Analysis and Implementation of the Bilayer Microfluidic Geometry

by

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B.S.E, University of Pennsylvania (2008)

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Abstract

Microfluidic devices form an important class of analytical platforms that have found wide use in the biomedical sciences. In particular, they have been used in cell culture systems, where they are used to monitor cell behavior in various environments. One challenge that has emerged, however, is the ability for a microfluidic device to uniformly deliver soluble factors to a given culture of cells without subjecting the cells to hydrodynamic shear stresses that could potentially alter their behavior in an unpredictable or undesirable way. This is especially true for a number of cell types, and striking a balance between solute transport and shear stress remains the subject of active research. In this thesis, we will consider a membrane bilayer device configuration in which the transport of a solute to a cell population is achieved by flowing solute through a proximate channel separated from the culture channel by a membrane and seek to characterize some of its hydrodynamic and transport characteristics. It will be shown analytically that this configuration affords greater flexibility over a more traditional single-channel setup, in terms of control over solute transport and applied shear. We will also discuss some topics related to the flow fields within such devices, as well as the fabrication and implementation of the bilayer microfluidic device in an experimental setting.

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Chapter 1

Introduction

1.1 Microfluidics

Microfluidic systems have emerged as revolutionary new platform technologies for a range of applications, from consumer products such as inkjet printer cartridges to lab-on-a-chip diagnostic systems. The development of microfluidic-based technologies over the past two decades has spawned advances in fields ranging from laboratory diagnostics to consumer devices, spurred by emerging requirements in molecular analysis, biodefense and microelectronics. Microfluidics have also found wide application in the field of bioengineering as platforms upon which tissue for a wide range of biological systems can be cultured and engineered for comprehensive and high-throughput analysis, and implantation and organ replacement. Microfluidic devices have given researchers the ability to develop more physiologically relevant assays by allowing for the concurrent study of mechanical and biochemical effects on tissue culture. Moreover, in light of shortages of organs available for transplantation, microfluidic devices have been used to develop architectures for implantable tissue for therapeutic purposes.

Broadly speaking, microfluidics encompasses systems involving the flow of flu-
ids within geometries with characteristic length dimensions on the order of ten to a hundred microns [4]. Microfluidics have become indispensable tools partly due to their size, since they can be made to accommodate anywhere between millions of cells to single cells. Moreover, they have also found favor with bioengineers and clinicians due to the presence of fluid flow, an important physiological condition which is naturally not present within static culture dishes. Microfluidic devices are relatively fast and easy to design and fabricate, and the most commonly used fabrication processes exploit techniques from the microelectronics and other industries and permit for the fast and easy fabrication of a large number of devices. These factors combine to make microfluidics an attractive technology upon which tissue engineering and related research can be conducted.

From an experimental point of view, a notable advantage of microfluidic cell culture is the potential to control concentrations of nutrients, growth factors, and other soluble regulatory molecules within the microfluidic environment; this stands in direct contrast to static cell culture dishes that are the norm. However, while microfluidic devices have developed into powerful, comprehensive experimental tools, there still remain several challenges if a microfluidic device is to be fully modular and capable of isolating important biochemical and mechanical factors in the in vitro environment.

1.2 Motivation and Scope of Thesis

In a typical microfluidic culture device, fluid is flowed directly over cells cultured in a single channel [Figure 1-1(a)], and as a consequence, fluidic shear is imparted directly on the cells. However, it is known that for certain cell types, metabolic activity, differentiation, and proliferation are sensitive to shear stresses [5, 6, 7, 8, 9]. It is possible, then, in the course of an assay, that cellular responses that are mea-
sured are altered by the presence of shear. Moreover, delivering the same amount of solute to all the cells cultured may become a challenge, for in a typical configuration, in which cells are cultured and medium flowed through a single channel configuration, equitable delivery may only occur by increasing the flow rate and consequently, shear. Hence, it is also preferable experimentally to deliver a controlled quantity of solute to the cell population while at the same time having independent control over the imparted shear. We may attempt, then, to seek an alternative device configuration. Some have already been analyzed in the literature, and utilize unique biologically-inspired or grooved geometries to shield cells from shear. Instead, we will analyze the bilayer construct, which affords itself to a much more straightforward analysis and offers greater simplicity in the way of fabrication and operation.

A bilayer device is one in which the cell culture region (the cell compartment) is separated from a flow channel by a semipermeable membrane. This allows for the possibility of decoupling solute transport to the cell culture and the fluidic shear imparted to the culture. In principle, the medium flow rate in the upper channel is set at a relatively high rate while modulation of the membrane transport characteristics and the flow rate in the flow channel then enables independent control of solute transport.

In this thesis, we will consider the application of the bilayer construct to a culture of mesenchymal stem cells (MSCs). A stem cell culture has been chosen for the model analysis for several reasons. First, stem cells have been the focus of a great deal of research in recent years due to their pluripotent nature, and their consequent potential for therapeutic uses in regenerative medicine. Thus, developing an experimental platform for their study has considerable practical interest. In terms of physiological variables, shear stresses have been shown to regulate activation of signaling pathways, gene expression, proliferation, and os-
Figure 1-1: Schematic of (a) single channel and (b) bilayer microfluidic device operating in a monoculture mode. In the single channel device configuration, medium flow is directly over culture. Solute, which is introduced at the left hand side of the channel, is convected forward and diffuses downwards towards the culture. In the bilayer configuration, medium flow is primarily in the upper flow channel, while the flow in the lower cell compartment is modulated depending on the shear requirements of the culture experiment. Solute is introduced at the left hand side of the flow channel, wherein it is convected forward while diffusing downwards and across the membrane; once solute enters the cell compartment, it continues to diffuse downwards towards the cell culture, where it is consumed by the cells.
teogenesis in MSCs [9, 5, 6, 14, 15]. Moreover, in response to low oxygen tensions (such as might be found during embryonic development [16], or in the bone marrow), MSCs upregulate hypoxia-induced factors that then control cell behaviors such as proliferation and differentiation into osteoblasts or chondrocytes [17, 18]. As such, stem cells lend themselves naturally to the study and validation in a bilayer microfluidic device.

The content of this thesis will be divided into three chapters, corresponding to Chapters 2, 3, and 4. In Chapter 2, we will apply a simple mathematical model describing the transport of oxygen within a bilayer device to a culture of mesenchymal stem cells (MSCs). In Chapter 3, we will touch upon some aspects of fluid flow within the bilayer construct and discuss the possibility of controlling transmembrane fluid flow that will be present should flow rates in the flow channel and cell compartment be different. We will conclude in Chapter 4 by discussing various considerations that go into the fabrication of a bilayer device and by demonstrating some preliminary experiments on a device that was actually fabricated. We note that in this thesis, due to the constraint of time, we only touch upon each of the aforementioned aspects. It is envisioned that this work will lay the foundation for a more comprehensive study of the characteristics of the bilayer geometry under a variety of biologically relevant conditions. Finally, it is hoped that this platform can be applied towards gaining a greater understanding of the differentiation and proliferation of stem cells under a greater variety of mechanical and biochemical cues.
Chapter 2

Analytical Investigation: Molecular Transport

2.1 Introduction

In this chapter, we will analytically investigate the transport characteristics of oxygen in a bilayer construct cultured with mesenchymal stem cells, and analyze some aspects of the bilayer’s transport properties. The investigation will proceed as follows: We will first consider the general equation of transport, then reduce it using scaling arguments to an analytically tractable form. Subsequently, we will consider the single channel case, in which cells are cultured at the bottom of the flow channel, and derive the general solution to the transport equation. We will then extend the general solution to the monoculture bilayer case (that is, the case in which cells are cultured solely in the cell compartment) by applying the appropriate boundary conditions, and finally, do the same for the coculture bilayer case, in which cells are cultured in both the flow channel and the cell compartment. The last case may be of interest in future studies in which different types of cells are cultured in one device and we are interested in (say) intercellular
communication between the populations. We will close by presenting the results of some calculations for each case and by comparing and discussing their implications.

Figure 2-1: Cross-sectional geometry and geometrical parameters of (a) single channel and (b) monoculture bilayer device constructs. It is assumed that the channel width \( w \) (into the page) is much greater than the channel heights, \( h, h_1, \) and \( h_{II}, \) for both cases.
2.2 Single Channel Case

2.2.1 Equations of transport

Generally, in two dimensions, the concentration of a given solute is governed by the transport equation:

$$\frac{\partial C}{\partial t} + u \frac{\partial C}{\partial x} + v \frac{\partial C}{\partial y} = D \left( \frac{\partial^2 C}{\partial x^2} + \frac{\partial^2 C}{\partial y^2} \right); \quad (2.1)$$

$u$ and $v$ are the velocities of the fluid in the horizontal ($x$) direction and in the vertical ($y$) direction, respectively. For a long, rectangular channel in which flow is sufficiently developed, we can take $v = 0$, and moreover, we may assume the configuration has reached steady state, so that $\partial C / \partial t = 0$. We will now assume that the channel is much longer than it is high; that is, if $L$ is the length of the channel and $h$ is the height (see Figure 2-1), then $h/L \ll 1$. Therefore, we may take $\partial^2 C / \partial x^2 = 0$. Eq. (2.1) will then reduce to

$$u(y) \frac{\partial C(x, y)}{\partial x} = D \frac{\partial^2 C(x, y)}{\partial y^2}. \quad (2.2)$$

2.2.2 Characteristic Time and Length Scales

We may opt to instead to justify the reduction to Eq. (2.2) on a more quantitative basis, rather than on qualitative, dimensional arguments. In considering the dominant terms in the momentum and transport equations, we must consider the time scales characteristic to each physical process. In the momentum equations (i.e., the Navier-Stokes equations), we are interested in knowing how long and far it takes the system to reach a steady state and full development, while in the transport equations, we wish to know, in a given direction, whether transport is diffusion-dominated or convection-dominated.
Momentum Transport

For fluid flux, the characteristic development time, $\tilde{t}_{dev}$ is given by

$$\tilde{t}_{dev} \sim \frac{h^2}{\nu} \quad (2.3)$$

where $h$ is the height of the channel and $\nu$ is the kinematic viscosity of the medium. For the development length $L_{dev}$ of the fluid flow (i.e. the length it takes for the boundary layers on all sides of an enclosed channel to meet), numerous correlations exist. One such expression reads [19]

$$L_{dev} = D_h \left[ \frac{0.6}{1 + 0.035 \text{Re}_L} + 0.056 \text{Re}_L \right] \quad (2.4)$$

where $\text{Re}_L$, given by $\rho_{\text{fluid}} u_0 L / \mu$, is the Reynolds number of the flow with respect to the channel length $L$, $u_0$ is the mean velocity of the flow, and $D_h$ is the hydraulic diameter of the channel, given by $2hw/(h + w)$.

Molecular Transport

The two means of molecular transport, diffusion and convection, have characteristic time scales, $\tilde{t}_{\text{diff}}$ and $\tilde{t}_{\text{conv}}$ respectively, given, along the length of the channel, by

$$\tilde{t}_{\text{diff}} = \frac{L^2}{D} \quad (2.5)$$

$$\tilde{t}_{\text{conv}} = \frac{L}{u_0} \quad (2.6)$$

where $D$ is the molecular diffusion coefficient for the medium; in the transverse direction, the expressions are the same, but with $h$ substituted for $L$. In the transverse direction, we will assume for now that there exists no momentum flux; transport is then solely by diffusion. The parameters used in this chapter are
typical as reported in the literature; they are summarized in Table 2.1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>h</td>
<td>50 µm [20, 21]</td>
</tr>
<tr>
<td>w</td>
<td>200 µm [20, 21]</td>
</tr>
<tr>
<td>Dh</td>
<td>80 µm</td>
</tr>
<tr>
<td>L</td>
<td>1.5 cm [20, 21]</td>
</tr>
<tr>
<td>D</td>
<td>$3.55 \times 10^{-5}$ cm$^2$/s [22]</td>
</tr>
<tr>
<td>$\rho_{\text{fluid}}$</td>
<td>1000 kg/m$^3$</td>
</tr>
<tr>
<td>$\mu$</td>
<td>$8.9 \times 10^{-4}$ kg/ms</td>
</tr>
<tr>
<td>$\nu$</td>
<td>$8.9 \times 10^{-7}$ m$^2$/s</td>
</tr>
</tbody>
</table>

Table 2.1: Parameters for channel.

For a volumetric flow rate $Q = 2.5$ nL/s (a value to be justified later on the basis of the maximum allowable shear to which the cell culture may be exposed), we have $u_0 = Q/(hw) = .25$ mm/s. The resulting characteristic times and lengths for this $Q$ are summarized in Table 2.2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{Re}_L$</td>
<td>4.2135</td>
</tr>
<tr>
<td>$\bar{t}_{\text{dev}}$</td>
<td>2.8 ms</td>
</tr>
<tr>
<td>$L_{\text{dev}}$</td>
<td>60.7 µm</td>
</tr>
<tr>
<td>$\bar{t}_{\text{diff}}$</td>
<td>63,380 s</td>
</tr>
<tr>
<td>$\bar{t}_{\text{conv}}$</td>
<td>60 s</td>
</tr>
</tbody>
</table>

Table 2.2: Characteristic times and development length for $Q = 2.5$ nL/s.

Clearly, $\bar{t}_{\text{conv}} \ll \bar{t}_{\text{diff}}$, which is to say that the time it takes for a molecule to travel the length of the channel by diffusion is several orders of magnitude greater than that for transport solely by convection; hence, along the length of the channel, transport may be considered to be solely by convection.

We may inquire as to the flow rate $\bar{Q}$ at which diffusive and convective effects
become comparable. By Eqs. (2.5) and (2.6), if \( \tilde{t}_{\text{diff}} \sim \tilde{t}_{\text{conv}} \),

\[
\tilde{Q} \sim \frac{hwD}{L} \tag{2.7}
\]

For the parameter values given above, \( \tilde{Q} \sim 2.37 \times 10^{-9} \text{cm}^3/\text{s} = 2.37 \text{ pL/s} \).

### 2.2.3 Single Channel Equation of Transport: Continued.

Physically, the transport Eq. (2.2) and the analysis above suggest that transport in the \( x \)-direction is convection-driven, while in the \( y \)-direction, it is diffusion-driven. We have indicated explicitly the dependence on \( y \) of \( u \). More precisely, it takes the well-known form

\[
u(y) = \frac{h^2}{2\mu} \left( -\frac{dp}{dx} \right) \left[ \frac{y}{h} \left( 1 - \frac{y}{h} \right) \right] \tag{2.8}
\]

In order to make the transport equation more generally applicable, we will now non-dimensionalize it. First, we let a characteristic fluid velocity \( u_0 \equiv (-dp/dx)h^2(8\mu)^{-1} \). Additionally, we let a characteristic concentration be \( C_0 \) and scale \( x \) and \( y \) against the height of the channel \( h \). Then, Eq. (2.2) becomes, in terms of non-dimensional coordinates and a scaled \( C \),

\[
4y(1 - y) \frac{\partial C(x, y)}{\partial y} = \frac{1}{\text{Pe}_h} \frac{\partial^2 C(x, y)}{\partial y^2}, \tag{2.9}
\]

where \( \text{Pe}_h \) is the well-known Peclet number, defined as \( u_0h/D \). It is clear that if \( L \) is the original length of the channel in the \( x \)-direction and \( h \) the height in the \( y \)-direction, that now \( x \in [0, L/h] \) and \( y \in [0, 1] \).

We may also note that \( \text{Pe}_h \) is dependent upon the volumetric flow rate \( Q \) of fluid into the channel. Assuming the velocity profile to be uniform across the width
$w$ of the device, it may be found that

$$\text{Pe}_h = \frac{3Q}{2wD}$$  \hspace{1cm} (2.10)

### 2.2.4 Solution

We will now derive the general solution to this equation before prescribing any relevant boundary conditions. First, let $C(x, y) = \xi(x) \eta(y)$. Inserting this into Eq. (2.9) and rearranging gives

$$4y(1 - y)\xi'(x)\eta(y) = \frac{1}{\text{Pe}_h} \xi(x)\eta''(y),$$  \hspace{1cm} (2.11)

so that separating leaves us with

$$\frac{4\text{Pe}_h}{\xi(x)} \xi'(x) = \eta''(y) \frac{1}{y(1 - y) \eta(y)} = -\lambda^2$$  \hspace{1cm} (2.12)

where $\lambda^2$ is some constant.

The left side of Eq. (2.12) is integrated immediately:

$$\xi(x) = A \exp \left( -\frac{\lambda^2}{4\text{Pe}_h} x \right),$$  \hspace{1cm} (2.13)

where $A$ is some constant, while the $\eta$ portion may be rearranged to give

$$\eta''(y) + \lambda^2 y(1 - y)\eta(y) = 0$$  \hspace{1cm} (2.14)

The solution for $\eta(y)$ will be found by method of power series. Since Eq. (2.14) is non-singular over the domain $[0, 1]$, we may assume $\eta(y) = \sum_{n=0}^{\infty} a_n y^n$. Substituting this into Eq. (2.14) and equating powers of $y$ yields a recurrence relationship
for the coefficients $a_n$ of the expansion:

$$a_n = \frac{-\lambda^2}{n(n-1)} (a_{n-3} - a_{n-4})$$

(2.15)

where $a_0$ and $a_1$ are to be determined and $a_2$ may be seen to be 0. First, we let $a_1 = 0$; then we may find the coefficients of the expansion $a_n^{(0)}$ (factoring out the common $a_0$) satisfying Eq. (2.14); they are given in Table 2.3.

<table>
<thead>
<tr>
<th>Coefficient, $a_n^{(0)}$</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a_0^{(0)}$</td>
<td>1</td>
</tr>
<tr>
<td>$a_1^{(0)}$</td>
<td>0</td>
</tr>
<tr>
<td>$a_2^{(0)}$</td>
<td>0</td>
</tr>
<tr>
<td>$a_3^{(0)}$</td>
<td>$-\frac{1}{6} \lambda^2$</td>
</tr>
<tr>
<td>$a_4^{(0)}$</td>
<td>$\frac{1}{12} \lambda^2$</td>
</tr>
<tr>
<td>$a_5^{(0)}$</td>
<td>0</td>
</tr>
<tr>
<td>$a_6^{(0)}$</td>
<td>$\frac{1}{180} \lambda^4$</td>
</tr>
<tr>
<td>$a_7^{(0)}$</td>
<td>$-\frac{1}{168} \lambda^4$</td>
</tr>
<tr>
<td>$a_8^{(0)}$</td>
<td>$-\frac{1}{472} \lambda^4$</td>
</tr>
<tr>
<td>$a_9^{(0)}$</td>
<td>$\frac{1}{12,990} \lambda^6$</td>
</tr>
<tr>
<td>$a_{10}^{(0)}$</td>
<td>$\frac{16}{25,131} \lambda^6$</td>
</tr>
<tr>
<td>$\vdots$</td>
<td>$\vdots$</td>
</tr>
</tbody>
</table>

Table 2.3: Coefficients of expansion for $a_1 = 0$.

Then, we may let $a_0 = 0$, so that after factoring out $a_1$, the coefficients for the expansion $a_n^{(1)}$ can be obtained; these are given in Table 2.4. Thus, $\eta(y)$ may be completely written as

$$\eta(y) = a_0 \sum_{n=0}^{\infty} a_n^{(0)} y^n + a_1 \sum_{n=0}^{\infty} a_n^{(1)} y^n$$

If we multiply this result by Eq. (2.13), we have the solution to Eq. (2.11). If we
do this and divide out by \( a_0 \), we have a general solution of

\[
C(x, y) = A \exp \left( -\frac{\lambda^2}{4 \text{Pe}_h} x \right) \left[ \sum_{n=0}^{\infty} a_n^{(0)} y^n + a_1 \sum_{n=0}^{\infty} a_n^{(1)} y^n \right]
\]

\[
= A \exp \left( -\frac{\lambda^2}{4 \text{Pe}_h} x \right) \eta(y)
\]

where \( 1/a_0 \) has been subsumed into the constant \( A \), \( a_1/a_0 \) redefined as \( a_1 \), and \( \eta(y) \) redefined as indicated.

**Boundary conditions**

In a dimensional system, we will suppose that at the left-hand boundary (the line \( x = 0 \)), we have a given fixed concentration \( C_0 \) of solute; that is, \( C(x = 0, y) = C_0 \).
In our non-dimensional system,

\[ C(x = 0, y) = 1 \]  \hspace{1cm} (2.17)

At the lower boundary, we will assume Michaelis-Menten kinetics for the consumption of the solute by the cells [23]. Therefore, in the dimensional system

\[
\left. \frac{D}{\partial y} \right|_{y=0} = \frac{V_{\text{max}} \rho_{\text{cells}} C(x, y = 0)}{K_m + C(x, y = 0)}
\]

where \( V_{\text{max}} \) is the maximum rate of consumption, \( K_m \) is the consumption rate at which the expression evaluates to \( V_{\text{max}}/2 \), and \( \rho_{\text{cells}} \) is the linear density of the cells. With our assumption of a low-oxygen fluidic environment, we expand the right hand side into a Taylor series:

\[
\frac{V_{\text{max}} \rho_{\text{cells}} C(x, y = 0)}{K_m + C(x, y = 0)} \approx \frac{V_{\text{max}} \rho_{\text{cells}}}{K_m} \left( C(x, y = 0) \right)
\]

Then the \( y \) boundary condition reads to the first order

\[
\left. \frac{D}{\partial y} \right|_{y=0} = V_{\text{max}} \rho_{\text{cells}} \frac{C(x, y = 0)}{K_m}.
\]

Non-dimensionalizing this gives then

\[
\left. \frac{\partial C}{\partial y} \right|_{y=0} = \text{Da} C(x, y = 0) \]  \hspace{1cm} (2.18)
where \( Da \equiv V_{max}\rho_{cell}h/(DK_m) \) is the Damkohler number. We have at the upper boundary a no-flux condition, so that in our non-dimensional system

\[
\frac{\partial C}{\partial y} \bigg|_{y=1} = 0
\]  

Eq. (2.19) gives a characteristic equation in the \( \lambda_i \), which is to be solved. Then the complete solution to Eq. (2.11) in non-dimensional form is given by

\[
C(x, y) = \sum_{i=0}^{\infty} A_i \exp \left( -\frac{\lambda_i^2}{4 Pe_h} x \right) \eta_i(y)
\]

where each \( \eta_i \) is distinguished according to \( \lambda_i \) and \( A_i \) is given by

\[
A_i = \frac{\int_{y=0}^{1} \eta(y) y(1-y) dy}{\int_{y=0}^{1} |\eta(y)|^2 y(1-y) dy},
\]

an elementary result in accordance with Sturm-Liouville theory, which may be derived by demonstrating the self-adjointness of Eq. (2.11) with the given boundary conditions. In dimensional form, the solution may be written

\[
C(x, y) = C_0 \sum_{i=1}^{\infty} A_i \exp \left( -\frac{\lambda_i^2 D x}{4u_0 h^2} \right) \eta_i(y/h)
\]

2.3 Monoculture Bilayer Case

2.3.1 Equations of Transport

We now consider the solution to the transport equation in the monoculture bilayer case. The region of interest is taken as a whole to be the union of two rectangular regions, both of length \( L \). The upper region is denoted \( \Omega_{II} \), and is characterized by a height of \( h_{II} \); the lower region is denoted \( \Omega_I \), and is characterized by a height
of \( h_I \). In applying the non-dimensionalization scheme to \( \Omega_{II} \) and \( \Omega_I \), we use as the reference height \( h_I \). Denote the concentrations of interest to be \( C_{II} \) and \( C_I \) for the upper and lower channels, respectively. Assuming no transmembrane fluid flux, the analogues for Eq. (2.11) for this bilayer configuration read

\[
\frac{4y}{\beta} \left( 1 - \frac{y}{\beta} \right) \frac{\partial C_{II}(x,y)}{\partial y} = \frac{1}{Pe_{II}} \frac{\partial^2 C_{II}(x,y)}{\partial y^2},
\]

\[x \in [0, L/h_I] \text{ and } y \in [0, \beta], \quad [0, L/h_I] \times [0, \beta] \equiv \Omega_{II}\]

and

\[
4y(1 - y) \frac{\partial C_I(x,y)}{\partial y} = \frac{1}{Pe_I} \frac{\partial^2 C_I(x,y)}{\partial y^2},
\]

\[x \in [0, L/h_I] \text{ and } y \in [0, 1], \quad [0, L/h_I] \times [0, 1] \equiv \Omega_I\]

where \( \beta \equiv h_{II}/h_I \), \( Pe_{II} \equiv u_{0,II}h_I/D_{II} \), and \( Pe_I \equiv u_{0,I}h_I/D_I \). \( u_{0,II} \) is equal to \((-dp_{II}/dx)h_{II}^2(8\mu_{II})^{-1}\) and \( u_{0,I} \) equal to \((-dp_I/dx)h_I^2(8\mu_I)^{-1}\). Again, in terms of flow rates \( Q_I \) and \( Q_{II} \) in \( \Omega_I \) and \( \Omega_{II} \) respectively, we may write the Peclet numbers as

\[
Pe_I = \frac{3Q_I}{2wD_I} \quad (2.23)
\]

\[
Pe_{II} = \frac{3Q_{II}}{2\beta wD_{II}} \quad (2.24)
\]
2.3.2 Solution

The general methodology used in Section 2.2.4 may be used to derive the general solutions for Eqs. (2.21) and (2.22). The general solution to Eq. (2.22) is then

\[
C_I(x, y) = A_I \exp \left( -\frac{\lambda_I^2}{4 \text{Pe}_I} x \right) \left[ \sum_{n=0}^{\infty} a_{I,n}^{(0)} y^n + a_{I,1} \sum_{n=0}^{\infty} a_{I,n}^{(1)} y^n \right] \\
= A_I \exp \left( -\frac{\lambda_I^2}{4 \text{Pe}_I} x \right) \eta_I(y) 
\]  

(2.25)

where the coefficients \(a_{I,n}^{(0)}\) and \(a_{I,n}^{(1)}\) are defined as in Tables 2.3 and 2.4. For Eq. (2.21), the presence of the factor of \(\beta\) requires slight modification:

\[
C_{II}(x, y) = A_{II} \exp \left( -\frac{\lambda_{II}^2}{4 \text{Pe}_{II}} x \right) \left[ \sum_{n=0}^{\infty} a_{II,n}^{(0)} \left( \frac{y}{\beta} \right)^n + a_{II,1} \sum_{n=0}^{\infty} a_{II,n}^{(1)} \left( \frac{y}{\beta} \right)^n \right] \\
= A_{II} \exp \left( -\frac{\lambda_{II}^2}{4 \text{Pe}_{II}} x \right) \eta_{II}(y) 
\]  

(2.26)

The coefficients \(a_{II,n}^{(0)}\) and \(a_{II,n}^{(1)}\) are found nearly as they were in the previous section, with the difference being that the coefficients must all be divided by a factor of \(\beta^2\); this may be verified easily by expanding the above power series, substituting into the transport equations, and equating terms.

In the \(x\)-direction, we will again have prescribed conditions; that is,

\[
C_{II}(x = 0, y) = C_0 \\
C_I(x = 0, y) = 0
\]

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In our non-dimensional system,

\[
C_{II}(x = 0, y) = 1 \quad (2.27)
\]

\[
C_I(x = 0, y) = 0 \quad (2.28)
\]

Let us assume that the media in both channels are the same; then \( D_I = D_{II} \equiv D \).

In \( \Omega_I \), we have the following \( y \) boundary conditions:

\[
D \left. \frac{\partial C_I}{\partial y} \right|_{y=0} = V_{\text{max}} \rho_{\text{cells}} \frac{C_I(x, y = 0)}{K_m}
\]

\[
D \left. \frac{\partial C_I}{\partial y} \right|_{y=h_I} = k [C_{II}(x, y = 0) - C_I(x, y = h_I)];
\]

\( k \) may be seen to be the diffusion coefficient of the membrane divided by the thickness of the membrane. Again, non-dimensionally:

\[
\left. \frac{\partial C_I}{\partial y} \right|_{y=0} = Da C_I(x, y = 0), \quad Da \equiv \frac{V_{\text{max}} \rho_{\text{cells}} h_I}{DK_m} \quad (2.29)
\]

\[
\left. \frac{\partial C_I}{\partial y} \right|_{y=1} = Sh [C_{II}(x, y = 0) - C_I(x, y = 1)], \quad Sh \equiv (kh_I/D) \quad (2.30)
\]

In \( \Omega_{II} \), we have a no-flux condition at the \( y = h_{II} \) boundary; the condition at \( y = 0 \) follows from the above, so that non-dimensionally,

\[
\left. \frac{\partial C_{II}}{\partial y} \right|_{y=0} = 0 \quad (2.31)
\]

\[
\left. \frac{\partial C_{II}}{\partial y} \right|_{y=\beta} = \left. \frac{\partial C_I}{\partial y} \right|_{y=1} \quad (2.32)
\]
2.3.3 Application of boundary conditions to the solution

For $\Omega_{II}$, the no-flux boundary condition on the top surface requires

$$\frac{\partial C_{II}}{\partial y} \bigg|_{y=\beta} = 0$$

But

$$C_{II}(x, y) = A_{II} \exp \left( -\frac{\lambda_{II}^2}{4 \text{Pe}_{II}} x \right) \left[ \sum_{n=0}^{\infty} a^{(0)}_{II,n} \left( \frac{y}{\beta} \right)^n + a_{II,1} \sum_{n=0}^{\infty} a^{(1)}_{II,n} \left( \frac{y}{\beta} \right)^n \right]$$

so

$$a_{II,1} = -\frac{\sum_{n=0}^{\infty} a^{(0)}_{II,n}}{\sum_{n=0}^{\infty} a^{(1)}_{II,n}}. \tag{2.33}$$

In $\Omega_{I}$, Eq. (2.29) gives $a_{I,1} = D$. We may now apply Eqs. (2.30) and (2.32). The first condition gives

$$\frac{\partial C_{I}}{\partial y} \bigg|_{y=1} = A_{I} \exp \left( -\frac{\lambda_{I}^2}{4 \text{Pe}_{I}} x \right) \eta_{I}(1)$$

$$= \text{Sh} \left[ A_{II} \exp \left( -\frac{\lambda_{II}^2}{4 \text{Pe}_{II}} x \right) - A_{I} \exp \left( -\frac{\lambda_{I}^2}{4 \text{Pe}_{I}} x \right) \eta_{I}(1) \right]$$

so that

$$\frac{A_{II}}{A_{I}} \exp \left( -\frac{\lambda_{II}^2}{4 \text{Pe}_{II}} x + \frac{\lambda_{I}^2}{4 \text{Pe}_{I}} x \right) = \frac{\text{Sh} \eta_{I}(1) + \eta_{I}'(1)}{\text{Sh}}.$$
The second gives
\[
\left. \frac{\partial C_{II}}{\partial y} \right|_{y=0} = A_{II} \exp \left( -\frac{\lambda_{II}^2}{4 \text{Pe}_I} x \right) a_{II,1} = A_I \exp \left( -\frac{\lambda_I^2}{4 \text{Pe}_I} x \right) \eta_I'(1)
\]
so that
\[
\frac{A_{II}}{A_I} \exp \left( -\frac{\lambda_{II}^2}{4 \text{Pe}_{II}} x + \frac{\lambda_I^2}{4 \text{Pe}_I} x \right) = \frac{\eta_I'(1)}{a_{II,1}}.
\]
Then
\[
\frac{\text{Sh} \eta_I(1) + \eta_I'(1)}{\text{Sh}} - \frac{\eta_I'(1)}{a_{II,1}} = 0 \tag{2.34}
\]
Since the quantity
\[
\frac{A_{II}}{A_I} \exp \left( -\frac{\lambda_{II}^2}{4 \text{Pe}_{II}} x + \frac{\lambda_I^2}{4 \text{Pe}_I} x \right)
\]
is equal to constants, the additional constraint
\[
\frac{\lambda_{II}^2}{\text{Pe}_{II}} = \frac{\lambda_I^2}{\text{Pe}_I}
\]
must be satisfied. Solving Eq. (2.34) will give a number of possible values for \(\lambda_{II}\) and subsequently \(\lambda_I\). In dimensionless form, the linear combinations
\[
C_{II} = \sum_{i=1}^{\infty} C_{II,i} = \sum_{i=1}^{\infty} A_{II,i} \exp \left( -\frac{\lambda_{II,i}^2}{4 \text{Pe}_{II}} x \right) \eta_{II,i} \tag{2.35}
\]
\[
C_I = \sum_{i=1}^{\infty} C_{I,i} = \sum_{i=1}^{\infty} A_{I,i} \exp \left( -\frac{\lambda_{I,i}^2}{4 \text{Pe}_I} x \right) \eta_{I,i} \tag{2.36}
\]
form the general solution, where each \(C_{I,i}\) and \(C_{II,i}\) is a set of solutions char-
acterized by the linked characteristic values $\lambda_I^2$ and $\lambda_{II}^2 = \Gamma \lambda_I^2$, where we define
$\Gamma \equiv \text{Pe}_{II} / \text{Pe}_I$. In a dimensional system, we may write the same as

$$C_{II} = C_0 \sum_{i=1}^{\infty} A_{II,i} \exp \left( -\frac{\lambda_{II,i}^2 D_{II} x}{4u_{0,II} h_I^2} \right) \eta_{II,i}(y/h_{II})$$  \hspace{1cm} (2.37)

$$C_I = C_0 \sum_{i=1}^{\infty} A_{I,i} \exp \left( -\frac{\lambda_{I,i}^2 D_I x}{4u_{0,I} h_I^2} \right) \eta_{I,i}(y/h_I)$$  \hspace{1cm} (2.38)

We have $A_{I,i} = A_{II,i} \frac{a_{II,i}}{\eta_I'(1)}$ so that, by Theorems 1 and 2,

$$A_{II,i} = \frac{\int_{y=0}^{\beta} u(y) \eta_{II,i} dy}{\int_{y=0}^{\beta} \eta_{II,i}^2 u(y) dy + \frac{1}{\Gamma} \left[ \frac{a_{II,i}}{\eta_I'(1)} \right]^2 \int_{y=0}^{1} \eta_{I,i}^2 u(y) dy}$$  \hspace{1cm} (2.39)

$$A_{I,i} = A_{II,i} \frac{a_{II,i}}{\eta_I'(1)}$$  \hspace{1cm} (2.40)

Eqs. (2.39) and (2.40) allow us to complete our description of the solution to Eqs. (2.21) and (2.22).

### 2.3.4 Average Outlet Concentration

The parameter that is most amenable to direct measurement is the average outlet concentration for each of the top and bottom channels, $\bar{C}_{II}$ and $\bar{C}_I$ respectively. These may be written in dimensional form as

$$\bar{C}_{II} = \frac{C_0}{\beta} \int_{0}^{\beta} \sum_{i=1}^{\infty} A_{II,i} \exp \left( -\frac{\lambda_{II,i}^2 D_{II} L}{4u_{0,II} h_I^2} \right) \eta_{II,i}(y/h_{II}) dy$$  \hspace{1cm} (2.41)

$$\bar{C}_I = \frac{C_0}{\beta} \int_{0}^{1} \sum_{i=1}^{\infty} A_{II,i} \exp \left( -\frac{\lambda_{I,i}^2 D_I L}{4u_{0,I} h_I^2} \right) \eta_{I,i}(y/h_I) dy$$  \hspace{1cm} (2.42)
2.4 Coculture Bilayer Case

A bilayer device may also have cells cultured on the membrane in the upper channel. These cells may in general be of a different type than those cultured on the bottom of the lower channel, and hence such a device is termed a “coculture device”. The extension for this case from that presented in the last chapter is straightforward. The inclusion of a layer of cells on the membrane will be negotiated as follows: First, assume that all the equations of interest have been non-dimensionalized as in the previous section. Then, in $\Omega_{II}$, along the line $y = 0$, consider a particular interval $[x, x + dx]$; it will be assumed that a certain portion ($\gamma : 0 \leq \gamma \leq 1$) of this interval is occupied by cells consuming at a first-order Michaelis-Menten rate of $Da_{II}$. The rest of the interval (a part $1 - \gamma$) will be open to the membrane below, which still has a transport coefficient $k$. The consumption rate for the cells in the lower channel, previously called $Da$, will now be called $Da_{I}$. The new set of boundary conditions will be as follows:

\[
\begin{align*}
C_{II}(x = 0, y) &= 1 \quad (2.43) \\
C_{I}(x = 0, y) &= 0 \quad (2.44) \\
\left. \frac{\partial C_{I}}{\partial y} \right|_{y=0} &= Da_{I} C_{I}(x, y = 0) \quad (2.45) \\
\left. \frac{\partial C_{I}}{\partial y} \right|_{y=1} &= (1 - \gamma) \text{Sh} [C_{II}(x, y = 0) - C_{I}(x, y = 1)] \quad (2.46) \\
\left. \frac{\partial C_{II}}{\partial y} \right|_{y=\beta} &= 0 \quad (2.47) \\
\left. \frac{\partial C_{II}}{\partial y} \right|_{y=0} &= \gamma Da_{II} C_{II}(y = 0) + \left. \frac{\partial C_{I}}{\partial y} \right|_{y=1} \quad (2.48)
\end{align*}
\]
2.4.1 Solution

The method of solving this system will be very similar to that presented in the previous section. We will have the following solutions to Eqs. (2.21) and (2.22) for regions $\Omega_{II}$ and $\Omega_I$, respectively:

\[
C_{II} = A_{II} \exp \left( -\frac{\lambda_{II}^2}{4Pe_{II}} x \right) \eta_{II}(y/\beta)
\]

\[
C_I = A_I \exp \left( -\frac{\lambda_I^2}{4Pe_I} x \right) \eta_I(y)
\]

where $\eta$ for each domain is defined as in Eq. (2.14). In light of Eqs. (2.46) and (2.48), the characteristic values $\lambda$ are found by solving the following equation:

\[
\frac{(1 - \gamma) Sh \eta_I(1)}{\gamma Da_{II} + (1 - \gamma) \chi - a_{II,1}} - \frac{\eta_I'(1) + (1 - \gamma) Sh \eta_I(1)}{(1 - \gamma) Sh} = 0 \quad (2.49)
\]

where $a_{II,1}$ is given as in Eq. (2.33). We have, as before, the relation

\[
\frac{\lambda_I^2}{Pe_I} = \frac{\lambda_{II}^2}{Pe_{II}} \quad (2.50)
\]

holding, while the coefficients $A_I$ and $A_{II}$ are related by

\[
A_I = A_{II} \frac{a_{II,1} - \gamma Da_{II}}{\eta_I'(1)} \quad (2.51)
\]

Note that both Theorems 1 and 2 still hold. Hence, we may develop a solution as in Eqs. (2.35) and (2.36), again each case $C_{I,i} = A_{I,i} \exp \left( -\frac{\lambda_{I,i}^2}{4Pe_{I}} x \right) \eta_{I,i}(y/\beta)$ and $C_{II,i} = A_{II,i} \exp \left( -\frac{\lambda_{II,i}^2}{4Pe_{II}} x \right) \eta_{II,i}(y/\beta)$ differentiated by the characteristic values $\lambda_{I,i}$ and $\lambda_{II,i}(= \lambda_{I,i} Pe_{II}/Pe_I)$ obtained by solving Eq. (2.49).
Using Theorems 1 and 2 and Eq. (2.51), we have

\[ A_{II,i} = \frac{\int_{y=0}^{\beta} u(y)\eta_{II,i}dy}{\int_{y=0}^{\beta} |\eta_{II,i}|^2 u(y)dy + \Gamma \left( \frac{a_{II,i}-\gamma Da_{II}}{\eta_{II}^{(1)}} \right)^2 \int_{y=0}^{\beta} |\eta_{II,i}|^2 u(y)dy} \]

\[ A_{I,i} = A_{II,i} a_{II,i} - \gamma Da_{II} \eta_{II}^{(1)} \]

The final expressions for the concentration profiles remain the same as in Eqs. (2.37) and (2.38), as do the expressions for the average outlet concentrations [Eqs. (2.41) and (2.42)].

\[ (2.52) \]

\[ (2.53) \]

2.5 Parameters

The parameters that we will use as input in the model are summarized in Tables 2.5 and 2.6. The geometrical parameters and physical constants are, in this chapter, chosen based on previously reported values. Table 2.6 summarizes three combinations of parameter values of interest, ranging from low (Case 1) to high (Case 3) uptake rates, given by the ratio \( V_{\text{max}}/K_M \), as well as varying cell seeding densities, ranging from sparse (Case 1) to confluent (Case 3). The range of suitable flow rates in the cell compartment can be determined by considering the threshold values that have been reported to affect MSC cell phenotypes. Proliferation and osteogenic differentiation, for instance, are affected by shear in the range between 0.3 and 2.7 dyne/cm\(^2\) [14, 24]. We therefore take as an upper threshold value for the shear in the lower compartment of \( \tau = 0.3 \) dyne/cm\(^2\). This constraint sets the maximum allowable volumetric flow rate in the lower channel to \( Q_I = 2.5 \) nL/s. Flow rates of this order have been used to culture stem cells in work that has successfully sustained cells and removed metabolic waste over a period of time on the order of days. There are no limits in the monoculture bilayer case to the flow rate.
in the flow channel, since, due to the shielding effect of the membrane, it can be modulated without imparting any shear stresses on the culture. A channel height of 50 μm and length of 1.5 cm for both the upper and lower channels have been selected based on experimental values for devices used in comparable cell culture studies [20, 21].
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_{I,II}$</td>
<td>$3 \times 10^{-5} \text{cm/s}$</td>
<td>Molecular diffusion coefficient of oxygen in water</td>
</tr>
<tr>
<td>$D_{\text{membrane}}$</td>
<td>$3.55 \times 10^{-5} \text{cm/s}$</td>
<td>Diffusion coefficient of oxygen in polymeric membrane [22]</td>
</tr>
<tr>
<td>$t$</td>
<td>10$\mu$m</td>
<td>Thickness of membrane</td>
</tr>
<tr>
<td>$\mu$</td>
<td>$1 \times 10^{-2} \text{dyne \cdot cm}^2/\text{s}$</td>
<td>Viscosity of water</td>
</tr>
<tr>
<td>$h_{I,H}$</td>
<td>50$\mu$m</td>
<td>Height of channel [20, 21]</td>
</tr>
<tr>
<td>$w$</td>
<td>200$\mu$m</td>
<td>Width of channel [20, 21]</td>
</tr>
<tr>
<td>$L$</td>
<td>1.5cm</td>
<td>Length of channel [20, 21]</td>
</tr>
<tr>
<td>$K_M$</td>
<td>$1 \times 10^{-7} \text{mol/cm}^3$</td>
<td>Michaelis-Menten parameter for oxygen uptake [7]</td>
</tr>
<tr>
<td>$C_{\text{sat}}$</td>
<td>$2.15 \times 10^{-1} \text{mol/cm}^3$</td>
<td>Oxygen concentration at saturation [5]</td>
</tr>
<tr>
<td>$C_0$</td>
<td>$2.15 \times 10^{-9} \text{mol/cm}^3$</td>
<td>Inlet oxygen concentration, taken to be 1$%$ $C_{\text{sat}}$ [5]</td>
</tr>
<tr>
<td>$\tau$</td>
<td>$.3 \text{dyne/cm}^2$</td>
<td>Shear, determined by initiation of osteogenic differentiation in MSCs [14, 24]</td>
</tr>
</tbody>
</table>

Table 2.5: Baseline parameters used.

<table>
<thead>
<tr>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{\text{max}}/K_M$</td>
<td>$5 \times 10^{-4} \text{cm}^3/10^6 \text{cells/s}$ [7]</td>
<td>$1 \times 10^{-4} \text{cm}^3/10^6 \text{cells/s}$</td>
</tr>
<tr>
<td>$\rho_{\text{cells}}$</td>
<td>$1 \times 10^4 \text{cells/cm}^2$ [7, 25, 26, 27]</td>
<td>$1 \times 10^6 \text{cells/cm}^2$ [7, 28]</td>
</tr>
</tbody>
</table>

Table 2.6: Combinations of parameters for various cases.
2.6 Results

2.6.1 Single Channel and Monoculture Bilayer

In Figure 2-2, model results for oxygen concentration at the cell surface for the bilayer and single channel case are shown. Using parameters for Case 2 and for flow rates $Q_I = 2.5 \text{ nL/s}$ (governed by the maximum allowable shear on the cell layer) with $Q_{II} = 25 \text{ nL/s}$ in the upper channel, the oxygen concentration profile is nearly invariant along the length of the cell compartment except for a small zone of depletion in the initial region, due to the assumption of zero concentration in the inlet. If, instead of using a bilayer membrane device, cells were cultured in a single channel configuration with the maximum allowable flow rate, given by $Q = 2.5 \text{ nL/s}$, the the oxygen concentration falls rapidly along the length of the channel. If we were to increase the flow rate in the single channel to a rate equal to that in the flow chamber of the bilayer ($Q = 25 \text{ nL/s}$), we see a comparable concentration profile, though at the cost of greater shear (an increase from $\tau = 0.3 \text{ dyne/cm}^2$ to $\tau = 3 \text{ dynes/cm}^2$).

The concentration profiles in the channels themselves can be visualized using colormaps over the domain of interest. We see in Figure 2-3 that, with $Q_I = 2.5 \text{ nL/s}$ and $Q_{II} = 25 \text{ nL/s}$ and with all other parameters as those in Case 2, the concentration field in the cell compartment for the bilayer case is nearly uniform. For the single channel configuration operating again at a flow rate of $Q = 2.5 \text{ nL/s}$, there exists considerable nonuniformity in the concentration profile along the length of the channel.

In Figures 2-4 and 2-5, we see the effect of changing the flow chamber flow rate $Q_{II}$ for Case 1, Case 2, and Case 3. In Figure 2-4, we have $Q_{II} = 10Q_I$, while in Figure 2-5, we have $Q_{II} = 50Q_I$. The effect of increasing the flow rate in the flow chamber is that the oxygen concentration at the cell surface is made more uniform.
along the length of the channel. This is especially clear for Case 3, in which cells are both confluent and uptaking oxygen at a high rate: by increasing the flow rate in the upper chamber, the end-channel concentration for Case 3 is increased by a factor of about 3, so that, instead of a nearly 75% decrease in concentration along the 1.5 cm device length, we have only about a 29% decrease.

In Figure 2-6, we consider the effect of increasing the inlet concentration. As expected, as the inlet concentration is increased, the overall concentration profile increases.
Average Outlet Concentrations

In Figures 2-7, 2-8, 2-9, and 2-10, we show the effect of increasing the flow rate in the upper chamber (equivalent to modulating $\Gamma$) on the average outlet concentrations for the single channel case and monoculture bilayer Cases 1, 2, and 3, respectively. For the single channel case, we see again the necessity of increasing shear in order to increase the outlet concentration. The same is not true for the single culture bilayer, in which the outlet concentrations are independent of the shear imparted on the cell layer.
2.6.2 Coculture Bilayer

We now consider the concentration profiles for the coculture bilayer configuration. In Figures 2-11, 2-12, and 2-13, we show the results of the model for cases in which the lower channel is cultured as in Cases 1, 2, and 3, respectively, with varying culture parameters in the upper channel. We see the considerable dependence of the lower channel’s concentration profile on that of the upper channel.

Average Outlet Concentrations in the Coculture Bilayer Case

Finally, we present some of the results of the average outlet concentrations for the coculture bilayer case (Figures 2-14 and 2-15).
2.7 Discussion

The results presented in the previous section describe the range of operating parameters that enable a bilayer microfluidic device to provide uniform, tailored levels of oxygen to various densities of cultured MSCs, while shielding cells from shear stresses induced by the flow rates that are necessary to transport solute along the device length. We have shown that, in the bilayer case, it is possible to achieve nearly uniform solute delivery without an increase in the shear stress to which the cells are exposed. Significantly, we have shown that the concentrations and hence, delivery profiles, are, except for a small entry zone, nearly uniform. Of course, the extent of this entry zone can, in practice, be modified, too, by varying the flow characteristics in both the cell compartment and the flow channel. For example, we may consider a baseline case in which $Q_I = 10.0 \text{ nL/s}$ and $Q_{II} = 100.0 \text{ nL/s}$; using the parameters from Case 2, the entry zone has an extent of approximately .25 cm (Figure 2-18). If, however, we decrease $Q_I$ to 2.5 nL/s and $Q_{II} = 43.75 \text{ nL/s}$, we effectively reduce the extent of this depletion zone by a factor of about 4. Nevertheless, the above analysis, derived from simple first principles considerations, demonstrates the potential for the bilayer construct to deliver uniform profiles of solute to a culture of cells independently of the shear stresses that are exerted on the culture.
Figure 2-2: Concentration at the cell surface as a function of $x$, in units of nanomole per cm$^3$: single channel (dotted and dashed lines) versus bilayer (solid black line). For the bilayer, the cell compartment flow rate is given by $Q_I = 2.5 \text{ nL/s (r = 0.3 dynes/cm}^2)$, while the flow channel flow rate is $Q_{II} = 25 \text{ nL/s}$. For the single channel, we have flow rates given by $Q = 2.5 \text{ nL/s},$ corresponding to $r = 0.3 \text{ dynes/cm}^2$ (dashed black line) and $Q = 25 \text{ nL/s},$ corresponding to $r = 3.0 \text{ dynes/cm}^2$ (dotted blue line). For the single channel case corresponding to $r = 0.3 \text{ dynes/cm}^2$, note the decaying consumption profile, while the bilayer consumption profile is, except for a small depletion zone at the beginning of the channel, nearly uniform. Other parameters are those for Case 2.
In (a), we have the concentration field for the single channel construct with $Q = 2.5 \text{ nL/s}$. In (b), we see the concentration field in the cell compartment of the bilayer construct with $Q_I = 2.5 \text{ nL/s}$ and $Q_{II} = 25 \text{ nL/s}$. The bilayer case demonstrates a far more uniform profile for a given flow rate and shear, while for the single channel configuration, the concentration of solute is depleted for much of the length of the channel. Other parameters are those for Case 2.
Figure 2-4: Concentration at cell surface as a function of $x$ for cases 1, 2, and 3, in units of nanomole per cm$^3$ with $Q_I = 2.5 \text{ nL/s}$ and $Q_{II} = 25 \text{ nL/s}$. The shear imparted on the cell culture is $\tau = .3 \text{ dyne/cm}^2$. 
Figure 2-5: Concentration at cell surface as a function of $x$ for Cases 1, 2, and 3, in units of nanomole per cm$^3$ with $Q_I = 2.5$ nL/s and $Q_{II} = 125$ nL/s. By increasing the flow rate in the upper chamber by a factor of 5, the consumption profile becomes more uniform. In particular, for the high uptake rate and confluent seeding case (Case 3), increasing the flow rate in the upper chamber prevents depletion along the length of channel caused by high uptake of oxygen. The shear imparted on the cell culture is $\tau = 0.3$ dyne/cm$^2$. 
Figure 2-6: Concentration at cell surface as a function of $x$ in units of nanomole per $cm^3$: $C_0$ varied as $1\%C_{sat}$ (solid blue line), $2\%C_{sat}$ (dashed red line), and $3\%C_{sat}$ (dotted black line). $Q_I = 2.5\ nL/s$ and $Q_{II} = 25\ nL/s$ while other parameters are those for Case 2.
Figure 2-7: Average outlet concentration $C$ for the single channel case for varying flow rates and hence shear, $\tau$.

Figure 2-8: Average outlet concentrations for flow channel ($\bar{C}_{II}$) and cell compartment ($\bar{C}_{I}$) for Case 1. $Q_I = 2.5 \, nL/s$ while $\Gamma$ and shear $\tau$ varies.
Figure 2-9: Average outlet concentrations for flow channel ($\bar{C}_{II}$) and cell compartment ($\bar{C}_I$) for Case 2. $Q_I = 2.5 \text{ nL/s}$ while $\Gamma$ and shear $\tau$ varies.

Figure 2-10: Average outlet concentrations for flow channel ($\bar{C}_{II}$) and cell compartment ($\bar{C}_I$) for Case 3. $Q_I = 2.5 \text{ nL/s}$ while $\Gamma$ and shear $\tau$ varies.
(a) Case 1 in lower channel and Case 1 in upper channel.

(b) Case 1 in lower channel and Case 3 in upper channel.

Figure 2-11: Concentration at cell surface as a function of $x$ in units of nanomole per cm$^3$ for various flow rate ratios $\Gamma$: Case 1 parameters in lower channel and Cases 1 and 3 parameters in the upper channel. $Q_l = 2.5 \, nL/s \, (\tau = .3 \, \text{dynes/cm}^2)$ while $\gamma = .5$. 

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(a) Case 2 in lower channel and Case 2 in upper channel.

(b) Case 2 in lower channel and Case 3 in upper channel.

Figure 2-12: Concentration at cell surface as a function of $x$ in units of nanomole per $cm^3$ for various flow rate ratios $\Gamma$: Case 2 parameters in lower channel and Cases 2 and 3 parameters in the upper channel. $Q_l = 2.5 \ nL/s$ ($\tau = .3 \ dynes/cm^2$) while $\gamma = .5$. 

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Figure 2-13: Concentration at cell surface as a function of $x$ in units of nanomole per $cm^3$ for various flow rate ratios $\Gamma$: Case 3 parameters in lower channel and Cases 1 and 3 parameters in the upper channel. $Q_f = 2.5 \text{ nL/s}$ ($\tau = .3 \text{ dynes/cm}^2$) while $\gamma = .5$. 

(a) Case 3 in lower channel and Case 1 in upper channel.

(b) Case 3 in lower channel and Case 3 in upper channel.
Figure 2-14: Average outlet concentration for coculture bilayer configuration with varying $\Gamma$: Case 1 in upper channel and Case 3 in lower channel. $Q_I = 2.5 \text{ nL/s}$ and $\gamma = 0.5$ while $\Gamma$ varies.

Figure 2-15: Average outlet concentration for coculture bilayer configuration with varying $\Gamma$: Case 2 in upper channel and Case 2 in lower channel. $Q_I = 2.5 \text{ nL/s}$ and $\gamma = 0.5$ while $\Gamma$ varies.
Figure 2-16: Average outlet concentration for coculture bilayer configuration with varying $\Gamma$: Case 3 in upper channel and Case 1 in lower channel. $Q_I = 2.5 \text{ nL/s}$ and $\gamma = .5$ while $\Gamma$ varies.

Figure 2-17: Average outlet concentration for coculture bilayer configuration with varying $\Gamma$: Case 3 in upper channel and Case 3 in lower channel. $Q_I = 2.5 \text{ nL/s}$ and $\gamma = .5$ while $\Gamma$ varies.
Figure 2-18: Reduction of the depletion zone in the bilayer construct. The solid blue curve is the concentration at the cell surface if \( Q_I = 10 \, nL/s \) and \( Q_{II} = 100 \, nL/s \). The depletion zone, which extends approximately .25cm, may be reduced in extent if we choose \( Q_I = 2.5 \, nL/s \) with \( Q_{II} = 43.75 \, nL/s \) (dashed black curve). The subsequent reduction is by a factor of about 4. Other parameters are those for Case 2.
Chapter 3

Analytical Investigation: Hydrodynamics

3.1 Introduction

In this chapter, we will investigate some properties of the bilayer construct relating to fluid flow. The reason for this is the presence of fluid flow across the membrane due to its natural porosity. In the previous chapter, we ignored such effects as an idealization, and it seems that in general, it would preferable to avoid such transmembrane flow for several reasons. Experimentally, it would be ideal to limit the amount of solute that enters from one flow stream to the other in order to maintain the integrity of the medium in each channel for later assay purposes, or in a more general sense, exercise control over the possible exchange of various biochemical factors in a culture's local fluidic environment. Moreover, the presence of additional fluid flux into the cell compartment could impart undue shear upon the cell culture. Thus, understanding and limiting transmembrane fluid flux is essential to maintaining precise control of the local fluidic environment and subsequently ensuring fidelity of experimental results. We will aim to derive several simple an-
alytical expressions that can be used as practical guidelines in the fabrication and implementation of bilayer devices.

3.2 Disturbances in the $y$-direction Flow Field

In the bilayer, the presence of transmembrane fluid flux will introduce a $y$-direction velocity that will perturb the flow field initially given by Eq. (2.8). Aside from the dimensions of the channel itself, one of the other parameters to be considered is the dimensions of the tubing that is necessary to deliver cell culture medium to the cell population. In this section, we will derive an expression that provides a guideline in choosing the geometrical parameters of the bilayer device and its associated tubing.

For a porous membrane, the hydraulic conductivity over a small surface element of area $\Delta A$ of the membrane is

$$\kappa = \frac{N\pi d_{\text{pore}}^2}{128\mu t} \quad (3.1)$$

with $t$ the thickness of the membrane, $N$ the number of pores present on the surface element, $\mu$ the fluid viscosity, and $d_{\text{pore}}$ the average diameter of a pore. $\kappa$ is defined by

$$Q = \kappa\Delta P \quad (3.2)$$

where $Q$ is the volumetric flow rate across the membrane and $\Delta P$ is the pressure drop across the membrane. The porosity $\varpi$ is given by

$$\varpi = \frac{N \pi d_{\text{pore}}^2}{4 \Delta A} \quad (3.3)$$

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so that

\[ \frac{N}{\Delta A} = \frac{4\varpi}{\pi d^2_{\text{pore}}}. \]  

(3.4)

Dividing both sides of Eq. (3.2) by \( \Delta A \) and letting \( \Delta A \to 0 \) gives for the local transmembrane velocity

\[ V = \sigma \Delta P \]  

(3.5)

where we define

\[ \sigma \equiv \frac{d^2_{\text{pore}} \varpi}{32\mu t}. \]  

(3.6)

To determine the magnitude of the disturbance the transmembrane flux has on the fluid flow in the cell compartment, consider both the flow channel (labelled “I”) and cell compartment (“II”) to be parts of a hydraulic network, with tube-device-tube elements. Then, across the circuit, \( \Delta P^{(i)} = R^{(i)}Q^{(i)} \) \((i = I, II)\), where \( R^{(i)} \) is the hydraulic resistance of the network and \( Q^{(i)} \) is the volumetric flow rate in the network. If the driving pressure in each network is \( P_0^{(i)} \) \((i = I, II)\) and each tubing opens out to \( P_{\text{atm}} (\equiv 0) \), then

\[ P_0^{(i)} = R^{(i)}Q^{(i)}. \]  

(3.7)

For elements in a series, \( R^{(i)} \) is written as a sum of individual elements: \( R^{(i)} = \)
\begin{equation}
R_{\text{tube},1}^{(i)} + R_{\text{device}}^{(i)} + R_{\text{tube},2}^{(i)} = \sum_j R_j^{(i)}, \text{ with}
\end{equation}

\begin{align*}
R_{\text{tube},1}^{(i)} &= \frac{128 \mu l_{\text{tube},1}}{\pi d_{\text{tube}}^4} \\
R_{\text{device}}^{(i)} &\approx \frac{12 \mu L_D}{wh^3} \left( 1 - \frac{h}{w} \cdot 0.630 \right)^{-1} \\
R_{\text{tube},2}^{(i)} &= \frac{128 \mu l_{\text{tube},2}}{\pi d_{\text{tube}}^4}
\end{align*}

(3.8)

where \( w \) is the width of the channel, \( h \) is the height of the channel, and \( L_D \) is the length of the channel (taken, as in Chapter 2 to be 200 \( \mu m \), 50 \( \mu m \), and 1.5 \( cm \), respectively; see Figure 3-1). For the pressure at the “beginning” of the device, \( P_{\text{device}}^{(i)} \),

\begin{equation}
P_0^{(i)} - P_{\text{device}}^{(i)} = R_{\text{tube},1}^{(i)} Q^{(i)}
\end{equation}

(3.9)

or

\begin{align*}
P_{\text{device}}^{(i)} &= P_0^{(i)} - R_{\text{tube},1}^{(i)} Q^{(i)} \\
&= \left( \sum_j R_j^{(i)} - R_{\text{tube},1}^{(i)} \right) Q^{(i)} \\
&= \left( R_{\text{device}}^{(i)} + R_{\text{tube},2}^{(i)} \right) Q^{(i)}
\end{align*}

(3.10)

Then, the maximum pressure difference across the membrane at the beginning of the device, \( \Delta P_{\text{device}} \), is given by (assuming the dimensions of the device are the same in both channels, as are those of the tubing coming out of the channels)

\begin{align*}
\Delta P_{\text{device}} &= (R_{\text{device}} + R_{\text{tube},2}) \Delta Q \\
&= \left[ \frac{12 \mu L_D}{wh^3} \left( 1 - \frac{0.630 \cdot h}{w} \right)^{-1} + \frac{128 \mu l_{\text{tube},2}}{\pi d_{\text{tube}}^4} \right] \Delta Q
\end{align*}

(3.11)
Figure 3-1: Overall device configuration with tubing and $l_{tube,1}$, $L_D$ and $l_{tube,2}$ defined.

where $\Delta Q$ is difference in flow rates in the two channels. For now, take $Q_{II} = 25 \, nL/s$ and $Q_I = 2.5 \, nL/s$.

The criterion for the disturbance caused by transmembrane flux to be minimal is given by [29]

$$\frac{h}{\delta} \lesssim 1$$

where $\delta$ is a length scale given by

$$\delta \equiv \frac{\nu}{V}$$

and where $\nu$ is the kinematic viscosity of the fluid and $V$ the transmembrane velocity ($= \sigma \Delta P_{device}$). Therefore,

$$\delta \sim \frac{\nu}{\sigma \left[ \frac{12\mu L_D}{\rho h^3} (1 - .630\frac{h}{w})^{-1} + \frac{128\mu L_{tube,2}}{\pi d_{tube}^4} \right] \Delta Q}$$

where $\sigma$ is given by Eq. (3.6).

For the membrane, we consider parameters consistent with some commercially available products. We consider membranes with two different pore sizes, both
available from GE Osmonics [30]: .8 μm and 8 μm. The associated thicknesses, porosities, and σ’s are summarized in Table 3.1. For tubing, we consider five tube diameter sizes available from Dow Corning: .30 mm, .51 mm, .76 mm, 1.02 mm, and 1.47 mm [31].

<table>
<thead>
<tr>
<th>d_pore</th>
<th>.8 μm</th>
<th>8 μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>t</td>
<td>9 μm</td>
<td>7 μm</td>
</tr>
<tr>
<td>N/ΔA</td>
<td>3 × 10^7 cm⁻²</td>
<td>1 × 10^5 cm⁻²</td>
</tr>
<tr>
<td>ω</td>
<td>.151</td>
<td>.050</td>
</tr>
<tr>
<td>σ</td>
<td>3.77 × 10⁻⁷ m²skg⁻¹</td>
<td>1.605 × 10⁻⁵ m²skg⁻¹</td>
</tr>
</tbody>
</table>

Table 3.1: Parameters for membranes with pore diameters d_pore = .8 μm and 8 μm.

Plots for δ on the basis of Eq. (3.14) for each of these cases are given in Figure 3-2. We can see from the ranges of δ that for relevant h (which we take to be in the range [50 μm, 200 μm]), that the disturbances should not interfere appreciably with the flow field save for the smallest tube diameter in the d_pore = 8 μm case.

3.3 How Well Does a Two-Dimensional Channel Approximate a Three-Dimensional One?

The two-dimensional transport problem we have considered in the previous chapter is much more tractable analytically than that of the full three-dimensional problem, and so, as an aside, we investigate to what extent we can approximate flow in a three-dimensional channel with that in two-dimensions. We shall compare the results for a two-dimensional flow versus that of a three-dimensional flow, and consider the aspect ratios for which the two-dimensional approximation remains valid.

We will assume that the origin is at the center of a rectangular channel, with
(a) $\delta$ as a function of tube length $l_{\text{tube,2}}$ for various diameters $d_{\text{tube}}$: $d_{\text{pore}} = 0.8 \, \mu m$.

(b) $\delta$ as a function of tube length $l_{\text{tube,2}}$ for various diameters $d_{\text{tube}}$: $d_{\text{pore}} = 8 \, \mu m$.

Figure 3-2: Characteristic disturbance length $\delta$ as a function of tube length $l_{\text{tube,2}}$ for various diameters $d_{\text{tube}}$: $d_{\text{pore}}$ is (a) $0.8 \, \mu m$ and (b) $8 \, \mu m$. 
dimensions spanning \( y \in [-a, a] \) and \( z \in [-b, b] \); in our problem, \( a = 1/2 \) and \( b = AR/2 \), where \( AR \) is the aspect ratio of the channel. The form of the velocity in three dimensions for laminar, fully developed flow is

\[
u(y, z) = \frac{4}{\mu \pi^3} \left( -\frac{\partial p}{\partial x} \right) \sum_{n=1,3,5,\ldots}^{\infty} \frac{(-1)^{n-1}}{n^3} \left\{ 1 - \frac{\cosh(n\pi z)}{\cosh(n\pi AR/2)} \right\} \cos(n\pi y) \tag{3.15}
\]

while in two dimensions, it takes the form

\[
u(y) = \frac{1}{2\mu} \left( -\frac{\partial p}{\partial x} \right) \left( y + \frac{1}{2} \right) \left[ 1 - \left( y + \frac{1}{2} \right) \right] \tag{3.16}
\]

This is simply Eq. (2.8) rewritten with the origin of the reference axis set at the middle of the channel in question. Eq. (3.16) may be expanded in a cosine series

\[
u(y) = \sum_{m=0}^{\infty} A_m \cos(m\pi y) \tag{3.17}
\]

where

\[
A_m = \frac{1}{2\mu} \left( -\frac{\partial p}{\partial x} \right) \int_{y=0}^{\frac{1}{2}} \left( y + \frac{1}{2} \right) \left[ 1 - \left( y + \frac{1}{2} \right) \right] \cos(m\pi y) dy \tag{3.18}
\]

Performing the integration, we find

\[
u(y) = \frac{4}{\mu \pi^3} \left( -\frac{\partial p}{\partial x} \right) \sum_{m=1,3,5,\ldots}^{\infty} \frac{(-1)^{m-1}}{m^3} \cos(m\pi y) \tag{3.19}
\]

that is, each term of the two-dimensional case differs from the three-dimensional case by a factor of

\[
1 - \frac{\cosh(n\pi z)}{\cosh(n\pi AR/2)}
\]

If we could ensure that this term was as close to unity as we desire, i.e. make
the ratio $\cosh(n\pi z)/\cosh(n\pi AR/2)$ small, we could well approximate the three-dimensional case with the two-dimensional case. Since $\cosh(n\pi z)/\cosh(n\pi AR/2) > 0$, we cannot recover the exact answer except at the plane $z = 0$ and in the limit $AR/2 \to \infty$, but we may stipulate, for instance, that the error between the two is within a certain value $\varepsilon$. We may write

$$\frac{u(y) - u(y, z)}{u(y)} \leq \varepsilon$$

or, rearranging,

$$\sum_{n=1,3,5,...}^{\infty} \frac{(-1)^{n-1}}{n^3} \left\{ 1 - \frac{\cosh(n\pi z)}{\cosh(n\pi AR/2)} \right\} \cos(n\pi y) \geq (1 - \varepsilon) \sum_{m=1,3,5,...}^{\infty} \frac{(-1)^{m-1}}{m^3} \cos(m\pi y)$$

Considering this term-by-term, we have

$$\left| \frac{\cosh(n\pi z)}{\cosh(n\pi AR/2)} \right| \leq \varepsilon \quad (3.20)$$

The $n = 1$ term will dominate, so we have

$$\left| \frac{\cosh(\pi z)}{\cosh(\pi AR/2)} \right| \leq \varepsilon \quad (3.21)$$

The permissible fraction of the central expanse of the channel is given by $2z^*/AR$, where $z^*$ is defined by

$$\left| \frac{\cosh(\pi z^*)}{\cosh(\pi AR/2)} \right| \equiv \varepsilon \quad (3.22)$$

A plot of the criterion Eq. (3.21) is given in Figure 3-3. The figure can be
used in order to determine the validity of future analyses in which flow in a three-
dimensional is approximated as two-dimensional.

Figure 3-3: Percentage of channel width that can be approximated by two-
dimensional flow field within error $\varepsilon$ for various channel aspect ratios. The solid
lines are from the analytical expression given by Eq. (3.21), while the dotted
lines are the exact percentages calculated numerically. The analytical expression
estimates the channel width percentage very well, particularly for aspect ratios
$\gtrsim 5$.

### 3.4 Perturbed flow field in the bilayer

In order to understand, at least to the next order of approximation, the effect of
the transmembrane fluid flux, we will now derive an expression for the velocity
field in the bilayer. The equation of motion for a long channel of height $h$ in two dimensions is, again, given by Eq. (2.8). The pressure gradient is constant (as may be found from an inspection of the Navier-Stokes equation for Poiseuille flow), and the pressure distribution is

$$P(x) = P_0 + \left( \frac{\partial p}{\partial x} \right) x$$

(3.23)

Let us suppose we have two channels now, so that, without any interaction between the fluid layers, we have (denoting the upper channel by subscript $II$ and the lower channel by subscript $I$)

$$\mu \frac{\partial^2 u_I}{\partial y^2} - \frac{\partial p_I}{\partial x} = 0$$

(3.24)

$$\mu \frac{\partial^2 u_{II}}{\partial y^2} - \frac{\partial p_{II}}{\partial x} = 0$$

(3.25)

Let us now perturb these velocities: $u_i(x,y) = u_{0,i}(y) + \delta u_i(x,y), i = I, II$. Additionally, let us consider the presence of a velocity in the $y$ direction, so that $v_i(x,y) = \delta v_i(x,y), i = I, II$. Let us suppose that the pressure field that exists in the channel dominates any perturbations that may exist thereof. Since the perturbed velocities are small, we may neglect the squares of them. In the limit of low Reynolds number, the inertial terms in the Navier-Stokes equations may be neglected, and then we will simply be left with

$$\nabla^2 \delta u_i = 0$$

(3.26)

$$\nabla^2 \delta v_i = 0$$

(3.27)

$$\frac{\partial \delta u_i}{\partial x} + \frac{\partial \delta v_i}{\partial y} = 0, \quad i = I, II$$

(3.28)
The boundary conditions are as follows, letting the height in the lower channel equal \( h_I \) and the height in the upper channel equal \( h_{II} \):

\[
\begin{align*}
\delta u_I(x=0,y) &= 0 \\
\frac{\partial \delta u_I}{\partial x} \bigg|_{x=L} &= 0 \\
\delta u_I(x,y=0) &= 0 \\
\delta v_I(x=0,y) &= 0 \\
\frac{\partial \delta v_I}{\partial x} \bigg|_{x=L} &= 0 \\
\delta v_I(x,y=0) &= 0
\end{align*}
\] (3.29)

and

\[
\begin{align*}
\delta u_{II}(x=0,y) &= 0 \\
\frac{\partial \delta u_{II}}{\partial x} \bigg|_{x=L} &= 0 \\
\delta u_{II}(x,y = h_{II}) &= 0 \\
\delta v_{II}(x=0,y) &= 0 \\
\frac{\partial \delta v_{II}}{\partial x} \bigg|_{x=L} &= 0 \\
\delta v_{II}(x,y = h_{II}) &= 0
\end{align*}
\] (3.30)

with the membrane condition

\[
\delta v_I(x,y = h_I) = \sigma [P_I(x) - P_{II}(x)] \\
\equiv \sigma [\Delta P_0 + (\Delta \delta P) x]
\] (3.33)

\[
\delta v_I(x,y = h_I) = \delta v_{II}(x,y = 0)
\] (3.34)
With these boundary conditions, we may expand the solutions for the \( y \)-direction perturbation velocity fields in Fourier series; consequently, we may write the solutions for \( \delta v_i \):

\[
\delta v_I(x, y) = \sum_{n=0}^{\infty} -2\sigma \left[ \Delta P_0 + \left( \Delta \delta P \right) \frac{(-1)^n}{\pi(n + \frac{1}{2})} \right] \sin \left[ \frac{(n + \frac{1}{2})\pi}{L} x \right] \sinh \left[ \frac{(n + \frac{1}{2})\pi}{L} h_I / L \right] \sinh \left[ \frac{(n + \frac{1}{2})\pi}{L} y \right]
\]

\[
\delta v_{II}(x, y) = \sum_{n=0}^{\infty} -2\sigma \left[ \Delta P_0 + \left( \Delta \delta P \right) \frac{(-1)^n}{\pi(n + \frac{1}{2})} \right] \sin \left[ \frac{(n + \frac{1}{2})\pi}{L} x \right] \sinh \left[ \frac{(n + \frac{1}{2})\pi}{L} h_{II} / L \right] \sinh \left[ \frac{(n + \frac{1}{2})\pi}{L} (h_{II} - y) \right]
\]

or, more concisely,

\[
\delta v_I(x, y) = \sum_{n=0}^{\infty} A_I^{(n)} \sin \left[ \frac{(n + \frac{1}{2})\pi}{L} x \right] \sinh \left[ \frac{(n + \frac{1}{2})\pi}{L} y \right] \tag{3.35}
\]

\[
\delta v_{II}(x, y) = \sum_{n=0}^{\infty} A_{II}^{(n)} \sin \left[ \frac{(n + \frac{1}{2})\pi}{L} x \right] \sinh \left[ \frac{(n + \frac{1}{2})\pi}{L} (h_{II} - y) \right] \tag{3.36}
\]

If we consider now the equation for the \( x \)-direction perturbed velocity, with the boundary conditions as given above, by Theorem 3, we cannot satisfy the equations as they are given. Instead, to solve the \( x \)-direction flow field, we consider the continuity equation

\[
\frac{\partial \delta u_i}{\partial x} + \frac{\partial \delta v_i}{\partial y} = 0 \tag{3.37}
\]

Integrating this

\[
\delta u_I = f_I(y) - \int \frac{\partial \delta v_I}{\partial y} \, dy
\]

\[
= f_I(y) + \sum_{n=0}^{\infty} A_I^{(n)} \cos \left[ \frac{(n + \frac{1}{2})\pi}{L} x \right] \cosh \left[ \frac{(n + \frac{1}{2})\pi}{L} y \right]
\]

\[
71
\]
and

\[ \delta u_{II} = f_{II}(y) - \int \frac{\partial \delta v_{II}}{\partial y} \, dx \]

\[ = f_{II}(y) - \sum_{n=0}^{\infty} A_{II}^{(n)} \cos \left( \frac{(n + \frac{1}{2})\pi}{L} x \right) \cosh \left( \frac{(n + \frac{1}{2})\pi}{L} (h_{II} - y) \right) \]

where \( f_{i}(y) \) is arbitrary. We will choose it to hold our boundary condition at \( x = 0 \), so that we have then

\[ \delta u_{I} = \sum_{n=1}^{\infty} A_{I}^{(n)} \left\{ \cos \left( \frac{(n + \frac{1}{2})\pi}{L} x \right) - 1 \right\} \cosh \left( \frac{(n + \frac{1}{2})\pi}{L} y \right) \]

\[ \delta u_{II} = \sum_{n=1}^{\infty} A_{II}^{(n)} \left\{ 1 - \cos \left( \frac{(n + \frac{1}{2})\pi}{L} x \right) \right\} \cosh \left( \frac{(n + \frac{1}{2})\pi}{L} (h_{II} - y) \right) \]

where \( A_{I}^{(n)} \) and \( A_{II}^{(n)} \) are as given above. We note that this solution holds up to the addition of a constant pressure term (\( \delta p \), say) to the Navier-Stokes equations.

### 3.5 A More Complete Stability Analysis of the Construct

A complete characterization of the stability of the flow field can in principle be carried out in accordance with the theory of hydrodynamic stability; in general, solving the equations of perturbation are very complex [32, 33, 34, 35, 36, 37, 38]. In our case, it requires a solution to the coupled Orr-Sommerfeld equations (one for each domain), connected by a pressure-dependent boundary condition. A numerical solution is necessary in carrying out the final calculation. The complex boundary condition at the membrane begs the question as to whether a truly linear stability analysis in the spirit of the traditional calculations exists for this problem. Nevertheless, we can attempt to extract some information by considering the
equations of stability in a simplified form. Of course, we would expect that under normal operating conditions, both flow fields would be stable. This is based on the following argument: In the case of a single channel Poiseuille flow, instability sets in at a high Reynolds number \( \text{Re} \approx 5700 \) \([38]\), much higher than would be the operating velocity for our microfluidic device [by comparison, our Reynolds number is, by reference to Table 2.2, \( \mathcal{O}(1) \)]. For low Reynolds numbers, however, we expect viscosity to have a stabilizing effect, and any perturbations should effectively be killed.

In order to conduct a simple linear stability analysis, we first need to consider the background flow field. We will do this slightly differently than in the previous section by deriving a solution that, in accordance with the traditional methods in linear stability analysis, is not \( x \)-dependent. Note that, for the following analysis, we will focus solely on the lower channel since, typically, this is where cells would be cultured. We consider this simple system in an attempt to determine a straightforward criterion for some system parameters that can be chosen so as to ensure stability of the flow field. Moreover, the method outlined can serve as a basis for a more comprehensive investigation of the topic.

We take the background flow fields \( \bar{u} \) and \( \bar{v} \) to be written as follows:

\[
\bar{u} = U + u' \quad (3.38)
\]
\[
\bar{v} = v' \quad (3.39)
\]

where \( U \) is the Poiseuille profile and the primed velocities are small changes in the field. Substituting these into the Navier-Stokes equations and ignoring small
quadratic quantities, we have

\[ U \frac{\partial u'}{\partial x} + v' \frac{\partial U}{\partial y} = \nu \frac{\partial^2 u'}{\partial y^2} \]

(3.40)

\[ U \frac{\partial v'}{\partial x} = \nu \frac{\partial^2 v'}{\partial y^2} \]

(3.41)

We note that applying dimensional arguments to Eq. (3.41) allows us to recover the condition for stability given earlier, (3.12). Now, we take any \( x \) dependency to be negligible. Then, we have

\[ v' \frac{dU}{dy} = \nu \frac{d^2 u'}{dy^2} \]

(3.42)

\[ 0 = \nu \frac{d^2 v'}{dy^2} \]

(3.43)

The boundary condition for \( v' \) will be that it is equal to zero at the lower wall and equal to a constant term \( \sigma \Delta P \) at the membrane. Hence, we have

\[ v' = \frac{\sigma \Delta P}{h} y \]

(3.44)

Now, we have by Eq. (2.8),

\[ U = \frac{h^2}{2\mu} \left( -\frac{dp}{dx} \right) \frac{y}{h} \left( 1 - \frac{y}{h} \right) \]

(3.45)

Let us non-dimensionalize \( y \) by defining \( y \equiv h\hat{y} \). Then,

\[ U = \alpha \hat{y} (1 - \hat{y}), \alpha \equiv \frac{h^2}{2\mu} \left( -\frac{dp}{dx} \right) \]

(3.46)
The equation for $u'$ then becomes

$$\frac{d^2 u'}{d\hat{y}^2} = \alpha \left( \frac{h \sigma \Delta P}{\nu} \right) \hat{y} (1 - 2\hat{y}) = \alpha \beta \hat{y} (1 - 2\hat{y}), \quad \beta \equiv \frac{h \sigma \Delta P}{\nu} \quad (3.47)$$

Integrating twice and applying the zero boundary conditions at $\hat{y} = 0$ and 1 gives

$$u' = \frac{\alpha \beta}{6} (\hat{y}^3 - \hat{y}^4) \quad (3.48)$$

so that

$$\tilde{u} = \alpha \left[ \hat{y} (1 - \hat{y}) + \frac{\beta}{6} (\hat{y}^3 - \hat{y}^4) \right] \quad (3.49)$$

We note that

$$\frac{d^2 \tilde{u}}{d\hat{y}^2} = \alpha [-2 + \beta \hat{y} (1 - 2\hat{y})] \quad (3.50)$$

With the background velocity field, we are in a position to analyze its stability.

Assume that the flow fields $\tilde{u}$ and $\tilde{v}$ and the pressure field $p$ are perturbed by disturbances $\tilde{u}$, $\tilde{v}$ and $\tilde{p}$, respectively, so that we take over the flow and pressure fields as follows:

$$\tilde{u} \rightarrow \tilde{u} + \tilde{u} \quad (3.51)$$
$$\tilde{v} \rightarrow \tilde{v} + \tilde{v} \quad (3.52)$$
$$p \rightarrow p + \tilde{p} \quad (3.53)$$

Substituting these into the Navier-Stokes equations, neglecting small quadratic
terms, and writing further

\[
\begin{align*}
\tilde{u} &= \tilde{u}(\tilde{y})e^{ik(x-ct)} \quad (3.54) \\
\tilde{v} &= \tilde{v}(\tilde{y})e^{ik(x-ct)} \quad (3.55) \\
\tilde{p} &= \tilde{p}(\tilde{y})e^{ik(x-ct)} \quad (3.56)
\end{align*}
\]

we get the following perturbation equations:

\[
\begin{align*}
[D^2 - k^2 - ikR(\bar{u} - c)] \dot{\tilde{u}} &= \text{Re}(D\tilde{u})\dot{\tilde{v}} + ikR\dot{\tilde{p}} \quad (3.57) \\
[D^2 - k^2 - ikR(\bar{u} - c)] \dot{\tilde{v}} &= \text{Re}D\dot{\tilde{P}} \quad (3.58)
\end{align*}
\]

Here, \(\text{Re}\) is the Reynolds number of the total flow \(\equiv \alpha h/\nu\) and we have written the differential operator \(d/d\tilde{y} \equiv D\). The boundary conditions are \(\tilde{u}, \tilde{v} = 0\) at the bottom wall and the membrane.

Along with the equations of motion, we also have the equation of continuity. By this, we can write

\[
\begin{align*}
\tilde{u} &= \frac{\partial \tilde{\phi}}{\partial \tilde{y}} \quad (3.59) \\
\tilde{v} &= -\frac{\partial \tilde{\phi}}{\partial x} \quad (3.60)
\end{align*}
\]

where \(x \equiv h\dot{x}\). Writing now

\[
\tilde{\phi} = \phi(\tilde{y})e^{ik(x-ct)} \quad (3.61)
\]

The perturbation equations then become a single fourth order differential equation, the Orr-Sommerfeld equation:

\[
(D^2 - k^2)^2 \phi = ik \text{Re} \left[ (\bar{u} - c) (D^2 - k^2) \phi - (D^2 \bar{u})\phi \right] \quad (3.62)
\]
The boundary conditions become \( \phi = D\phi = 0 \) at the lower wall and the membrane.

As mentioned, in general, this equation is exceptionally difficult to integrate, even for the simplest of background velocity fields. To get a basic idea of the stability, however, we will consider the limiting case of very great Reynolds number, corresponding to inviscid flow. We consequently argue that the conditions for instability for viscous flow be "less stringent" than for inviscid flow. In the limit of Re very large, the Orr-Sommerfeld equation reduces to the Rayleigh equation:

\[
(\ddot{u} - c) \left( D^2 - k^2 \right) \phi - (D^2 \ddot{u})\phi = 0 \tag{3.63}
\]

In the disturbance equations, we note that if we write the wave speed \( c = c_r + ic_i \), that if the imaginary part \( c_i > 0 \), the amplitude of the disturbance will grow arbitrarily large, and hence the flow is unstable.

We now demonstrate a sufficient criterion for instability in the flow field. If we take Eq. (3.63), multiply all terms by the complex conjugate of \( \phi \), \( \phi^* \), and integrate from \( \hat{y} = 0 \) to 1, we have (after integrating by parts)

\[
\int_{0}^{1} \left( |D\phi|^2 + k^2 |\phi|^2 \right) d\hat{y} + \int_{0}^{1} \frac{D^2 \ddot{u}}{\ddot{u} - c} |\phi|^2 d\hat{y} = 0 \tag{3.64}
\]

The first term is obviously real. For the second term, multiply by \((\ddot{u} - c^*)/(\ddot{u} - c^*)\) and then take the imaginary part of the above equation; we get

\[
c_i \int_{0}^{1} \frac{D^2 \ddot{u}}{|\ddot{u} - c|} |\phi|^2 d\hat{y} = 0 \tag{3.65}
\]

Assuming that the flow is unstable, \( c_i \neq 0 \). Therefore, the only way the integral can evaluate to 0 is if \( D^2 \ddot{u} \) changed signs throughout the interval \( \hat{y} \in [0, 1] \). Hence, a necessary condition for instability is that \( D^2 \ddot{u} = 0 \) somewhere in the flow field;
this result is known as the Rayleigh inflection point theorem. We note that since it does not guarantee us that \( c_i \neq 0 \), or if it is nonzero, what the sign of \( c_i \) would be, it is only a necessary condition, and not a sufficient one.

A somewhat stronger statement can be made by taking the real part of the above equation to get

\[
\int_0^1 \frac{D^2 \bar{u}}{|\bar{u} - c|} (\bar{u} - c_r) |\phi|^2 \, d\hat{y} = - \int_0^1 (|D\phi|^2 + \kappa^2 |\phi|^2) \, d\hat{y} < 0 \quad (3.66)
\]

We assume that Rayleigh’s inflection point criterion is satisfied; hence, \( D^2 \bar{u} = 0 \) at some value \( \hat{y} = y_c \) between 0 and 1. Multiplying Rayleigh’s result by \(-[\bar{u}(y_c) - c_r]\) and adding it to the above result gives then

\[
\int_0^1 \frac{D^2 \bar{u}}{|\bar{u} - c|} [\bar{u}(\hat{y}) - \bar{u}(y_c)] |\phi|^2 \, d\hat{y} < 0 \quad (3.67)
\]

Therefore, the necessary condition for instability is that

\[
D^2 \bar{u} [\bar{u}(\hat{y}) - \bar{u}(y_c)] < 0 \quad (3.68)
\]

somewhere in the flow. This result is Fjortoft’s theorem, and it gives a somewhat stronger result than the Rayleigh inflection point criterion.

The inflection point in \( \bar{u} \) may be found easily to be

\[
y_c = \frac{1}{4} \left( 1 \pm \sqrt{1 - \frac{16}{\beta}} \right) \quad (3.69)
\]

Evaluating the Fjortoft criterion, it is found that a sufficient condition for
instability to occur is that

\[ \beta > 16 \]  

(3.70)

or, in terms of the original variables,

\[ \frac{h\sigma \Delta P}{\nu} > 16 \]  

(3.71)

In the case that the upper channel flows at a greater speed than the lower channel, the quantity \( \sigma \Delta P \) evaluates to a positive number, which is to say, the transmembrane flux is in the positive \( \hat{y} \)-direction. We have then that, increasing the membrane permeability beyond the critical value \( \sigma_c \equiv (16\nu)/(h\Delta P) \) renders the flow unstable. Physical intuition suggests that, for finite Reynolds numbers, the critical value of \( \beta \) must be greater than 16, due to the stabilizing effect of the background viscosity. That this is the case is not entirely straightforward, but our result for \( \beta_c \equiv 16 \) is a good starting point and may be used as a first-order rule of thumb.
Chapter 4

Implementation of a Bilayer Device

4.1 Introduction

The actual fabrication and implementation of the bilayer device is obviously of practical interest. In this chapter, we will briefly outline some of the techniques that may be used to fabricate a bilayer microfluidic device, as well as some of the parameters that should be considered when doing so. We will demonstrate the actual fabrication of a bilayer microfluidic device, and the results of some initial experiments that have been carried out to characterize some properties of the device.
4.2 Overview: Materials, Design and Fabrication

The materials, design methodologies, and fabrication techniques utilized in the manufacture of a microfluidic device encompasses in general the microfabrication techniques employed in the microelectronics and other industries, though with certain extensions and modifications. It seems plausible that it is partly for this reason that microfluidics have become so pervasive; and, with the continual move in microelectronics towards cheaper and faster means of microfabrication capable of generating more complex geometries with finer resolutions, it is fair to say that the same trend applies to microfluidics. The basic manufacturing protocol can be summarized in the following steps:

1. Design the channel and auxiliary geometry using a computer design tool. By auxiliary geometry we mean support structures, fiducial markings, and the like.

2. Use any of a number of techniques to transfer this design onto a master or mold. This step will depend on the material being used for the final device.

3. Use the mold to transfer the channel geometry to any number of patterned devices, which will then serve as the final microfluidic device. Any additional parts, such as mounting or tubing, are added to the device.

We can expand on some of the options for each step below.

Step 1: There exist a number of computer-aided design (CAD) tools available for master generation depending on the geometry of the fluidic device. If the characteristic dimensions of the device lend themselves to photolithography or electron-beam lithography, the design can be generated in a circuit layout tool. If
it would be more suitable to manufacture the master using, say, a computer numerical control (CNC) mill, then it is reasonable to use a solid modeling package, such as Pro/Engineer or SolidWorks. In developing a design, it is, of course, imperative that issues related to the material and the entire manufacturing process of the device is taken into consideration.

**Step 2**: This step has the greatest amount of variability. A typical way of transferring the design to a mold would be to print the computer design to a photomask, then to use standard photolithographic techniques to transfer the design into a wafer coated in photoresist. If, as mentioned above, it is possible to use a mill to create the master, this will require generating an engineering drawing or an electronic file to input into a CNC machine. For the most popular method of fabrication, so-called soft lithography, it is suitable to transfer the design to a silicon wafer coated in photoresist which may or may not be further etched. If the material to which the design is being transferred is not suitable as a master, it will be necessary to introduce extra processes in this step. One such example of this would be if the final device material was chosen to be a cyclic olefin copolymer (COC)—a material which is becoming increasingly popular—and the material to which the design was initially transferred was a silicon wafer. In order to transfer a design to a COC, it must be embossed. Silicon is not suitable for this, though other materials such as copper sheet metal and Conapoxy (Cytec Industries) are suitable. This necessitates an extra transfer step of the design from the silicon to copper, or, in the case of Conapoxy, from the silicon to polydimethysiloxane (PDMS) and then to Conapoxy. It will subsequently be necessary to ensure that during the first transfer, the geometry has the correct polarity so that the final device has positive or negative features as desired.
Step 3: In this step, the microfluidic device is directly patterned. Again, the way in which this is done depends upon the material being considered, and care must also be taken to consider the way in which the patterned device is demolded. If the final device is to be made from a COC, then this step will be the embossing of a blank piece of COC against a copper sheet or Conapoxy piece in a laminator or hot embossing machine. In the soft lithography technique, a liquid PDMS mixture is poured upon the wafer and then cured. The PDMS can then be easily demolded from the wafer, and the wafer subsequently reused. This step is also when any additional details of fabrication are completed: the bonding of different layers (in order to create a closed construct, for instance), the addition of tubing and connectors, and so on.

4.2.1 Materials

A key question that naturally arises is what material ought to be used. There are a number of things that need to be considered in this regard. Materials that have been used in microfluidic culture systems include glass, biologically-based materials such as collagen, thermoplastics, and synthetic and thermosetting polymers. In general, it is essential that materials used in issue engineering microfluidic systems be biologically compatible. Attention must be paid to material cost, machinability, reusability, and part interchangeability, especially with regards to tubing and other interconnections that may be a part of the experimental setup. The ability to image the cell-culture system is of considerable importance, and thus the material should be at least transparent in the visible spectrum. The mechanical and chemical characteristics of materials must be considered, as well. The greater understanding of the role that the mechanical environment plays in cell behavior that has emerged in recent years means that purely mechanical and geometrical characteristics (such as stiffness and topology) must be taken into account when choosing suitable
materials, while functionalizing surface chemistries allows for the greater control and flexibility of the local chemical environment.

One of the first requirements often considered is the ability to image the device and its contents. This represents one of the moves away from the standard microelectronic fabrication techniques—silicon is opaque to optical and ultraviolet wavelengths and therefore not generally useful for microfluidic devices themselves. Moreover, certain components that are increasingly found in microfluidics, such as pumps and valves, are more easily fabricated from elastomeric materials than from, say, glass. For analytical microfluidic platforms, the most popular material used is polydimethylsiloxane (PDMS), a thermosetting polymer whose optical transparency from ultraviolet to the near infrared wavelengths, biocompatibility, and non-toxicity make it an attractive choice for researchers. One issue with PDMS is that it is susceptible to non-specific protein adsorption; this, however, may be remedied by surface treating the surface with an oxygen plasma or poly(ethylene oxide) to render it hydrophilic. Moreover, PDMS is known to be readily permeable to oxygen and some organic solvents, issues that must also be taken into account depending on the researcher’s needs. Other materials that have been used in microfluidics include poly(methyl methacrylate) (PMMA), polycarbonate (PC), and polystyrene, the material traditionally used in static tissue culture dishes. Each has its advantages and disadvantages, an excellent review of which is provided by Nunes, et al. [39]. It is possible that, for the focus of this particular study, COCs may be useful in the future due to its low O₂ permeability, which would provide for greater control of oxygen concentrations within the cell. Nevertheless, for stem cells, PDMS has a well-established history of use in microfluidic constructs [5, 6], and for this reason and those given above, PDMS is used in this study.
4.3 Implementation of Design

In this section we will discuss the implementation of a bilayer design based on the principles outlined in the previous section. The device was sketched [Figure 4-1(a)], and the layout of both the cell compartment and the flow channel designed in L-Edit (Tanner EDA) [Figure 4-1(b) shows an overlay of both]. The Y-configuration of both channels is so that other solutes or fluids can be introduced other than just cell growth medium; the staggering of the Y’s is so that tubing can be attached at the attach points (the circular areas) without interference. The actual geometrical specifications are as follows: The cell compartment [corresponding to the narrower Y shape in Figure 4-1(b)] was designed to 200 \( \mu m \) wide, while the flow channel was designed to a slightly larger value of 300\( \mu m \); this was done so that, during the bonding of both pieces, there is some flexibility in aligning both channels with one another. The overall length of the cell compartment was designed to 1.5 cm, while the length of the overlap of the cell compartment and the flow channel was designed to 1.15 cm.

The individual cell compartment and flow channel layouts were then patterned so as to fit on a 10 cm silicon wafer [Figure 4-2(a)], and a mylar photomask ordered (CAD/Art Services, Bandon, Oregon). Using the process outline in Appendix B, the silicon mask was patterned with the channel geometries using standard photolithographic techniques [Figure 4-2(b)].

The channel depths can be decided upon during the patterning stage, depending on the thickness of the SU-8 photoresist spun onto the silicon wafer. In our case, it was decided that, for a first design, the channel depths would be between 100 and 150 \( \mu m \); this was done primarily to ensure subsequent ease of culturing cells. The final channel height was measured, using a profilometer, to be about 137.5 \( \mu m \) (Figure 4-3).

The integration processes for the bilayer device is shown schematically in Figure
Figure 4-1: (a) Design concept sketch and (b) channel layouts designed in Tanner L-Edit software.

4-4. It was necessary to choose both an appropriate membrane and tubing, both of which were chosen based on the analysis in Section 3.2: 8 μm-pore PVC-free polycarbonate membranes from GE Osmonics [30], and .51 mm-diameter Silastic laboratory tubing from Dow Corning [31]. PDMS molds were made from the wafer master by first pouring the elastomer mixture onto the wafer, degassing the
mixture in a vacuum chamber to remove any bubbles, then allowing the mixture to cure in an oven set to 65°C. The membranes were treated with oxygen plasma prior to bonding with the PDMS pieces. Bonding of the membrane to the PDMS pieces was achieved by distributing a thin layer of Dow Corning 3140 silicone RTV coating to the PDMS pieces and placing the membrane onto the PDMS. The assembled pieces were allowed to cure at 65°C. Tubing was inserted into holes made at the attach points using a 1 mm biopsy punch. The tubing was attached using Dow Corning 3140 silicone RTV coating, the assembly again allowed to cure at 65°C. Completed bilayer devices are shown in Figure 4-5.
Figure 4-3: Channel cross-section measured by profilometer. Height is approximately 137 μm.

Figure 4-4: Soft lithography fabrication process for microfluidic device. (a) A silicon wafer is fabricated (see Appendix B); (b) liquid polymer (PDMS) is poured directly onto the wafer and placed in a vacuum chamber to eliminate any bubbles; (c) the PDMS is allowed to cure in an oven set to 65°C; (d) the PDMS is demolded from the wafer to reveal the channel geometries (e); (f) two PDMS pieces are bonded to a polycarbonate membrane using Dow Corning 3140 silicone RTV coating; and (e) tubing is attached to the device, again using Dow Corning 3140 coating.
4.4 Some Initial Experiments to Determine Membrane Parameters

In this section, we will outline some simple experiments which were considered in order to characterize two membrane parameters of interest: the diffusion coefficient of oxygen in the membrane, $D_{\text{membrane}}$, and $\sigma$.

4.4.1 Determining $D_{\text{membrane}}$

We must consider the diffusion coefficient of oxygen in the membrane. In order to do this, we will consider an analytical expression as well as experimental data, and compare the results. The experimental setup is as follows: the upper channel of the microfluidic device is flowed with water saturated with oxygen; the lower channel with water with negligible oxygen content (the deoxygenated water was obtained by mixing 100 mL of water with 1 g of sodium sulfite $\text{Na}_2\text{SO}_3$). The water was flowed through the device using a syringe pump; the rate at which
water is flowed is not especially important to the analysis, so long as it is sufficient that the concentration in the upper channel can be taken constant. An oxygen-sensing probe was placed at the outlet of the lower channel’s tube where oxygen concentration was measured as a function of time. In order to measure the diffusion coefficient of the membrane, we make use of the following relation:

\[ C = C_{0,\text{sat}} \left[ 1 - \exp \left( -\frac{D_{\text{membrane}} t}{\theta h} \right) \right] \]  

(4.1)

where we have written \( \theta \) for the membrane thickness in order to avoid confusion with the time \( t \) and \( h \) for the height of the channel. Eq. (4.1) may be obtained in the following manner: First, denote the concentration in the upper chamber as \( C_{\text{II}} \), that in the lower chamber \( C_I \), and that in the membrane \( C_M \). We suppose that the concentration within the membrane has reached steady state; that is, it has become linear. The concentration within the membrane is given by

\[ C_M = C_{\text{II}} - \frac{x}{\theta} [C_{\text{II}} - C_I] \]  

(4.2)

At this point, \( C_I = 0 \), since we have assumed that there is no solute in the flow stream entering the lower chamber. However, once this steady state has been reached, we can assume that solute begins entering the lower channel. Hence, \( C_I \) will have an explicit time dependence, and the rate of change of \( C_I \) will be given by the following application of the conservation of mass:

\[ V \frac{dC_I}{dt} = -D_{\text{membrane}} A \left. \frac{\partial C_M}{\partial x} \right|_{x=\theta} \]  

\[ = \frac{D_{\text{membrane}} A}{\theta} [C_{\text{II}} - C_I(t)] \]  

(4.3)

(4.4)

Here, \( A \) is the area of the membrane exposed to each chamber and \( V \) is the volume of the lower chamber, equal to \( A \) multiplied by the height of the chamber \( h \). Eq.
4.4 can then be integrated to give Eq. (4.1). We note again that the rate at which the water is pumped through the device is not important so long as the rate of convection is much greater than the rate of oxygen diffusion in water, since we can draw an arbitrarily long control volume down the length of the exit tubing to where the sensor is placed. We note for reference, however, that in our experiment, fluid was flowed through both tubes at 10 μL/min.

The sensor output is given in Figure 4-6; the concentration values have been normalized against the maximum value attained during the experimental run. We note that the concentration profile is not exactly of the form expected from Eq. 4.1; this, it is believed, was primarily due to the difficulty of placing the oxygen sensors in close proximity to the tube outlet and holding it steady. Nevertheless, the output allows us to obtain an order of magnitude estimate for $D_{\text{membrane}}$ based on the characteristic time scale required to achieve the maximum concentration value. Thus,

$$D_{\text{membrane}} \sim O(1) \times \frac{\theta h}{88 \, s}$$

$$= 1.09375 \times 10^{-7} \, \text{cm}^2/\text{s}$$

where we have used $\theta = 7 \, \mu m$ and $h = 137.5 \, \mu m$. We may compare this to previously published data in which, at a temperature of 25°C, $D_{\text{membrane}} = 5.4 \times 10^{-8} \, \text{cm}^2/\text{s}$ [40].

### 4.4.2 On the experiment for determining $\sigma$

**Experiment**

We may outline a simple experiment for determining the value of the permeability constant for a given membrane. Consider a container which is divided in half by the membrane. The width of the container is given by $w$. On one side, say the
Figure 4-6: Outlet concentration in terms of percent saturation measured as a function of time.

Figure 4-7: Experimental setup.
left side, fluid is poured to a height of $h_0$; then, we allow time to pass and allow the water to flow from the left side to the right. By measuring the height of the fluid on the right, $h_R$, with time, or that of the filled side, $h_L$, we may determine an approximate value for the permeability constant by comparing the measured height with the analytical expression obtained below.

**Formulation**

Let us formulate an approximate equation describing the time evolution of the heights. Consider the heights on the left and right side chambers, $h_L(t)$ and $h_R(t)$, respectively. The if $y$ is the dimension measured from the bottom of the chamber up, then the pressure in each chamber will be given by

$$
\begin{align*}
  p_L(y,t) &= \rho g [h_L(t) - y] \\
  p_R(y,t) &= \rho g [h_R(t) - y]
\end{align*}
$$

The difference in pressure $\Delta p$ across the membrane will then be given by

$$
\Delta p(y,t) = \begin{cases} 
\rho g [h_L(t) - y], & y > h_R \\
\rho g [h_L(t) - h_R(t)], & 0 < y < h_R
\end{cases}
$$

The transmembrane velocity $v(t)$ is given by

$$
v(t) = \sigma \Delta p = \begin{cases} 
\rho g \sigma [h_L(t) - y], & y > h_R \\
\rho g \sigma [h_L(t) - h_R(t)], & 0 < y < h_R
\end{cases}
$$
Let $A_L$ and $A_R$ be the cross-sectional areas of the chambers if viewed from above. Then, by the Reynolds transport theorem,

$$- A_L \frac{dh_L}{dt} = w \int_0^{h_L} v(t) \, dy$$  \hspace{1cm} (4.10)

$$A_R \frac{dh_R}{dt} = -A_L \frac{dh_L}{dt}$$  \hspace{1cm} (4.11)

Henceforth, we assume $A_L = A_R = A$. We can immediately integrate (4.11).

Using the fact that $h_R(t = 0) = 0$ while $h_L(t = 0) = h_0$, we have

$$h_R(t) = -[h_L(t) - h_0] \hspace{1cm} (4.12)$$

We may substitute Eq. (4.12) into Eq. (4.10), and, after using (4.9), obtain

$$\frac{dh_L}{dt} = - \frac{w \rho g \sigma}{A} \left\{ \int_0^{h_R(t)} [h_L(t) - h_R(t)] \, dy + \int_{h_R(t)}^{h_L(t)} [h_L(t) - y] \, dy \right\} \hspace{1cm} (4.13)$$

which, after substituting Eq. 4.12 simplifies to

$$\frac{dh_L}{dt} = - \frac{w \rho g \sigma}{A} \left[ h_L^2(t) - 3h_0 h_L(t) + \frac{3}{2} h_0^2 \right] \hspace{1cm} (4.14)$$

which can be integrated to give

$$h_L(t) = \frac{h_0}{2} \left\{ 3 - \sqrt{3} \tanh \left[ \frac{\sqrt{3} w \rho g \sigma h_0}{2} t + \tanh^{-1} \left( \frac{1}{\sqrt{3}} \right) \right] \right\} \hspace{1cm} (4.15)$$

Experimental results are given in Figure 4-8, and the analytical solution above fit to the data points. The value for $\sigma$ found in this way may be compared, too, to
the formula given earlier for a membrane comprised of cylindrical pores:

\[ \sigma = \frac{d_{\text{pore}} \varpi}{32 \mu t} \]  

(4.16)

Using the values given in Table 4.1, we find \( \sigma_{\text{theoretical}} = 1.61 \times 10^{-5} \text{ m}^2\text{skg}^{-1} \). This is about an order of magnitude away from the value obtained by obtaining an approximate fit to the data points, wherein \( \sigma_{\text{experimental}} = 1.3 \times 10^{-6} \text{ m}^2\text{skg}^{-1} \).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( d_{\text{pore}} )</td>
<td>8 ( \mu \text{m} ) [30]</td>
</tr>
<tr>
<td>( t )</td>
<td>7 ( \mu \text{m} ) [30]</td>
</tr>
<tr>
<td>( \varpi )</td>
<td>0.05 [30]</td>
</tr>
<tr>
<td>( h_0 )</td>
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<tr>
<td>( A )</td>
<td>18 cm(^2)</td>
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<tr>
<td>( w )</td>
<td>6 cm</td>
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Table 4.1: Parameters for membrane experiment.

### 4.5 Culturing hTERT MSCs in the Device

Cells were cultured in the devices for a very preliminary investigation in order to determine if cells could indeed be cultured in the device. The cells which were chosen for culture were mesenchymal stem cells transduced with human telomerase reverse transcriptase, otherwise known as hTERT MSCs. Using the protocol outlined in Appendix C, hTERT MSCs were cultured in a standard tissue culture Petrie dish to confluence (achieved after a period of approximately 4 days), resuspended, and then injected into the device tubing using a 1 mL syringe. The cell culture medium was replaced within the device daily by reinjecting medium; the cells were subsequently observed. Images of the cells are shown in Figures 4-9.
Analytical expression for $h_L$:

$$a = 1.3 \times 10^{-6} \text{ m}^2 \text{skg}^{-1}$$

Experimental data, averaged over 5 trials

Figure 4-8: Experimental data for $h_L$ compared to fit with $\sigma = 1.3 \times 10^{-6} \text{ m}^2 \text{skg}^{-1}$. See Appendix E for raw data.

4-10, 4-11, and 4-12. The cells were observed to have spread considerably despite the fact that the PDMS surfaces were not functionalized (for instance, with collagen) prior to seeding. Considerable cell spreading and evidence of subcellular structure suggest the cells were functional.
Figure 4-9: Cells in microfluidic device after one day. Scale bar on left is 100 \( \mu m \) and scale bar on right is 200 \( \mu m \).

Figure 4-10: Cells in microfluidic device after two days. Note evidence of subcellular structure. Scale bars are 100 \( \mu m \).
Figure 4-11: Cells in microfluidic device after two days. Scale bar is 200 $\mu m$.

Figure 4-12: Cells in microfluidic device after two days. Scale bar is 200 $\mu m$. 

(c)
Chapter 5

Conclusions

In this thesis, we have considered both analytically and experimentally several aspects of the membrane bilayer geometry. In particular, we have demonstrated the ability of the bilayer microfluidic device to deliver solute to a culture of cells more uniformly than possible with a typical, single channel device and, most importantly, independently of the shear stress imparted on the cell culture. Furthermore, we conducted simplified analyses and provided some guidelines in order to understand and control the transmembrane fluid flux that may be expected during the operation of the device. Finally, we discussed and demonstrated the actual fabrication of a membrane bilayer device from PDMS. We conducted several simple experiments in order to determine membrane properties and showed that the device can indeed sustain cells.

That being said, however, there is a great deal that still can be done in order to fully establish and characterize this particular device configuration; indeed, it is hoped that the work presented in this thesis will provide a foundation for more comprehensive studies of the bilayer geometry as applied to cell culture systems. The bilayer device has the potential of a great deal of power in the analytical study of biochemistry at the cellular level, and it seems to be an ideal experimen-
tal platform. A deeper understanding of its properties will ensure experimental robustness in the future.

5.1 Future Work and Goals

Each aspect of the bilayer presented in this thesis—its transport characteristics, the effect of transmembrane fluid flux in perturbing the flow field, and the implementation of the device itself—can be investigated and expanded upon significantly. Below, we will outline some possible routes that may be taken by future investigators.

5.1.1 Short-Term Goals

In the short-term, we may wish to focus on and expand upon some aspects of the device related to fluid flow, chemical transport, fabrication, and cell culture sustenance. This includes, for instance, conducting a more precise study of the hydrodynamical characteristics of the device, including an exact determination of the combination of membrane parameters and Reynolds number for which instability sets in. Moreover, it may be of pedagogical interest to attempt to develop an analytical theory of stability for flows in connected domains that goes further than the first order investigation presented in this thesis. We may extend the calculation for transmembrane flow to include complex porous materials, such as hydrogels, that are likely to be implemented in future studies (see “Long-Term Goals” below). At this point, it seems likely that the bulk of the calculations would be carried out computationally using, for instance, a commercially available package capable of solving coupled partial differential equations.

With regards to chemical transport, we may begin to develop models that take into account the intrinsically coupled nature of transport, differentiation,
and proliferation by developing models that include each of these in addition to binding kinetics for several solutes. From an experimental point of view, it is desirable to integrate directly oxygen sensors into the device, rather than placing the oxygen sensors downstream of the outlet tubing. Obviously, this would provide us with spatial resolution of chemical gradients within the device itself, so that, after assaying the cells, we may gain a greater level of fidelity in elucidating the relationship between chemical gradients and cell behavior (see Figure 5-1 below for a possible means of incorporating an oxygen sensor into a device).

We may, as mentioned in Chapter 4, consider fabricating our devices from materials other than PDMS. This will, however, certainly require the consideration of fabrication techniques different from the soft lithography method presented in Chapter 4.

Finally, a complete experimental investigation into the behavior of cells as cultured in the microfluidic device may be carried out. In principle, this should be relatively straightforward, and includes assaying the cells for movement and metabolic function in real-time. Specific metabolic function may be assessed by isolating medium after it has been perfused through the device, collected, and assayed as required.

### 5.1.2 Long-Term Goals

In contrast to some of the short-term goals outlined above, in the long-term, it is envisioned that studies involving the bilayer will focus on exploiting the design geometry in order to understand problems in intercellular and intracellular communication in order to develop robust communication network models to better understand biological behaviors in both normal and pathological contexts. Accomplishing this necessitates the inclusion of powerful, engineered biochemical tools, the development of which is, at the time of this writing, taking place in several
labs at MIT [41].

One potential route is to incorporate, into the membrane or the channel geometry itself, protein-specific biosensors that have the potential to allow for real-time temporal and spatial study of cellular communication networks. In this case, the presence of various proteins or cytokines (such as TNFα, which is implicated in inflammatory response) in the extracellular environment would quench a fluorescent signal from a target-specific sensor molecule, and therefore indicate the presence of the target molecule in quantities proportional to the decrease in the fluorescent signal.

Once such sensors have been incorporated into the device architecture, we may then expose the cell culture or cultures to chemical gradients and shear in what we envision to be a comprehensive, physiologically-relevant microfluidic platform for understanding cell communication networks.
Figure 5-1: Possible future microfluidic device configurations. Each demonstrates ways in which oxygen sensors and flow inlet and outlets can incorporated into the device itself while allowing for the possibility of multiple membranes and modularity between different compartments. Each compartment may, in principle, be used to culture a different type of cell.
Appendix A

Some Theorems for Partial Differential Equations

A.1 Determination of $A_i$s

In developing the theory presented in Chapter 2 for transport in domains connected by boundary conditions, it was necessary to extend the notion of self-adjointness beyond that given in usual Sturm-Liouville theory. Furthermore, elucidating a notion of orthogonality for the solutions $C_{I,i}$ and $C_{II,i}$ within the domains $\Omega_I$ and $\Omega_{II}$ is somewhat complicated by the fact that the boundary conditions linking the two solutions are dependent upon the $x$-direction as well. These difficulties were overcome by means of Theorems 1 and 2 below.

**Theorem 1** With the given boundary conditions (2.29), (2.30), (2.31), and (2.32),

$$\langle \mathcal{L}[C_i], C_j \rangle = \langle C_i, \mathcal{L}[C_j] \rangle$$

where $\mathcal{L} \equiv \frac{\partial^2}{\partial y^2}$ and $\langle \varphi_i, \varphi_j \rangle \equiv \int_I \varphi_{I,i} \varphi_{I,j} + \int_{II} \varphi_{II,i} \varphi_{II,j}$. 

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Proof.

\[
\langle \mathcal{L}[C_i], C_j \rangle = \int_{y=0}^{1} \frac{\partial^2 C_{1,i}}{\partial y^2} C_{1,j} dy + \int_{y=0}^{\beta} \frac{\partial^2 C_{11,i}}{\partial y^2} C_{11,j} dy
\]

\[
= \frac{\partial C_{1,i}}{\partial y} C_{1,j} \bigg|_{y=0}^{1} - \int_{y=0}^{1} \frac{\partial C_{1,i}}{\partial y} \frac{\partial C_{1,j}}{\partial y} dy + \frac{\partial C_{11,i}}{\partial y} C_{11,j} \bigg|_{y=0}^{\beta} - \int_{y=0}^{\beta} \frac{\partial C_{11,i}}{\partial y} \frac{\partial C_{11,j}}{\partial y} dy
\]

\[
= \frac{\partial C_{1,i}}{\partial y} C_{1,j} \bigg|_{y=0}^{1} - C_{1,i} \frac{\partial C_{1,j}}{\partial y} \bigg|_{y=0}^{1} + \int_{y=0}^{1} C_{1,i} \frac{\partial^2 C_{1,j}}{\partial y^2} dy
\]

\[
+ \frac{\partial C_{11,i}}{\partial y} C_{11,j} \bigg|_{y=0}^{\beta} - C_{11,i} \frac{\partial C_{11,j}}{\partial y} \bigg|_{y=0}^{\beta} + \int_{y=0}^{\beta} C_{11,i} \frac{\partial^2 C_{11,j}}{\partial y^2} dy
\]

\[
= \int_{y=0}^{1} C_{1,i} \frac{\partial^2 C_{1,j}}{\partial y^2} dy + \int_{y=0}^{\beta} C_{11,i} \frac{\partial^2 C_{11,j}}{\partial y^2} dy
\]

\[
= \langle C_i, \mathcal{L}[C_j] \rangle
\]

after repeated integration by parts and cancellation from the boundary terms. ■

From Theorem 1, we may state the following

Theorem 2

\[
A_{11,i} A_{11,j} \int_{y=0}^{\beta} \eta_{11,i} u(y) \eta_{11,j} dy + \frac{Pe_i}{Pe_{11}} A_{1,i} A_{1,j} \int_{y=0}^{1} \eta_{1,i} u(y) \eta_{1,j} dy = 0, \quad (i \neq j)
\]
Proof. We have

$$\int_{y=0}^{1} \frac{\partial^2 C_{1,i}}{\partial y^2} C_{1,j} dy + \int_{y=0}^{\beta} \frac{\partial^2 C_{11,i}}{\partial y^2} C_{11,j} dy - \int_{y=0}^{1} C_{1,i} \frac{\partial^2 C_{1,j}}{\partial y^2} dy - \int_{y=0}^{\beta} C_{11,i} \frac{\partial^2 C_{11,j}}{\partial y^2} dy = 0$$

$$\text{Pe}_I \int_{y=0}^{1} u(y) \frac{\partial C_{1,i}}{\partial x} C_{1,j} dy + \text{Pe}_{II} \int_{y=0}^{\beta} u(y) \frac{\partial C_{11,i}}{\partial x} C_{11,j} dy - \text{Pe}_I \int_{y=0}^{1} C_{1,i} \frac{\partial C_{1,j}}{\partial x} dy -$$

$$\text{Pe}_{II} \int_{y=0}^{\beta} C_{11,i} u(y) \frac{\partial C_{11,j}}{\partial x} dy = 0$$

$$-\lambda^2_{I,i} A_{I,i} A_{I,j} e^{\left(\frac{\lambda^2_{I,i}}{4 \text{Pe}_I} + \frac{\lambda^2_{I,j}}{4 \text{Pe}_I}\right) x} \int_{y=0}^{1} \eta_{I,i} u(y) \eta_{I,j} dy$$

$$-\lambda^2_{II,i} A_{II,i} A_{II,j} e^{\left(\frac{\lambda^2_{II,i}}{4 \text{Pe}_{II}} + \frac{\lambda^2_{II,j}}{4 \text{Pe}_{II}}\right) x} \int_{y=0}^{\beta} \eta_{II,i} u(y) \eta_{II,j} dy$$

$$+\lambda^2_{I,j} A_{I,i} A_{I,j} e^{\left(\frac{\lambda^2_{I,i}}{4 \text{Pe}_I} + \frac{\lambda^2_{I,j}}{4 \text{Pe}_I}\right) x} \int_{y=0}^{1} \eta_{I,i} u(y) \eta_{I,j} dy$$

$$+\lambda^2_{II,j} A_{II,i} A_{II,j} e^{\left(\frac{\lambda^2_{II,i}}{4 \text{Pe}_{II}} + \frac{\lambda^2_{II,j}}{4 \text{Pe}_{II}}\right) x} \int_{y=0}^{\beta} \eta_{II,i} u(y) \eta_{II,j} dy = 0$$

Since $\frac{\lambda^2_{I,i}}{\text{Pe}_I} = \frac{\lambda^2_{II,i}}{\text{Pe}_{II}^2}$,

$$(\lambda^2_{II,j} - \lambda^2_{II,i}) \left[ A_{II,i} A_{II,j} \int_{y=0}^{\beta} \eta_{II,i} u(y) \eta_{II,j} dy + \frac{\text{Pe}_I}{\text{Pe}_{II}} A_{I,i} A_{I,j} \int_{y=0}^{1} \eta_{I,i} u(y) \eta_{I,j} dy \right] = 0$$

The cases $i$ and $j$ are distinguished by their different characteristic values $\lambda^2$; therefore, $\lambda^2_{II,i} \neq \lambda^2_{II,j}$, and the quantity in the brackets is equal to zero. $\blacksquare$
A.2 Possible solutions to Laplace’s equations with certain boundary conditions

Theorem 3 For Laplace’s equation

\[ \nabla^2 \psi = 0 \]

with boundary conditions \( \psi(x = 0, y) = 0, \frac{\partial \psi}{\partial x}|_{x=L} = 0, \psi(x, y = 0) = 0 \) and \( \psi(x, y = h) = 0 \), the solution vanishes identically over \( \Omega \equiv [0, L] \times [0, h] \).

Proof. By Green’s identity

\[
\int_{\Omega} \psi \nabla^2 \psi \, dV = \int_{\partial \Omega} \psi \frac{\partial \psi}{\partial n} \, dS - \int_{\Omega} \left\{ \left( \frac{\partial \psi}{\partial x} \right)^2 + \left( \frac{\partial \psi}{\partial y} \right)^2 \right\} \, dV
\]

But the left side and the first term on the right equal zero, so that we are left with

\[
\int_{\Omega} \left\{ \left( \frac{\partial \psi}{\partial x} \right)^2 + \left( \frac{\partial \psi}{\partial y} \right)^2 \right\} \, dV = 0
\]

which is satisfied if and only if

\[
\frac{\partial \psi}{\partial x} = 0 \text{ in } \Omega; \quad \frac{\partial \psi}{\partial y} = 0 \text{ in } \Omega
\]

Since we have \( \psi(x = 0, y) = 0 \) and \( \psi(x, y = 0) = 0 \), the solution is identically zero throughout. ■
Appendix B

Fabrication Protocol for Silicon Wafer

1. 100 mm diameter, 1000 µm-thick silicon wafers are obtained.

2. Wafers are cleaned for 20 minutes using a fresh Piranha bath, with composition 1 H₂SO₄ : 1 H₂O₂ by volume. The wafers are rinsed with DI water for 5 minutes, and then spin rinse dried with N₂.

3. Wafers are dehydrated for 30 minutes in an oven set at 110°C.

4. SU8 negative photoresist (MicroChem Corp., Newton, MA) is spun upon the silicon wafer at a spin rate and ramp rate appropriate to the desired thickness. After each spin, the SU8 is softbaked at 95°C for a period of 25 minutes.

5. The photomask is cleaned. For a mylar mask (used in this study), the mask is sprayed with acetone and isopropyl alcohol (IPA) before being dried with an N₂ gun. The mask and wafer are placed in a photomask aligner [in this case, a Suss MA-6 (SUSS MicroTec AG, Garching, Germany)].
6. The wafer is exposed for 40 seconds at a dosage of 24 $W/cm^2$ with an alignment gap of 200 $\mu m$.

7. The wafers are given a post-exposure bake, first at 65°C for 1 minute, then at 95°C for 8 minutes. The wafers are allowed to cool for 10 minutes in an N$_2$ box.

8. The wafers are developed using SU8 developer from the same manufacturer (MicroChem Corp.) for 14 minutes. They are placed in a sonicator for 1 minute afterwards. After this, the wafers are placed for 30 seconds in a clean bath of SU8 developer.

9. The wafers are rinsed with IPA and dried with an N$_2$ gun.
Appendix C

Cell Culture Protocols for hTERT MSCs

C.1 Ingredients Used for hTERT MSC Growth Medium

Below, we list the ingredients necessary to create growth medium appropriate for MSCs:

1. 430 mL Dulbecco’s Modified Eagle Medium, sterile filtered using a .22 μm filter.

2. 5 mL sodium pyruvate, 100 × 10 mM.

3. 50 mL fetal bovine serum (FBS).

4. 5 mL Penicillin and Streptomycin.

5. 5 mL L-Glutamine, 200mM.
After the cells have been cultured in a dish, it is necessary to both feed them and split them after they have reached confluence. The steps required for this are outlined below.

C.2 Protocol Used for Splitting hTERT MSCs

1. Pre-warm all reagents (i.e. Phosphate-buffered saline (PBS), pH7.2 – 7.4; Trypsin-EDTA (0.05% trypsin); Serum-containing cell culture medium) to 37°C in water bath.

2. After reagents are warmed, spray bottles down with ethanol and prepare the hood as for routine feeding.

3. Aspirate spent culture media from the cell culture vessel.

4. Wash the cells once with PBS. Add 5 ml of PBS for every 25 cm² of culture area.

5. Aspirate the PBS.

6. Add 1 – 2 ml per 25 cm² of trypsin-EDTA into the culture flask (i.e., 5ml of trypsin-EDTA for a T-75 culture flask), and return the sealed flask to the incubator for 5 minutes.

7. Once the cells have detached, add serum-containing medium to the flask in an amount approximately 2 – 3× that of the trypsin. Trypsin will start to act on the excess serum proteins instead of harming the cells.

8. Collect the harvested cells and pipet into an appropriately sized centrifuge tube.

9. Centrifuge cells for approximately 5 minutes at 1000 RPM.
10. Following centrifugation, aspirate the media above the cell pellet and resuspend the cells in a logical volume (5 – 10 ml).

11. Resuspend pelleted cells in an appropriate volume of growth medium and dispense into sterile flasks or onto your experimental surfaces.

C.3 Protocol Used for Feeding hTERT MSCs

1. Pre-warm medium to 37°C in water bath.

2. After reagents are warmed, thoroughly spray the hood with ethanol.

3. Take cells out of the incubator and examine them with a table-top phase microscope. Ensure there are no signs of contamination or unusual cell morphology before feeding and placing cells in the hood.

4. Aspirate spent culture media from the cell culture vessel.

5. Carefully pipette the appropriate amount of growth medium to the cells. Slowly pipette the medium down the side of the culture flask so not to disrupt the cell layer with shear flow. Medium volume is highly dependent on cell culture vessel size. Too low a volume will not contain enough nutrients for the cells, and too high a volume will inhibit oxygen diffusion.
Appendix D

Backflow in Y-Channel

In this appendix, we provide a criterion for ensuring there exists no backflow in a Y-channel such as that implemented in Chapter 4.

The mass and momentum conservation laws are

\[
\frac{d}{dt} \int_{CV(t)} \rho dV + \int_{CS(t)} \rho \mathbf{v} \cdot \mathbf{n} dA = 0 \quad \text{(D.1)}
\]

\[
\frac{d}{dt} \int_{CV(t)} \rho \mathbf{v} dV + \int_{CS(t)} \rho \mathbf{v} \cdot \mathbf{n} dA = F_{CV(t)}, \quad \text{(D.2)}
\]

respectively. Assuming an outflow pressure of \( p_{atm} \) and the same cross-sectional dimensions for each channel, these simplify then to

\[
v_1 + v_2 = v_{out} \quad \text{(D.3)}
\]

\[
h_3 v_{out}^2 - (h_2 v_2^2 + h_1 v_1^2) = \frac{1}{\rho} \{ h_1 p_1 + h_2 p_2 - p_{atm} (h_1 + h_2 - h_3) \} \quad \text{(D.4)}
\]

where \( v_1, v_2, \) and \( v_{out} \) are the average velocities at inlet ports 1 and 2, and the outlet, respectively. Rearranging and substituting Eq. (D.3) into Eq. (D.4) then
gives

\[(h_3 - h_2)v_2^2 + (2h_3v_1)v_2 + (h_3 - h_1)v_1^2 - \frac{1}{\rho} \{h_1p_1 + h_2p_2 - p_{atm}(h_1 + h_2 - h_3)\} = 0\]

So that solving gives

\[v_2 = \frac{-(2h_3v_1) \pm \sqrt{(2h_3v_1)^2 - 4(h_3 - h_2)[(h_3 - h_1)v_1^2 - \frac{1}{\rho} \{h_1p_1 + h_2p_2 - p_{atm}(h_1 + h_2 - h_3)\}]}{2(h_3 - h_2)}\]

\[v_{out} = v_1 + v_2\]

Backflow is given by the condition \(v_2 < 0\), so

\[(h_3 - h_1)v_1^2 - \frac{1}{\rho} \{h_1p_1 + h_2p_2 - p_{atm}(h_1 + h_2 - h_3)\} > 0\]  \hspace{1cm} (D.5)

Corresponding to this we have

\[p_2 < \frac{\rho(h_3 - h_1)v_1^2 - h_1p_1 + p_{atm}(h_1 + h_2 - h_3)}{h_2}\]  \hspace{1cm} (D.6)

as the backflow pressure condition at the upper inlet.
Appendix E

Data from measurements to determine $\sigma$

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Table E.1: Data for $h_L$
References


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