MULTIPHASE MODELING OF MONOCYTE MIGRATION AND DIFFERENTIATION IN A FLOW BIOREACTOR SYSTEM: AN *IN-SILICO* STUDY

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Abstract: The objective of this study was to model monocyte migration and differentiation into dendritic cells under a variety of conditions in a novel designed plate bioreactor with flow using Computational Fluid Dynamics (CFD) simulation software. The model was developed to study fluid flow phenomena throughout the geometry such as shear rate patterns and velocity streamlines that can be expected over a range of parameters. Additionally, monocyte and dendritic cell concentrations were predicted for the same conditions to provide insight for actual experimental conditions and make recommendations for an optimal setup. To model the endothelial cell layer that was cultured in the plate bioreactor during experiments, this region of the bioreactor was set up in the model as a porous media with viscous and inertial resistance calculated based on cell properties and porosity of the layer. Using preliminary experimental data for the plate bioreactor, a monocyte death rate correlation was developed as a function of local shear rate and a first-order kinetics reaction correlation was developed to describe the differentiation of monocytes into dendritic cells. These correlations were incorporated into the developed model in the form of User-Defined Functions (UDFs) so that cell concentrations could be monitored over the duration of actual experimental time. The plate bioreactor was simulated for 48 hours at an inlet Reynolds number (Re_{in}) ranging from 2.5 to 25 and inlet monocyte concentration ranging from 250,000 cells/mL to 1,000,000 cells/mL. Concentrations of both monocytes and dendritic cells were greatly affected by the changing of $Re_{in.}$ Results showed that a Re_{in} of 5.0 provides the most optimal conditions in an experimental setting. As Re_{in} increases beyond 5.0, it is predicted that the number of still living monocytes and differentiated dendritic cells will greatly decrease in a 48-hour experiment. Simulation results were validated by measuring monocyte and dendritic cell concentrations over a range of Re_{in} tested experimentally and recommendations were made for potential future works.

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CHAPTER I

INTRODUCTION AND OBJECTIVES

1.1 Introduction to CFD Studies

Flow bioreactors are commonly used equipment in the field of tissue engineering to culture three-dimensionally engineered constructs and expose them to conditions that mimic physiological processes experienced in the body [1]. As experimental studies become more sophisticated, it is of the utmost importance that these systems are designed and the experiments are carried out under extremely precise and closely monitored conditions. A technology that allows for quick and efficient prediction of possible outcomes in an experimental setting without actually having to carry out the studies in the lab is the use of Computational Fluid Dynamics (CFD).

By developing a model that accurately describes the geometry, flow phenomena, and properties of the tissue engineered construct, CFD studies can have many advantages in tissue engineering applications [2, 3]. CFD allows for a significant reduction in not only the time, but also the cost required in bioreactor and experimental design. If simulation results suggest that certain experimental conditions may provide largely unfavorable results, researchers can avoid carrying out a number of experiments that would not meet the goals of the study. This can save a great deal of time in carrying out these experiments, as they often take days or even weeks to complete. Similarly, a quick

parametric analysis of experimental conditions can point researchers in the right direction and help them to optimize experimental conditions that may not otherwise have been considered. Perhaps most of all, CFD simulations allow one to study a system with a level of detail that is practically impossible in an experimental setting. Concerning tissue engineering and flow bioreactor systems, simulations allow for predictions of local shear rates, velocity and pressure profiles, and cell behavior, such as migration or differentiation that may be occurring in the developed system and cannot otherwise be measured in the middle of an experiment.

Without the use of CFD in these flow bioreactor studies, experimental researchers are left to carry out their studies with significantly less insight into the likely outcomes of any given experiment. The advantages discussed highlight the need for CFD simulation in flow bioreactor studies, an area where precision is crucial to the successful outcome of an experimental study. This study will focus on the development of a computationally efficient model that can accurately predict fluid flow patterns and final results for monocyte and dendritic cell concentrations in the bioreactor, along with expected distribution throughout the geometry under a variety of inlet conditions.

1.2 Hypothesis and Objectives

This study had multiple main areas of focus: The development of a Computational Fluid Dynamics (CFD) model for a flow bioreactor system, the validation of the developed model with experimental studies, and an in-depth analysis of inlet conditions. As such, the goals of this project had multiple objectives. With regards to the experimental studies, the underlying goal is to generate dendritic cells from a tissue-

engineered construct within a bioreactor with flow conditions, with the goal of producing dendritic cells that are more functional than those that are generated by standard 2D cell culture. To meet this objective, three specific aims were formulated for the experimental component of this research. With these in mind, the three aims were the basis for the development of this studies' numerical modeling objectives The overall goal of the numerical modeling study is to develop a sufficient model that is experimentally validated and can predict monocyte concentration, migration, and finally differentiation into dendritic cells. Both the experimental and computational aims in this study were outlined below with a description of the end goal of each of these specific aims.

1.2.1 Experimental Objectives

1) Characterize the 3D tissue construct within a bioreactor with flow conditions.

A vascular tissue construct was integrated into the parallel plate bioreactor with multiple flow conditions. The endothelial cells were characterized via cell viability, surface marker expression, and release of soluble products that are relevant to monocyte transmigration. Endothelial cells were characterized via flow cytometry and immunohistochemistry. It is worth noting that the characterization of the endothelial cell layer is the region of the geometry in which cells migrate through and differentiate into the desired dendritic cell product. Additionally, these regions of the geometry will have different properties in the developed CFD simulation, so it is critical for both the experimental and CFD aspects of this project to effectively and accurately characterize these regions.

2) Characterize monocyte migration within the bioreactor

Upon successful characterization of the endothelial cell layer in the flow bioreactor, monocytes were introduced into the experiments at time points that were deemed appropriate. For this aim, initial monocyte migration was characterized in different regions of the bioreactor after the cells were allowed to continuously circulate in the bioreactor system for 48 hours. To achieve a measure of the number of monocytes migrated and the characteristics of monocyte migration, monocytes that had successfully migrated through the endothelial cell layer were counted, in addition to counting the number of monocytes located on the ring-insert, and those still in the free-flowing region of the bioreactor above the endothelial layer. In addition to making a monocyte cell count in different regions of the bioreactor, cell adhesion molecule expression was also measured on the monocytes to aide in the determination of the possible factors driving cell migration.

3) Characterize dendritic cells generated within the bioreactor

After completion of Aim #2 of this experimental study, the final aim was to collect dendritic cells from the bioreactor and characterize the cells based on their phenotype, analyzed by flow cytometry, and to examine their functionality by Ag processing and presentation to T cells. The outcome for this aim is an understanding of the impact of a more physiologically realistic system that induces shear on the cells to generate dendritic cells on their overall functionality, compared to that of the traditionally used static systems. Since there is not one cell marker that is specific just for dendritic cells, a combination of markers and the expression of them was taken into account when

considering which of the remaining cells had successfully differentiated into functional dendritic cells.

1.2.2 Computational Fluid Dynamics Objectives

1) Develop a CFD simulation that predicts cell migration and differentiation

Due to the goals of the experimental study, not only was it desirable to produce a model that can accurately predict shear rates and velocity profiles in the geometry, but also to monitor the distribution and mass fraction of multiple cell types of interest in this study. A death rate correlation for monocytes based on first-order kinetics was developed from some of the preliminary experimental results, such that final species mass fractions would account for the inherent cell loss that occurs during these experiments. The death rate correlation was developed as a function of the local shear rate and the local cell concentration throughout the bioreactor volume. Additionally, a correlation was developed based on first-order kinetics and implemented into the model which describes the differentiation of monocytes into dendritic cells, which is ultimately the desired product in this study. Again, using data from experimental studies, a reaction term was included to describe this differentiation process of monocytes into dendritic cells. Based on collected data, the cell death rate and differentiation correlations combined to produce a model that could predict complicated phenomena that is occurring within the bioreactor, and provide an estimation to the experimenters as to the expected final species mass fraction at a given set of inlet conditions.

2) Validate the flow bioreactor simulations with experimental results

After the development of the flow bioreactor geometry, the setup of the tissue-engineered construct, and the inclusion of relevant fluid properties, the cell-specific correlations that were developed could be included in the model. Subsequently, simulations were carried out with inlet conditions the same as that of those used in experimental studies. Essentially, the measure of the differentiation of monocytes into dendritic cells was the determining factor for the accuracy of the simulation studies, as this was the end product measured in the lab setting. By recreating the geometry of the bioreactor to the exact specifications it was actually produced to, developing a correlation to describe the cell media's Non-Newtonian behavior, and incorporating cell death and differentiation, this allowed for accurate validation of the simulation studies with experimental results that were carried out in a lab setting. Simulation studies were carried out for all of the experimental setups that were to be tested to validate the CFD model and to determine the degree to which the model can predict final results of the monocyte differentiation experiments.

3) Perform a parametric analysis to recommend idealized experimental conditions

Lastly, a parametric analysis of many different feasible experimental conditions was performed. Inlet Reynolds Number and inlet species mass fraction, specifically, were tested over a range of values to determine the optimal run conditions in an experimental setting. By performing a parametric analysis, the results and information provided allow for conclusions to be drawn about the effect of changing flow conditions and inlet mass fraction of monocytes in the designed bioreactor geometry. For example, a study on the range of feasible inlet velocities could provide a range that indicates not much of the flow reaches the endothelial layer, thereby decreasing the amount of cell migration and

differentiation that will take place in the reactor. Thus, experimenters can quickly determine if a set of inlet conditions would even be worth exploring in an actual lab setting. Finally, parametric analyses allow for an optimized set of conditions to be predicted and recommended for further experimental study in the production of dendritic cells.

CHAPTER II

BACKGROUND

2.1 Immune System and Dendritic Cells

The immune system is responsible for protecting the body by identifying and killing pathogens and tumor cells, while at the same time being able to distinguish them from healthy cells [4]. If the immune system could be manipulated in such a way that cancers, infections, autoimmune diseases, and a host of other medical problems could be cured by creating custom made treatments to meet the needs of an individual patient, then the landscape of medicine and patient health would be forever changed. Dendritic cells are antigen presenting cells which function to initiate and modulate the immune response, making them appealing for use to manipulate the immune system [5]. The unique manner in which dendritic cells can initiate adaptive response and mediate immunity to infection makes them an attractive candidate for use to develop dendritic cell-based therapeutics and treat a variety of diseases [6, 7].

Although it has been shown that there is a potential for the use of dendritic cells to treat disease, there are still challenges that need to be addressed before these possible treatments can be realized. One of the major issues facing researchers is the lack of available dendritic cells in a patient to be used for treatment. Although only a relatively small number of dendritic cells are needed to stimulate the immune system response,

researchers face challenges producing the quantity or even the type of cell needed to use as treatment for disease [8]. Thus, it would be beneficial to develop a method of producing dendritic cells in great quantity to be used for research purposes, and later for the development of dendritic cell based therapeutics. Most importantly, it is crucial to develop a method of producing dendritic cells that are functional and perform similarly to those found in natural physiological conditions [9]. If a novel method of producing functional dendritic cells were developed, it could contribute in a major way to the clinical research of cancer immunotherapy.

2.2 Previous Studies on Dendritic Cell Systems

There have been several studies completed regarding the production of dendritic cells under appropriate conditions that provide the cellular interactions necessary for dendritic cell production. Generally, since the dendritic cells circulating in the body make up only about 0.1 – 1% of peripheral blood mononuclear cells (PBMCs), if large amounts of dendritic cells are desired for production, they must be obtained via the differentiation of CD14+ monocytes [10].

Nair et al. [11] focused on the generation of dendritic cells and demonstrated the ability to produce dendritic cells and culture them to maturation in a standard tissue culture flask. Randolph et al. [12] also developed a system that exploited the ability of monocytes to migrate across an endothelial matrix and differentiate into endothelial cells. This study also utilized a static system to culture an endothelial tissue construct before introducing monocytes to migrate and differentiate within the system. Essentially, to date most studies on dendritic cell production that have been completed were done in a static

system under standard cell culture conditions. Thus, there has been little research completed on the production of dendritic cells in systems that are more similar to the expected physiological conditions within the human body, and a gap in research could be filled by completing such studies.

2.3 Cell Migration and Differentiation

The creation of an intricate system that can produce functional dendritic cells, along with a developed CFD model that can accurately and reliably predict the results of these experiments requires an understanding of certain cell mechanisms that are taking place. The goal of aims 2 and 3 of the experimental study were to characterize monocyte migration within the bioreactor, and to characterize dendritic cells generated within the bioreactor, which requires a knowledge of cell migration and cell differentiation, and especially how to develop a system that can induce these cell mechanisms. As such, a background knowledge of these specific phenomena that are taking place in the system was required in the development of the model and design of experiments.

Because of a monocyte's ability to differentiate into different types of cells, monocytes will engage in many different innate and adaptive immune responses and will be recruited to different sites based on the activation of certain integrins by specific chemokines and other relevant stimuli [13]. Since monocytes express most of their functions away from the vascular tissue network, they are almost always required to be trafficked and migrated across blood vessel walls [14]. Recruitment of monocytes outside of blood vessel walls involves migration across endothelial cell layers, which is usually mediated by an inflammatory response in the endothelial tissue construct. Inflammatory

cytokines like tumor necrosis factor-α (TNF-α) will activate the endothelium and induce the expression of adhesion molecules signaling leuokocytes, such as monocytes, to traffic towards and migrate through the endothelial layer [15, 16]. This is exploited in experimental studies such as ours, where a tissue-engineered endothelial construct is prepared and activated by TNF-α to induce migration once the monocytes are introduced. This phenomenon allows us to study and characterize the monocyte's migration within the bioreactor system.

In addition to the induction of monocyte migration through the 3-D tissue engineered endothelial construct created within the bioreactor geometry, monocyte differentiation is also an important cell mechanism in these studies. Since monocytes can differentiate into a broad spectrum of other cell types, whether it be dendritic cells or some type of tissue macrophage, it is critical to have an understanding of cell differentiation and how to induce the desired product in tissue engineering studies [17]. A study by Chapuis et al. [18] investigated the relationship of dendritic cell and macrophage pathways to determine what experimental conditions might result in different cell morphology and gene expression. Results showed that dendritic cells were reliably differentiated from PBMCs when cultured in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) and in conjunction with interleukin (IL-4). The results demonstrated that the presence of these two factors with or without the addition of TNF-α would allow for the cells to acquire dendritic cell morphology via differentiation, whereas if these factors were not present in experiments, the cells may still differentiate into macrophages or would retain their monocyte function [18].

With the knowledge of the importance of inducing the cell migration and differentiation mechanisms in this study, it is also vital to understand the reasoning for and importance of introducing physiological flow on the cells in this study [19]. The addition of fluid shear stress acting on endothelial cells plays a key role in the regulation of cell adhesion molecules that guides the interaction of monocytes with the endothelium [19, 20]. Many of these cell adhesion molecules on the endothelial cells play a vital role in the tethering, rolling, and activation of leukocyte transmigration [21]. In the design of these experiments and bioreactor system, the introduction of flow can help regulate much of the conditions necessary to produce functional dendritic cells, providing an improvement over the aforementioned methods of using static conditions to generate dendritic cells. The experimental and simulation studies developed will make it possible to study the effects of flow conditions on monocyte migration and subsequent differentiation to dendritic cells.

2.4 Bioreactor Systems

Bioreactor systems are widely used *in vitro* models that function to culture tissue engineered constructs and expose them to conditions that are normally experienced under physiological conditions [22]. Generally, these systems are designed to accommodate biological processes that develop under a closely controlled environment with specific operating conditions such as pH, pressure, temperature, nutrient supply, and waste removal [18]. Ideally, bioreactors are designed to have a high degree of reproducibility and some degree of automation, so that these biological processes easily be scaled up to larger applications. In addition to creating a reproducible biological environment for

cells, these systems can explain much of the fundamental workings of a 3-dimensional cell environment [23].

For many bioreactor systems, fluid flow is commonly utilized to introduce certain cell types to mechanical stresses to achieve the desired functionality of the cells [24]. By introducing flow into these systems, the design incorporates a feature that is crucial to the success of creating an environment that can continually produce functional cell types [25]. Additionally, depending on the application, by adding flow to a bioreactor, the system can create an environment that better mimics *in vivo* conditions, creating a better response in the stimulated cells or tissue-engineered construct that is involved in the experiments. It is also of the utmost importance to design a bioreactor with a sensible geometry, especially if flow in involved, such that the constructs within the geometry are receiving an adequate amount of contact with the induced flow. The bioreactor used in this study is a parallel-flow bioreactor, which allows flow horizontally above the cell-seeded region in the reactor. The geometry for the plate bioreactor was designed with rounded wells, which contains less dead space than if a rectangular well was used.

Combining the usefulness of flow bioreactor systems with the knowledge that dendritic cells have an ability to migrate between tissue and organs, a novel tissue-engineered construct could hypothetically be developed to aide in the production of functional dendritic cells [26]. By designing a bioreactor geometry that mimics the interface between a blood vessel and surrounding tissue, an environment could be created that allows for cell migration through a tissue-engineered construct. In doing so, the system developed could induce cell differentiation and the desired functionality for further use.

2.5 CFD In Tissue Engineering

As stated in the introduction, a tool that many researchers have begun to employ in flow bioreactor research to better understand the effect of fluid flow is the use of Computational Fluid Dynamics (CFD) to predict flow patterns and shear rates acting on tissue-engineered constructs locally [27]. CFD can often be a quick and cost-effective option to predict phenomena that will be experienced in the lab for flow bioreactor systems. Visualization of the phenomena that occur within the working volume of these systems can provide great insights that otherwise would be difficult or impossible to gain through experimental research [28]. By utilizing CFD to perform a parametric analysis on important experimental conditions, researchers can predict, or at the very least estimate a range of experimental conditions that would provide the most optimal results with regard to the desired functionality of the biological system. One of the most beneficial features of simulation is that parameter changes can quickly be made, as opposed to carrying out all of these changes in experimental settings, which would greatly increase the cost and time involved in these studies. Knowledge gained in these simulation studies allows researchers to design more controllable experiments and devise strategies to produce desirable results in extremely sensitive cell culturing experiments [29].

Although many studies have begun to take advantage of CFD to predict shear rates and flow patterns, there have been few studies completed regarding the multiphase modeling of cells migrating through a tissue-engineered construct. Numerical methods can not only be applied to calculate local shear rates, but can also be used to predict cell deposition, migration, and differentiation within a flow bioreactor. By developing an experimentally optimized and novel CFD model, numerical simulations can make

predictions about experimental outcomes that would otherwise be unavailable when designing an experiment for the production of dendritic cells.

This work not only utilized CFD to predict flow patterns and shear rates in the customized in-house bioreactor, but the numerical study also predicted monocyte migration and differentiation into dendritic cells. By developing a correlation to describe the death rate of monocytes over time and including a reaction term in the model to represent the differentiation of the remaining monocytes into dendritic cells, the model can provide not only more accurate details regarding shear rates and fluid flow patterns, but expected results regarding the end concentrations of monocytes and dendritic cells at any given set of experimental parameters. CFD can evaluate the feasibility of an experiment and provide concise information regarding the expected outcome of a trial under a specific set of parameters.

CHAPTER III

MODEL DEVELOPMENT

3.1 Introduction to Numerical Studies

Since the experimental conditions are vital for the survival of the cells in the flow bioreactor system, the experimental setup must be optimized to produce the desired results and cell functionality, while also minimizing cell death. Recommended run conditions were optimized via the development of a CFD model and the variation of several different parameters. Since there has been little research regarding the differentiation of monocytes into dendritic cells in a flow bioreactor system, this model is a novel contribution and can provide a basis for future studies of this nature.

To fulfill the objectives of this study, several parameters such as inlet velocity and inlet monocyte mass fraction were varied. In each analysis, the local shear rates, velocity streamlines, local monocyte mass fraction, and local dendritic cell mass fraction were evaluated from the simulation results. Based on the results of these studies, an experimental setup that is suitable for producing the maximum number of dendritic cells was selected as the optimized case. Based on the analyses performed, recommendations can be made and changes can be incorporated into future experiments and bioreactor designs. The results of these simulations provide information on the limitations of the

system designed and direction on the range of parameter values that are worthy of experimental analysis.

3.2 Numerical Setup

The numerical setup of this model included multiple steps to arrive at the final iteration of the model. Initially, the bioreactor geometry was created, followed by a mesh generation and independence test. Next, the initial and boundary conditions were determined, followed by a determination of the optimal discretization schemes. Then, incorporation of the Non-Newtonian cell media properties, along with the porous media properties for the location of the endothelial cells was included in the model. Finally, the correlations determined for monocyte death rate and monocyte differentiation could be included in the model, allowing for prediction of final species concentrations in the bioreactor. The sections below outline the work completed in the numerical model setup in further detail.

3.2.1 Bioreactor Geometry

A parallel-plate flow bioreactor was simulated in this study. The geometry for one of the plate bioreactor wells where the flow domain is relevant for analysis was created using SolidWorks 3D design software. The smaller, bottom cylinder has a height of 7.7 mm and a diameter of 9.00 mm. The larger top cylinder has a height of 13.41 mm and a diameter of 15.88 mm. Additionally, there is an inlet and outlet segment with a length of 17.07 mm that introduces and removes flow to the area where the endothelial construct is located. The wells have custom made ring-inserts that are the diameter of the larger top cylinder and rest in the bioreactor wells at the bottom of the larger cylinder. These ring

inserts are coated with a fibronectin/collagen solution which cells are then seeded on. This region is denoted by the red section in the figure below, and has an approximate height of 2 mm. Additionally, this red region of the bioreactor geometry is set up as a separate zone and defined as a porous media, such that the properties of the region where the ring is coated and seeded with cells can be described by a set of different, more realistic properties than just the free flowing media. Figure 3.1 below is a rendering of the geometry with some of the relevant dimensions of the reactor and the highlighted porous region.

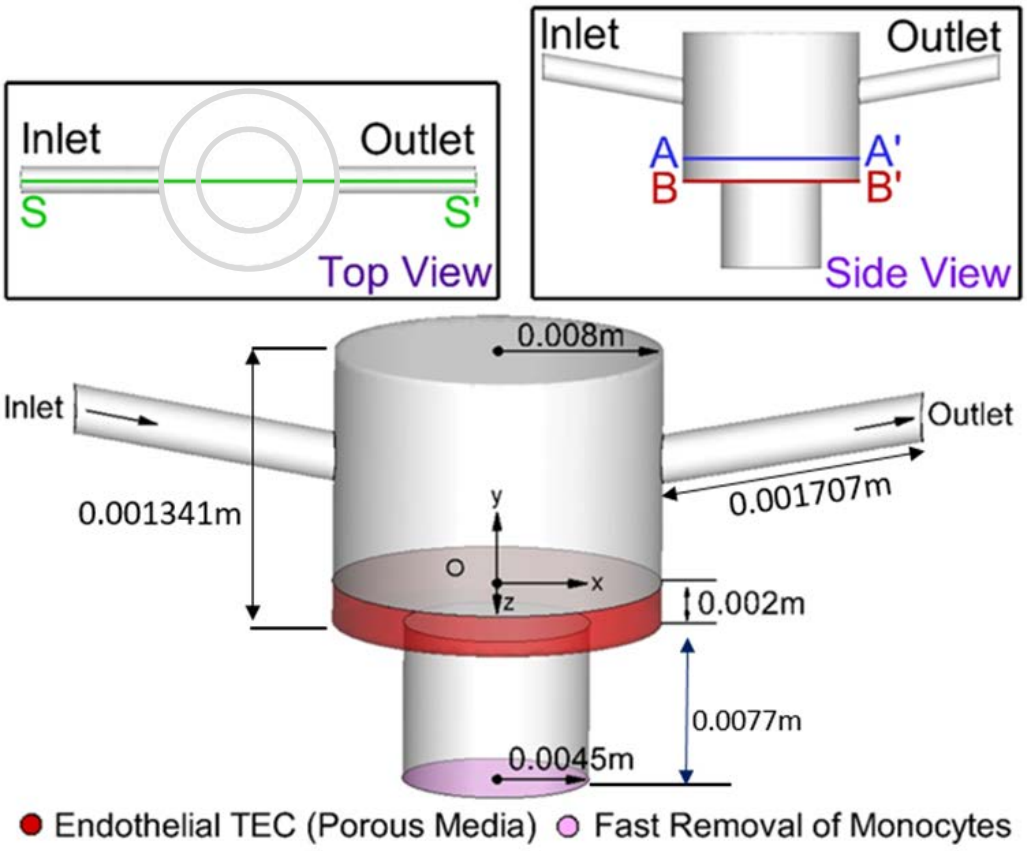


Figure 3.1: Plate Bioreactor Geometry with Highlighted Porous Region

Figure 3.2 below is a CAD drawing of the bioreactor outlining more of the relevant dimensions measured in inches of the entire bioreactor along with more of the specific dimensions of an individual well. For the purposes of this study, the second inlet and outlet at the bottoms of each well that is shown was plugged and did not allow for flow through those regions of the wells.

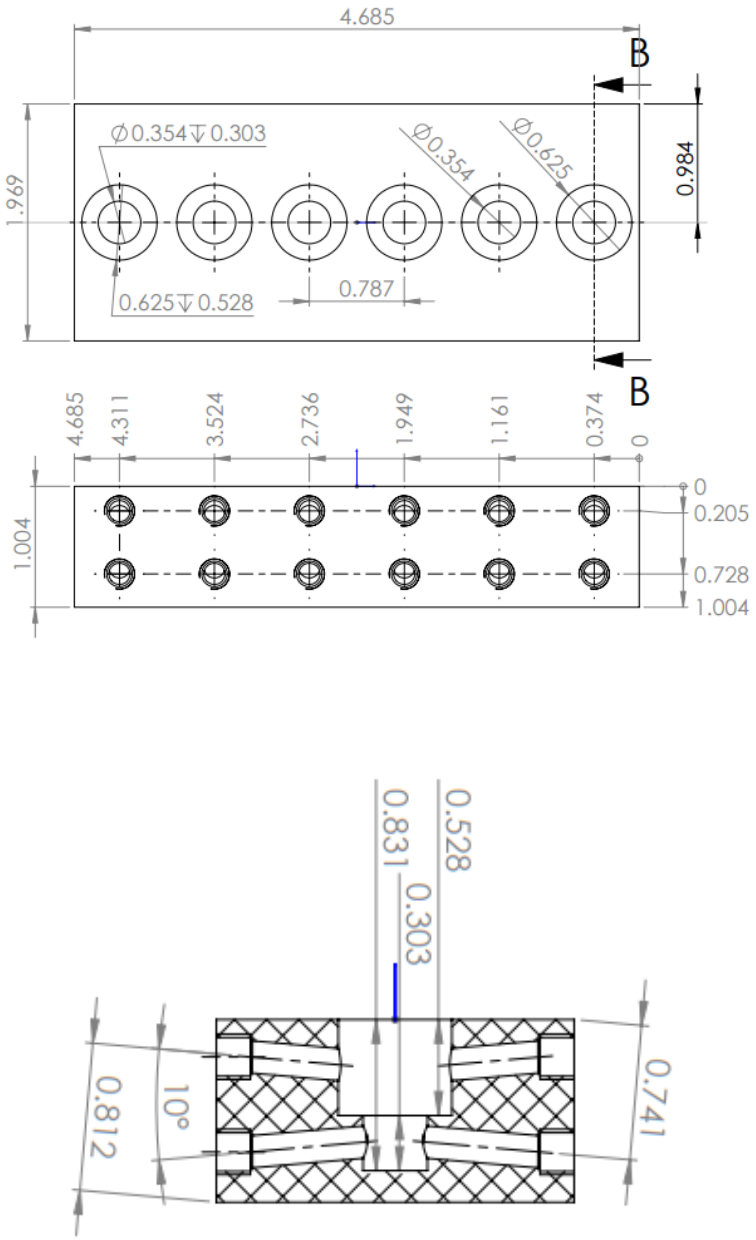


Figure 3.2: CAD Drawing of Plate Bioreactor

3.2.2 Mesh Generation and Independence Test

Meshing of the bioreactor geometry was done using ANSYS ICEM CFD meshing. Meshes were created using tetrahedral and prism elements for the parallel-plate geometry. Six near-wall prism layers were generated with a refined thickness to capture the flow phenomena more accurately at the geometry walls. A mesh independence test was performed by increasing the number of nodes and decreasing the element size until the percent difference of average velocity values in the working volume was less than 1% between mesh iterations.

Mesh independence tests were performed using a steady-state water simulation with an inlet flow rate of 0.4 ml/min. The percent difference in average velocity values was less than 1% for all recorded values, indicating that the mesh quality had been refined enough such that the results of the simulation would not be affected by the mesh. The optimized mesh for the parallel plate bioreactor had 203,025 nodes and minimum skew of about 0.35. Figure 3.2 below shows a plot of the nondimensionalized velocity versus the nondimensionalized position across the plane where velocity magnitudes were measured. The length across line AA’ denoted in the previous section in Figure 1 was nondimensionalized from zero to 1 to simply express the general location of measurements taken in the mesh independent test.

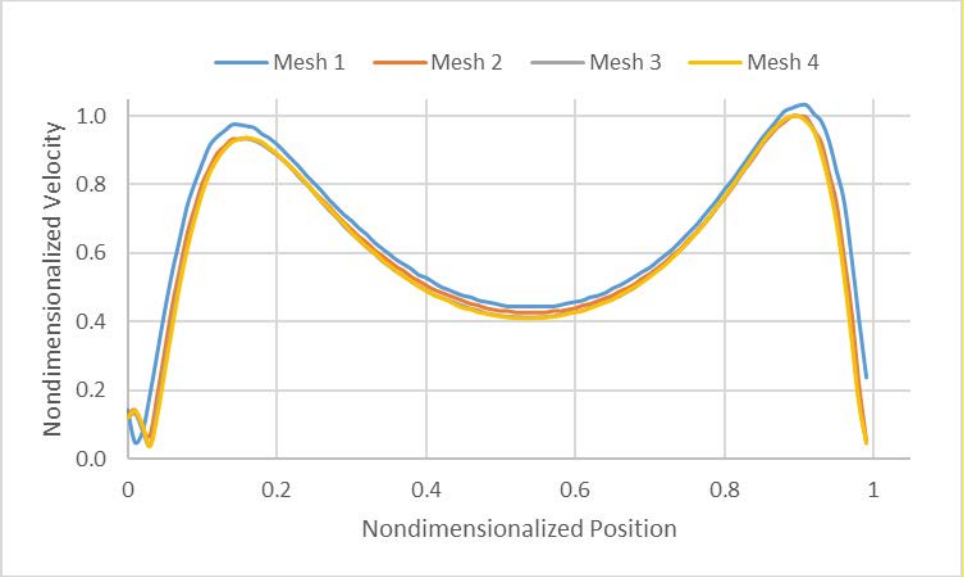


Figure 3.3: Mesh Independence Test

In addition to the mesh independent plots shown above, a table was generated which gives information regarding the mesh size and number of elements in each successive refined mesh that is plotted in the figure above. The relevant details of each mesh are given below in Table 3.1.

Table 3.1: Mesh Refinement Details

Mesh	Total Elements	Total Nodes	Growth Ratio	Avg. Element Size (mm^3)
1	718,488	134,133	1.1	0.00315
2	**1,153,124**	**203,025**	**1.1**	**0.00275**
3	2,105,225	473,597	1.1	0.00159
4	3,111,942	625,979	1.1	0.00117

As the plots demonstrate, mesh 2 was to a sufficient degree of refinement and was thus selected moving forward in the simulation since differences in velocity magnitudes were less than 1% for all points measured.

3.2.3 Initial and Boundary Conditions

As inlet conditions, different inlet velocity and inlet species mass fraction were assigned based on previous research that indicated the range of shear rates and monocyte concentrations that may be feasible in an actual experimental study. Experimentally, a flow rate of 0.4 ml/min was used for the majority of the trials. The inlet flow rate was converted to a velocity which was applied uniformly at the reactor inlet in these simulations. A range of inlet flow rates were analyzed from 0.2 ml/min to 2.0 ml/min, indicating a possible range of conditions that could achieve desired results. Additionally, monocyte concentration is another inlet condition that was varied in the simulation studies. Experimentally, a concentration of 500,000 cells/ml was typically used. This was converted to an inlet species mass fraction of 0.000883 which was the input within Fluent. A range of inlet concentrations were also analyzed from 250,000 cells/ml up to 1,000,000 cells/ml to determine an optimal inlet monocyte concentration. Additionally, the inlet species mass fraction of dendritic cells is set to zero, since they are formed via differentiation during experiments. The wall boundary condition was set with a specified flux of zero for both monocytes and dendritic cells.

3.2.4 Discretization Schemes

The numerical solution of the governing equations with appropriate boundary conditions was performed by using a user-enhanced, commercial finite-volume based

program, i.e., ANSYS Fluent 19.1 (ANSYS Inc., Canonsburg, PA). All variables, including velocity components, pressure, shear rates, and species concentrations were calculated and located at the centroids of the discretized mesh cells. Numerical simulations were performed on a local 64-bit Dell Precision T7810 workstation with 128 GB RAM. The pressure-velocity coupling scheme was set as SIMPLE, while the second-order upwind scheme was used for the terms in the momentum equation calculation as well as the species transport equation, except for pressure where the solution was discretized using just a second-order solution method. The monocyte death rate and differentiation source terms were written in C and compiled as user-defined functions (UDFs) in ANSYS Fluent. These User-Defined Scalars were set with a first-order upwind solution method. The time step is 30 seconds, which was chosen after an analysis to determine a time step that would allow the solution to converge in no more than 20 iterations per time step.

3.2.5 Non-Newtonian Cell Media

It is important that not only the geometry be accurately defined, but that the medium which is flowing through the geometry be well defined also. Typically, CFD studies of flow bioreactor systems assume the medium to have the properties of water at 37°C. However, most cell media have a cocktail of additives that can change the makeup of the solution such that the properties differ from water. The cell media used in the lab was Promocell Endothelial Growth Media MV2, with approximately a 5% Fetal Bovine Serum (FBS) solution. This FBS solution can increase the viscosity, thus changing the behavior of the media when it is exposed to shear. Hence, it was determined that it could be beneficial to the development of the model if shear stress versus shear rate data was

collected and a correlation was developed to describe the fluid's behavior under varying conditions.

Using in-house rheometry tools and techniques, shear stress vs. shear rate data was collected and fitted to the generalized Herschel-Bulkley fluid flow model for the cell media that was used in the experiments. It was determined that for a shear rate up to 148 s^{-1}, the data fits to the Herschel-Bulkley model with an R^2 value of 0.999, indicating that at low shear rates the cell medium behaves as a Non-Newtonian fluid. At shear rates above 150 s^{-1} the shear stress vs. shear rate plot is almost linear, indicating that the cell media behaves as a Newtonian fluid at these higher shear rates. Interestingly, physiological shear rates experienced by the cells of interest in this study is less than 100 s^{-1} for most of the simulations performed. Thus, it was determined that the inclusion of the developed Herschel-Bulkley correlation would be beneficial in more realistically defining the fluid in the bioreactor. Further details regarding the fitting of the shear stress vs. shear rate data to the Herschel-Bulkley correlation is given in section 3.3 which explicitly describes the relevant governing equations in the model setup.

3.2.6 Porous Media Regions

To more precisely define the regions where the endothelial construct was located in the bioreactor, these regions were described using ANSYS Fluent's porous media conditions. This allows for the model to determine the proper value of pressure drop across the porous material [30]. This model can be used for a number of different multiphase applications, and flow through a cell layer grown on a fibronectin/collagen matrix is one of the potential applications for porous media. The inclusion of a porous

media zone in the geometry requires the addition of a momentum source term, which includes an inertial and a viscous resistance term, which are generally based on the superficial velocity of the fluid within the porous media [31] The inertial loss term can essentially be taken as a loss per unit length along the flow direction, while the viscous loss term largely takes into consideration the porous media properties with regards to the viscosity of the porous area. The required user inputs for Fluent's porous media model with regards to this study was a definition of the fluid material flowing through the porous medium, viscous and inertial resistance coefficients, porosity of the medium, and the relevant reaction mechanisms in the porous regions of the geometry.

3.2.7 Monocyte Death Rate Term

Whenever monocytes are introduced into the flow bioreactor system, they are allowed to circulate throughout the flow domain for 48 hours. This time was chosen based on previous studies that indicated this is a sufficient amount of time to allow interaction of monocytes with the endothelial construct, and then subsequent differentiation into dendritic cells. To accurately portray the cell environment in the numerical setup when monocytes are established in the system, the model must include a secondary phase. This was done in the CFD model setup by utilizing a User-Defined Scalar (UDS) within ANSYS Fluent to describe a secondary phase, which allows the user to input a specific inlet species mass fraction [31].

It should be noted, however, that by allowing the monocytes to circulate throughout the bioreactor for this amount of time, there is inherent cell death that occurs, both due to the flow rate, along with general cell death. To capture this cell death in the

model, several experiments were run at different flow rates to develop a correlation that would describe the monocyte death rate as a function of the bioreactor's inlet Reynolds Number and the local cell concentration. Trials were run at inlet Reynolds numbers of 0, 5, and 10. After developing a death rate correlation for the monocytes, a User- Defined Function (UDF) was written in C to incorporate this death rate source term. It was assumed that this death rate was the same for all regions of the bioreactor, so the UDF was applied for each zone of the working volume, including the porous zone.

3.2.8 Monocyte Differentiation Term

As has previously been discussed, differentiation of monocytes into dendritic cells is the ultimate goal of experimental studies, and hence, incorporation of this phenomena into the CFD model is the final goal of this study. Similar to the monocyte death rate term, previous studies have utilized first-order kinetics to describe the differentiation of cells into a different cell type. A study by Wang et al. described the differentiation of monocytes into macrophages and derived a series of first-order equations to depict this phenomena in their model [32]. It should be noted that while these studies did attribute first-order kinetics to the cell differentiation process in their numerical models, neither of these studies were modeling monocyte differentiation into dendritic cells. Thus, one limitation of this study is the scarce amount of data regarding monocyte differentiation, and that there essentially no CFD studies to date on cell migration and differentiation.

To arrive at the correlation determined for this CFD study, multiple trials were run to obtain a measure of the dendritic cell concentration in the bioreactor at set time points. This allowed for a fit of the data to a first-order kinetics equation as a measure of

cell concentration versus time. Again, similar to the monocyte death rate, this correlation was written in the form of a UDF and imported into ANSYS Fluent. This UDF was applied as a species reaction term throughout the bioreactor volume, allowing for differentiation to occur within the flow regions of the plate.

3.2.9 Model Assumptions

There are several assumptions made and used in the flow simulations of this study and they are listed below:

- The porosity of the porous regions of the bioreactor (regions where the geometry is coated and cells are grown) was set to a constant 40% [33]
- The system is symmetric in both cases from the central axis
- The diffusivity was isotropic throughout the flow bioreactor
- There is zero flux at the exterior boundaries of the geometry except for at the inlet and outlet

3.3 Governing Equations for Bioreactor Model

The circulating cell media and different cell types form a mixture at conditions of 37°C and standard atmospheric pressure, and can be modeled as a continuous mixture phase. The governing equations can be given as:

Continuity Equation

$$\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \vec{u}) = 0 \quad (1)$$

in which u_i represents velocity vector of the mixture [34].

Cauchy Momentum Equation

$$\frac{\partial(\rho\vec{v})}{\partial t} + \nabla \cdot (\rho\vec{v}\vec{v}) = -\nabla p + \nabla \cdot \vec{\vec{\tau}} + \rho\vec{g} \quad (2)$$

where ρ is the density of the mixture, $\vec{v}$ represents the velocity vector, p is the pressure, $\vec{\vec{\tau}}$ is the stress tensor, and $\vec{g}$ represents the gravitational body forces. At the typical flow rates that could feasibly be analyzed and compared in flow bioreactor studies, it was determined that only the laminar flow regime would be observed, and therefore the laminar fluid flow model was adopted in this study [35].

Non-Newtonian Model Fitting

As previously stated, in general the properties of the cell media are assumed to be equivalent to those of water at 37°C, which is a Newtonian fluid. By assuming cell media to have the properties of water, this allows for the fluid behavior to be assumed to follow the incompressible Navier-Stokes equation, thus simplifying the setup of the model and saving some efforts. However, inaccuracy arises when the fluid properties are described to be Newtonian, when in reality the shear stress versus shear rate relationship is not linear.

Since the in-house results suggested that the fluid behaves in a Non-Newtonian manner when the shear rate applied is in the range that is expected in experimental studies, there was concern regarding the assumption of a Newtonian fluid for the cell media. After utilizing rheometry equipment to collect shear stress vs. shear rate information, the data was used to develop a correlation to describe this non-linear behavior with the Herschel-Bulkley fluid model. The Herschel-Bulkley model is a

generalized Non-Newtonian fluid model that utilizes flow index, yield shear stress, and consistency parameters to describe the relationship between the effective viscosity, fluid shear stress, and shear rate [36]. The generalized Herschel-Bulkley model is shown in the equations below for calculating the effective viscosity, μ_{eff}, of the fluids.

$$\begin{cases} \mu_{eff} = k * |\dot{\gamma}|^{n-1} + \tau_0 * |\dot{\gamma}|^{-1}, & |\dot{\gamma}| \leq \dot{\gamma}_o \\ \mu_{eff} = \mu_o, & |\dot{\gamma}| > \dot{\gamma}_o \end{cases} \quad (3)$$

Where k is the consistency index, τ is the shear stress, γ is the shear rate, and n is the flow index of the fluid of interest. The required indices were determined along with a critical shear rate where the fluid's behavior no longer follows the Herschel-Bulkley model, and then performed as a Newtonian fluid. At shear rates higher than this critical value, a constant viscosity value is observed. The fitted Herschel-Bulkley model for the cell media used in the experiments is given in the equation below.

$$\begin{cases} \mu_{eff} = 0.0747825 * |\dot{\gamma}|^{1.32231-1} + 1.79x10^{-4} * |\dot{\gamma}|^{-1}, & |\dot{\gamma}| \leq 148s^{-1} \\ \mu_{eff} = 1.46x10^{-3}\ Pa{\cdot}s, & |\dot{\gamma}| > 148s^{-1} \end{cases}$$

Porous Media Regions

In addition to the continuity, momentum, and Non-Newtonian governing equations discussed in the development of this model, there is a region of the bioreactor volume that is described as a porous region, which is a feature available in ANSYS Fluent. This porous region, which is shown by the highlighted red volume of the geometry in Figure 3.1, essentially incorporates a flow resistance into regions of the model that are described as such and adds a momentum sink into the aforementioned governing equations. As is outlined in the ANSYS Fluent manual [31], the porous media

model assumes that there is thermal equilibrium between porous media solids and multiphase flows and that the momentum resistance and heat source terms are calculated separately. An additional limitation of the model is that since the volume of the actual blockage, in this case a cell layer, is not physically present in the model, ANSYS Fluent uses a superficial velocity inside the porous medium to ensure the continuity of velocity vectors across the porous medium. The additional source term consists of a viscous loss term (the first term on the left), and an inertial loss term (the second term on the right) in the equation below.

$$S_i = -(\sum_{j=1}^{3} D_{ij}\mu v_j + \sum_{j=1}^{3} C_{ij}\frac{1}{2}\rho|v|v_j) \tag{4}$$

where S_i is the source term for the *i*th momentum equation in the x,y, or z direction, $|v|$ is the magnitude of the velocity and D and C are prescribed matrices for the viscous and inertial resistance, respectively [31]. These terms were then calculated for the endothelial cell region and included in the numerical setup to best describe this region during experiments. The equations that were used to calculate the inertial and viscous resistance coefficients are given below

$$D = 150 * \frac{(1-\varepsilon)^2}{\emptyset^2} * D^2 * \varepsilon^3 \tag{5}$$

$$C = 2 * \frac{1.75 * (1-\varepsilon)}{\emptyset} * D * \varepsilon^3 \tag{6}$$

where ε, $\emptyset$, and D are the porosity of the medium, sphericity, and diameter of cells making the medium.

UDS Advection-Diffusion Equation

Monocytes and dendritic cell products have been described in the simulation setup using Fluent's user-defined scalar (UDS) transport equations. As such, two separate UDSs were assigned in the fluid flow simulations, one of which is the monocyte concentration, and the other is dendritic cell concentration differentiated from monocytes, which is the final product. The generalized scalar transport equation for a UDS is given below.

$$\frac{\partial \emptyset_k}{\partial t} + \frac{\partial}{\partial x_i}\left(F_i \emptyset_k - \Gamma_k \frac{\partial \emptyset_k}{\partial x_i}\right) = S_{\emptyset_k} \quad (7)$$

Where $\emptyset_k$ represents an arbitrary scalar k, $\frac{\partial \emptyset_k}{\partial t}$ is the unsteady state term, $F_i \emptyset_k$ is the convection term with F_i representing the convective momentum , $\Gamma_k \frac{\partial \emptyset_k}{\partial x_i}$ is the diffusion term with Γ_k is the diffusion coefficient, and lastly, $S_{\emptyset_k}$ is the source term that can be customized for each scalar k [37]. In the case of monocyte death rate and differentiation, a customized source term was developed in the form of a UDF and applied to relevant regions of the bioreactor. Further details and governing equations regarding these source terms are outlined in the sections that follow.

Cell Death Rate

Multiple trials were carried out to obtain a correlation of the monocyte death rate based on the inlet flow rate. A trial was completed with no flow, a flow rate of 0.4 ml/min, and a flow rate of 0.8 ml/min was completed, corresponding to a Re_{in} of 0,5, and 10. Cell death rate was measured at pre-determined time points to monitor the death of cells over the 48-hour duration that they would be allowed to circulate in cell

differentiation experiments. The results of the three trials were plotted as a function of $\ln(\rho_{monocyte})$ *vs.* time, where $\rho_{monocyte}$ is a measure of monocytes/m^3. This plot was constructed so that the change in monocyte concentration could be visualized over time. In Figure 3 below, the results of these three trials are shown as data was recorded over the 48 hour trials.

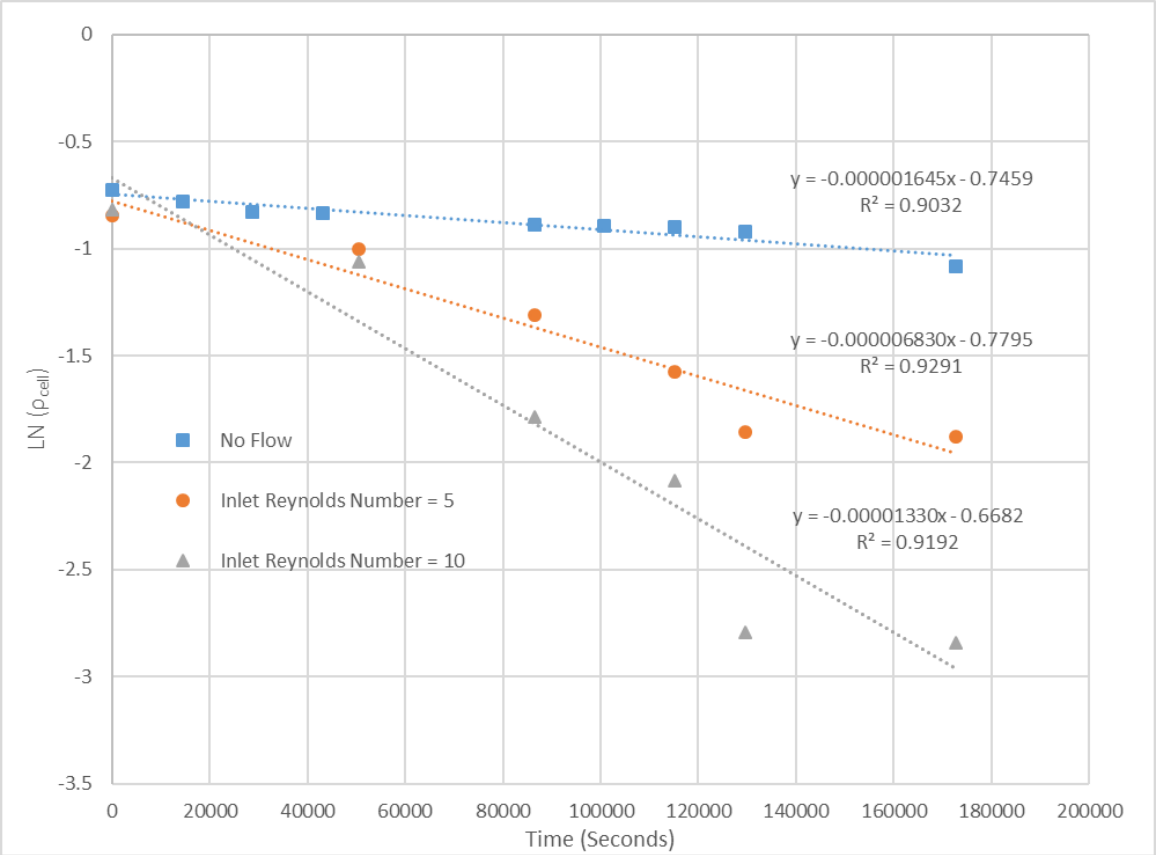


Figure 3.4: Natural Log of Monocyte Concentration vs. Time at Different Experimental Flow Rates

After data collection, it was determined that a linear function best fits the cell death rate versus inlet Reynolds number. Data from the three trials at 24 hours was taken

from Figure 3.3 above and was then plotted using the curve fitting tool within MATLAB R2018a. This allowed for a linear fit as a function of inlet Reynolds Number to describe the monocyte death rate over time. It was determined that to accurately measure the cell's death rate throughout the reactor over time, the local shear rate would better capture the death rate at any point in the geometry, so it was adopted in the final death rate equation. The fit of the data had an R-squared value of 0.944, and the final equation is given below.

$$\dot{C}_{dr} = (-1.296x10^{-6} * \dot{\gamma}_{local} - 0.000001645) * C_{monocyte} \quad (8)$$

where $\dot{C}_{dr}$ is the monocyte death rate term, $\dot{\gamma}_{local}$ is the local shear rate in the plate bioreactor, and $C_{monocyte}$ is a measure of the local monocyte concentration at any given location of the bioreactor.

Cell Differentiation

As previous studies have shown, there has only been scarce research regarding the mathematical modeling of cell differentiation. However, studies have shown that by developing a correlation in the form of first-order kinetics, cell differentiation can be adequately modeled for a number of other cell types. This was also assumed for monocyte to dendritic cell differentiation, and a form was derived to describe this phenomenon based on preliminary time trials that measured cell differentiation at set time-points. The data was fit to a plot of ln ($\rho_{dendritic}$) vs. time (seconds), which can be found in Figure 3.4 below.

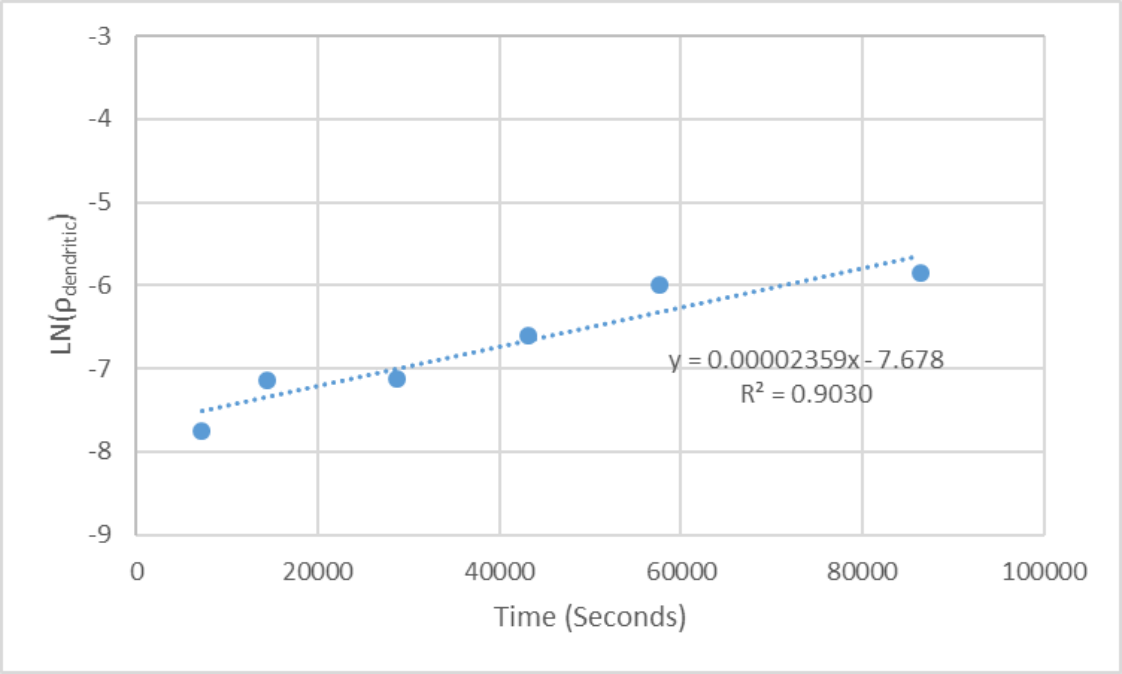


Figure 3.5: Natural Log of Dendritic Cell Concentration vs. Time at Re_{in} = 5

The derived correlation had R^2 of approximately 0.90 and the final equation is given below.

$$\dot{C}_{diff} = (2.359x10^{-5}) * C_{monocyte} \quad (9)$$

where $\dot{C}_{diff}$ is the monocyte to dendritic cell differentiation term, t is the time in seconds, and $C_{monocyte}$ is the local monocyte concentration at any given location in the bioreactor.

CHAPTER IV

MODEL OPTIMIZATION

4.1 Overview of Optimization Methods

In order to gain insight regarding the usefulness of the high-resolution numerical results, experimental optimization of monocyte and dendritic cell concentration predictions is necessary. Experimental studies that were completed either in parallel with or after development of the model were compared to the results of simulation studies to prove the utility of the numerical setup so that further simulation could be performed, and recommendations for future experimental setups could be made. Since very few studies have been completed to date regarding monocyte differentiation in flow bioreactors, there was little research published to validate a developed model with. Thus, correlations were derived from preliminary trials such that they could be implemented into the model and predict outcomes for future studies. The following section outlines the process that was completed to optimize the numerical model with experimental results.

3.2 Monocyte Death Rate and Differentiation Optimization

While the actual experiments with monocytes in circulation throughout the reactor were run for 48 hours, it was determined that the simulation reached steady-state in terms of flow patterns and cell concentrations at an end time of 5 hours. At this point, the monocyte and

dendritic cell concentrations no longer changed, and the fluid flow patterns were at steady-state such that shear rate and velocity had reached equilibrium. The figure below shows the comparison of the simulation results utilizing the developed correlations described in section 3.3 with the preliminary experimental data that was collected at the same inlet conditions.

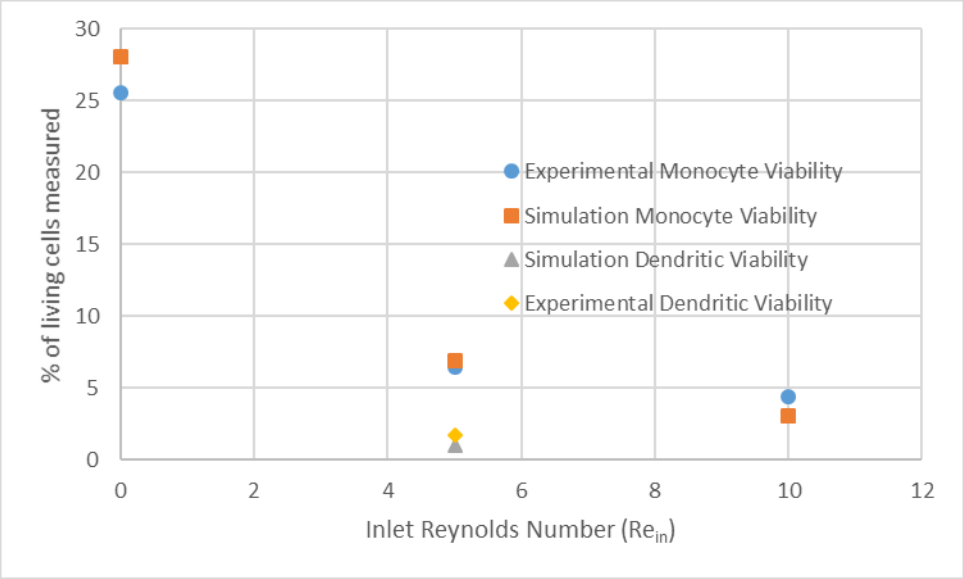


Figure 4.1: Optimization of Monocyte and Dendritic Cell Concentrations

Figure 4.1 shows a measure of the percentage of living cells measured versus the inlet Reynolds Number (Re_{in}). The percentage of living cells was calculated as a ratio of the number of monocytes and dendritic cells counted to the initial number of monocytes that were introduced into the system at the beginning of the experiment or simulation. Results showed good agreement with both monocyte and dendritic cell final concentrations, indicated that an experimentally optimized model has been developed for moving forward in an in-depth parametric analysis of potential experimental conditions.

To provide some context into the numbers predicted, a simulation performed at the conditions used in the majority of experiments predicted that 6.89% of the monocytes introduced

into the system would still be living after 48 hours, while the experiments showed about 6.5% of the monocytes were actually still living. With regards to dendritic cells, the model predicted that of the monocytes that were introduced at the beginning of the experiment, about 1.04% of the monocytes would successfully differentiate into dendritic cells.

CHAPTER V

RESULTS AND DISCUSSION

5.1 Fluid Flow Patterns in the Plate Bioreactor

Initial simulations that were performed for the plate bioreactor geometry was a comparison of Non-Newtonian versus Newtonian fluid flow patterns for the cell media being circulated throughout the volume of the reactor, since it was hypothesized that including a Non-Newtonian model for the cell media would provide more accurate results for the fluid flow patterns. It was determined that for both cases, there was a recirculation zone right around the area that the endothelial tissue construct was located, as indicated by the red line through the volume. However, it was also noted that the recirculation zone was much more pronounced in the Newtonian simulation. Figure 5.1 below shows this recirculation region in the bioreactor volume when water is assumed as the cell media for a single-phase simulation. This inlet flow rate of 0.4 ml/min, or a velocity of 0.001844 m/s, was the flow rate ultimately determined to be used moving forward in experiments.

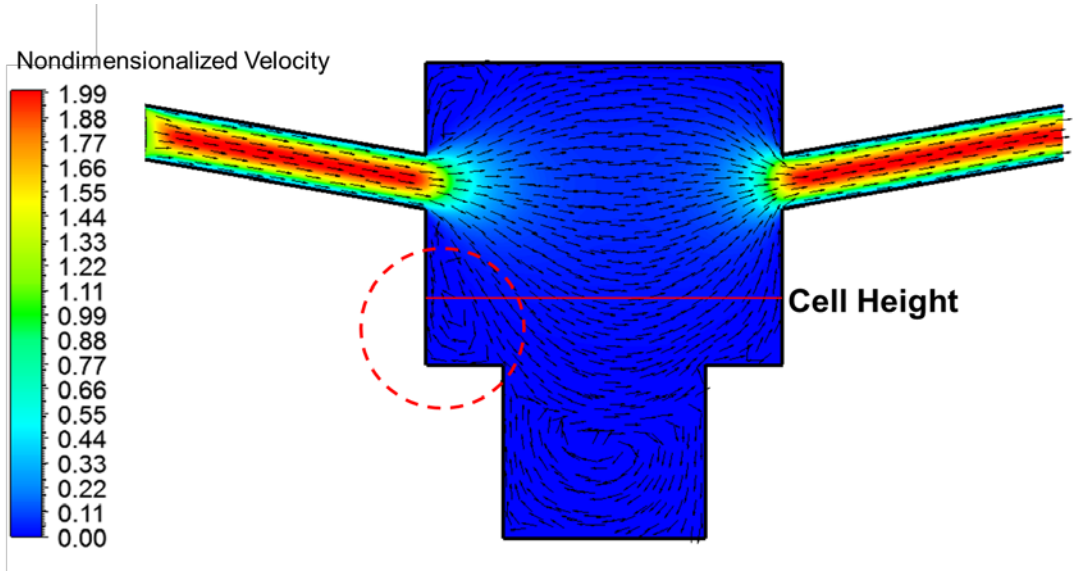


Figure 5.1 Nondimensionalized Velocity with Vectors for Newtonian Fluid

When the Herschel-Bulkley model that was fitted to the data collected for cell media was applied to this simulation, the view of the sagittal plane was similar, but the recirculation zone was smaller at the location of the endothelial construct. This indicated that it would be beneficial to adopt the Non-Newtonian model for further simulation studies, as it is of the utmost importance that these delicate biological systems be described accurately. The figure below shows the same sagittal view of the bioreactor, and outlines a smaller recirculation zone near the cell layer that is indicated by the red line.

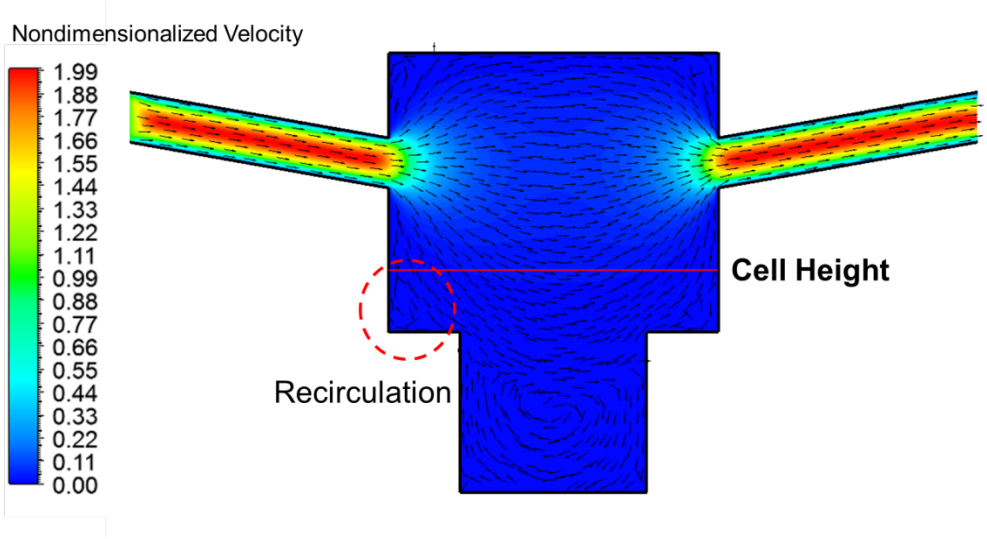
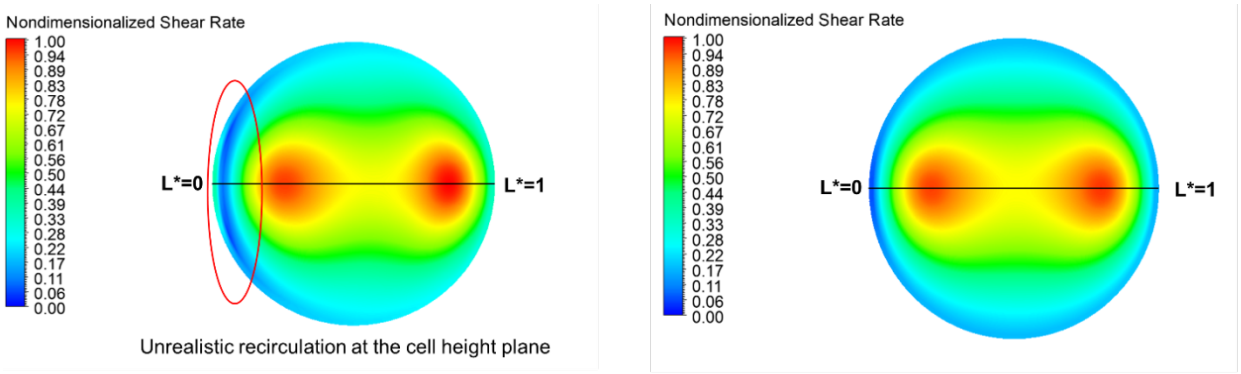


Figure 5.2: Nondimensionalized Velocity with Vectors for Non-Newtonian Fluid

In addition to the velocity contours that were generated and displayed above, shear rate contours were generated at the location of the cell layer. Nondimensionalized shear rate is shown in figure 5.3 below, and further confirms that the Newtonian simulation has a recirculation zone, and hence creates unrealistic shear rate magnitudes at the location of the endothelial cells.



(a) Contour of Newtonian Simulation

(b) Contour of Non-Newtonian Simulation

Figure 5.3: Shear Rate Contour of Newtonian/Non-Newtonian Simulations

In addition to confirming the advantage of incorporating the Non-Newtonian simulation into the final model, it was determined that shear rate magnitudes at the proposed flow rate of 0.4 ml/min were not of concern to kill or shear off the endothelial cells. Experimentally, flow rates of 0.3, 0.4, and 0.5 mL/min were tested, which were all shown in simulations to have shear rates within a reasonable range such that they would not shear off or kill the endothelial cells. Experimental results were most favorable for the 0.4 mL/min, which was why this was chosen in future experiments and development of the simulation. Inlet shear rates for this 0.4 mL/min flow rate were a maximum of about 18 s^{-1}, which is well within the range of physiological conditions experienced by endothelial cells, monocytes, and dendritic cells.

It is also beneficial to understand the velocity profile throughout the bioreactor to gain a further understanding as to the effect of the geometry on the flow path of the cell media, and to gain insight into the flow's effect on the endothelial cell region lower in the geometry. In figure 5.4 below, a general image of the bioreactor streamlines from inlet to the outlet at a Re_{in} of 5 is shown below to outline the flow path and areas of higher magnitude velocity.

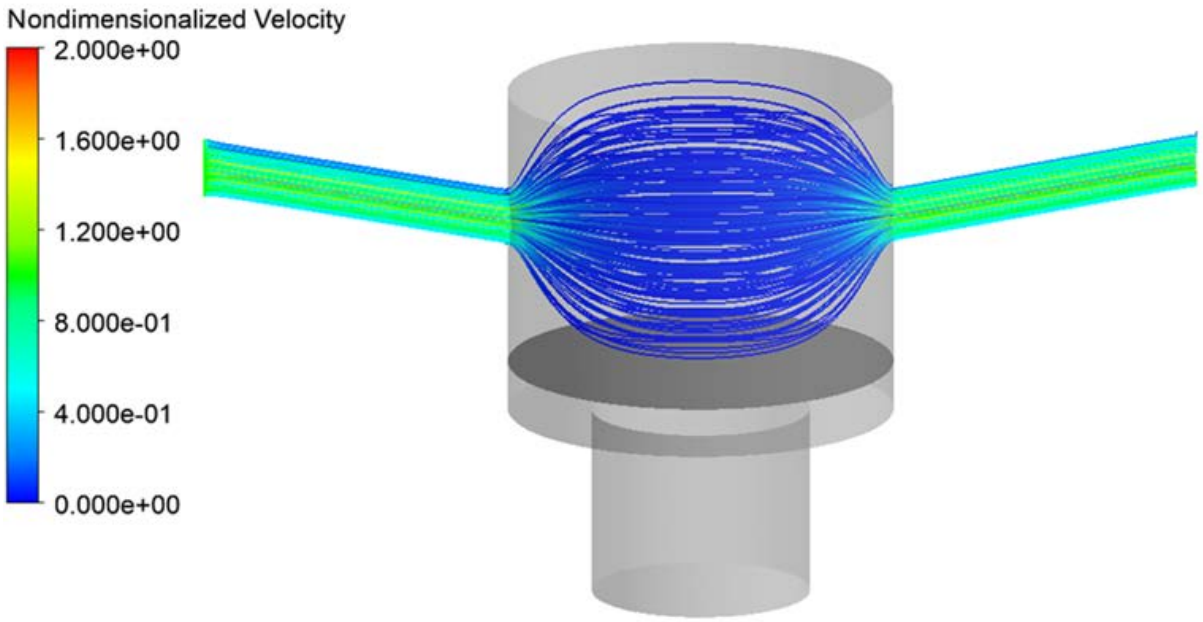


Figure 5.4: Streamlines of Nondimensionalized Velocity from Inlet to Outlet of Plate Bioreactor at Re_{in} = 5

This shows that the velocity magnitude, as expected, is much higher in the inlet and outlet sections of the geometry. In the bulk regions of the geometry the velocity magnitude quickly declines as the fluid gets lower in the reactor. It can be seen that most of the flow is above the endothelial cell layer, while some of the lower magnitude flow would move across the top of the endothelial layer, indicating contact between monocytes and the tissue-engineered construct. The flow that does reach the porous region has a much less uniform and defined path than the upper free flow region, which is because of both the very low velocity magnitudes, and the viscous and inertial resistance that was defined in this region. Thus, there is some recirculation in this region, likely due to the specific geometric design of the reactor, but the flow largely slows down and gradually flows straight down through this region of the reactor. Figure

5.5 below shows the nondimensionalized velocity streamlines in the lowermost section of the bioreactor, just below the porous region of the bioreactor.

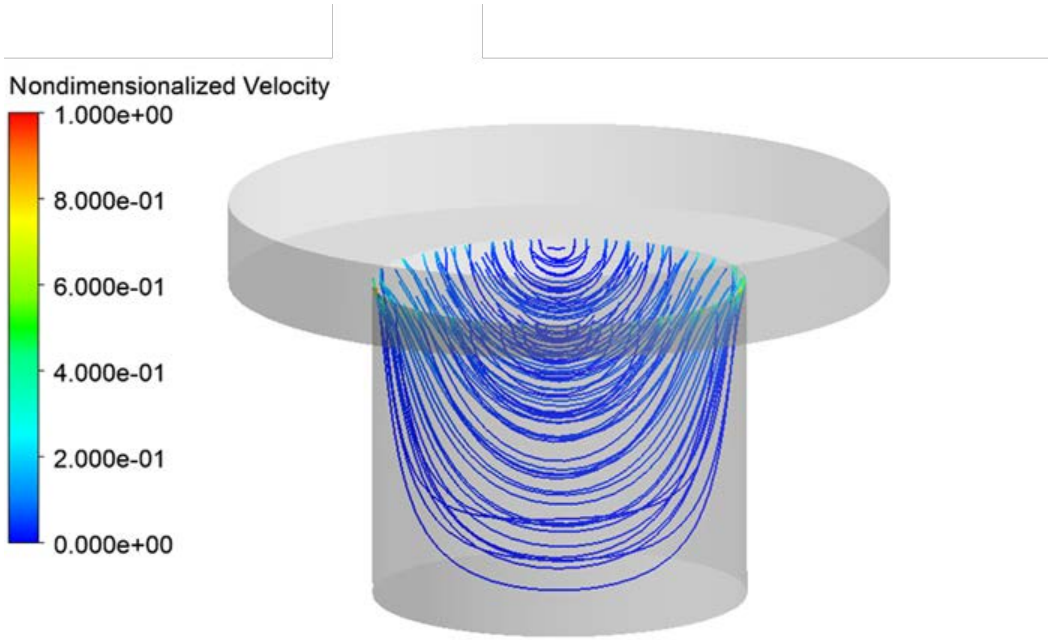


Figure 5.5: Streamlines of Nondimensionalized Velocity for Lower Bioreactor Region

Figure 5.5 shows that the flow leaves the porous region of the bioreactor and moves downward from the outer edge of the bottom cylinder. It can be seen that this flow, albeit at a low magnitude, gradually recirculates in this region and would eventually move upwards in the geometry and be continually pumped through the reactor. Together, these streamline figures provide information regarding the general flow path of the cell media, monocytes, and dendritic cells over the range of Re_{in} examined in these studies.

5.2 Parametric Analysis of Inlet Conditions

It was determined that for the experimental setup the inlet monocyte concentration for all of the trials would be 500,000 cells/mL, which corresponds to an inlet monocyte mass fraction of

0.000883 in the Fluent setup. Additionally, the flow rate used in the final trials was 0.4 mL/min, or a velocity of 0.001844 m/s and Re_{in} of about 5.0 in the Fluent setup. After simulating these experimental conditions with the developed model, a range of inlet conditions were tested to determine the potential outcomes of an experiment under a variety of conditions. The inlet monocyte concentration was varied from 250,000 cells/mL to 1,000,000 cells mL to give an idea of the final concentration of monocytes and dendritic cells expected under those scenarios. In addition to altering the inlet monocyte concentration, Re_{in} was varied from 2.5 to 25 to predict the effect of changing flow rate on the final cell concentrations at the end of an experiment. This parametric analysis of inlet conditions provided key insights into the expected outcomes of an experiment under any combination of the feasible experimental conditions. Thus, the results that are to follow in this section can provide recommendation of optimal experimental conditions, or expected results if a new set of conditions were to be tested.

In order to gain an in-depth understanding of the flow path and fluid flow patterns, streamline figures were generated at reach Re_{in} to determine the degree to which the flow reaches the porous endothelial region, and hence how well the monocytes come into contact with this cell layer. The figures outline the flow path of the bulk fluid flow in the center region of the geometry, and also demonstrate the effect of the porous media region in the reactor. It can be seen that the flow reaches just around the interface in the plate bioreactor geometry that represents the top of the porous endothelial layer. The flow then continues toward the bioreactor wall where the velocity increases through to the outlet of the geometry. In general, the flow tends to go mostly straight through from the inlet to the outlet of the reactor, but some of the cell media does flow lower toward the endothelial region, indicating contact of the monocytes with the endothelial cells.

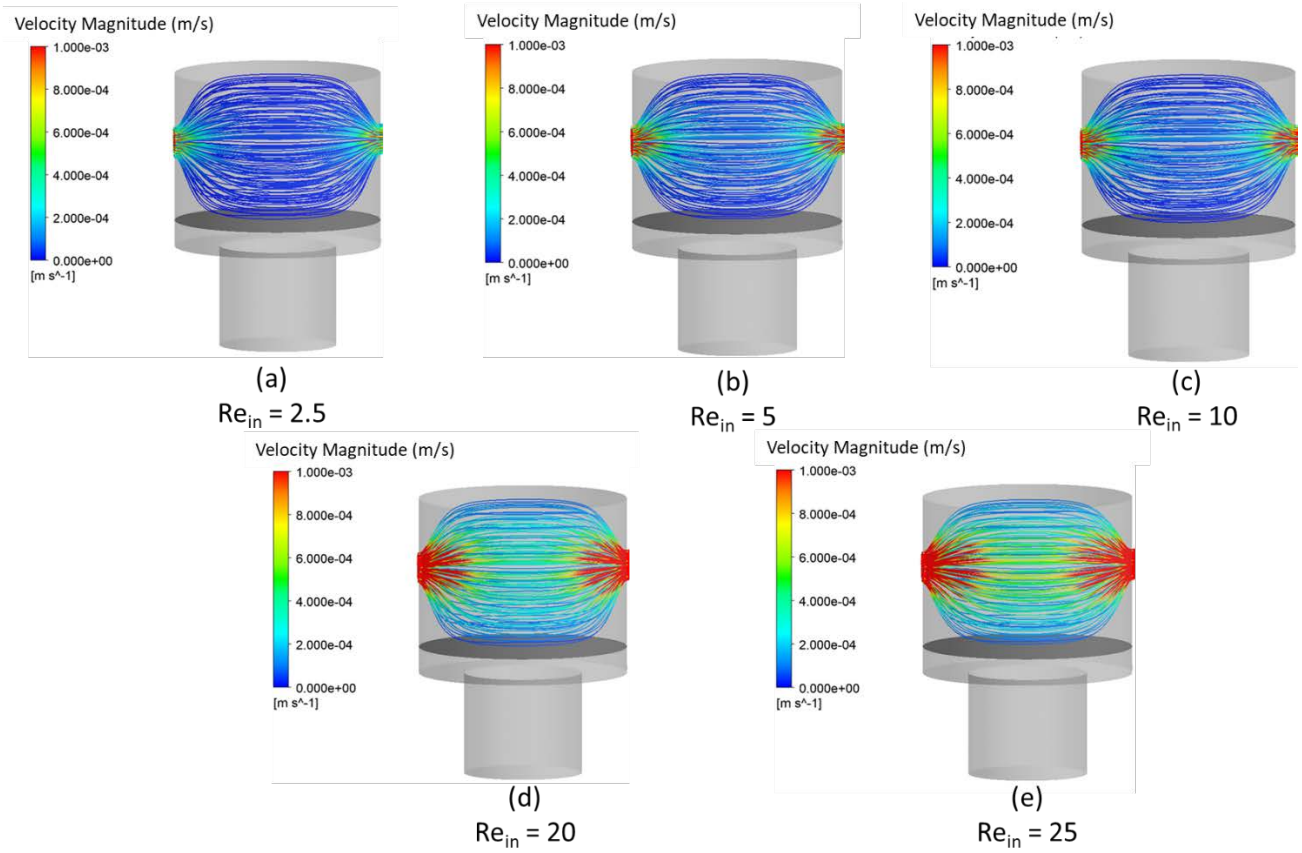


Figure 5.6: Streamlines of Velocity Magnitude through Center Region of Bioreactor at Varying Re_{in}

While the velocity magnitudes and streamlines are helpful to understand the flow path of the cell media in this geometry and areas of high flow, shear rate magnitudes experienced by the monocytes, dendritic cells, and endothelial layer are the biggest factor in the feasibility of using any specific Re_{in}. The figure below shows shear rate contours at the location of the endothelial layer, giving insight into how much the shear rate magnitude and pattern changes in the porous region as Re_{in} is increased.

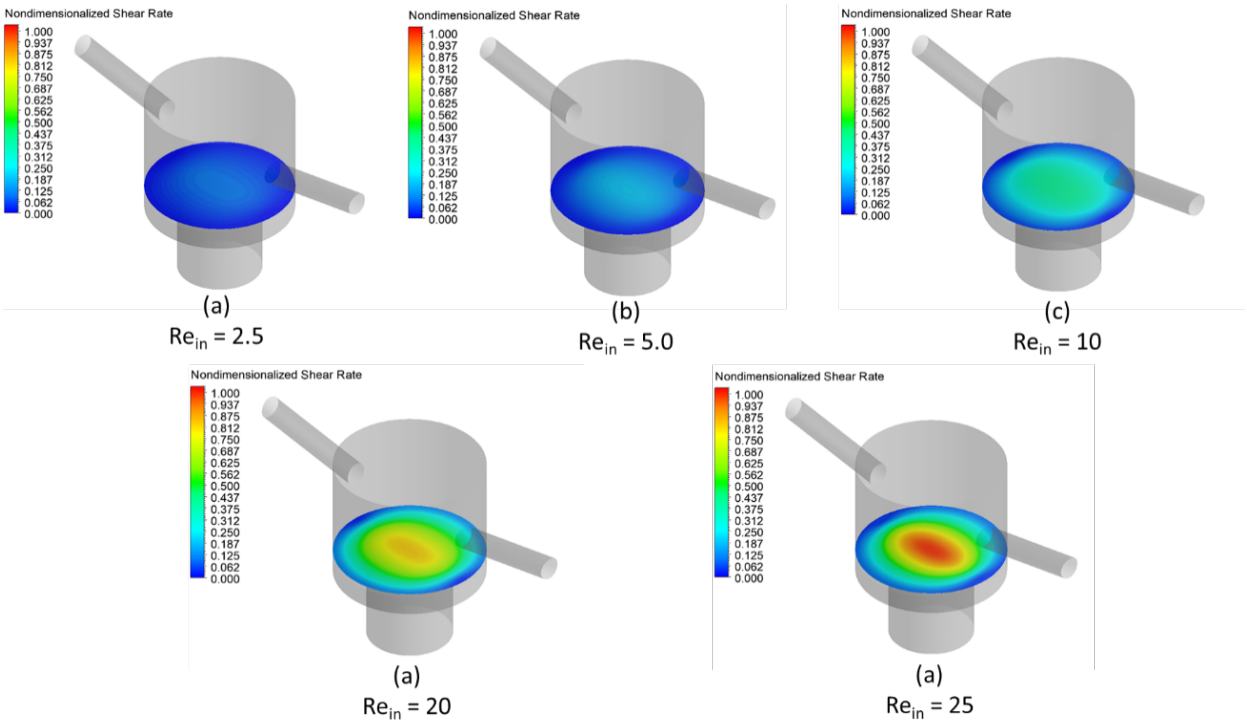


Figure 5.7: Nondimensionalized Shear Rate contour at Endothelial Cell Layer

Figure 5.7 above shows a shear rate contour at each Re_{in} that was simulated. As can be seen in the figure, shear rate magnitudes at the endothelial layer are quite low for a Re_{in} of 2.5-5, while a Re_{in} of 10 sees a larger jump in magnitude and a more uniform distribution of this higher magnitude at the location of the endothelial layer. As Re_{in} increases further, the shear rate magnitude increases as expected, but the magnitude is much less uniform moving across the geometry, although it is symmetric. This shear rate pattern is due to the angled entrance in the geometry, and as flow rate is increases results in a higher magnitude region in the center of the geometry. Overall, the shear rate magnitudes at the endothelial layer are quite low in magnitude, less than 1 s^{-1} for all Re_{in} that were simulated in this study.

As expected, shear rates are much higher at the inlet and entrance to the center region of the bioreactor. Along this region of the geometry, the shear rates do reach higher magnitudes that

could be detrimental to the circulating cells over the range of Re_{in} that were simulated. As was previously mentioned in this study, shear rates should ideally mimic physiological conditions in this system, and in general this would result in magnitudes less than $100s^{-1}$. While the shear rate pattern looks the same for the simulations at the inlet and outlet, once the Re_{in} is increased to 10.0, shear rate at the inlet and outlet begins to reach magnitudes above $100s^{-1}$ in some areas of the bioreactor. Up to a Re_{in} of 25.0, shear rate magnitudes reach close to $300s^{-1}$, which provides some insight into the great increase in monocyte death as flow rate increases.

In addition to the fluid flow pattern analysis that was completed after the simulations were run, figures were generated that predicted both the final monocyte and dendritic cell concentrations in the reactor. Figure 5.8 below shows the predicted final monocyte concentration throughout the reactor after a 48-hour experiment at any given starting concentration or Re_{in}. Cell concentration is plotted as a measure of monocytes/mL. This was calculated by pulling the volume-averaged monocyte concentration from Fluent and converting it to a cells/mL basis.

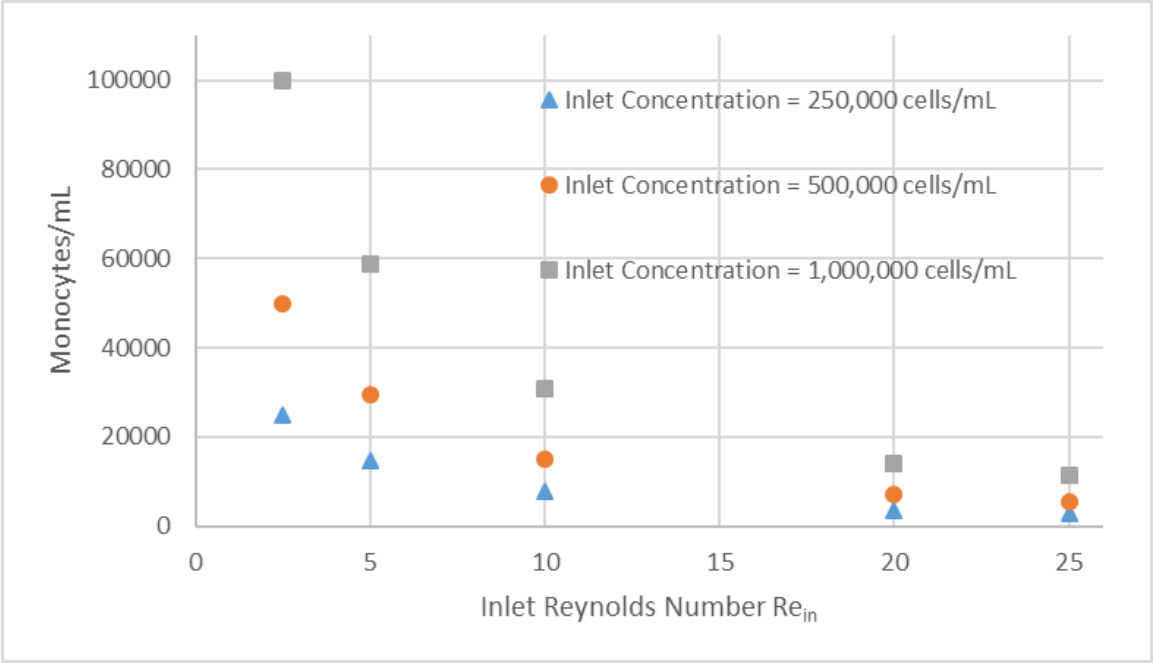


Figure 5.8: Predicted Monocyte Concentration vs. Re_{in} after 48-hour experiment

As the results demonstrate, the predicted final concentration of monocytes sharply declines as Re_{in} increases. Since the death rate is a function of the local shear rate, as Re_{in} increases, so does the local shear rate throughout the reactor, and thus the overall death rate. Results show that at the experimental conditions that were used at a concentration of 500,000 cells/mL and Re_{in} of 5, the final monocyte concentration predicted is about 30,000 cells/mL, or 6% of the original starting concentration. This number continues to decline up to a Re_{in} of 25, where the predicted final concentration is only around 1.1% of the starting monocyte concentration at 500,000 cells/mL.

In addition to the predicted final monocyte concentration, the same procedure was carried out to predict the dendritic cell concentration at the end of a 48-hour experiment. Figure 5.9 below shows the predicted concentration of monocytes that will successfully differentiate into dendritic cells at any given inlet conditions.

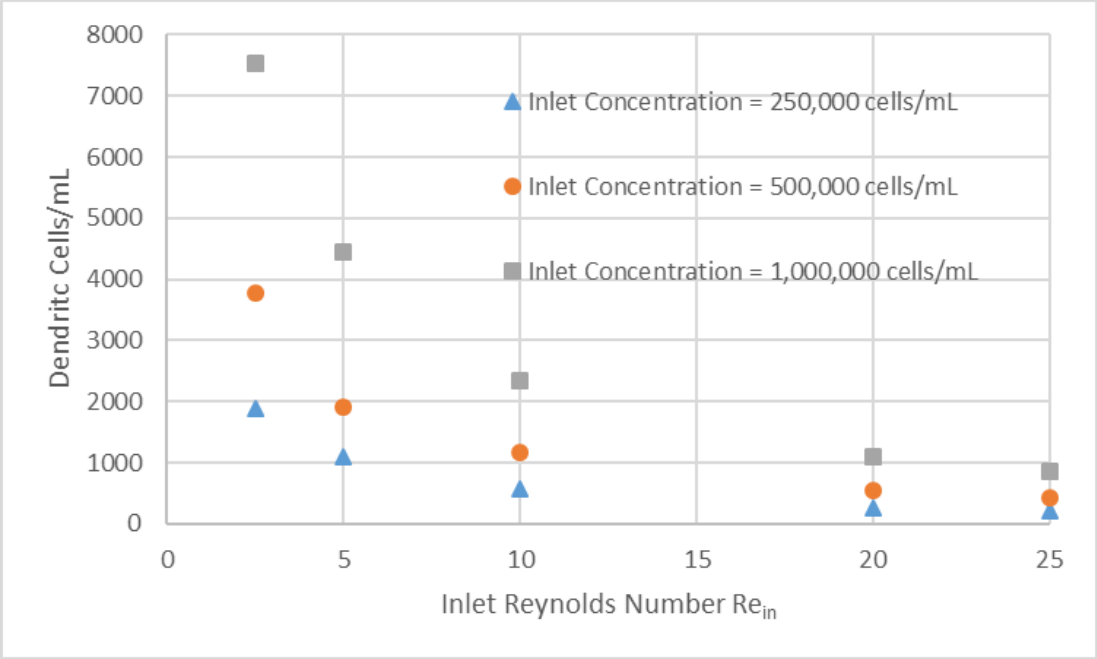


Figure 5.9: Predicted Dendritic Cell Concentration vs. Re_{in} after 48-hour experiment

Since the correlation for monocyte differentiation into dendritic cells is simply a function of the local concentration of monocytes, the profile of dendritic cell concentration (cells/mL) vs. Re_{in} is the same as that of the monocyte concentration (cells/mL) vs. $Re_{in,}$ except that the concentration of these cells is far lower. Essentially, preliminary experiments showed that about 7% of the remaining monocytes differentiate into dendritic cells at the conditions that were utilized in trials.

Together, these figures demonstrate that when deciding on inlet conditions for an experiment that a balance needs to be met between the desired final dendritic cell concentration and the exposure to shear that the cells will experience. The hypothesis of the experimental study, after all, is that the dendritic cells exposed to flow will be more functional than those produced under static conditions. So while a lower flow rate will likely result in more living monocytes at the end of the experiment, and hence more dendritic cells, it may not result in the

functionality of dendritic cells that is desired. Thus, these simulation results can provide information into the expected number of cells that will be produced so that the researcher can choose a set of inlet conditions at their discretion.

Another method of visualizing the monocyte and dendritic cell concentration throughout the reactor after the duration of an experiment is to use a volume rendering of the scalars within CFD-Post. By using the volume rendering feature, this enables us to visualize field variables throughout the entire domain to give the viewer an idea of the distribution of the monocytes and dendritic cells at varying conditions. The figure below shows isometric views of the plate bioreactor at a monocyte inlet condition of 500,000 cells/mL and varying Re_{in} from 2.5 to 25.

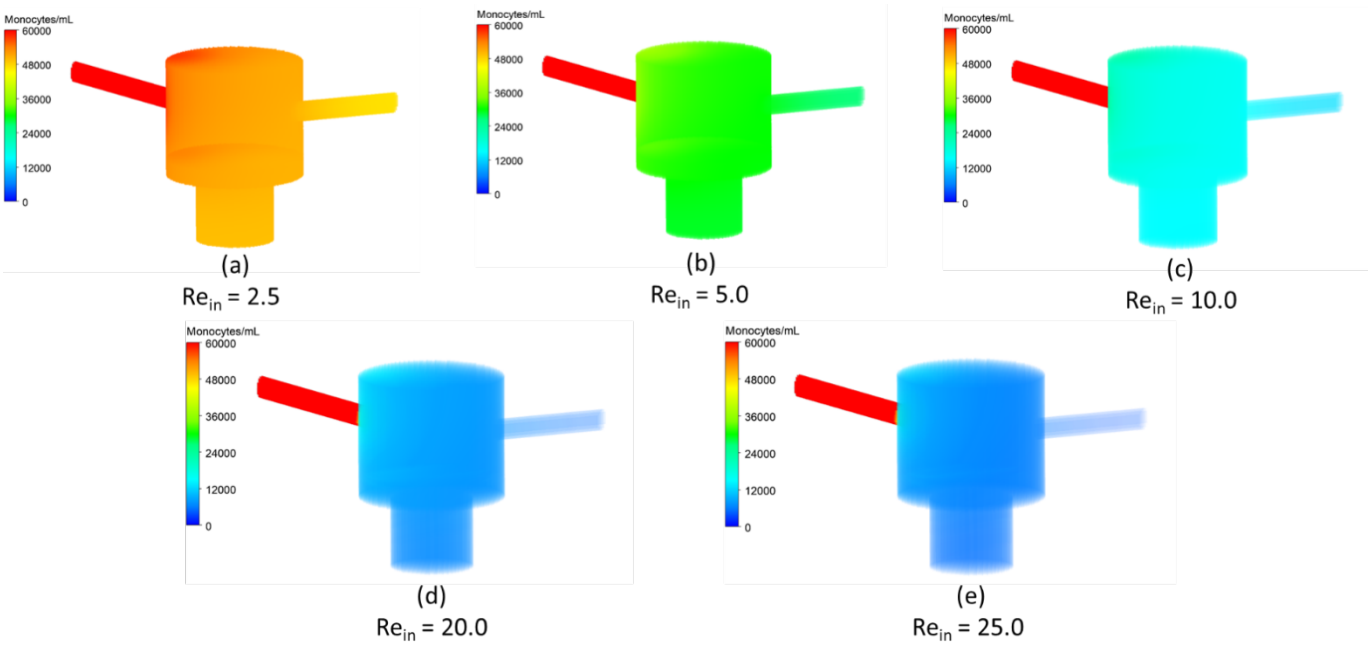


Figure 5.10: Volume Rendering of Monocyte Concentration at inlet monocyte concentration of 500,000 cells/mL after 48 hours

This figure demonstrates that in Figure 5.10(a), the monocyte concentration throughout the bioreactor geometry is the highest, at a Re_{in} of 2.5 and a concentration of approximately

50,000 cells/mL, or about 10% of the inlet concentration throughout much of the reactor. Figure 5.10(b) is a Re_{in} of about 5.0 and inlet monocyte concentration of 500,000 cells/mL, which were the conditions typically carried out in experiments. Results show that at these conditions, the monocyte concentration throughout the reactor is certainly lower than Figure 5.10(a) at about a value of 30,000 cells/mL throughout the bulk of the geometry, but this is the point at which the flow rate begins to have a significant effect on the resulting monocyte mass fraction throughout the geometry. As Re_{in} increases beyond 5.0, the monocyte concentration rapidly decreases in the reactor, and the distribution becomes less uniform. This gives insight into the expected number of monocytes that are available to differentiate into dendritic cells as Re_{in} increases. So while a lower flow rate would leave more living monocytes for differentiation, a flow rate that is too low would result in near static conditions, which would not accomplish the goals of this study.

In addition to the volume rendering of monocyte mass fraction at various Re_{in} conditions, a volume rendering could be generated and provide useful information for the differentiation of monocytes into dendritic cells. Figure 5.11 below shows the volume rendering of dendritic cells in the plate bioreactor at the five different Re_{in} that were studied.

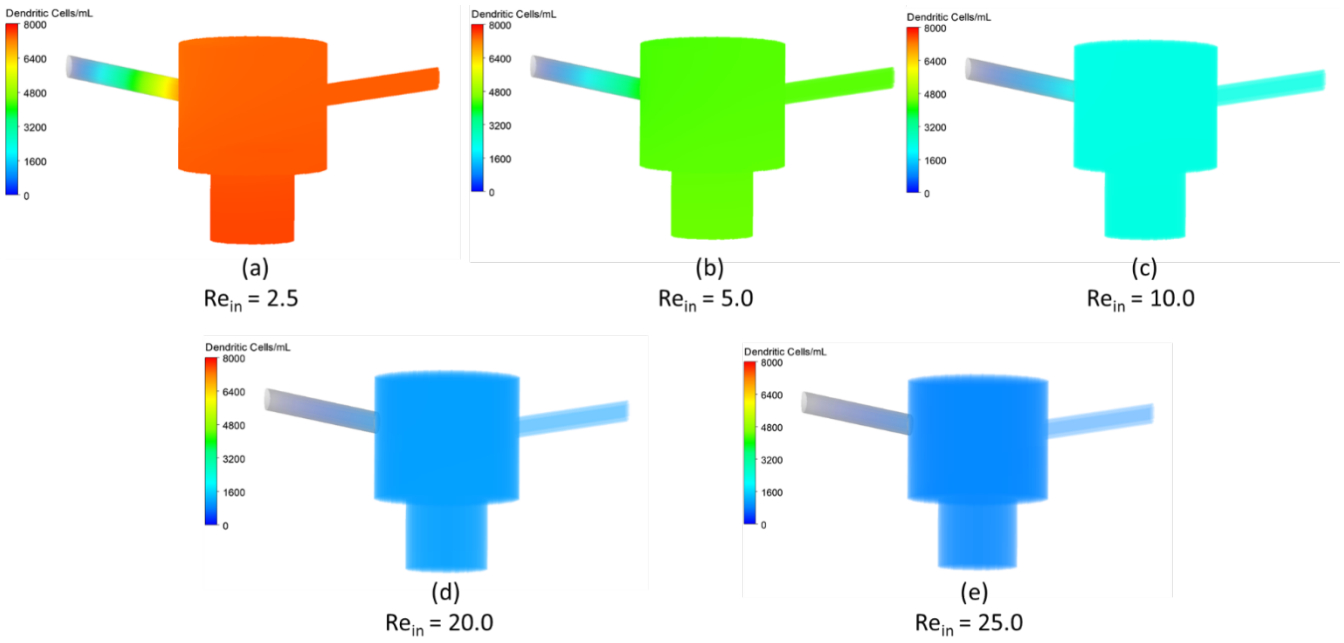


Figure 5.11: Volume Rendering of Dendritic Cell Concentration at inlet monocyte concentration of 1,000,000 cells/mL after 48 hours

These results give an idea as to the predicted concentration and distribution of dendritic cells throughout the bioreactor after a 48-hour simulation. This particular figure utilizes the 5 different Re_{in} values at an inlet concentration of 1,000,000 cells/mL. At the Re_{in} of 5.0, the predicted concentration of dendritic cells throughout the bulk of the reactor is about 4,500 cells/mL. To put in perspective this prediction, the total volume in the plate bioreactor well is about 2.7 mL. Thus, this predicted concentration in the well would give an estimate of approximately 1.04% of the monocytes introduced to the system differentiating into dendritic cells. As flow rate increases beyond 0.4 mL/min, or Re_{in} of 5.0, this number will begin to sharply decrease even further. These results in Figures 5.10 and 5.11 give an idea as to the expected number of monocytes still living after 48 hours, and the expected number of monocytes that have differentiated into dendritic cells along with a predicted distribution throughout the geometry.

All results confirm a decrease in monocyte and dendritic cell concentrations with an increasing flow rate, while even at a low Re_{in} of 2.5, the death rate of monocytes predicted is still about 90%, and dendritic cell production is predicted to be about 1.5% of the monocytes that were originally introduced into the system.

5.3 Summary

Simulations were performed to determine the effects of bioreactor geometry and volumetric flow rate on the monocyte death rate and subsequent differentiation into dendritic cells. Inlet conditions were simulated at monocyte concentrations ranging from 250,000 to 1,000,000 cells/mL, and Re_{in} values ranging from 2.5 to 25. Monocyte concentrations were significantly decreased after a 48-hour simulation for all cases, but it was determined that at a threshold Re_{in} of 5.0, or flow rate of 0.4 mL/min, the monocyte death rate greatly increases, and therefore reduces the number of potential dendritic cells. In addition to the cell concentration that was measured after each simulation, shear rates and general fluid flow patterns were also investigated due to concerns for environmental conditions for the cells. Additionally, since it was desired to emulate physiological flow conditions, a balance needed to be found between the desired shear rate and expected rate of dendritic cell production. It was concluded that at a range of Re_{in} from about 2.5 to around 10, a reasonable number of dendritic cells were expected, and the shear rates produced did not exceed those expected under physiological conditions, indicating that significant damage to the cells would not occur due to the flow. Overall, it was determined that the simulation developed could quickly and precisely predict experimental results at a variety of conditions for the custom-designed parallel plate bioreactor.

CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

A CFD model was developed to investigate the fluid flow patterns, shear rate and velocity distributions, and monocyte migration and differentiation into dendritic cells in a flow bioreactor system under a variety of inlet conditions. Incorporating a CFD model into the experimental studies can precisely predict the fluid behavior throughout the entire volume of the reactor, while also providing predictions as to the final concentration of dendritic cells in the bioreactor. It was determined that for the experimental conditions that were tested, both the monocyte death rate and differentiation correlations predicted the results of experiments to a high degree of accuracy based on the data provided. Ultimately, it was determined that going above a threshold Re_{in} of 5.0 was the point at which the flow rate had a detrimental effect on the viability of the cells. Thus, the recommended experimental Re_{in} would be 5.0 for this geometry. In terms of the inlet cell concentration, results provide predictions of final cell counts over a range of different inlet conditions. Researchers performing the experiments should take into consideration the potential effects of increasing the inlet monocyte concentration when deciding on experimental run conditions moving forward.

6.2 Recommendations

In order to more accurately develop correlations for the monocyte death rate and the differentiation of monocytes into dendritic cells, it would be desirable to have more data at a number of different experimental conditions to develop a more definitive correlation, especially with regard to monocyte differentiation. In terms of the measurement of differentiation of monocytes, only a flow rate of 0.4 mL/min, or Re_{in} of 5.0 was run experimentally to provide data when developing this correlation. By measuring the differentiation of monocytes into dendritic cells over a range of flow rates similar to the method that the monocyte death rate was measured, a correlation could be developed that is more likely to be a good fit for the differentiation over a range of flow rates.

In addition to more comprehensive experimental studies, it would be beneficial to incorporate parameters to better describe the actual biological makeup and behavior of the cells in this study. As it currently stands, the endothelial layer is described as a porous media region with viscous and inertial coefficients and an estimated porosity. When this layer is exposed to an inflammatory stimulus like TNF-α, the properties of this layer would change. Currently, the model assumes the porous region maintains the same properties under all experimental scenarios. Incorporation of the changing cell layer properties as a function of available binding sites is a potential improvement for the model.

The monocytes and dendritic cells are set up as User-Defined Scalars where the inlet mass fraction is specified, the specified flux at boundaries, and their own User-Defined functions to describe cell death and differentiation. However, if information regarding cell receptors, such as mechanisms of receptor-ligand binding, and more definitive

kinetic parameters that accurately express migration or differentiation patterns and mechanisms that may affect these phenomena could be incorporated, then a more realistic model would be developed.

6.2.1 Future Work

In addition to the plate bioreactor that was designed and created for experimental and simulation studies, a tubular geometry was also designed and created for potential experimental studies. Future works should involve an analysis of conditions for this tubular geometry and provide recommendations should the tubular geometry become adopted in future experimental works. Currently, the correlations developed are based on preliminary data that was generated from the plate bioreactor experiments. Thus, future works with the tubular reactor would benefit from performing several preliminary experiments with the new reactor so that geometry specific correlations could be developed.

Lastly, besides for the previously mentioned incorporation of improved kinetic parameters and correlations that include cell specific receptor-ligand binding mechanisms, it would be beneficial to explore the effects of improved plate geometry. Currently, the monocytes have a very large cell death rate in the plate bioreactor, preventing a large number of dendritic cells from being produced, so it may be worthwhile to explore other geometries with varying fluid flow patterns to arrive at an optimal geometry in future flow bioreactor studies. Currently, shear rates are much higher at the inlet and outlet than at the site of the tissue-engineered construct. Additionally, there is some recirculation and non-uniform flow around the site of the endothelial layer

which may affect both the monocytes and the tissue-engineered construct in a negative way. If different plate geometries were explored such that shear rates and flow patterns are more uniform throughout the geometry so that the desired shear could be produced at the location of the endothelial layer without being detrimental to the cells flowing in other regions of the bioreactor, then perhaps more reliable results regarding monocyte migration and differentiation could be achieved.

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APPENDICES

APPENDIX A: User-Defined Functions

Below is the User-Defined Function (UDF) code that was written to describe the death of monocytes as a function of the local shear rate. This correlation was developed from preliminary experimental studies that were carried out with monocytes in circulation at different flow rates. These preliminary experiments were carried out at flow rates of 0, 0.4, and 0.8 mL/min to get a range of data over a likely choice for an experimental flow rate. The change in concentration of monocytes over time was plotted, and using MATLAB's curve fitting tool, the data was fitted to a linear correlation as a function of the inlet Reynold's number. This correlation was then adapted to calculate the death rate as a function of the local shear rate so that the rate would be more accurate throughout the entire geometry.

```
#include "udf.h"
DEFINE_SOURCE(cell_deathrate,c,t,dS,eqn)
{
        real source;
        source = (-.000001296*C_STRAIN_RATE_MAG(c,t)-
0.000001644)*C_UDSI(c,t,0);
        /* derivative of source term with respect to time. */
```

```
    dS[eqn]=(-.000001296*C_STRAIN_RATE_MAG(c,t)-0.000001644);
    return source;
}
```

In addition to the UDF developed for the monocyte death rate, it was necessary to develop a correlation for the differentiation of the still living monocytes into dendritic cells. The data used to develop this correlation was quite limited at only a flow rate of 0.4 mL/min, but the available data was fitted to first-order kinetics with R^2 of about 0.9. The code for the UDF describing monocytes differentiating into dendritic cells is given below, where the source term is a function of UDSI 0, which is the User-Defined Scalar for the monocytes.

```
#include "udf.h"

#define k 0.00002302

DEFINE_SOURCE(cell_differentiation,c,t,dS,eqn)

{

    real source;



    source=k*C_UDSI(c,t,0);

    /* derivative of source term with respect to time. */

    dS[eqn]=k;
```

```
return source;
```

VITA

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