Design, Modeling, and Validation of an Apical Flow Transwell Insert for Small Intestinal Models

by

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Submitted to the Department of Mechanical Engineering in partial fulfillment of the requirements for the degree of Master of Science in Mechanical Engineering at the MASSACHUSETTS INSTITUTE OF TECHNOLOGY

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Abstract

This thesis presents the design, modeling and experimental validation of a device that allows for apical flow over an in vitro small intestine model. The device is designed to interface with commercially available hanging Transwells. Design considerations and specifications are discussed, based on user considerations, mechanical requirements, and biological requirements. To create a more informed design, multiple phenomena are modeled and results are used to modify design characteristics and give insight into viable operating parameters, such as apical flow rate. Within the device, velocity profiles of media flow as well as oxygen transport and cellular oxygen consumption are modeled. External to the device, heat transfer from a warm incubator into room temperature tubing containing media is modeled to ensure proper media warming. Experimental validation of the flow models is done by tracking dye flow through the insert, and preliminary flow experiments appear to be in agreement with modeling results. The design, modeling, and experimental results discussed in this thesis present a promising step toward a device capable of maintaining oxygen gradients that recapitulate specific aspects of the complex, dynamic environment within the small intestine.

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Chapter 1

Introduction

The small intestine is a remarkably complex organ. Functionally, it plays a crucial role in nutrient absorption: food that is mostly digested by the stomach is passed to the small intestine, where essential nutrients (e.g. sugars, amino acids, fatty acids) are transported out of the intestine and into the bloodstream. This process, however, does not only apply to food; the small intestine also plays a large role in drug absorption, as drug metabolism in the intestinal epithelial layer (followed by further metabolism in the liver, in many cases) significantly affects the bioavailability of orally administered drugs, as illustrated in Figure 1-1 [1, 2].

For this reason, among others, there is a growing body of work focused on further understanding the small intestine’s barrier and drug metabolism roles. In this chapter, we will discuss approaches to creating in vitro models of the small intestine, which are particularly useful for testing during early stage drug discovery in the drug development process.
ORAL DRUG INTAKE

Drug Disintegration and Dissolution

Loss via Luminal Degradation

Diffusion through Gastrointestinal Fluids

Membrane Permeation

Loss via Mucosal Metabolism

Uptake into Blood or Lymph

Loss via Hepatic Metabolism

Drug in Systemic Circulation

Bound Drug ← Unbound Drug

Clearance ← Free Tissue Drug Concentrations

Bound Tissue Drug Concentrations

Active Site Concentrations

EFFECT

Figure 1-1: Diagram of the relationship between an orally administered drug and its ultimate effect. Note the multiple paths for drug loss, particularly through mucosal metabolism of the small intestine followed by further metabolism in the liver. From [2]. Reprinted with permission by John Wiley & Sons, Inc.

1.1 The Gut Microenvironment

We begin with a brief description of the anatomy and microenvironment of the small intestine. As previously stated, partially digested food (called “chyme”) enters the small intestine from the stomach. This chyme slowly passes through the lumen of the small intestine, pushed forward through peristalsis—a wave of muscle contractions that travel down the length of the intestine. Intestinal epithelial cells known as enterocytes line the inner wall and form finger-like protruding microstructures called villi, as illustrated in Figure 1-2. It is widely accepted that the purpose of this villus structure is to increase the available surface area for absorption.

Immediately underneath the epithelium is a layer called the lamina propria, which
houses several types of immune cells. The immune component strongly contributes to the barrier function of the small intestine, as it must prevent pathogenic microorganisms from crossing the epithelium and entering the bloodstream. However, not all microbes are pathogenic—there exists a host of commensal microbes within the lumen that assist with further digestion of chyme.

The oxygen tension distribution in the gut is worth noting here. For the most part, tissue near the intestinal wall is oxygenated as a result of the proximity to blood vessels within the lamina propria and submucosa. As oxygen diffuses through the epithelium and into the lumen, it is consumed by immune cells, enterocytes, microbes near the epithelium, and any other aerobic constituents in the diffusion path. As a result of this dense concentration of oxygen-consuming species, oxygen tension quickly decreases as one moves from the submucosal layer to the lumen, to the point where the environment is nearly anoxic 1 mm away from the epithelium. As annotated in Figure 1-2, this creates a complex distribution of microbes: microaerophiles and facultative anaerobes will cluster near the oxygenated epithelium, while obligate anaerobes remain in the

Figure 1-2: Overview of intestinal villi anatomy, with oxygen concentration in relation to radial position drawn on the left. Note the steep gradient from the oxygenated submucosa to the nearly anoxic lumen. Reprinted from [3], ©2013, with permission from Elsevier.
hypoxic and anoxic areas deeper within the lumen. Thus, the steep, decreasing oxygen
gradient in the gut results in a population density gradient of microbes—similarly
decreasing for facultative anaerobes, and increasing for obligate anaerobes [3].

1.2 Previous Work

When attempting to translate the complex, dynamic environment of the small intesti-
tine into an in vitro model, the prime question to ask is which in vivo characteristics
should be recapitulated in vitro. For example, if one wishes to study drug absorp-
tion, an epithelial culture is essential. However, there are deeper choices to consider:
a monolayer of enterocytes may be simpler to culture (and, in turn, simpler to scale
for a high-throughput test), but it would be more physiological to recreate the high
surface area villi structure seen in vivo. To recapitulate barrier function, a number
of additions can be made, including goblet cells to produce mucus and immune cells
underneath the epithelium. The addition of commensal microbe strains to create a
microbe/mammalian cell coculture may also drive the culture toward a more phys-
iological state. We have yet to mention mechanical stimuli, such as shear on the
intestinal wall from luminal flow, buckling/compression of the epithelial layer dur-
ing peristaltic contraction, and the proper transport conditions to create a realistic
oxygen gradient.

Clearly, there are a myriad of potential approaches to creating an in vitro small
intestine model, and the end approach depends on the phenomena to be studied, as
well as consideration for balancing system complexity with ease of use and scalability.
In this section, we give an overview of selected work in this field, including static
cultures, dynamic devices that produce mechanical stimuli, and the current model
under development in our lab.
1.2.1 Static *In Vitro* Small Intestine Models

Caco-2, a human colon carcinoma cell line, is commonly used as a model for drug absorption in the small intestine. Although the Caco-2 cell line is not derived from enterocytes, the cells are still commonly used as a model for enterocytes due to their similar morphology and function [4, 5]. In fact, the highly cited, seminal paper published in 1989 on using the Caco-2 cell line as a small intestine barrier model has been credited with catalyzing the eventual migration from *in vivo* and *ex vivo* pharmaceutical studies to *in vitro* studies on cell lines for drug discovery [6].

Since then, many studies have improved on the Caco-2 monolayer to create *in vitro* models that more closely approximate the *in vivo* intestinal barrier. An increasingly common approach is the addition of a gel such as collagen or Matrigel on the apical side of a Transwell membrane to facilitate the growth of 3D cultures. For example, Caco-2 cells in coculture with mucus-producing cells and stromal cells (e.g. fibroblasts) are seeded on and/or in the gel before it cures, creating a 3D culture. One such demonstration of this was published by Li et al., resulting in a structure where fibroblasts were distributed among a collagen matrix while Caco-2 and mucus-producing cells were concentrated on the apical surface of the matrix, not unlike the epithelium and its underlying lamina propria [7].

Although Caco-2 is a reliable, widely used cell line, there have also been efforts to use primary enterocytes in their place, as their function would ideally be more physiological than Caco-2 cells. However, primary cells tend to be much harder to maintain than established cell lines; although short-term (on the order of two weeks) primary enterocyte cultures have been demonstrated, culture techniques for long-term maintenance of primary enterocyte cultures are still in development to the present day [8]. A fairly recent publication by DiMarco et al. describes a system composed of organoids assembled from murine small intestine explants and myofibroblasts, seeded
on a collagen gel that was set on a Transwell membrane or at the bottom of a well [9]. Organoids assembled in the gel on the Transwell demonstrated more contractile behavior, potentially as a result of the increased oxygen transport available in a suspended Transwell.

### 1.2.2 Devices with Flow

The examples described in Section 1.2.1 all utilize some form of an epithelial culture on a Transwell suspended in a multiwell plate, such as a 12-well plate. Although the coculture and surrounding matrix may be complex, the media environment in these situations is fairly simple as it is static over the course of the culture lifetime (apart from handling operations such as media replacement).

![Figure 1-3: Assembled (top) and exploded (bottom) solid models of device for exposing epithelial cultures to wall shear stress. Reproduced from [10] with permission from Springer.](image)

Attempting a variety of cocultures under a myriad of matrix conditions is not the only way to coax cells into a more physiological state. For example, stimulating the cells through shear may activate a mechanotransduction pathway that leads to more
realistic behavior, particularly among cell types that typically receive such stimuli *in vivo*—including enterocytes. To this effect, there have been many unique approaches for introducing shear to *in vitro* cultures. One such approach is illustrated in Figure 1-3, where a custom device has been designed to house several cultures arranged collinearly [10]. When the device is assembled, the apical sections of the cultures are sealed off from the surrounding atmosphere, and the only access is through an inlet and outlet channel on the apical side. When air flows through the channel (and technically media could work here as well), the cell cultures at the boundary of the channel experience a wall shear stress that could be modeled or calculated fairly easily, and the amount of shear can be controlled simply by varying flow rate.

Another flow device that recently gained some attention is the “gut-on-a-chip”
by Kim et al., which is illustrated in Figure 1-4. The device utilizes a microfluidic approach to an in vitro intestinal model; the cell culture is seeded on a membrane suspended in a microfluidic chamber, and media flow on both sides of the culture is made possible by flowing through inlet connections to the chamber. In contrast to the shear-inducing device in Figure 1-3, this device can be thought of as fully-enclosed and fully-integrated. There are no sub-components to assemble and disassemble; the device is, for all intents and purposes, a single part. This approach has its share of benefits and drawbacks. For example, while it is simpler for a user to physically handle the device, it is also harder to initially operate during an experiment, as cell seeding is not as straightforward as seeding on an open insert which can be done with the shear-inducing device.

1.2.3 Our In Vitro Model

![In Vitro Small Intestine Model Diagram]

Figure 1-5: Illustration of in vitro small intestine model currently under development in our lab. Basal flow is accomplished by a multi-tissue culture platform, also developed in-house. This culture model will form the basis for the device presented in this thesis.

The in vitro small intestine model currently under development in the Griffith lab [12], in collaboration with Rebecca Carrier at Northwestern University and Doug
Lauffenburger at MIT, is illustrated in Figure 1-5. Briefly, a coculture of enterocytes and mucus-producing goblet-like cells are grown on the apical side of the membrane of a Transwell while macrophages are seeded on the basal side. Thus, the apical media is analogous to the lumenal environment, while the basal media is analogous to systemic circulation. The Transwell can be placed in a well within a multi-tissue culture platform, developed in-house, that allows for basal media flow. This flow comes in the form of recirculation, media transfer from one well to another on the platform, or both.

We note here that this approach, in contrast to the examples given in Section 1.2.2, is a particularly beneficial mix of custom-developed solutions and commercial-off-the-shelf components; by using the nearly ubiquitous Transwell, the barrier to adoption of such a system is reduced, as many research groups already use Transwells to develop their own in vitro models.

However, there is currently no apical flow within our model, as the platform is currently designed only for basal flow within and between cultures. If the capability for apical flow on our model could be achieved, a more physiological apical microenvironment could be created, which in turn may drive our culture toward more in vivo-like behavior.
Chapter 2

Designing the Apical Insert

As a step toward improved control of the apical microenvironment within our \textit{in vitro} small intestine model, we present an apical insert that fits over a commercially available Transwell and allows for increased functionality. Namely, the insert allows for continuous apical media replenishment and control over local oxygen tension, capabilities that are not typically available in a standard Transwell in a static well.

This chapter describes various aspects of the design process behind the apical flow insert. We begin by illustrating the ideal concept behind the apical insert, followed by an examination of the commercially available Transwell and how particular features can be used advantageously for compatibility with the apical insert. The apical flow insert itself is then presented, with general notes on overall design and operation. To better elucidate the design process behind the insert, we then delve into a detailed discussion of design and operational specifications, both from a mechanical and biological perspective.
2.1 Concept

Figure 2-1 illustrates a theoretical \textit{in vitro} gut microenvironment, based on the current gut model described in Section 1.2.3. A co-culture of enterocytes and mucus-producing goblet-like cells are cultured on the apical side of a hanging Transwell. Macrophages—which serve as a model for immune response—are seeded on the basal side of the Transwell membrane. A group of microbe strains that are minimally representative of an \textit{in vivo} microbiome are introduced on the apical side.

Also seen in the schematic is the presence of media flow on both apical and basal sides. As previously described, basal flow is accomplished through a multi-tissue culture platform developed independently of the apical flow insert, and will not be extensively discussed in this thesis, while apical flow is accomplished through the
apical insert presented here. Apical flow in this tissue model serves not only as a means for media replenishment, but also for microbe population control as well as mucin shearing to maintain a relatively constant mucin concentration. Additionally, if the insert seals the apical side of the Transwell from the surrounding environment, this would allow for reduced oxygen tension in media as the cell/microbe culture consumes oxygen faster than it can diffuse into media through the basal side. Depending on the balance of oxygen consumption and diffusion, a microenvironment that more closely recapitulates the hypoxic domain within the lumen of the small intestine may be achieved.

2.2 The Transwell

![Figure 2-2: (a) Rendering of 12-well Transwell above a 12-well multiwell plate. The Transwell flange and well annulus form a contact pair when the Transwell is inserted into the plate. The locating feature assists with alignment during insertion. (b) Cutaway view of inserted Transwell.](image)

The hanging Transwell used in the experiments described here is a 12-well Transwell with 0.4 μm pores in a polyethylene terephthalate (PET) membrane (#3460, Corning Life Sciences, Tewksbury, MA). Figure 2-2(a) shows a rendering of a Transwell hovering above a 12-well plate, while Figure 2-2(b) illustrates a cross sectional view of the Transwell inserted into a well. As seen in the figure, the flange at the top
of the Transwell is the contact surface that allows it to hang over a well—specifically, the bottom of the flange contacts the top of an an extruded annulus above the well, and this contact pair is the only source of contact between the Transwell and multiwell plate. The solid model for the Transwell was created by measuring critical components of a physical Transwell with calipers multiple times. Each dimension that was input into the solid model was the mean of all measurements recorded for that particular dimension. The solid model for the 12-well plate was created from dimensions provided by Corning Life Sciences [13].

Within the Transwell, there are several characteristics of note. As these components are injection molded, the inner wall of the Transwell has a slight draft angle to assist with removal of the polystyrene shell from the mold. This draft angle must be accounted for to ensure the apical insert fits properly when placed into the Transwell. Another interesting design feature is the presence of three discrete flange supports (as opposed to, for example, a single tapered cylindrical shell) that connect the “well” of the Transwell to the flange. The supports are rotationally symmetric of order 3 (i.e. by 120°) about the centerline of the Transwell. One added benefit of these discrete supports is that they can be easily grabbed with tweezers during handling, thus serving as a stable point of contact for tweezers.

![Figure 2-3: Illustration of Transwell locating feature. In addition to preventing an off-center placement, the angled boss also guides the user toward the center of the well.](image-url)
As Transwells are typically used in a sterile environment due to the presence of live cell cultures, they are almost never handled directly—as previously mentioned, tweezers are commonly used for handling. Though this method may assist with precision, one disadvantage is that it lacks an element of tactile feedback that is normally present in manual handling. To facilitate tasks such as placing a Transwell into a multiwell plate, the Transwell has features that assist with locating—the outer face of the three flange supports have an angled boss that leads to the flange itself, as seen in Figures 2-2(a) and 2-3. The purpose of this angled feature is to assist with locating the Transwell toward the center of the well, as it is unlikely that users will be able to place a Transwell precisely in the center on their first attempt. As illustrated in Figure 2-3, an off-center placement is corrected as the angled boss guides the user toward the well center.

2.3 The Apical Flow Insert

With a better understanding of the design features of the commercial Transwell, we now present the features of the novel apical flow insert developed in this thesis work. We begin with an overview of the components used to achieve apical flow within a Transwell, along with instructions for device assembly and operation during an experiment.

2.3.1 Components and Geometry

Figure 2-4 shows exploded and assembled views of the components used to create flow along the apical side of a Transwell. As labeled in the figure, the component directly above the Transwell is the apical flow insert itself. When inserted into the Transwell, the cylindrical boss on the bottom of the apical insert protrudes into the Transwell, coming to a stop before colliding with the Transwell membrane. An o-ring
groove machined into the apical insert holds two 11mm ID x 1mm CS Viton o-rings (9263K549, McMaster-Carr, Robbinsville, NJ), which create a radial seal between the insert and the inner wall of the Transwell. The apical insert has two internal channels which form an inlet and outlet path for media flow; details of the flow profile will be described further in Section 3.2. Below the Transwell is the lower ring, a simple annulus with four 0-80 tapped holes that align with four clearance holes in the apical insert.

During assembly, the apical insert and lower ring fasten together, sandwiching the Transwell between them in the process. This ensures that the apical insert is fully extended into the Transwell, and also prevents the insert from becoming loose. Finally, two compression fittings with flat-bottom ferrules (P-844X, Upchurch Scientific, Oak Harbor, WA) can be screwed into ports on the top surface of the apical insert, thus allowing more rigid tubing (such as PTFE or PEEK tubing) to interface with the insert.
Figure 2-5: Cross-section view of apical flow system, illustrating the fluid flow path and contact surfaces between the apical insert, Transwell, and lower ring. A selection of critical dimensions are labeled: the total height of the system is 1" (neglecting fittings and tubing), the height of the apical flow channel created by the insert is 2 mm, and there is a 0.020" vertical gap between the Transwell rim and apical insert to prevent overdetermined contact between the components. Dimensions are in inches unless otherwise specified.

Figure 2-5 shows a cross-section view of the assembled system. The media flow path through the inlet fitting, insert, apical channel, and outlet fitting is highlighted. To create flow, tubing coupled to the inlet fitting can be connected to a syringe pump, although other methods for inducing gradual flow (e.g. gravity driven flow) may also be viable. The height of the apical channel, a critical dimension which will be further discussed in Section 2.4.2, is defined to be 2 mm. Other critical features labeled in Figure 2-5 include the o-ring seal and the contact surfaces between the insert, Transwell, and ring. The 0.020" gap between the top of the Transwell and the apical insert ensures that the system is not overdetermined by an abundance of contact surfaces—the Transwell/insert contact surface adjacent to the o-rings is sufficient to
determine the vertical position of the insert relative to the Transwell.

### 2.3.2 Operation

Because these components must be assembled in a sterile environment, it is necessary to develop an assembly procedure that minimizes the risk of contamination during this phase. Broad guidelines for working in a sterile environment such as a biosafety cabinet include the following:

1. Keep sterile parts sterile; sterile surfaces should only be in contact with other sterile surfaces. For example, the underside of a plate lid is considered sterile, but the top is not. If the tips of sterile tweezers rest on the top of a lid, the tweezers are now non-sterile.

2. When working with sterile substances (e.g. media) or cultures, minimize exposure time (such as when the cap is removed from a bottle, or the lid is removed from a plate).

3. Do not pass anything non-sterile (e.g. your hand or arm) over any sterile substance or culture that is exposed.

4. Minimize amount of time handling parts in general.

Additional guidelines may exist depending on the situation or laboratory; standard operating procedure varies from place to place.

With these guidelines in mind, a sterile assembly procedure can be constructed. The following procedure assumes all components are already sterilized (either by autoclaving or EtO sterilization):

1. Place the lower ring on a 12-well plate, concentric to one of the wells. The ring should sit on top of the well annulus.

2. Place a 12-well Transwell with a live culture onto the lower ring. The Transwell flange should be hanging on the lower ring, and the Transwell itself should be suspended in the well.
3. If there is apical media in the Transwell, aspirate it.

4. Gently place the apical insert (with an o-ring in the groove) into the Transwell. There is no need to press the insert down; the screws will create that force when fastened.

5. Place an 0-80 screw into each of the four clearance holes in the apical insert and fasten them to the lower ring (using a screwdriver that has either been autoclaved or sprayed with 70% ethanol).

6. Thread a pair of PEEK compression fittings and ferrules with sterile tubing, one for the inlet and one for the outlet (this step can also be completed in advance). If using a syringe pump, the inlet tubing should be connected to the syringe at this stage.

7. Fasten both compression fitting/tubing complexes to the apical insert. Set up outlet tubing for desired sample collection method (see Figure 2-6).

8. Begin priming the apical chamber. Preliminary results suggest that a relatively high flow rate of 30 mL h⁻¹ will prime the chamber in approximately one minute.

9. Switch to a slow flow rate (e.g. 500 µL d⁻¹) for continuous gradual media replacement.

Figure 2-6 illustrates two possible setups of the entire apical flow system. Because a vast majority of the system is fully sealed, there is one primary variable that determines hydrostatic pressure: height of the media at the outlet relative to the cell layer. Because the effects of hydrostatic pressure on these cells is unknown, it is desirable to maintain the media height at the outlet close to the height of the cell layer. Figure 2-6(a) is simple to set up and straightforward to collect media samples from; however, hydrostatic pressure will slowly build up as media height in the collection vial rises over time. In contrast, Figure 2-6(b) maintains a constant hydrostatic pressure, but retrieving media samples will require extra work from the user as the entire volume of sampling media is contained in a long coiled tube. It is worth noting that the setup in
Figure 2-6: Schematic of entire apical flow system. Two possible setups that minimize hydrostatic pressure on the cell layer are shown: (a) Outlet goes into a collection vial, where media level in the vial is at or near the height of the cell layer in the Transwell. (b) Outlet tubing is a long section of tubing that coils at the same height as the cell layer.

Figure 2-6(b) has the additional benefit of sample segregation by time; older samples will be more downstream in the tube, while newer samples will be upstream.

2.4 Apical Flow Insert Requirements

There are several requirements that must be considered in the course of designing the insert. In addition to standard mechanical considerations such as machining tolerances, fit, and designing for simple user handling, there are biological considerations to keep in mind as this device will be used to maintain a live cell culture. These requirements will be discussed in this section, then summarized in a requirements table at the end.
Figure 2-7: Design guidelines for a flat-bottomed port intended to interface to a 10-32 fitting with a flat-bottom ferrule. Note the callouts for a 32 microinch RMS surface finish and flatness on the bottom surface of the port to ensure a robust seal between the port and ferrule. Dimensions are in inches. Illustration adapted from a drawing made available by Upchurch Scientific [14].

2.4.1 Mechanical Considerations

Fabrication Tolerances

A typical machining tolerance for most dimensions is ±0.005", or about ±125μm from the nominal dimension—it is not uncommon to see a drawing block within a technical drawing specifying a “default” tolerance of ±0.005", and in fact many machine shops are capable of achieving higher precision during standard operation without loss of manufacturing speed. Nonetheless, designing for a general tolerance of ±0.005" is a safe approach, and callouts for higher precision in certain critical features can be specified as needed.

One such callout is detailed in Figure 2-7, which illustrates design guidelines for a flat-bottomed port that interfaces with a compression fitting. The compression
fitting, in turn, allows rigid tubing to connect to the apical flow insert for media replenishment. To prevent leaks, the fitting essentially applies a constant downward force on a flat-bottomed ferrule as it is screwed in, and the ferrule seals against the bottom surface of the port. Therefore, a sufficient "smoothness" must be specified on the bottom surface to ensure sealing against a reasonable pressure (generally on the order of 100+ PSI). Figure 2-7 specifies this smoothness through two callouts at the bottom of the port specifying a 32 microinch RMS surface finish and flatness within 0.002".

Figure 2-8: Rotational constraints during and after assembly. The first two renders in this series illustrate the location of the cross-sectional cut to better understand the context of the third render. The third render highlights the 120° rotational symmetry of the apical insert as well as the 4° buffer for rotational error between the Transwell and apical insert.

**Design for Assembly (DFA)**

Coupling the apical flow insert to a 12-well Transwell must be done by the user, so increased effort must be extended in designing an insert that assembles with a Transwell easily with minimal room for assembly error.

The apical flow components have several features to assist with assembly. The ID of the lower ring, which is the first component to be placed during assembly, is very close to the ID of a well from a 12-well plate. This allows the user to take advantage of the locating features on the Transwell (as described in Section 2.2) to center the
Transwell on the lower ring.

The apical insert, Transwell, and lower ring are translationally fixed relative to each other once assembled—in other words, if one component is translated in space, the other two components are translated with it by the same magnitude. However, in some designs it is possible that the components are not rotationally fixed to each other. Since the apical insert is fastened to the lower ring and not directly to the Transwell, the Transwell could technically slip and rotate about its centerline independently of the insert/ring, which would be undesirable. Therefore, a feature has been designed into the apical insert to fix all three components rotationally, as seen by the three green radial protrusions in Figure 2-8. The feature utilizes the three flange supports on the Transwell—or specifically, the gaps between them—to constrain rotation of the Transwell. If the Transwell does slip from the insert/ring, it can only rotate 4° before one of the flange supports collides with the apical insert. This feature, which is rotationally symmetric of order 3, allows for three possible ways to place the insert into the Transwell. If the user initially misplaces the insert, they could simply rotate it in either direction until the insert feature lines up with the gaps between the Transwell flange supports. In the absolute worst case, the user would have to rotate by 56° before the insert falls into place.

2.4.2 Biological Considerations

As this is a component that will be used in conjunction with a live cell culture, there are several biological constraints that must be considered in the design. For example, any materials used with the insert must be biocompatible and ideally low in protein adsorption, as the user should be able to use media with a known concentration of supplements without concern for losing a significant amount of their reagents to surface partitioning. In addition to material choice, the volume of media (and therefore, total amount of available nutrients) supported by the insert is highly applicable to
cell viability, and there are certain critical dimensions to the insert that directly affect total apical media volume.

**Material Choices**

There are several material considerations involved in designing a device to be used in tandem with cell cultures. Any device that interacts with cultures must be sterilizable to minimize risk of contamination. This sterilization is commonly done through autoclaving, where components to be sterilized are placed in a chamber that undergoes cycles of high heat and pressure to kill a majority of potential contaminants. There also exist other sterilization methods for components that cannot withstand the high temperatures and pressure of an autoclave, such as ETO sterilization and gamma ray irradiation. However, these methods tend to be more time consuming for biologists, as autoclaves are by far the most common (and typically the most convenient) form of sterilization. Thus, it is beneficial to choose materials that can survive the autoclaving process.

Polysulfone, a thermoplastic that is fairly machinable and autoclavable, has been used in cell culturing devices such as the previously developed LiverChip [15]. Therefore, there is precedence in using polysulfone for the apical flow insert as there exists a body of knowledge to build upon. Generally, polysulfone surfaces that will come in contact with culture media are first passivated by submerging or priming the respective surfaces with media, so the apical insert must be amenable to a passivating procedure that is easy for the user. Potential approaches involve partially submerging the insert in media in a 6-well multiwell plate while continually priming any internal channels with media.
Media Volumes and Replenishment Rates

Apical and basal volumes were determined based on considerations in media replenishment. For this *in vitro* model (and generally for many static Transwell cultures), media is manually replaced every 2-3 days to replenish media nutrients that have been depleted by the culture. This manual media replacement rate is generally found experimentally, and depends on both the culture’s consumption rate and the volume of media used. In the case of fast consumption and/or low volumes, media replacement must be more frequent, and correspondingly less frequent in the opposite case of slower consumption and large media volumes.

The volume in the apical chamber is primarily determined by how far the insert protrudes into the Transwell, and can be characterized by a height $h$ between the Transwell membrane and the bottom of the apical insert. Given a membrane surface area of 112 mm$^2$, a height of $h = 2$ mm was chosen to create an apical volume of 224 µL. Thus, if a flow rate of $0.5\text{mL/day}$ is used, the apical media will be completely replaced twice a day. Though this may seem aggressive, this value was chosen to mimic a protocol developed in our lab consisting of half-volume media replacement every 6 hours on the static microbiome co-culture, as certain microbe strains have the potential to quickly proliferate and dominate the culture.

2.4.3 Design Parameter Reduction

With many points to consider both on the mechanical and biological side, it is helpful to create a table of relevant parameters to guide design and operation. Table 2.1 shows such a table for the apical flow insert, with variable names defined for each parameter, a list of dependencies (if any), a desired range of values for each parameter, and justifications for each range. In an effort toward dimensional reduction, parameters are distinguished by those that are “driving,” or explicitly determined by the user or...
designer, and those that are "driven," or implicitly determined via relations to sets of driving parameters. With these parameter values in mind, a more informed design can be created.
Table 2.1: DESIGN AND OPERATION PARAMETERS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Name</th>
<th>Dependencies</th>
<th>Priority</th>
<th>Desired Range</th>
<th>Justification/Notes</th>
</tr>
</thead>
</table>
| Culture Surface Area | A |  | Driving | 100–200 mm² | • 12-well transwell is 112 mm² as a starting point  
• Scaling recommendation is to maximize surface area |
| Apical Channel Height | h |  | Driving | 1–5 mm | • <1mm may cause issues with priming, general operation  
• “Tall” channels (5mm or more) can create large O₂ gradients, possibly capable of housing both obligate anaerobes and microaerobes |
| Apical Volume | V_{top} | A, h | Driven | 100–500 µL | Calculated from A, h |
| Basal Volume | V_{bot} |  | Driving | 500–1000 µL | • Current basal volume is 1.5 mL  
• Scaling recommendation is to minimize basal volume  
• 500 µL is a potential minimum for nutrient delivery |
| Apical Flow Rate (volumetric) | Q_{top} | V_{top}, nutrient requirements | Driven | 100–1000 µL d⁻¹ | Wide range due to uncertainty—experimental validation may be best approach to narrow range |
| Apical Flow Rate (linear) | u_{top} | Q_{top}, A | Driven | Highly dependent on inlet geometry | Useful to consider for wall shear stress |
| Apical Dilution Rate | D | Q_{top}, V_{top} | Driven | 1–5 d⁻¹ | Defined as \( \frac{Q_{top}}{V_{top}} \). One of the key characteristics of a chemostat; helpful for planning microbe dilution |
| Oxygen Tension of Feed | P_{O₂} |  | Driving | 160 mmHg  
30–40 mmHg  
0.05 mmHg | • 160 mmHg is ambient P_{O₂}  
• 30–40 mmHg is \textit{in vivo} P_{O₂} throughout all three sections of small intestine  
• 0.05 mmHg is \( \sim 75 \) ppm (anaerobic chamber) |

Table of parameters to guide design and operation of apical insert. Parameters are distinguished by those that are “driving,” explicitly determined by the user or designer, and “driven,” those that are implicitly determined \textit{via} relations to sets of driving parameters.
Figure 2-9: Technical drawing of apical insert.
Chapter 3

Computational Models of Fluid Dynamics, Oxygen Transport and Reaction, and Heat Transfer

As discussed in Section 1.1, there exists a steep radial oxygen gradient within the small intestine, from the oxygenated submucosa to the nearly anoxic lumen. In an effort to recapitulate this phenomenon in vitro, a major design goal for the apical flow insert is the capability to control apical oxygen tension. There are many factors that affect oxygen tension within the system: the enterocyte and goblet cell coculture on the Transwell membrane will consume oxygen, and in a microbiome culture any aerobic microbes suspended in the apical chamber will consume oxygen as well. These microbes will also proliferate as long as there exists a sufficient nutrient supply, which in turn further increases the total oxygen consumption rate. To balance this removal of oxygen from the system, oxygenated media can be introduced through the apical or basal inlets, and the act of flowing new media through the apical channel may also reduce the population of oxygen-consuming microbes as suspended microbes are
washed away.

As this is a fairly complex environment, it is helpful to model this system to gain insight into the relationship between parameters such as apical and basal flow rate, unknowns such as growth rate and oxygen consumption rate for the microbe strains of interest, and the resulting oxygen gradients within the system from these parameters and unknowns. In this chapter, we present a first step toward this complex oxygen model, beginning with models of computational fluid dynamics to understand velocity profiles through the device, as well as an oxygen transport and reaction model incorporating the cell coculture (without microbes) to gain insight into what oxygen gradients are achievable within the device when only mammalian cells are present. Additionally, we present a heat transfer model to determine whether the warm air of the incubator is sufficient to warm up room temperature media before it comes in contact with the culture. All finite element models presented here were created and simulated in COMSOL (4.3a, COMSOL Inc., Burlington, MA).

Before presenting the models, we begin with a discussion of the geometry used for these finite element models, as there are characteristics that can be simplified for the purpose of optimizing model run time.

### 3.1 Model Geometry

To model characteristics of flow, oxygen transport, and oxygen consumption through the system, we wish to model the assembled apical insert and Transwell as presented in Figure 2-4. However, though it may be possible to directly import the geometry of the apical flow insert as designed and create finite element models based on that geometry, it is more efficient in the long run to consider what simplifications can be done while maintaining a realistic representation of the geometry of interest.

The first area to consider is the inlet/outlet region that connects tubing to the
Figure 3-1: Exploded view of geometry used for flow and oxygen transport models. The geometry is split into 3 domains: the apical chamber, the cell/membrane layer, and the basal chamber.

Apical insert. The inlet and outlet have a fairly high aspect ratio—the inlets are approximately 1/2” in length, but a notable portion of the inlet diameter is 1/16”. It is reasonable to assume that media flowing through the inlet and outlet will reach fully developed flow well before it travels the entire 1/2” length. This “length to fully developed flow,” or entrance length, is defined by $L_e = 0.05 \cdot Re \cdot D$, where Re is the Reynolds number and $D$ is the diameter of the inlet. Using $D = 1.59\text{mm}$ and a calculated Reynolds number of $Re = 0.005$, we find $L_e \approx 4\text{mm}$, an extremely short entrance length due to a slow volumetric flow rate of $500\mu\text{L.d}^{-1}$. Thus, before any meaningful modeling is done, we can already assume that the long inlets are not necessary for modeling purposes and can be drastically shortened in the model (though they do serve the functional purpose of guiding media from tubing to the apical side of the Transwell).

Another area to consider is the cell monolayer on the Transwell membrane, as this region plays a critical role in transport of various species—for example, oxygen
will be consumed as it diffuses from media through the cell layer, and the thickness of this cell layer affects total consumption. Additionally, the cell layer itself ideally models the barrier between the intestinal lumen (the apical side) and the basolateral membrane/circulatory system (the basal side), and its efficacy at barrier function in turn affects the permeability of larger molecules such as diclofenac or various lipopolysaccharides. To simplify the complex geometry in this area, the cell layer and membrane are modeled as a single 50 μm thick disc that separates the apical and basal domains, based on the thickness of the Transwell membrane and the approximate height of Caco-2 cells, which are columnar epithelial cells.

With these considerations in mind, we create the model geometry seen in Figure 3-1. For modeling purposes, the entire geometry is split into three domains; the apical and basal chambers both represent the regions where media can flow, while the cell/membrane layer represents the cell coculture and Transwell membrane.

### 3.2 Flow Modeling

As one of the core features of this device is that it allows for apical flow in a Transwell, one pertinent question is how exactly the flow behaves in the device. To create a more controlled environment, the inlets to the apical chamber must be designed such that flow is fairly even across the Transwell, which in turn allows for an even distribution of fresh media across the cells. In this section, two designs for the apical insert inlets are presented and their respective velocity profiles in the apical chamber are modeled.

#### 3.2.1 Setup

In this model, the apical domain is the only domain studied as our main interest here is the velocity profiles of media in the region directly above the cells. The governing
equation for fluid flow in this system is the Navier-Stokes equation [16]:

\[
\rho \left( \frac{\partial \vec{v}}{\partial t} + (\vec{v} \cdot \nabla) \vec{v} \right) = -\nabla P + \mu \nabla^2 \vec{v}.
\]  \hspace{1cm} (3.1)

Here, \( \rho \) is fluid density, \( \mu \) is fluid viscosity, and \( \nabla P \) is the pressure gradient. In practice, the system will likely be run at a constant flow rate, and in the absence of turbulence or any elements that create oscillatory flow, we can assume steady-state flow. Specifically, this means there is no local acceleration of fluid, but convective acceleration can occur due to changes in geometry as fluid travels through the system:

\[
\rho \left( \frac{\partial \vec{v}}{\partial t} + (\vec{v} \cdot \nabla) \vec{v} \right)_{\text{local}} = -\nabla P + \mu \nabla^2 \vec{v}.
\]

However, this can be further simplified. For example, explicitly writing the convective acceleration term and the viscous term in the x-direction gives:

\[
\rho \left( u \frac{\partial u}{\partial x} + v \frac{\partial u}{\partial y} + w \frac{\partial u}{\partial z} \right) = -\nabla P + \mu \left( \frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial y^2} + \frac{\partial^2 u}{\partial z^2} \right). \hspace{1cm} (a)
\]

We perform a scaling analysis on terms (a) and (b), defining a characteristic length \( L \) for \( \frac{\partial}{\partial x} \):

\[
\frac{\rho u^2}{L} \sim \mu \frac{u}{L^2}
\]

\[
\frac{\rho u L}{\mu} \sim 1. \hspace{1cm} (3.2)
\]

The left side of Equation 3.2 is the Reynolds number, the ratio of inertial forces to viscous forces. If the Reynolds number is close to 1, both forces are comparable and neither may be neglected.
When we consider practical usage of the apical insert, volumetric flow rates (and therefore velocities) will be very slow to maintain steady, constant media replenishment during operation. The suggested flow rate mentioned in Section 2.4.2 is 500μL d⁻¹, which can result in a linear velocity on the order of nm s⁻¹. This results in a very low Reynolds number, which suggests that viscous forces dominate in this system during normal operation; inertial forces can be neglected. Similar scaling arguments can be made in the y- and z-direction, reducing the Navier-Stokes Equation seen in Equation 3.1 to the simpler Stokes Equation:

\[ 0 = -\nabla P + \mu \nabla^2 \vec{v}. \] (3.3)

Therefore, with the simplifications explained in this section thus far, we are justified in using Equation 3.3 as the governing equation for the finite element models of flow that are conducted throughout this chapter.

### 3.2.2 Inlet and Boundary Conditions

Conditions for the finite element model are seen in Figure 3-2. As previously mentioned, the apical chamber is the only geometry considered for this particular model, so this system is notably easier to work with. As the apical chamber is fully enclosed, a no-slip condition (i.e. a wall) is defined on all surfaces other than the inlet and outlet. For simplicity, media is modeled as water, using the viscosity and density of water. Values used for the various parameters in this model are seen in Table 3.1.

In an effort to create a more even velocity profile directly above the Transwell membrane, two different geometries are modeled with these conditions. The first geometry, based on the first apical insert design that was created, is seen on the left side of Figure 3-3. If we imagine a fluid particle traveling through this volume from the inlet to the apical chamber, there are some domains that we can define—there is...
Figure 3-2: Conditions for modeling flow in the apical chamber (domain 1 in Figure 3-1). The surfaces in this figure are the boundaries of fluid flow. A volumetric flow rate is set at the inlet and a standard 0 PSIG (i.e. no head pressure) condition is set at the outlet. Other than the inlet and outlet, all surfaces in the apical domain are no-slip surfaces.

The vertical cylindrical channel that forms the inlet, followed by a horizontal channel, then a vertical curved slot that connects the horizontal channel to the apical chamber. The purpose of the curved slot is to spread out the velocity profile, as fluid exiting the horizontal channel will initially be concentrated around its cylindrical shape. Thus, the vertical curved slot essentially creates room for momentum diffusion to occur, ideally creating a more even velocity profile by the time the fluid reaches the apical chamber.

The second geometry to be modeled can be seen on the right side of Figure 3-3. This geometry is similar to the first geometry with one key difference: rather than a horizontal bore to connect the inlet to the vertical curved slot, the second geometry uses a wide slot to connect the two domains. From the perspective of the fluid, the vertical inlet connects to a half-circle perpendicular to the inlet itself, thus allowing for even more room to spread out incoming fluid. Velocity profiles in both geometries...
Table 3.1: PARAMETERS FOR MODELING MEDIA FLOW

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value Used in Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>( Q_{top} )</td>
<td>Volumetric apical flow rate</td>
<td>5.79 ( \text{mL s}^{-1} )</td>
</tr>
<tr>
<td>( \rho )</td>
<td>Density of water</td>
<td>1000 ( \text{kg m}^{-3} )</td>
</tr>
<tr>
<td>( \mu )</td>
<td>Viscosity of water</td>
<td>8.90 ( \times 10^{-4} ) ( \text{Pa s} )</td>
</tr>
</tbody>
</table>

Table of values used to model media flow through the apical insert.

Figure 3-3: Illustrations of different geometries of the apical flow domain. The geometry on the left has a simple horizontal bore connecting the inlet to the vertical slot, while the geometry on the right utilizes a horizontal slot instead, further spreading out flow.

will be further explored in Section 3.2.3.

3.2.3 Results

Results from the flow model given the previously defined geometries and parameters are seen in Figures 3-4 and 3-5, for the straight horizontal and slotted horizontal channels respectively.

Subfigures 3-4b and 3-4c present a colormap visualization of the velocity profiles in the apical chamber. The curved vertical slot does influence flow spreading to some degree—if the connections to the vertical inlets were simply cylindrical channels, we would see a velocity profile that more closely follows the shape of a paraboloid in the apical chamber. Although these subfigures can be very elucidating at a glance,
Figure 3-4: Velocity profiles obtained from finite element model. (a) Illustration of geometry used. Note the straight horizontal connections from vertical inlets to vertical curved slots. (b) 3D visualization of velocity profiles in apical chamber. Slices parallel to the \( yz \) plane are deformed in the \(-x\) direction by an amount proportional to their velocity magnitude. (c) 2D visualization of velocity profile in apical chamber, as seen from the top view. A single slice parallel to the \( xy \) plane at a height 1 mm above the Transwell membrane (i.e. the center of the apical channel) is shown. (d) Top view of the same \( xy \) slice in subfigure c. Red lines indicate probes where 1D velocity profiles are computed and plotted in subfigure e. (e) 1D velocity profiles in the \(-x\) direction, along lines parallel to the \( y\)-axis as seen in subfigure d. Curve 1 refers to the rightmost red line while curve 7 refers to the leftmost red line.
Figure 3-5: Velocity profiles obtained from finite element model. (a) Illustration of slotted inlet geometry used. (b) 3D visualization of velocity profiles in apical chamber. Slices parallel to the yz plane are deformed in the -x direction by an amount proportional to their velocity magnitude. (c) 2D visualization of velocity profile in apical chamber, as seen from the top view. A single slice parallel to the xy plane at a height 1 mm above the Transwell membrane (i.e. the center of the apical channel) is shown. (d) Top view of the same xy slice in subfigure c. Red lines indicate probes where 1D velocity profiles are computed and plotted in subfigure e. (e) 1D velocity profiles in the -x direction, along lines parallel to the y-axis as seen in subfigure d. Curve 1 refers to the rightmost red line while curve 7 refers to the leftmost red line.
they are 2D/3D representations of velocity profiles and it is hard to pull quantitative results from them. By looking at velocity profiles along a 1D domain, we can gain a more quantitative understanding of how even the flow is in this region. Subfigure 3-4d shows a top view of the system, with red lines indicating the domains where velocity profiles will be evaluated. Finally, Subfigure 3-4e plots the velocity profiles along each red line. Although there is still a higher velocity in the center of each line (not unlike a parabola), it is promising that we see clear signs of momentum diffusion as the velocity profile partially flattens out when we move toward the center of the apical chamber.

However, these results can be even further improved. The slotted horizontal geometry described earlier and its modeling results can be seen in Figure 3-5. Similar to Figure 3-4, subfigures 3-5b and 3-5c are colormap visualizations of the velocity profiles in the apical chamber. It is especially clear in subfigure 3-5c that flow profiles with this geometry are much more even. Using the same technique as previously described, we can plot 1D velocity profiles as seen in subfigure 3-5e to gain a more quantitative understanding of velocity distribution across the apical chamber. When compared to the design with cylindrical inlets as seen in subfigure 3-4e, these velocity profiles have a more desirable wide even area. Thus, these models suggest that the insert design with slotted inlets is worth fabricating and validating as it may result in an even velocity distribution throughout a majority of the apical chamber.

3.3 Oxygen Transport and Reaction with Mammalian Culture

When considering oxygen transport and consumption within the system, it would be ideal to model the environment described at the beginning of this chapter: oxygen in the media is consumed by both the mammalian cell layer and the population
of microbes suspended in the apical chamber, and this consumption is balanced by introduction of oxygenated media via apical and basal inlets; the difference in these rates determines the steady state oxygen tension within the system. Rather than oxygenated media, deoxygenated media could be introduced through the apical inlet in case cellular consumption alone is not capable of creating a hypoxic environment.

In this section, we present a simplified model where oxygen consumption occurs only from the mammalian cells (e.g. enterocytes, goblet cells) on the Transwell membrane; no microbes are considered in this model. This matches the anticipated pilot experiments with the apical flow insert where only mammalian cells will be cultured. As we are interested in the potential to create a hypoxic apical environment, there should be a means to remove oxygen from the system. While this can be done through external means, such as introducing deoxygenated sparged media, we should not neglect oxygen consumption by the cell layer, as that could be a more natural way to create a hypoxic environment.

Although microbe oxygen consumption is currently not implemented, the oxygen model presented here is a first step toward a more complex model that considers not only oxygen consumption by microbes, but also microbe growth as a function of local microbe population, oxygen, and available nutrients.

### 3.3.1 Setup

Figure 3-6 shows a schematic of the oxygen transport and reaction model. The apical geometry with slotted inlets is used due to its more even velocity distribution (and thus more even oxygen transport across the cells). In contrast to the velocity model, all three domains (apical, cell/membrane layer, basal) are used in this model as they all play a crucial role in determining the oxygen distribution: both the apical and basal chambers transport oxygen through media flow, and the cellular layer is responsible for oxygen consumption.
Figure 3-6: Schematic of conditions for oxygen modeling. A cross-section of the geometry is shown for easier visualization. In addition to the flow conditions modeled in Section 3.2, these conditions add a basal chamber with a faster basal flow rate. Oxygenated media is introduced through both apical and basal inlets, and oxygen consumption occurs at the cell layer.

**Advection**

The governing equation for flow in this system is the Stokes equation, as derived in Section 3.2.1. In terms of flow boundary conditions, there is a set volumetric flow rate for the apical and basal inlets, notated as $Q_{\text{top}}$ and $Q_{\text{bot}}$ respectively. The top surface of the basal domain is specified as a slip surface; this is because in practice, basal media in the platform will be open to air, thus creating a free surface at the air-liquid interface. Finally, both the top and bottom surfaces of the cell/membrane layer are specified as no-slip surfaces. This is an important condition as it prevents advective transport between the apical and basal chambers—only diffusive transport across the cell layer can occur.

**Diffusion**

Oxygen transport not only occurs through bulk advection, but simultaneously through diffusion. The governing equation in this case is Fick’s second law of diffusion, which relates concentration of a species over time at a point to the Laplacian of its concent-
dC
Dt = D∇^2 C.

C is the concentration of the species of interest (oxygen in this case) and D is the diffusion coefficient of the species in a specified medium. We add concentration boundary conditions to the existing flow boundary conditions in our model: media in the apical and basal inlets enter the system at a constant oxygen concentration \( C_{O_2} \), and the free surface of the basal chamber is fixed at \( C_{O_2, atm} \) as it is exposed to air at ambient oxygen levels. We also set an oxygen diffusion coefficient \( D_{O_2, H_2O} \) throughout the apical and basal domains; it is common practice in the literature to use values for oxygen diffusion in water to represent oxygen diffusion in media, which we will use in this model as well. Finally, it is anticipated that oxygen diffusion is slower through the cell layer; we set a slower diffusion coefficient \( D_{O_2, cell} \) throughout the cell/membrane domain.

Consumption

The final component of this transport model is oxygen consumption by the cells. Essentially, oxygen diffuses into the cell layer from bulk media in both the apical and basal chambers; within the cellular domain, oxygen consumption occurs at a specified rate. We use Michaelis-Menten kinetics to determine consumption rate:

\[
R_{O_2} = R_{max} \left( \frac{C_{O_2}}{C_{MM, O_2} + C_{O_2}} \right) \cdot \delta(C_{O_2} > C_{ct}).
\]

\( C_{ct} \) is a critical oxygen concentration where oxygen consumption no longer occurs due to cellular death at severely low oxygen. This behavior is modeled as a Heaviside step function; as long as the local oxygen concentration remains above the critical level, consumption follows standard Michaelis-Menten kinetics. When oxygen con-
centration falls below the critical level, consumption immediately halts. In practice, this behavior is not as strictly binary as described, but this model is a reasonable first step toward considering reduced function in severely hypoxic conditions, and has previously been used in the literature [9, 17, 18].

An important parameter to this equation is $R_{\text{max}}$, the maximum oxygen consumption rate. In the absence of measured oxygen consumption rates from our cultures, one sensible approach to converge on a value is to look at reported consumption rates across the literature to gain a sense of reasonable ranges for these values. Table 3.2 shows several values for consumption rates that were found in the literature, including hepatocytes, HeLa cells, and colonocytes. As values are reported in different units, such as moles per cm$^2$ or per mg protein, certain assumptions (given in the table) must be made to convert these values into a standardized per cell rate.

Table 3.2: Comparison of Oxygen Consumption Values

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Reported Consumption Rate</th>
<th>Assumptions</th>
<th>Converted Value (mol cell$^{-1}$ s$^{-1}$)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary human small intestinal organoids</td>
<td>0.0006 mol m$^{-3}$ s$^{-1}$</td>
<td>$10^5$ cells/cm$^2$ and 50 μm cell height = 2 $\cdot$ $10^7$ cells/cm$^3$</td>
<td>$3.00 \cdot 10^{-17}$</td>
<td>[9]</td>
</tr>
<tr>
<td>Rat hepatocytes</td>
<td>2.25 μmol mg protein$^{-1}$ h$^{-1}$</td>
<td>$10^6$ cells/mg protein</td>
<td>$6.94 \cdot 10^{-16}$</td>
<td>[15]</td>
</tr>
<tr>
<td>Mouse hepatocytes</td>
<td>18 pmol cm$^{-2}$ s$^{-1}$</td>
<td>$10^5$ cells/cm$^2$</td>
<td>$1.80 \cdot 10^{-16}$</td>
<td>[19]</td>
</tr>
<tr>
<td>HeLa</td>
<td>1.4-2.0 μmol mg protein$^{-1}$ h$^{-1}$</td>
<td>$10^6$ cells/mg protein</td>
<td>$4.72 \cdot 10^{-16}$</td>
<td>[20]</td>
</tr>
<tr>
<td>Rat hepatocytes</td>
<td>3.94 μmol g wet wt$^{-1}$ min$^{-1}$</td>
<td>$10^8$ hepatocytes/g wet wt</td>
<td>$6.57 \cdot 10^{-16}$</td>
<td>[22]</td>
</tr>
<tr>
<td>Human colonocytes</td>
<td>8.29 μmol g dry wt$^{-1}$ min$^{-1}$</td>
<td>$10^9$ cells/g wet wt</td>
<td>$1.38 \cdot 10^{-16}$</td>
<td>[23]</td>
</tr>
</tbody>
</table>

Table of cellular oxygen consumption rates found in the literature across several cell types. Reported consumption rates are converted to the same units (mol cell$^{-1}$ s$^{-1}$) for a more straightforward comparison.

Clearly, the consumption rate measured from small intestinal organoids is the...
slowest oxygen consumption rate in the list—the next slowest rate, from human colonocytes, is about four times faster. It is promising that the two rat hepatocyte values agree with each other, as this potentially validates the assumptions made to convert their values into the same units. It is also reasonable that hepatocytes in general have higher oxygen consumption rates than other cell types, in agreement with additional literature [24, 25].

Despite the variation in reported consumption rates across cell types, all values reported in Table 3.2 fall within about one order of magnitude. The human colonocyte consumption rate from [23], nearly in the center of these two bounds, is used in the following transport model.

**Parameter Values**

Parameter values used in the oxygen transport and consumption model are seen in Table 3.3. The volumetric apical flow rate, 5.79 nL s⁻¹, corresponds to 500 μL d⁻¹ as previously established. Volumetric basal flow rate was varied across several models to investigate its effect on oxygen gradients across the cell layer; the results will be discussed in Section 3.3.2. For practical considerations, oxygen tension in both apical and basal feeds are assumed to be equal to atmospheric oxygen tension. As mentioned in the introduction to this section, it is possible to prepare deoxygenated media (e.g. through N₂ sparging) if needed; however, it is worth investigating whether a sufficiently hypoxic environment can be created purely by the culture.

The remaining parameters are the oxygen diffusion coefficients and the cellular consumption constants. In the apical and basal domains where there is media, the diffusion coefficient for oxygen in water is used, in general agreement with the literature (1–5 \(\cdot\) 10⁻⁵ cm² s⁻¹ from 10–50°C) [26]. Within the cell/membrane layer, the diffusion coefficient is decreased by an order of magnitude—however, it turns out this difference in diffusion rate does not significantly impact oxygen transport due to the
small thickness of the layer (50 μm). It is noted that this is only the case for oxygen; larger molecules may be more severely impacted by the decreased permeability of the cell/membrane layer. The maximum cellular consumption rate of oxygen was chosen based on a wide literature search as described previously. The Michaelis-Menten half-saturation constant and the critical oxygen threshold were chosen from literature values [9, 18].

Table 3.3: PARAMETERS FOR FLOW AND OXYGEN TRANSPORT

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value Used in Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Q_{\text{top}}$</td>
<td>Volumetric apical flow rate</td>
<td>5.79 nL s$^{-1}$</td>
</tr>
<tr>
<td>$Q_{\text{bot}}$</td>
<td>Volumetric basal flow rate</td>
<td>0.1–1 μL s$^{-1}$</td>
</tr>
<tr>
<td>$C_{\text{top}}$</td>
<td>Apical inlet oxygen concentration</td>
<td>0.2 mol O$_2$ m$^{-3}$</td>
</tr>
<tr>
<td>$C_{\text{bot}}$</td>
<td>Basal inlet oxygen concentration</td>
<td>0.2 mol O$_2$ m$^{-3}$</td>
</tr>
<tr>
<td>$D_{O_2,\text{H}_2O}$</td>
<td>Oxygen diffusion coefficient in water</td>
<td>$2 \cdot 10^{-9}$ m$^2$ s$^{-1}$</td>
</tr>
<tr>
<td>$D_{O_2,\text{cell}}$</td>
<td>Oxygen diffusion coefficient in cell layer</td>
<td>$2 \cdot 10^{-10}$ m$^2$ s$^{-1}$</td>
</tr>
<tr>
<td>$R_{\text{max}}$</td>
<td>Maximum oxygen consumption rate</td>
<td>$3 \cdot 10^{-3}$ mol m$^{-3}$ s$^{-1}$</td>
</tr>
<tr>
<td>$C_{\text{MM,}O_2}$</td>
<td>Michaelis-Menten half-saturation constant</td>
<td>$1 \cdot 10^{-3}$ mol m$^{-3}$</td>
</tr>
<tr>
<td>$C_{\text{ct}}$</td>
<td>Critical oxygen threshold</td>
<td>$1 \cdot 10^{-4}$ mol m$^{-3}$</td>
</tr>
</tbody>
</table>

Table of values used to model media flow and oxygen transport through the advanced gut model.

### 3.3.2 Results

Results from the oxygen transport/consumption finite element models are seen in Figure 3-7. Simulations were run with basal flow rates of 1 μL s$^{-1}$, 0.25 μL s$^{-1}$, and 0.1 μL s$^{-1}$ and steady state oxygen concentration throughout the system was computed in each case. Subfigures 3-7a through 3-7c show colormap visualizations of oxygen concentration for the three basal flow rates, while Subfigure 3-7e plots oxygen
concentration across a 1D line drawn through the center of the cell/membrane layer from inlet to outlet.

There is a general trend that slower basal flow rates result in lower average oxygen across the cell layer, and intuitively this makes sense: higher basal flow rates will remove deoxygenated media from the system faster and replace it with oxygenated media, as we have determined for this model that both inlets introduce fully oxygenated media. At 0.1 $\mu$L s$^{-1}$, oxygen concentration in the cell layer approaches a minimum of 0.03 mol m$^{-3}$, or about 23 mmHg. There are two particularly interesting observations that can be gleaned from these results: the concentration value at 0.1 $\mu$L s$^{-1}$ is in line with values for in vivo concentrations in the small intestine, and there is a gradient of decreasing oxygen in the cell layer from inlet to outlet, itself also a phenomenon seen in vivo as one travels from the duodenum through the jejunum and ileum [3]. These results are promising, as they imply that controlling the volumetric basal flow rate is in fact a viable mechanism for controlling oxygen tension within the system.

We note that in practice the basal flow rate may not be an operational parameter that can be freely chosen for the sole purpose of controlling oxygen tension within the small intestine model; there may be other factors driving the basal flow rate such as interaction rates between the small intestine and other organ models on the platform. However, oxygen tension can still play an influence as one of several factors driving this parameter.

**Characteristic Numbers**

Similar to how the Reynolds number can be a useful metric for evaluating flow behavior in a system, there are characteristic numbers for transport that we can calculate to summarize transport behavior in this model. The first characteristic number is the Peclet number, the ratio of advective transport to diffusive transport:
Figure 3-7: Results from finite element model of oxygen transport and consumption throughout the system. (a)–(c) Colormap visualizations of steady state oxygen concentration at basal flow rates of 1, 0.25, and 0.1 \( \mu \text{L/s} \) respectively. (d) Illustration of 1D probe line used in model to determine oxygen concentration across the cell/membrane layer. (e) Oxygen concentration plots along probing line drawn in subfigure d at the three simulated basal flow rates.

\[
\text{Pe} = \frac{Lu}{D},
\]

\( L \) is a characteristic length for the geometry of interest, \( u \) is velocity, and \( D \) is the diffusion coefficient for the species of interest. Because geometries and flow rates differ between the apical and basal chambers, we must calculate Peclet numbers in both domains.

Given \( L = 1.5 \text{ mm} \), \( u = 0.5 \text{ \mu m/s} \), and \( D_{O_2} = 2 \cdot 10^{-9} \text{ m}^2 \text{s}^{-1} \) in the apical chamber, we have \( \text{Pe}_{\text{top}} = 0.4 \). Though slightly in favor of the denominator, this value is relatively close to unity. Thus, from the Peclet number we cannot definitively state whether transport in the apical chamber is advection or diffusion dominated. This appears reasonable; although the height of the apical chamber does not approach microfluidic scales (so diffusive mixing is not fast), the flow rate of the apical feed is
remarkably slow, resulting in similarly low advective transport. This is by design, as there are other factors (shear stress on cells, microbe replacement rate) that we must be sensitive to when determining the apical feed rate.

In contrast, the basal domain has a slightly larger characteristic length and a basal feed rate that is 20–200 times faster (at $0.1 \mu$L s$^{-1}$ and $1 \mu$L s$^{-1}$, respectively). Given $L = 3$ mm, $u = 2 \mu$m s$^{-1}$, and $D_{O_2} = 2 \cdot 10^{-9}$ m$^2$ s$^{-1}$, we have $\text{Pe}_{\text{bot}} = 3$ at the slowest flow rate and $\text{Pe}_{\text{bot}} = 30$ at the fastest flow rate. Thus, depending on the basal feed rate, oxygen transport in the basal domain may be balanced between advective and diffusive transport (at $0.1 \mu$L s$^{-1}$) or advection dominated (at $1 \mu$L s$^{-1}$). This result is illuminating; it was found in Section 3.3.2 that the basal flow rate can noticeably affect steady state oxygen gradients along the cell layer, and from the Peclet number we can begin to construct an explanation: advection dominated transport of oxygenated media into the system (and transport of deoxygenated media out of the system) is directly tied to basal feed rate.

Another characteristic number to consider in this situation is the Thiele modulus, which gives insight into the concentration profile across a slab of tissue (in this situation, the 50 \mu m thick cell layer) by comparing species transport and reaction rate throughout the tissue [27]. The Thiele modulus is defined as the ratio of reaction rate to diffusion rate across the cell layer:

$$\Phi^2 = \frac{L^2 R}{C_0 D}.$$  

$L$ is the thickness of the slab, $R$ is the cellular consumption rate, $C_0$ is the surface concentration, and $D$ is the species diffusion coefficient in the cell layer. Before we evaluate the Thiele modulus, we attempt to simplify the cellular consumption rate given results from our finite element models. The Michaelis-Menten half-saturation constant is given in Table 3.3 as $C_{\text{MM},O_2} = 1 \cdot 10^{-3}$ mol m$^{-3}$. From results shown in Figure 3-7, we see that even at the slowest basal flow rate, the minimum oxygen
concentration in the cell layer is well above the half-saturation constant; when this occurs, we are justified in recasting Michaelis-Menten kinetics as a simple zeroth-order reaction, \( R = R_{\text{max}} \).

Given \( L = 50\mu\text{m} \), \( R = R_{\text{max}} = 3 \times 10^{-3} \text{ mol m}^{-3} \text{ s}^{-1} \), \( C_0 = 0.2 \text{ mol m}^{-3} \), and \( D_{O_2} = 2 \times 10^{-10} \text{ m}^2 \text{ s}^{-1} \), we find \( \Phi^2 = 0.02 \). As this number is much lower than unity, this implies that diffusion across the cell layer occurs at a much faster rate than consumption. The main contributing factor for this phenomenon is the thin cell layer; even if we assume an oxygen diffusion rate an order of magnitude faster \( (2 \times 10^{-9} \text{ m}^2 \text{ s}^{-1} \), equal to oxygen in water), the Thiele modulus remains well below unity. Thus, we can reasonably assume that there is little to no oxygen gradient along the thickness of the cell layer; \( \frac{\partial C}{\partial z} \approx 0 \). It is noted that this does not tell us about oxygen gradients along the length of the cell layer, in the direction of flow; indeed, we have seen earlier from the finite element model results in Figure 3-7 that there is a negative gradient as we travel from inlet to outlet.

### 3.4 Heat Transfer in an Incubator

So far, we have considered flow and transport phenomena within the apical insert, the results of which have been insightful in terms of creating a well-designed system capable of tunable oxygen gradients along the culture. However, there are some aspects external to the system that must also be considered; for example, media temperature is an important attribute that must be controlled upstream of the intestinal culture.

In the course of manual cell seeding and media replacement, it is common practice to warm media up to 37°C before it is introduced to any culture. This is typically done by submerging a container of media in a temperature controlled water bath until media temperature has reached equilibrium with the surrounding bath.

However, this media warming procedure is a batch process, and integrating it with
continuous media replacement may be complex. During operation, there will be a syringe of fresh media in a syringe pump placed outside the incubator. To ensure that the culture is initially exposed to warm media, the syringe could be warmed in a media bath to 37 °C immediately prior to priming the apical flow device. However, this syringe will cool down to room temperature over time. Therefore, we must consider approaches to continuous media warming.

Fortunately, the incubator itself is temperature controlled at 37°C. Although media initially enters the incubator at room temperature, it is certainly worth evaluating whether the warm incubator air is sufficient for warming media as it travels through tubing toward the apical insert. Particularly, we wish to investigate whether media will reach 37°C before it reaches the insert, as we do not want to introduce cold media to the cells.

3.4.1 Setup

This problem involves heat transfer from the warm, surrounding incubator air to the PTFE tubing, and in turn from PTFE tubing to media flowing inside. The governing equation in this situation is the aptly named heat equation:

\[
\frac{\partial T}{\partial t} - \alpha \nabla^2 T = 0. \tag{3.4}
\]

\[\alpha = \frac{k}{\rho C_v}\] is the thermal diffusivity for the material of interest. The setup for the finite element model combines heat transfer through the heat equation and media flow via the Stokes equation, previously described in Section 3.2. Figure 3-8 illustrates the setup conditions: the outer surface of the PTFE tubing is fixed at incubator temperature, and media is fed through tubing at a given flow rate and initial temperature. Over time, conductive heat transfer occurs through the tubing and media, and this rate is partially determined by intrinsic features such as thermal diffusivity. Table
Figure 3-8: Schematic of system setup for media warming in an incubator. Media, initially at room temperature \((T_{in})\), flows through PTFE tubing at a slow constant volumetric flow rate \((Q_{in})\). Heat is transferred from the surrounding incubator air \((T_{out})\) to the PTFE tubing which in turn transfers heat to the media within. Intrinsic properties of the materials, including thermal conductivity \(k\), density \(\rho\), and specific heat capacity \(C_p\), affect heat transfer rates through that material.

3.4 summarizes values used for all parameters.

### 3.4.2 Analytical Solution

Similar to our process with modeling flow, it is good practice to attempt an analytical solution to this problem to verify that our numerical solutions are set up properly. We begin by simplifying the problem based on experimental conditions. Though the finite element model is set up to consider both fluid flow and heat transfer, volumetric flow rate is very slow at 500 \(\mu\)L d\(^{-1}\), or about 5.79 \(\times\) \(10^{-3}\) \(\mu\)L s\(^{-1}\). Assuming media flows within cylindrical tubing with a \(1/32"\) ID (or a radius of \(1/64" = 0.397\) mm), this translates to a linear flow rate of \(v = \frac{5.79 \times 10^{-3} \text{ mm}^3 \text{s}^{-1}}{\pi(0.397\text{mm})^2} = 11.7 \text{ nm s}^{-1}\) through the tube, and can be neglected for these calculations. Thus, our problem becomes a static heat transfer problem, where the entire tube/media system begins at 293 K, the external temperature is fixed at 310 K, and heat transfer occurs until the system reaches an equilibrium at 310 K.

We begin with the cylindrical form of the heat equation as written in Equation 3.4:

\[
\rho C_p \frac{\partial T}{\partial t} = \frac{1}{r} \frac{\partial}{\partial r} \left( k r \frac{\partial T}{\partial r} \right) + \frac{1}{r^2} \frac{\partial}{\partial \theta} \left( k \frac{\partial T}{\partial \theta} \right) + \frac{\partial}{\partial z} \left( k \frac{\partial T}{\partial z} \right). \tag{3.5}
\]
Table 3.4: Parameters for Heat Transfer

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value Used in Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r_1$</td>
<td>Inner wall radius of tubing</td>
<td>0.794 mm (⅝ in.)</td>
</tr>
<tr>
<td>$r_2$</td>
<td>Outer wall radius of tubing</td>
<td>1.588 mm (⅛ in.)</td>
</tr>
<tr>
<td>$T_{in}$</td>
<td>Temperature of inlet feed</td>
<td>293 K</td>
</tr>
<tr>
<td>$T_{out}$</td>
<td>Temperature of surrounding air (in incubator)</td>
<td>310 K</td>
</tr>
<tr>
<td>$Q_{in}$</td>
<td>Volumetric basal flow rate</td>
<td>500 μL d⁻¹</td>
</tr>
<tr>
<td>$k_{H_2O}$</td>
<td>Thermal conductivity of water</td>
<td>0.6 W m⁻¹ K⁻¹</td>
</tr>
<tr>
<td>$\rho_{H_2O}$</td>
<td>Density of water</td>
<td>1000 kg m⁻³</td>
</tr>
<tr>
<td>$C_{p,H_2O}$</td>
<td>Specific heat capacity of water</td>
<td>4186 J kg⁻¹ K⁻¹</td>
</tr>
<tr>
<td>$k_{PTFE}$</td>
<td>Thermal conductivity of PTFE</td>
<td>0.24 W m⁻¹ K⁻¹</td>
</tr>
<tr>
<td>$\rho_{PTFE}$</td>
<td>Density of PTFE</td>
<td>1050 kg m⁻³</td>
</tr>
<tr>
<td>$C_{p,PTFE}$</td>
<td>Specific heat capacity of PTFE</td>
<td>2200 J kg⁻¹ K⁻¹</td>
</tr>
</tbody>
</table>

Table of values used to model heat transfer and fluid flow as room temperature media flows through PTFE tubing within an incubator.

Fortunately, there are a number of simplifications that can be done. As this is a static system without flow, we do not expect major temperature variations along the length of the tube ($\frac{\partial}{\partial z} = 0$). Additionally, the tubing will be suspended in incubator air as it travels from the incubator door to the inlet of the apical insert. Thus, heat transfer should be equal along all sides of the tubing; we should not expect angular variations in temperature ($\frac{\partial}{\partial \theta} = 0$):

$$\rho C_p \frac{\partial T}{\partial t} = \frac{1}{r} \frac{\partial}{\partial r} \left( kr \frac{\partial T}{\partial r} \right) + \frac{1}{r^2} \frac{\partial}{\partial \theta} \left( k \frac{\partial T}{\partial \theta} \right) + \frac{\partial}{\partial z} \left( k \frac{\partial T}{\partial z} \right).$$

This simplifies Equation 3.5 into an unsteady 1D heat transfer problem in polar coordinates. Because there are two domains with different materials (PTFE tubing
and media), we require two equations to describe the system:

\[
\begin{align*}
\frac{1}{\alpha_{\text{PTFE}}} \frac{\partial T}{\partial t} &= \frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial T}{\partial r} \right) & r \in [r_1, r_2] \quad (3.6) \\
\frac{1}{\alpha_{\text{H}_2\text{O}}} \frac{\partial T}{\partial t} &= \frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial T}{\partial r} \right) & r \in [0, r_1]. \quad (3.7)
\end{align*}
\]

As well as a condition for continuity at the interface; \( T_{\text{H}_2\text{O}}(r_1, t) = T_{\text{PTFE}}(r_1, t) \). It is noted that the main differences between Equations 3.6 and 3.7 are their domains and thermal diffusivities. Using the values listed in Table 3.4, we find \( \alpha_{\text{PTFE}} = 0.104 \text{ mm}^2 \text{s}^{-1} \) and \( \alpha_{\text{H}_2\text{O}} = 0.143 \text{ mm}^2 \text{s}^{-1} \), a nontrivial difference but also noticeably less than an order of magnitude; \( \frac{\alpha_{\text{H}_2\text{O}}}{\alpha_{\text{PTFE}}} = 1.375 \).

At this point we keep in mind that we pursue an analytical approach as a first effort to gain a general sense of temperature distribution over time; to do this effectively, a judicious balance between equation complexity and model accuracy must be found. By combining the domains of Equations 3.6 and 3.7 and choosing a single effective thermal diffusivity, \( \alpha_{\text{eff}} = 0.112 \text{ m}^2 \text{s}^{-1} \) (on the basis that \( \frac{3}{4} \) of the material is PTFE and \( \frac{1}{4} \) is water), we approach a more workable equation:

\[
\frac{1}{\alpha_{\text{eff}}} \frac{\partial T}{\partial t} = \frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial T}{\partial r} \right) & r \in [0, r_2]. \quad (3.8)
\]

Subject to the following conditions on \( T(r, t) \):

\[
\begin{align*}
T(r_2, t) &= 310 & \text{(Dirichlet condition)} \\
\left. \frac{\partial T}{\partial r} \right|_{r=0} &= 0 & \text{(Neumann condition)} \\
T(r, 0) &= 293 & \text{(Cauchy condition)}
\end{align*}
\]

Where \( r = 0 \) is the center of the system and \( r_2 \) is the radius of the outer wall as previously illustrated in Figure 3-8.
This reduces our 3D cylindrical heat and mass transfer problem with two domains into a 1D polar static heat problem with a single effective thermal diffusivity. This problem has been previously solved, and the general solution is:

\[ T(r, t) = 310 + \frac{-17 \cdot 2}{r_2} \sum_{n=1}^{\infty} \frac{J_0(\lambda_n r)}{\lambda_n J_1(\lambda_n r_2)} e^{-\alpha \lambda_n^2 t}. \] (3.9)

\( J_i \) is a Bessel function of the first kind of order \( i \), and \( \lambda_n \) are the roots of the Bessel function of the first kind of order 0. This function can be programmed in MATLAB to create plots of the analytical solution. Listing 3.1 shows MATLAB code that implements Equation 3.9 with 1000 Bessel function roots evaluated, and Figure 3-9 shows two plots created by Listing 3.1.

```matlab
1 % Plots analytical solution of heat equation for tubing in an incubator
2 % Dependencies: besselzero.m
3 %
4 % Tanson Nguyen
5
6 clear all
7 close all

8 numroots = 1000; % number of bessel function roots to find
9 r2 = (1/16)*25.4; % radius of outer wall (mm)
10 alpha = .1137; % thermal diffusivity (mm^2/s)
11 r = linspace(0,r2); % radial domain (mm)
12 t = 0:1:60; % time domain (s)
13
14 j0roots = besselzero(0,numroots,r2)/r2; % find roots of bessel function
15 besselterm = zeros(length(r),length(t));
16 for j = 1:length(t) % for each timestep
17     rcol = zeros(1,length(r));
18     for i = 1:numroots % sum through each bessel root
19         rcol = rcol + besselj(0,r*j0roots(i))...
20             ./ (j0roots(i)*besselj(1,r*2*j0roots(i))) ...
21             + exp(-alpha*j0roots(i)^2*t(j));
22     end
23     besselterm(:,j) = rcol;
24 end

25 T = 310 - 34/r2 - besselterm; % general solution
26
27 hold on
28 for i = 1:20; plot(r,T(:,i)); end % plot one curve per timestep
29 axis([0 1.6 292 310.5])
```

60
Listing 3.1: MATLAB code for plotting analytical solution of heat transfer through a tube. Equation 3.9 is evaluated up to 1000 Bessel function roots and the two plots seen in Figure 3-9 are drawn.

3.4.3 Finite Element Model Results

With an analytical solution found, we can run a more complex finite element model to compare results. The setup for the finite element model has a few key differences compared to the analytical model: both domains (tubing and media) with their differing thermal diffusivities are considered, and media is flowing through the tube at the previously suggested volumetric flow rate of 500 µL.d⁻¹.

Results from the finite element model are seen in Figure 3-10. Subfigures 3-10a through 3-10c are colormap visualizations of temperature distribution in the tube/media system at various points in time. Subfigures 3-10d and 3-10e are analogous to 3-9a and 3-9b; the first plot shows the evolution of radial temperature distribution over time, while the second plot shows the temperature at the center of the tube/media system over time; this point is the lowest temperature of the entire system at any point in time, so this is helpful as it serves as a lower bound for media temperature.

Promisingly, we see general agreement between the analytical and finite element models. Temperature equilibrium is established slightly faster in the analytical model, and this can be attributed to the fact that the finite element model considers the lower thermal diffusivity of the PTFE tubing as well as the constant introduction of colder room temperature media. Nonetheless, the general heat distribution profile

```matlab
32 title('Radial Temperature over Time (up to 20 seconds)')
33 xlabel('Radius (mm)'); ylabel('Temperature (K)')
34 hold off
35
36 figure;
37 plot(t,T(1,:))
38 axis([0 60 202 310.5])
39 title('Temperature of Centerline over Time')
40 xlabel('Time (s)'); ylabel('Temperature (K)')
```
is in agreement between the two models, and from the finite element data we can conservatively estimate that room temperature media will be sufficiently warmed after one minute in the incubator. Using the parameters suggested in this thesis, this is not a problem at all—for example, with a volumetric flow rate of 500 μL d\(^{-1}\) through 300 mm (about 1 ft) of \(\frac{3}{8}\)" ID tubing, the residence time is about 7 h. However, if faster flow rates, smaller bore tubing, and shorter tubing lengths are used in future experiments, care must be taken to ensure a media residence time of at least one minute before entering the apical flow system.
Figure 3-9: Plots of analytical solution of heat transfer from an incubator to PTFE tubing filled with media. (a) Temperature of system, from $r = 0$ (center) to $r = 1/16''$ (outer wall). Each curve represents temperature distribution at a point in time, from $t = 0s$ to $t = 20s$ in 1 second increments. (b) Temperature in the center over time.

Figure 3-10: Results of finite element model of heat transfer. (a)-(c) Cutaway view of temperature distribution of system at 5, 10, and 20 seconds respectively. The cooler region in the left side of the tube section is due to constant feed of room temperature media. (d) Temperature of system, from $r = 0$ (center) to $r = 1/16''$ (outer wall). Each curve represents temperature distribution at a point in time, from $t = 0s$ to $t = 20s$ in 1 second increments. See Figure 3-9(a) for comparison. (e) Temperature at the furthest downstream point on the centerline of the system over time.
Chapter 4

Preliminary Experimental Validation

After a few iterative rounds of component design coupled with finite element modeling to better inform the design process, we can move forward with the fabrication and testing of actual prototypes to ensure the models and design features work as intended. This section largely focuses on the initial fabrication runs and preliminary tests that were run on the physical inserts. We begin with a brief discussion on the process required to bring a design to fabrication, followed by results on priming and initial flow experiments.

Preparing for Fabrication

When a design is ready for manufacturing, it is good engineering practice to create a Bill of Materials, or BOM, that lists all sub-components and materials needed to create a particular component. For each sub-component, the BOM typically lists critical information such as a suggested vendor (if the component is off-the-shelf), a part number, and the quantity required to create an assembled device. The BOM for the apical flow insert can be seen in Appendix A; optional columns present in this
BOM include a description of each subcomponent and their usage in relation to the assembly. Analogous to commenting lines of code in a program, the purpose of these columns is to facilitate faster knowledge transfer to any person that is previously unfamiliar with the apical insert.

For any component that will be machined, technical drawings must be drafted and sent to the machinist. Aside from displaying crucial information such as the dimensions of each feature, the technical drawing also conveys any special instructions, or “callouts”; this includes surface finishes, non-standard tolerances, or even critical information that would be hard to discern in the drawing if not called out, such as the thread length or thread type on a tapped hole. Technical drawings for the apical flow insert and lower ring can be seen in Appendix A.

4.1 Priming

Though the finite element models have given some insight into fluid behavior within the device, the models have been run under the assumption that the apical insert is fully primed from the beginning. In practice, this is not the case during assembly, as outlined in Section 2.3.2. Thus, it is important to verify that the assembled Transwell/apical insert device is self-priming, or at least sufficiently self-priming to the point where any introduced bubbles do not significantly interfere with flow.

Fortunately, the even flow profiles created by the inlet geometries appear to apply during priming as well. Figure 4-1 shows an image time series of the assembled device as it is primed with dye in water. The components seen in the images were fabricated via stereolithography of a methacrylic acid resin, so it should be noted that priming should also be validated on the machined polysulfone components. Nonetheless, it is seen in the time series that the assembled device will successfully self-prime, as no bubbles in the flow path were visible upon inspection.
Figure 4-1: Time series of assembled apical insert as it is primed. The device appears to be self-priming as there are no visible bubbles obstructing flow. The drop of dye at the bottom of the last image is due to water slowly leaching through the porous membrane at the bottom of the Transwell.

4.2 Flow

As previously mentioned, the flow and transport models in Sections 3.2 and 3.3 proved to be helpful during the design process as it suggested which inlet geometries would presumably create a more even flow profile. However, the modeling results alone are only theoretical, and validation must be done to determine whether they reliably predict actual fluid behavior.

One way to test this is to introduce a bolus of dye into the system and compare the profile of the dye to that of a finite element model. Figure 4-2 shows an image time series of the results of this test. The model snapshots on the left of each image
Figure 4-2: Time series of dye flow in apical chamber. All images were taken from the bottom of the Transwell, looking up through the membrane. For each image, finite element model of dye transport is on the left, and image of Transwell is on the right. Overall, there is good agreement between model and experiment.

Combine both the flow and diffusion models for a dye solution (with $D_{\text{dye}} = 1 \cdot 10^{-9}$ m$^2$/s$^{-1}$) introduced through the apical inlet. The right side of each image are pictures taken through the bottom of an assembled Transwell/apical insert device, looking up through the Transwell membrane. Over time, dye is eventually introduced through the inlet and flows over the apical side of the membrane.

As seen in Figure 4-2, there is very good agreement between the modeling results and experimental results. There appears to be a larger diffusion boundary compared to the model, but this can be attributed to the experimental setup: the device is initially primed with water, then the inlet tubing is disconnected from a syringe filled
with water and connected to a syringe filled with blue dye on a syringe pump. As soon as this connection happens, diffusion at the dye/clear water boundary immediately occurs. The additional time required for dye to travel from the syringe pump to the apical chamber also results in additional time for the diffusion boundary to grow. Qualitatively, the general profile of the dye bolus is quite similar between model and experiment, which is a promising validation of the model.
Chapter 5

Discussion and Conclusions

5.1 Future Work

In this thesis, we have covered the design process and all the biological and mechanical considerations that come with it; several models of flow, oxygen transport, and heat transfer, both analytical and numerical; and a brief glimpse into experiments that validate the design and models. However, as we move toward more practical uses of the devices for biology, there remains some work to be done.

This section will cover various aspects of future development for the apical insert; some tasks, such as creating a sterile lid, are in the critical path and must eventually be tackled. Other tasks, such as alternate sealing approaches, are optional but will lead to a more robust, user-friendly device.

5.1.1 Lid Design

A reasonable first milestone to aim for is a pilot biology experiment where the current small intestine model is cultured on a Transwell and interfaced with the apical insert under flow, with cell viability as the key metric to ensure that the device does not
somehow harm the culture. Before we reach this point, there is one more component that must be created: a lid.

As previously addressed in Chapter 2, maintaining sterility at all times is key with any cell culture experiment. When cultures are maintained in a multiwell plate, the plate itself comes with a sterile polystyrene lid that fits over the plate while allowing a thin, slightly tortuous path for ambient oxygen to diffuse through. While the apical insert assembly is compatible with a standard 12-well multiwell plate, it is unfortunately not compatible with the associated plate lid as the assembly sticks out well above the height of the plate. Thus, a lid must be designed that accommodates the insert (or multiple inserts) while remaining true to the function of the original plate lid: preventing contamination while still allowing for oxygen transfer.

On the surface, this appears relatively simple: design the lid to be very similar in dimension to a plate lid, and add large bores where the apical inserts will be. This is a reasonable approach, although there are a number of details that require careful thought. For example, the bores must seal or at least somehow isolate the multiwell plate from the surrounding environment when the apical flow inserts are placed into their wells—otherwise, there exists a path for contaminants to easily fall down through the lid and into media. Also, there must be some mechanism to prevent the inserts from rotating out of place, as the collection of rigid tubing connected to the inlets has the potential to apply an undesired torque to the system during platform handing. Finally, fabrication and material choice must be considered—much like any other component in this system, the material used for the lid must be sterilizable, preferably via autoclaving for ease of use.

Figure 5-1 shows renderings of a custom lid that may address these three issues. The outer dimensions of the lid are similar to that of a standard plate lid, thus allowing for oxygen transfer through the sides of the plate as originally intended. The insert itself is bolted to the lid, which prevents undesired torque during user handling unless
the applied torque is high enough to lift the lid with the inserts. This potential issue can be mitigated by creating a heavier lid, which brings us to the final task of material choice. 316 stainless steel is a grade of stainless steel that is commonly used in medical applications and can be autoclaved for sterility. At a density of 8000 kg m⁻³ versus polysulfone’s 1250 kg m⁻³, it is much more capable of resisting undesired torques that would otherwise displace the system. If this approach is still not sufficient, then directly clamping the lid to the plate itself (e.g. with flat c-clamps) would clearly fix the lid relative to the plate, and would be more deterministic than relying on the weight of the lid.

5.1.2 Contact and Sealing Improvements

When assembled, the currently designed apical insert creates a radial seal with the inner wall of the Transwell via two off-the-shelf 11mm ID × 1mm CS Viton o-rings. In terms of user handling, it is certainly possible to push the apical insert into the Transwell to eventually create the radial seal, but more force than intuitively expected is required to do so. This is due to the fact that the o-ring groove is directly adjacent to the surface that contacts the Transwell, as seen in the illustration on the left of
Figure 5-2: Illustrations of contact and sealing improvements. Both illustrations show a cross section of the apical insert coupled to a 12-well Transwell. The illustration on the left is the current iteration of the insert, while the illustration on the right utilizes a different contact surface and a lower o-ring groove to assist with assembly.

Figure 5-2. There are two straightforward design changes that can be done to improve this.

The first change is to simply shift the o-ring groove slightly downward such that it is not directly adjacent to the protruding apical insert features. This allows the o-ring to move further down the Transwell during assembly so that it is fully covered by the Transwell’s inner wall. The second change is to define a different contact surface; rather than using the radially protruding features, another viable choice is near the top of the insert, which in turn contacts the top of the Transwell. Both of these changes are summarized in Figure 5-2.

5.1.3 Apical Flow Dimensions

In the current design, the bottom of the apical insert rests 1.5 mm away from the Transwell membrane, essentially creating a 1.5 mm tall apical channel for flow. The models and preliminary validation experiments show that this apical height is certainly usable as is—a fairly even flow distribution is possible, and there is a slightly decreasing oxygen gradient from inlet to outlet, similar in principle to the decreasing oxygen gradient from the duodenum down to the ileum [3].

Assuming cell viability experiments are well validated with this current design,
there are some additional oxygen gradients that can be explored by changing the height of the apical chamber. Increasing the height would increase the Peclet number in the apical domain, which in turn reduces the slight preference for diffusive transport over advective transport. As this trend continues, we would at some point see an oxygen gradient in the z-direction, perpendicular to the Transwell membrane; if compared to an in vivo system, this would be analogous to a radial oxygen gradient in the small intestine. This would be particularly interesting as it would allow for segmentation of microbe cocultures—anaerobic microbes would thrive in the more hypoxic regions, while facultative anaerobes would be denser in the more oxygenated regions—not unlike the microbe segmentation seen in vivo. This would be a very complex in vitro model, and would serve as a good long-term goal to aim for.

5.1.4 Sensor Integration

Another eventual goal is the integration of various sensors with the apical insert to bolster the set of metrics. For example, transepithelial electrical resistance, or TEER, is currently used as a quality control metric to gain a general sense of the culture’s barrier function. However, TEER measurements are currently done as discrete events where a Transwell containing culture is removed from its plate, placed on a TEER measuring device, and then placed back in its plate. If electrodes were directly integrated into the apical insert and multi-organ platform, more frequent TEER measurements would be possible with minimal perturbation to the cell layer. On the surface, this appears to be a complex task that could turn into its own project, as there are a multitude of factors to consider.

On-board oxygen sensing is another desired platform feature, as oxygen levels in media can be used as a metric for cellular metabolism, which can be a marker for cell viability. A promising proof of principle for oxygen sensors has previously been developed within the lab and is currently in a state of further development [28]. From a
mechanical perspective, integration with the sensors would be fairly straightforward—
given oxygen probe dimensions, fixtures that accommodate the probes within the
apical flow insert can certainly be designed. However, as oxygen levels can vary spa-
tially within the device, the exact position of the oxygen probe tips is an interesting
question that can be approached with a combination of finite element modeling and a
series of experiments. Another important point to consider is resistance to microbial
fouling, as microbe cocultures can form a biofilm on the surface of the oxygen probe
tips as they are exposed to the culture over time.

5.2 Conclusions

The process of design, modeling, and experimental validation presented in this thesis
is a strong first step toward the creation of a robust apical flow device for commercial
Transwells. By designing the device to be compatible with commonly used Tran-
swells, user adoption is considerably easier compared to a fully custom approach, and
reducing “user friction,” i.e. creating a simple handling experience, is key to user re-
tention. The models that were presented have been essential for gaining insight into
potential velocity profiles and oxygen gradients within the device before any physical
testing can be done. This design/model feedback loop, when utilized properly, re-
sults in a higher probability that the physical device will perform as intended. This is
particularly helpful as it has the potential to reduce the number of design/build/test
iterations in the hardware development process, thus saving time and money. Finally,
the preliminary flow experiment presented in this thesis is a promising glimpse toward
the future, as initial tests show good agreement with model predictions.

As discussed in Section 5.1, there are a number of areas for improvement in future
iterations, including easier handling, more reliable operation, and the capability for
more complex experiments. However, this initial iteration is quite promising on its
own and is fully capable of the core feature it was designed for: apical flow on a Transwell. With the capability of this apical insert, as well as the future iterations and improvements planned for this device, the ability for controlled apical media replenishment and tunable oxygen gradients in cell cultures is on the horizon.
# Appendix A

## A.1 Bill of Materials

<table>
<thead>
<tr>
<th>Part #</th>
<th>Vendor</th>
<th>Description</th>
<th>Usage</th>
<th>Qty. needed per system</th>
</tr>
</thead>
<tbody>
<tr>
<td>7400A11</td>
<td>McMaster-Carr</td>
<td>0.050&quot; hex driver</td>
<td>hex driver for 0-80 screws</td>
<td>1</td>
</tr>
<tr>
<td>92196A070</td>
<td>McMaster-Carr</td>
<td>1/2&quot; 0-80 socket cap 18-8 stainless steel screws</td>
<td>fastens apical insert to lower ring</td>
<td>3</td>
</tr>
<tr>
<td>5239K23</td>
<td>McMaster-Carr</td>
<td>PTFE tubing, 1/32&quot; ID 1/16&quot; OD</td>
<td>carries media for apical flow</td>
<td>6 ft.</td>
</tr>
<tr>
<td>25995A359</td>
<td>McMaster-Carr</td>
<td>set of three 10-32 taps</td>
<td>for hand tapping 3d printed parts</td>
<td>1</td>
</tr>
<tr>
<td>25995A117</td>
<td>McMaster-Carr</td>
<td>set of three 0-80 taps</td>
<td>for hand tapping 3d printed parts</td>
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</tr>
<tr>
<td>25605A63</td>
<td>McMaster-Carr</td>
<td>T-handle tap wrench</td>
<td>for hand tapping 3d printed parts</td>
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</tr>
<tr>
<td>9319K46</td>
<td>McMaster-Carr</td>
<td>FEP-encapsulated silicone o-rings, -012</td>
<td>seals apical insert and transwell</td>
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</tr>
<tr>
<td>9263K549</td>
<td>McMaster-Carr</td>
<td>Viton o-rings, 1mm CS, 11mm ID</td>
<td>seals apical insert and transwell</td>
<td>1</td>
</tr>
<tr>
<td>75165A123</td>
<td>McMaster-Carr</td>
<td>stainless steel luer lock needles, 20 gauge, 1/4&quot; length</td>
<td>connects syringe to tubing</td>
<td>1</td>
</tr>
<tr>
<td>7510A653</td>
<td>McMaster-Carr</td>
<td>10 mL luer lock syringe</td>
<td>holds media</td>
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</tr>
<tr>
<td>21001-090</td>
<td>VWR</td>
<td>10-32 flat bottom fitting + flangeless ferrule for 1/16&quot; OD tubing</td>
<td>connects tubing for apical flow</td>
<td>2</td>
</tr>
<tr>
<td>NE-1000</td>
<td>syringepump.com</td>
<td>syringe pump</td>
<td>syringe pump</td>
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</tr>
<tr>
<td>N/A</td>
<td>All Dimensions (machined)</td>
<td>apical insert, polysulfone</td>
<td>apical insert, polysulfone</td>
<td>1</td>
</tr>
<tr>
<td>N/A</td>
<td>All Dimensions (machined)</td>
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<td>lower ring, polysulfone</td>
<td>1</td>
</tr>
<tr>
<td>N/A</td>
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<td>apical insert, likely ABS</td>
<td>apical insert, likely ABS</td>
<td>1</td>
</tr>
<tr>
<td>N/A</td>
<td>DangerAwesome (3D printed)</td>
<td>lower ring, likely ABS</td>
<td>lower ring, likely ABS</td>
<td>1</td>
</tr>
</tbody>
</table>
12-well attachment lip

DIMENSIONS ARE IN INCHES
THREE PLACE DECIMALS 
ANGULAR TOLERANCES 
INTERPRET GEOMETRIC TOLERANCES PER AMERICAN NATIONAL STANDARDS INSTITUTE POLYSULFONE

4 x 0.047 THRU ALL
0-80 UNF THRU ALL

UNLESS OTHERWISE SPECIFIED:

NAME DATE

DRAWN
CHECKED
ENG. DPR
MFG. DPR

POLYSULFONE

TITLE:

SIZE DWG. NO. REV

SCALE: 2:1

SHEET 1 OF 1

A

NAME DATE

DRAWN
CHECKED
ENG. DPR
MFG. DPR

POLYSULFONE

TITLE:

SIZE DWG. NO. REV

SCALE: 2:1

SHEET 1 OF 1

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Bibliography


