2}$
**Figure S-4:** The velocity along line 3 is shown for various stiffnesses of gels with dimensions: Pore Diameter of 20µm and membrane thickness of 30µm.

**Figure S-5:** The velocity along line 5 is shown for various stiffnesses of gels with dimensions: Pore Diameter of 20µm and membrane thickness of 30µm.
**Figure S-5:** The O₂ concentration along line 3 is shown for various stiffesses of gels with dimensions: Pore Diameter of 20µm and membrane thickness of 30µm.

**Figure S-6:** The O₂ concentration along line 5 is shown for various stiffesses of gels with dimensions: Pore Diameter of 20µm and membrane thickness of 30µm.
Figure S-7: The CO$_2$ concentration contour along the plane is shown for different gel stiffness, with dimensions: Pore diameter of 20µm, membrane thickness of 30µm. Gel shown have viscous resistances of (A) 5x10$^{14}$ m$^{-2}$, (B) 5x10$^{13}$ m$^{-2}$, and (C) 5x10$^{12}$ m$^{-2}$.
Figure S-8: The CO₂ concentration along line 3 is shown for various stiffnesses of gels with dimensions: Pore Diameter of 20µm and membrane thickness of 30µm.

Figure S-9: The CO₂ concentration along line 5 is shown for various stiffnesses of gels with dimensions: Pore Diameter of 20µm and membrane thickness of 30µm.
Figure S-10: The Glucose concentration contour along the plane is shown for different gel stiffness, with dimensions: Pore diameter of 20µm, membrane thickness of 30µm. Gel shown have viscous resistances of (A) $5 \times 10^{14}$ m$^{-2}$, (B) $5 \times 10^{13}$ m$^{-2}$, and (C) $5 \times 10^{12}$ m$^{-2}$. 
Figure S-11: The Glucose concentration contour along Line 3 is shown for different gel stiffnesses, with dimensions: Pore diameter of 20µm, membrane thickness of 30µm.

Figure S-12: The Glucose concentration contour along Line 5 is shown for different gel stiffnesses, with dimensions: Pore diameter of 20µm, membrane thickness of 30µm.
Figure S-13: The velocity magnitude along line 2 is shown for various middle membrane thicknesses with dimensions: Pore Diameter of 20µm and hydrogel viscous resistance of 5\times10^{14} \text{ m}^{-2}.

Figure S-14: The velocity magnitude along line 5 is shown for various middle membrane thicknesses with dimensions: Pore Diameter of 20µm and hydrogel viscous resistance of 5\times10^{14} \text{ m}^{-2}.
**Figure S-14:** The O$_2$ concentration along line 3 is shown for various middle membrane thicknesses with dimensions: Pore Diameter of 20µm and hydrogel viscous resistance of 5x10$^{14}$ m$^{-2}$.

**Figure S-15:** The O$_2$ concentration along line 5 is shown for various middle membrane thicknesses with dimensions: Pore Diameter of 20µm and hydrogel viscous resistance of 5x10$^{14}$ m$^{-2}$.
Figure S-16: The CO$_2$ concentration along line 3 is shown for various middle membrane thicknesses with dimensions: Pore Diameter of 20µm and hydrogel viscous resistance of 5x10$^{14}$ m$^{-2}$.

Figure S-17: The CO$_2$ concentration along line 5 is shown for various middle membrane thicknesses with dimensions: Pore Diameter of 20µm and hydrogel viscous resistance of 5x10$^{14}$ m$^{-2}$.
Figure S-18: The Glucose concentration along line 3 is shown for various middle membrane thicknesses with dimensions: Pore Diameter of 20µm and hydrogel viscous resistance of 5x10^{14} m^2.

Figure S-19: The Glucose concentration along line 3 is shown for various middle membrane thicknesses with dimensions: Pore Diameter of 20µm and hydrogel viscous resistance of 5x10^{14} m^2.
Figure S-20: The velocity profile along Line 3 is shown for different pore sizes, membrane thickness of 40µm, and a viscous resistance(hydrogel) of $5 \times 10^{14} \text{ m}^2$.

Figure S-21: The O$_2$ concentration along Line 5 is shown for different pore sizes, membrane thickness of 40µm, and a viscous resistance(hydrogel) of $5 \times 10^{14} \text{ m}^2$. 
Membrane Pore Size – Glucose, O\textsubscript{2} and CO\textsubscript{2} Profile

**Figure S-22:** The Glucose concentration along Line 3 is shown for 40 \(\mu\)m and 10 \(\mu\)m pore sizes, membrane thickness of 40\(\mu\)m, and a viscous resistance(hydrogel) of \(5 \times 10^{14}\) m\(^{-2}\).

**Figure S-23:** The CO\textsubscript{2} concentration along Line 5 is shown for 40 \(\mu\)m and 10 \(\mu\)m pore sizes, membrane thickness of 40\(\mu\)m, and a viscous resistance(hydrogel) of \(5 \times 10^{14}\) m\(^{-2}\).
Figure S-24: The CO$_2$ concentration along Line 5 is shown for 40 µm and 10 µm pore sizes, membrane thickness of 40µm, and a viscous resistance(hydrogel) of $5 \times 10^{14}$ m$^{-2}$.