MODELING OF MASS TRANSFER AND FLUID FLOW IN PERUFUSION BIOREACTORS

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ABSTRACT

Tissue engineering is an emerging field with the aim to produce artificial organs and tissues for transplant treatments. Cultivating cells on scaffolds by means of bioreactors is a critical step to forming the organ or tissue substitutes prior to transplantation. Among various bioreactors, the perfusion bioreactor is known for its enhanced convection through the cell-scaffold constructs. The enhanced convection will significantly increase the mass transport and at the same time, will increase the shear stress acting on the cells and scaffolds. To manipulate the scaffold-based cell culture process, knowledge of the mass transport and fluid flow (featured by flow velocity and shear stress) in bioreactors is required. Due to the complicated microstructure and multiphase flow involved in this process, the development of models for capturing the aforementioned knowledge has proven to be a challenging task. In this research, the mass transport and fluid flow in scaffolds cultivated in perfusion bioreactors was studied using numerical methods. In the first stream of this research, a novel mathematical model was developed to represent the nutrient transport and cell growth within three-dimensional scaffolds. Based on the developed model, the effect of such factors as the scaffold porosity, the culture time, and the flow rate were investigated. In the second stream, the flow field within the scaffold was studied with an emphasis on representing the shear stress distribution over the scaffold surface. The commercial computational fluid dynamics software ANSYS-CFX was used to simulate and represent the effect of factors, such as the diameter of the scaffold strand, the horizontal span between two strands, and the flow rate, on the shear

stress distribution. Results showed that the nutrient concentration and cell volume fraction are time dependent and sensitive to the porosity and flow rate. The diameters of the strands, the horizontal span and the flow rate affect the magnitude of the shear stress. The knowledge obtained in this study provides new insight into the scaffold-based cell culture process in perfusion bioreactors and allows for potential optimization of the cell culture process by regulating the process parameters as well as the scaffold structure during its fabrication.

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CHAPTER 1

INTRODUCTION

1.1 Background

1.1.1 Tissue engineering

Tissue engineering is an interdisciplinary field that applies the principles of engineering and the life sciences to provide a new solution to tissue loss, replacement or restoration of tissue, or organ function with scaffold constructs that contain specific populations of living cells [1]. Fig.1 shows the five steps typically involved in the process of tissue engineering, which include:

1) Isolating: cells are isolated from a living animal or obtained through human donation;

2) Expanding: the isolated cells are expanded in the laboratory to have a sufficient number of cells for applications;

3) Seeding: the cells are seeded into a three-dimensional (3D) polymeric scaffold;

4) Culturing: cells are cultured in the scaffold using bioreactors or incubators. During this process, growth factors, enzymes, nutrients and/or mechanical stimulation may be added to the cultivating culture to increase cell growth, thus forming constructs with required biological functions; and

5) Implanting: the formed constructs are implanted into the *in vivo* environment, such as an animal or human body, to repair or replace the damaged/diseased tissues or organs [1, 2].

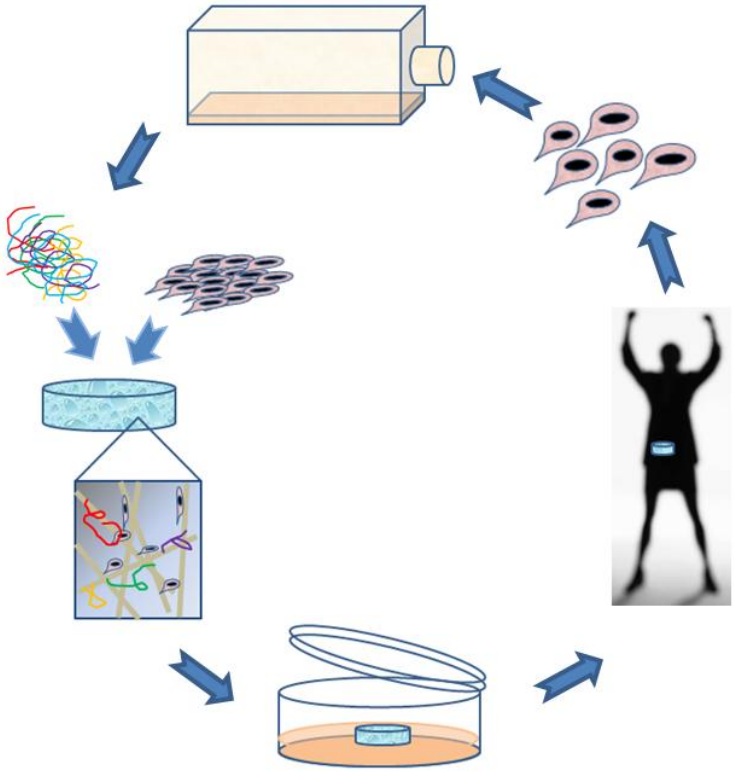

Fig. 1 Major steps involved in tissue engineering.

Obviously, the cell culturing process is a key step in the tissue engineering cycle. For its success, scaffolds are of great importance and must possess the properties as detailed in the following section.

1.1.2 Scaffold and perfusion bioreactor

In tissue engineering, scaffolds play a critical role in supporting cell growth and

differentiation, cell migration, and eventually the formation of tissue constructs [3]. Generally speaking, tissue scaffolds should meet the following requirements [2]:

1) the scaffold material must be biocompatible and biodegradable to match cell/tissue growth *in vitro* and *in vivo*;

2) the scaffold surface must be suitable for cell attachment, proliferation and differentiation;

3) the mechanical properties of the scaffolds should match the tissue at the proposed implant site; and

4) the scaffold should be highly porous to allow cell growth and movement as well as the transport of nutrients and metabolic waste.

Typically, tissue scaffolds are made from either natural or synthetic material, such as chitosan, collagen, polyglycolic acid (PLA) and polycaprolactone(PCL). The scaffolds can be fabricated by different methods and as a result, many exhibit diverse inner structures. The following are common methods presently used in the scaffold fabrication:

(i) Porogen leaching: a polymer solution with dispersed templates, such as particles, is gelled or fixed; and then the templates are removed, forming a scaffold with a porous structure [4];

(ii) Phase separation: a polymer solution can separate into two phases, the polymer-rich phase and polymer-lean phase, with the variation of thermal conditions due to the lower system energy in a thermodynamically unstable state. For example, cooling can result in phase separation of a polymer solution into high and low concentration

regions. The high concentration region (the polymer-rich phase) solidifies, while the low concentration region (the polymer-lean phase) forms the pores [1, 4];

(iii) Gas foaming: A gas, such as CO_2, can be pressurized in a molded polymer and then the pressure released, resulting in the nucleation and growth of air bubbles within the polymer [5];

(iv) Textile technology: the methods originally developed in the textile industry can be used in the fabrication of scaffolds, with a textile structure for tissue engineering applications [1];

(v) Solid free-form fabrication (SFF) and rapid prototyping (RP): in this method, the scaffolds are built through selectively adding materials layer-by-layer as controlled by computers [6].

Among the above, the fabrication methods (i)-(iv) are referred as to conventional techniques in the literature and have been widely used to fabricate scaffolds with different porosities (a ratio of the void space to the entire volume of the scaffold) and pore sizes for various tissue engineering applications. Common features of these methods include that the inner pores of the scaffold are randomly distributed and that, by regulating the fabrication conditions, such as the temperature, pressure, etc., the level of porosity and range of pore size can be controlled. Nonetheless, the local porosity and pore geometry cannot be controlled accurately. Thus, the scaffolds fabricated with conventional methods have irregular internal structures (Fig. 2 (a)). In contrast to conventional methods, the SFF and RP methods can produce scaffolds with a regular inner structure (Fig. 2 (b)). With the

help of computers, the scaffold parameters can be adjusted and controlled readily and accurately. As such, the SFF and RP method has shown great promising in tissue engineering.

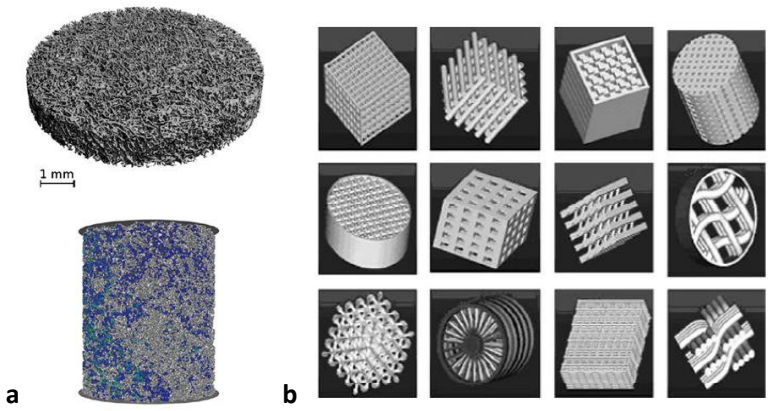

Fig. 2 Scaffolds with: a) irregular inner structure [7, 8], and b) regular inner structure [9].

The bioreactor plays a significant role in the *in vitro* experiments in tissue engineering. Bioreactors are generally defined as devices in which biological and biochemical processes develop under closely monitored and tightly controlled environmental and operating conditions. By using bioreactors, engineered tissue and cells can obtain adequate nutrient supply, timely waste removal, sufficient gaseous exchange, temperature regulation and mechanical simulation [3]. The main functions of bioreactors include improving cell seeding in a scaffold, increasing mass transfer during the cultivation process, providing mechanical stimuli, and eventually promoting the formation of tissues or organs [3, 10, 11]. Compared with steady-state cultivation, bioreactors can provide a dynamic environment to

stimulate cells and enhance proliferation and matrix secretion.

There are different types of bioreactors in terms of configuration or the mechanical stimuli methods employed, which include:

(i) Spinner flask: Cells are seeded on the scaffolds that are placed on the side arms and during culture, the stir bar at the bottom stirs the flow to enhance mass transfer (Fig.3 (a));

(ii) Rotating wall vessels: Rotation provides a dynamic culture environment for constructs with low shear stresses and high mass-transfer rates (Fig.3 (b));

(iii) Holly-fibre systems: Mass transfer during the culture is enhanced by the systems for highly metabolic and sensitive cell types, such as hepatocytes (Fig. 3 (c));

(iv) Perfusion bioreactors: The medium flow is forced directly through the pores of the scaffold. As a result, the enhanced mass transfer occurs both at the periphery and within the internal pores of the scaffold, so that relatively uniform mass transfer occurs (Fig. 3 (d));

(v) Compression-loading systems: Controlled mechanical forces are applied to engineered constructs to simulate physiological loading conditions (Fig.3 (e));

(vi) Concentric cylinder bioreactors: Two concentric cylinders, an outer one and an inner one, move relative to each other in order to enhance mixing (Fig. 3 (f)) [3, 12-14];

(vii) Non-perfusion bioreactors: Although there is no flow passing directly through the scaffold, the scaffold is immersed into an environment with uninterrupted nutrient

replenishment and waste removal (Fig. 3 (g)).

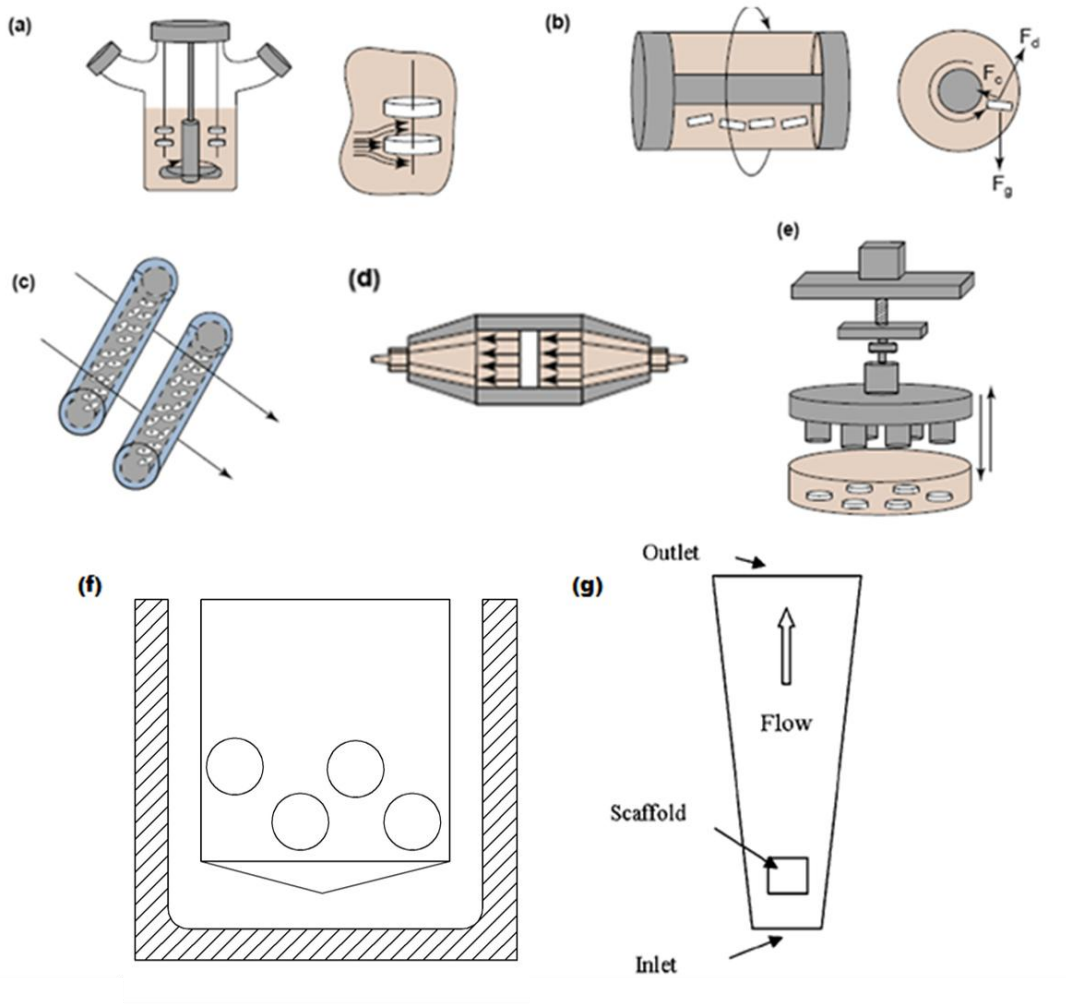

Fig. 3 Bioreactors of different configurations: a) spinner flask [3], b) rotating wall vessel [3], c) holly-fibre system [3], d) perfusion bioreactor [3], e) compression-loading system [3], f) concentric cylinder bioreactor, and g) non-perfusion bioreactor[15].

Among the above bioreactors, the perfusion bioreactor has been widely used in tissue engineering since it allows the culture medium to flow directly through the scaffold pores,

as seen in Fig. 4. As a result, a perfusion bioreactor can enhance mass transfer not only around the scaffold construct periphery as do some other kinds of bioreactors, but also within the internal pores [16]. However, due to the medium flow within the scaffolds, the shear stress level can be elevated in a perfusion bioreactor.

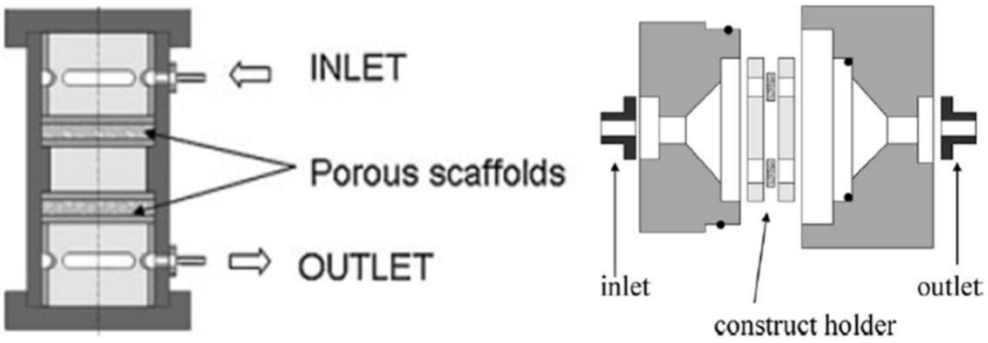

Fig.4 Perfusion bioreactors: (a) fluid flow in the vertical direction [16], and (b) fluid flow in the horizontal direction [17].

For *in vitro* cell culture, the nutrients including glucose and oxygen which are necessary for cell metabolism and proliferation must be supplied. If the supply of nutrients is inadequate [3] , then a hypoxic, necrotic center surrounded by a rim of viable cells may be formed, which was exemplified by R.M. Sutherlan [18]. Similar observations have been made for the cell culture in 3D scaffolds under static conditions [19, 20]. Besides, waste products, such as lactate may also accumulate within scaffolds, which can suppress cell growth and eventually cause non-uniform cell distributions in scaffolds [41]. Therefore, mass transfer must be adequate to provide sufficient nutrient

and eliminate waste materials within scaffolds in cell culture *in vitro*.

Enhancing nutrient transport in scaffolds is the most attractive feature of the perfusion bioreactor. For optimal control of the cell culture process in a perfusion bioreactor, knowledge of nutrient transport and the corresponding cell response in scaffolds is desired. Unfortunately, due to the complicated scaffold structure and the different phases or components (i.e., solid, fluid, and gas) involved in the cell culture process, capturing such knowledge becomes difficult. Nowadays, research based on numerical methods, in addition to experimental methods, has shown promise in providing knowledge on nutrient transport within the scaffolds.

Besides the enhanced nutrient transfer, the other unique characteristic of perfusion bioreactors is the increase in shear stress on the cells and scaffolds due to the perfused flow through the scaffold pores. Depending on applications, the shear stress might have either positive or negative effects on the cell culture process. Previous studies [19, 21] show a moderate shear stress is essential to cell growth and metabolism since it can help shape the engineered tissue and glycosaminoglycan (GAG) in cartilage tissue engineering. In contrast, if the shear stress exceeds the physiological range, negative effects may become apparent, causing the decrease in matrix synthesis, damaging cell structure or even killing the cells [22]. Negative cell response to the flow-induced shear stress may also include the changes in cell shape and alignment at the earlier stage. With the increase in the magnitude of shear stress and exposure time, cell metabolism varies and the cell viability become an issue in the cell culture process [15, 22, 23]. Another negative effect

of higher shear stress is that cells may not be able to adhere on the surfaces of the scaffolds and, instead, be washed away from the scaffold. As such, to ensure the shear stress is at an appropriate level for a given application, knowledge of mass transfer and shear stress in cell culture process in perfusion bioreactors is desired. The capture of such knowledge is the focus of the present study.

1.2 Literature review

In this section, a literature review is presented on the following aspects of this field of study: experimental methods versus computational methods, and the mass transfer and fluid flow within scaffolds cultivated in bioreactors.

1.2.1 Experimental methods and computational methods

In order to study mass transfer and fluid flow in scaffolds cultured in perfusion bioreactors, both experimental and computational methods have been used in the past.

For mass transfer in 3D tissue scaffolds, source insight comes from cell culture experiments. In several experimental studies [19, 21, 24-26], cells were seeded in scaffolds and then cultured in bioreactors; after a period of time, changes in the cell density and metabolism component concentration, such as the glycosaminoglycan (GAG), in the tissue scaffold were examined. To investigate fluid flow in a bioreactor, particle image velocimetry (PIV) and laser-doppler velocimetry (LDV) have been used to measure

the fluid velocity in bioreactors [27-30]. For the flow field within the scaffold, micro particle image velocimetry (μ-PIV) has been applied to capture the flow characteristics in the inner pores [31].

Computational fluid dynamics (CFD) is a method to solve the equations governing the fluid flow based on numerical methods. It has proven to be a powerful tool for studying the fluid flow in such diverse areas as aerospace engineering, chemical engineering, and civil engineering [32]. In tissue engineering, the application of CFD has shown promise for studying the flow phenomena in bioreactors, thus providing detailed information and insight that is difficult, even impossible, to obtain through experiments. CFD is reviewed in the following sections in terms of mass transfer and fluid flow in cell culture applications.

1.2.2 Mass transfer in a scaffold

Cell culture *in vitro* is a key process for tissue engineering, in which the cells are expanded on the scaffolds to form the constructs, for implantation to the animal or human patients. For success, nutrients and growth factors have to be provided adequately to the cells. Researchers have applied different methods to study the cell culture process in an attempt to understand and characterize the process qualitatively and quantitatively. Experimental methods are perhaps most widely used to investigate the mechanism of cell culture within the scaffold in bioreactor. Freed *et. al.* [33-35] developed empirical

equations based on the cell culture in Petri dish and bioreactors. Experimental data, such as cell density and composition are the most reliable for evaluating the cell culture process. However, the experimental results are of limited value when experimental parameters and conditions are changed.

Modeling the mass transfer in a tissue scaffold is a challenging task due to the complicated scaffold microstructure. Local volume average theory (LVA), which only considers the average properties of each representative element volume instead of the specific distribution in each phase, provides one approach to meet this challenge. Based on LVA, Galban and Locke developed a model to represent the distribution of glucose and cell density [36, 37]. With a focus on the effective diffusivities for biofilms and tissues, Wood *et. al.* also used the volume average principle to predict the effective diffusivity of a cellular system [38, 39].

As a multiphase porous medium, a tissue scaffold includes a solid phase which is the scaffold frame, a liquid phase which is the nutrient solution, and a gas phase which is necessary for cell metabolism, such as oxygen. In contrast to other porous media, the tissue scaffold includes a special phase, i.e., the cell phase, so modeling mass transfer in the tissue scaffold becomes more challenging. Lemon and King [40] developed a multiphase model to describe the growth tissue comprising motile cells and water in a solid frame. A limitation of this model is the neglect of the gas phase. Tristan *et al.* [41] considered the effect of the cell density on the effective oxygen diffusivity in their model.

Yu et al. developed a mathematical model to represent fluid flow and oxygen

transport in a micro-bioreactor [42]. They also used an improved model to examine the influence of cell density and relate it to the permeability [43]. However, the cell responses to the transport of the nutrients, including cell growth, cell migration and cell apoptosis, were not considered in their study.

Cell response is another challenge for modeling. This is due to the complexity of biological processes in the tissue scaffold. Chung *et al.* [44] developed a mathematical model to describe cell growth in a porous scaffold considering cell mortality, cell growth rate and cell nutrient consumption rate. However, in their research, the scaffold was assumed to be cultivated in a steady-state environment, which only involved diffusion mass transfer without the consideration of convection. The same research group made an effort to improve the numerical models to give more realistic description of cell culture process in the scaffolds. The highlights of their research are: including convection transfer in the model, treating extracellular matrix (ECM) and cells separately, and considering the influence of chemotaxis [45-47].

A major drawback of these studies is that the porosity of the scaffold is assumed to be higher than 95% so that the solid frame of the scaffold can be ignored. This is further limited by ignoring the scaffold degradation. For improvement, Coletti *et. al.* developed a mathematical model for the cell culture in a three-dimensional perfusion bioreactor including the solid phase. However, their work is still limited by the fact that the effect of the initial porosity and the degradation of the solid frame is not included [48]. It is known that one major function of scaffolds is to provide mechanical support for cell attachment

and growth. A higher porosity (>90%) may provide a greater pore volume for cell infiltration and extracellular matrix formation, but conversely it decreases the mechanical properties [49]. As such, scaffolds designed with a higher porosity may not be appropriate for some tissue engineering applications. As such, the porosity and degradation of the scaffold need to be considered in the mathematical models developed for the cell culture process in a bioreactor.

In the present research, the existence of the solid phase and its degradation will be included in the model development with a focus on the effect of porosity on the mass transfer. Also, the environment is considered to be a multiphase one which includes the response to both glucose and oxygen. The detailed modeling process of mass transfer in the scaffold in a perfusion bioreactor is discussed in a paper documented in Chapter 2 In this paper, the effects of porosity, cell culture time and flow rate are considered.

1.2.3 Fluid flow in bioreactors

With enhanced mass transfer in a perfusion bioreactor due to convection, increased shear stress levels can exist on the surfaces of the scaffold. Due to the tiny size and the complicated internal structure of the tissue scaffold, it is difficult and expensive to use the sensors to measure the surface shear stress on a scaffold strand. However, this knowledge of shear stress distribution is crucial for researchers and engineers because it is used to identify the shear stress on the cells which can significantly impacts cell distribution and

metabolism.

Originally, scaffolds were treated as impermeable constructs in a development of CFD models. Based on simulation results, an improved design of the bioreactor and scaffold construct was reported by assuming that the shape of the pore is sphere [29, 50-52]. The drawback of these studies is that while the shear stress on the external surfaces is represented, there is no description of the shear stress at the surfaces of the pores inside the scaffold where the cells are actually attached.

In the following studies, the structure inside the scaffold was taken into consideration. For the irregular scaffolds fabricated by conventional fabrication methods, micro-computed tomography (μCT) was used to reconstruct a 3-D model from 2-D images in the model development [16, 17, 53-55]. However, because of the random internal structure, the models established in this way can only describe the shear stress magnitude and distribution on the specific areas where the 2-D images are taken. Moreover, for a different scaffold, a new model has to be established through the use of μCT reconstruction.

Another strategy to deal with the irregular internal structure is to limit the study to the specific pores in the scaffold and in this way, the irregular pores can be treated as the regular ones [56, 57]. Computer aided design methods can be used to establish geometric models of these specific pores, referred as to as the region of interest (ROI). To avoid imposing boundary conditions directly on the ROI, Boschetti *et al.* [56-58] included the neighbor cells of the ROI in their research and developed a model to study flow inside a

scaffold in a perfusion system. For such models, details of the shear stress magnitude and distribution can be captured with the use of defined numerical meshes. However, the accuracy of the simulation becomes questionable due to the following two reasons. Firstly, the model geometry itself is an approximation of the realistic scaffold pores which appeared randomly in fabrication process. Secondly, applying the boundary conditions on the neighboring cells is an acceptable approach for the unit in the center of the scaffolds. However, it is not accurate for the pores near the surfaces or for the scaffolds within which the flow field varies significantly. An example of the latter situation is a scaffold which has a small scale in one dimension, where the boundary conditions for cells in different locations vary dramatically. As a result, this method may not be reliable.

For the scaffolds with a regular internal structure, the models can be developed by means of the computer aided design method. Singh *et al.* [59, 60] utilized commercial CFD software to create models of such scaffolds in bioreactors and studied the influence of mechanical stimuli on the velocity and shear stress distribution. Unfortunately, their studies are limited to non-perfusion bioreactors.

From the discussion above, it can be seen that models representative of the complete scaffolds are needed to study the fluid flow and shear stress distribution. This is of particularly significance for regular scaffolds fabricated by means of SFF RP fabrication techniques.

1.3 Objectives

The aim of this research work is to carry out a comprehensive study on the scaffold-based cell culture process in perfusion bioreactors using CFD. The two research objectives to be achieved in this research are presented below, along with the methods used.

The first objective is to develop a model to represent the mass transfer process in the tissue scaffolds in perfusion bioreactors. For this, a mass transfer model will be developed by taking into account scaffold degradation and cell response to both glucose and oxygen. Based on the developed models, the effect of porosity, culture time and flow rate on the mass transfer will be studied and examined.

The second objective is to develop a CFD model to represent the fluid flow through the scaffolds in perfusion bioreactors and to provide quantitative information of the velocity and shear stress distribution within the scaffold. By taking advantage of commercial software, simulations are to be carried out to determine the shear stress distribution over the scaffold surfaces. The effect of parameters which can be controlled in the scaffold fabrication process and cell culture process, such as the diameter of the strand, the horizontal span between the two strands and the flow rate, are also to be investigated.

1.4 Thesis organizations

In this thesis, the study of mass transport and fluid flow in tissue scaffold in perfusion bioreactors is carried out using numerical methods. The layout of the thesis consists of

four chapters that include two journal manuscripts. The present chapter introduces the research background, literature review and objectives. Chapters two and three contain the two journal manuscripts that address the two objectives of the thesis as follows. Chapter two presents the model development for mass transfer in tissue scaffolds cultured in bioreactors. Chapter three presents a numerical study on the flow field and shear stress within the scaffold cultured in perfusion bioreactor based on commercial software. Chapter four presents the conclusions that are drawn from the present study and a discussion of future work. An explanation of some of the technical terms used in tissue engineering is presented in Appendix A.

The journal manuscripts included in Chapter 2 and Chapter 3 are co-authored by Xin Yan, Prof. Bergstrom and Prof. Chen. All of the research work documented in the manuscripts was performed by Xin Yan with Prof. Bergstrom and Prof. Chen providing some technical guidance and advice. The first draft of each manuscript was also written by Xin Yan.

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CHAPTER 2

MODELING OF CELL CULTURES IN PERFUSION BIOREACTORS

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Contribution of this Chapter to the Thesis

The research work presented in this chapter aims at achieving the first objective of the thesis. More specifically, the chapter addresses the model development for mass transfer within the scaffold in a perfusion bioreactor. The effect of porosity and flow rate are investigated.

Modeling of Cell Cultures in Perfusion Bioreactors

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ABSTRACT

Cultivating cells and tissue in bioreactors is a critical step to forming artificial organs or tissues prior to transplantation. Among various bioreactors, the perfusion bioreactor is known for its enhanced convection through the cell-scaffold constructs. Due to the intrinsic complexity of biological systems, knowledge of the mass transfer process is required for better moderating cell culture in vitro. In this research, a novel mathematical model is developed to describe nutrient transport and cell growth in a three-dimensional scaffold cultivated in a perfusion bioreactor. Numerical methods are employed to solve the model equations, with a focus on identifying the effect on cell cultures of such factors as porosity, culturing time, and flow rate, which are controllable in the scaffold fabrication and culturing process. To validate the new model, the results from the model simulations were compared to experimental data reported in the literature. With the validated model, further simulations were carried out to investigate the glucose and oxygen distributions and the cell growth within the cell-scaffold construct in a perfusion bioreactor, thus providing additional insight into the cell culture process.

Keywords: Mathematical model, Perfusion, Mass transfer, Convection

NOMENCLATURE

$\langle C_g \rangle^c$	average glucose concentration in cell phase, kg/m^3
$\langle C_g \rangle^f$	average glucose concentration in fluid phase, kg/m^3
$\langle C_o \rangle$	average oxygen concentration in fluid phase, mol/m^3
D_c	molecular diffusivity of glucose in cell phase, m^2/s
D_{cell}	cell diffusivity (random walk coefficient), m^2/s
$D_{effcell}$	effective cell diffusivity, m^2/s
D_f	molecular diffusivity of glucose in fluid phase, m^2/s
D_{geff}	effective glucose diffusivity in the tissue scaffold, m^2/s
D_{geffm}	effective diffusivity of glucose in the fluid and cell phase, m^2/s
D_o	molecular diffusivity of oxygen, m^2/s
D_{oeff}	effective diffusivity of the oxygen in the tissue scaffold, m^2/s
D_{oeffm}	effective diffusivity of oxygen in the fluid and cell phases, m^2/s
d_d	diameter of the inlet, m
K_{eq}	equilibrium coefficient
K_{gm}	saturation coefficient of glucose, kg/m^3
K_{om}	saturation coefficient of oxygen, mol/m
R_d	cell death rate, 1/s
R_g	cell growth rate, 1/s

R_{gm}	maximum glucose metabolic rate, kg/(m^3·s)
R_{om}	maximum oxygen metabolic rate, mol/(m^3 s)
u_D	Darcy velocity, m/s
V_c	cell phase volume, m^3
V_f	fluid phase volume, m^3
$\langle v \rangle_f$	average medium velocity, m/s

Greek Symbols

ε_c	cell volume fraction
ε_f	fluid volume fraction
ε_o	initial porosity
μ_{max}	maximum cell growth rate, 1/s
ρ_{cell}	single cell mass density, kg/m^3
σ	degradation rate, s
τ	tortuosity of the scaffold

2.1 Introduction

Tissue engineering is an interdisciplinary field that applies the principles of engineering and life sciences to provide new solutions to tissue loss, replacement or restoration of tissue, or organ function with scaffold constructs that contain specific

populations of living cells [1]. By providing a favorable environment with controlled mechanical and chemical stimuli, bioreactors play an important role in the in vitro experiments of cell-based tissue engineering. The perfusion bioreactor, in which the culture medium is controlled to flow through the pores of the scaffold, is superior compared to other non-perfusion or static bioreactors by enhancing mass transfer within the scaffold. As such, the scaffold in the perfusion bioreactor can obtain adequate nutrient supply, timely waste removal, and sufficient gaseous exchange, thus promoting the cell growth and proliferation on the scaffold.

To properly design the cell culture process, it is of paramount importance to obtain knowledge of flow and transport phenomena in bioreactors. While experiments have shown the advantages of perfusion bioreactors in improving cell seeding and increasing nutrient transfer [2-4], mathematical modeling of the cell culture process has proven promising to quantitatively describe the complex chemical, mechanical and biological mechanisms behind the improvement and at the same time to cast light on further experimental design.

In a bioreactor, the cell-scaffold construct is exposed to a multiphase environment which involves a solid phase which is the scaffold frame, a liquid phase which is the nutrient solution and a gas phase - such as oxygen - which is necessary for cell metabolism. Distinct from other porous media, the tissue scaffold includes one more phase, i.e., the cell phase. As such multiphase models are required to represent the cell culture process in bioreactors [5].

Development of models to represent the mass transport in the scaffolds in bioreactors has attracted the attention of numerous researchers. Yu *et al*. developed a mathematical model to describe fluid dynamics and oxygen transport in a micro-bioreactor using the finite volume method [6]. However, the cell response was not considered in their study. They also used an improved model to examine the influence of cell density and relate it to the permeability [7]. Cell response to mass transport and distribution of cells is another important phenomenon in a tissue scaffold. It is known that cell growth and distribution can be affected by the supply of glucose and oxygen, cell density, pH values, etc. Due to the complexity involved, modeling the cell response has proven to be a significant challenge. Chung et al. [8] developed a mathematical model to describe cell growth in a porous scaffold considering cell mortality, cell growth rate and cell consumption rate of nutrients. On this basis, further studies have been carried out to develop a more comprehensive transport model by considering convection transfer, treating extracellular matrix (ECM) and cells separately, and including the influence of chemotaxis [9-11]. A major drawback of these studies is that the porosity of the scaffold is assumed to be higher than 95%，and the existence of the scaffold is ignored as well as the degradation of the scaffold. It is known that one major function of scaffolds is to provide mechanical support for cell attachment and growth. A high porosity (>90%) may provide a greater pore volume for cell infiltration and extracellular matrix formation, but may conversely decrease the mechanical properties [12]. As such, scaffolds designed with a higher porosity may not be appropriate for some tissue engineering applications. Hence, the

actual porosity and degradation of the scaffold need to be considered in the mathematical models developed for the cell culture process in a bioreactor. From the aspect of scaffold design, a good scaffold design should provide an appropriate channel for nutrient transport and a relatively low shear stress environment for cell attachment. The most suitable porosity of the scaffold needs to be determined, and then ensured during the scaffold fabrication process. Thus, there is a compelling need to study the effect of porosity on the cell culture process.

Oxygen availability throughout the tissue is also of importance in the development of tissue-engineered constructs. The oxygen distribution in the tissue scaffolds has been shown to vary with time [13]. However, some of the existing studies ignored the mass transport of oxygen, or treat it separately without considering the effect of other nutrients. This paper presents the development of a novel mathematical model, by taking into account the multi-phase mass transfer within a scaffold in a perfusion bioreactor. Based on the improved model, simulations were carried out to investigate the effect of parameters which are controllable in the scaffold design and fabrication (i.e., scaffold porosity) and during the cultivation process (i.e., flow rate) on the cell culture process.

2.2 Model development

2.2.1 Governing equations

Consider a scaffold in a perfusion system, as shown in Fig. 1a, with a diameter of 10

mm (D) and a height of 3 mm (H). The local volume average (LVA) theory was adopted for the model development, by which the average properties in each representative elemental volume (REV) of characteristic length (ℓ) are considered instead of the specific property at each point [14]. In order to apply the LVA method, the characteristic length must be much smaller than the scale of the scaffold (H) and greater than the internal structure scale (d). In this research, the length scale of the cell colony (d) varies from several nanometers to more than fifty micrometers, and the minimum length in the three spatial dimensions (L) is H. Taking 100 μm as the characteristic length of a REV, then $d < \ell << L$, so that the LVA approach is valid.

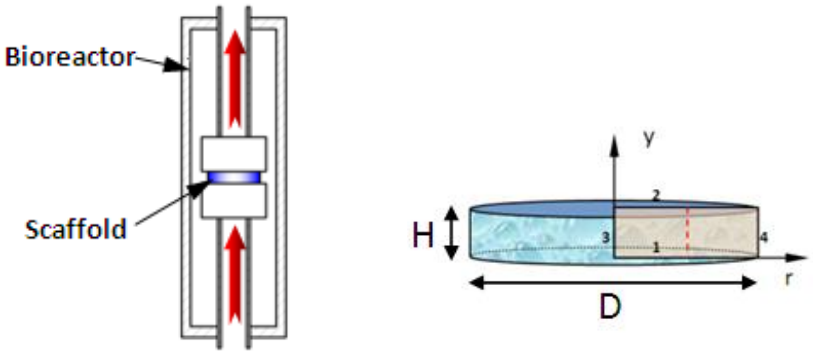
In the present study, the following assumptions are made: 1) the cell phase comprises both cells and extracellular matrix (ECM) and the difference in the mass diffusivity between them is neglected; 2) cells are uniformly seeded on the scaffold before culturing; 3) the velocity is uniform within the scaffold; 4) the volume of the gas phase is ignored since the gas is assumed to be dissolved in solution; 5) once entering the cell phase, oxygen is immediately consumed; 6) the tissue scaffold is symmetric about the center line such that cylindrical coordinates can be used (Fig. 1b); 7) convection has no influence on cell attachment and the cells are only distributed within or on the scaffold; 8) convection in the r-direction is ignored; and 9) the glucose transfer across the interface of the fluid and cell phases is much faster than diffusion, so that there is an equilibrium relationship between the intercellular glucose concentration and extracellular glucose concentration. The average properties over the volume of the phase are defined by

$$\langle C_g \rangle^c = \frac{1}{V_c} \int_{V_c(t)} C_g \, dV \tag{1}$$

$$\langle C_g \rangle^f = \frac{1}{V_f} \int_{V_f(t)} C_g \, dV \tag{2}$$

$$\langle C_o \rangle = \frac{1}{V_f} \int_{V_f(t)} C_o \, dV \tag{3}$$

where $\langle C_g \rangle^c$ is the average glucose concentration in the cell phase; $\langle C_g \rangle^f$ is the average glucose concentration in the fluid phase; and $\langle C_o \rangle$ is the average oxygen concentration in the fluid phase. V_c and V_f are the phase volume of cell phase and fluid phase, respectively.



a **b**

Fig. 1. a) schematic of a perfusion system, and b) solution domain with center line and boundary surfaces labeled.

Glucose is the primary energy source for cell metabolism and appears in both the cell phase and fluid phase. The continuity equation for glucose includes diffusive transport in both phases and is governed by

$$\frac{\partial}{\partial t}\left[\varepsilon_c\langle C_g\rangle^c+\varepsilon_f\langle C_g\rangle^f\right]+\langle v\rangle_f\frac{\partial\left[\varepsilon_f\langle C_g\rangle^f\right]}{\partial y}=\frac{1}{r}\frac{\partial}{\partial r}\left(D_{geff}r\,\frac{\partial\langle C_g\rangle^f}{\partial r}\right)+\frac{\partial}{\partial y}\left(D_{geff}\,\frac{\partial\langle C_g\rangle^f}{\partial y}\right)-S_1 \qquad (4)$$

According to Assumption 9, $\langle C_g\rangle^f$ and $\langle C_g\rangle^c$ in the above equation can be related by $\langle C_g\rangle^c = K_{eq}\langle C_g\rangle^f$, in which K_{eq} is the equilibrium coefficient. In Equation (4), ε_c and ε_f are the volume fraction of the cell and fluid phases and are defined by $\varepsilon_f = \frac{V_f}{V}$ and $\varepsilon_c = \frac{V_c}{V}$, respectively. It is noted that $\varepsilon_f + \varepsilon_c = \varepsilon$, where ε is the total volume fraction of cell and fluid. Due to the degradation of the scaffold frame with time, the value of ε is not constant, but increases during the cell culture process.

Let the initial porosity of the scaffold is denoted by ε_o and assume that the degradation of the scaffold is described by $\varepsilon = 1-(1-\varepsilon_o)e^{-\frac{t}{\sigma}}$ based on a previous study [15]. In this equation, σ is the degradation coefficient. Fig. 2, for example, shows the degradation profile and porosity profile of Polyglycolic acid (PGA) in a cell culture process (in which σ is degradation coefficient). When $t = 0$, ε is equal to the initial porosity ε_o, and as t approaches infinity, ε becomes close to 1, which implies that the scaffold has completely degraded.

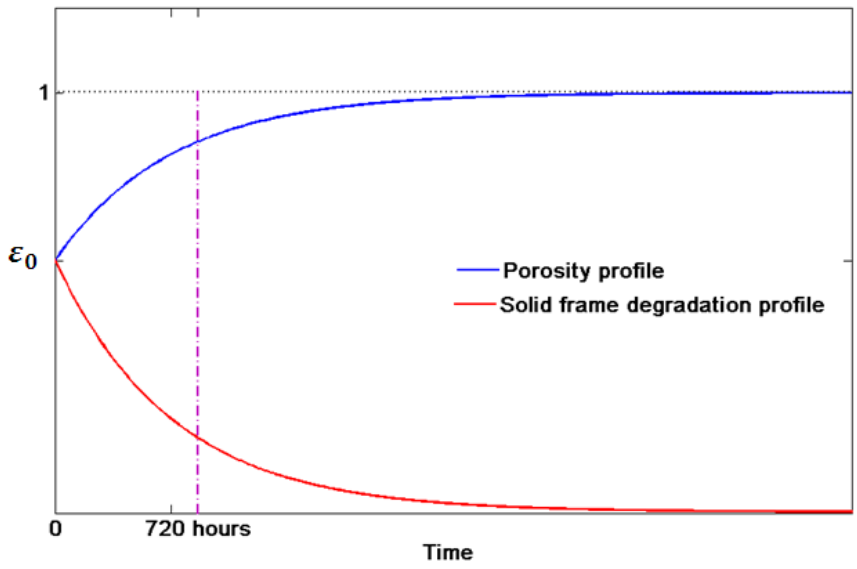


Fig. 2. Solid degradation profile and porosity profile in cell culture process.

In Equation (4), D_{geff} is the effective glucose diffusivity in the tissue scaffold, which represents the effective diffusivity in the fluid and cell phase modified to incorporate the structural effects of the tissue scaffold [16]. The effective diffusivity in the fluid and cell phases (D_{geffm}) depends on the cell and fluid phase properties, and the equilibrium constant. The relationship between the effective diffusivity in the fluid and cell phase and the effective diffusivity in the scaffold is given by:

$$D_{geff} = \frac{D_{geffm} \cdot \varepsilon}{\tau} \tag{5}$$

where D_{geffm} is evaluated from the Maxwell formula [17], i.e., $D_{geffm} = D_f \frac{3\alpha - 2(\varepsilon_f/\varepsilon)(\alpha-1)}{3+(\varepsilon_f/\varepsilon)(\alpha-1)}$, where $\alpha = \frac{K_{eq} D_c}{D_f}$, and D_f and D_c are the molecular diffusivities of glucose in the fluid phase and cell phase, respectively. In Equation (5), τ

is the tortuosity of the scaffold which can be modeled as a function of the porosity ε [18], i.e., $\tau = \frac{(2-\varepsilon)^2}{\varepsilon}$. In this way, the effect of the solid frame on the diffusivity is included in the model through the porosity ε and tortuosity τ.

In Equation (4), S_1 is a term to describe the consumption of glucose as given by the Michaelis-Menten kinetics [9], i.e., $S_1 = \frac{R_{gm}\langle C_g\rangle^f}{K_{gm}+\langle C_g\rangle^f}\varepsilon_c$, where K_{gm} is the saturation coefficient of glucose and R_{gm} is the maximum glucose metabolic rate.

The gas phase, oxygen, is also included in the model, and the transport of oxygen is governed by

$$\frac{\partial}{\partial t}\langle C_o\rangle + \langle v\rangle_f \frac{\partial\langle C_o\rangle}{\partial y} = \frac{1}{r}\frac{\partial}{\partial r}\left(D_{oeff} r \frac{\partial\langle C_o\rangle}{\partial r}\right) + \frac{\partial}{\partial y}\left(D_{oeff}\frac{\partial\langle C_o\rangle}{\partial y}\right) - S_2 \qquad (6)$$

In the above equation, D_{oeff} is the effective diffusivity of the oxygen in the tissue scaffold, which is related to the diffusivities of oxygen in the fluid and cell phase by the expression,

$$D_{oeff} = \frac{D_{oeffm}\cdot\varepsilon}{\tau}. \qquad (7)$$

Here D_{oeffm} is the effective diffusivity in the fluid and the cell phases which can be calculated from $D_{oeffm} = \frac{D_o * 2(1-\varepsilon_c/\varepsilon)}{2+\varepsilon_c/\varepsilon}$ [17], and D_o is the molecular diffusivity of oxygen in the medium. The effective diffusivities of both glucose and oxygen in the fluid and cell phase are evaluated from the Maxwell formula. The difference in the way these

two terms are calculated is due to their respective transport characteristics in the fluid and cell phase. For glucose, the mass transfer across the interface of the fluid phase and cell phase is much faster than diffusion (Assumption 9). However, for oxygen, it is assumed that the extracellular transport is faster then trans-membrane transport [17].

In Equation (6), S_2 is used to represent the cell consumption of oxygen and is also specified by the Michaelis-Menten kinetics: $S_2 = \frac{R_{om}\langle C_o\rangle}{K_{om}+\langle C_o\rangle}\varepsilon_c$ in which R_{om} is the maximum oxygen metabolic rate and K_{om} is the saturation coefficient of oxygen. Similar to the glucose conservation equation, via the diffusivity and source terms, the conservation of oxygen is coupled to mass conservation of cells.

In both Equation (4) and Equation (6), the second term on the left is the convection term where $\langle v\rangle_f$ is the Darcy velocity ($\langle v\rangle_f = u_D$), which can be calculated from the flow rate, i.e., $u_D = \frac{4Q}{\pi {d_d}^2}$. Here d_d is the inlet diameter, which equals to 10 mm for the bioreactor considered in the present study. The flow rate is a controllable parameter for culturing in perfusion bioreactors.

Cell proliferation and migration are affected by both oxygen and glucose. The conservation of cells is governed by

$$\frac{\partial \varepsilon_c}{\partial t} = \frac{1}{r}\frac{\partial}{\partial r}\left(D_{effcell} r \frac{\partial \varepsilon_c}{\partial r}\right) + \frac{\partial}{\partial y}\left(D_{effcell}\frac{\partial \varepsilon_c}{\partial y}\right) + \left[R_g - R_d\right]\varepsilon_c \qquad (8)$$

where $D_{effcell}$ is the effective cell diffusivity and $D_{effcell} = \frac{D_{cell}\cdot\varepsilon}{\tau}$, where D_{cell} is the cell diffusivity, which can be represented using a random walk model [19]; R_g is the cell growth rate, which is mediated by both glucose and oxygen concentrations as

$$R_g = \mu_{max} \cdot \frac{\langle C_o \rangle}{K_c \rho_{cell} \varepsilon_c + \langle C_o \rangle} \cdot \frac{\langle C_g \rangle^f}{K_{eq}^{-1} K_c \rho_{cell} \varepsilon_c + \langle C_g \rangle^f} \quad (9)$$

where μ_{max} is the maximum cell growth rate; and R_d in Equation (8) is the dying rate of cells used to describe cell apoptosis.

In Equation (9), the second part on the right side describes oxygen regulation using Contois kinetics while the last part, glucose regulation, is a modified Contois kinetics for cell growth [20, 21]

2.2.2 Boundary conditions and initial conditions

As shown in Fig. 1b, surface 1 is the inlet and surface 2 is the outlet. The scaffold is symmetric about the center line, implying use of symmetric boundary conditions for surface 3. Surface 4 is the lateral external face of the scaffold and the nutrients are diffused through this surface. The concentrations of glucose and oxygen are fixed at their supply values at this surface, and according to Assumption 7, cells are confined in the space enclosed by this surface. Homogeneous cell seeding is assumed to have resulted in an initial cell volume fraction of 0.00868 [22] prior to the culturing process, and initially there is no oxygen and glucose inside the scaffold. The mathematical statement of the boundary and initial conditions is given in Table 1.

Table 1. Boundary and initial conditions

Surface	$\langle C_g \rangle^f$	$\langle C_o \rangle$	ε_c
1	$\langle C_g \rangle^f = C_{glu}$	$\langle C_o \rangle = C_{oxy}$	$\frac{\partial \varepsilon_c}{\partial r} = 0$
2	$\frac{\partial \langle C_g \rangle^f}{\partial y} = 0$	$\frac{\partial \langle C_o \rangle}{\partial y} = 0$	$\frac{\partial \varepsilon_c}{\partial y} = 0$
3	$\frac{\partial \langle C_g \rangle^f}{\partial r} = 0$	$\langle C_o \rangle = C_{oxy}$	$\frac{\partial \varepsilon_c}{\partial r} = 0$
4	$\langle C_g \rangle^f = C_{glu}$	$\langle C_o \rangle = C_{oxy}$	$\frac{\partial \varepsilon_c}{\partial r} = 0$
Initial condition	$\langle C_g \rangle^f = 0$	$\langle C_o \rangle = 0$	$\varepsilon_c = \varepsilon_{c0}$

2.2.3 Computational method and parameter values

An implicit finite difference method was used to discretize the governing equations. MATLAB was used to write an in-house code which solves the discrete equation set using the Gauss-Seidel method. The values for the transport coefficients and other model parameters were adopted from the literature and listed in Table 2. The culturing solution is Eagle's minimal essential medium (DMEM), which is typically used in tissue engineering.

Table 2. Values of main coefficients

Definition	Value	Reference
Glucose diffusivity in fluid phase	$D_f = 1.0 \times 10^{-9}\ m^2/s$	[20]
Glucose diffusivity in cell phase	$D_C = 1.0 \times 10^{-10}\ m^2/s$	[20]
Equilibrium coefficient	$K_{eq} = 0.1$	[23]
Cell random walk coefficient	$D_{Cell} = 1.7 \times 10^{-14}\ m^2/s$	[24]

Oxygen diffusivity in fluid phase	$D_o = 3.093 \times 10^{-9}\,m^2/s$	[25]
Maximal cell growth rate	$\mu_{max} = 3.7 \times 10^{-6}\,1/s$	[22]
Cell diameter	$d_{cell} = 2 \times 10^{-5} m$	[26]
Maximum glucose consumption rate	$R_{gm} = 8 \times 10^{-3}\,kg/(m^3 \cdot s)$	[27]
Saturation coefficient of glucose	$K_{gm} = 6.3 \times 10^{-2}\,kg/m^3$	[28]
Maximum oxygen consumption rate	$R_{om} = 1.77 \times 10^{-3}\,mol/(m^3 \cdot s)$	[25]
Saturation coefficient of oxygen	$K_{om} = 6 \times 10^{-3}\,mol/m^3$	[25]
Contois saturation coefficient	$K_c = 0.154$	[9]
Single cell mass density	$\rho_{cell} = 182 kg/m^3$	[29, 30]
Cell death rate	$R_d = 3.3 \times 10^{-7}\,1/s$	[10]
Degradation constant	σ=2098800 s	[15]

2.2.4 Numerical solution

The solution domain as shown in Fig. 1b) was divided into 120 elements in the r direction and 90 elements in the y direction. The first element and last element in each coordinate direction are fictitious nodes used only to set up the boundary conditions at the exterior surfaces.

2.3 Model validation

The average cell volume fraction was simulated with the developed model and aforementioned method. This simulation was carried out under the same conditions as the

experiments reported in [22], in which Freed et al. investigated the growth kinetics of chondrocytes in polymer implants with different thicknesses. The simulation results are presented in Fig. 3, along with the experimental data reported in [22] for comparison. Freed et al. did the experiment with different scaffold heights; the red triangle with the error bar is the experimental data for the scaffold with the height of 0.307 cm. Similarly, the purple triangle with the error bar and the brown triangle with the error bar are the data for the scaffold with heights of 0.168 cm and 0.116 cm, respectively. Based on the experimental data, the average cell volume fraction kept increasing during most of the culture time, however, at the end of the experiment, the growth rate is reduced and even becomes negative which means that the cell volume decreased somewhat. Overall, the simulation results agree with the experimental results. The blue line which is the simulation for the scaffold with the height of 0.307 cm agrees well with the experimental data during most of the culture time except the final period. For different scaffold heights, the simulation model captures the main feature of the experimental results as given by the green line (scaffold height of 0.168) and the sky blue line (scaffold height of 0.116). The difference between the simulation and experiment is caused by the influence of such factors as collagen, which was not included in the present model but was measured in the experiment. The experimental results indicate that the collagen played a dual role in cell growth, as a promoter when it was first secreted by the cells and as an inhibitor when collagen gradually increases in amount [31, 32]. Another possible reason for the discrepancy between the simulation and experimental results in the final period may be the role of oxygen, since the

oxygen concentration in the cell culture was not documented in Freed's study [22].

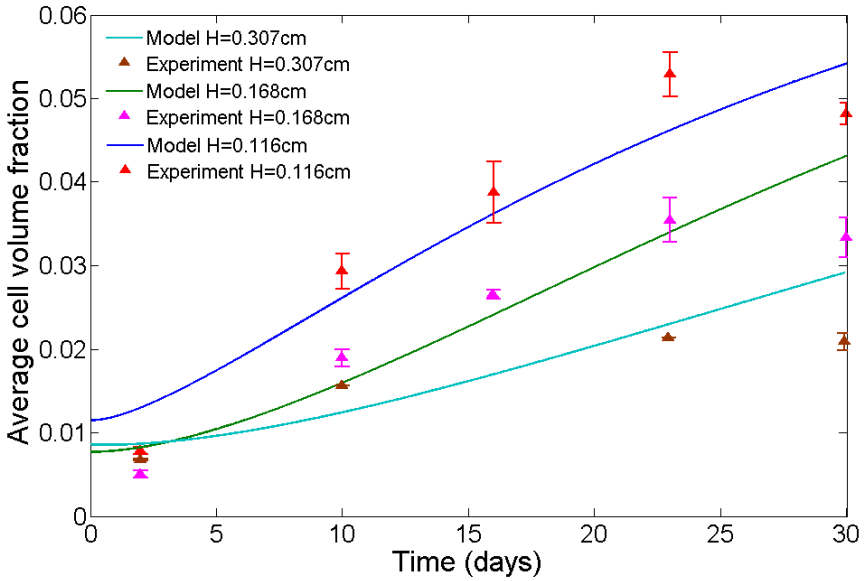


Fig. 3. Comparison of cell volume fraction between the model predictions and the data reported in [15].

2.4 Simulation results and discussion

With the validated model, additional simulations were carried out, in which the scaffold was assumed to have an initial porosity of 80%, and then due to degradation increase to 94.17% by the end of test period (720 hours). In this case, the culturing solution was DMEM with a constant glucose concentration of 4.5 kg/m^3 and oxygen concentration of 0.119 mol/ m^3. The results of mass transfer at the end of the test period are shown in Fig. 4. The effects of perfusion can be clearly seen with strong convective mass transfer along the y-direction, resulting in a high concentration of oxygen (Fig. 4a) and glucose (same trend as oxygen, not shown) at the inlet, and a high cell volume fraction (Fig.

4b) in the same region. Note that Fig. 4a also shows a steep gradient near the outer lateral surface (r = 0.005 m), suggesting that diffusion is also an important transport mechanism within the scaffold. Finally, the cell volume fraction drops significantly near the outlet, which agrees with the effect of the lower glucose and oxygen concentrations.

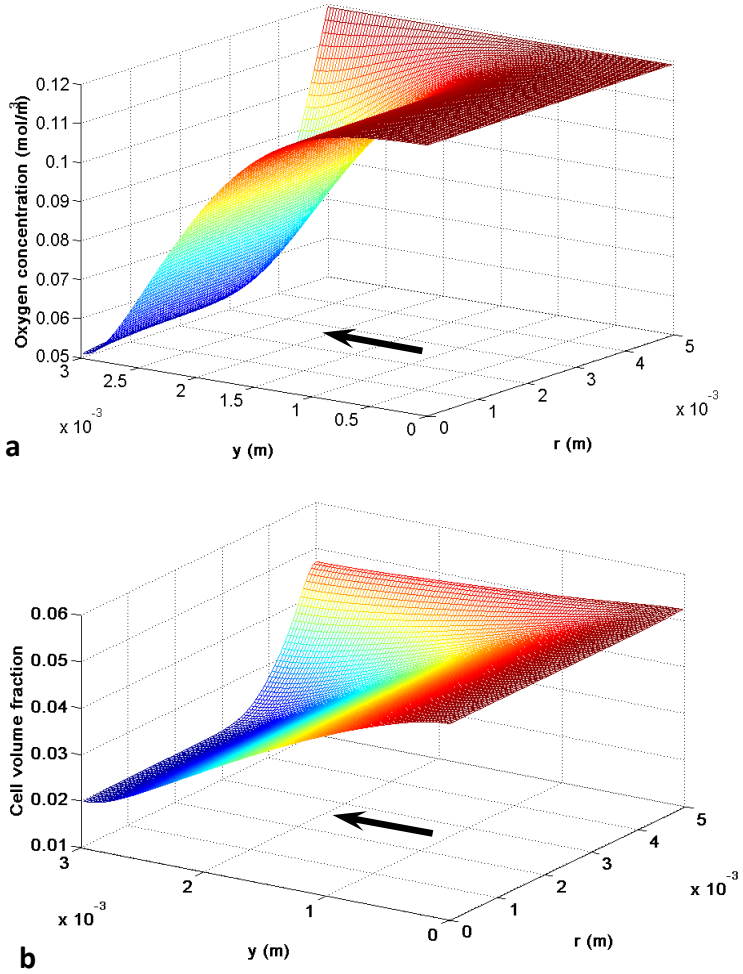


Fig. 4. Oxygen concentration (a) and cell volume fraction (b) distribution for a scaffold in perfusion bioreactor after 720 hours.

The average glucose concentration and average cell volume fraction within the scaffold, simulated by the model as a function of time, are shown in Fig. 5. The average quantities

are obtained by averaging each property over the entire construct. The glucose concentration (blue line) initially increased at the beginning of culturing until it reached a maximum value, and then decreased slowly due to the increase in cell volume fraction (red line).

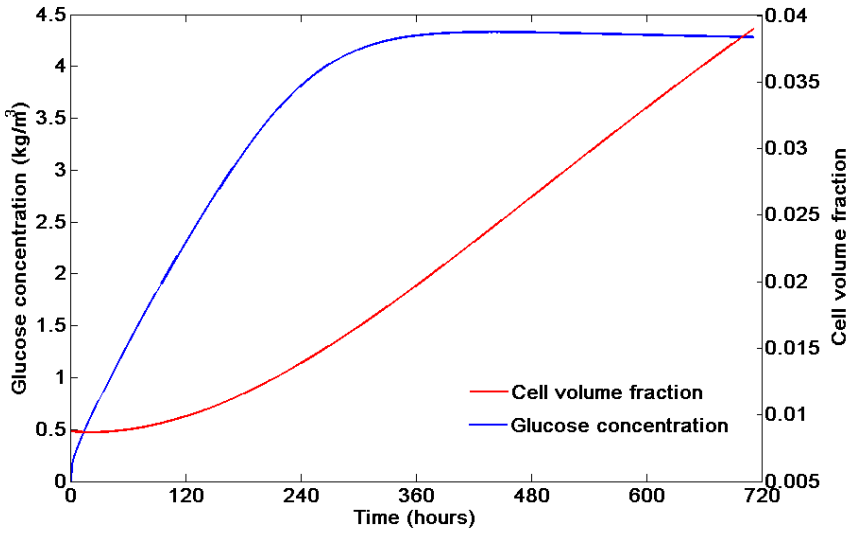
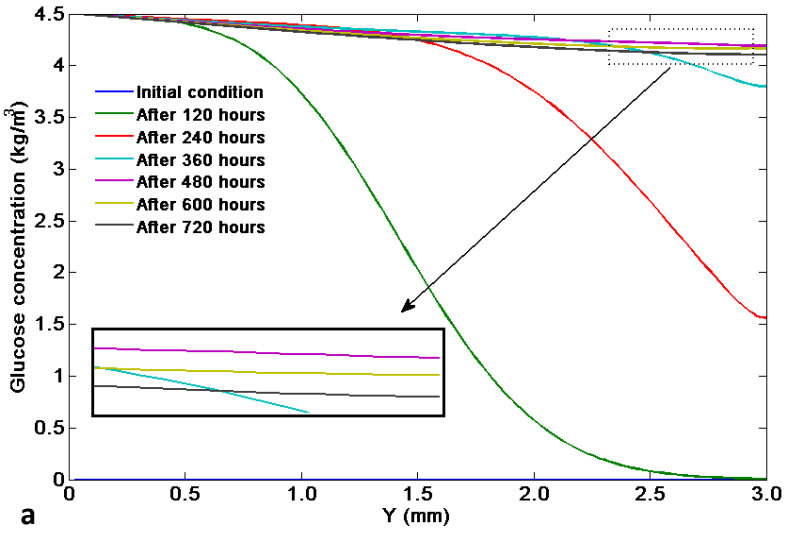


Fig. 5. Variation of glucose concentration and cell volume fraction with time over test period.

As mentioned previously, cell culture is a time-dependent process: the details are shown in Fig. 6. The data is for the center line location (i.e., the red dashed line in Fig. 1b) of the scaffold. In Fig. 6a, the initial glucose concentration in the scaffold is zero. Due to the strong convection along the y-direction, the area near the inlet reaches almost the same concentration as the exterior medium. The glucose concentration keeps increasing along the center line until around 360 hours and at this time the glucose concentration is almost uniform. However, after 480 hours, the glucose concentration begins to decrease with time

and a possible reason is the increase in the cell numbers (cell volume fraction show in Fig. 6c) and corresponding glucose consumption. The oxygen concentration which is shown in Fig. 6b behaves somewhat differently. Similar to the inchoate period of the glucose concentration, the oxygen concentration increases over time. Turning attention to the cell volume fraction (Fig. 6c), originally, it is uniform with a value of 0.00868. As cell culturing proceeds, the cell volume fraction increases in the area near the inlet because of the relatively abundant nutrient supply; conversely, at the area near the outlet, the cell volume fraction decreases. After around 480 hours, strong convection brings the nutrients, especially the oxygen, throughout the scaffold, and the cells all across the scaffold begin to proliferate dramatically. For the case considered in the simulation, the oxygen supply is the main restriction for cell culture.



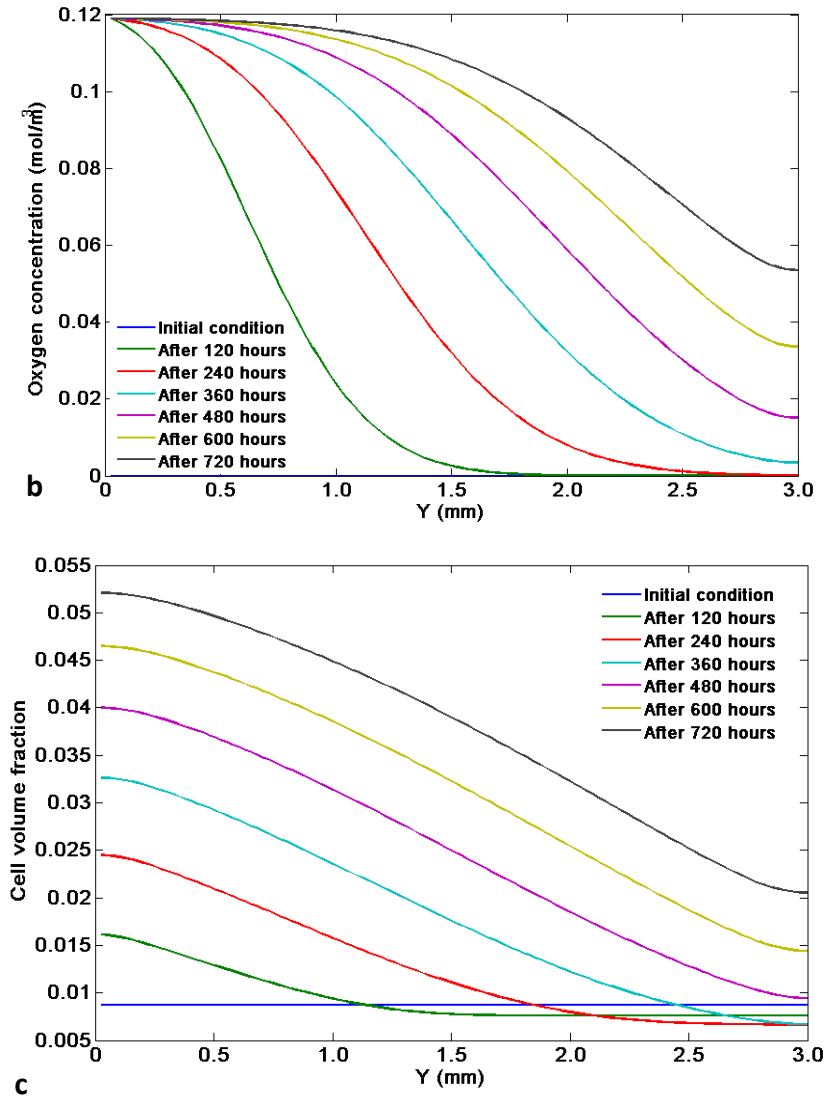
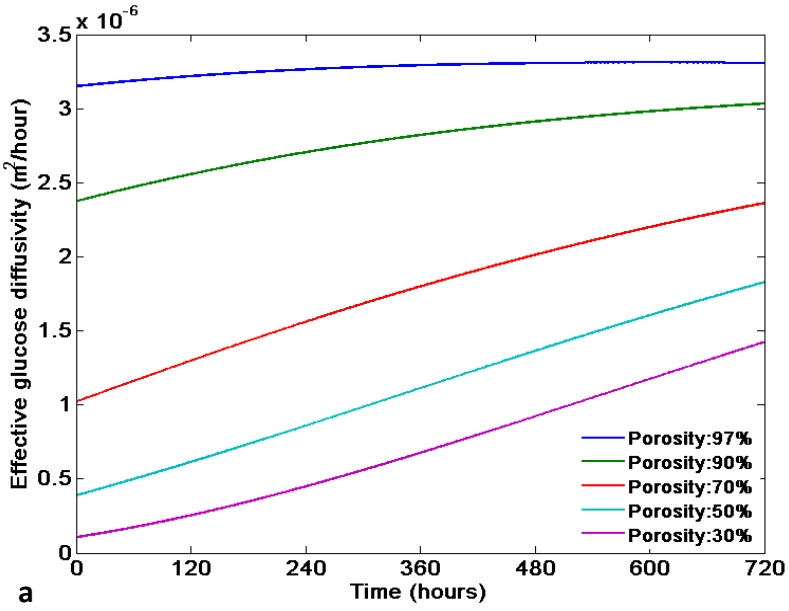


Fig. 6. Temporal variation of glucose concentration (a), oxygen concentration (b) and cell volume fraction (c) along the center line.

Recall that in the present simulation, the scaffold porosity increased in time due to the scaffold material degradation. For a variation in the porosity from 30% to 97%, the effective diffusivities of glucose, oxygen and cells (cell random walk coefficient), are affected significantly, as shown in Fig. 7. For the effective glucose diffusivity (Fig. 7a) and

effective oxygen diffusivity (same trend as glucose, not shown), the increase in porosity (which means more space for the fluid medium) has a significant effect. Even for the same porosity, take the case of 30% initial porosity as an example (pink line), when the solid frame degrades over time, the porosity increases as shown in Fig. 2, and the corresponding effective diffusivity increases as well. In contrast to the effective glucose/oxygen diffusivities, the effective cell diffusivity (Fig. 7b) reduces with the increase in porosity. The probable reason for this interesting phenomenon is that the reduced solid frame provides less surface area for cells to attach. For a given initial porosity value, the effective cell diffusivity decreases as the solid frame degrades with the elapse of time. Note that the effective cell diffusivity in Fig. 7b is represented by a logarithmic scale, so that the difference between 97% porosity and 30% porosity is almost 1000 times. Such a dramatic variation in properties indicates that the effect of porosity on diffusivity cannot be ignored.



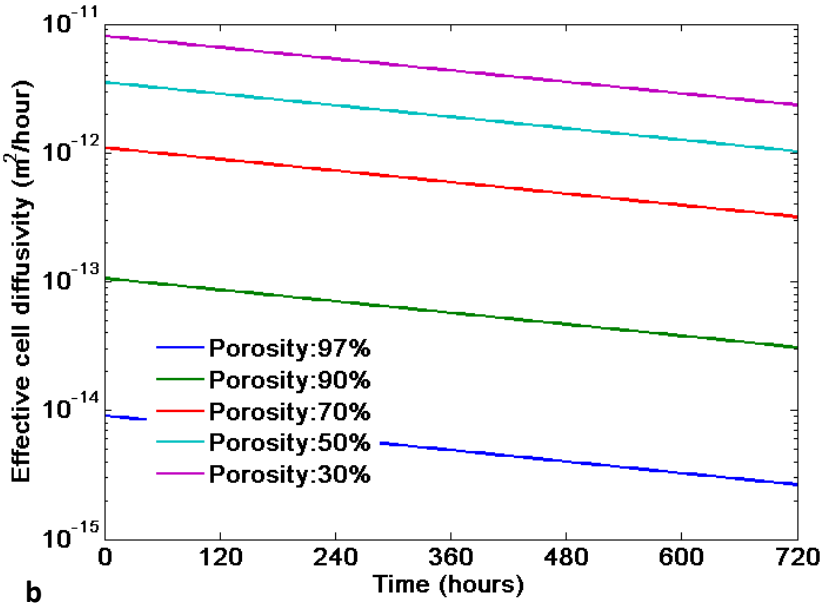
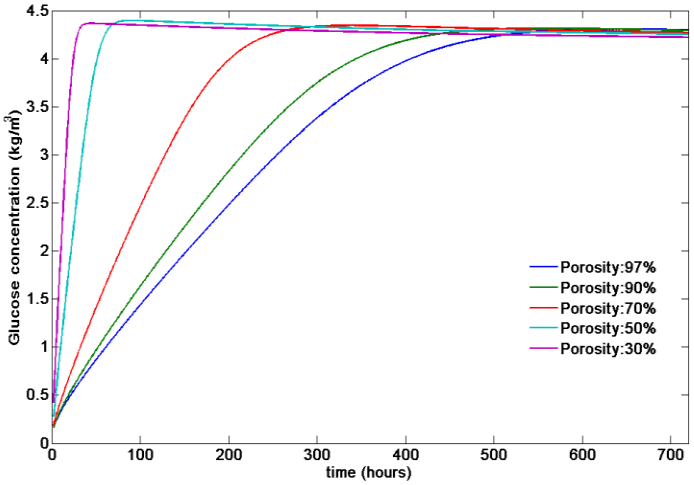


Fig.7. (a) Variation of effective glucose diffusivity with time for different porosities; (b) Variation of effective cell diffusivity variation with time for different porosities (the values are bulk values at any instant of time).

The temporal variation of the glucose concentration during the cell culture process as a function of porosity is shown in Fig. 8a, while the variation of the oxygen concentration is given in Fig. 8b. The corresponding cell volume fraction is presented in Fig. 8c. It is interesting to note in Fig. 8a that the lower porosity gave a higher average concentration of the glucose in the scaffold up until approximately 600 hours. Thereafter, the concentration of glucose in the scaffold with the higher porosity is slightly higher than for the scaffold with the lower porosity. For oxygen, the behavior is similar, except that the peak concentration is not reached within the test period for porosities of 70% or greater. One possible explanation for this behavior is that as the porosity increases, the time required to

saturate the water within the scaffold with the nutrient component increases. The cell volume fraction showed similar behavior with respect to porosity: with an increase in porosity, the cell volume fraction decreased. Note that in Fig. 8, the maximum difference due to porosity (i.e., the maximum difference between the parameter value for 97% porosity and the value for 30%, normalized by the variation of parameter value over the whole scaffold) is 86.75% for the glucose concentration, 71.76% for the oxygen concentration and 44.28% for the cell volume fraction. This suggests that the influence of the solid frame is important and should be considered in model development, especially for the scaffolds with lower porosities (70% or less).



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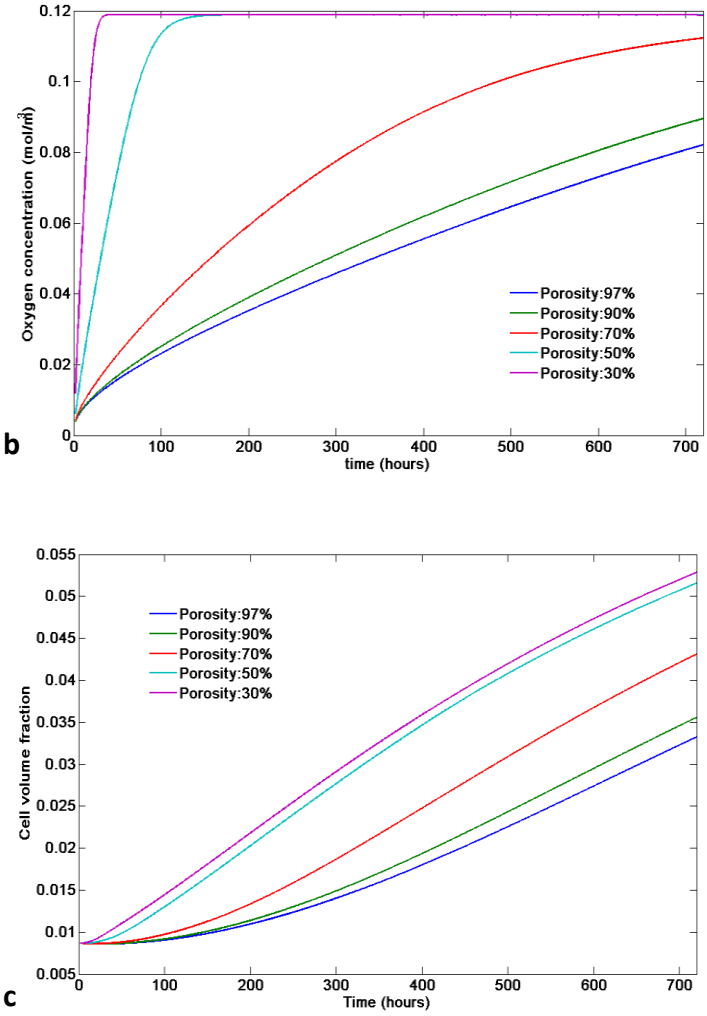

Fig. 8. Variation of glucose concentration (a), oxygen concentration and (b) cell volume fraction (c) with time for different porosities (the values are bulk values at any instant of time).

Finally, the flow rate is an important parameter to modulate the cell culture process in a bioreactor. Fig. 9 shows the variation of nutrient concentration and cell volume fraction along the center line for three different flow rates, i.e., 0.05ml/min, 0.10ml/min, and 0.15 ml/min. It is seen that both the glucose and oxygen concentrations increase with flow rate (Fig. 9a), which leads to a more uniform distribution of cell volume fraction as shown in

Fig. 9b. On the other hand, any substantial increase in flow rate may also cause large shear stresses within the scaffold, which may in turn wash out the attached cells, influence the cellular metabolism, and even cause physical damage to the cells. Thus, the optimal flow condition should provide a compromise between enhanced mass transfer and sufficiently low shear stress. This issue is currently being pursued by the authors.

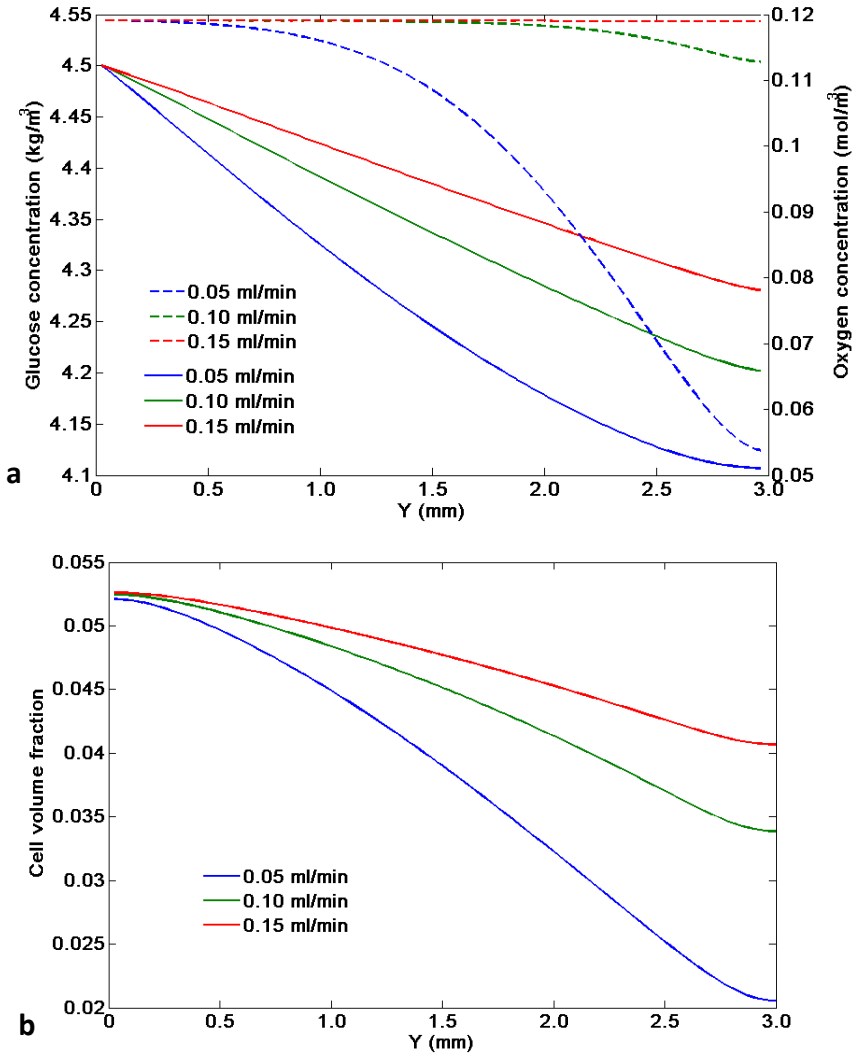

Fig. 9. Effect of flow rate on glucose concentration (solid line) and oxygen concentration (dash line) (a), and cell volume fraction (b) for scaffold in perfusion bioreactor after 720 hours.

2.5 Conclusions

This paper reports on the development of a novel mathematical model to describe mass transfer in tissue scaffolds cultured in a perfusion bioreactor, by taking into account mass transfer and scaffold degradation. The model was validated using data extracted from the literature. Simulations were then carried out for cell culture typically taking place in a perfusion bioreactor. The results demonstrate that perfusion bioreactors with enhanced convection transport can increase mass transfer rates inside the tissue scaffold. The results also show that the nutrient concentration and cell volume fraction are time dependent, but in different fashions. Specifically, in contrast to the steady increase in cell volume fraction over the test period, a peak or maximum value appears in the profile of the nutrient concentration. The effects of controllable factors in scaffold fabrication and cell culturing were also investigated using the numerical model. It was found that an increase in porosity can reduce the inhibiting effect of the solid scaffold on nutrient transport represented by an increase in the nutrient effective diffusivity. In addition, increasing the flow rate can enhance convection, thus promoting a more uniform distribution of both nutrient concentration and cell volume fraction. The contribution of this research pertains to the use of a comprehensive model to explore and explain the complex temporal transport in a perfusion bioreactor. The knowledge obtained based on the model simulations provides insight into the cell culture process, which would not be possible to obtain from experiments. This insight can be used to significantly improve the design of in vitro cell

culture.

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CHAPTER 3

MODELING OF THE FLOW WITHIN SCAFFOLDS IN PERFUSION BIOREACTORS

Submitted as

X. Yan, D. J. Bergstrom, and X. B. Chen, Modeling of cell cultures in perfusion bioreactors, IEEE Transactions on Biomedical Engineering, 2011

Contribution of this Chapter to the Thesis

The research work presented in this chapter aims at achieving the second objective of the thesis. More specifically, the chapter addresses the model development for fluid flow within scaffolds in perfusion bioreactors. The effect of the diameter of the strand, the horizontal span and the flow rate are investigated.

Modeling of the flow within scaffolds in perfusion bioreactors

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ABSTRACT

Tissue engineering aims to produce artificial organs and tissues for transplant treatments, in which cultivating cells on scaffolds in bioreactors is of critical importance. To control the cultivating process, the knowledge of the fluid flow inside and around a scaffold in the bioreactor is essential. However, due to the complicated microstructure of a scaffold, it is difficult, or even impossible, to gain such knowledge experimentally. In contrast, numerical methods employing computational fluid dynamics (CFD) have proven promising to alleviate the problem. In this research the fluid flow in perfusion bioreactors is studied with numerical methods. The emphasis is on investigating the effect of the controllable parameters in both the scaffold fabrication (i.e., the diameter of scaffold strand and the distance between two strands) and cell culture process (i.e., the flow rate) on the distribution of shear stress within the scaffold in a perfusion bioreactor. The knowledge obtained in this study will allow for improved control strategies in scaffold fabrication and cell culturing experiments.

Keywords: Perfusion bioreactor, CFD, Velocity, Wall shear stress

NOMENCLATURE

D	strand diameter, mm
h_{xy}	horizontal distance, mm
h_z	vertical pore size, mm
Q	flow rate, mL/min
Y	horizontal span, mm

Greek Symbol

ε	porosity
τ_e	elastic limit stress, Pa

3.1 Introduction

Tissue engineering is an emerging field with the aim of repairing or creating new tissues. It is evident that the scaffold plays a critical role in forming the required constructs in a bioreactor [1]. In bioreactors, biological and biochemical processes occur under closely monitored and tightly controlled environmental or operating conditions. As such, bioreactors play a significant role in the in vitro experiments of cell-based tissue engineering [2]. The perfusion bioreactor, in which the culture medium continuously flows through the pores of the scaffold, is superior compared to other bioreactors (e.g. the spinner flask bioreactor and the rotating wall vessel bioreactor) since mass transfer is

enhanced within the scaffold. The scaffold in a perfusion bioreactor can have adequate nutrient supply, timely waste removal, and sufficient gaseous exchange, thus promoting cell growth and proliferation within the scaffold [3]. However, increased flow rates can create large shear stresses on the scaffold strands, which can in turn wash away the attached cells, adversely influence the cellular metabolism, and even damage the cells. It is noted in the literature [4, 5] that a moderate shear stress is highly beneficial to the formation of glycosaminoglycan (GAG) and thus cartilage tissues. Therefore, a compromise between the mass transfer and the shear stress must be made in the cell culture for a given application.

Due to the lack of adequate sensors, it is difficult, even impossible, to measure the local shear stress distribution within a scaffold [6]. Computational fluid dynamics (CFD) shows promise in solving this problem. CFD has been widely used in various fields because it often requires less time and fewer resources than experiments. In tissue engineering, CFD has recently shown promise in visualizing the flow phenomena within bioreactors, thus providing the detailed information and insight, which would be difficult to gain by experiments.

The local volume average approach is one method to evaluate the average shear stress in a porous media, for which specific mathematical models are required [6]. The limitation of this method is that only the averaged shear stress, rather than its distribution, can be obtained. To overcome this limitation, various approaches have been developed and reported in the literature, though at their early stage. In the earliest studies, scaffolds

were treated as impermeable constructs in the development of CFD models. Based on simulation results, improved designs for bioreactors and scaffold constructs were reported [7-10]. In subsequent studies, the scaffold structure was taken into consideration. For scaffolds with irregular structures such as those fabricated by means of conventional fabrication methods, micro-computed tomography (μCT) was used to create 3-dimensional (3D) geometric models [3, 11-13]. In addition to μCT, another method to deal with irregular geometry is to treat the inner structure as a repetitive pattern of units by means of computer-aided-design (CAD) methods. The shear stress distribution in such a unit has been studied with the identified effect of pore size and porosity on it [6, 11, 14]. In these studies, the scaffolds with irregular internal structures were simplified for the model development, thus contributing to the errors in the following simulation. Currently, scaffolds manufactured by rapid prototyping (RP) techniques have shown promising in various tissue engineering applications due to their controllable microstructure [15, 16] For such scaffolds, the structure is regular and the geometry can be readily modelled by CAD methods. Singh et al. [17, 18] utilized commercial CFD software to create models of such scaffolds in bioreactors and studied the influence of mechanical stimuli on the velocity and shear stress distribution. Unfortunately, their studies were limited to non-perfusion bioreactors.

In this research, a cylindrical section of a regular scaffold structure, fabricated through the RP technique, is modelled under both perfusion and non-perfusion situations. This study specifically focuses on the flow field within the scaffold and the influence on

the wall shear stress distribution of the controllable parameters in scaffold fabrication and cell culture process.

3.2 Methodology

3.2.1 Bioreactor configuration

Both the perfusion and non-perfusion bioreactors considered in this study are shown schematically in Fig. 1, with the difference in the inlet and outlet locations. A cell seeded tissue scaffold is placed between the two struts and the chamber allows for circulation of the fluid medium. A perfusion system occurs when the inlet flow comes directly through the channel inside the struts and enters the bottom surface of the scaffold (Fig.1 (a)). A non-perfusion system occurs when the inlet is located at the wall of the chamber (Fig. 1(b)). In the present study, the inlet diameter is 10 mm and the height, length and width of the chamber are 140, 50 and 50 mm, respectively. Taking advantage of the symmetry of the chamber, only one-fourth of the bioreactor chamber is modeled to reduce the computational time.

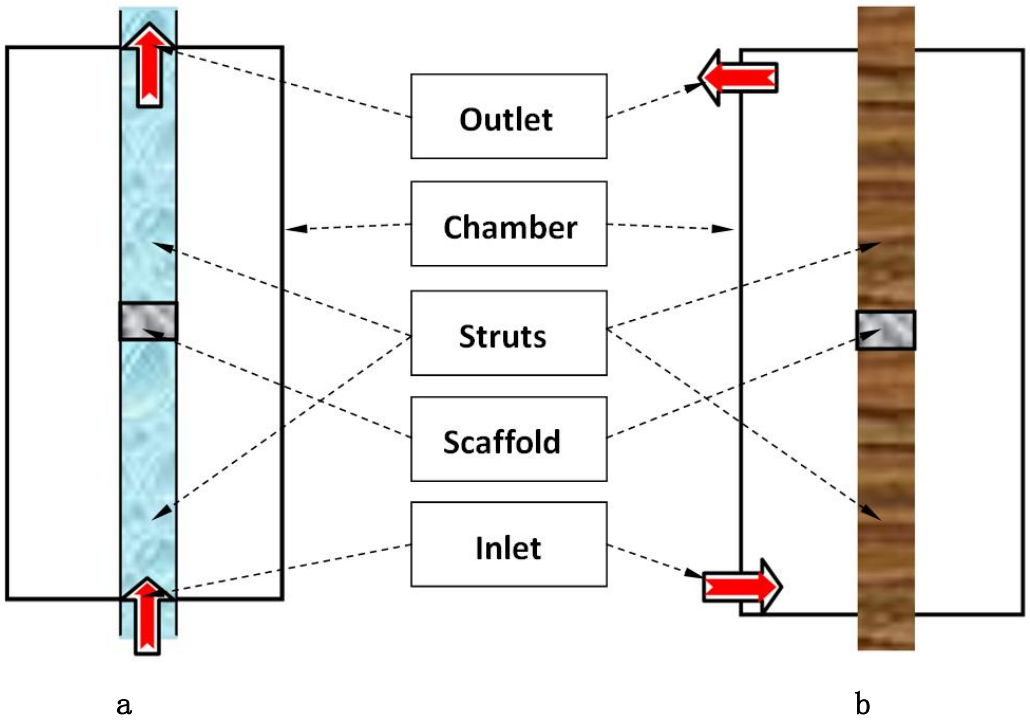


Fig. 1 Schematic of bioreactors: (a) perfusion bioreactor, and (b) non-perfusion bioreactor.

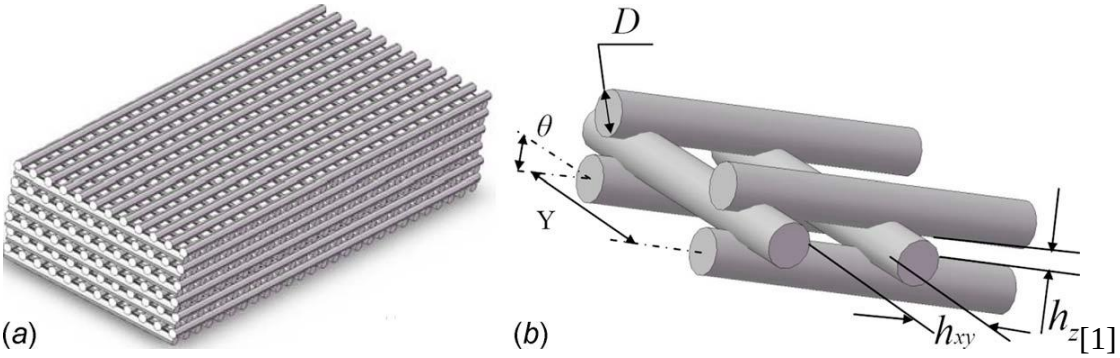
3.2.2 Scaffold used for model development

The scaffold for the model development which can be fabricated through RP techniques is shown in Fig. 2; the strand diameter (D) and the horizontal span (Y) are controllable during the scaffold fabrication [16]. Also shown in Fig. 2 are the distance between two adjacent horizontal (h_{xy}) and vertical (h_z) strands, which together represent the pore size. While both the vertical pore size (h_z) and the horizontal distance (h_{xy}) are associated with the strand diameter (D) and the horizontal span (Y), respectively, the vertical pore size (h_z) is also affected by the scaffold material properties due to the fusion

of the two strands. Based on previous research in our group [16], the vertical pore size (h_z) is determined by the diameter of the strand (D), the density of the scaffold material (ρ), the elastic limit stress (τ_e), the horizontal span (Y) and the angle between the two layers (θ) (Fig. 2). The approximate relationship can be described as follows:

$$h_z = D \cdot \sqrt{1 - \frac{\rho g Y}{2\tau_e} \cdot \sin\theta}$$

The values of the density (ρ) and elastic limit stress (τ_e) are different for different scaffold materials. In the present study, a chitosan solution with 40% hyroxylapatite (HA) gel (40g HA in 100 mL water) is assumed to be used for the scaffold fabrication and its elastic limit stress (τ_e) is 11.0 Pa as identified in [16]. In the present study, the strand diameter D was varied from 0.2 to 0.4 mm, while the horizontal span Y was varied from 0.5 to 0.9 mm. The corresponding vertical pore sizes are given in Table 1. With this information, the geometric model was constructed in SOLIDWORKS. From the geometric model, the porosity, which is defined as the ratio of the void volume to the total volume, was calculated for each scaffold. The calculated porosity values are also listed in Table 1.



[1]

Fig. 2. Geometric parameters for tissue scaffold.

Table 1. Pore size, hz, and porosity, ε, for different scaffolds

Horizontal Span, Y (mm)	Strand Diameter, D (mm)					
	0.2		0.3		0.4	
	h_z (mm)	ε (%)	h_z (mm)	ε (%)	h_z (mm)	ε (%)
0.5	0.179	66.9	0.268	49.9	0.358	34.0
0.7	0.170	75.8	0.255	63.9	0.340	51.7
0.9	0.160	80.6	0.240	71.3	0.320	61.2

3.2.3 Computational method

When a tissue scaffold is submerged in the fluid environment within a perfusion reactor, the fluid not only flows around the outside of the scaffold but also within the scaffold itself. The fluid deformation then results in the development of fluid stresses: of specific interest in this study are the shear stresses exerted on the surface of the strands of the scaffold. The model geometry created in SOLIDWORKS was imported into the commercial CFD package ANSYS-CFX, which was used to solve the Navier-Stokes equations to determine the velocity field and also the shear stress exerted on the scaffolds. In this case, the flow was treated as three-dimensional, incompressible flow of a Newtonian fluid.

CFX-Mesh as used to create three unstructured meshes with 284681, 622261 and 1142781 elements, respectively. The difference in the calculated maximum wall shear stresses between the last two meshes was approximately 1.5%. Therefore, the mesh with 622261 elements, shown in Fig 3(b), was assumed to be fine enough to accurately

determine the flow field. The simulation used a non-uniform unstructured mesh or grid in which the element size was varied for different parts of the bioreactor. Local grid refinement was used to resolve the tissue scaffold geometry, as shown in Fig. 3(c). Near the scaffold surface, the grid size ranged from 0.1 to 0.15 mm, while the maximum grid length near the wall of the bioreactor chamber was 7 mm.

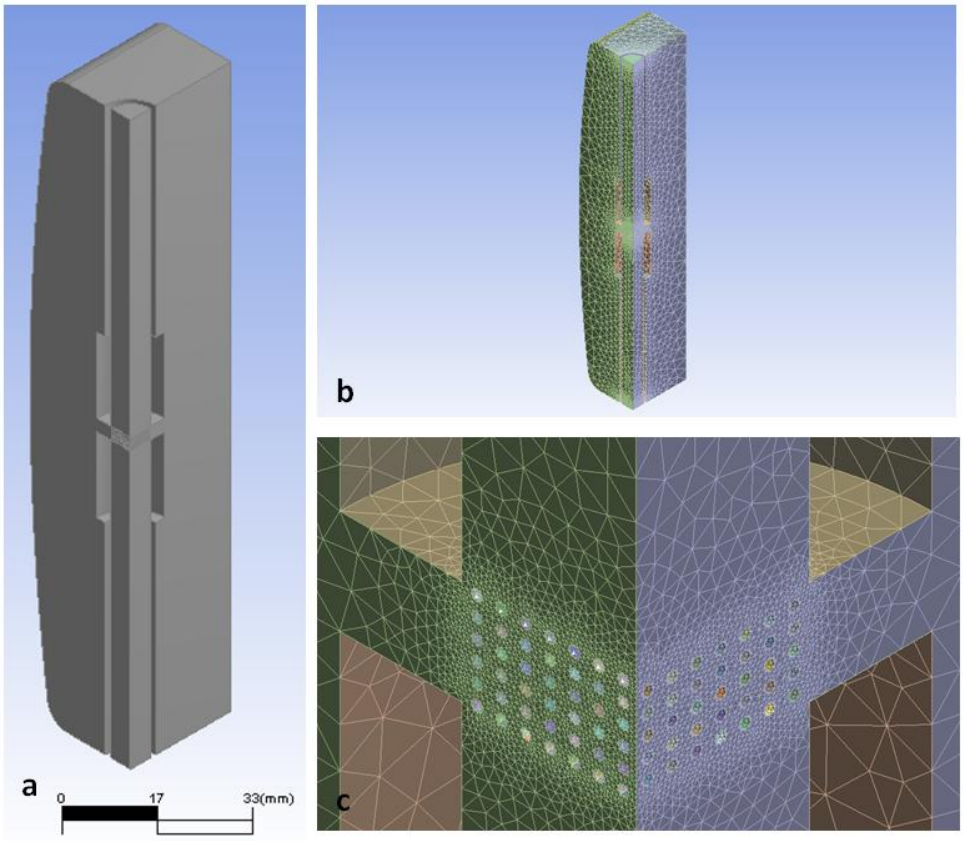


Fig. 3 a) Geometric model, b) mesh, and c) refined mesh around tissue scaffold.

3.2.4 Boundary conditions

As shown previously in Fig.1 (a), for a perfusion bioreactor, the fluid enters the

scaffold through the bottom strut. A constant mass flow rate boundary condition was specified at an inlet section located upstream of the scaffold within the supporting strut. In this way, the flow can develop within the channel inside the channel to simulate the actual experimental condition. Note that the internal flow can connect with the fluid outside the scaffold through the open channels of the scaffold, and in this way, the internal fluid creates a small disturbance in the fluid contained in the bioreactor. The outlet was placed at an exit plane located within the channel inside the strut. In this case, the average pressure at the outlet was set to zero. The walls of the chamber the struts and the scaffold were assumed to be no-slip, solid walls.

The simulations were first performed for a scaffold with D = 0.3 mm and Y = 0.7 mm. To investigate the effect of flow rate, three different flow rates were considered: 0.05ml/min, 0.1ml/min and 0.15ml/min. In order to assess the effect of geometry, additional simulations explored scaffolds in which the strand diameter (D) and horizontal span (Y) were independently varied, as shown in Table 1.

3.3 Results and Discussion

3.3.1 Comparison of flow field for perfusion and non-perfusion bioreactors

Simulations were initially carried out for a tissue scaffold with a strand diameter of D = 0.3 mm and horizontal span of Y = 0.7 mm, for the case of both perfusion and non-perfusion bioreactors. Fig. 4 shows the simulation results for the case of the

perfusion bioreactor. In this figure, it is seen that the majority of the streamlines go through the tissue scaffold, implying that there is strong perfusion inside the scaffold. In contrast, Fig. 4(a) shows that there is minimal fluid motion in other areas of the bioreactor. As a result, the strong perfusion produces relatively high shear stresses on the surfaces of the strands in some regions of the scaffold as shown in Fig.4(c). The shear stress typically is larger near the outer edge of the scaffold. This suggests that when seeding cells, one strategy might be to seed more cells in the center area of the scaffold to avoid the regions of high shear stress created by the perfusion flow.

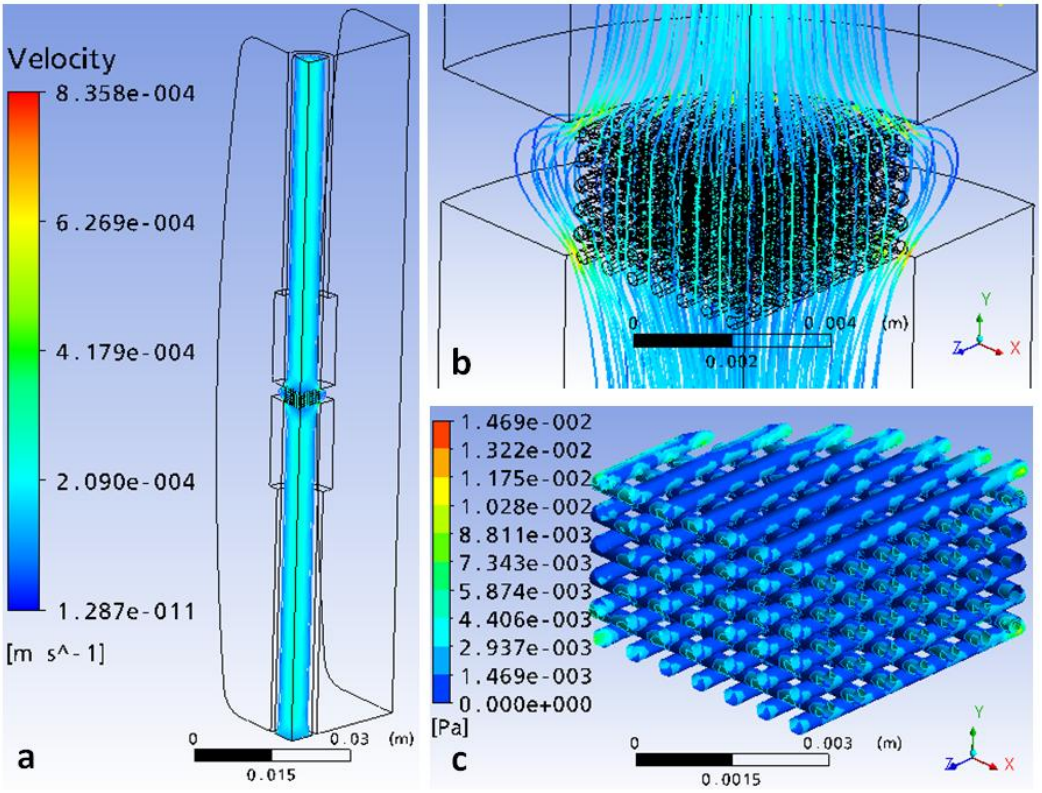


Fig. 4 Simulation results for the scaffold with D = 0.3 mm and Y = 0.7 mm in a perfusion bioreactor: a) velocity streamlines in bioreactor, b) velocity streamlines around the tissue scaffold, and c) surface shear stress distribution in the scaffold.

To better highlight the flow characteristics of a perfusion bioreactor a simulation of the same scaffold in the non-perfusion bioreactor, was carried out as shown in Fig. 5. Recall that for the non-perfusion bioreactor, the inlet and outlet were located in the wall of the bioreactor chamber, as shown in Fig.1 (b). It is seen from Fig. 5(a) that the flow occurs throughout the bioreactor. The average velocity in the scaffold area is 5.84×10^{-11} m/s and the average Reynolds Number based on the diameter of the scaffold strand is 6.54×10^{-8}. For the relatively low velocity levels near the scaffold, the shear stress in Fig. 5 (b) is almost zero.

Based on the comparison between Fig. 4 and 5, it is seen that in the perfusion bioreactor, the convection and hence mass transfer enhanced, which also results in increased levels of shear stress on the internal walls. These results suggest that for cell culture, the most suitable bioreactor depends on the specific situation and cell type. For example, if the cells require more nutrients and growth factors during the cell culture process, the perfusion bioreactor is more effective; however, the non-perfusion bioreactor is a safer choice if the cells are especially sensitive to the shear stress level. If the perfusion bioreactor is used, then the flow rate must be set to ensure acceptable levels of wall shear stress within the scaffold. In this context, the factors which affect the shear stress distribution and magnitude are considered in the next section.

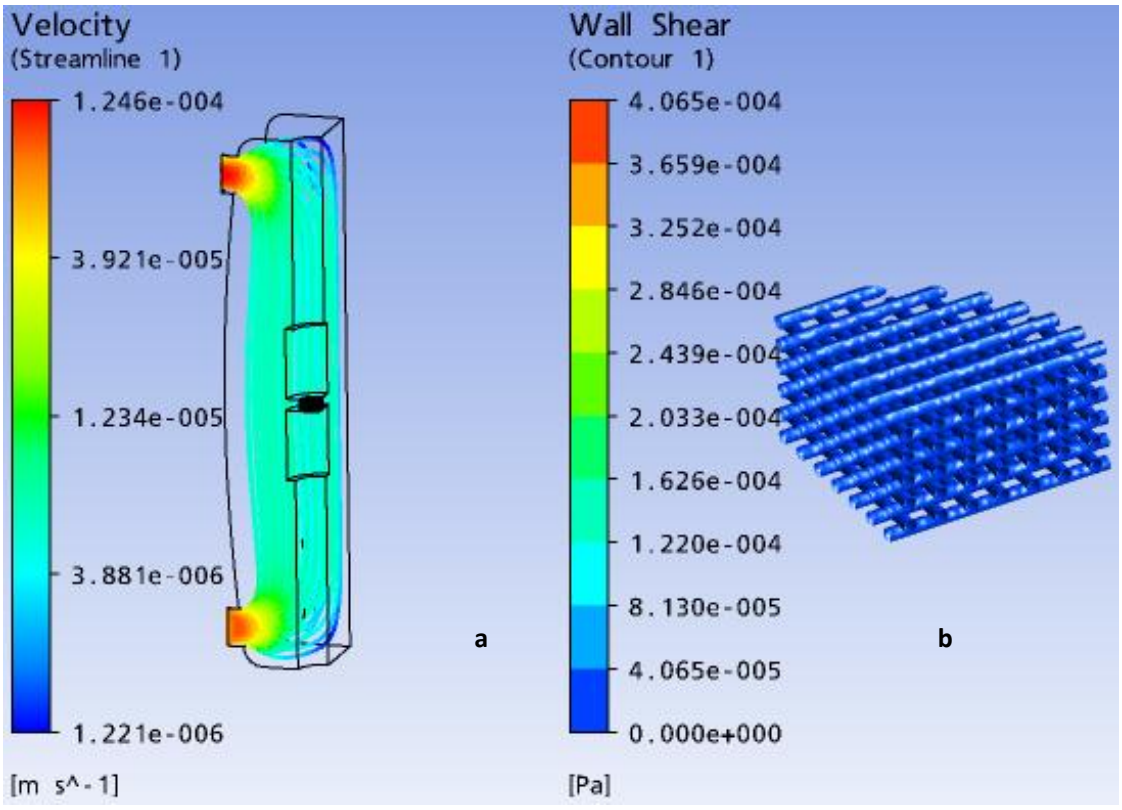


Fig. 5 Simulation results for the scaffold with D = 0.3 mm and Y = 0.7 mm in a non-perfusion bioreactor: a) velocity streamlines, and b) wall shear stress distribution in the tissue scaffold.

3.3.2 Flow field within the scaffold in the perfusion bioreactor

With the help of CFD, the flow field within the internal pores of the scaffold can be captured. The average velocity in the scaffold is 3.4768×10^{-4} m/s and the average Reynolds Number based on the diameter of the scaffold strand is 1.17×10^{-2}. In order to illustrate the details of the fluid motion within the scaffold, the velocity fields for cross-sections at two different locations were investigated. As shown in Fig.6, section I is

a plane section through the scaffold strand and represents the flow which is blocked by the scaffold strands; section II represents a plane section located between the two lines of strands and hence represents the flow which has a direct path through the scaffold channel. The simulation results presented below are for the case in which D = 0.3 mm and Y = 0.7 mm.

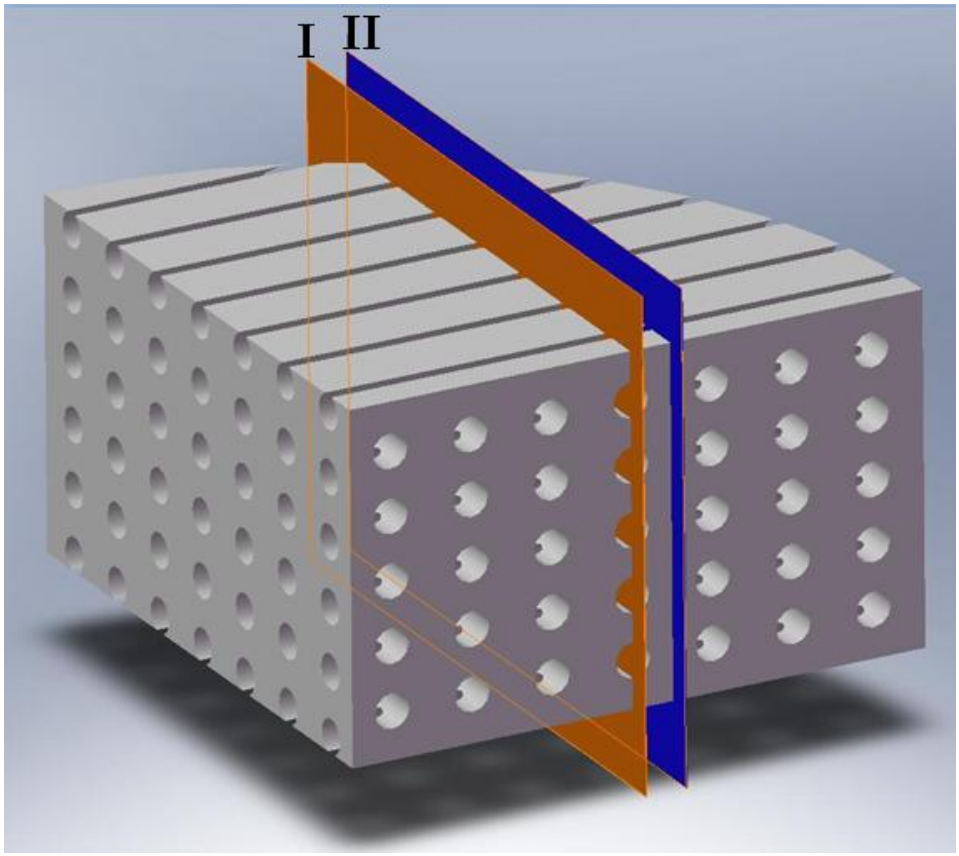


Fig. 6The location of the two sections used to visualize the flow.

To visualize the local flow, the velocity vectors in section I and section II are presented in Fig. 7 (a) and (b), respectively. Note that small arrows are used to show the local flow directions and the colors represent the magnitude. For section I, due to the

obstruction of the scaffold strands, fluid is squeezed out near the lateral surfaces of the scaffold, especially near the top and bottom of the scaffold, which are also the locations of enhanced velocity. Some fluid is observed to exit the scaffold and then re-enter the scaffold prior to exiting the outlet channel of the bioreactor located within the strut. For section II, the open channel within the scaffold provides a direct passage for the perfused medium. The local velocity magnitude is shown by color contours in Fig. 7 (c) and (d) for section I and section II, respectively. From these simulation results, it is seen that due to the shielding provided by the scaffold strands in section I, the velocity in the area between two horizontal strands is relatively low, implying that that this area would be suitable for cell attachment. In contrast, from Fig. 7 (d), it is clear that strong perfusion exists in the channel between two series of strands, which creates relatively high local velocities. Based on a comparison between Fig.7 (c) and (d), the region enclosed by the red line in Fig. 7(c) would be a favorable area for cells to adhere due to the lower flow velocity levels. When seeding cells, if priority is given to seeding in this area, especially on the top and bottom walls of the strands, the cells will have less likelihood to be washed out by the perfused medium.

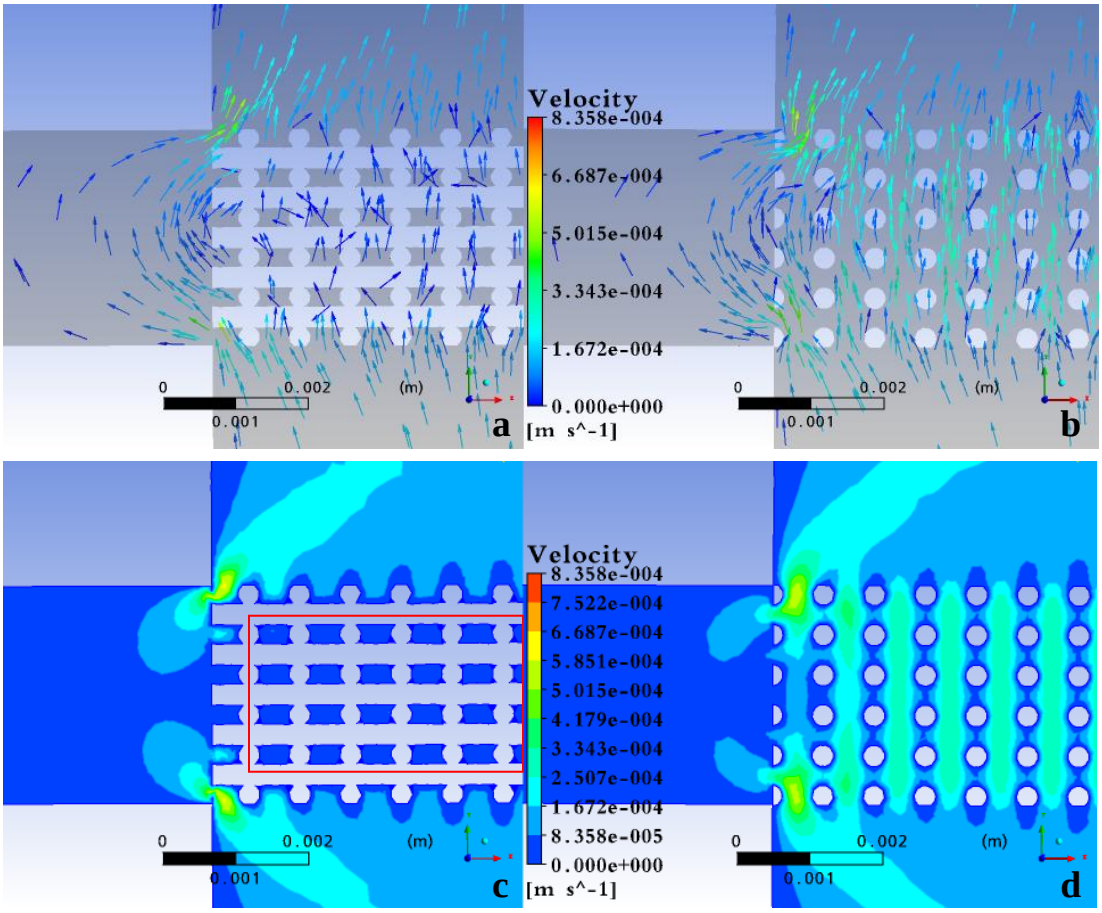


Fig. 7 Velocity distribution for two cross-sections at different locations within the scaffold: a) velocity vectors in section I, b) velocity vectors in section II, c) velocity magnitude in section I, and d) velocity magnitude in section II.

3.3.3 Wall shear stress within the scaffold in the perfusion bioreactor

The local wall shear stress within the scaffold can be affected by the scaffold geometric parameters including the strand diameter and the horizontal span as well as the flow rate of the circulated medium. Numerical simulations were performed for the cases presented in Table 1 and the results were compared to illustrate the effect of D and Y on

the wall shear stress distributions. Fig. 8 presents the discrete probability distribution for the magnitude of the wall shear stress for different values of D and Y. From the results, it is seen that the level of the wall shear stress values mostly appear in the bin centred on 1 mPa. With an increase in D (from top to bottom), the distribution tends to extend to higher pick values and the mean values also increases. Looking Fig.8 from left to right for a given value of D, with an increase in the value of Y, the wall shear stress has probability of appearing in the bin centred on 1 mPa. A similar conclusion can be drawn from Fig. 9, which shows the dependence of the average surface shear stress for scaffolds with different values of D and Y. This suggests that scaffolds with a smaller strand diameter can be used in cell culture in a perfusion bioreactor to limit the wall shear stress levels within the scaffold. Another summary conclusion is that so long as the mechanical strength criterion is satisfied, the horizontal span can be used to adjust the shear stress level within the scaffold. Specifically, for a given flow rate, a larger span will result in a reduction in the average shear stress level.

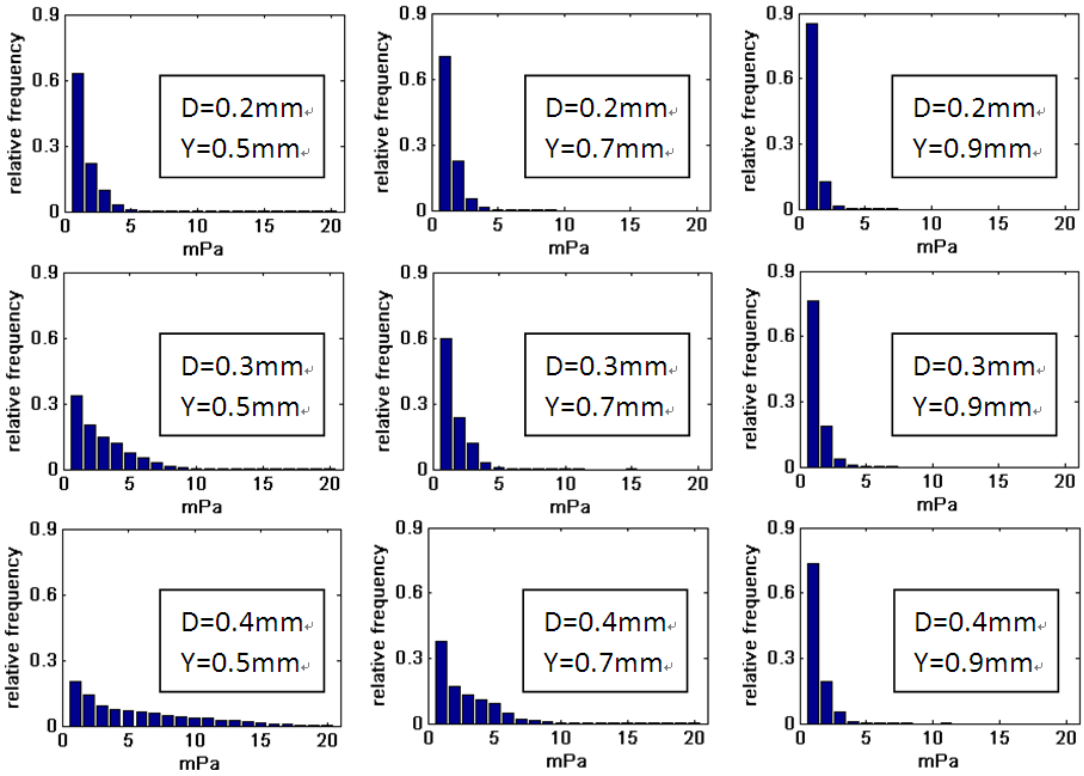


Fig. 8 Distribution of surface shear stress for scaffold in perfusion bioreactor.

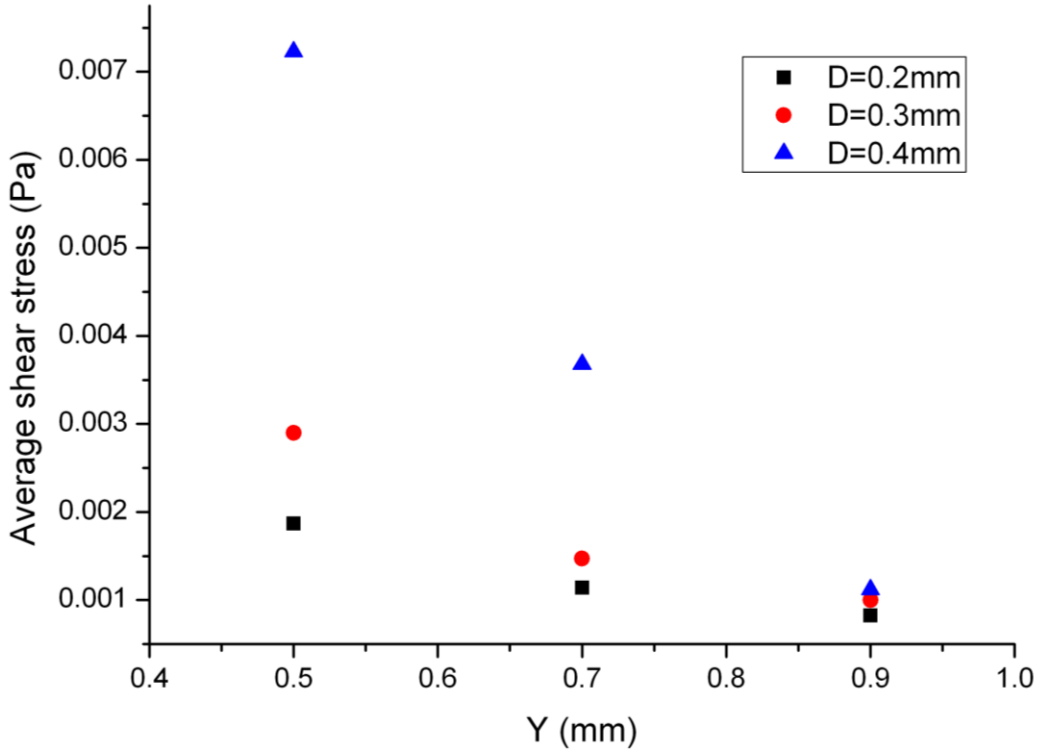


Fig. 9 Average shear stress distribution versus D and Y.

Fig. 10 demonstrates the variation of the wall shear stress with the flow rate of circulated medium for the scaffold with D = 0.3 mm and Y = 0.7 mm. The discrete distribution function indicates that as the flow rate increases, the wall shear stress values become smaller in magnitude (Fig. 10 (a)-(c)).This results in a decrease in the average wall shear stress (Fig. 10 (d)) , which has a linear relationship with volume flow rate. The approximate expression in Fig.10 (d) for the dependence of the shear stress magnitude on flow rate can be used to select the appropriate operating condition for a perfusion bioreactor for the specific scaffold parameters being considered.

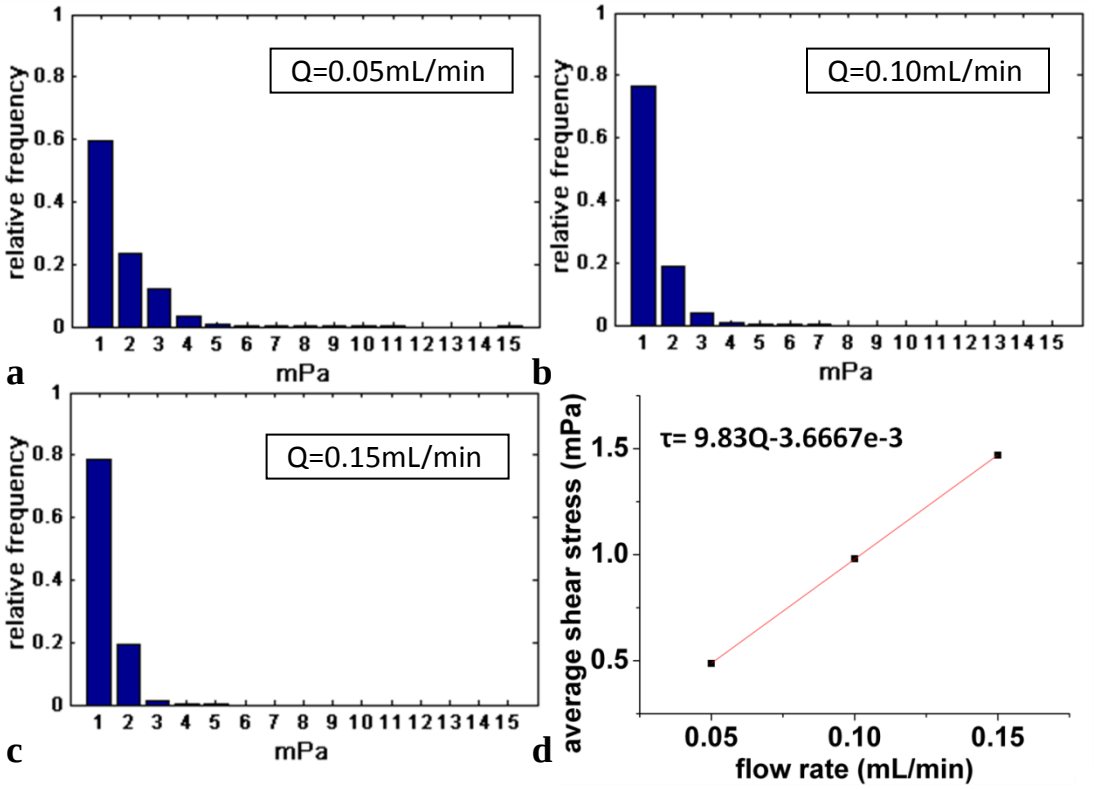


Fig.10 Shear stress distribution within scaffolds with different flow rates (Q): a) Q = 0.05 mL/min, b) Q = 0.10 mL/min, c) Q = 0.15 mL/min, and d) average wall shear stress versus Q.

3.4 Conclusions

The fluid flow inside and around a scaffold in a bioreactor is complex. This paper reports an investigation into such a flow within scaffolds cultured in both perfusion and non-fusion bioreactors. The simulation results demonstrate that the perfusion bioreactor provides a strong flow within the tissue scaffold, thus increasing the shear stress on the scaffold surface as compared to the non-perfusion bioreactor. The results also show that the value of the strand diameter and horizontal span affect the shear stresses on the scaffold surface. Generally, with an increase in the diameter, the shear stress level also increased; with an increase in the horizontal span, the shear stress decreased. The effect of flow rate, a controllable parameter in the cell culture process, was also investigated and it was found that the average shear stress level increased linearly with flow rate.

The knowledge obtained from this research provides insight into the velocity field within the scaffold and the corresponding shear stresses that occur during cell culture in a perfusion bioreactor. The effects of the controllable factors described here can be used to guide future scaffold design, as well as experimental studies.

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CHAPTER 4

CONCLUSIONS AND FUTURE WORK

4.1 Summary and conclusions

This thesis presents a study on the scaffold-based cell culture process using numerical methods, with a focus on modeling the mass transport and fluid flow. The main work and conclusions of this research are summarized as follows.

(i) In Chapter 2, a novel mathematical model to describe mass transfer in tissue scaffolds cultured in a perfusion bioreactor was developed, by taking into account the mass transfer and scaffold degradation. The model was validated using the data extracted from the literature. Based on the new model, simulations were carried out for the cell culture typically taken place in a perfusion bioreactor. The results demonstrated perfusion bioreactors can increase mass transfer within the tissue scaffold due to enhanced convection. The nutrient concentration and cell volume fraction are time dependent, but in different fashions. The controllable factors during both scaffold fabrication and cell culturing, such as porosity and flow rate, have a significant effect on the mass transport and cell distribution. It was found that an increase in porosity can reduce the inhibiting effect of the solid scaffold on nutrient transport, resulting in

an increase in the nutrient effective diffusivity. In addition, increasing the flow rate can enhance convection, thus promoting a more uniform distribution of both nutrient concentration and cell volume fraction. By means of the model developed, the nutrient transport and cell distribution can be predicted quantitatively.

(ii) Chapter 3 studied the flow within the scaffolds being cultured in both perfusion and non-fusion bioreactors by means of commercial CFD software. Also the effects of scaffold geometrical properties such as the diameter of the strands and the horizontal span, which can be accurately controlled in fabrication process, are investigated with the developed model. The results demonstrate that higher shear stress occurs on the surface of the scaffold strands in perfusion bioreactors compared to those cultured in non-perfusion bioreactors. The results also show that the strand diameter and horizontal span have a significant effect on the shear stress distribution within the scaffold. Specifically, the magnitude of shear stress increases with the strand diameter, while the shear stress is distributed with the lower magnitude as the horizontal span increases. The effect of flow rate, a controllable parameter in the cell culture process, was also investigated. It was found that the flow rate had a large effect on the maximum magnitude of shear stress. Based on the model developed, the shear stress magnitude and distribution can be predicted for different scaffolds and culture conditions.

4.2 Future work

To overcome the limitation of the present work, future work would be generally carried out from two streams: one stream is to improve the current model developed for the scaffold-based cell culture process and the other one is to conduct experiments to validate the simulation results.

In the present model, scaffold degradation was assumed to be a function of time; however, the degradation is also affected by the nutrient concentration, cell distribution, and temperature. Thus, the mass conservation equation of the solid frame needs to be included in the future research, along with the mass conservation equations of glucose, oxygen and cell. Other factors such as temperature and pH value also have an effect on mass transport and cell growth, so these factors need to be included in the model development.

To validate the simulation results from the present research, two types of experiments need to be carried out. For the mass transport in tissue scaffolds as presented in Chapter 2, one way to validate the simulation is to conduct the corresponding cell culture tests on the scaffolds, which are fabricated with the same structure as the one used in simulation and seeded with chondrocytes. For the cell culture tests, the medium with the desired glucose and oxygen concentration will be perfused through the scaffold under the flow rate specified in Chapter 2. The Bose biodynamic test machine may be used as the perfusion bioreactor and its boundary conditions are established in Chapter 2. If the experimental conditions, such as the material of the scaffold, the concentration of the

nutrients, the culture time are different from those used in the present study, the corresponding changes need to be made in the simulation.

To validate the simulation results of fluid field presented in Chapter 3, experiments are also required. To measure the flow velocity profile, the advanced micro velocimetry, such as micro particle image velocimetry (micro PIV), will be appropriate for use. Particles with a diameter of several hundred nm are suggested for use to capture the fluid characteristics in the scaffold pores, which are in the range 150 - 300 μm. To ensure the laser can go through the bioreactor and the scaffold, the scaffolds for experiments and the chamber of bioreactor need to be fabricated from the transparent material, e.g., acrylic. The flow rate can be controlled by regulating the pump that supplies the media for circulation. The velocity field measured by the micro PIV can be used to validate the results from numerical models.

APPENDIX A

The following explanations may be useful for mechanical engineers not familiar with tissue engineering terminology:

Apoptosis: the normal, genetically regulated process leading to the death of cells, triggered by the presence or absence of certain stimuli, such as DNA damage.

Chemotaxis: oriented movement toward or away from a chemical stimulus.

Extracellular matrix (ECM): the intercellular substance of body tissue.

Hypoxic: relating to a deficiency in the amount of oxygen delivered to the body tissues.

Lactate: salt or ester of lactic acid. Lactate is a product of fermentation and is produced during cellular respiration as glucose in broken down.

Glycosaminoglycan (GAG): any of a group of polysaccharides with high molecular weight that contain amino sugars and often form complexes with proteins.

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3 messages

Xin Yan <xiy562@mail.usask.ca> **Wed, Dec 7, 2011 at 1:11 PM**
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The information of paper:
Title:The effect of hydrodynamic shear on 3D engineered chondrocyte systems subject to direct perfusion
Author: Manuela T. Raimondi[1], Matteo Moretti[1], Margherita Cioffi[1], Carmen Giordano[2], Federica Boschetti[1], Katia Laganà[1], Riccardo Pietrabissa[1]
Published in: Biorheology Volume 43, Number 3-4 / 2006

My thesis:
Title: Modeling of mass transfer and fluid flow in perfusion bioreactors.

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The original paper:

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My thesis:

Title: Modeling of mass transfer and fluid flow in perfusion bioreactors.

--
Xin Yan

Master student

Department of Mechanical Engineering

University of Saskatchewan

Canada

--
Xin Yan

Master student
Department of Mechanical Engineering
University of Saskatchewan
Canada

Manuela T. Raimondi <manuela.raimondi@biomed.polimi.it> **Thu, Dec 8, 2011 at 11:55 AM**
To: Xin Yan <xiy562@mail.usask.ca>

Dear Xin Yan,

yes, you have my permission to use one of the figures of my paper in your Master Thesis.

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Best regards,
MTR

_____
Manuela T. Raimondi
Department of Structural Engineering
Politecnico di Milano
http://www.labsmech.polimi.it/index.php?id=119

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Xin Yan <xiy562@mail.usask.ca> **Thu, Dec 8, 2011 at 12:05 PM**
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