Development of a Mathematical Model for a 3-D Perfused Bone Marrow Culture System

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Abstract

Bone marrow (BM) is a three-dimensional tissue and the site of blood formation. The successful reconstruction of the BM has enormous potential benefits to the society. Its success depends, partly, on the development of a suitable culture system necessary for its growth. In the present work, we have developed a mathematical model for a 3-D perfused BM culture system that captures the growth characteristics of the BM microenvironment, and as a first approximation, mimics the structural entity of *in vivo* BM. The model has been used to investigate cellular growth, fluid flow, shear stress and nutrient distribution within a Rotating Wall Perfused Bioreactor - RWPB (Synthecon Inc.). Based on our preliminary results, for a 2 week culture, it was observed that the total cell number increased by 30 and 38 fold for a scaffold with 90% and 80% porosity, respectively. Further analysis showed that, with a 20% oxygen supply, the oxygen tension was sufficiently maintained to avoid hypoxic regions, but with increasing axial distance in the bioreactor some areas experienced low oxygen levels; though this effect varied with porosity of the scaffold.

1.0 Introduction

With more than 5000 cancers being diagnosed in the UK and 200 000 in the US per week [1, 2], there is an unprecedented need for bone marrow transplants. Due to numerous complications (donor availability, immunogenic compatibility, etc.), the demand exceeds the supply. To overcome this difficulty, researchers are attempting to reconstruct functional human tissues *ex vivo* [3]. There has been a great deal of attention and resources devoted to the bone marrow in an attempt to realise this goal. Developing an ex vivo system able to mimic the in vivo haematopoietic system would provide sufficient amount of cells for transplantation, but unfortunately most of the culture systems provide a growth environments that differ significantly from physiologically realistic conditions [3].

Bone marrow, in adults, is the site of blood formation (haematopoiesis), and has an intricate three dimensional (3-D) architecture with an intravascular and extravascular component. The intravascular space consists of varying vessel types, including the nutrient arteries, radial arteries, sinusoidal network and the central sinus (Figure 1A). The extravscular space is the site for haematopoiesis, which is a complex process that is controlled precisely by the 3-D bone marrow microenvironment. The constituents of the BM microenvironment include, the extracellular matrix (ECM) and the stromal cells, and the latter are responsible for producing and secreting the cytokines [4]. The eight different lineages of bloods cell originate from the haematopoietic stem cells (HSC). These stem cells have the capability to self-renew, proliferate and differentiate. In the process of an asymmetrical division, an undifferentiated stem cell and a differentiated daughter cell are produced. The daughter cell is committed to two lineages: myeloid and lymphoid. The myeloid cells further differentiate into erythrocytes (red blood cells), monocytes, monocytes, granulocytes (neutrophils, eosinopils, and basophils), and platelets, while the lymphoid lineage mature into T-cells, B-cells and NK cells [5].

Malfunctions of either the intra/extra-vascular space can lead to several BM diseases, such as leukaemia. At present, the treatment for such diseases is bone marrow transplantation. The lack of suitable donors and the complications of allogeneic transplants have led the research community to focus on developing an ex vivo expansion BM culture; offering a great promise for clinical use [6]. However, there are still several questions that remain requited for the development of new methodologies for producing cultures of clinically relevant populations of haematopoietic stem cells (HSC).

Traditionally, ex vivo bone marrow culture systems have been two dimensional (2-D) involving the use of flask cultures. The static nature of these systems does not facilitate mass transfer of nutrients and metabolites [7]. In addition, they can only support monolayer growth which is not suitable for the necessary cell-cell contact, as observed in the *in vivo* microenvironment [8]. Bioreactor systems such as stirred, perfusion and airlift bioreactors have been developed and used for the growth of HSC cultures to allow optimum control of culture parameters, further enhance mass transport and to support high density growth [9-12]. Moreover, they allow more efficient control of culture parameters. However, the drawbacks of such systems include high shear stress, and in certain cases they are also unable to provide suitable 3-D growth environment. To overcome some of these deficiencies, porous scaffolds have been used to support the 3-D growth of cellular components. In addition, porous scaffold offers a large surface area to sustain high cell density growth [8, 13]. Scaffold properties, such as porosity, interconnectivity and pore size can influence cell orientation and medium transport efficiency, which ultimately affect the degree of cell expansion. It is apparent that the use of scaffold is advantageous [8, 14, 15], as it has been shown to have increased cell proliferation in comparison to flask culture, but most of the previous studies were performed under static conditions and consequently suffered from limited mass transport [14, 16].

In addition to the 3D microenvironment, culture parameters are also crucial and need special consideration in the development of haematopoietic culture systems. More specifically, inoculum density, medium exchange rate, nutrient concentration (oxygen, growth factors and glucose) play an important role in the growth of continuous HSC cultures. Among these parameters, special consideration is required when cultivating HSC that are known to have a high growth rate, for example, (1) inoculum density should be kept at a minimum to avoid overcrowding and inadequate nutrients supply, (2) as high levels of oxygen tension leads to cellular toxicity and thus can lead to cell growth inhibition (or even death), and (3) high medium exchange rate allows an increase in nutrient supply with concomitant removal of waste (e.g. CO_2, ammonia). Such control mechanism has led to significant improvement in stem cell maintenance and proliferation, and thus further supporting the need for efficient control of flow conditions and culture parameters within the ex vivo culture system.

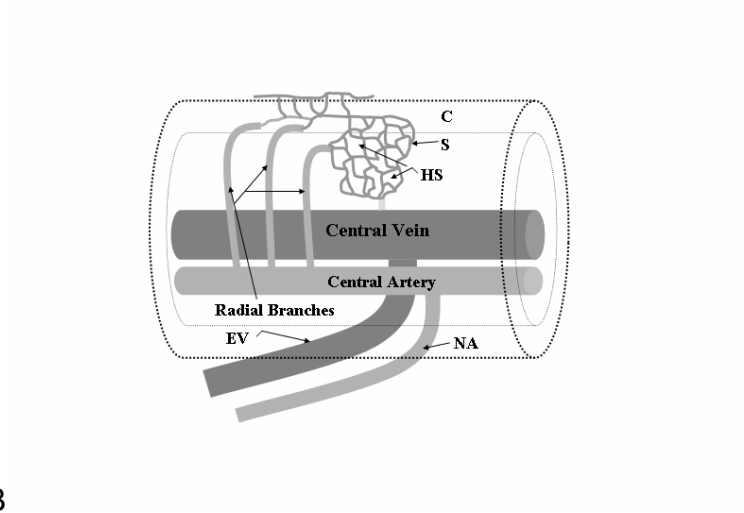
Utilising experimental methods to obtain culture parameters to sustain the normal haematopoietic functions is considered to be cost inefficient, and thus mathematical modelling is an attractive alternative that provides optimised conditions for the growth of BM cells. It also allows, (1) prediction of the underlying transport mechanisms within the system, (2) analysis of the spatial nutrient distribution and cellular growth and (3) visualisation of the flow patterns within the vessel of interest. Various mathematical models have been developed to describe the associations between the growth of the stem cells, and the flow conditions and nutrient concentrations within a specified culture system. However, these models are limited to the homogeneous system, and do not account for BM heterogeneity.

In the present work, the Navier-Stokes equations were combined with the porous media theory to develop a numerical model that captures the multiscale nature of the BM intravascular arrangement (qualitatively) and the BM functionality based on its known physiology. This paper reports our preliminary results on the investigation of multi-lineage cellular growth within a scaffold (with varying architecture), distribution of the fluid flow, shear stress and nutrient distribution within a rotating wall perfused bioreactor' (RWPB). The bioreactor is capable of providing efficient mass transport of nutrients and metabolites as well as low mechanical stress necessary for cellular growth [17].

2.0 Mathematical Model

The model equations presented here describe the fluid, oxygen and glucose transport coupled with the cellular growth kinetics in a specified reactor configuration. The bioreactor geometry is shown in Figure 1B, and all the necessary dimensions in Table 1.

A



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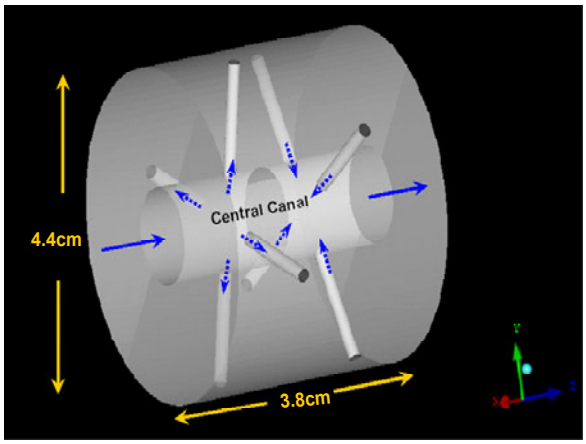

Figure 1. (A) Architecture of the bone marrow. (B) Architecture of the scaffold.

Table 1. Bioreactor and scaffold parameters

Bioreactor Parameters	**Value**
Vessel Volume	$50.41cm^3$
Vessel Length	3.8cm
Central canal radius	0.8cm
Vessel radius	2.2cm
Scaffold Parameters	
Scaffold Volume	$50.06cm^3$
Scaffold Length	3.8cm
Outer Tube Radius	2.2cm
Inner Tube Radius	0.8cm
Branches Diameter	0.2cm
Branches Position	1.15cm, 3cm

2.1 Transport equations

The fundamental transport equations for fluid flow are the continuity and Navier-Stokes equations [18]:

$$\frac{\partial}{\partial t}\rho + \nabla \bullet (\rho \cdot v) = 0 \tag{1}$$

$$\rho\left(\frac{\partial v}{\partial t} + \vec{v} \bullet \nabla v\right) = -\nabla P + \mu \nabla^2 v \tag{2}$$

where ρ is the density of the fluid, v is the velocity of the fluid, μ is the viscosity of the fluid and P is the pressure in the system. The flow inside the vessel is assumed to be laminar, incompressible and Newtonian for both porous and non-porous regions. The density and viscosity of the fluid is assumed to have the same properties as water at 37°C.

In the scaffold region, a generalized transport model which accounts for the convective term is used to represent flow inside the porous media [19]:

$$\frac{\partial}{\partial t}(\gamma\rho U) + \nabla \bullet (\rho(K \cdot U) \otimes U) - \nabla \bullet K\sigma = -\gamma R \cdot U - \gamma \nabla P \tag{3}$$

where γ is the porosity, K is the permeability, σ is the stress tensor and R is the resistance term. Permeability of the porosity region is calculated using Brinkman's theory [20]:

$$K = \frac{D_P^2}{72}[3 + \frac{4}{1-\gamma} - 3(\frac{8}{1-\gamma})^{\frac{1}{2}} - 3] \tag{4}$$

where D_p is the particle diameter. The porous structure is extremely complex and is arranged in a random manner, therefore, simple assumptions were made to facilitate the analysis of the

internal perfusion dynamics. The physical properties of the scaffold, which represents the extra-vascular region of the BM, are assumed to be isotropic and homogenous, and the dimensions of the scaffold do not change with time (i.e. there is no flow blockage or deposition within the scaffold).

2.2 Nutrient Transport Model

Glucose and oxygen are the two crucial nutrients to support the growth of cells and thus both are considered in this model. Equation 5 is a general mass transport equation used to describe the transport for each nutrient, with the consumption kinetics being represented by the Double Monod Kinetics-DMM (Equation 6):

$$\frac{\partial}{\partial t}\rho C + \nabla \rho UC = \nabla \cdot D\nabla C - k_m \tag{5}$$

where D is the diffusion coefficient of the substrate in the medium, in this case, the glucose and the oxygen; k_m is the metabolic consumption by cells and it is described by a DMM equation [21]:

$$k_m = K_{\max}\frac{C_O C_G}{(1+k_O C_O)(1+k_G C_G)} \tag{6}$$

where C_o and C_G are the oxygen and glucose concentration; k_o and k_G are the kinetic coefficients for oxygen and glucose respectively; $K_{\max}$ is the rate constant in the Michaelis-Menten type expression. The specific uptake rate is dependent on the cell number, and therefore the value $K_{\max}$ is coupled to the growth kinetics model to account for the dynamic behaviour in the bioreactor. Although a heterogeneous population of BM cells is considered, progenitor granulocytes dominate the extravascular compartment and therefore, the value of the consumption term used in all metabolism equations is specific to this cell type.

2.3 Cellular Growth

Due to the complexity of the four stage haematopoietic process, we assume that cellular growth can be divided into two subunits: progenitor and mature cells. In accordance with this approach, equations 7 and 8 can be adapted to describe either the growth of progenitor or the mature cells. The particular cells under consideration include Pluripotent Stem Cell (PSC), Stem Cell (SC), Myeloid Stem Cell (MySC), Lymphoid Stem Cell (LySC), Colony Forming Unit – Granulocyte, Macrophage (CFU-GM), Colony Forming Unit – Megakaryocyte (CFU-MEG), Burst Forming Unit – Erythroid (BFU-E), Colony Forming Unit – Eosinophils (CFU-Eo), and the mature cells such as erythrocytes, granulocytes, lymphocytes, megakaryocytes and macrocytes. The growth kinetics for all the cell types is represented by the following equation [22]:

$$\frac{d[C_1]}{dt} = k_e^X \gamma[C_1] + k_d^X[C_0] - \sum_Y k_d^Y[C_1] - k_k^X[C_1] \tag{7}$$

where C_0 and C_1 are the cell concentrations for the previous and the present stage of the differentiation process, respectively. The constants k_e^X , k_d^X , k_d^Y , and k_k^X represent self-expansion, generation, consumption and death term respectively. γ is the limiting coefficient defined as:

$$\gamma = \left[\frac{C_{MAX} - \sum_{\text{all types of cells}} C}{C_{MAX}} \right] \tag{8}$$

where γ ranges between 0.4-0.8 and is dependent on nutrient and space availability.

In both models, there are no convection and diffusion of cells, assuming all cells are attached to and grow around the scaffold. The boundary conditions for the above model include no-slip condition for the fluid at the bounding walls, a uniform inlet concentration for the oxygen and the glucose and finally a no flux condition on the exterior domain for all components. The parameter values used in the simulation are given in Table 2 and 3. Where possible, these values correlate well with haematopoietic cultures.

3.0 Results and Discussions

The results presented here represent only preliminary data obtained from simulations of growth kinetics and nutrient distribution in haematopoietic cultures, using the model described earlier. From the fluid flow calculation, the maximum shear stress in the scaffold is 0.111 dyne/cm^2, which is significantly lower than the threshold values (0.92-10 dyne/cm^2) that may cause cell damage, and thus leading to reduced cell viability [23, 24]. In regards to the velocity distribution, our data indicate that the average velocity is approximately 0.09 mm/s in the scaffold region. This is consistent with the known BM sinusoidal data, where the blood flow velocity ranges between 0.01-0.3 mm/s.

In Figure 2, we present the data for the haematopoietic culture after two weeks. From an initial cell seeding of 10^6 CD 34+ (cells/ml), the increase in cell number is around 2.9×10^7 (cells/ml) and 3.8×10^7 (cells/ml), representing a 30 and 38 folds increase for a 90% and 80% porosity, respectively. These cell populations are consistent with experimental observations [25]. Furthermore, the total CFU-GM number obtained is 3.68×10^8 (per reactor @ 90% porosity, data not shown). Data in the literature suggest that the minimum CFU-GM number required for a 70kg patient transplantation is 1.4×10^7 [26, 27], indicating that with the conditions of the simulated haematopoietic culture sufficient cellular expansion is achieved.

In contrast to static cultures, perfusion system facilitates oxygen (and other nutrients) transport, which in turn promotes the optimum growth of haematopoietic cells. Figure 2 shows the average oxygen tension (per day) inside the bioreactor, and from this datum, it is evident that oxygen tension is depleted with increasing cell number; at the end of the simulated culture, the oxygen tension is around (5-6)%. Ishikawa and Ito showed that the average oxygen tension in the human BM is around 7% oxygen [28]; thus further substantiating our data.

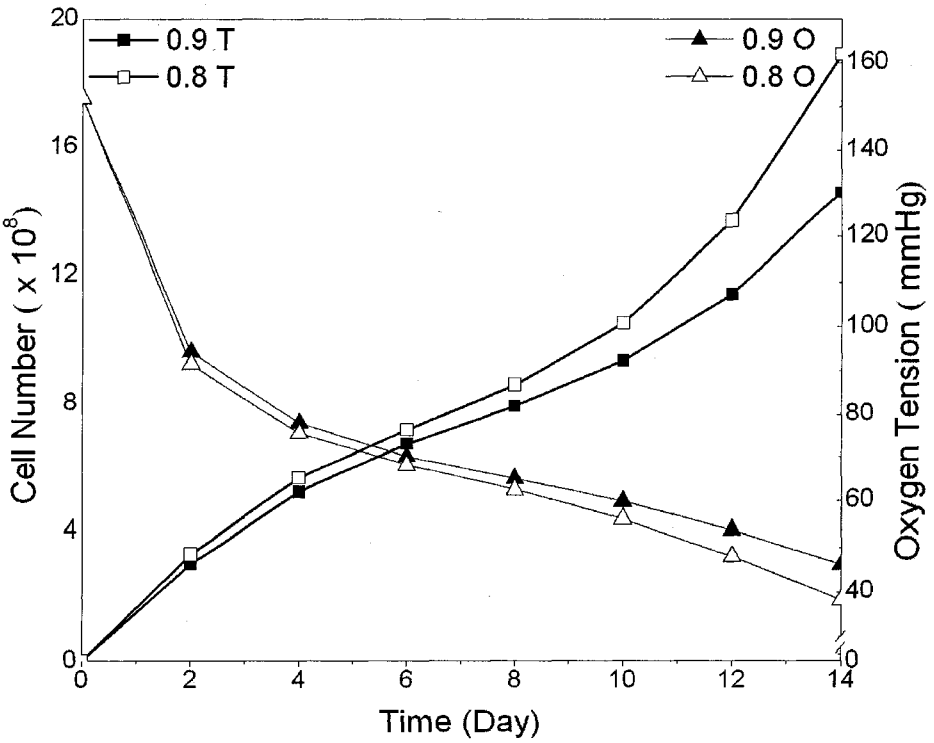

Figure 2. Cellular growth and oxygen tension within RWPB. Solid symbols represent 90% porosity, and clear symbols represent 80% porosity. T: Total cell number. O; Oxygen Tension. Parameters from Table 2.

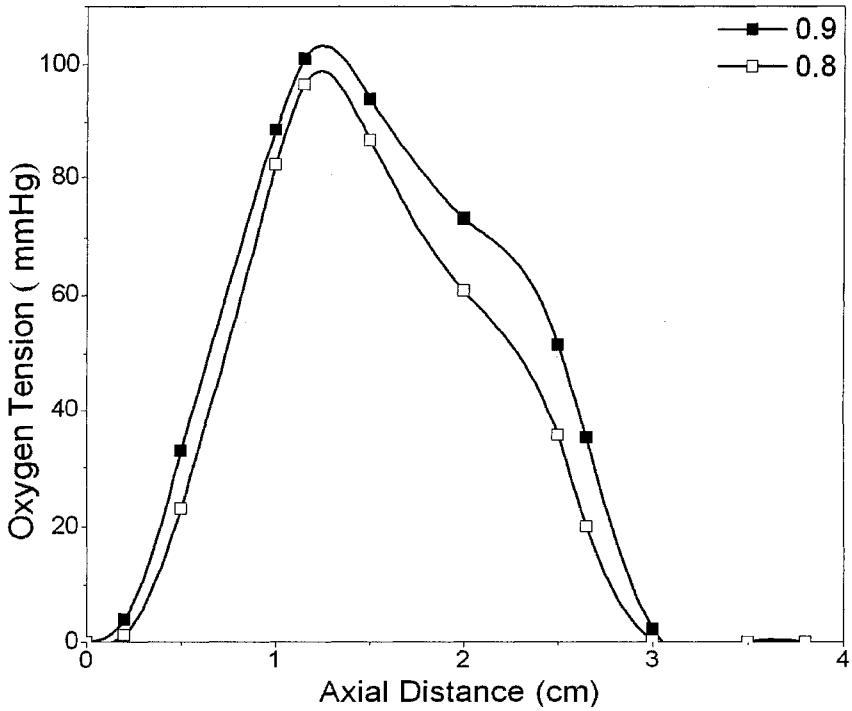

Figure 3. Variation of Oxygen level with axial position at Day 14. v = 1.0E-02m/s; C_G = 4.4mM; C_O =152mmHg; V_{max}=33.55mmHg; k_G=0.2mM^{-1}; k_O=3.77mmHg^{-1}; inoculum density=10^{6}cells/mL. Solid Symbols represent 90% porosity, and clear symbols represent 80%.

The geometry of the bioreactor necessitates the consideration of oxygen distribution from a spatial perspective. Figure 3 shows the radially averaged oxygen tension distribution (at different porosities) in the axial direction on day 14. The inlet and outlet of the reactor are positioned at 1.15 cm and 3.0 cm along the axial direction. The oxygen variation is palpable based on the reactor configuration, which further highlights the need to design and place the scaffold within these limits, lest, the cells are likely to experience hypoxic and anoxic conditions that is, inadvertently, detrimental to the cells.

Conclusions

The present work is a first attempt to combine heterogeneous cellular growth to represent the true nature of haematopoiesis. The present model simulates the cell growth pattern and the oxygen tension distribution within a perfused vessel. The results obtained so far are consistent with data in the literature, but would require experimental corroboration for further model validation. Furthermore, the results suggest that, the operating condition within the reactor is able to support *ex vivo* expansion of HSC. This work is intended to support the effort towards the reconstitution of an ex vivo bone marrow system for expansion of bone marrow cells for clinical use.

Table 2. Model Parameters

Parameter	Symbol	Value	Reference
Flow Parameters			
Viscosity	μ	6.92×10^{-4} kg/m·s	[29]
Density	ρ	993.37kg/m^3	[29]
Temperature	T	37^{o}C	
Perfusion Rate	v	10mm/s	-
Porous Material Parameters			
Volume Porosity	γ	0.8 & 0.9	-
Pore Sizes (Diameter)	d_p	200 μm	-
Resistance	R	μ / k	-
Cell Parameters			
Cell Diameter (Granulocytes)		10-25 μm	[30]
Specific Cell Volume ($x10^9$) (Granulocytes)		2.8 cm^3/cell	[30]
Inoculum Cell Density*		10^6cells/mL	-
Mass Transfer Parameters (Glucose)			
Glucose Diffusivity	D_G	6.6×10^{-10}m^2/s	[31]
Michaelis-Menten constant	K_G	5mM	[32]
Initial Concentration	C_G	4.4mM	-
Mass Transfer Parameters (Oxygen)			
Oxygen Diffusivity	D_O	1.5×10^{-9}m^2/s	[33]
Michaelis-Menten constant	K_O	0.52-2.6 mmHg	[30]
Specific oxygen uptake rate ($x10^{14}$) (for granulocytes cell)	V_{max}	2.2-64.9 mol/cell/h	[30]
Initial oxygen tension	C_O	20%	-
Reaction Parameters			
Rate constant in Michaelis-Menten equation	K_{max}	$\frac{V_{max}}{KG * KO}$	[30]
Rate constant in Michaelis-Menten expression for glucose	k_G	1.1 kg/m^3	[32]
Rate constant in Michaelis-Menten expression for oxygen	k_O	1161 kg/m^3	[30]
Growth Kinetics of Mature Cells			
Rate of Mature Cell Growth	k_d^X	0.35 day^{-1}	[34]
Rate of Mature Cell Death	k_k^X	1 day^{-1}	[34]

* stromal-free CD34+ cells

Table 3. Growth Kinetics of progenitor cells [22]

Units in day^{-1}		**PSC**	**SC**	**MySc**	**LySc**	**CFU-GM**	**CFU-MEG**	**BFU-E**	**CFU-Eo**
Self Expansion Term	k_e^X	3.6	9.0×10^{-2}	7.3×10^{-1}	1.1×10^{-2}	7.4×10^{-1}	2.1×10^{-1}	3.4×10^{-1}	1.0×10^{-1}
Cell Death Term	k_k^X	9.4×10^{-5}	9.9×10^{-4}	1.9×10^{-3}	1.9×10^{-4}	8.7×10^{-2}	8.1×10^{-2}	9.6×10^{-2}	1.0×10^{-1}
Differentiation Term	k_d^X	-	3.7	8.3×10^{-2}	1.0×10^{-1}	1.3	4.5×10^{-4}	3.7×10^{-4}	2.0×10^{-4}

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