THE DETRIMENTAL EFFECT OF LOW VELOCITY STREAM-LINES ON AIR DIFFUSION TO TISSUE CONSTRUCTS IN A PERFUSION BIOREACTOR

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Abstract

Perfusion bioreactors are used to grow and condition blood vessel replacements in a biomimetic environment. Within the bioreactor environment, variables such as heat, pressure, gaseous diffusion and nutrients are accurately controlled with a view to supplying blood vessels and tissue constructs that which is required for their growth and development. In this study, a bioreactor for growth and mechanical conditioning of tissue engineered vascular constructs was constructed, and the oxygen diffusion characteristics within it were modelled for a time period from start-up. Mass, momentum and energy transfer were modelled using computational fluid dynamics for a 3D transient model of the bioreactor. Conditions that exist within a bioreactor for tissue-engineered vessels should be uniform throughout in order to promote the homogenous growth and development of the construct. This study found that the agitation of culture medium within the particular bioreactor created streamlines which had a detrimental effect on the oxygen diffusion through to the construct. Variation of agitation provided the ability to control how the streamlines in the chamber were described, enabling control of the oxygen diffusion characteristics. Computational fluid dynamics can be used to optimise the nature of oxygen diffusion characteristics within perfusion bioreactors.

Introduction

Bioreactors for blood vessel tissue engineering applications are complex systems designed to replicate the biochemical, mechanical and thermal environments which exist *in vivo* [1]. Numerous studies have investigated the biomimetic environment within bioreactors. Studies have been conducted that investigate the role of various growth factors, cell types and other factors on the growth and development of tissue constructs [2,3]. Work has also investigated the effects of fluid induced wall shear stresses on the tissue constructs [4] and how oscillating pressures can strengthen certain cells, such as smooth muscle cells [5,6].

Perfusion bioreactors have been developed to subject cell-seeded vascular scaffolds to fluid induced shears thereby increasing the supply of nutrients and the removal of waste [4]. Systems manufactured to apply varying or oscillating pressures on constructs and within tubular constructs have been found to increase the growth and proliferation of cells and the mechanical strength of the construct [6]. At present, the preconditioning of constructs is capable of producing blood vessel replacements with the required strength to perform their function within potential *in vivo* hosts without rupturing.

Oxygen is required for the respiration of cells within bioreactors and this oxygen diffuses into the culture medium from an oxygen source [7]. To promote homogenous growth and development of cells throughout the construct, it is necessary that the gaseous exchange, mechanical loads, supply of nutrients and removal of waste for each cell is uniform and that no adverse gradients occur. Uneven diffusion of oxygen may lead to non-uniform cell growth with resulting implications for the homogeneity of the tissue construct [8,9]. The objective of this study was to design and build a perfusion bioreactor suitable for growing and conditioning tissue constructs and to investigate the effects of agitation on oxygen diffusion through to the construct using computational fluid dynamics (CFD). The study investigated a transient numerical model of the mass, momentum and energy transport for the bioreactor for a certain time from start up.

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The nature of the streamlines within the bioreactor and their effect on oxygen diffusion was assessed, and modifications were made to the numerical model of the bioreactor to promote more even distribution of oxygen.

Materials and Methods

All culture medium and gas contacting materials were selected to be biocompatible. The bioreactor chamber was machined from a block of PEI 1000 (polyetherimide) with chamber dimensions of 70 × 70 × 80mm. The block was then split into three sections, a base section, to contact a stainless steel heating plate, a mid-section, to mount the tissue construct on polyurethane supports, and a lid, containing the gas inlet ports. A feedback agitator loop was used to connect, using a silicone rubber pipe, the outlet of the tissue construct, to the chamber medium, via a port on the base section. The inlet to the tissue construct and the outlet of the chamber were connected to each other using a compliant silicone rubber tube and a computer controlled peristaltic pump was used to pump the medium through the system. The gas inlet ports were connected to gas reservoirs (carbon dioxide and oxygen) via computer controlled one-way valves through 2μm miocrobiological filters. A pressure release valve was mounted onto the lid of the bioreactor to enable the periodic flushing of the chamber atmosphere. Figure 1 contains two views of the manufactured bioreactor.

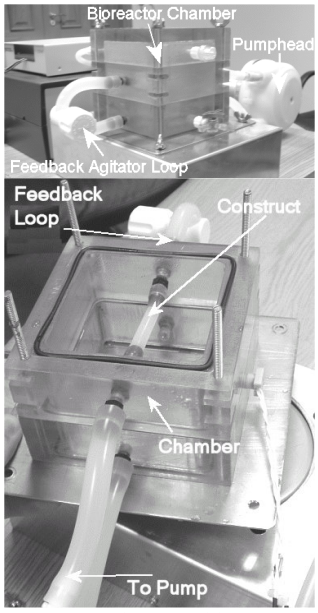

Figure 1. The experimental bioreactor chamber under investigation in this study.

The bioreactor stainless steel base-plate is maintained at 310K. The working fluid in the bioreactor is a tissue culture medium consisting of M199 culture medium with 10% fetal bovine serum. The atmosphere in the chamber of the bioreactor above the medium is 5% CO2 (to maintain the Ph) with 95% O2. As the culture medium M199 is a solution of salts, amino acids, vitamins and sugars dissolved in a distilled water solvent, it was assumed to have a specific heat at constant pressure of 4186j/kg.K and a thermal conductivity of 0.58W/m.K. The viscosity of M199 is batch dependant though a general value of 0.012Poise was determined using a cone and plate viscometer. The density was determined to be 1000kg/m^3. The specific heat of the CO_2-O_2 mixture was estimated at 900j/kg.K. The mass diffusivity of oxygen into the mixture is 2.8×10^{-9}m^2/s. The oxygen consumption of mammalian cells varies from 0.02 to 0.2kg/m^3 per hour for a culture of 10^6 cells/ml [7]. Oxygen diffuses into the medium from the atmosphere through the medium surface. Therefore, it is critical to ensure that sufficient oxygen is reaching the cells. The solubility of oxygen in an air-saturated aqueous solution is 0.22mM at 37ºC.

A solid model of the experimental bioreactor chamber was created using Gambit 2.0 (Fluent Europe). Due to the axi-symmetric nature of the bioreactor, the model was split through the chamber/construct centreplane and only one-half of the model was exported for solution. The model was meshed using 0.3mm quadrilateral elements within the construct, and 0.7mm quadrilateral elements within the chamber. Approximately 250,000 8-node quadrilateral elements were required to mesh the model using a paved scheme. All elements were found to have an aspect ratio less than 10 and an equi-angle skew less than 0.85. Grid independence was defined as being within 2% of limiting values of velocity and WSS through the construct and within the chamber. The resulting mesh was exported to Fluent 6.0 (Fluent Europe) for solution. A transient, 3D, Newtonian analysis was performed for solution of the governing equations, namely, the continuity, Navier-Stokes, convection-diffusion and energy equations. Pressure velocity coupling was performed using the PISO method with the momentum, species and energy equations solved using the QUICK scheme. The model was run for 600 seconds with a time-step interval of 5 seconds. Data was acquired every 12 steps (60 seconds). Convergence criteria was 0.001 of the residuals for continuity, velocity, species and 0.000001 for the energy equation.

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Models were solved on a Dell Precision 670 3.6 GHz dual processor workstation with 3Gb RAM.

Four models were investigated, namely, a model for oxygen diffusion with no flow, and three different oxygen diffusion with flow models. The oxygen diffusion with no flow was to be used as a control for the three models with flow. Figure 2 shows the computer solid model used in the investigations, together with a drawing of the centre-plane cross-section. The three different outlet positions used in the investigations are shown in the drawing located in the lower half of model. A steady inlet velocity of 0.2m/s was applied at the inlet to the tissue construct. This velocity represents the peak flow through a vessel of comparable size [10]. A steady flow investigation was performed as a preliminary to more detailed pulsatile flow analyses due to the insight steady flow computations give without the inherent complexity and computational expense associated with pulsatile investigations (11).

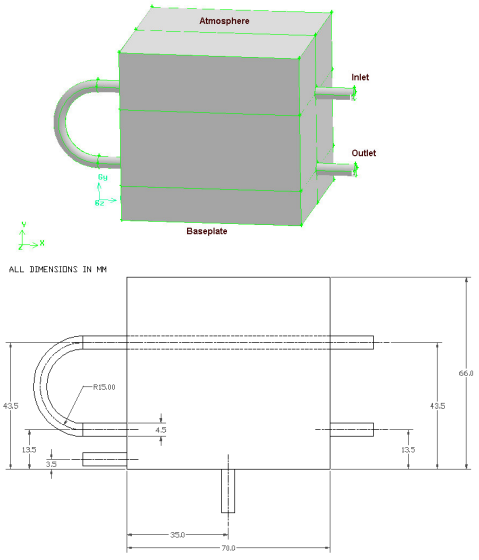

Figure 2. Solid model of the experimental bioreactor showing the inlet and outlet to the tissue construct/chamber. The line drawing shows the inlet, the mounted tissue construct and the three outlets investigated in this study.

Two fluids are modelled as being in contact, namely, the culture medium, M199, and the oxygen gas. The medium is modelled with a viscosity of 0.0012Pa.s and a density of 1000Kg/m^3. The specific heat at constant pressure is assumed 4186j/kg.K with a thermal conductivity of 0.58W/m.K. The molecular weight is 18.1 kg/kg.mol. The atmosphere is modelled with 95% O_2 with the gas having a specific heat of oxygen equal to 900j/kg.K, a density of 1.3kg/m^3 and a molecular weight of 32kg/kg.mol. For the mixture, the density is calculated using the volume weighted mixing law and the viscosity is determined using the mass weighted mixing law. A mass diffusivity of 2.8×10^{-9}m^2/s is set for oxygen [12].

The construct mounted in the bioreactor is initially a porous material with 95% porosity. It is necessary to model the porosity of this material in order to establish how construct porosity affects the mass, momentum and heat transfer in the bioreactor. There is a significant pressure drop across the construct which results in fluid passing through the construct into the surrounding media and also deceleration of the fluid passing through the construct and therefore a reduction in wall shear stress along the lumen wall.

In laminar flows through porous media the pressure drop is typically proportional to the velocity and the pressure-drop coefficient can be set to zero, resulting in Darcy's law:

$$\Delta P = -\frac{\mu}{\alpha}\vec{v} \qquad (1)$$

Therefore, the permeability and the thickness of the material are sufficient to define the pressure-jump permeability model in FLUENT. A 97% porous scaffold has been previously reported as having a permeability of 1×10^{-6}m^2 [13] and the wall thickness was set to 0.5mm. The pressure jump permeability model was found to produce velocity motions exterior to the construct wall which influenced the localised motion of flow. Porosity had a significant effect on decreasing the magnitude of the low velocity streamlines generated within the bioreactor due to dropping the pressure within the construct and the egress of fluid through the walls of the construct. However, due to the poor quality nature of the flow within the bioreactor and the inconclusivity of the results, the porosity model was not used in the detailed analyses following.

Results

The following results are for the four numerical models described in the previous section. By comparing the centre-plane streamlines and oxygen diffusion contour plots of the three varying outlet models to that of the control model, it was expected that insight into the effect that low velocity streamlines was having on the diffusion of oxygen in the system, would be attained.

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The results for the control test are shown in figure 3. It may be seen that after 600 seconds the 0.1kg/m^3 oxygen contour line has diffused evenly across the bioreactor toward the construct. There are no adverse gradients present.

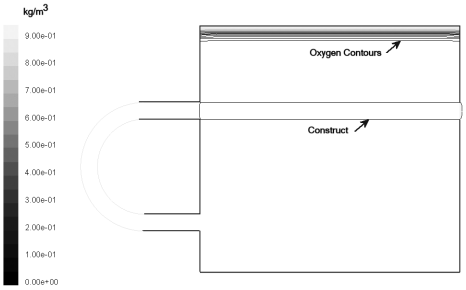

Figure 3. The oxygen concentration contours (kg/m^3) for the no-flow model after 600s. Oxygen is diffusing through at the same rate.

The next test involved enabling flow through the construct, around the feedback agitator loop, through the chamber with egress through oulet number 1. Figure 4 shows a velocity vector plot for the region above the construct. It may be seen that love velocity vectors are occurring of approximately 0.3 – 0.8mm/s.

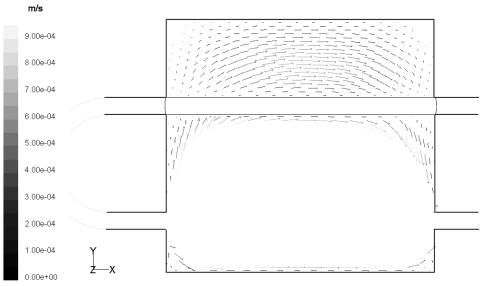

Figure 4. Velocity vector plot of model 1 at t = 600s. Velocities of up to 0.8mm/s are present in the region above the construct.

Figure 5 then presents the streamlines and the oxygen contours associated with these low magnitude velocity vectors. It is evident from figure 5 that the low velocity streamlines present in the chamber are causing oxygen build up to the rightside of the chamber with depletion on the leftside. In passing through the bioreactor, the medium is causing motion of the dissolved oxygen around the bioreactor.

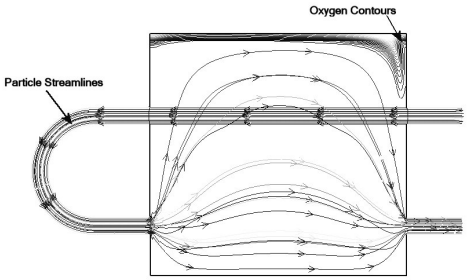

Figure 5. The effect of the low velocity streamlines on the oxygen concentration contours for fluid egress through outlet 1. It may be seen that low velocity streamlines are causing undesirable gradients to occur within the chamber. The oxygen is diffusing through to the construct at a faster pace to the rightside of the bioreactor than at the leftside.

The egress of fluid is then changed from outlet 1 to outlet 2 and finally to outlet 3. It may be seen from figure 6 that streamlines of sufficient magnitude are occuring within the bioreactor to cause similar non-uniformities as for flow egress through outlet 1. The nature of the disturbance is less than in the previous figure, nevertheless, it is significant.

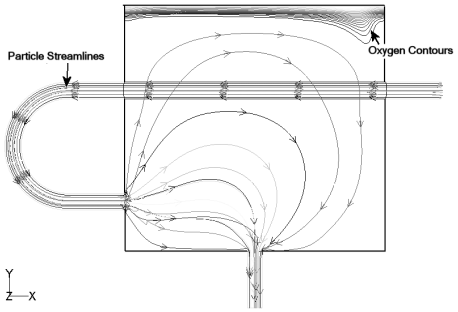

Figure 6. The effects of low velocity streamlines on oxygen concentration for egress via outlet 2.

Figure 7 shows the flow through outlet 3. With minimal agitation occurring, the streamlines so pronounced within the two previous models are no longer present above the construct. Within this model, the low velocity vectors occurring above the construct were found to be less than 0.01mm/s. While there is a slight disturbance of the oxygen diffusion through to the construct, this disturbance is not significant.

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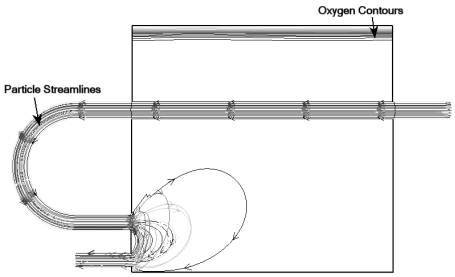

Figure 7. The effects of low velocity streamlines on oxygen concentration for egress via outlet 3.

The maximum and minimum penetration of the 0.1kg/m^3 oxygen contour was then measured for each of the 4 models. Penetration was defined as the displacement during the 600 seconds interval of the 0.1kg/m^3 contour line. The 0.1kg/m^3 contour line represents a concentration of oxygen required for normal growth of cells in the bioreactor.
The total distance from the atmosphere/medium interface to the construct surface was found to be 22mm and the following figures show the displacement of the contour line over the time interval.

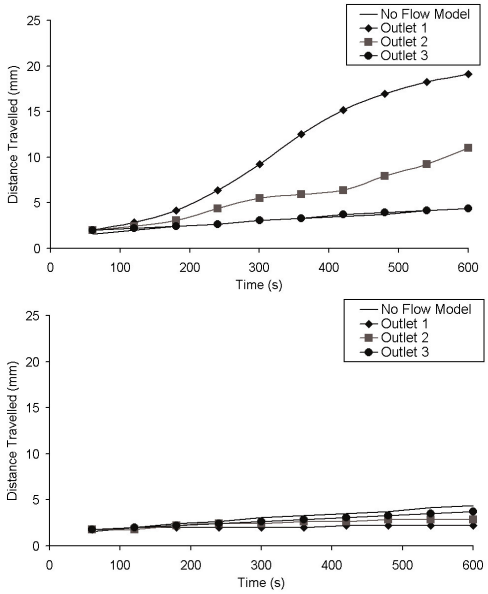

Figure 8. The displacement of 0.1kg/m^3 contour through the bioreactor (maximum and minimum distances for each) over the course of the simulation. It may be seen that the least disturbance to the oxygen diffusion occurs with medium egress through outlet 3.

The results for contour penetration show that with fluid egress through outlet 3, the least amount of oxygen diffusion disturbance is occurring. After 600 seconds, outlet 1 exhibits a maximum contour displacement of 19mm while outlet 2 is 11mm and outlet 3 is 4.4mm. The penetration for the no flow model is 4.4mm. On the minimum displacement, outlet 1 displaces 2.2mm, outlet 2 displaces 2.9mm and outlet 3 displaces 3.7mm. The no flow model displacement is 4.4mm. It is clear from these results that flow through outlet 3 gives the least significant difference to that which occurs in the control model.

Discussion

Perfusion bioreactors are widely used to grow and condition tissue engineered vascular constructs. Bioreactors accurately control the various biochemical, mechanical and thermal environments required for the growth and development of the cells constituting the construct. Investigative studies have been performed on the bioreactor environment and the conditions required to grow constructs suitable for use in blood vessel replacement. While a variety of different biochemical and mechanical contributory factors have been investigated, little attention has been paid to the actual design of the chamber in which the constructs are grown [14]. This study aimed to address this. A typical perfusion bioreactor designed to grow tubular constructs was modelled using CFD in order to gain insight into the fluid dynamic nature that occurs within the bioreactor. Due to the complexity of the system and its variables, a simplified model using a steady inlet velocity, was investigated for a period of time from start-up. It was found that during this start-up phase, that low velocity streamlines which occur due to the agitation of the medium, can have a detrimental effect on the oxygen diffusion through to the construct. Indeed, significant differences were found in the diffusion of oxygen through the medium for a variety of different fluid egress points. It has been observed that low velocities in the range of 0.1 to 1mm/s can influence oxygen diffusion. While oxygen diffusion is influenced during the start-up phase, it is possible that following an initial start-up period, the conditions throughout the medium will homogenise due to the diffusive nature of oxygen and the bioreactors agitation mechanism. Nevertheless, there exists a start-up phase for the bioreactor investigated in which oxygen gradients may be created which can have an adverse effect on the uniform growth and development of cells. Therefore, in the future use of a bioreactor of this type, it is preferable that oxygenation of the medium be allowed take place prior to placing the construct within the bioreactor.

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Conclusions

CFD can be used to model oxygen diffusion in perfusion bioreactors. Low velocity streamlines occurring within bioreactors can have a detrimental effect on oxygen diffusion during the start-up phase. A specific bioreactor chamber can be re-engineered to produce optimum oxygen diffusion through to tissue construct. Agitated bioreactors should be designed using CFD in order to optimise the biochemical environment within the bioreactor. Transient conditions exist within a bioreactor upon start-up and these should be allowed to minimise prior to placing constructs inside.

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