Numerical study of fluid flow, mass transfer and cell growth in a three-dimensional bioreactor for bone marrow culture

Chi Yip Ma

A thesis submitted in partial fulfilment of the requirements of the degree of Doctor of Philosophy of the University of London and for the Diploma of Membership of Imperial College

Department of Chemical Engineering and Chemical Technology
Imperial College London

2008
Abstract

The three-dimensional (3-D) architecture of the bone marrow (BM) supports the renewal, maintenance, proliferation and differentiation of haematopoietic stem cells (HSCs). Efforts have been made towards the reconstruction of an ex vivo HSC culture system, which could have several potential applications, such as the detailed understanding of normal and leukaemic haematopoiesis and expansion of haematopoietic stem cells for use in bone marrow transplantation (BMT). In this thesis, a numerical model has been developed to investigate the fluid flow, shear stress, and nutrient distribution within a purpose-designed scaffold placed inside a Rotating Wall Perfused Bioreactor (RWPB). A multi-lineage cellular growth model of haematopoietic cells was incorporated in order to mimic the complexity of the BM. Subsequently, the model has been extended further to investigate the advanced aspects of haematopoiesis such as the regulatory effects of growth factors including granulocyte colony-stimulating factor (G-CSF), thrombopoietin (TPO) and erythropoietin (EPO) on the production of chronic myeloid leukaemia (CML) cells. The simulation results demonstrate that: (1) the average fluid velocity in the scaffold is 0.1 mm/s, which is qualitatively similar to sinusoidal blood flow found in the BM, (2) the inlet conditions (oxygen concentration = 20% v/v, inoculum density = $2 \times 10^5$ cells/cm$^3$), transport properties (scaffold porosity = 95% and pore size = 250 μm) and reaction parameters (consumption rates) are sufficient to support the growth of haematopoietic cells required for BMT applications, (3) different combinations and concentrations of cytokines can alter the self-renewal and differentiation of CML cells and this effect is also dependent upon operation conditions and scaffold properties, (4) the cellular growth kinetics are in good agreement with both published data and experimental results, validating the major finding predicted by
the computation model. The numerical model presented here enables the optimisation of scaffold design parameters, fluid dynamics and mass transfer environment necessary for developing a suitable \textit{ex vivo} haematopoietic system.
Acknowledgements

First of all I would like to thank my supervisors, Dr A. Mantalaris and Dr X.Y. Xu, who have given me supervision and supportive for my research these last few years. The invaluable experience has been personally and academically rewarding for me. In addition, I sincerely thank Dr R Kumar for his guidance throughout this journey, and helped me to understand and identify my strengths and weaknesses.

Special thanks to Dr Isabel Hwang who inspired me to start this endeavour and gave me the momentum that keeps me going.

Thanks to all the friends who came to visit me during my course of study: Queenie, Veronica C, Kirk, Penelope, Veronica M, Alice, Shane, Karen, Bianca, Noah, Carolyn and Angelia. Thanks to my Paulinian friends (the Form 3L bunch) in Hong Kong who fill me up with nice food and various entertainment every time I go home for vacations, especially Veronica, Patricia, Gigi, Karen, Pamela, Winnie and Kuen. Thanks to the CK Life colleagues, especially Dr SF Pang, Dr B. Chan, Dr K. Hui, Jo, Pat and Dr HY Poon, who gave me valuable research “tips”. Thanks to Natalie who always cooks for me during weekends.

I am very lucky to have many supporting group members, Dr R. Kumar, Dr F. Sidoli, Dr MH Wu, Dr YS Ho, Dr IM Chung, Dr Y He, Sandeep, Maya, Carolyn, Teresa, Yunyi, Jae, Dr M Placzek, and David, who share their experience with me and broaden my research horizons. Big thanks to the entire Chemical Engineering Department staff who helped smooth my path, especially Dr P Valluri for his CFX help and N. Ly for his PC technical support.

I would like to extend my thanks to my dinner and movie buddies, Michelle Tang, Dr ISS Hwang and Soon Ju Tok, who provide me with company when I get bored, share my fun and happiness, worries and troubles.

Lastly, thanks to my parents, Iau Kin Ma and Hiu Fei Chu, and my brothers, Karl and Fred. Without their love and support, this would not have been possible.
# Contents

Abstract .......................................................................................................................... 1  

Acknowledgements ....................................................................................................... 3  

Nomenclature .................................................................................................................. 8  

Chapter 1 ....................................................................................................................... 16  

Introduction .................................................................................................................... 16  

1.1 Motivation .............................................................................................................. 17  

1.2 Why Modelling? .................................................................................................... 18  

1.3 Research Objectives ......................................................................................... 19  

1.4 Thesis Outline .................................................................................................... 20  

Chapter 2 ....................................................................................................................... 21  

Background ................................................................................................................... 21  

2.1 Bone Marrow Structure ................................................................................... 21  

2.2 Haematopoietic Cell Hierarchy: Stem Cell Differentiation ................................. 22  

2.3 Bone Marrow Microenvironment ....................................................................... 25  

2.3.1 Stromal Cells ..................................................................................................... 25  

2.3.2 Cytokines .......................................................................................................... 27  

2.3.3 Location of Haematopoietic Cells within the Marrow Cavity ......................... 28  

2.4 Chronic Myeloid Leukaemia .............................................................................. 29  

2.5 Development of ex vivo Bioreactor for Haematopoietic Cell Culture .................. 31  

2.5.1 Operational and Cultural Parameters .............................................................. 32  

2.5.1.1 Inoculum Density (ID) .................................................................................. 32  

2.5.1.2 Dissolved Oxygen Tension ............................................................................. 33  

2.5.1.3 Endogenous Growth Factors ......................................................................... 34  

2.5.2 Bioreactor Design and Operation ..................................................................... 35  

2.5.2.1 Shear Stress ................................................................................................... 36  

2.5.2.2 Surface Area ................................................................................................ 37
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2</td>
<td>Model Formulation</td>
<td>79</td>
</tr>
<tr>
<td>4.3</td>
<td>Model Equations</td>
<td>79</td>
</tr>
<tr>
<td>4.3.1</td>
<td>Fluid Flow</td>
<td>79</td>
</tr>
<tr>
<td>4.3.2</td>
<td>Porous Flow</td>
<td>79</td>
</tr>
<tr>
<td>4.3.3</td>
<td>Nutrient Transport</td>
<td>81</td>
</tr>
<tr>
<td>4.3.4</td>
<td>Cellular Growth Kinetics</td>
<td>82</td>
</tr>
<tr>
<td>4.4</td>
<td>Initial and Boundary Conditions</td>
<td>83</td>
</tr>
<tr>
<td>4.5</td>
<td>Computational Details and Model Parameters</td>
<td>83</td>
</tr>
<tr>
<td>4.6</td>
<td>Results and Analysis</td>
<td>84</td>
</tr>
<tr>
<td>4.6.1</td>
<td>Velocity &amp; Shear Stress Profiles</td>
<td>84</td>
</tr>
<tr>
<td>4.6.2</td>
<td>Nutrient Distribution and Cellular Growth Kinetics</td>
<td>85</td>
</tr>
<tr>
<td>4.6.3</td>
<td>Multi-lineage Cell Distribution</td>
<td>90</td>
</tr>
<tr>
<td>4.7</td>
<td>Summary</td>
<td>91</td>
</tr>
</tbody>
</table>

Chapter 5 ................................................................................................................... 99

Model Testing – Part I ................................................................................................. 99

5.1 Model Formulation and Parameter Values ........................................................... 99
5.2 Results and Discussion                                                        100
5.2.1 Total Cell Number                                                          100
5.2.2 CFU-GM expansions                                                          102

Model Testing – Part II............................................................................................. 104

5.3 Material and Method                                                           104
5.3.1 Cell Line Culture                                                          104
5.3.2 Glucose Consumption                                                          105
5.3.3 Statistical Analysis                                                        105
5.3.4 Mathematical Model and Equations Development                               105
5.4 Results and Discussions                                                        106
5.5 Summary                                                                      106

Chapter 6 ................................................................................................................... 110

Simulation of ex vivo Bone Marrow Culture: Application to Chronic Myeloid
Leukaemia Growth Model ................................................................................................. 110

6.1 Geometry                                                                      112
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.2 Model Formulation</td>
<td>112</td>
</tr>
<tr>
<td>6.2.1 Model Equations</td>
<td>112</td>
</tr>
<tr>
<td>6.2.1.1 Fluid Flow and Mass Transport</td>
<td>112</td>
</tr>
<tr>
<td>6.2.1.2 Growth Kinetics</td>
<td>112</td>
</tr>
<tr>
<td>6.3 Initial and Boundary Conditions</td>
<td>117</td>
</tr>
<tr>
<td>6.4 Re-circulation Medium – Periodic Boundary Conditions (PBCs)</td>
<td>118</td>
</tr>
<tr>
<td>6.5 Results and Discussion</td>
<td>118</td>
</tr>
<tr>
<td>6.5.1 Perfusion Without Re-circulation</td>
<td>118</td>
</tr>
<tr>
<td>6.5.2 Perfusion With Re-circulation</td>
<td>132</td>
</tr>
<tr>
<td>6.6 Summary</td>
<td>142</td>
</tr>
<tr>
<td>Chapter 7</td>
<td>143</td>
</tr>
<tr>
<td>Final Remarks</td>
<td>143</td>
</tr>
<tr>
<td>7.1 Conclusion</td>
<td>143</td>
</tr>
<tr>
<td>7.2 Future Work</td>
<td>145</td>
</tr>
<tr>
<td>7.2.1 3-D BM and Scaffold</td>
<td>145</td>
</tr>
<tr>
<td>7.2.2 Cytokine Interaction</td>
<td>145</td>
</tr>
<tr>
<td>7.2.3 Rotational Effect</td>
<td>146</td>
</tr>
<tr>
<td>7.2.4 Fluid Flow Validation</td>
<td>147</td>
</tr>
<tr>
<td>Appendix A</td>
<td>148</td>
</tr>
<tr>
<td>Model Parameters and Nomenclature</td>
<td>148</td>
</tr>
<tr>
<td>Appendix B</td>
<td>157</td>
</tr>
<tr>
<td>Model Implementation and Validation</td>
<td>157</td>
</tr>
<tr>
<td>B1 Comparison with Analytical Solutions</td>
<td>158</td>
</tr>
<tr>
<td>B2 Model Implementation Using CFX</td>
<td>163</td>
</tr>
<tr>
<td>B3 Grid Sensitivity Test</td>
<td>164</td>
</tr>
<tr>
<td>B4 Validation Using gPROMS</td>
<td>165</td>
</tr>
<tr>
<td>Appendix C</td>
<td>168</td>
</tr>
<tr>
<td>Sample CFX Files</td>
<td>168</td>
</tr>
<tr>
<td>Bibliography</td>
<td>197</td>
</tr>
</tbody>
</table>
Nomenclature

\( A_n \) Amplification factor of leukocyte
\( A_r \) Amplification factor of erythrocyte
\( A_p \) Amplification factor of platelet
\( a_{EPO} \) Hill coefficient in Eq. 3.40, 6.10
\( a_{TPO} \) Hill coefficient in Eq. 6.9
\( B \) Total number of stages
\( B \) Body force
\( b \) Tube thickness
\( b \) Number of stages
\( C \) Mass concentration
\( C \) Cell density
\( C_G \) Glucose concentration
\( C_0 \) Cell concentration for the previous stage
\( C_i \) Cell concentration for the present stage
\( C_{O_2} \) Oxygen concentration
\( C_{LS} \) Concentration of limiting substrate
\( C_S \) Concentration of secondary substrate
\( C \) Kozeny constant
\( D \) Diffusivity of the species
\( D_\phi \) Diffusivity of \( \phi \)
\( d_p \) Pore diameter
\( E \) Concentrations of EPO
\( f \) Component of the convective or diffusive flux vector in the direction normal to the CV face
\( f_e \) Value of \( f \) at location 'e'
$f^d$  Diffusive flux

$f^c$  Convective flux

$f_o$  Hill coefficient in Eq. 3.38, 6.2

$G$  Concentrations of G-CSF

$H_n$  Hydraulic radius

$h_1$  Hill coefficient in Eq. 3.38, 6.2

$h_2$  Hill coefficient in Eq. 3.40, 3.41, 6.4, 6.10

$h_3$  Hill coefficient in Eq. 3.43, 6.3, 6.9

$K_{EPO}$  Hill coefficient in Eq. 3.40, 6.10

$K_{TPO}$  Hill coefficient in Eq. 3.43, 6.9

$K_m$  Monod constant

$K_{mLs}$  Monod constant for limiting substrate

$K_{ms}$  Monod constant for secondary substrate

$K_{max}$  Rate limiting constant

$\kappa$  Area porosity tensor/permeability

$k, k_1, k_2$  Reaction term

$k_G$  Kinetic coefficients for glucose

$k_N$  Dissociation constant of neutrophils

$k_c$  Growth kinetics

$k_m$  Cellular consumption

$k_o$  Maximum recruitment rate from non-proliferative phase

$k_{O2}$  Kinetic coefficients for oxygen

$k_{GCSF}$  Hill constant used in Eq. 6.2

$k_{EPO}$  Hill constant used in Eq. 6.4

$k_{TPO}$  Hill constant used in Eq. 6.3
$S_x, S_y, S_z$: Shear stresses in the x, y and z-direction

$T$: Concentration of TPO

$T_r$: Time delay

$T'$: Transpose

$t$: Time

$V$: Control volume

$V_{\text{max}}$: Rate constant describing the maximal cell-specific nutrient uptake

$V_{\text{max,LS}}$: Rate constant for the limiting substrate in the reaction

$V_G^{\text{max}}$: Maximum production rate of G-CSF

$v$: Velocity

$x$: Position on x-coordinate in Cartesian space

$y$: Position on y-coordinate in Cartesian space

**Greek Letters**

$\alpha_1, \alpha_2$: Constants

$\beta$: Re-entry rate of CML stem cells into proliferating phase

$\delta$: Death rate

$\delta_M$: Specific death rate of mature cells in the terminal stage

$\delta_N$: Death rate of leukocyte

$\delta_P$: Death rate of platelet

$\delta_Q$: Death rate of stem cell

$\delta_R$: Death rate of erythrocyte

$\delta_{\text{EPO}}$: Destruction or decaying rate of EPO

$\delta_{\text{GCSF}}$: Elimination of G-CSF by neutrophils

$\delta_{\text{TPO}}$: Half-life of TPO

$\varepsilon$: Hill coefficient in Eq. 3.36
\[ \varepsilon_M \] Number of mature cells
\[ \varepsilon_a \] Number of active cells
\[ \varepsilon_{n,0} \] Number of quiescent cells
\[ \phi \] Any conserved intensive property
\[ \phi_E \] Any conserved intensive property at location 'E'
\[ \phi_P \] Any conserved intensive property at location 'P'
\[ \phi_e \] Any conserved intensive property at location 'e'
\[ \Gamma \] Diffusivity of the quantity
\[ \gamma \] Porosity
\[ \eta_d \] Differentiation rate
\[ \eta_g \] Cell specific growth rate
\[ \eta_r \] Self renewal probability
\[ \eta^a \] Active to quiescent transition rate
\[ \eta^q \] Quiescent to active transition rate
\[ \kappa_N \] Differentiation rates of stem cells into leukocytes
\[ \kappa_P \] Differentiation rates of stem cells platelets
\[ \kappa_R \] Differentiation rates of stem cells into erythrocytes
\[ \overline{\kappa}_r \] Hill coefficient in Eq. 3.41, 6.4
\[ \overline{\kappa}_p \] Hill coefficient in Eq. 3.43, 6.3
\[ \lambda_x \] Linear interpolation factor
\[ \mu \] Viscosity of the fluid
\[ \mu_x \] Effective viscosity
\[ \theta_1 \] Hill coefficient in Eq. 3.38
\[ \theta_2 \] Hill coefficient in Eq. 3.36
\[ \rho \] Density
\[ \sigma \] Stress tensor
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\sigma_L$</td>
<td>Limiting coefficient</td>
</tr>
<tr>
<td>$\tau_N$</td>
<td>Time delay for leukocyte maturation</td>
</tr>
<tr>
<td>$\tau_{PM}$</td>
<td>Time delay for platelet maturation</td>
</tr>
<tr>
<td>$\tau_{PS}$</td>
<td>Time delay for platelet aging due to senescence</td>
</tr>
<tr>
<td>$\tau_{RM}$</td>
<td>Time delay for reticulocyte</td>
</tr>
<tr>
<td>$\tau_S$</td>
<td>Time delay for stem cell proliferation</td>
</tr>
<tr>
<td>$\tau_{sum}$</td>
<td>Time delay for erythrocyte</td>
</tr>
<tr>
<td>$\Omega$</td>
<td>Angular velocity</td>
</tr>
<tr>
<td>$\Omega_D$</td>
<td>Extend of differentiation ranging between 0 and 1</td>
</tr>
<tr>
<td>$\varsigma$</td>
<td>Bulk viscosity</td>
</tr>
</tbody>
</table>

**Abbreviations**

- **2-D** Two-dimensional
- **3-D** Three-dimensional
- **AEA** Atomic Energy Authority
- **BFU-E** Burst forming unit-erythroid (erythroid progenitor)
- **BM** Bone marrow
- **BMT** Bone marrow transplantation
- **CB MNCs** Cord blood mononuclear cells
- **CDS** Central-difference approximation/ linear interpolation
- **CFC** Colony forming cells
- **CFD** Computational fluid dynamics
- **CFU-C** Colony forming units
- **CFU-Eo** Colony forming unit-eosinophils
- **CFU-GM** Colony forming unit-granulocyte, macrophage
- **CFU-mast** Mouse mast cell progenitors
- **CFU-MEG** Colony forming unit-megakaryocyte
- **CFU-mix** Multipotential progenitor
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF</td>
<td>Colony stimulating factor</td>
</tr>
<tr>
<td>CV</td>
<td>Control volume</td>
</tr>
<tr>
<td>DDE</td>
<td>Delay differential equation</td>
</tr>
<tr>
<td>DMM</td>
<td>Double Monod kinetics</td>
</tr>
<tr>
<td>DO</td>
<td>Dissolved oxygen</td>
</tr>
<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
</tr>
<tr>
<td>ECS</td>
<td>Extracapillary space</td>
</tr>
<tr>
<td>EPO</td>
<td>Erythropoietin</td>
</tr>
<tr>
<td>FBS</td>
<td>Fetal bovine serum</td>
</tr>
<tr>
<td>FD</td>
<td>Finite difference</td>
</tr>
<tr>
<td>FE</td>
<td>Finite element</td>
</tr>
<tr>
<td>FV</td>
<td>Finite volume</td>
</tr>
<tr>
<td>G-CSF</td>
<td>Granulocyte colony stimulating factor</td>
</tr>
<tr>
<td>GF</td>
<td>Growth factor</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Granulocyte/macrophage colony stimulating factor</td>
</tr>
<tr>
<td>HIM</td>
<td>Haematopoietic inductive microenvironment</td>
</tr>
<tr>
<td>HSC</td>
<td>Haematopoietic stem cell</td>
</tr>
<tr>
<td>ID</td>
<td>Inoculum density</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IMDM</td>
<td>Iscove's modified Dulbecco's medium</td>
</tr>
<tr>
<td>LTC</td>
<td>Long-term culture</td>
</tr>
<tr>
<td>LTC-IC</td>
<td>Long-term culture initiating cell</td>
</tr>
<tr>
<td>LySC</td>
<td>Lymphoid stem cell</td>
</tr>
<tr>
<td>M-CSF</td>
<td>Monocytes colony stimulating factor</td>
</tr>
<tr>
<td>MGF</td>
<td>Mast cell growth factor</td>
</tr>
<tr>
<td>MNC</td>
<td>Mononuclear cell</td>
</tr>
<tr>
<td>MySC</td>
<td>Myeloid stem cell</td>
</tr>
<tr>
<td>NK</td>
<td>Natural killer</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>PB</td>
<td>Peripheral blood</td>
</tr>
<tr>
<td>PBCs</td>
<td>Periodic boundary conditions</td>
</tr>
<tr>
<td>PET</td>
<td>Polyethylene terephthalate</td>
</tr>
<tr>
<td>Ph+</td>
<td>Philadelphia</td>
</tr>
<tr>
<td>PIV</td>
<td>Particle-image velocimetry</td>
</tr>
<tr>
<td>QUICK</td>
<td>Quadratic upwind interpolation</td>
</tr>
<tr>
<td>Re</td>
<td>Reynolds number</td>
</tr>
<tr>
<td>RWPB</td>
<td>Rotating wall perfused bioreactor</td>
</tr>
<tr>
<td>RWV</td>
<td>Rotating wall vessel</td>
</tr>
<tr>
<td>SC</td>
<td>Committed stem cell</td>
</tr>
<tr>
<td>SCF</td>
<td>Stem cell factor</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SF</td>
<td>Steel factor</td>
</tr>
<tr>
<td>SIMPLE</td>
<td>Semi-implicit method for pressure-linked equation</td>
</tr>
<tr>
<td>SIMPLER</td>
<td>Revised semi-implicit method for pressure-linked equation</td>
</tr>
<tr>
<td>TCPB</td>
<td>Tantalum coated porous biomaterial</td>
</tr>
<tr>
<td>TPO</td>
<td>Thrombopoietin</td>
</tr>
<tr>
<td>UDS</td>
<td>Upwind interpolation scheme</td>
</tr>
</tbody>
</table>
Chapter 1

Introduction

An ex vivo system capable of mimicking the in vivo environment of bone marrow (BM) can provide a valuable model for studying blood cell formation under normal and pathological conditions. Such a culture system could have potential applications in new drug screening, and toxicity studies, as well as stem cell expansion for bone marrow transplantation (Koller and Palsson 1993; Chapman et al. 1999). However, mimicking haematopoiesis ex vivo poses many challenges as haematopoietic cultures are complex because there are many interplaying factors which influence blood cell formation. Simulation methods, such as computational fluid dynamics (CFD), can be utilized to obtain detailed flow pattern and species concentration, thereby helping to optimize the design and operational parameters of ex vivo culture system for haematopoietic cell cultures.
1.1 Motivation

Aleix Carrel, a French scientist working at the Rockefeller Institute (New York) in the early 1900s, is generally credited with starting the current revolution in Tissue Engineering. With over a century's worth of experimentation, it is now believed that stem cells are the key to the growth of tissues. There are many scientists, clinicians and industrialists who believe that haematopoietic stem cells are the way forward to delivering therapeutic strategies for treating bone marrow disease, as it is more effective to manipulate the blood's primitive components ex vivo than through factors or small molecules in vivo.

Furthermore, drug resistance and lack of bone marrow donors have always limited the choice of treatment for bone marrow diseases. As a result, there is an increasing demand for research into haematopoiesis and the development of new curative strategies. Consequently, there have been continued efforts towards the development of novel haematopoietic stem cell expansion systems. By providing a comprehensive level of monitoring and control over specific parameters in 3-D cultures, bioreactors can provide the technological means to perform controlled studies aimed at understanding the role that specific biological, chemical or physical parameters plays in a defined tissue.

Apart from stem cell isolation, one of the most challenging aspects in the reconstitution of tissue/organs is the ex vivo expansion of stem cells. Originally, it was assumed that the expansion stage would be relatively simple based on the Dexter culture system (see Chapter 2) (Dexter et al. 1973; Dexter et al. 1977). In reality, human haematopoietic cells die within 10 weeks (Moore and Sheridan 1979). The following key factors have been identified to cause the failure of haematopoietic stem cells to reproduce in an ex vivo environment: 1) lack of three-dimensional (3-D) geometry to recreate the haematopoietic inductive microenvironment (HIM), 2) poor medium exchange of fresh media and metabolic waste, 3)
limited space for the highly proliferative cells and 4) lack of stroma cells or endogenous growth factors.

Considerable efforts have been made within this area to overcome the limitation highlighted above. In particular, it is important to integrate the knowledge acquired from a variety of disciplines to aid the development of haematopoietic stem cell expansion systems. For example, *in vivo* BM physiological characteristic obtained through modelling means, such as the significance of delivery system (BM Microcirculation) and transportive properties (convection and diffusion) (Kumar et al. 2004) can aid the development of the *ex vivo* system. Contributions from Material Engineer's characterising scaffold properties (Safinia et al. 2007) provide an opportunity to analyse and mimic the *in vivo* microenvironment. Stem cell engineers utilise their expertise to optimise the 'right' combination of cytokines necessary for the haematopoietic stem cell expansion (Lim et al. 2007).

1.2 Why Modelling?

The development of a stable *ex vivo* system for the growth of haematopoietic cells would be invaluable for the study of the mechanisms controlling haematopoiesis. However, the complex kinetics and transient nature of the cells and intricate interactions between culture parameters make it difficult to define the optimum factors and culture conditions that can enhance production of haematopoietic cells. An optimisation methodology using experimental techniques would be impractical due to time and cost concerns. Thus a modelling approach is undertaken as an alternative to experimental method to design the optimal experimental condition(s) to study haematopoiesis.

Detailed knowledge of the momentum and mass transport characteristics of the bioreactor in different operating conditions is necessary for the successful operation of the system (Williams et al. 2002). This can be done by using a computational fluid dynamic (CFD) software to simulate the flow conditions in the bioreactor and to define the properties of the
model environment wherein biological tissues are grown. It can also be used to optimize the specifications of the culture system and operating conditions, such as the porosity and perfusion flow rate so as to achieve the best possible conditions for cell growth, resulting in improved quality and quantity of the generated tissue (Begley and Kleis 2000).

1.3 Research Objectives

The main objective of this thesis is to investigate the fluid dynamical aspects (e.g. fluid flow and shear stress), mass transport (oxygen, glucose and growth factors) and cellular growth within the bioreactor for BM culture. The Rotating Wall Perfused Bioreactor (RWPB-Synthecon, Inc) has been chosen to be a suitable culture system for this study. The perfusion and rotation operation conditions of this culture system provide a dynamic culture environment to the cellular components with low shear stress and high mass transfer rates.

The model presented in this work does not, at present, consider rotational aspects of the bioreactor.

Furthermore, a scaffold is designed and embedded in the bioreactor to provide a three-dimensional environment mimicking the in vivo bone marrow microenvironment for stem cell expansion. Finally, to simulate the growth characteristics of HSCs under various operating conditions, different stem cell models are incorporated to mimic the complexity of the BM. This is achieved by taking the following steps:

i) To develop a multi-lineage model to capture the complex functionality of the BM according to its known physiology.

ii) To extend the above model by incorporating the regulatory effects of growth factors in the production of different cell types.

iii) To apply the model to diseased BM; specifically chronic myeloid leukaemia.
1.4 Thesis Outline

This thesis presents the numerical study of fluid flow, mass transfer and cell growth in a three-dimensional bioreactor for bone marrow culture. A comprehensive literature review of the bone marrow structure, blood formation process and haematopoietic cell culture systems is given in Chapter 2. The modelling aspect of the work is presented in Chapter 3, where modelling techniques are introduced and available haematopoiesis models are presented and analysed. Chapter 4 presents a fluid dynamics, mass transport and multi-lineage cell growth model. In Chapter 5, comparison of numerical results with existing published data and additional experimental work is presented to serve as a preliminary validation. Chapter 6 extends the previous model application into BM under pathological condition, such as chronic myeloid leukaemia. The conclusions of the work are given in Chapter 7 supplemented by further recommendations.
Chapter 2

Background

2.1 Bone Marrow Structure

Bone marrow is one of the largest organs in the human body located inside the bone cavities. Structurally, BM has an intricate three dimensional architecture that consists of an intravascular (blood vessel network) and an extravascular (haematopoietic) space (Lichtman 1981; Abboud and Lichtman 2001), as shown in Figure 2.1. The intravascular space comprises of the arteriolar and the sinusoidal networks, with the major arterial supply to the marrow coming from the nutrient artery. The nutrient artery bifurcates into ascending and descending arteries with radial branches extending towards the cortex. On returning towards the central bone cavity, these vessels form sinusoids, which supply blood to the extravascular space. The marrow sinus system is a highly branched network providing the channels where
blood flows through the marrow cavity and drains into the central sinus and the emissary veins (Brookes 1971; Lichtman 1981).

The extravascular space is the site of haematopoiesis or blood cell formation. Haematopoiesis is a complex process involving a variety of specialized blood cell types, including as white and red blood cells. These cells are generated and differentiate continuously throughout adult life from a relatively small population of multipotent haematopoietic stem cells (HSCs), located primarily in the bone marrow (Zandstra et al. 1994; Koller et al. 1998; Nielsen 1999) (Figure 2.2).

2.2 Haematopoietic Cell Hierarchy: Stem Cell Differentiation

On average, an adult produces nearly 400 billion mature blood cells per day in order to replace the natural turnover and to maintain homeostasis (Koller and Palsson 1993; Nielsen 1999). This continuous regeneration of mature cells involves complex and highly regulated differentiation pathways that begin from a common precursor-HSC. HSC is multipotent and has the capacity to differentiate into any type of mature blood cells. It divides by asymmetrical division that results in one differentiated daughter cell and one undifferentiated stem cell (Koller et al. 1998). The differentiated daughter cell commits to enter either the lymphoid or the myeloid lineage (Williams 2000). The common lymphoid progenitors generate B and T lymphocytes, and natural killer (NK) cells, while the common myeloid progenitors give rise to red cells, platelets, granulocytes, and monocytes. Further downstream, the myeloid and lymphoid stem cells form more mature progenitors, and with each differentiation stage, they lose proliferative potential, i.e. become more restricted in the number and the type of lineages they can generate (Koller and Palsson 1993).
Figure 2.1: The blood supply of bone marrow [modified from Lichtman 1981]. Blood comes from nutrient artery, bifurcates into medullary arteries and travels to the cortex through radial artery. In the cortex, arterial blood from nutrient artery mixes with blood from periosteal arteriole. On returning towards marrow cavity, cortical capillaries form sinusoids, which supply blood to the extravascular region (shown in pink). These sinuses collect into a large central sinus and drain into the emissary vein.
Figure 2.2: The haematopoietic process [modified from da Silva et al. 2003]. A single committed HSC can produce approximately $10^6$ mature blood cells after 20 divisions (Hoffbrand et al. 2001).
Progenitor cells give rise to monopotential blast cells called precursor cells that can only differentiate into a single mature cell type. After the blood cells mature, they exit the sinusoids and enter the blood stream. Ultimately, the terminally differentiated cells produced can neither divide nor undergo apoptosis after a period of time, which ranges from hours (for neutrophils) to decades (for some lymphocytes) (Smith 2003). The series of differentiations highlighted above involves many levels of control (Koller and Palsson 1993; Zandstra et al. 1994). These intricate processes are regulated by the haematopoietic inductive microenvironment (HIM), a 3-D structural and chemical microenvironment that regulates, through positive and negative signals, the survival, proliferation, differentiation and maturation of HSCs (Smith 2003; Mantalaris et al. 2004). Abnormalities in the regulation of haematopoiesis may lead to the development of bone marrow disorders, such as leukaemia.

2.3 Bone Marrow Microenvironment

The bone marrow provides a HIM that promotes and regulates the haematopoiesis (Chertkov 1986). This HIM comprises of a complex network of stromal cells and their products, such as cytokines and extracellular matrix (ECM) (Hoffbrand et al. 2001) (Figure 2.3).

2.3.1 Stromal Cells

Marrow stromal cells promote and regulate stem cell self-renewal, commitment, differentiation and proliferation through their secreted cytokines. Other components, such as collagen, glycoproteins (fibronectin and thrombospondin) and glycosaminoglycans (hyaluronan and chondroitin derivatives) form the extracellular matrix (ECM) (Hoffbrand et al. 2001). In addition, the haematopoietic stroma supports the vasculature (Weiss 1976; Weiss 1995; Verfaillie 2000; Williams 2000).
ECM Growth factor receptor

Figure 2.3: Components of bone marrow microenvironment. Stromal cells interact with HSCs via cell-cell contact, and produce ECM and cytokines. Cytokines may present to the HSC during the direct interaction between i) stromal cell and HSC or ii) HSC and ECM. This unique microenvironment regulates the self-renewal, proliferation and differentiation of HSCs.

Reticular and endothelial cells are the major component of stromal cells. A complete luminal layer of endothelial cells and an incomplete abluminal layer of adventitial reticular cells line the wall of the vascular sinuses. On maturation, the blood cells from the haematopoietic compartments pass through the adventitial reticular cells, and the endothelial cells to enter the sinus lumen. Adventitial reticular cells regulate cell passage across the venous sinuses by covering or uncovering the endothelium as needed (Weiss 1995). The other components of the stromal cells include, macrophages, which are responsible for the homeostasis of the haematopoiesis and the production of cytokines and ECM.

Through long-term bone marrow cultures (LTC), stromal-mediated haematopoiesis has been shown (Dexter et al. 1977). In such systems, the stromal cells form an adherent layer to which the haematopoietic cells attach loosely. More primitive progenitors, termed long-term
culture initiating cells (LTC-IC), can be detected between or under the stromal cells. During maturation, blood cells are then released into the non-adherent compartment, and as a consequence differentiated progeny, including colony-forming cells (CFC), and more mature precursors can be found in or suspended above the stromal layer (Wang et al. 1995). Further studies have also shown the development of an adherent layer of stromal cells supporting the expansion of progenitor cells (Bagley et al. 1999) and the forming of cell colonies (Dorshkind et al. 1985).

The haematopoietic stroma makes major contributions in the construction of a 3-D haematopoietic microenvironment (Weiss 1995); the intimate association facilitates cell-to-cell and cell-to-matrix interactions (Glowacki et al. 1998; Li et al. 2001). It is believed that this 3-D geometry with close structural and functional relationship with the stromal cells of the bone marrow plays a crucial role in maintaining haematopoietic progenitor viability and pluripotency (Bagley et al. 1999).

### 2.3.2 Cytokines

Cytokines are a group of glycoproteins that act as messengers regulating haematopoiesis, namely the proliferation and differentiation of haematopoietic stem progenitor, and precursor cells (Bain and Gupta 2003). The process is controlled by the feedback mechanisms of the cytokines that transmit positive or negative signals to maintain the number of blood cells and return to normal conditions post any upset. Essentially, the biological effects of cytokines are mediated through the specific receptors on the surface of the cells.

Over 20 haematopoietic regulators have been identified including erythropoietin (EPO), thrombopoietin (TPO), interleukin (IL)-1, IL-3, IL-6, IL-11, granulocyte/macrophage (GM)-CSF, granulocyte (G)-CSF, monocytes (M)-CSF, and c-kit ligand (stem cell factor (SCF), Steel factor (SF), or mast cell growth factor (MGF)). Major production sources of the growth factors are the stromal cells in the bone marrow except for erythropoietin and
thrombopoietin, which are largely synthesised in the kidneys and liver, respectively (Hoffbrand et al. 2001). Secretion of cytokines can vary significantly with changes in in vivo oxygen tension, pH, and shear stress (Nielsen 1999). In vivo studies have demonstrated that cytokines facilitate the formation of colonies (Tun et al. 2002). Additionally, cytokines act to either activate or inhibit the proliferation, differentiation, maturation, and apoptosis of blood cell during the blood formation process.

2.3.3 Location of Haematopoietic Cells within the Marrow Cavity

Within the marrow cavity, haematopoietic cells show distinct patterns of lodgment. Studies have demonstrated that the more differentiated cells lie against the vascular (sinuses) wall, while the more undifferentiated cells are the most distant from the venous vasculature. Nilssen et al. (2001) reported that the most primitive haematopoietic stem cells are enriched within the endosteal (bone) region, whereas terminally mature differentiated and lineage-committed cells are selectively and predominantly located in the central marrow region (Nilsson et al. 2001).

Spatial distribution of lineage-specific cell types has also been described in the bone marrow (Nilsson et al. 2001). Maturing red cells (erythroblasts) are produced near sinuses; clusters of erythroblasts surround a central macrophage in the bone marrow to form erythroblastic islands (erythroblastic islets), which occurs in close proximity to the adventitial surface of vascular sinuses. Megakaryocytes and lymphocytes lie over the adventitial surface of the vascular sinuses and in clusters surrounding the radial arteries, respectively (Weiss 1995; Nilsson et al. 2001). Granulocytes are usually produced at sites close to arterioles and away from the sinuses wall; when they become mature, they migrate towards the sinuses wall to be released into the circulation. These well-defined structures suggest the existence of haematopoietic cell "niches" that are involved in the self-renewal, proliferation and differentiation of the cells.
2.4 Chronic Myeloid Leukaemia

Each year, 24,000 new cases of leukaemia are diagnosed in the United Kingdom, and among these, about 750 were of the Chronic Myeloid Leukaemia type (Leukaemia Research Fund 2006). CML is a clonal myeloproliferative disorder that is characterised by the abnormal amplification of myeloid lineages, especially granulocytes, and sometimes platelets. Mutation of normal haematopoietic stem cells into leukaemic cells at the cellular level contributes to the excessive production of granulocytes. This increase in cell production leads to increased cellularity in the bone marrow, approximately 3-5 fold more than healthy individuals. The unique feature of CML is the presence of the Philadelphia (Ph+) chromosome that directs the production of a fusion protein which is responsible for most of the physiological alterations of the cells. The resultant overproduction of granulocytes in \textit{in vivo} and \textit{in vitro} is a consequence of such genetic abnormality which attributed is to the alteration in cell kinetics, adhesion/homing and feedback mechanism. A summary of the abnormalities of CML cells are described in Table 2.1.

Regulation of activation, proliferation, differentiation, maturation and apoptosis of haematopoietic cell is controlled by cytokines. It plays an important role in up and down regulation of the number of cells in BM and blood. In certain forms of CML, regular oscillation of blood cell numbers has been observed, ranging between 70-80 days (Adimy et al. 2006b). The leukocyte lineages show the most frequent oscillation, of approximately 40-80 days (Fortin and Mackey 1999). Similar oscillatory patterns have been observed in the platelet and reticulocyte lineages. In healthy individuals, certain cytokines are known to 'emit' either a positive or negative signal to maintain normal blood cell levels, and, consequently, it is believed that the oscillations are due to the abnormalities in the feedback mechanism between the regulators and blood cells (Colijn and Mackey 2005).
<table>
<thead>
<tr>
<th>Functional alteration</th>
<th>Growth characteristics in CML cells</th>
</tr>
</thead>
</table>
| Adhesion and homing   | • Impaired cell surface adhesion receptors lead to and facilitate the release of premature cells into the circulating blood (Gordon et al. 1987).  
  • Abnormal sinus endothelium layer has more pores compared to normal BM cells, therefore allowing passage of immature CML cells through the sinus. These contribute to the expansion in the premature progenitor and precursor circulation population in blood (Petrides and Dittmann 1990). |
| Cell kinetics         | Apoptosis                           |
|                       | • Reduced apoptosis: it has been observed that the circulating granulocytes have longer life span than normal mature granulocytes (Ogawa et al. 1970).  
  • Abnormal gene in CML patients suppress apoptosis and enhance survival (Bedi et al. 1994). |
|                       | Self renewal and proliferative potential |
|                       | • CML leads to the decrease in self-renewal capability and concomitant increase in differentiation probability (Petzer et al. 1997)  
  • Majority of the primitive progenitors, LTC-IC, are Ph-, whereas, many of the differentiated progeny, CFCs, are Ph+ in patients’ cells (Podesta et al. 1997).  
  • *In vivo* study shows long term culture–initiating leukaemic cells disappear rapidly from the culture (Udomsakdi et al. 1992; Luna-Bautista et al. 2003), whereas the colony-forming leukaemic cells population increase significantly (Petzer et al. 1997). |
| Feedback Mechanism    | Distorted                            |
|                       | Response to cytokines is less dependent |

Table 2.1: Abnormalities of cell kinetics in CML
Various studies have shown that CML cells altered proliferation and maturation patterns in response to cytokines stimulation. It has been shown that CML cells are growth factor dependent and the addition of different combinations of cytokines can facilitate CML cells to differentiate into different lineages (Luna-Bautista et al. 2003). However, a recent study demonstrated that CML cultures were less dependent on external cytokines and showed better maintenance than normal BM cells without cytokines (Chavez-Gonzalez et al. 2004). The possible explanation is that CML cells have enhanced sensitivity to growth factors in inducing division and maturation, thus contributing to the abnormal expansion of CML cells compared to their normal counterparts (Bhatia et al. 2000). Furthermore, it has been demonstrated that CML cells have the ability to produce growth factor IL-3 and G-CSF under culture conditions (Jiang et al. 1999).

2.5 Development of ex vivo Bioreactor for Haematopoietic Cell Culture

Successfully constructing an ex vivo haematopoietic cell culture system would serve as a valuable tool for understanding the mechanisms of haematopoiesis under normal or pathological states. Further, such a system can be use for drug screening studies of the dynamics and mechanisms of pharmacological and toxicological actions and also has potential clinical applications in bone marrow transplantation (BMT) (Kirschstein and Skirboll 2001; Wu et al. 2006).

In order to reconstitute the bone marrow in an ex vivo environment, the ideal design of an ex vivo culture system should structurally and biologically simulate the in vivo state to match in vivo haematopoiesis as closely as possible. The design of such a system poses challenges because of the complex kinetics of the heterogeneous cell populations and the intricate interactions between culture parameters. A lot of effort has been made to investigate the crucial design parameters (e.g. reactor type and materials) and operational conditions (e.g.
oxygen level, feeding schedule) for the ex vivo expansion of stem cell cultures. In the following section, key design parameters that are known to successfully establish primary haematopoietic cell cultures are reviewed and discussed.

2.5.1 Operational and Cultural Parameters

2.5.1.1. Inoculum Density (ID)

Seeding density has been demonstrated to influence the performance of haematopoietic cultures. Zandstra et al. (1994) showed that inoculum density might play a key role in the prolonged production of CFC and total cells in suspension cultures. High inoculum cell density cultures (1x10^6 cells/ml) do show significant increase in LTC-ICs and CFCs (Zandstra et al. 1994). However, other researchers reported that the expansion of total cell number and colony forming unit–granulocyte, macrophage (CFU-GM) was inversely proportional to the seeding density (Palsson et al. 1993). Erythropoietic Burst Formation cells (BFU-E), however, did not follow this pattern and were not influenced significantly by the seeding density. Palsson and co-workers proposed that the optimal seeding density appeared to depend on the cell type(s) desired.

To obtain the maximum expansion or number of cells, consideration needs to be given not only to inoculum density but also surface area. Surface area can become a limiting factor for generating the desired number of haematopoietic cells (Palsson et al. 1993). Koller et al. (1996) reported lower inoculum density favored cell expansion ratio and higher ID favored total cell output. As ID increased, cells (CFU-CM and LTC-IC) output increased, but the actual expansion efficiency decreased due to the fact that there was limited availability in the culture system (Koller et al. 1996). Similarly, Takagi et al. (2002) demonstrated that limited surface area of each porous carrier restricted cell growth; the final cell concentration did not show dependency on the inoculum cell density even when cells were supplied with extra glucose, glutamine and dissolved oxygen. Therefore, special attention is required when
cultivating the more primitive cells, such as CD34-enriched cell cultures; they must be inoculated at relatively low density because of their high expansion potential (Koller et al. 1998). The typical reported inoculum density of mononuclear cells (MNC) is 1-3x10^6 cells/ml and 0.5-1x10^4 cells/ml for CD34+ cells (Brugger et al. 1993; Zandstra et al. 1994; Koller et al. 1996; Collins et al. 1998b; Bagley et al. 1999).

2.5.1.2. Dissolved Oxygen Tension

Control and regulation of dissolved oxygen level is especially important because undesirable oxygen level can cause toxicity or anoxia, and hence influence the expansion of haematopoietic cells (Palsson et al. 1993).

Several studies have reported that oxygen tension in the bone marrow is between 10 and 50 mmHg (Ishikawa and Ito 1988; Koller and Palsson 1993). That means, approximately, 5% O_2 will be sufficient to provide an environment similar to the in vivo bone marrow microenvironment (Lindop and Rotblat 1960; Wright and Bewley 1960). On the other hand, in in vitro studies, different oxygen concentration levels produced various results in the pattern of cell expansion. Studies have shown that low oxygen concentration increases cell expansion, causes the formation of more and larger cell colonies in colony assay cultures, favours the differentiation of progenitor cells (Koller et al. 1992a), enhances colony forming units (CFU-C), erythroid progenitors (BFU-E), multipotential progenitors (CFU-mix) and mouse mast cell progenitors (CFU-mast) (Ishikawa and Ito 1988). Other studies have showed that, when comparing culture performance in different oxygen levels in the gas phase, 20% oxygen level resulted in enhanced total cell, BFU-E and CFU-GM expansion than the 5% and 60% oxygen (Palsson et al. 1993). Recently very low oxygen concentration (0.5-5%) has been shown to favor the expansion and maintenance of CD34+ by controlling the cell cycle of these primitive HSCs (Ivanovic et al. 2000; Hermitte et al. 2006)
Most experiments in expanding bone marrow cultures (and other animal cell cultures) are carried out using 20% oxygen. Although high concentration of oxygen in the gas phase may enhance the mass transfer rate, the oxygen concentration may reach an inhibitory level, which can be toxic to cells (Ishikawa and Ito 1988; Palsson et al. 1993). Thus, a well-designed system with optimal gas phase oxygen concentration at non-inhibitory levels and sufficient driving force for good mass transfer of oxygen to accommodate the higher cell density prolific cultures is needed. In addition, special attention is necessary on the diffusion rate of dissolved oxygen (DO) in 3-D culture systems, especially when cells are maintained at a high density (Glowacki et al. 1998).

2.5.1.3. Endogenous Growth Factors

Cytokines play an important role in the regulation of cell expansion and differentiation. Different combinations of recombinant molecules or human growth factors have been utilised in ex vivo cultures of HSCs to achieve an increased expansion of haematopoietic stem cells and do enable maintenance of long term bone marrow cultures (Koller et al. 1993a; Poloni et al. 1997; Balducci et al. 2003).

In general, some growth factors are more selective, acting on either progenitor cells or their differentiated progenies; while other growth factors are less specific, acting on most types of cells. For example, CSFs act primarily on relatively committed cells that are not pluripotent except for IL-3, while C-kit ligand appears to be one of the critical factors required for early cell expansion (Koller and Palsson 1993). Other haematopoietic regulators, such as erythropoietin (EPO) and thrombopoietin (TPO), have been identified to mediate the production of erythrocytes and platelets, respectively (Metcalf and Nicola 1995). The growth factor, granulocyte colony-stimulating factor (G-CSF), is a major growth factor that controls the neutrophil regulatory system by controlling the apoptosis rate of neutrophil precursors and the rate of HSC differentiation into the neutrophil precursor compartment (Metcalf and
Nicola 1995; Bernard et al. 2003). *In vitro* studies also show that different combination and concentration of exogenous cytokine affects HSC expansion and differentiation; thus cytokine consumption and supply is an important parameter that requires careful control (Koller et al. 1992b; Brugger et al. 1993; Koller et al. 1995a)

### 2.5.2 Bioreactor Design and Operation

As highlighted in the previous sections, the idea of cultivating haematopoietic cells along with stromal cells in two-dimensional (2-D) flask cultures was initially suggested by Dexter in the late 1970s (Dexter et al. 1973; Dexter et al. 1977). He and his co-workers postulated that stromal cells could provide the appropriate growth environment and the required signalling molecules for the survival of the haematopoietic cells. Flask cultures with a monolayer growth environment limit the proliferation potential and the viability of cells, and though stromal cells were used as the feeding layer, it lack of the 3-D microenvironment observed in *in vivo* system. The 2-D system leads to significant changes in cell-cell contact and cell-substrate interactions (Tun et al. 2002). Furthermore, Dexter cultures operate predominantly at static conditions and therefore cannot provide effective mass transfer, leading to accumulation of metabolic waste and insufficient nutrient supply for cell growth (Wang et al. 1995; Glowacki et al. 1998; Bagley et al. 1999). Although experimental work on culture dishes and flasks has provided invaluable information, there are important limitations in 2-D systems, which ultimately result in the lack of long-term culture maintenance and multi-lineage differentiation. These limitations include the inability to provide a controlled culture environment, which can be overcome through the use of bioreactors.

Bioreactor culture systems have been developed to overcome the limitations of 2-D static cultures for haematopoietic stem cell expansion (Koller and Palsson 1993). Most research has been focused on developing a bioreactor that mimics the haematopoietic system *ex vivo* with a 3-D arrangement and perfusion features (Wang et al. 1995; Glowacki et al. 1998;
Mantalaris et al. 1998; Jelinek et al. 2002). It is reasonable to assume that more effective cultures of these cells could occur in an environment that resembles the architecture and function of the bone marrow as closely as possible (Highfill et al. 1996).

The development of a bioreactor system has several advantages over the traditional 2-D static methods of culturing haematopoietic cells. (Nielsen 1999): 1) An automatic control of bioreactors can minimize labour-intensive daily feeding and daily culture condition cycles. Less physical disruption and contamination can significantly improve the productivity and longevity of haematopoietic cultures (Koller and Palsson 1993). Also, some automatic systems can monitor pH, O₂, nutrients and temperature levels. 2) Higher surface area to volume ratio is able to support high cell density growth, in contrast to traditional culture systems. This is especially important for high proliferative haematopoietic stem cell research for BMT. 3) Dynamic environment with low shear stress conditions within the bioreactor, and 4) Allow rapid medium exchange to minimize accumulation of metabolites and to increase mass transport.

2.5.2.1. Shear Stress

Due to the lack of a cell wall, mammalian cells are susceptible to forces in their environment, such as shear stress. Exposure to high shear stress can lead to cell denaturation and degradation. However, to achieve cell growth, agitation and aeration may be necessary to supply cells with sufficient nutrients and oxygen thus creating shear forces in the culture environment.

Cherry and Papoutsakis (1986) investigated the hydrodynamics effects on cells bounded on microcarriers in a stirred reactor, they noted that the possible mechanisms of cell damage appeared to result from collision of microcarriers with another microcarriers or the reactor walls (Cherry and Papoutsakis 1986). They suggested that a level beyond the threshold of 3-10 dynes/cm² would be enough to cause cell damage. Cherry and Papoutsakis further
suggested the importance of this consideration in the design and operation of bioreactors. Among the convectional bioreactors, it was demonstrated that the Rotating Wall Vessel (RWV) design is able to provide low shear stress environment for cellular growth while supplying sufficient nutrient and oxygen. Goodwin et al. (1993) demonstrated that increasing the calculated shear stress from 0.51 to 0.92 dynes/cm² in the RWV decreased the proliferation and aggregation of cells on microcarriers. They suggested that under low shear environment, cells focused on the structural and functional growth rather than constantly repairing damaging cells in the high shear condition.

2.5.2.2. Surface Area

Bone marrow tissue is an extremely prolific tissue; insufficient surface area or space will restrict cell growth (Oh et al. 1994), therefore, estimating the proliferative capacity of human bone marrow is an important step before designing a culture system.

In 2-D culture systems, inadequate space causes the cells to saturate with some cell densities showing 5-10 cell layers of thickness, so that cells in the bottom layers are far from the nutrient and oxygen source. To overcome this limitation, Oh and others (1994) suggested periodically to harvest half of the cell population, thereby increasing surface area for cell expansion. Increased surface area can effectively reduce cell surface densities, therefore improving oxygen and nutrients transfer to the expanding cells. Their study showed that frequent harvesting allowed longer growth periods and greater cell expansion than the previous single batch expansion protocol (Oh et al. 1994). Another parameter that needs to be considered along with periodic harvesting is the time of harvest because different types of progenitors show expansion maxima time points. CFU-GM progenitor cell density increases with time and reaches a maximum at day 10, while BFU-E reaches a maximum expansion point on day 7 (Palsson et al. 1993).
Monolayer 2-D growth limits the opportunity for studying high cell density growth in the 3-D environment (Glowacki et al. 1998). Many studies have now emphasized the significance of the use of 3-D polymeric scaffolds for the growth of HSCs both in static and dynamic culture (Bagley et al. 1999; Holy et al. 2000; Tomimori et al. 2000; Banu et al. 2001; Li et al. 2001; Tun et al. 2002; Ehring et al. 2003).

2.5.2.3. Feeding Schedule and Medium Exchange

Medium exchange rate is a key variable in ex vivo human bone marrow cultivation. Effective mass transport can avoid depletion of nutrients and accumulation of metabolites. Martiat et al. (1987) found that in vivo perfusion rate in the bone marrow is approximately 10 ml blood/100 cm³ marrow/minute (Martiat et al. 1987). To capture such features, the culture medium is generally replaced on a daily basis when using the Dexter culture inoculum density. However, traditional Dexter cultures require the manual exchange of culture medium once or twice a week (Dexter et al. 1977). This medium exchange rate is inadequate, which results in maintenance of human haematopoietic cells that rarely exceeds 12 weeks.

The Dexter protocol does not provide an optimal environment for maintaining haematopoietic cells. However, modifications can be made to the process to enhance cell differentiation by increasing the medium exchange rate (Schwartz et al. 1991b). This is achieved by developing an automated bioreactor based system to perform the necessary medium exchange for the supply of nutrients and the removal of metabolic wastes (Palsson et al. 1993). Studies have demonstrated that frequent feeding enhances progenitor cell expansion over traditional static cultures and also increases the production of growth factors by human stroma (Caldwell et al. 1991; Schwartz et al. 1991a; Schwartz et al. 1991b). Similarly, Schwartz’s group also demonstrated that rapid perfusion can improve stem cell maintenance and proliferation (Schwartz et al. 1991b). When Dexter-type cultures were carried out with rapid medium exchange rates, they supported progenitor cell production for
at least 20 weeks, which was considerably longer than the static cultures that were previously reported by Hocking and Toogood's group (Hocking and Golde 1980; Toogood et al. 1980; Schwartz et al. 1991b).

2.5.2.4. Bioreactors

Varying types of bioreactors exist for the expansion of human haematopoietic cells. These include: airlift (Sardonini and Wu 1993; Highfill et al. 1996), hollow fibre (Sardonini and Wu 1993), suspension (Sardonini and Wu 1993; Zandstra et al. 1994) and perfusion bioreactors (Koller et al. 1993a; Koller et al. 1993b; Palsson et al. 1993; Sardonini and Wu 1993; Koller et al. 1995a; Sandstrom et al. 1995; Wang et al. 1995; Sandstrom et al. 1996; Mantalaris et al. 1998; Liu et al. 2006). However, the success rates for such applications are varied. Interestingly, the geometry and operating conditions of the bioreactors have been reported to affect the cell culture performance (Peng and Palsson 1996; Cabrita et al. 2003). A summary of different bioreactors used for haematopoietic cell cultures is shown in Table 2.2.

**STIRRED/SUSPENSION CULTURE SYSTEM.** BM, peripheral blood (PB) and cord blood mononuclear cells (CB MNCs) have been successfully propagated in stirred culture systems (Sardonini and Wu 1993; Zandstra et al. 1994; Pierson et al. 1996; Collins et al. 1998b; Jelinek et al. 2002). Sardonini et al. (1993) reported that BM MNC have the largest relative expansion in a suspension bioreactor as compared to hollow fibre and airlift culture systems (Sardonini and Wu 1993). Stirred suspension culture system has also been used by Zandstra et al. (1994) to support haematopoiesis using normal human bone marrow cells. The results from these studies demonstrated that such systems supports continuous and significant net expansion of primitive normal human haematopoietic progenitor cells and stromal cell precursors over extended periods (Zandstra et al. 1994). Collin et al. (1998) further demonstrated that haematopoietic cultures, such as peripheral blood and cord blood cells, grow well in spinner vessels with or without serum medium (Collins et al. 1998b).
<table>
<thead>
<tr>
<th>Type of Bioreactor</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspension/Stirred</td>
<td>• Homogenous environment</td>
<td>• Do not permit cell-cell interaction</td>
</tr>
<tr>
<td></td>
<td>• Easy Sampling</td>
<td>• Mechanical agitation induce shear stress</td>
</tr>
<tr>
<td></td>
<td>• Easy to scale up</td>
<td></td>
</tr>
<tr>
<td>Airlift</td>
<td>• Easy to scale up</td>
<td>• Bubble breakage cause cell damages</td>
</tr>
<tr>
<td></td>
<td>• Homogenous environment</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Support high density growth</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Support stromal layer</td>
<td></td>
</tr>
<tr>
<td>Hollow-fibre</td>
<td>• Continuous process</td>
<td>• Difficulty in retrieving cells</td>
</tr>
<tr>
<td></td>
<td>• Shear-free</td>
<td>• Difficulty in monitoring parameters</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Spatial inhomogeneous environment</td>
</tr>
<tr>
<td>Perfusion</td>
<td>• Continuous process</td>
<td>• Scale up difficulties</td>
</tr>
<tr>
<td></td>
<td>• Support stromal layer</td>
<td>• Difficult to monitor the parameters</td>
</tr>
<tr>
<td></td>
<td>• Clinical Application</td>
<td></td>
</tr>
</tbody>
</table>

Table 2.2: Summary of different culture systems which has been used for haematopoietic cell culture.
One of the advantages of stirred suspension culture systems is that it can provide a homogenous environment of O\textsubscript{2} tension (Ferziger and Peric 2001), cytokine concentrations, serum components, medium exchange rates and other culture parameters for haematopoietic cells to proliferate and differentiate. In addition, a stirred culture system is more amenable for sampling, data collection, control of medium conditions and scale-up (Collins et al. 1996). Although they offer several advantages of homogeneity (Zandstra et al. 1994), suspension cultures do not permit cell-cell or cell-matrix interactions which are known to be important, in particular for haematopoietic activities (Highfill et al. 1996). Also, the fluid dynamics conditions inside the vessel are far from the in vivo environment (Highfill et al. 1996), mechanical agitation and fluid shear in the suspension cultures may negatively affect the cell growth pattern (Sardonini and Wu 1993). Haematopoietic cells are relatively sensitive to shear (Collins et al. 1996). High shear rate is thought to be unsuitable for ex vivo expansion of stem cells and it is very likely that the cells will suffer physical damages (Nielsen 1999).

**Airlift Bioreactor.** Sardonini and others employed an airlift bioreactor for the cultivation of human haematopoietic stem cells but experienced poor expansion (Sardonini and Wu 1993). The undesirable results may be due to gas sparging that leads to bubble shear and breakage, which may cause cell damages. Though it failed to support human haematopoietic bone marrow cultures, Highfill et al. (1996) demonstrated that this system supports the co-culture growth of murine bone marrow and stromal cells (Highfill et al. 1996).

The advantage of an airlift bioreactor system is that it has low shear stress and can be scaled up. Further, it can provide a well-mixed environment, which eliminates many of the undesirable aspects of common tissue culture methods such as gradient difference, intermittent feeding, static fluid conditions, and limited pH and dissolved oxygen controls.

**Hollow-Fibre Bioreactor.** Sardonini and co-workers compared the expansion of total and progenitor cell populations in different bioreactors (Sardonini and Wu 1993). The results from the hollow fibre reactor system showed neither growth nor expansion of haematopoietic
cells; possibly due to difficulties and/or inefficiencies in retrieving cells from the extracapillary space (ECS) of the bioreactor. They suggested optimizing parameters such as fiber type, flow rates and improved methods for cell retrieval could lead to an increase in cell yields (Sardonini and Wu 1993).

2-D PERFUSION BIOREACTOR. Koller et al. (1993) has demonstrated that continuous perfusion in the flatbed bioreactor has the ability to expand human haematopoietic LTC-IC and progenitor cells from BM MNC. Their results showed that total cell number increased 10 fold by day 14, CFU-GM number expanded 21 fold by day 14, BFU-E number expanded 12 fold by day 8 and LTC-IC number expanded 7.5 fold. A continuously perfused system can in fact support the full spectrum of marrow cell development ex vivo and is able to selectively expand the number of stem and progenitor cells to transplantable levels (Koller et al. 1993b). Research by Palsson et al. (1993) has also shown that a flatbed perfusion bioreactor system inoculated with human MNCs give rise to a 20- to 25-fold and a 10- to 30-fold expansion in human MNC and CFU-GM progenitor cells, respectively, over a two-week period (Palsson et al. 1993). By frequent harvesting of human bone marrow cells from the perfusion system, Oh et al. reported a 100-fold expansion of the total cell population and a 30-fold increase in CFU-GM over a 27-day period (Oh et al. 1994). Sandstrom et al. (1996) developed a modified perfusion reactor with a "grooved" chamber for the expansion of PB MNCs. The system can retain cells while allowing for rapid medium exchange. Their results also showed that the perfusion system enhanced CFU-GM expansion and LTC-IC maintenance with the addition of IL-3, IL-6, G-CSF and stem cell factor (SCF) (Sandstrom et al. 1996).

A flow perfusion bioreactor can maintain a uniform distribution of cells on the scaffolds and provide adequate levels of oxygen, nutrients and growth factors (Bancroft et al. 2002; Gomes et al. 2003). Perfusion bioreactor systems such as flat bed and groove design are able to support the stromal layer. However, each perfusion chamber can only sustain limited cell densities and scale up is difficult (Nielsen 1999).
3-D PERFUSION CULTURE SYSTEM As highlighted previously, an important feature of the bone marrow’s haematopoietic inductive environment is its 3-D organisation, which is required in controlling \textit{in vivo} haematopoietic process. Consequently, the utilisation of support matrices that support cells a 3-D growth environment by enhancing cell-cell contact, promoting the spatial arrangement of cells, and supporting a high cell density due to their high surface area to volume ratio, has been explored (Rosenzweig et al. 1997; Bagley et al. 1999; Agrawal and Ray 2001; Banu et al. 2001; Li et al. 2001; Tun et al. 2002).

To mimic this 3-D cell growth configuration, a 3-D packed bed bioreactor was developed for culturing human bone marrow cells on porous microspheres (Wang et al. 1995; Glowacki et al. 1998; Mantalaris et al. 1998; Jelinek et al. 2002). The culture system is continuously perfused with medium to avoid cycling of the cell growth pattern caused by weekly medium exchange (Toksoz et al. 1980). It has also been shown that this type of culture system is able to support the differentiation of different lineages \textit{in vitro} for several weeks (Wang et al. 1995; Mantalaris et al. 1998). Further, perfusion of medium through 3-D collagen sponges enhances viability and functionality of both marrow stromal and haematopoietic cells. This perfused system also supports stromal layer and enhances matrix production and cellular migration (Glowacki et al. 1998; Mantalaris et al. 1998; Jelinek et al. 2002).

Recently, rotating wall vessel (RWV) was used to culture cord blood cells (Figure 2.4). The design of this bioreactor overcomes the major limitations of other types of bioreactors: high shear stress, inhomogeneous environment, and bubble formation/breakage. RWV is able to provide laminar flow, low shear stress and efficient mass transport environment to support cell growth (Begley and Kleis 2000; Begley and Kleis 2002). Liu et al. (2006) demonstrated that with supply of low dose cytokines and frequent harvest, total expansion increased more than 400 times in this type of bioreactor within 10 days (Liu et al. 2006).
2.6 Scaffold Material and Architecture

The utilization of support matrices that support cells’ 3-D growth underpins by reducing cell-cell contact, promoting the spatial arrangement of cells, and supporting a high cell density due to their high surface area to volume ratio, has been explored extensively (Li et al., 1997; Ng et al., 1997; Agrawal and Ray, 1991; Ikoma et al., 2004; Li et al., 2001; Van et al., 2002). Solutions such as collagen (Agrawal et al., 1991), vessels coated with silicon carbide (CSCI), (Hartl et al., 1997), and directed matrix synthesis (van Hove et al., 1998; Bollinger et al., 1998) and threedimensional perfusion culture systems (Figure 2.4) have been developed to support high density of cultures (van Hove et al., 1998; Bollinger et al., 1998). These methods offer a promising approach to achieve 3-D growth and promote the spatial arrangement of cells, as shown in Figure 2.4.

Figure 2.4: Rotating-Wall Perfused Vessel Bioreactor System (Synthecon, Inc).
2.6 Scaffold Material and Architecture

The utilisation of support matrices that support cells a 3-D growth environment by enhancing cell-cell contact, promoting the spatial arrangement of cells, and supporting a high cell density due to their high surface area to volume ratio, has been explored (Rosenzweig et al. 1997; Bagley et al. 1999; Agrawal and Ray 2001; Banu et al. 2001; Li et al. 2001; Tun et al. 2002). Scaffolds such as Cellfoam™ (Banu et al. 2001), tantalum coated porous biomaterial (TCPB) (Rosenzweig et al. 1997; Bagley et al. 1999), porous cellulose microspheres (Mantalaris et al. 1998), macroporous collagen carriers (Jelinek et al. 2002) and non-woven polyethylene terephthalate (PET) matrix (Li et al. 2001) have been used to facilitate a 3-D environment that resembles the BM microstructure and promotes expansion of haematopoietic cells, albeit under static conditions.

3-D scaffold architecture can enhance cell proliferation (Li et al. 2001; Tun et al. 2002) because of the highly porous structure which can provide large surface areas to support high-density culture growth (Tun et al. 2002). It can also affect the homogeneity of formed tissue (Gomes et al. 2003) because the scaffold's interconnecting pores can facilitate the rearrangement of dispersed cells (Tun et al. 2002). Tomimori et al. (2000) have observed that stromal cells have different morphology on different porous carriers, affecting their haematopoiesis-supporting activity (Tomimori et al. 2000).

Therefore, scaffold should possess adequate porosity, interconnectivity, pore size and permeability to allow the ingress of cells, growth, and mass transfer (Bagley et al. 1999; Agrawal and Ray 2001; Tun et al. 2002). An appropriate surface chemistry is also important for cell attachment and proliferation similar to the 3-D haematopoietic microenvironment (Tomimori et al. 2000). A summary of different scaffold used for haematopoietic cell cultures is shown in Table 2.3.
Table 2.3: Summary of 3-D scaffold used for the growth and expansion of haematopoietic cells

2.7 Summary

The development of a stable *ex vivo* system for the growth of haematopoietic cells would be valuable for the study of the mechanisms controlling haematopoiesis in BM. The successful operation of the system lies between the optimisation of bioreactor design and operational parameters. Among the various culture systems, the Rotating Wall Perfused Bioreactor (RWPB-Synthecon, Inc) is able to provide a low shear stress and high mass transfer culture environment to the cellular components with promising results. However, the complex kinetics and transient nature of the cells and intricate interactions between culture parameters make it difficult to define the optimum factors and culture conditions to enhance production of haematopoietic cells. An optimisation methodology using experimental techniques would be impractical due to cost issues. Thus, a modelling approach is utilized to obtain detailed knowledge of the momentum and mass transport characteristics of the bioreactor and to assist in designing culture vessel and optimising experiment condition (Williams et al. 2002).
Chapter 3

Simulations and Mathematical Models

In this chapter, computational fluid dynamics modelling strategies and techniques for the implementation of the various mathematical models (Chapters 4, 5 and 6) developed for this work are detailed. In addition, fundamental aspects of porous media, mass transport and reaction kinetics (for both normal and CML BM) are emphasised. Furthermore, computational models developed by several groups are reviewed and their applicability to bone marrow tissue is discussed.
3.1 Simulation Environment

A true understanding of haematopoietic cultures necessitates amalgamating in vivo characteristics of the bone marrow and contributory transport phenomena. The BM characteristics were detailed in earlier sections; in the present section, fundamental description of Computational Fluid Dynamics (CFD) is offered. As the focus of the present work is on determining and highlighting key parameters for ex vivo haematopoiesis, the use of ‘ready made’ CFD packages is deemed ‘fit for purpose’. A plethora of ‘ready made’ packages are available, CFX, Fluent, Numeca, Phoenics, Star-CD to name but a few. The basis of CFD is the Navier-Stokes equation, and the main consideration is treatment of continuous fluid in a discretised fashion computationally. The CFD solution procedures described in the sections below focus on the discretisation method utilises by the CFD software used, CFX 4.4 (Ansys®).

3.2 Discretisation Method

Discretisation is a class of numerical methods of approximating differential equations using a system of algebraic equations set at different discrete locations in space and time. These discrete equations give information at each spatial point in the domain, termed a grid. There are different techniques for discretisation, including finite difference (FD), finite volume (FV) and finite element (FE) methods. The main differences between these approaches lie in the way the flow variables are approximated; the method of approximation varies with the problem type. Of the methods highlighted, only the finite volume method will be discussed as the CFX 4.4 (Ansys®) code has adopted this method.
3.2.1 Finite Volume Method

The finite volume method uses the integral form of the conservation equations as the starting point:

$$\frac{\partial}{\partial t} \int_V \rho \phi dV + \int_S \rho \phi \nu \cdot ndS = \int_S \Gamma \text{grad} \phi \cdot ndS + \int_V k_\phi dV$$

where \( t \) is the time, \( \rho \) is the density, \( \phi \) is any conserved intensive property (for mass conservation, \( \phi = 1 \); for momentum conservation, \( \phi = v \); for conservation of a scalar, \( \phi \) represents the conserved property per unit mass), \( V \) is the control volume (CV), \( S \) is the surface enclosing CV, \( n \) is the unit vector normal to \( S \), \( v \) is the velocity, \( \Gamma \) is the diffusivity of the quantity \( \phi \), and \( k_\phi \) is the sink/source of \( \phi \).

The solution domain is subdivided into a finite number of small control volumes (or cells) by a grid (or mesh). The usual approach is to define CVs by a suitable grid and assign the computational node to the CV centre (Figure 3.1).

The integral conservation equation applies to each CV, as well as to the solution domain as a whole. The global conservation equation is obtained on summing the equations for all CVs, the surface integrals over inner CV faces balance each other. Typical 2-D Cartesian control volumes are shown in Figure 3.1 together with the notation. The CV surface consists of four (in 2-D) or six (in 3-D) plane faces, denoted by lower-case letters corresponding to their direction (For a 2-D system this equates to East (e), West (w), North (n), South (s)) with respect to the central node (P) (Figure 3.1).

The net flux through the CV boundary is the sum of integrals over the four (in 2-D) or six (in 3-D) CV faces:
where $f$ is the component of the convective or diffusive flux vector in the direction normal to the CV face. To calculate the surface integral, one would need to know the integrand $f$ everywhere on the surface $S_k$. This information is not available since only values at nodal points are known. Therefore, this integral is approximated as a product of the integrand at the cell-face centre and the cell-face area using the midpoint rule:

$$\int f dS = \sum_k \int f dS = f_{e} \Delta a_{e} \approx f_{e} S_e$$  \hspace{1cm} (3.3)
\( f \) represents the product of several variables and or variable gradients at those locations:

\[ f^e = \rho \phi v \cdot n \] for the convective flux and

\[ f^d = \nabla \phi \cdot n \] for the diffusive flux. This approximation to the integral requires that the value of \( f \) at location 'e' is known. The value of \( f_e \) is obtained by interpolation. For volume integrals, the equation becomes the product of the mean value of the integrand and the CV volume.

\[
Q_p = \frac{1}{V} \int_q dV = \bar{q} \Delta V \approx q_p \Delta V \quad (3.4)
\]

where \( q_p \) stands for the value of \( q \) at the CV centre.

The approximations to the integrals require the values of variables at locations other than computational nodes (CV centres). To calculate the convective or diffusive fluxes, the value of \( \phi \) and its gradient normal to the cell face at one more locations on the CV surface are needed. Volume integrals of the source terms may also require these values.

Upwind Interpolation Scheme (UDS) and linear interpolation or central-difference approximation (CDS) are the commonly used schemes for discretising the convection-diffusion equations. Other discretising methods have been proposed and can be found in the book by Ferziger and Peric 2001. UDS is a first-order scheme, approximating \( \phi_e \) by its value at the node upstream of 'e' is equivalent to using a backward or forward difference approximation for the first derivative.

\[
\phi_e = \begin{cases} 
\phi_p & \text{if } (v \cdot n)_e > 0; \\
\phi_E & \text{if } (v \cdot n)_e < 0.
\end{cases} \quad (3.5)
\]

Another straightforward approximation for the value at CV-face centre is linear interpolation between the two nearest nodes. At location 'e' on a Cartesian grid,
\[ \phi_e = \phi_E \lambda_e + \phi_P (1 - \lambda_e) \]  

(3.6)

where the linear interpolation factor, \( \lambda_e = \frac{x_e - x_p}{x_E - x_P} \)

Examples of approximating the volume integrals of convective, diffusive and source term of Equation 3.1 are presented below, only the details of discretisation for the ‘e’ face of a 2-D CV are considered (see Fig 3.1).

**APPROXIMATION OF DIFFUSIVE FLUXES:** Midpoint rule and linear interpolation (CDS) approximation is applied to the integrated diffusive flux integral:

\[ f_e^d = \int_{S_e} \Gamma \text{grad} \phi \cdot n dS \approx (\Gamma \frac{\partial \phi}{\partial x})_e \Delta y = \frac{\Gamma \Delta y}{x_E - x_P} (\phi_E - \phi_P) \]  

(3.7)

where \( x_E = \frac{1}{2} (x_{i+1} + x_i) \) and \( x_P = \frac{1}{2} (x_i + x_{i-1}) \)

**APPROXIMATION OF CONVECTIVE FLUXES:** Convective flux is can be evaluated using midpoint rule and either upwind interpolation (UDS) or linear interpolation (CDS):

\[ f_e^c = \int_{S_e} \rho v \phi \cdot n dS \approx \dot{M}_e \phi_e \]  

(3.8)

where \( \dot{M}_e \) is the mass flux through the ‘e’ face \( \dot{M}_e = \int_{S_e} \rho v \cdot n dS = (\rho u_e) \Delta y \), thus the flux approximation is:
\[
\psi = \begin{cases} 
\max(\dot{M}_e, 0.)\phi_p + \min(\dot{M}_e, 0.)\phi_E & \text{for UDS;} \\
\dot{M}_e(1 - \lambda_e)\phi_p + \dot{M}_e\lambda_e\phi_E & \text{for CDS.}
\end{cases} 
\]

**Approximation of Source Terms:** For a given control volume \( V \), source term is represented by means of a linear form \( k_\phi = S_U + S_P\phi \). Approximation of the volume integral of the source term by the midpoint rule gives,

\[
Q_\phi^p = \int_V k_\phi dV \approx k_\phi^p \Delta V = (S_U + S_P\phi^p)\Delta V
\]

where \( S_U \) is the constant part of the source term, \( S_P \) is the proportional part of source term and it denotes the coefficient of \( \phi^p \).

### 3.2.2 Numerical Grid

Depending on the coordinate systems and the basis vectors used, the conservation equations can be written in Cartesian, cylindrical, spherical, curvilinear orthogonal or non-orthogonal coordinate systems.

As mentioned above, calculations for a set of algebraic equations can be made over a collection of discrete grid points. The arrangement of the discrete points throughout the flow field is termed the 'grid' through the process of mesh or grid generation. Two of the most commonly used meshes/grids in FV calculation are structured mesh/grid and unstructured mesh/grid.

A structured regular grid is relatively a more simplified grid structure, and it has consistent geometrical regularity. Such grid types are generally restricted to amenable geometries with the limited control over the grid distribution. A multi-block grid is a collection of structured grids that together fill the domain. In these grids, there are two or more levels of subdivisions.
of the solution domain. On the coarse level, there are blocks which are relatively large segments of the domains, on the finer level (within each block), a structure grid is defined. It is easier to grid a complex geometry with a multi-block than a structured grid and allows finer grids in the regions which required greater resolutions. Unstructured grid is another meshing method where grid points are placed in the flow field in an irregular fashion. Unstructured methods are very flexible and can be used in complex geometries. Most often, the 2-D grids are made of triangles or quadrilaterals, while 3-D grids are tetrahedral and hexahedra. Due to its irregular nature, node point location and neighbours connection need to be defined explicitly, however, unstructured meshing requires longer solution time.

3.2.3 Finite Approximations

Several methods for discretising a generic conservation equation (for a diffusion and convection problem) in FV scheme are available, such as central differencing, upwind differencing, hybrid differencing, and quadratic upwind interpolation (QUICK). For the Navier-Stokes equations (for momentum and continuity equations, the pressure-velocity coupling), Semi-Implicit Method for Pressure-Linked Equation (SIMPLE) and SIMPLE Revised (SIMPLER) are the commonly used schemes. Details of the specific schemes given above are covered in depth in books by (Patankar 1980; Versteeg and Malalasekera 1995)) and Ferziger and Peric (2001).

3.2.4 Solution Method

Discretisation yields a large system of non-linear algebraic equations. The method of solution depends on the problem. For steady flow problems, pseudo-time marching or an equivalent iteration scheme are usually employed. These methods use successive linearization of the equations and the resulting linear systems are solved by iterative techniques. In general, the
solution involves two levels of iterations: an inner level, where the linear equations are solved, and an outer level that deals with the non-linear equations.

Due to the nature of the iterative procedure, exact solutions to CFD problem are not known, and consequently a ‘convergence’ criterion is normally supplied. Convergence can be the proximity of the final numerical outcome to a known solution, however, in CFD software the convergence is generally set by specifying a ‘minimum residual tolerance’ (like mass residual) expected or by just fixing the maximum number of iterations or both. The iteration scheme stops when the specified conditions have been met.

3.3 Modelling Environment

CFX 4 (Ansys®), formerly known as Flow3D, was commercialised in the late eighties based on an ‘in-house’ code developed by the Atomic Energy Authority (AEA). The code is a multi-block structured solver based on the staggered SIMPLE technique. Although this version of this code is no longer available to developers, the code does permit the end-user to modify the equations solved.

There are several physical models available within the CFX 4 environment, including multiphase, heat transfer, moving mesh, etc. The model properties within CFX 4 used in this work are outlined below, and these are consistent with the physical model detailed in Chapters 4 and 6.

3.3.1 CFX 4 Model Properties

LAMINAR FLOW: Biological cellular components are very sensitive to the surrounding environment and, therefore, it is important to maintain laminar flow within the scaffold to avoid damage to the cells. To provide a criterion to distinguish between laminar and turbulent
flow, the Reynolds number (Re), a dimensionless number, is used to quantify the flow condition. The Re defined for flow through porous media is given as (Scheidegger 1960):

\[ Re = \frac{v \rho d_p}{\mu} \]  

(3.11)

where \( v \) is the fluid velocity, \( \mu \) the viscosity of the fluid, \( d_p \) is the pore diameter and \( \rho \) is the fluid density. Flow within the scaffold that has a value of Re<10 is considered laminar (Bear 1972).

**ISOTHERMAL AND INCOMPRESSIBLE FLOW:** The circulating medium within the bioreactor is normally assumed to have the properties of water; an incompressible Newtonian fluid. Additionally, the standard experimental condition for culturing HSCs is maintenance at 37°C, thus isothermal properties are invoked.

**SCALAR TRANSPORT MODELS:** These are used to describe nutrient transport as well as multicellular growth. To simulate such features, CFX 4 allows the use of scalar transport equations to represent the specific entity under consideration. In addition, source terms are incorporated into the scalar equations that describe the nutrient consumption and growth kinetics.

**POROUS MODEL:** As mentioned in Chapter 2, scaffolds are used to mimic the 3-D architecture of the bone marrow. To model the fluid flow through the scaffold, the porous property utilises a Navier-Stokes equation and Darcy's law. Specific simplifying assumptions have been adopted with respect to the volume porosity; it is assumed to be isotropic and homogenous. Furthermore, an additional body force term can be implemented to represent the resistance to flow in the porous media as a function of the scaffold pore size and porosity.

**TRANSIENT FLOW:** To simulate the time dependent cellular growth within the vessel, transient conditions are employed.
CONVERGENCE CRITERIA: By testing the effect of converging to different levels of residual on the parameters of interest, a criterion of $10^{-15}$ for mass source tolerance is assumed to be sufficient to determine solution convergence.

3.3.2 Geometries and Meshes Generation

The 3-D geometries and the dimensions of the bioreactor were defined and the physical domains were discretised using the ANSYS ICEM CFD and AI*Environment Version 5.0 (Ansys®). All model geometries used within the work were created with body-fitted coordinates and structured hexahedral grids.

![Typical grid and boundary conditions of the domain of interest.](image)

Figure 3.1: Typical grid and boundary conditions of the domain of interest.
3.3.3 Boundary Conditions

INLET (DIRICHLET BOUNDARY CONDITIONS): All variables such as velocities and scalars are specified values at this boundary condition.

OUTLET (NEUMANN BOUNDARY CONDITIONS): The mass flow boundaries correspond to the exit which has been placed sufficiently far from the entrance. All variables, such as velocities and scalars, are extrapolated from upstream.

WALL: No-slip boundary condition is assumed at all solid walls. A common boundary conditions applied in fluid flow, and it applies to the velocity components. For scalars variables, no flux boundary condition is applied.

INITIALISATION: Good convergence to a problem can be achieved if the starting point, or 'initial guess', is considered to be suitable. In the present work, experimental knowledge is to be used to estimate a reasonable initial guess for all problems that are considered. For example, initial nutrient concentration and cellular loading are known at the start of the culture, thus such values will used with the simulation set-up. In cases where non-simplified initial guesses are required, CFX 4 allows the use of external subroutines (e.g. USRINT) to implement the necessary function(s).

PERIODIC: An important feature of the work presented in this thesis is the use of recirculating media in perfused bioreactors. To represent such features, periodic boundary conditions are utilised. This essentially allows for all variables and coefficients from both ends of the computational or physical domain to assume the same value. The implementation of this boundary condition in CFX 4 requires two steps. These steps have been validated by others (Valluri 2003). A similar strategy has also been adopted in this work (See Figure 3.2).
Figure 3.2: Periodic Boundary Conditions (PBCs) implementation procedure in CFX 4 (AEA Technology plc 1999; Valluri 2003).
3.3.4 Solution Quality

MODELLING ERRORS. All simulations involve simplifying assumptions with regards to the domain of interest, geometry, fluid properties and boundary conditions. Consequently errors can be introduced at each step in the process and eventually impact on the overall solution. However, careful analysis of all of these variables, such as suitable boundary conditions and reasonable representation of the cellular growth kinetics, can minimize introduction of avoidable errors.

DISCRETISATION ERRORS. Discretisation allows approximations of the governing equations (such as approximation of volume and time integrals) using an algebraic system of equations. Reducing the size of the discrete element (grids) can increase the accuracy, but at the same time, this increases the computational time and storage. Grid dependency on the solution of the flow field is a common procedure to investigate the influence of the grid quality on the numerical solution.

PROGRAMMING AND USER ERRORS. To ensure user errors are eliminated and any software "bugs" identified, numerical validation via analytical method is often employed. As an initial form of validation, the numerical output is compared with a known analytical solution of fluid flow in an annular cylinder (see Appendix B1 for details). Additionally, the solution from the CFX 4 numerical scheme has been cross checked with an alternative popular commercial software-gPROMS (by Process Systems Enterprise); see Appendix B4. However, a more thorough form of validation arises from experimental comparison with the 'real' numerical simulation. Such forms of validation are presented in Chapters 5.
3.4 Mathematical Models

3.4.1 Fluid Flow Model

The governing equations of the fluid motion comprise equations for conservation of mass and momentum. The continuity equation is given by:

\[ \frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \mathbf{v}) = 0 \]  \hspace{1cm} (3.12)

and the momentum equation is as follows:

\[ \frac{\partial}{\partial t} \rho \mathbf{v} + \nabla \cdot (\rho \mathbf{v} \otimes \mathbf{v} - \mu \nabla \mathbf{v}) = \mathbf{B} - \nabla P' + \nabla \cdot (\mu (\nabla \mathbf{v})^T) \]  \hspace{1cm} (3.13)

where \( P' \) is the modified pressure given by:

\[ P' = P + (\frac{2}{3} \mu - \sigma) \nabla \cdot \mathbf{v} \]  \hspace{1cm} (3.14)

Here \( \rho \) is the fluid density, \( \mathbf{v} \) is the fluid velocity, \( P \) is the pressure, \( \mathbf{T} \) is the transpose notation and \( t \) is the time. Further, \( \mathbf{B} \) is body force (such as buoyancy, rotational and resistance forces), \( \mu \) is the molecular viscosity and \( \sigma \) is the bulk viscosity. If the flow is incompressible, the modified pressure, \( P' \), is equal to the pressure. Additionally, the molecular viscosity \( \mu \) is constant, hence the contribution from the stress divergence to the right hand side vanishes. Assuming incompressible flow and constant viscosity for a Newtonian fluid, the equation becomes:

\[ \frac{\partial}{\partial t} \rho \mathbf{v} + \nabla \cdot (\rho \mathbf{v} \otimes \mathbf{v} - \mu \nabla \mathbf{v}) = \mathbf{B} - \nabla P \]  \hspace{1cm} (3.15)
which also known as the Navier-Stokes equation.

### 3.4.2 Porous Model

Scaffolds have been used to support the growth of haematopoietic cells cultures. Scaffolds are solid materials containing either connected or non-connected voids inter-dispersed in regular or random manner. Due to the random character of the porous material, the velocity of a fluid changes rapidly from point to point along the tortuous path. The model describing the flow in the porous regions in CFX 4 is the Navier-Stokes equation and Darcy’s law (AEA Technology plc 1999):

\[
\frac{\partial}{\partial t} (\rho \mathbf{v}) + \nabla \cdot (\rho (\mathbf{K} \cdot \mathbf{v}) \mathbf{v}) - \nabla \cdot (\mu (\mathbf{v} (\nabla \mathbf{v} + (\nabla \mathbf{v})^T))) = -\mathcal{R} \mathbf{v} - \gamma \nabla P
\]  

(3.16)

where \( \mu_e \) is the effective viscosity, \( \mathbf{K} \) is the area porosity tensor, \( \mathbf{v} \) is the velocity, \( \mathcal{R} \) is the resistance to flow in the porous medium and \( \gamma \) is the porosity. In case of large resistance to flow through the porous medium, a large pressure gradient is needed to counterbalance the resistance, thus the convective and diffusive terms on the left hand side are negligible, and Equation 3.16 reduces to Darcy’s Law:

\[
\mathbf{v} = -\mathcal{R}^{-1} \cdot \nabla P
\]

(3.17)

Darcy’s Law describes the flow within a porous medium by defining the relationship of pressure drop to flow rate through the use of an empirical coefficient (Collins 1976).

\[
q = \frac{K}{\mu} \left( \frac{\Delta P}{L} \right)
\]

(3.18)

where \( q \) is the flow rate, \( K \) is the permeability coefficient which contains all the information about the “architecture” of the consolidated porous medium, \( \frac{\Delta P}{L} \) is the pressure drop within
sample length $L$ and $\mu$ is the viscosity of the fluid. Darcy’s Law can also be extended to obtain a general expression for the law of flow in three dimensions in differential form:

$$
\nu = -\frac{K}{\mu} \nabla p
$$

(3.19)

In porous flow, permeability is an important parameter because the flow patterns and the transport phenomena depend on the geometrical arrangement of the pores, internal surface area, etc. Many attempts have been made to describe the realistic phenomena inside the porous medium using empirical correlations between permeability and other properties, such as porosity, capillary pressure curve and internal surface area. One of the most widely used models is the Kozeny theory, which describes the relationship of permeability and porosity. The model assumes that the bundles of capillary tubes of equal lengths but the cross-sections of these tubes have varied radii distribution. Therefore, this model is sometimes treated as a capillary tube model instead. The Kozeny equation is expressed as:

$$
K = \frac{C\gamma^3}{S_A^2}
$$

(3.20)

where $K$ is the permeability, $\gamma$ is the porosity, $C$ is a numerical coefficient called the ‘Kozeny’s constant’ and $S_A$ is the surface area per unit bulk volume/porous medium.

The basic Kozeny equation has been modified to suit different applications. Carman used Kozeny’s equations and replaced ‘$C$’ with 1/5, which gives the best agreement with the experiments in packed beds (Bear 1972). The Carman-Kozeny equation gives:

$$
K = \frac{\gamma^3}{5S_A^2 (1-\gamma)^2}
$$

(3.21)
where $S_A$ is the surface area per unit volume of solid (not porous) material.

### 3.4.3 Mass Transfer Model

As mentioned previously, oxygen, glucose and growth factor concentration play important roles in the growth of haematopoietic cells. Due to the multi-cellular nature of the haematopoietic environment, knowledge of the varying concentration of the aforementioned nutrients is critical, as it will assist in the true design of the ex vivo haematopoietic cultures. To investigate the spatial and the temporal changes in concentration of these nutrients, the general form of mass transport equation is used (Bird et al. 1960):

$$\frac{\partial}{\partial t} \rho C + \nabla \rho v C = \nabla \cdot D \nabla C - k$$

where $\rho$ is the density, $C$ is the mass concentration, $D$ is diffusivity of the species, and $k$ is the sink/source term which corresponds to the reaction rate.

The nutrient consumption (the reaction term $k$) such as Monod-type can be either be zero-order, first-order or non-linear order kinetics. However, based on the assumption that the cell density is constant throughout the culture, the reaction term can be of zero-order. In the majority of cases such assumptions are oversimplified and the representation of the dynamic pattern of nutrient consumption and cellular growth is inadequate. Consequently, the reaction term can be represented by either the single Monod (Equation 3.23) or double Monod (Equation 3.24) kinetics. Monod-type kinetics has been used in computational models to describe the cytokine-dependency of haematopoietic cells lines (Chaudhry et al. 2004), oxygen consumption by cells in bone marrow (Chow et al. 2001) and oxygen distribution in perfusion bioreactors (Pathi et al. 2005; Coletti et al. 2006).
where $V_{\text{max}}$ is the rate constant describing the maximal cell-specific nutrient uptake, and $K_m$ is the Monod constant. The double Monod kinetics (DMM) is applied to multi-components reactions, such as couple reaction between two substrates.

$$k = \frac{V_{\text{max},C}}{(K_m + C)}$$  \hspace{1cm} (3.23)

where $V_{\text{max},C}$ is the rate constant for the limiting substrate in the reaction. $C_{LS}$ and $C_S$ are the concentration of limiting substrate and secondary substrate, $K_{m_{LS}}$ and $K_{m_S}$ are the Monod constant for limiting substrate and secondary substrate, respectively. Double Monod kinetics has particular applicability in nutrient transport models for tissue cultures where the metabolism of two (or more) metabolites are co-dependent (Howell and Atkinson 1976; Kirkpatrick et al. 2003).

### 3.4.4 Normal BM Cells Models

Haematopoiesis through the development of mathematical models has been studied over the last three-four decades. During this period, the various mathematical models have aided further understanding of the underlying mechanisms for blood cell formation. Most importantly, these models have offered a tool to elucidate the cell renewal, proliferation, differentiation, maturation and maintenance of haematopoiesis.

The total cell production in an \textit{ex vivo} BM system can be modelled as an ‘unilineage kinetic’ model (Peng et al. 1996), which allows the study of cell expansion at each subpopulation (compartment) as a function of self-renewal probability, specific growth rate and mature cell death rate over time. The model is represented by the following set of equations:

$$k = V_{\text{max},LS} \frac{C_{LS}C_S}{(K_{m_{LS}} + C_{LS})(K_{m_S} + C_S)}$$  \hspace{1cm} (3.24)
\[
\frac{d[C_1]}{dt} = (2\eta_r - 1)\eta_g [C_1]
\]

\[
\frac{d[C_2]}{dt} = 2(1 - \eta_r)\eta_g [C_1] - \eta_g [C_2]
\]

\[
\frac{d[C_b]}{dt} = 2\eta_g [C_{b-1}] - \eta_g [C_b] \quad \text{where } b = 3, 4, ..., B - 1
\]

\[
\frac{d[C_B]}{dt} = 2\eta_g [C_{B-1}] - \delta [C_B]
\]

where \( C \) is the cell density, \( \eta_r \) is the self-renewal probability, \( \eta_g \) is the cell specific growth rate, \( \delta \) is the death rate, \( b \) is the number of stages and \( B \) is the total number of stages.

In this model, the process of blood cell formation is represented by a series of compartments as shown in Figure 3.3. The first compartment contains the stem cells which undergo either self-renewal or differentiation. The committed cells further proliferate or differentiate to generate mature cells; represented by the subsequent compartments. There are specific model assumptions that include: 1) Cell specific growth rate is obtained from in vitro experiments; 2) Haematopoiesis is a series of discrete states of expansion; 3) The stem cell undergoes either self-renewal or differentiation and the rate is represented by probability; 4) The cell division and growth rates are constant; 5) Death rate only occurs in the final phase, random loss during the process is ignored.

There are a number of limitations to the model. Firstly, it restricts the study to only a single lineage. Secondly, the assumptions about constant growth rate, death rate and discrete stages of differentiation oversimplify the process of haematopoiesis, since each lineage has its distinct growth and death kinetics, as well as number of cell division stages. Thus this model is insufficient to capture the true kinetics of haematopoietic cells.
Unilineage modelling has been taken further to incorporate the complex dynamics in a single lineage at different stages of differentiation (Nielsen et al. 1998). The population of individual lineages is described as a continuous and deterministic process instead of compartmentalized cells in discrete states. The model assumes the members of active cells, quiescent and mature cells are controlled by four processes: the growth, differentiation, death and cell cycle.

The active cell population:

\[
\frac{\partial [\varepsilon_n]}{\partial t} = \left[ -\frac{\partial (\eta_d \varepsilon_n)}{\partial \eta_d} \right] + \left[ \alpha^q \varepsilon_{n,0} + (\eta_g - \delta - \eta^g) \varepsilon_n \right]
\]  
(3.29)

The quiescent cell population:

\[
\frac{\partial [\varepsilon_{n,0}]}{\partial t} = \left[ \eta^q \varepsilon_n - \eta^q \varepsilon_{n,0} \right]
\]  
(3.30)

The mature cell population:

\[
\frac{\partial [\varepsilon_M]}{\partial t} = \left[ \eta_d (t,1) \varepsilon_n (t,1) \right] - [\delta_M \varepsilon_M] 
\]  
(3.31)
where \( e_a \) is the number of active cells, \( t \) is time, \( \Omega_0 \) is the extent of differentiation ranging between 0 and 1, \( \eta_d \) is the differentiation rate, \( e_{n,0} \) is the number of quiescent cells, \( \eta_q \) is the quiescent to active transition rate, \( \eta_g \) is the specific growth rate, \( \delta \) is the specific death rate, \( \eta^* \) is the active to quiescent transition rate, \( e_M \) is the number of mature cells and \( \delta_M \) is the specific death rate of mature cells in the terminal stage. The framework of the above model has been applied to investigate the effects of a negative acting growth factor (TGF-\( \beta \)) on erythropoiesis and on the study of granulopoiesis under different culture environments (Nielsen et al. 1998; Hevehan et al. 2000).

The above unilineage models focus on a single population or single lineage expansion, and as an improvement on these earlier models, more recent work has been proposed which describes the kinetics of different lineages of human haematopoietic stem cells in an ex vivo BM culture system (da Silva et al. 2003):

\[
\frac{d[C_1]}{dt} = k^X \sigma_L [C_1] + k^X_g [C_0] - \sum_y k^Y_y [C_1] - k^X_C [C_1] \tag{3.32}
\]

where, \( C_0 \) and \( C_1 \) are the cell concentrations for the previous and present stages of the differentiation process, respectively. The rate constants \( k^X_c \), \( k^X_g \), \( k^Y_y \), and \( k^X_C \) represent the self-expansion, generation, differentiation and death terms, respectively. \( \sigma_L \) is the limiting coefficient and is dependent on nutrient and space availability:

\[
\sigma_L = \left[ \frac{C_{\text{MAX}}}{C_{\text{MAX}}} \sum_{\text{all types of cells}} C \right] \tag{3.33}
\]

This kinetic model describes the kinetics of major haematopoietic cells: haematopoietic stem cells (HSCs), committed stem cells (SCs), myeloid stem cells (MySCs), lymphoid stem cells...
(LySCs), colony forming unit–granulocyte, macrophage (CFU-GM), colony forming unit–megakaryocyte (CFU-MEG), burst forming unit–erythroid (BFU-E) and colony forming unit–eosinophils (CFU-Eo). Concentration of each type of cells as a function of time depends on cell expansion, differentiation and death rate. All the rate constants for cell expansion, differentiation and death were obtained from experimental results using a primitive type of haematopoietic stem cell, CD34+, as a starting population.

For example, the balance for MySCs is:

\[
\frac{d[MySC]}{dt} = k_{MySc}^{sc}[MySc] + k_{MySc}^{sc}[SC] - (k_{CFU-GM}[MySc] + k_{CFU-MEG}[MySc] + k_{BFU-E}[MySc] + k_{CFU-Eo}[MySc]) - k_{MySc}[MySc]
\]

(3.34)

The concentration of MySc as a function of time depends on its own self expansion, differentiation from SC, differentiation into CFU-GM, CFU-MEG, BFU-E, CFU-Eo and self-destruction.

Despite the usefulness of the above model, it does not, however, explicitly account for other parameters, such as glucose concentration, oxygen tension, and growth factor levels. Furthermore, the kinetics parameters were obtained from only one single experiment, thus, the rate constant may not be sufficient or truthfully represent the actual kinetics. Moreover, this type of model is limited to the study of in vitro cell expansion only.

### 3.4.5 Periodic Haematological Disease - CML Cells Model

The advancement of mathematical models has contributed to the enhanced understanding of regulatory mechanisms of normal haematopoiesis. Using such models, and with further modifications, it is also possible to extend the applicability to haematopoiesis under pathological conditions. A particular group of haematological conditions are known as
periodic, such as chronic myeloid leukaemia. This condition is of particular interest due to the unique genetic abnormality and the periodic oscillation in cell numbers.

A number of mathematical models available in the literature have accounted for the complex kinetics oscillation behaviour of CML cells and elucidate the haematopoietic process under CML. The main assumptions for these models typically fall into two categories: 1) oscillation originated from the stem cell compartment, and 2) oscillation is due to the destabilisation of the feedback control.

In earlier studies, models of periodic haematological disease focused on the oscillation in a single lineage system. An age-structured model for erythropoiesis in autoimmune haemolytic anaemia has been developed (Mahaffy et al. 1998), which incorporated the effect of EPO on the production of erythrocytes. Following on from this work, the age-structure model for cyclical thrombocytopenia was developed (Santillan et al. 2000), which incorporated a negative feedback loop to examine the production of platelets under the effects of TPO.

Feedback mechanisms of different lineages can contribute to the oscillation of blood cells, but it is also known that the destabilisation of HSC regulation mechanism can be another source of blood cell oscillation. A two compartment model developed by Bernard and co-workers investigated the production and regulation of neutrophils in cyclical neutropenia (Bernard et al. 2003). In addition to the feedback mechanism between G-CSF and neutrophil numbers, the model also incorporated the G₀ cell cycle in the stem cell compartment; representing the feedback mechanism between stem cell recycling and stem cell proliferation/differentiation.

A more comprehensive model has been proposed recently to study the dynamics of CML, taking into account the G₀ cell cycle of stem cells, along with leukocytes, platelets and erythrocytes lineages (see Figure 3.4) (Colijn and Mackey 2005). This model takes the form of delay differential equations based on the previous G₀ stem cell model, age-structured
model of erythropoiesis, model of leukopoiesis in cyclical neutropenia and age-structured model of cyclical platelet disease (Mahaffy et al. 1998; Santillan et al. 2000; Bernard et al. 2003). Furthermore, this model incorporates implicitly the feedback mechanisms between the different lineages and growth factors.

3.4.5.1. Stem Cell Compartment

The G_0 stem cell model describes the proliferating and resting state of the stem cells that occurs in the cell cycle. Stem cells either stay in the resting phase or undergo mitosis and proliferate into another daughter cell. During this process, stem cells can be destroyed by apoptosis. The G_0 stem cell model is used for CML application:

$$\frac{dQ}{dt} = -\beta(Q)Q - (\kappa_N + \kappa_R + \kappa_P)Q + 2e^{-\delta\tau_s} \beta(Q(t - \tau_s))Q(t - \tau_s)$$ (3.35)

where Q is the CML stem cells population, t is the time, \(\delta\) is the death rate of stem cell, \(\tau_s\) is the time delay for stem cell proliferation, \(\kappa_N\), \(\kappa_R\), \(\kappa_P\) are the differentiation rates of stem cells into leukocytes, erythrocytes and platelets, respectively. \(\beta(Q)\) is the recycle phase of resting CML stem cells into proliferating phase and can be represented by the Hill function (see Bernard et al. 2003):

$$\beta(Q) = k_o \frac{\theta^2}{\theta^2 + Q^e}$$ (3.36)

where \(k_o\) is the maximum recruitment rate from non-proliferative phase, \(\theta_2\) and \(\varepsilon\) are the Hill coefficients. The coefficient is estimated based on the experimental data of re-entry rate of cells into the cell cycle, cytokines involved in the division signalling and the proliferation rate of HSC at steady-state (for specific details see Bernard et al. 2003).
Figure 3.4: Haematopoiesis model of CML cells including Go stem cells (Q), leukocytes (L), erythrocytes (R) and platelets (P) lineages. Stem cell is either in resting phase or entering \((\beta)\) into proliferating phase \((Q_p)\) and differentiation into erythrocytes, leukocytes and platelets with the differentiation rate of R \((\kappa_R)\), N \((\kappa_N)\) and P \((\kappa_P)\). \(A_R\), \(A_N\) and \(A_P\) are the amplification factors of R, N and P, respectively. \(\tau_{HN}\), \(\tau_{RN}\), \(\tau_{PN}\) are the time delay for stem cells, leukocyte, reticulocyte, erythrocyte and platelet maturation, respectively. \(\tau_{PS}\) is the time delay for platelet aging due to senescence. \(\delta_Q\), \(\delta_R\), \(\delta_N\) and \(\delta_P\) are the death rate of Q, R, N and P, respectively. Adapted from Colijn et al. (Colijn and Mackey 2005)
3.4.5.2. Leukocyte Compartment

The daughter cells from stem cells further differentiate into leukocyte, erythrocyte and platelet lineages. These cells continue to divide, mature and eventually die. These lineages differentiation and expansion processes are represented by another three components in the model. The process of leukocytes (\(N\)) production is represented as:

\[
\frac{dN}{dt} = -\delta_N N + A_N \kappa_N(N_{\tau_\kappa})Q_{\tau_\kappa}
\]  

(3.37)

where \(\delta_N\) is the death rate of leukocyte, \(A_N\) is the amplification factor of leukocyte, \(\tau_\kappa\) is the time delay for leukocyte maturation and \(\kappa_N(N)\) represents the feedback loop between the leukocyte compartment and the rate of stem cell differentiation into the leukocyte lineage. It is proposed that G-CSF binds to the receptor on leukocytes and the relationship between the activities of leukocytes is proportional to the bounded active site:

\[
\kappa_N(N) = f_0 \frac{\theta_h N^h}{\theta_1^h + N^h}
\]

(3.38)

where \(f_0\), \(\theta_1\) and \(h\) are the Hill function coefficients estimated based on the differentiation activity of leukocytes under G-CSF administration and the proliferation rate of leukocytes under steady-state (for specific details see Bernard et al. 2003).

3.4.5.3. Erythrocyte Compartment

A delay model replaced the age-structured model for erythropoiesis and this is written as:

\[
\frac{dR}{dt} = -\delta_R R + A_R \left\{ \kappa_R(R_{\tau_\kappa})Q_{\tau_\kappa} - \exp^{-\delta_{R_{\tau_\kappa}}} \kappa_R(R_{\tau_\kappa})Q_{\tau_\kappa} \right\}
\]

(3.39)
where \( R \) is the number of erythrocytes, \( \delta_R \) is the death rate of erythrocyte, \( A_R \) is the amplification factor of erythrocyte, \( \kappa_R(R) \) is the differentiation rate of stem cells into erythrocytes, \( \tau\text{R,i} \) and \( \tau\text{R,m} \) are the time delay for immature erythrocyte (reticulocyte) and erythrocyte, respectively, \( Q\text{R,i} \) and \( Q\text{R,m} \) are the time delay for the stem cells to mature into reticulocyte and erythrocyte, respectively. When stem cells differentiate into reticulocytes, these committed cells continue to divide and mature into erythrocytes. Ultimately, erythrocytes then enter into the circulation. During this process, the erythrocytes can be lost through senescence and random death.

The negative feedback mechanism of EPO for the control of erythrocytes can be represented by:

\[
\frac{dE}{dt} = \frac{a_{EPO}}{1 + K_{EPO}R^h} - \delta_{EPO}E \tag{3.40}
\]

where \( a_{EPO} \), \( h \), and \( K_{EPO} \) are the estimated Hill function coefficients from the data presented in Erslev et al. (1991), \( E \) is the concentration of EPO, \( R \) is the erythrocyte number and \( \delta_{EPO} \) is the destruction or decaying rate of EPO (Erslev 1991; Mahaffy et al. 1998). Assuming the number of stem cells differentiating into the erythrocyte lineage is proportional to the concentration of EPO under steady state condition, the differentiation rate of stem cells into erythrocytes becomes:

\[
\kappa_R(R) = \frac{\bar{\kappa}}{1 + K_{EPO}R^h} \tag{3.41}
\]
where $\bar{K}_r$ is the Hill coefficient. The coefficient is estimated based on the steady-state values of erythrocyte mortality, stem cell concentration, and erythrocyte concentration (for specific details see Mahaffy et al. 1998).

### 3.4.5.4. Platelet Compartment

Similarly, the age-structured model for platelet production and apoptosis can be replaced by the differential delay equation:

$$\frac{dP}{dt} = -\delta_P P + A_p \left( \kappa_p (P_{thru}) Q_{thru} - e^{-\delta_P t_{PS}} \kappa_p (P_{thru+t_{PS}}) Q_{thru+t_{PS}} \right)$$

where $P$ is the number of platelets, $\delta_p$ is the death rate of platelet, $A_p$ is the amplification factor of platelet, $t_{PM}$ is the time delay for platelet maturation, $t_{PS}$ is the time delay for platelet aging due to senescence and $\kappa_p(P)$ is the rate of differentiation into the platelet lineage. Similarly, assuming that the number of stem cells differentiating into the platelet lineages is proportional to the concentration of TPO under steady state, the differentiation rate of stem cells into platelets, $\kappa_p(P)$, becomes:

$$\kappa_p(P) = \frac{K_{TPO} P_{h_b}}{1 + K_{TPO} P_{h_b}}$$

where $K_{TPO}$ and $h_b$ are the estimated Hill function coefficients from the data presented in Kuter, D.J (1996) and $\bar{K}_p$ is the Hill coefficient obtained using the same approach as $\bar{K}_r$ (for specific details see Santillan et al. 2000) (Kuter 1996; Santillan et al. 2000).

Combining the four cell compartments along with the six delay differential equations, given above, forms the basis for the study of CML dynamics.
3.5 Delay Differential Equation (DDE)

Delay differential equations are a special type of functional differential equations similar to ordinary differential equations, but their evolution involves past values of the state variable. The solution of delay differential equations, therefore, requires knowledge of not only the current state, but also of the state a certain time previously.

In the literature, for such problems the following approach is normally employed and implemented (Asachenkov et al. 1994).

The previous DDE equations have a general form:

\[
\frac{\partial \phi}{\partial t} = -\nabla \cdot \phi + \nabla \cdot D_\phi \nabla \phi + k_1 + k_2 \tag{3.44}
\]

where

\[
k_1 = \alpha_1 \left[ \phi_T \right] \phi_T, \phi_T = \phi(t) \tag{3.45}
\]

\[
k_2 = \alpha_2 \left[ \phi_{T_s} \right] \phi_{T_s}, \phi_{T_s} = \phi(t - T_s) \tag{3.46}
\]

The terms \(\alpha_1\) and \(\alpha_2\) are constants, \(\phi\) is any conserved intensive property, \(D_\phi\) is the diffusivity of \(\phi\) and \(T_s\) is the time delay. Replace \(\phi_{T_s}\) with \(\psi_s\) and solve the DDE equations using the following form:

\[
\frac{\partial \psi_s}{\partial t} = -\nabla \cdot \phi + \nabla \cdot D_\phi \nabla \psi_s + \left( \frac{\phi - \psi_s}{T_s} \right) \tag{3.47}
\]
Equation 3.47 is the form in which it is implemented in CFX. Essentially, the variable $\psi_s$ follows the variable $\phi$ with a time lag of $T_s$. The time lag need not be a fixed constant, but a distribution with a mean around $T_s$. (Asachenkov et al. 1994).
Chapter 4

Bone Marrow Culture Growth Model

4.1 Introduction

A number of mathematical models have been developed to study the fluid dynamics and nutrient distribution in perfusion bioreactors (Horner et al. 1998; Begley and Kleis 2000; Begley and Kleis 2002; Williams et al. 2002; Pathi et al. 2005; Coletti et al. 2006), as well as haematopoietic cell growth dynamics (Peng et al. 1996; Hevehan et al. 2000; McNiece and Briddell 2001; da Silva et al. 2003) (See Chapter 3 for details). These models have served to not only enhance our understanding of the hydrodynamic and mass transfer environments in various bioreactors under different operating conditions, but also to demonstrate the utility and potential of computational models in designing bioreactors for optimal growth of cells.
and/or tissue-engineered constructs. However, none of these models accounted for the cellular growth of different lineages of HSCs in a dynamic flow bioreactor. In the present study, a mathematical model has been developed to study the growth characteristics of haematopoietic cells under various operating conditions in a low-shear perfused bioreactor, embedded with a porous scaffold (Figure 4.1). The model is used to obtain data on the 3-D flow field, shear stress patterns and nutrient distribution alongside simulated multi-cellular growth and cellular functions.

### 4.2 Model Formulation

The assumptions made in the present model include a laminar flow regime within the vessel, Newtonian and incompressible fluid properties, and an isotropic and homogenous porous scaffold. In addition, the scaffold is assumed to be free from blockage and deposition. The dimensions for the bioreactor (Rotating Wall Perfused Bioreactor-RWPB, Synthecon, USA) and the scaffold are given in Appendix A Table A1 and the geometry of the bioreactor is shown in Figure 4.1 (B). All the model equations, below, are presented in their general forms for convenience and expandability.

### 4.3 Model Equations

#### 4.3.1 Fluid Flow

The continuity and Navier-Stokes equations presented in Chapter 3 are used to describe the flow within the RWPB (Bird et al. 1960).

#### 4.3.2 Porous Flow

Fluid flow inside the scaffold is described by the general porous flow equations (AEA Technology plc 1999),
Figure 4.1: (A) 3-D architecture of the bone marrow showing the intravascular and extravascular components (modified from Abboud and Lichman 2001). The intravascular space consists of the nutrient arteries, radial arteries, sinuses, central arteries and the central sinus. The extravascular component represents the haematopoietic space. (B) 3-D scaffold architecture. The structure of the porous scaffold mimicking the intra and extra-vascular region of the BM is incorporated inside the vessel. (C) Rotating wall perfused bioreactor. A Couette-Taylor mixing vessel which is able to provide a low shear environment and high mass transfer rates required for cellular growth.
\[
\frac{\partial}{\partial t} (\gamma \rho v) + \nabla \cdot (\rho (K \cdot v) \otimes v) - \nabla \cdot K \sigma = -\gamma \rho A \cdot v - \gamma \nabla P
\] (4.1)

where, \(\gamma\) is the porosity, \(K\) is the permeability, \(\sigma\) is the stress tensor and \(A\) is the resistance term. The scaffold is representative of a porous medium whose resistance is given by the Carmen-Kozeny Theory (Bear 1972):

\[
K = \frac{\gamma^3}{5 S_{s1}^2 (1 - \gamma)^2}
\] (4.2)

where, \(S_{s1}\) is the specific surface defined by \(S_{s1} = \gamma / H_s\) and \(H_s\) is the hydraulic radius, defined as \(H_s = r_e / 2\) with \(r_e\) being the pore radius. The mean shear stress in the scaffold region is given by Begley et al. 2000 (Begley and Kleis 2000),

\[
S_m = \frac{1}{3} (S_x^2 + S_y^2 + S_z^2)^{0.5}
\] (4.3)

where, \(S_m\) is the mean shear stress and \(S_x, S_y, S_z\) are the shear stresses in the x, y and z-direction, respectively.

### 4.3.3 Nutrient Transport

The spatial distribution of nutrients, \(C\), (we focused on oxygen and glucose) within the scaffold is described by the general mass transport equation (Bird et al. 1960),

\[
\frac{\partial}{\partial t} \rho C + \nabla \rho v C = \nabla \cdot D \nabla C - k
\] (4.4)

where, \(D\) is the diffusion coefficient of the substrate (oxygen and glucose) in the medium and \(k\) is the source term to describe the cellular consumption \(k_m\) or growth \(k_g\) kinetics.
Metabolic consumption by the cells, $k_m$, is described by the Double Monod Kinetic (DMM), which is derived by coupling two single Monod kinetics for oxygen and glucose (Howell and Atkinson 1976),

$$k_m = K_{max} \frac{C_{O_2} C_G}{(1 + k_{O_2} C_{O_2})(1 + k_G C_G)} \quad (4.5)$$

where, $C_{O_2}$ and $C_G$ are the oxygen and glucose concentrations, respectively, $k_{O_2}$ and $k_G$ are the kinetic coefficients for oxygen and glucose, respectively, and $K_{max}$ is the rate limiting constant in the expression (Kirkpatrick et al. 2003).

4.3.4 Cellular Growth Kinetics

The growth kinetics for the cells is analysed by dividing all cells into two groups: progenitor and mature cells. The progenitor cell group consists of 8 cell types: haematopoietic stem cells (HSCs), committed stem cells (SCs), myeloid stem cells (MySCs), lymphoid stem cells (LySCs), colony forming unit–granulocyte, macrophage (CFU-GM), colony forming unit–megakaryocyte (CFU-MEG), burst forming unit–erythroid (BFU-E) and colony forming unit–eosinophils (CFU-Eo). Although, this study focuses on the growth of different progenitor cells, a mature cell group is included in the model to provide a fuller description of the BM cellular hierarchy. To reduce the number of equations to be solved, only a single type of mature cells is considered. Specifically, the consumption term used in the mature cell group metabolism equations refers to granulocytes since they dominate the extravascular compartment (Chow et al. 2001). Cellular growth kinetics ($k_c$) of both compartments is given by (da Silva et al. 2003),

$$k_c = k_c^X \sigma_x [C_1] + k_c^X [C_0] - \sum_y k_d^Y [C_1] - k_f^X [C_1] \quad (4.6)$$
where, $C_0$ and $C_1$ are the cell concentrations for the previous and present stages of the differentiation process, respectively. The constants $k_e^X$, $k_d^X$, $k_d^T$, and $k_i^X$ represent the self-expansion, generation, differentiation and death terms, respectively. $\sigma_L$ is the limiting coefficient defined as,

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

(4.7)

where, the $\sigma_L$ range is dependent on nutrient and space availability.

### 4.4 Initial and Boundary Conditions

No slip and no flux conditions are applied at all boundary walls. The inlet perfusion rate is at 10 mm/s. The operation parameters were chosen to match the experimental conditions: the culture medium was assumed to have aqueous properties at 37 °C and 1 atm; inlet oxygen and glucose concentrations were 20% and 5.5 mM, respectively; inoculum density ranged from $0.1 \times 10^6$ cells/ cm$^3$; and the duration of the culture simulation was limited to 14 days. In this study, rotation of the vessel is not included.

### 4.5 Computational Details and Model Parameters

Structure and hexahedral grids were used to discretise the physical domain using ICEM CFD 5.0 meshing software developed by ANSYS, Inc. Sensitivity analysis was performed to optimise the computational mesh with approximately 220,000 cell nodes being chosen as an acceptable compromise between accuracy and computational time. The mathematical models for the 3-D flow/reaction/transport problem were developed and implemented within a computational fluid dynamics environment (CFX4.4, ANSYS®). The biophysical parameters
are given in Appendix A Table A2 and the cellular growth kinetics of progenitors and mature cells are given in Appendix A Tables A3 and A4, respectively.

4.6 Results and Analysis

4.6.1 Velocity & Shear Stress Profiles

The flow field and shear stress in a bioreactor affect nutrient distribution and cellular growth. Furthermore, the velocity field can be optimised to control the extent to which seeded cells can penetrate the scaffold core. It is pertinent, therefore, that these properties are analysed. Figure 4.2 shows the velocity contours within the RWPB embedded with a scaffold for varying scaffold porosities. In general, the fluid velocity was found to be low, which is consistent with the hydrodynamic conditions in the bone marrow in vivo and also justifies the laminar flow assumption. The average flow velocity in the scaffold is 0.1 mm/s, which is within the range of sinusoidal blood flow (0.01-0.3 mm/s) found in the BM (Wickramasinghe 1975). The velocity contours show that the flow improves with increasing scaffold porosity, with the maximum velocity occurring in the central region of the bioreactor (or scaffold). Furthermore, the highest velocity was found in the lowest porosity (80%) scaffold. This is due to the fact that low porosity restricts the volume of space available to the fluid, which creates a higher pressure drop resulting in an elevated fluid velocity within the scaffold.

A low shear environment can induce and facilitate cell-cell contact and cell aggregation as well as cell-matrix associations that may subsequently assist in the tissue formation process. Our results show that the shear stress in the RWPB was between $6.6 \times 10^{-8}$ to $0.086$ dynes/cm$^2$. The minimum value corresponds to the low fluid flow near the wall of the bioreactor, whereas the maximum value was found at the inlets of the RWPB where the highest fluid velocity was observed. Figure 4.3 shows the partial shear stress contour profiles ($1.6$ to $8.0 \times 10^3$ dynes/cm$^2$) in the RWPB for various pore sizes (250-450 μm). It can be noted that shear stress is relatively high near the centre of the scaffold and gradually
decreases towards the vessel wall. The predicted maximum shear stress within the vessel is lower than the threshold value (0.92 dynes/cm²) that has been reported to be detrimental to the animal cells (Goodwin et al. 1993). The low shear stress in the bioreactor is able to provide a suitable environment that facilitates the formation of a 3-D microenvironment and supports the growth and maintenance of haematopoietic cell cultures.

4.6.2 Nutrient Distribution and Cellular Growth Kinetics

The haematopoietic space in the BM is occupied by various types of blood cells that have different metabolic demands, such as oxygen and glucose. In order to develop more realistic and complex multi-cellular models, a comparison in the oxygen tension between the single and multi-cellular models was performed, as shown in Figure 4.4. In the single cell type growth model, it is assumed that the culture consists of only granulocytes, which are the most abundant type of cells in the BM (Chow et al. 2001). Rapid proliferation and the high nutrient consumption rate of granulocytes result in a low gas phase oxygen concentration throughout the scaffold. Furthermore, these simulations show that some regions of the scaffold experience less than the optimum oxygen tension (4-20%) required for BM cellular growth; therefore it is likely that a certain proportion of growing cells encountered hypoxic and anoxic conditions, which in turn influences their growth profiles (Ishikawa and Ito 1988; Cipolleschi et al. 1993; Palsson et al. 1993). In contrast, the multi-cellular growth model, which includes the 8 different cell types, confirmed that the oxygen tension levels are maintained above the known hypoxic conditions throughout the scaffold. These results demonstrate that single cell lineage development is not appropriate for the ex vivo reconstruction of the BM and therefore it is insufficient to represent the complex nature of haematopoiesis in BM. Thus, the multi-lineage growth model has been adopted in this work.

Nutrient mass transfer to metabolically active cells is controlled by convection and diffusion; nutrient utilisation kinetics was represented by the DMM model, as given in Eqs. 4.4 & 4.5.
Figure 4.2: Velocity contours (mm/s) in the mid-plane of the bioreactor for different scaffold porosities: (A) 80%, (B) 85%, (C) 90%, and (D) 95%. The operating conditions are: $\gamma = 80-95\%$, $d_{e} = 250 \mu m$ and $v = 10 \text{ mm/s}$.
Figure 4.3: Shear stress contours ($10^3$ dynes/cm$^2$) in the mid-plane of the bioreactor for different pore sizes: (A) 250μm, (B) 350μm, and (C) 450μm. The operating conditions are: $\gamma = 90\%$, $d_p = 250-450 \mu$m and $v = 10$ mm/s.
Cellular growth and oxygen concentration profiles are illustrated in Figure 4.5 for different inoculum densities and porosities. These results highlight the expansion patterns of the haematopoietic cell lineages and their oxygen demand during a 14 day culture period. It is shown that a reduction in the oxygen concentration is accompanied by an increase in the cell numbers for all inoculum densities and porosities. While such results are expected, interestingly, the rate of expansion of the haematopoietic cell lineages under the simulated environmental conditions is a function of both the inoculum density and porosity. Oxygen is regarded as the limiting substrate in cell growth process due to its low solubility in the culture media. In contrast, glucose has a higher solubility than that of oxygen and therefore glucose depletion is less likely to occur.

Inoculum densities of 0.1, 0.2, and 1 (x 10^6 cells/cm^3) showed a 42, 38 and 24 fold increase in total cell numbers at 95% porosity, respectively. These results are consistent with the experimental data available in the literature for HSC (stirred) cultures; for a 14 day culture period, a 40-50 fold total expansion has been achieved (Collins et al. 1998a). In the present case, the higher the inoculum density the lower the rate of expansion. This can be attributed to the limited space available within the scaffold to accommodate the increased number of cells, as reported previously by other researchers (Palsson et al. 1993; Poloni et al. 1997). Consequently, when selecting scaffold properties, it is important to choose a suitable porosity factor, as this is critical to the proliferation rate for the cells. An increased porosity factor provides a larger surface area for cell growth and thus it is able to support higher cell density growth (Palsson et al. 1993; Oh et al. 1994; Poloni et al. 1997). As demonstrated in Figure 4.5A, the 95% porosity scaffold supported a total cell number of 4.1 x 10^6 cells/cm^3 compared to a total cell number of 3.5 x 10^6 cells/cm^3 for an 80% porosity scaffold. These results highlight the importance of the porosity factor in the oxygen transport process. High porosity scaffolds facilitate higher average oxygen concentration in the bioreactor than low porosity scaffolds for the same inoculum density. Therefore, the greater the area available for oxygen transport, the higher the rate of cell proliferation.
Figure 4.4: Average oxygen concentration for various axial positions of the scaffold. Oxygen consumption of single and multiple cell types were compared at day 14. The operating conditions are: \( v = 10 \text{ mm/s}, C_{O_2} = 20\%, d_p = 250 \mu m \), inoculum density = \( 1 \times 10^5 \) cells/cm\(^3\) and \( \gamma = 80\% \). (Open Circle: Single cell type, Square: Multiple cell types).
4.6.3 Multi-lineage Cell Distribution

To incorporate the dynamic nature of the haematopoietic process, a multi-lineage growth model for HSC cultures was formulated that provided a transient distribution of the different cell types, as shown in Figure 4.6. With an initial CD34+ cell concentration of $2 \times 10^5$ cells/cm$^3$, the 14 day culture simulation shows that CFU-GM and CFU-Eo dominate the primitive cell compartment corresponding to 3.5% and 3% (on average) of the total population, respectively. Furthermore, the total number of CFU-GM ($1.5 \times 10^7$ cells) represents a 16-fold increase. These simulation results are consistent with the work of Sandstrom et al. (Sandstrom et al. 1995), who showed in their experimental study that CFU-GM increased 18 folds after a 15 day culture period. Our results are significant as they indicate the minimum number of CFU-GM required for transplantation for an average patient. CFU-GM number is a customary indicator of assessing the adequacy for transplantation (McAdams et al. 1996).

The spatial distribution of oxygen tension within the bioreactor is important for the development and production of the haematopoietic cellular components. The oxygen tension within the bioreactor (gas phase oxygen) is presented as frequency plots in Figure 4.7. At the start of the culture, the oxygen tension distribution within the bioreactor is homogeneous at 20% (v/v), which corresponds to the inlet condition (Figure 4.7A). As the culture progresses, the gas phase oxygen concentration in the bioreactor varies to values between 3% to 20% (v/v), which is consistent with the oxygen requirements by the distributed cell types. By the end of culture at day 14, the simulation results show a heterogeneous oxygen tension distribution in the vessel (as a percentage of scaffold volume), where about 53% of the scaffold volume is at higher than 14% (v/v) oxygen tension, 19% of the scaffold volume is between 7-14% (v/v) oxygen tension, and 28% of the scaffold volume is at lower than 7% (v/v) oxygen tension for the 90% porosity case.
Ishikawa and Ito have indicated that a spatial variation of oxygen tension within the microenvironment of the BM does indeed exist, ranging between 4-21% (v/v) (Ishikawa and Ito 1988), corresponding to the different oxygen tension requirements by the varying cell types, which is in agreement with the present study. High oxygen tension (20%) supports the growth of mature cells, while low oxygen tension (5%) enhances the growth of primitive cells and favour the differentiation of particular progenitor cells (Ishikawa and Ito 1988; Koller et al. 1992b; Palsson et al. 1993). From our simulations, it is evident that the distribution of oxygen tension obtained during the 2-week culture period should support self-renewal, differentiation and proliferation of all types of haematopoietic cells in a manner that resembles the in vivo BM. As highlighted earlier, porosity affects the oxygen tension within the scaffold. Our results confirm the effect of porosity on the gas phase oxygen concentration distribution in the vessel (Figure 4.7). By the end of the culture at day 14, the highest porosity (95%) scaffold maintains 26% of its volume at 20% (v/v) oxygen concentration. Whereas, for the lowest porosity (80%) scaffold, a larger proportion (32%) of its volume is maintained at an oxygen level of 20%. This is consistent with the view that higher porosity scaffolds support higher cellular densities and therefore higher levels of oxygen consumption for cellular metabolism. In contrast, lower porosities provide less space for cellular growth and thus a lower oxygen tension requirement.

4.7 Summary

Haematopoietic stem cell cultures have generated intense interest due to their potential applications in cell-based therapies (Nielsen 1999). However, achieving controllable and consistent production of clinically relevant numbers of haematopoietic cells requires the optimisation of several important bioprocess culture parameters and bioreactor specification factors. The work presented in this chapter aimed at developing a model that is able to simulate the growth of HSCs in a low-shear, perfused, 3-D bioreactor system embedded with a porous scaffold. The unique aspect of this study is the inclusion of the multi-cellular growth
Figure 4.5: Cellular growth (solid symbol) and oxygen consumption (open symbol) for different inoculum densities: (A) $1 \times 10^5$ cells/cm$^3$ (B) $2 \times 10^5$ cells/cm$^3$, (C) $1 \times 10^6$ cells/cm$^3$, and porosities (square: $\gamma = 80\%$, circle: $\gamma = 85\%$, triangle: $\gamma = 90\%$, inverse triangle: $\gamma = 95\%$). The operating conditions are, $v = 10$ mm/s, $C_{O_2} = 20\%$ (v/v), $d_{p} = 250$ μm. G: Cellular growth curves, O: Oxygen concentration curves.
Figure 4.6: Cell population distribution at various time points. The operating conditions are: $v = 10 \text{ mm/s}$, $C_{O_2} = 20\% (\text{v/v})$, $\gamma = 95\%$, $d_p = 250 \mu\text{m}$, inoculum density of $2 \times 10^5$ cells/cm$^3$. 
Gas Phase Oxygen Concentration (% v/v)

Frequency (%)
Figure 4.7: Oxygen concentration frequency for different porosities (γ = 80-95%) during the culture at various culture points: (A) Day 3, (B) Day 6, (C) Day 9, (D) Day 12 and (E) Day 14. The operating conditions are: \( v = 10 \text{ mm/s} \), \( C_{O_2} = 20\% \text{ (v/v)} \), \( d_p = 250 \mu\text{m} \), inoculum density of \( 2 \times 10^5 \text{ cells/cm}^3 \).
model for different cell lineages that attempts to account for the complexity of the BM functionality. This particular characteristic is crucial given the mass production ability of the BM, which has been absent from other studies involving bioreactor design for BM reconstruction. Although experimental validation of this work is yet to be carried out, the model presented here is able to demonstrate a number of key points: (1) the fluid dynamics environment inside porous scaffolds offers a low shear stress environment for cellular growth and velocity that resemble the known sinus blood velocities within the in vivo BM environment; (2) within the range of practical initial seeding densities, the present reactor design and configuration can accommodate HSC expansion that is normally required for clinical applications; (3) the spatial average oxygen concentration in the vessel ranges from 4-21%, which is close to known physiological BM conditions in vivo. This result further substantiates the importance of local oxygen concentration experienced by the cells over the widely used gas-phase concentration; spatial oxygen distribution is also more informative. Our data highlight the close relationship between flow characteristics, inoculum density, perfusion rate, pore size, porosity, and nutrient concentration, enabling their a priori optimisation that could lead to a more favourable environment for cell growth and maintenance. Furthermore, the simulation results are substantiated by the available literature data. The computational model developed is sufficiently general for further model expansion and incorporation of an even more realistic haematopoietic process and parameters, such as inclusion of growth factors and metabolites. In addition, the model can also be expanded to study other types of cultures important for clinical investigations, such as leukaemia (Colijn and Mackey 2005).
Chapter 5

Model Testing – Part I

To assess the validity of the model, numerical solutions for nutrients transport and cell growth in the bioreactor are compared with experimental results obtained by Liu et al. (2006). Their work demonstrated the expansion of umbilical cord blood cells in a RWPV using combinations of low level cytokines and cell-dilution feeding protocol. Quantitative analysis of the differences between the experimental and computational results was carried out.

5.1 Model Formulation and Parameter Values

As the experimental work of Liu and others does not correspond to the work presented in Chapter 4 directly, some minor modifications were implemented in the model to capture the experimental work for the expansion of haematopoietic stem cells in a perfusion vessel using
a cell-dilution feeding protocol (Liu et al. 2006). Due to the high prolific rate of HSC, Liu et al. (2006) utilised the cell-dilution feeding protocol in order to provide sufficient space for cell expansion, avoid depletion of nutrients and accumulation of metabolites in the culture. Cells are diluted on day 3, 5, and 7 to maintain the cell density below $1.5 \times 10^6$ cells/ml by partial removal of excess cell suspension and replenish the vessel with fresh medium. To simulate this feeding schedule, the simulation is re-started on day 3, 5, and 7 with an initial cell density at $2 \times 10^5$ cells/ml. Furthermore, in contrast to the previous case, where a scaffold was utilised in the bioreactor, the work of Liu and others dealt with suspension cultures, thus, porous flow parameters are redundant and set the porosity to 1.0.

Other biophysical parameters and growth kinetic parameters are as in the case of Chapter 4, see Appendix A, Table A2-4. All experimental results reported in the following section are shown as the mean ± standard deviation. The coefficient of determination, $R^2$, was used to assess the variability between the model and experimental values of total cells and G-CSF.

### 5.2 Results and Discussion

#### 5.2.1 Total Cell Number

Figure 5.1A compares the total cell density for 8 days with an inoculum density of $2 \times 10^5$ cells/ml. The results showed that the predicted total cell density is within the range of all experimental values, at first glance, it does appear that the experimental and numerical results agree well.

In the work of Liu et al. (2006), the cells removed from the experimentation are assumed to have the same expansion capability as the cells in the vessels, and therefore, the total expansion also accounts for these cells. For a suitable comparison of cell expansion pattern, the results obtained from this work capture such features and are presented in Figure 5.1B. It can be seen that the simulation results are very close to experimental results until day 7, after
Figure 5.1: Comparison of (A) total cell density and (B) cumulative total cell expansion between published experimental results (○) and numerical predictions (■).
which the difference become larger. This is mainly due to the value obtained from simulation on day 3 is considerably lower than the mean experimental values (Figure 5.1A), thus when calculating the cumulative expansion of cells, this effect become noticeable after day 7. However, in general, the simulation results do capture the trend of the cell expansion obtained from the experimentation.

5.2.2 CFU-GM expansions

As mentioned previously, the number of CFU-GM is an important indicator for assessing the BMT possibilities. Figure 5.2 compares the simulations and experimental results (Liu et al. 2006) of the CFU-GM expansion. The simulations results predict that there is a 9 fold increase in CFU-GM, whereas the results of Liu and others (Liu et al. 2006) report a 23 fold increases. As the work within Chapter 4 does not take into account specific cytokines for the growth of the CFU-GM, it is possible that this is the reason for the large difference in the growth between the experimental and the simulation results. It is well understood that G-CSF and GM-CSF stimulate the production of CFU-GM, additions of these cytokines in ex vivo environment has been shown to increase the CFU-GM output by 2-3 folds in 10 days (Koller et al. 1995b; Poloni et al. 1997). The cellular growth kinetics parameters used within the simulation work were obtained from the work of da Silva et al. (2003), where neither G-CSF or GM-CSF were utilised, thus the differences between the simulation work and the experimental work of Liu et al (2006) are considered reasonable.

The sum of square errors between the experimental data and model predictions can be shown to be less than 10%; signifying a good level of predictability from the model. However, further improvements to the model are clearly necessary in order to capture the effects of cytokines towards the cell growth (which is the focus of the Chapter 6).
Figure 5.2: Comparison of CFU-GM expansion between published results (Liu et al. (2006)) and numerical predictions (Present Work).
Model Testing – Part II

In the earlier part of this chapter, normal haematopoietic cell growth model has been tested against the experiment data from Liu et al. (2006). To further assess the validity of the BM model, specific experiments were carried out using a human myeloid leukaemia cell line to obtain growth and metabolism data. The purpose of this section is to compare data generated from the model to the experimental data.

5.3 Material and Method

5.3.1 Cell Line Culture

K562 human leukaemia cells (ATCC) were grown and maintained in Iscove's modified Dulbecco's medium (IMDM, Invitrogen) in the presence of 10% fetal bovine serum (FBS, Invitrogen) at 37°C in a humidified atmosphere with 5% CO2 in air, supplemented with 50 units/ml penicillin, and 50 mg/ml streptomycin (1% Pen/Strep, Invitrogen). Inoculum density of the cell cultures were 1x10⁴ cell/ml in a T-75 flask. Due to the high proliferative nature of the cell, a cell-dilution protocol was adopted to reduce the cell concentration. At day 2, 4, 6 and 8, half of the medium (including cells) were removed and discarded and replaced with the same volume of fresh media.

Cell growth was studied by determining the cell number/ml at different days of in vitro cell culture. Cell number was counted every other day. The viable and non-viable cells in the culture were distinguished with the use of trypan blue dye (Sigma) and a bright-line haemocytometer (Assistant, Germany).
5.3.2 Glucose Consumption

The glucose concentration in the culture medium was measured every other day by using a BioProfile 400 (Nova Biomedical, U.S.A.). Medium was removed from the culture and centrifuged at 120g for 7 minutes at 25°C. 1.5ml of sample in supernatant was collected for analysis.

5.3.3 Statistical Analysis

All experimental data are reported as means ± standard deviation (SD). Statistical analysis for comparing the variability of total cells and glucose level between the computational and experimental results are performed using the coefficient of determination, R².

5.3.4 Mathematical Model and Equations Development

The underlying mathematical model presented in Chapter 4 has been adapted used to simulate the expansion of K562, a human myeloid leukaemia cell line, in the bioreactor. Generally speaking, the use of primary cells is a standard practice for accessing cell properties in vitro, however, primary cells activities vary greatly, thus it is not realistic to use primary cell to validate the existing model. Furthermore, no human haematopoietic cells have been successfully derived into a cell line, for this reason, we have employed K562, a cell line derived from the human leukaemic haematopoietic cells for the experiment.

Cellular growth kinetics equations are given in section 4.3.3 (Equations 4.6); these are in general form and can be used to study either multi-cellular growth or single cell type growth. To model this cell dilution protocol, the simulation is re-started on day 2, 4, 6, and 8 with half of the cell population and medium (from the previous simulation). The various parameters and constants within the model are similar to the values given for Section 5.1 (See Appendix A Table A2-4), with suitable adjustments made where necessary (see Appendix A Table A7).
5.4 Results and Discussions

Comparisons between simulation and experimental results for K562 cells are presented in Figures 5.3 and 5.4. In Figure 5.3A, there are observable differences between the model and the experimental data, however, statistical analysis (based on R-squares) shows that the sum of square error was less than 3% between the two sets of data. Although, overall the model is able to predict the experimental with a good level of accuracy, the relatively large difference between the two types of data during the initial culture periods can be attributed to the model not being able to capture and account for the ability of the K562 cells to ‘handle’ shocks in the system where there is a sudden change in environmental conditions. Therefore, while the cells within the experimental set-up are adjusting and acclimatising accordingly to the ‘new’ conditions, the model is not yet designed for such features. However, with the increase in the culture time, the cell density approaches to that of the experimental value (day 6 and beyond), which is possibly due to the culture conditions not affecting the growth characteristics as greatly as observed during the early stages of the culture. In Figure 5.3B, the total cell number between the numerical results and the experimental data are compared. Similar strategy is adopted by Liu et al. (2006) to obtain total cell expansion as mentioned previously. It is evident that the observable differences are minimal ($R^2 = 0.971$).

Glucose consumption is an indication of cell metabolism and growth. In Figure 5.4, the glucose consumption of K562 in the experiment is comparable to the model predictions. The sum of square errors between the experimental data and model predictions was less than 10%.

5.5 Summary

The validity of growth and metabolic model of haematopoietic stem cells was investigated. In summary, the model predictions are in good overall agreement with the published work and
experimental data ($R^2>0.9$). These results demonstrate that the model is capable of describing the growth of the progenitor cells (CFU-GM), total cell growth and metabolic behaviour of K562 cells with a good level of accuracy. This work herein formed the basis for the model presented in Chapter 6.
Figure 5.3: Comparison of (A) cell density and (B) cumulative total cell number for K562 between experimental results (■) and simulations predictions (○) under cell-dilution feeding protocol. Experimental values shown are the means ± standard deviation (SD) for data from 3 independent experiments.
Figure 5.4: Comparison of total glucose consumption between experimental results (■) and simulation predictions (○).
Abnormalities in the feedback mechanisms for the haematopoietic process lead to bone marrow diseases, such as chronic myeloid leukaemia. There are many variants of CML, including the periodic form, as characterized by oscillating cell numbers. Studies suggest that the destabilization of the feedback loop between blood cells and growth factors (GF) contributes to periodic haematological diseases (Adimy et al. 2006a).

Considerable work has been carried out in the 2-D (flask) CML cultures attempting to explicate the cell functionalities within an ex vivo environment. However, CML cells are
unable to sustain growth in 2-D (flask) cultures for lengthy periods, normal cells have the selective growth advantage and outgrow the CML cells \textit{in vitro} (Udomsakdi et al. 1992; Petzer et al. 1997; Luna-Bautista et al. 2003; Chavez-Gonzalez et al. 2004). Recently, Wu et al. (2006) developed a 3-D bioreactor and successfully generated functional leukaemia cells for at least four weeks (Wu et al. 2006). As in the case for normal haematopoiesis, it is plausible that the lack of a three-dimensional (3-D) microenvironment, a key contributor to the growth patterns, results in the varying properties of the BM cells. More specifically, the cell-cell and cell-matrix interactions are known to be crucial in sustaining long term haematopoietic cell cultures.

A number of mathematical models developed to study the mechanisms involved in CML growth were highlighted in Chapter 3. The work of Colijn and Mackey (2005) was particularly emphasised. Colijn and Mackey's model failed to explicitly include the G-CSF feedback control; therefore, how G-CSF affects granulopoiesis in CML could not be investigated. The recent work of Adimy et al. (2006) studied the effects of growth factors, G-CSF on the dynamics of neutrophil production (Adimy et al. 2006a). Though the G-CSF is explicitly accounted for in the model, other important cell lineages such as erythrocytes and platelets were not included, and therefore limited the study to certain lineages, affecting the stability of the system.

The present work deals with the development of a mathematical model for the study of CML cellular growth patterns in an \textit{ex vivo} culture system using a 3-D perfusion bioreactor. This work is an extension of the work presented by Colijn et al. (2005), with the inclusion of specific growth factors, such as G-CSF, TPO and EPO, to account for the feedback mechanisms of different lineages. Furthermore, the effect of different cytokine combinations and concentrations on CML growth is investigated, along with different operating conditions (re-circulating and non-re-circulating media).
6.1 Geometry

The vessel comprises of two main regions: non-scaffold and scaffold, see Chapter 4, Figure 4.1. The non-scaffold region consists of inlet/outlet, central canal and radial branches. The scaffold is the region where the cells are localised and is assumed to have a 80-90% porosity with a pore size of 250–350 μm as well as isotropic and homogenous pore structure. In addition, the scaffold is assumed to be free from blockage and deposition.

6.2 Model Formulation

The basic model equations were presented in Chapter 3; in this chapter specific model equations pertaining to the work described herein are offered.

6.2.1 Model Equations

6.2.1.1. Fluid Flow and Mass Transport

As in the case of Chapter 4, the continuity, Navier-Stokes and porous flow equations are used to describe the flow within the Rotating Wall Perfused Bioreactor (RWPB). The spatial distribution of nutrients within the scaffold is described by the general mass transport equation and the growth factors consumption is described by Monod-type kinetics.

6.2.1.2. Growth Kinetics

Four compartments (stem cells, leukocytes, platelets and erythrocytes) and the relative feedback system (Figure 6.1) are represented by a system of differential equations described below.
Figure 6.1: CML haematopoiesis model. Stem cell (Q) is either in resting phase or entering (\( \beta \)) into proliferating phase (\( Q_p \)) and differentiation into erythrocytes (R), leukocytes (N) and platelets (P). The differentiation rate of R (\( \kappa_R \)), N (\( \kappa_N \)) and P (\( \kappa_P \)) are regulated by the amount of EPO, G-CSF and TPO, respectively. \( A_R, A_N \) and \( A_P \) are the amplification factors of R, N and P, respectively. \( \delta_Q, \delta_R, \delta_N \) and \( \delta_P \) are the death rate of Q, R, N and P, respectively. \( \delta_{EPO}, \delta_{G-CSF} \) and \( \delta_{TPO} \) are the degradation rate of EPO, G-CSF and TPO, respectively.
i) Stem Cell compartment

Under normal conditions, the majority of HSCs and progenitor cells are quiescent in the $G_0$ phase of the cell cycle. Colijn et al. (2005) employed a $G_0$ model to illustrate the proliferating and resting phase of stem cells (Colijn and Mackey 2005). It is a typical delay model to accounts for the time that the cells spend in completing the proliferative phase of the cell cycle.

$$\frac{dQ}{dt} = -\beta(Q)Q - (\kappa_N + \kappa_R + \kappa_p)Q + 2e^{-\delta_Q} \beta(Q(t - \tau_x))Q(t - \tau_x)$$

(6.1)

where $Q$ is the CML stem cell population, $t$ is the time, $\beta(Q)$ is the re-entry rate of resting CML stem cells into proliferating phase and is represented by the Hill function described in equations 3.36, $\delta_Q$ is the death rate of stem cells, $\tau_x$ is the time delay for stem cell proliferation, $\kappa_N$, $\kappa_R$ and $\kappa_p$ are the differentiation rates into the leukocyte, erythrocyte and platelet compartment, respectively and are describe by the following Hill function expression:

$$\kappa_N(G) = \kappa_0 \frac{G^h}{G^h + k_{GCSF}}$$

(6.2)

$$\kappa_R(T) = \kappa_p \frac{T^h}{T^h + k_{TPO}}$$

(6.3)

$$\kappa_R(E) = \kappa_r \frac{E^h}{E^h + k_{EPO}}$$

(6.4)
where $G$, $T$, $E$ are the concentrations of G-CSF, TPO and EPO, respectively. $f_o$, $\bar{k}_p$, $\bar{k}_r$, $h_1$, $h_2$, and $h_3$ are the Hill function coefficients. Estimation of these values are described in Chapter 3. $k_{GCSF}$, $k_{EPO}$, $k_{TPO}$, are Hill constants calculated from the differentiate rate of leukocyte, erythrocyte and platelet compartment respectively (Appendix A, Table A5).

ii) Leukocyte, erythrocyte and platelet compartment

Similarly, leukocyte $(N)$, erythrocyte $(R)$ and platelet $(P)$ cellular components are represented by the following equations.

\[
\frac{dN}{dt} = -\delta_N N + A_N \kappa_N (N_{g_N}) Q_{r_N} \tag{6.5}
\]

\[
\frac{dR}{dt} = -\delta_R R + A_R \kappa_R (R_{g_R}) Q_{r_R} - e^{-\delta_R \tau_{r_R}} \kappa_R (R_{g_R}) Q_{r_R} \tag{6.6}
\]

\[
\frac{dP}{dt} = -\delta_P P + A_P \kappa_P (P_{g_P}) Q_{r_P} - e^{-\delta_P \tau_{r_P}} \kappa_P (P_{g_P}) Q_{r_P} + \tau_{r_P} \tag{6.7}
\]

where $\delta_N$, $\delta_R$, $\delta_P$ are the death rates of leukocytes, erythrocytes and platelets, respectively. $Q_{r_N}$, $Q_{r_R}$, $Q_{r_P}$, $Q_{r_P}$, $Q_{r_P} + \tau_{r_P}$ are the delay time for stem cells to mature into leukocytes, reticulocytes, erythrocytes, platelets and senescence platelets respectively. $A_N$, $A_R$, $A_P$ are the amplification factors of leukocytes, erythrocytes and platelets, respectively. $\tau_N$, $\tau_{RM}$, $\tau_{sum}$ and $\tau_{PM}$ are the time delay for leukocyte, reticulocyte, erythrocyte and platelet maturation, respectively. $\tau_{PS}$ is the time delay for platelet aging due to senescence.

Additional complexity lies in the modelling of erythrocyte and platelet lineages in comparison to the leukocyte lineage. The leukocyte lineage matures and dies at a constant
rate, while the erythrocytes and platelets mature and go into senescence. Therefore, an extra delay term has been incorporated into the respective equation to account for the loss via senescence in blood (Colijn and Mackey 2005).

iii) G-CSF

Change in the G-CSF concentration represents the difference between the production and elimination of G-CSF as suggested by Shochat et al. (Shochat et al. 2006):

\[
\frac{d[G]}{dt} = \frac{V_G^{\text{max}} k_N}{k_N + N} - \frac{\delta_{\text{GCSF}} N}{k_N + N} [G] \tag{6.8}
\]

where \( G \) is the concentration of G-CSF, \( V_G^{\text{max}} \) is the maximum production rate of G-CSF, \( k_N \) is the dissociation constant of neutrophils and \( \delta_{\text{GCSF}} \) the elimination of G-CSF by neutrophils.

iv) TPO

In a similar fashion to G-CSF, the changes in the TPO concentration represent the difference between the production and elimination of TPO represented by (Santillan et al. 2000):

\[
\frac{d[T]}{dt} = \frac{a_{\text{TPO}}}{1 + K_{\text{TPO}} P^h} - \delta_{\text{TPO}} [T] \tag{6.9}
\]

where TPO is the concentration of TPO, \( a_{\text{TPO}} \), \( K_{\text{TPO}} \) and \( h \) are the parameters in the Hill function and \( \delta_{\text{TPO}} \) is the half-life of TPO.

v) EPO

EPO feedback mechanism has a similar form to the TPO’s (Mahaffy et al. 1998):
where EPO is the concentration of EPO, \( a_{EPO} \), \( K_{EPO} \) and \( h_2 \) are the parameters in the Hill function and \( \delta_{EPO} \) is the half-life of TPO.

### 6.3 Initial and Boundary Conditions

The initial conditions were: the inlet perfusion rate is at 15 mm/s; the inoculum density is set at \( 1 \times 10^9 \) cells/cm³ with the duration of the culture simulation set at 10 days; and the inlet growth factor concentrations and combinations being: 1) G-CSF (10 ng/ml), 2) TPO (10 ng/ml) 3) EPO (3 U/ml) 4) G-CSF (10 ng/ml) + TPO (10 ng/ml), 5) G-CSF (10 ng/ml) + EPO (3 U/ml) 6) G-CSF (10 ng/ml) + TPO (10 ng/ml) + EPO (3 U/ml) 7) G-CSF (30 pg/ml) + TPO (5 mU/ml) + EPO (10 mU/ml). The kinetics of CML lineages and growth factors are given in Appendix A Table A5 and the biophysical parameters are given in Appendix A Tables A6.

As indicated previously, this chapter is divided into two parts: with and without re-circulation. In simulations without re-circulation, the inlet and outlet boundary conditions are declared as Dirichlet boundary and outlet mass flow boundary conditions, respectively. While for the simulations with the re-circulation, the boundary conditions are set as periodic boundaries conditions (PBC). The standard velocity condition of 'no slip' is applied at the wall boundaries, with no-flux condition across the walls. The flow assumptions applied in Chapter 4 are carried forward here.
6.4 Re-circulation Medium – Periodic Boundary Conditions (PBCs)

In a perfusion culture system, fresh medium are introduced into the vessel continuously while the metabolic waste is removed to prevent waste ‘build-up’. However, for effective use of cytokines, as they are costly materials, the medium is usually recycled back into the vessel; fresh medium is only replaced at various time points during the culture. To simulate medium re-circulation, periodic boundary conditions are applied. The approach for the implementation procedure was described in Chapter 3.

6.5 Results and Discussion

6.5.1 Perfusion Without Re-circulation

CASE 1: NORMAL BM CELLS VS. CML CELLS

In the previous chapter, the growth characteristics of normal haematopoietic cells were investigated under varying operating conditions in a low-shear perfused bioreactor embedded with a porous scaffold. As a further advancement of this previous work, in this chapter the former model is utilised, which further incorporates the effects of cytokines on cellular growth. This model is then applied to CML cells in pathological BM. Growth rates of specific lineages of normal and CML cells are provided in Table A5 (Appendix A).

Comparison of normal BM and CML cell growth in a 3-D perfusion culture system are shown in Figure 6.2. At the stem cell level, CML cells rapidly decline to an undetectable level, whereas normal BM cells decrease more slowly (Figure 6.2A). This observation is in line with the work carried out by Udomsakdi et al. (1992), who showed that the long-term culture initiating cells of CML cells decline more rapidly than the normal BM cells due to a defect at the CML stem cell level (Udomsakdi et al. 1992).
At higher levels of maturity, CML cells demonstrate a significant increase in leukocytes as compared to normal BM culture cells (Figure 6.2B). Throughout the simulation period, CML cultures have three times more leukocytes than the normal BM cells culture. Platelets in the CML cultures show a higher expansion rate than in normal haematopoiesis, whereas, almost no difference (<1%) is observed between the number of erythrocytes CML and normal BM cultures (Figure 6.2C,D). At the end of the 10 days simulation period, CML cultures have approximately 5% more cells than the normal BM cultures (Figure 6.2E). These cell proliferation patterns of CML are also observed in clinical BM: amplification in granulocytes lineages, decrease in erythropoiesis, steady or an increased in the number of platelets. Some patients also display hyper-cellular bone marrow; correlated with increasing number of cells in the marrow (Lichtman and Liesveld 2000). These results also agree with the previous ex vivo findings of other investigators, who compare the expansion of normal and CML cell and demonstrate that the primitive CML cells (LTC-IC) lost the ability to expand regardless of the addition of cytokines; while the relatively more mature cells showed amplification in their population (Petzer et al. 1997).

**CASE 2: EFFECT OF SINGLE VS. MULTIPLE GROWTH FACTORS ON CELL EXPANSION PATTERNS**

Cytokines can be specific or non-specific, as they can act on different types of cells such as primitive cells, progenitor cells, or committed cells and on different lineages. Some growth factors also have synergistic or additive effects when used with other growth factors (Metcalf and Nicola 1995). In this study, the individual effects of single cytokines (G-CSF, TPO or EPO) as well as their combined effects (G-CSF+TPO, G-CSF+EPO, TPO+EPO, G-CSF+EPO+TPO) on the expansion of different lineages are examined.

In all cases, stem cell numbers show a continuous decline throughout the 10 day cultures, especially in the case of cytokine cocktails (Figure 6.3A). Previous studies showed that G-CSF, TPO and EPO stimulate the growth of leukocytes, platelets and erythrocytes,
A. Fold increase in stem cells

- Normal
- CML

Time (days)

B. Fold increase in leukocytes

- Normal
- CML

Time (days)
**Figure C**

Fold increase in platelets over time for normal and CML samples.

**Figure D**

Fold increase in erythrocytes over time for normal and CML samples.
Figure 6.2: Expansion of different lineages in normal and CML cells. Both cases have the same conditions: 90% porosity, pore size at 250 μm, perfusion rate of $1.5 \times 10^{-3}$ m/s, G-CSF, TPO and EPO concentration at 10 ng/ml, 10 ng/ml, and 3 U/ml, respectively.
respectively (Metcalf and Nicola 1995). As expected, in the single cytokine studies, G-CSF amplified and maintained the number of leukocytes while platelet and erythrocyte expansion was dependent on the addition of TPO and EPO respectively (Figure 6.3B,C,D). Interestingly, in the cytokine cocktail studies, combining G-CSF with TPO, EPO or TPO+EPO did not indicate better expansion in leukocytes than by using G-CSF alone; the GF combinations indeed decrease leukocyte expansion to some extent (Figure 6.3E). This observation is also found in the erythrocytes lineages (Figure 6.3D). Whether this effect is cell lineage specific or due to growth factor competition is unclear and needs further analysis.

The effects of either single or combinations of growth factors on cell dynamics of different lineages varied. Though adding TPO+EPO gives similar total number of cells as G-CSF+EPO+TPO, there is no expansion in the leukocytes lineages (Figure 6.3B). The growth factors combination of G-CSF, TPO and EPO seems to be the more effective in increasing the total numbers of each cell types (Figure 6.3A-E). These results are in line with a previous study by Bhatia’s group demonstrated total CML cells expansion is significantly higher under the combinations of cytokines than using single cytokines (Bhatia et al. 2000).

The addition of cytokines promotes the proliferation, differentiation and maturation of early pre-progenitors through terminal differentiation, thereby amplifying the total cell population. However, at the same time, this process increased GF-induced maturation and decreased self-renewing divisions of primitive progenitors. This has also been demonstrated in other studies, where the combination of growth factors within the culture significantly reduced the primitive population that has multilineages colony formation ability (Brugger et al. 1993; Bhatia et al. 2000).

**CASE 3. EFFECT OF GROWTH FACTOR CONCENTRATION ON CML CELL EXPANSION**

Previous studies have demonstrated that high concentration of cytokines can enhance the self-renewal, proliferation, differentiation and maintenance of CML haematopoietic cells.
A G-CSF
TPO
EPO
G-CSF+TPO
G-CSF+EPO
TPO+EPO
G-CSF+TPO+EPO

0.9
0.6
0.4
0.2

Time (days)

Fold increase in stem cells

G-CSF
TPO
EPO
G-CSF+TPO
G-CSF+EPO
TPO+EPO
G-CSF+TPO+EPO

B

15
10
5
0

Time (days)

Fold increase in leukocytes

G-CSF
TPO
EPO
G-CSF+TPO
G-CSF+EPO
TPO+EPO
G-CSF+TPO+EPO

124
Figure 6.3: Expansion of stem cells, leukocytes, platelets and erythrocytes under single and combined cytokines (G-CSF, TPO, EPO). The culture conditions: I.D. = $1 \times 10^3$ cells/cm$^2$, $\gamma$ = 90%, $d_p$ = 250$\mu$m, $v$ = 1.5 mm/s, G-CSF = 10 ng/ml, TPO = 10 ng/ml and EPO = 3 U/ml.
(Luna-Bautista et al. 2003; Chavez-Gonzalez et al. 2004). However, Bhatia et al. (2000) demonstrated that using high doses of cytokines does not further increase CML cells expansion. To further investigate the effect of varying growth factor levels on the growth of CML cells, two different concentrations of cytokines cocktails were chosen. The first concentration (normal level: G-CSF = 10 ng/ml, TPO = 10 ng/ml, EPO = 3 U/ml) typically represents the values used in in vitro cultures, whereas the second concentration (low level: G-CSF = 30 pg/ml, TPO = 5 mU/ml, EPO = 10 mU/ml) corresponds to the in vivo levels of cytokines.

Figure 6.4 compares the growth kinetics of stem cells, leukocytes, platelets and erythrocytes under normal (in vitro level) and low (in vivo level) growth factor concentrations. Under normal GF concentration, stem cell numbers declined to an undetectable level after the first day, whereas, in the low GF concentration culture, the stem cells is able to maintained higher numbers for a longer period (Figure 6.4A). A previous study by Bhatia et al. (2000) has also demonstrated that increasing cytokines concentration fail to expand the primitive CML LTC-IC (Bhatia et al. 2000).

At normal GF concentrations, leukocytes show a marked increase in numbers followed by a decline (Figure 6.4B), whereas, under low GF conditions, leukocytes show a continuous decrease in cell numbers. Platelet numbers show similar expansion patterns under both conditions, though platelet numbers under normal conditions has about 22% more cell numbers than the low conditions (Figure 6.4C). Erythrocyte numbers under normal growth factor concentration showed a continuous increase throughout the culture as compared to low GF conditions (Figure 6.4D). Under normal GF concentration, there are four times more total cell number than the low GF concentration at the end of 10 days culture simulation (Figure 6.4E). Interestingly, among these lineages, leukocytes and erythrocytes are relatively more sensitive to the cytokines concentration. At the end of the culture, over 10 times difference of
A

Fold increase in stem cells

Time (days)

B

Fold increase in leukocytes

Time (days)
Figure 6.4: Expansion of stem cells, leukocytes, platelets, erythrocytes and total cell number under normal and low growth factors concentration. The operational conditions are $\gamma = 90\%$, $d_p = 250$ $\mu$m, $v = 1.5$ mm/s, $ID = 1 \times 10^6$ cells/cm$^3$. Normal GF: G-CSF = 10 ng/ml, TPO = 10 ng/ml and EPO = 3 U/ml, Low GF: G-CSF = 30 pg/ml, TPO = 5 mU/ml and EPO = 10 mU/ml.
Figure 6.5: CML cells population distribution at various time points. The operational conditions are \( \gamma = 90\% \), \( d_p = 250 \) \( \mu \)m, \( v = 1.5 \) mm/s, \( ID = 1 \times 10^9 \) cells/cm\(^3\). Normal GF: G-CSF = 10 ng/ml, TPO = 10 ng/ml and EPO = 3 U/ml, Low GF: G-CSF = 10ug/ml, TPO = 5 mU/ml and EPO = 10 mU/ml.
leukocytes and erythrocytes numbers were found between two different GF conditions, whereas, only 1.3 times difference is shown in platelet numbers. The expansion patterns of individual lineages under normal and low GF concentrations are shown in Figure 6.5. From the distribution pattern of the lineages, it is demonstrated that under normal GF concentrations, erythrocytes dominate the cellular composition with over 80% occurrence whereas under the low GF concentrations, platelets are the most abundant cell type, which contribute to 60% of the total cell population (Figure 6.5). This further demonstrates that platelet expansion is less responsive to GF level and showed better expansion among the lineages in the low GF condition.

Different levels of cytokine have drastic effects on the stem cell dynamics, which in turn, influences the distribution of cell types. Based on this observation, optimising the cytokine level could be an effective strategy to maintain cell numbers and cell types distribution, thus, to re-create an environment similar to in vivo steady state for expansion and maintenance.

6.5.2 Perfusion With Re-circulation

CASE 4: EFFECT OF RE-CIRCULATION MEDIUM ON CELL EXPANSION PATTERN

Without re-circulation of the medium in the perfusion bioreactor, the average cytokine concentration in the vessel remains the same throughout the 10 days culture (Figure 6.6A-C); signifying that cytokines are not utilized in an efficient manner. Consequently, the use of medium re-circulation avoids unnecessary disposal of cytokines. Results from this study demonstrate that the total amount of growth factor utilised in the re-circulation culture simulation was limited to 1500 ng of G-CSF, 1500 ng of TPO and 450 U of EPO, while in the culture without any re-circulation, approximately 100 times greater concentration of growth factors was required. Additional effects of re-circulation and non re-circulation on cell lineages kinetics are shown in Figure 6.7. Although there are limited differences between
Figure 6.6: Concentration of G-CSF (A), TPO (B) and EPO (C) in the perfusion bioreactor with re-circulation and without re-circulation. The operational conditions are I.D. = 1x10⁵ cells/cm³, γ = 90%, dₚ = 250 μm, v = 1.5 mm/s, G-CSF = 10 ng/ml, TPO = 10 ng/ml and EPO = 3U/ml. Arrow represents the time when new medium is added to the culture in the re-circulating condition.
Figure 6.7: Kinetics of different lineages in the perfusion bioreactor with re-circulation and without circulation. The operational conditions are I.D. = 1x10^5 cells/cm³, γ = 90%, dₚ = 250 µm, v = 1.5 mm/s, G-CSF = 10 ng/ml, TPO = 10 ng/ml and EPO = 3 U/ml.
cell numbers under the two conditions (Figure 6.7A, B, C), it does appear that all cell lineages have lower expansion potential under re-circulation mode. At the end of the simulation, the total number of cells has 1.3 fold difference between the two operating conditions (Figure 6.7D). The decline in cell proliferation in all lineages is attributed to the lower levels of cytokines being delivered to the cells in the re-circulation operation. Different types of operation can influence the expansion pattern of CML cells, by replacing the depending GFs more frequently and combined with re-circulation of medium, GFs can be more effectively used.

**CASE 5: SPATIAL DISTRIBUTION OF GROWTH FACTORS AND CELLS WITHIN THE SCAFFOLD**

The design of scaffold architecture can affect the transport profiles in the bioreactor and therefore can affect cellular growth. The spatial distribution of growth factors, EPO, and erythrocytes in the 3-D perfusion culture vessel are shown in Figure 6.8. In figure 6.8A, the spatial distribution of EPO shows a high level of growth factors around the centre part of the bioreactor which corresponds to the highest velocity area, as observed in Chapter 4 (Figure 4.2). During the transport of growth factors, these are likely to be consumed or degraded naturally, but with high flow rate, the growth factors are replenished. Low GF concentration were observed around the boundary areas at two ends, it is possible that due to the slow flow of the media around these areas, as a result, the consumption and degradation rate is faster than the replenishing rate, thus creating this gradient level. In Figure 6.8B, the distribution of erythrocytes showed a similar contour as the distribution of EPO. Comparing two contour plots, numbers of erythrocytes are relative to the concentration of growth factor, this implies that cellular growth is dependent on the growth factor concentration.

The delivery of GFs for cellular growth is governed by scaffold porosity and pore size. Thus, the effect of scaffold properties on CML cell expansion was investigated and the results
Figure 6.8: Contour plot of EPO and Erythrocytes concentrations on axial positions on day 3 under re-circulation operation. Planes are drawn at the X-Z orientation on the axial direction of the RWPB (Figure 4.1B). The operation conditions are I.D. = 1x10^5 cells/cm^3, γ = 90%, \(d_r = 250 \mu m\), \(v = 1.5 \text{ mm/s}\), G-CSF =10 ng/ml, TPO = 10 ng/ml and EPO = 3 U/ml. Arrows represent the inlet and outlet respectively.
Figure 6.9: Expansion of total number of cells for different porosities and pore sizes. The operation conditions are I.D. = $1 \times 10^3$ cells/cm$^3$, $\gamma = 80$ or $90\%$, $d_p = 250$ or $350$ $\mu$m, $v = 1.5$ mm/s, inlet GF concentration: G-CSF = 10 ng/ml, TPO = 10 ng/ml and EPO = 3 U/ml.
Figure 6.10: G-CSF (A), TPO (B) and EPO (C) concentrations within different scaffold architecture during 10 days with culture under re-circulation operation. The operation conditions are I.D. = 1x10^5 cells/cm^2, \( \gamma \) = 80 or 90\%, \( d_r \) = 250 or 350 \( \mu \)m, \( v \) = 1.5 mm/s, initial GF concentration: G-CSF = 10 ng/ml, TPO = 10 ng/ml and EPO = 3 U/ml.
demonstrated the largest number of total cell expansion was observed in the high porosity (90%) and large pore size (350 μm) case (Figure 6.9). This finding is in agreement with the results from Chapter 4 that higher porosity scaffolds provides more space for cellular growth thus, resulting in a higher demand for growth factor. For this reason, the least GF concentration is observed in the scaffold with 90% porosity 350 μm (Figure 6.10A,B,C). Furthermore, the low flow resistance in the large pore size scaffold facilitate the transport of fresh growth factors into the vessel, as a result, increase the cell proliferation rate.

6.6 Summary

The work presented in Chapter 4 has been extended to investigate the proliferation capability of BM cells under pathological conditions. The proposed model is applied to simulate the expansion of CML cells in a perfusion vessel using cytokines as the stimulant. The result demonstrates the in vivo and in vitro phenomena observed in CML cells. Additionally, this study has shown that individual lineages CML cells grow better under single growth factors as compared to growth factor cocktails, whereas, for total CML cells expansion, a mixed growth factor condition is preferred. CML cells are sensitive to GF concentration, GF concentration normally utilised within in vitro cultures showed higher expansion (four times) in total CML cell numbers. Furthermore, scaffolds with high porosity (90%) and large pores size (350 μm) offers a more efficient way of transporting growth factor for CML proliferation. Finally, non re-circulation operation provides a more stable growth factors gradient in the vessel for greater cell expansion, nevertheless, by optimising the re-circulation condition, GFs can be economically used. The developed model improves the understanding of nutrients transport and CML cells expansion pattern in a perfusion culture system, thus, can assist and optimize the design of the CML culture system for pre-clinical and clinical use.
Chapter 7

Final Remarks

7.1 Conclusion

In the present work, a mathematical model has been developed for the study of the growth of haematopoietic cells in a 3-D perfused bioreactor system embedded with a scaffold. Momentum, mass and porous transport equations were solved simultaneously to obtain the shear stress, velocity, nutrients concentration (oxygen and glucose) and cellular growth distribution inside the scaffold. The simulation results show that the bioreactor system can provide a low shear stress ($6.6 \times 10^{-8}$ to $0.086$ dynes/cm$^2$) environment for cellular growth, while having an average velocity ($0.1$ mm/s) that resemble the known sinus blood velocities within the in vivo BM environment. The multi-cell-types study showed that oxygen content within the bioreactor is sufficient to avoid hypoxic conditions (<4% v/v) which can be
detrimental to the cells (Ishikawa and Ito 1988; Cipolleschi et al. 1993). The simulation work provided in chapter 4 can be utilised in a number of ways to not only aid the development of the most favourable design of haematopoietic culture systems, but also to provide a sound basis for the advancement of haematopoietic culture models for its application to diseased BM. In particular, the oxygen and nutrient concentration profiles attained offer the starting point for culture conditions in the study of pathological BM (see Chapter 6). Thus, this work supports further studies presented in the chapter 6 of this thesis.

With any simulation efforts, it is critical that the outputs are verified so as to provide a degree of confidence with the obtained results. Consequently, the simulation results are compared with the experimental work by Liu and others, where haematopoietic cells were cultured using a similar perfusion system (Liu et al. 2006). Following the methodology given in their work, the work was simulated and the results compared to the experimental results provided. The simulated results for the CFU-GM numbers and total cell numbers were within an acceptable range of error (the sum of square errors between the experimental data and model predictions was less than 10%), therefore suggesting that the computational model is able to capture the growth kinetics of haematopoietic cells. To further assess the validity of the model, experimental work was carried out using K562, a type of human myeloid leukaemic cells. With a pre-defined experimental set-up, total cell density and nutrient (glucose) utilisation were obtained. Comparing the experimental output with the simulation output, it is evident that the model data are in good agreement with the experimental data ($R^2>0.9$), thus further validating the major findings predicted by the computational model.

The validation of results obtained from the mathematical model described in Chapter 4 formed the basis to extend the application of this model into diseased BM. A more complete picture of haematopoiesis and feedback regulations by growth factors has been incorporated into a known stem cell model (Chapter 6). The proposed model is applied to simulate the expansion of CML cells in a perfusion vessel using cytokines as the stimulant. The result
demonstrates the *in vivo* and *in vitro* phenomena observed in CML cells: CML stem cells rapidly decline whereas leukocyte lineages increase significantly, this observation is in line with work carried out by Petzer et al. (1997) and Lichtman et al. (2000). Furthermore, the self-renewal, differentiation and expansion pattern are affected and altered by the concentration and varying combinations of cytokines. By combining non-recycled perfusion with the presence of haematopoietic growth factors G-CSF, TPO and EPO, a remarkable amplification of the cell number was observed. This model provides a better understanding of the combined effects of some important culture parameters such as flow rate, scaffold properties, growth factors concentrations and operation mode. Identification of the parameters controlling haematopoiesis of CML cells can assist and optimize the design of the CML culture system for pre-clinical and clinical use.

Although the usefulness of the models presented in this work cannot be discounted, significant amounts of work pertaining to either normal or CML haematopoietic cultures remain to be carried out. These models provide a basis for future developments and enhancement of viable haematopoietic culture models for aiding the reconstitution of healthy and pathological BM in an *ex vivo* environment.

7.2 Future Work

In this sections, suggestions for advancing the present models and future implementation in the CFD simulation software are offered.

7.2.1 3-D BM and Scaffold

The 3-D haematopoietic inductive microenvironment controls the renewal, differentiation, maintenance, maturation and the egress of the blood cells in haematopoiesis. This intricate process involves interactions between proteins, cells and cytokines in this unique 3-D niche. In the present study, the growth of blood cells in a bioreactor has been simulated with an
assumption that the 3-D scaffold is homogeneous and isotropic. However, as cells grow, they tend to occupy the void spaces in the scaffold, and consequently, the scaffold can no longer be assumed to be isotropic, which in turn is likely to affect the momentum and mass transport in the bioreactor. In addition to considering the scaffold properties, it also pertinent that the cell-cell and cell-matrix interactions information should be incorporated into the model. However, how the cell-cell and cell-matrix interacts is yet to be fully elucidated. Nevertheless, the combination of such data should prove to be very useful in design of a tissue-specific scaffold for the cells to recreate the necessary HIM.

7.2.2 Cytokine Interaction

Three different cytokines, G-CSF, EPO and TPO, have been studied in the production of CML cells in a perfused BM culture system. There are many other major growth factors such as stem cell factor, Interleukin-3 and 6, etc involved in blood formation process that are specific or non-specific on a cell lineage, and/or act on the different maturity levels. In addition, these cytokines can be synergistic and/or compete with each others (Koller et al. 1992b; Brugger et al. 1993; Koller et al. 1995b; Poloni et al. 1997; Chaudhry et al. 2004). It has been demonstrated that cytokines can stimulate the rates of cellular glycolysis and show different expansion cell patterns under varying oxygen tensions (Koller et al. 1992b; Laluppa et al. 1998; Bauer et al. 2004). Thus far, in the present work, such effects have been neglected. Further advancements of BM culture models would therefore attempt to incorporate the synergistic and/or competitive effects of the different cytokines involved in cell growth.

7.2.3 Rotational Effect

Although the rotating wall bioreactor has been utilised, the rotational aspect of the reactor has yet to be studied in the context of BM cultures, and how it actually affects shear stress levels,
mass transport and cellular growth. In a previous study, Kohler et al. (1999) found there were no significant improvement in the CD34+ and CFU-GM expansion using rotating culture vessels (Kohler et al. 1999). However, other studies have shown that rotating the vessel can improve fluid flow and mass transport, while maintaining the low shear stress levels (Begley and Kleis 2000; Begley and Kleis 2002). Further, simulated microgravity conditions can also allow cells to aggregate into a 3-D structure (Goodwin et al. 1993). In regards to this, studies have shown that haematopoietic bone marrow cells produce greater number of cells and progenitors in a microgravity environment than 'normal' 2-D flask cultures (Plett et al. 2001). Additionally, under microgravity, human malignant melanoma tissue has similar immunohistochemistry to the in vivo situation (Licato et al. 2001). A simplified analytical model to study the fluid flow in a rotating tube is offered. (See Appendix B1). For completeness and further verify such observations, it is a worthwhile effort to investigate the rotational effects of the vessel on cellular growth.

7.2.4 Fluid Flow Validation

Despite the fact that the results presented in Chapter 6 demonstrate good overall agreement with the published and our own experimental data, one important aspect of future work will be the spatial verification of the simulated flow field. For such purposes, it is recommended to employ either particle-image velocimetry (PIV) or nuclear magnetic resonance (NMR) technique to obtain detailed experimental data around or within the scaffold (Williams et al. 1997; Sucosky et al. 2004).
Appendix A

Model Parameters and Nomenclature

The following tables contain the configurations of the bioreactor and model parameters used in Chapter 4, 5 and 6. These include the cell kinetics of normal and CML cells, the uptake rate of oxygen, glucose and cytokines.
<table>
<thead>
<tr>
<th>Bioreactor Parameters</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vessel Volume</td>
<td>50.41</td>
<td>cm³</td>
</tr>
<tr>
<td>Vessel Length</td>
<td>3.8</td>
<td>cm</td>
</tr>
<tr>
<td>Central canal radius</td>
<td>0.8</td>
<td>cm</td>
</tr>
<tr>
<td>Vessel radius</td>
<td>2.2</td>
<td>cm</td>
</tr>
<tr>
<td><strong>Scaffold Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scaffold Volume</td>
<td>50.06</td>
<td>cm³</td>
</tr>
<tr>
<td>Scaffold Length</td>
<td>3.8</td>
<td>cm</td>
</tr>
<tr>
<td>Outer Tube Radius</td>
<td>2.2</td>
<td>cm</td>
</tr>
<tr>
<td>Inner Tube Radius</td>
<td>0.8</td>
<td>cm</td>
</tr>
<tr>
<td>Branch Diameter</td>
<td>0.2</td>
<td>cm</td>
</tr>
<tr>
<td>Branch Position</td>
<td>1.15/3</td>
<td>cm</td>
</tr>
</tbody>
</table>

Table A1: Bioreactor and scaffold parameters
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Flow Parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viscosity</td>
<td>( \mu )</td>
<td>( 6.92 \times 10^{-5} \text{ kg/m s} )</td>
<td>(Perry and Green 1997)</td>
</tr>
<tr>
<td>Density</td>
<td>( \rho )</td>
<td>( 993.37 \text{ kg/m}^3 )</td>
<td>(Perry and Green 1997)</td>
</tr>
<tr>
<td>Temperature</td>
<td></td>
<td>37 °C</td>
<td>-</td>
</tr>
<tr>
<td>Perfusion Rate</td>
<td>( v )</td>
<td>10 mm/s</td>
<td>-</td>
</tr>
<tr>
<td><strong>Porous Material Parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume Porosity</td>
<td>( \gamma )</td>
<td>80, 85, 90, 95 (%)</td>
<td>-</td>
</tr>
<tr>
<td>Pore Sizes (Diameter)</td>
<td>( d_p )</td>
<td>250, 350, 450 ( ( \mu \text{m} ))</td>
<td>-</td>
</tr>
<tr>
<td><strong>Cell Parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specific Cell Volume</td>
<td></td>
<td>2.8 cell/ cm(^3)</td>
<td>(Chow et al. 2001)</td>
</tr>
<tr>
<td>(Granulocytes)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inoculum Cell Density</td>
<td></td>
<td>0.1-1 ( \times 10^6 \text{ cells/cm}^3 )</td>
<td>-</td>
</tr>
<tr>
<td><strong>Mass Transfer Parameters (Glucose)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose Diffusivity</td>
<td>( D_G )</td>
<td>( 6.6 \times 10^{-10} \text{ m}^2/\text{s} )</td>
<td>(Gruetter et al. 1998; Botchwey et al. 2003)</td>
</tr>
<tr>
<td>Initial Concentration</td>
<td>( C_0 )</td>
<td>3000 mg/l</td>
<td></td>
</tr>
<tr>
<td><strong>Mass Transfer Parameters (Oxygen)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxygen Diffusivity</td>
<td>( D_O2 )</td>
<td>( 1.5 \times 10^{-9} \text{ m}^2/\text{s} )</td>
<td>(Chow et al. 2001)</td>
</tr>
<tr>
<td>Specific Oxygen uptake rate</td>
<td>( V_{max} )</td>
<td>2.2-64.9 mol/cell/h</td>
<td>(Chow et al. 2001)</td>
</tr>
<tr>
<td>(for granulocytes cell)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial Oxygen tension</td>
<td>( C_{O2} )</td>
<td>20%</td>
<td>-</td>
</tr>
<tr>
<td><strong>Reaction Parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate constant in Monod type kinetics equation</td>
<td>( k_{max} )</td>
<td>( V_{max}/k_O )</td>
<td>-</td>
</tr>
<tr>
<td>Rate constant in Monod type kinetics expression for glucose</td>
<td>( k_0 )</td>
<td>1.1 m(^3)/kg</td>
<td>(Carruthers 1990)</td>
</tr>
<tr>
<td>Rate constant in Monod type kinetics expression for oxygen</td>
<td>( k_{O2} )</td>
<td>1161 m(^3)/kg</td>
<td>(Chow et al. 2001)</td>
</tr>
</tbody>
</table>

Table A2: Model parameters for normal BM cells in a 3-D perfused vessel.
### Table A3: Growth kinetics of normal BM progenitor cells (per day) (da Silva et al. 2003)

<table>
<thead>
<tr>
<th>Terms</th>
<th>Symbol</th>
<th>HSC</th>
<th>SC</th>
<th>MySc</th>
<th>LySc</th>
<th>CFU-GM</th>
<th>CFU-MEG</th>
<th>BFU-E</th>
<th>CFU-Eo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Self Expansion</td>
<td>$k_s$</td>
<td>3.8</td>
<td>1.3</td>
<td>$2.8 \times 10^2$</td>
<td>$1.0 \times 10^3$</td>
<td>$2.1 \times 10^4$</td>
<td>$1.7 \times 10^5$</td>
<td>$4.2 \times 10^6$</td>
<td>$4.9 \times 10^7$</td>
</tr>
<tr>
<td>Cell Death</td>
<td>$k_F$</td>
<td>$9.5 \times 10^4$</td>
<td>$8.2 \times 10^4$</td>
<td>$1.5 \times 10^4$</td>
<td>$1.4 \times 10^5$</td>
<td>$1.0 \times 10^8$</td>
<td>$2.9 \times 10^9$</td>
<td>$3.2 \times 10^9$</td>
<td>$9.1 \times 10^9$</td>
</tr>
<tr>
<td>Differentiation</td>
<td>$k_f$</td>
<td>-</td>
<td>4.2</td>
<td>1.6</td>
<td>$3.3 \times 10^2$</td>
<td>$9.1 \times 10^3$</td>
<td>$1.4 \times 10^4$</td>
<td>$1.2 \times 10^4$</td>
<td>$4.4 \times 10^4$</td>
</tr>
</tbody>
</table>

**Rate of Granulocytes Growth**
- $k_s$: 0.69 day$^{-1}$ (Hevehan et al. 2000)
- $k_F$: 0.35 day$^{-1}$ (Peng et al. 1996)

**Rate of Mature Cell Death**
- $k_f$: 0.5 day$^{-1}$ (Peng et al. 1996)

Table A4: Growth kinetics of normal BM granulocytes and mature cells
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of CML stem cells</td>
<td>$Q$</td>
<td>$1.1 \times 10^9$ cells/kg</td>
<td>(Bernard et al. 2003)</td>
</tr>
<tr>
<td>Death rate of proliferation normal BM stem cells</td>
<td>$\delta_Q$</td>
<td>0.1/day</td>
<td>(Bernard et al. 2003)</td>
</tr>
<tr>
<td>Death rate of proliferation CML stem cells</td>
<td>$\delta_Q$</td>
<td>0.04-0.18/day</td>
<td>(Colijn and Mackey 2005)</td>
</tr>
<tr>
<td>Number of leukocytes</td>
<td>$N$</td>
<td>$3.55-7 \times 10^9$ cells/kg</td>
<td>(Beutler et al. 1995)</td>
</tr>
<tr>
<td>Amplification factor for leukocytes in normal BM</td>
<td>$A_N$</td>
<td>75200</td>
<td>(Bernard et al. 2003)</td>
</tr>
<tr>
<td>Amplification factor for leukocytes in CML BM</td>
<td>$A_N$</td>
<td>68800-763000</td>
<td>(Colijn and Mackey 2005)</td>
</tr>
<tr>
<td>Death rate of leukocytes</td>
<td>$\delta_N$</td>
<td>2.4/day</td>
<td>(Bernard et al. 2003)</td>
</tr>
<tr>
<td>Number of leukocytes</td>
<td>$R$</td>
<td>$3.5 \times 10^7$ cells/kg</td>
<td>(Mahaffy et al. 1998)</td>
</tr>
<tr>
<td>Amplification factor for leukocytes in normal BM</td>
<td>$A_R$</td>
<td>563000</td>
<td>(Beutler et al. 1995)</td>
</tr>
<tr>
<td>Amplification factor for leukocytes in CML BM</td>
<td>$A_R$</td>
<td>42100-559700</td>
<td>(Colijn and Mackey 2005)</td>
</tr>
<tr>
<td>Death rate of leukocytes</td>
<td>$\delta_R$</td>
<td>2.4/day</td>
<td>(Bernard et al. 2003)</td>
</tr>
<tr>
<td>Number of erythrocytes</td>
<td>$R$</td>
<td>$3.5 \times 10^6$ cells/kg</td>
<td>(Mahaffy et al. 1998)</td>
</tr>
<tr>
<td>Amplification factor for erythrocytes</td>
<td>$A_R$</td>
<td>282000</td>
<td>(Santillan et al. 2000)</td>
</tr>
<tr>
<td>Amplification factor for erythrocytes</td>
<td>$A_R$</td>
<td>42100-559700</td>
<td>(Colijn and Mackey 2005)</td>
</tr>
<tr>
<td>Death rate of erythrocytes</td>
<td>$\delta_E$</td>
<td>0.001/day</td>
<td>(Mahaffy et al. 1998)</td>
</tr>
<tr>
<td>Number of platelets</td>
<td>$P$</td>
<td>$2.94 \times 10^6$ cells/kg</td>
<td>(Santillan et al. 2000)</td>
</tr>
<tr>
<td>Amplification factor for platelets in normal BM</td>
<td>$A_P$</td>
<td>282000</td>
<td>(Santillan et al. 2000)</td>
</tr>
<tr>
<td>Amplification factor for platelets in CML BM</td>
<td>$A_P$</td>
<td>42100-559700</td>
<td>(Colijn and Mackey 2005)</td>
</tr>
<tr>
<td>Death rate of platelets</td>
<td>$\delta_P$</td>
<td>0.15/day</td>
<td>(Santillan et al. 2000)</td>
</tr>
<tr>
<td>Time delay for stem cells proliferation</td>
<td>$r_s$</td>
<td>2.8 days</td>
<td>(Bernard et al. 2003)</td>
</tr>
<tr>
<td>Time delay for leukocyte maturation</td>
<td>$r_N$</td>
<td>3.5 days</td>
<td>(Bernard et al. 2003)</td>
</tr>
<tr>
<td>Time delay for erythrocyte maturation</td>
<td>$r_{ER}$</td>
<td>6 days</td>
<td>(Mahaffy et al. 1998)</td>
</tr>
<tr>
<td>Time delay for reticulocyte maturation</td>
<td>$r_{RS}$</td>
<td>2.8 days</td>
<td>(Beutler et al. 1995)</td>
</tr>
<tr>
<td>$r_{RS} + r_{PM} + r_{ER}$</td>
<td>$r_{sew}$</td>
<td>120 days</td>
<td>(Mahaffy et al. 1998)</td>
</tr>
<tr>
<td>Time delay for platelet maturation</td>
<td>$r_{PM}$</td>
<td>7 days</td>
<td>(Santillan et al. 2000)</td>
</tr>
<tr>
<td>Time delay for platelet aging to senescence</td>
<td>$r_{ps}$</td>
<td>9.5 days</td>
<td>(Santillan et al. 2000)</td>
</tr>
<tr>
<td>$k_n$ in normal BM</td>
<td>$k_n$</td>
<td>3.0/day</td>
<td>(Bernard et al. 2003)</td>
</tr>
<tr>
<td>$k_n$ in CML BM</td>
<td>$k_n$</td>
<td>0.98-3.88/day</td>
<td>(Colijn and Mackey 2005)</td>
</tr>
<tr>
<td>$\theta_1$</td>
<td>$\theta_1$</td>
<td>0.5$ \times 10^8$ cells/kg</td>
<td>(Bernard et al. 2003)</td>
</tr>
<tr>
<td>$\varepsilon$</td>
<td>$\varepsilon$</td>
<td>4</td>
<td>(Bernard et al. 2003)</td>
</tr>
<tr>
<td>$f_n$ in normal BM</td>
<td>$f_n$</td>
<td>0.4/day</td>
<td>(Colijn and Mackey 2005)</td>
</tr>
<tr>
<td>$f_n$ in CML BM</td>
<td>$f_n$</td>
<td>0.53-20.48/day</td>
<td>(Colijn and Mackey 2005)</td>
</tr>
<tr>
<td>$\theta_1$</td>
<td>$\theta_1$</td>
<td>0.36$ \times 10^8$ cells/kg</td>
<td>(Bernard et al. 2003)</td>
</tr>
<tr>
<td>$h_1$</td>
<td>$h_1$</td>
<td>1</td>
<td>(Bernard et al. 2003)</td>
</tr>
<tr>
<td>$\bar{K}_P$ in normal BM</td>
<td>$\bar{K}_P$</td>
<td>1.17/day</td>
<td>(Colijn and Mackey 2005)</td>
</tr>
</tbody>
</table>
Table A5: Kinetics of CML BM cells.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value/Unit</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\bar{K}_p$ in CML BM</td>
<td>0.05-5.06/day</td>
<td>(Colijn and Mackey 2005)</td>
</tr>
<tr>
<td>$K_{TPO}$</td>
<td>11.66x10^6 cells/kg</td>
<td>(Santillan et al. 2000)</td>
</tr>
<tr>
<td>$h_3$</td>
<td>1.29</td>
<td>(Santillan et al. 2000)</td>
</tr>
<tr>
<td>$\bar{K}_r$</td>
<td>1.1/day</td>
<td>(Colijn and Mackey 2005)</td>
</tr>
<tr>
<td>$K_{EPO}$</td>
<td>0.0382x10^13 cells/kg</td>
<td>(Mahaffy et al. 1998)</td>
</tr>
<tr>
<td>$h_2$</td>
<td>6.96</td>
<td>(Mahaffy et al. 1998)</td>
</tr>
</tbody>
</table>

**Growth Factors**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value/Unit</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCSF MM constant</td>
<td>$k_{GCSF}$</td>
<td>427 pg/ml</td>
</tr>
<tr>
<td>TPO MM constant</td>
<td>$k_{TPO}$</td>
<td>0.095 mU/ml</td>
</tr>
<tr>
<td>EPO MM constant</td>
<td>$k_{EPO}$</td>
<td>28 mU/ml</td>
</tr>
<tr>
<td>G-CSF at in vivo state</td>
<td>G</td>
<td>30 pg/ml</td>
</tr>
<tr>
<td>G-CSF at ex vivo state</td>
<td>G</td>
<td>10 ng/ml</td>
</tr>
<tr>
<td>N dissociation constant</td>
<td>$k_N$</td>
<td>0.5x10^6 cells/ml</td>
</tr>
<tr>
<td>Elimination rate of G-CSF</td>
<td>$\delta_{GCSF}$</td>
<td>6 pg/grams/cell/day</td>
</tr>
<tr>
<td>TPO at in vivo state</td>
<td>T</td>
<td>5 mU/ml</td>
</tr>
<tr>
<td>TPO at ex vivo state</td>
<td>T</td>
<td>10 ng/ml</td>
</tr>
<tr>
<td>Elimination rate of TPO</td>
<td>$\delta_{TPO}$</td>
<td>0.69/day</td>
</tr>
<tr>
<td>EPO at in vivo state</td>
<td>E</td>
<td>10 mU/ml</td>
</tr>
<tr>
<td>EPO at ex vivo state</td>
<td>E</td>
<td>3 U/ml</td>
</tr>
<tr>
<td>Elimination rate of EPO</td>
<td>$\delta_{EPO}$</td>
<td>6/hr</td>
</tr>
</tbody>
</table>
* Calculation of $k_{GCSF}$, $k_{TPO}$, $k_{EPO}$

The feedback function for G-CSF is represented by:

$$F(N) = f_o \frac{\theta_{h}^h}{\theta_{A}^h + N^h}$$

as describe in Chapter 3, Equation 3.38. Bernard et al. suggested (Bernard et al. 2003), $F(G) = F(N)$, and

$$F(G) = f_o \frac{G^h}{G^h + k_{GCSF}^h}$$

where $k_{GCSF}$ is the reaction coefficient.

Using $F(N)$ value obtained value from Colijn et al. (2005), we have $F(N) = 0.026 \text{day}^{-1} = F(G)$ and $f_o = 0.4 \text{ day}^{-1}$. According to Shochat’s paper, G-CSF normal value (steady state value) is 30 pg/ml, therefore,

$$F(G) = 0.4 \text{day}^{-1} \frac{30 \text{pg/ml}}{30 \text{pg/ml} + k_{GCSF} \text{pg/ml}} = 0.0263 \text{day}^{-1}$$

$k_{GCSF} \approx 427 \text{ pg/ml}$

The same approach has been used to calculate $k_{EPO}$ and $k_{TPO}$. 
** Calculation of Diffusivities of G-CSF, TPO and EPO

Diffusivities of different growth factors were calculated using the Polson equation suggested by Zandstra (Zandstra et al. 1999):

\[
D = \frac{9.4 \times 10^{-15} T}{\mu M^{0.5}}
\]

where \( T \) is the absolute temperature, \( M \) is the dimer molecular weight and \( \mu \) is the viscosity of the medium.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume Porosity</td>
<td>γ</td>
<td>1.0</td>
<td>-</td>
</tr>
<tr>
<td><strong>Porous Material Parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inoculum Cell Density</td>
<td></td>
<td>1x10^5 cells/ml</td>
<td></td>
</tr>
<tr>
<td>K562 Growth Rate</td>
<td></td>
<td>0.582</td>
<td>(McGahon et al. 1994)</td>
</tr>
<tr>
<td><strong>Cell Parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose Diffusivity</td>
<td>D_0</td>
<td>6.6 x 10^{-10} m²/s</td>
<td>(Gruetter et al. 1998; Botchwey et al. 2003)</td>
</tr>
<tr>
<td>Initial Concentration</td>
<td>C₀</td>
<td>22.5 mmol/l</td>
<td>Obtained from experiment</td>
</tr>
</tbody>
</table>

Table A7: Model parameters for K562 cells in a RWPB using dilution protocol.
Appendix B

Model Implementation and Validation

In the following sections the underlying implementation procedure for the models presented in Chapters 4, 5 and 6 are presented. Firstly, in addition to the model validation presented in Chapter 5, a simplified analytical model of the fluid flow in a rotating tube is offered (See Chapter 7, Section 2.3). Secondly, the procedure for implementing the model equations in CFX 4.4 is described. Thirdly, the grid sensitivity aspects of the numerical scheme are presented. Finally, simplified models implemented in other commercial software (gPROMS) are compared to the results obtained from CFX4.4. All the data provided here further support the model results and validation offered in the earlier part of this thesis.
B1 Comparison with Analytical Solutions

This section offers a simplified analytical solution to the flow through a rotating annular tube. In the bioreactor, nutrients are transported by fluid flow and molecular diffusion and it is possible that molecular diffusion may result from both axial and radial gradients. The analysis for nutrient transport requires the knowledge of flow conditions within the bioreactor. The velocity profile depends upon the rheological properties of the culture medium, pressure drop and flow rate through the system.

To study the flow inside the annular tube in Figure B1.1, the Navier-Stokes equation is solved with a set of boundary conditions (Equations B1.1) and the following assumptions: laminar flow, incompressible fluid and no slip velocity at the walls of the inner and outer tubes.

\[
\begin{align*}
\text{B.C.1 at } r &= R, \ v_z = 0 \\
\text{B.C.2 at } r &= \kappa R, \ v_z = 0 \\
\text{B.C.3 at } z &= 0, \ v_z = 0.000135 \text{m/s} \\
\text{B.C.4 at } z &= 10, \ \frac{\partial z}{\partial n} = 0
\end{align*}
\]  

where \( n \) is the normal outward vector. With the above conditions and the assumptions, the analytical solution is shown to be (Bird et al. 1960):

\[
v_z = \frac{(P_2 - P_L)R^2}{4\mu L} \left[ 1 - \left(\frac{r}{R}\right)^2 + \left(\frac{1 - \kappa^2}{\ln(1/\kappa)}\right) \ln\left(\frac{r}{R}\right) \right]
\]  

(B1.2)

The above model was implemented in CFX 4.4 with the geometry meshed using hexahedral grid; the velocity profile within the annulus cylinder is shown in Figure in B1.2. It is seen that Poiseuille flow was achieved in the studied model geometry.
The analytical solution represented by Equation B1.2 was compared to that of the CFX numerical model. It is evident in Figure B1.3 that the numerical results are accurate and the numerical error was found to be less than 1%.

To incorporate the rotation effect, the classical problem presented in Bird, et al (1960) has been employed here (Figure B1.4).

The analytical solution to the above model was shown to be (Bird et al. 1960):

\[ v_r = \left( \frac{x}{b} \right) V \quad \text{(B1.3)} \]

where \( V = r_0 \Omega \), \( r_0 \) is the outer radius, \( \Omega \) is the angular velocity, \( b \) is the slit width, \( V \) is the velocity of top surface.

The analytical result (Equation B1.3) has been compared to that of CFX and is shown in Figures B1.5; the error between the two results is less than 1%.

Figure B1.1: Annular geometry. Symbols: \( R \), outer tube radius; \( \kappa R \), inner tube radius. The structure dimensions are, \( R = 0.03182 \) m; \( \kappa R = 0.0105 \) m; length =10m.
Figure B1.2: Velocity profile at the mid-section of the annulus cylinder using CFX 4.4.
Figure B1.3: Comparing analytical solution (Equation B1.2) and numerical flow model using CFX 4.4. The parameters used are, $\mu = 6.92 \times 10^{-4} \text{kg/m} \cdot \text{s}$; $\rho = 993.37 \text{kg/m}^3$; $v = 1.35 \times 10^{-4} \text{m/s}$.

![Diagram of outer cylinder moving with angular velocity $\Omega$](image)

Figure B1.4: Rotating annular cylinder [taken from Bird et al. 1960]. Symbols: $b$, tube thickness or slit. The dimensions are, $b = 0.005 \text{ m}$; $z = 0.2 \text{ m}$.
Figure B1.5: Analytical and numerical rotational model comparison for velocity. Parameters: 
\( \mu = 1 \times 10^{-5} \text{kg/m} \cdot \text{s} \); \( \rho = 1 \text{ kg/m}^3 \); Inlet/outlet mass flow rate = 10kg/s.
B2 Model Implementation Using CFX

The mass transfer equation of oxygen within the vessel can be expressed as (Pathi et al. 2005; Coletti et al. 2006):

\[
\frac{\partial C_{O_2}}{\partial t} + \nabla \cdot \vec{v}C_{O_2} = \nabla \cdot D_{O_2} \nabla C_{O_2} - k
\]  \hfill (B2.1)

where \( C_{O_2} \) is the oxygen concentration, \( \vec{v} \) is the fluid velocity and \( k \) is the source term, the consumption kinetics of cells which depends on the concentration of oxygen.

The scalar equation in CFX 4.4 was used to model mass transport:

\[
\rho \left( \frac{\partial \phi}{\partial t} + \vec{v} \cdot \nabla \phi \right) = \nabla \cdot \Gamma \nabla \phi + \phi
\]  \hfill (B2.2)

where \( \rho \) is the density, \( \phi \) is any conserved intensive property (for mass conservation, \( \phi = 1 \); for momentum conservation, \( \phi = \vec{v} \); for conservation of a scalar, \( \phi \) represents the conserved property per unit mass), \( \Gamma \) is the diffusivity and \( k \) is the source/sink term.

Comparing the oxygen transport equation with scalar equation in CFX 4.4, it gives:

\[
\begin{align*}
C_{O_2} &= \phi \\
\rho &= 1 \\
D_{O_2} &= \Gamma \\
-k &= \phi
\end{align*}
\]  \hfill (B2.3)

The same method has been applied to the mass transport of other nutrients and the porous model presented in Chapters 4 and 5.
B3  Grid Sensitivity Test

The size of numerical grids determines the accuracy of the model results and also the computational time, therefore, it is necessary to find a suitable compromise between the accuracy and time.

Grid sensitivity analysis was carried out with 4 different grid densities. Grid 1 is the coarse grid with the lowest number of nodes and elements, whereas Grid 4 is the finest grid with the highest number of nodes and elements in this study (Table B3.1). In Figure B3.1, percentage differences were calculated by comparing the results of average velocity in the vessel obtained from Grid 2 to Grid 1, Grid 3 to Grid 2, and Grid 4 to Grid 3 for various axial positions. Though, there is a huge difference between Grid 2 and Grid 1 at the two ends, the differences are small and less than 5% between Grid 3 to Grid 2, and Grid 4 to Grid 3 (Figure B3.1). Furthermore, the average velocity, oxygen and total cell number in the vessel are compared between each successive grid (Figure B3.2). It is shown that the least change in the solution was between Grids 4 and 3, therefore, Grid 3 was thought to be adequate for accurate prediction of the flow and mass transfer within the bioreactor culture system. Thus, final grid chosen for the simulation is Grid 3. This grid sensitivity test was carried out for every geometry used in this work. A set of files is listed in Appendix C to show the implementation methods of the model in CFX 4.4.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Number of nodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grid 1</td>
<td>109424</td>
</tr>
<tr>
<td>Grid 2</td>
<td>193896</td>
</tr>
<tr>
<td>Grid 3</td>
<td>230204</td>
</tr>
<tr>
<td>Grid 4</td>
<td>300888</td>
</tr>
</tbody>
</table>

Table B3.1: Information of different grid geometries with varying grid densities
Comparing the numerical scheme of CFX 4 to other readily available and independently validated commercial software; such studies are merely to add further validity of the numerical scheme within CFX 4.4. gPROMS, a process modelling, process simulation and optimisation software, was chosen for this purpose. The interface is a more user friendly environment to implement a large set of differential equations. There are deficiencies with using gPROMS, but as the solution time is comparatively much lower, such an approach provides a 'quick' way checking the accuracy of the implementation process. Growth kinetics models are implemented in both CFX and gPROMS environment and the simulations solution of total cell numbers were checked and compared.

Figure B4.1 shows a comparison between the total cell numbers output from CFX and the gPROMS. It is demonstrated that results between the two codes are in good agreement, providing further confidence in using the numerical models implemented in CFX 4.4.
Figure B3.1: Comparison of solution (velocity) of different grids for various axial positions of the scaffold.

Figure B3.2: Comparison of average solutions (velocity, oxygen and total cell number) of different grids.
Figure B4.1: Numerical verification of total cell numbers using CFX 4.4 and gPROMS.
Appendix C

Sample CFX Files
/* A COMBINED FLUID DYNAMICS, MASS TRANSPORT AND CELL GROWTH MODEL */
/* FOR A THREE-DIMENSIONAL PERFUSED BIORECTOR */
/* FOR TISSUE ENGINEERING OF HAEMATOPOIETIC CELLS */
/* POROSITY = 90%, PORE SIZE = 250um, ID = 1E7 cells/cm^3 */

/*
   FLDPOR = Fluid Porosity
   PORITY = Porosity
   WATDEN = Water Density
   VISCOS = Viscosity
   NRMVEL = Velocity
   ITER = Iteration
   MST = Mass source tolerance
   INGLUCON = Initial Glucose Concentration
   INOXYCON = Initial Oxygen Concentration
   GLUDIF = Glucose Diffusivity
   OXYDIF = Oxygen Diffusivity
   RESIST = Body Force
*/

#CALC

FLDPOR = 1.0;
PORITY = 0.9;
VISCOS = 6.90E-04;
WATDEN = 1;
NRMVEL = 1.0E-02;
ITER = 5;
MST = 1.0E-15;
INGLUCON = 1.0;
INOXYCON = 0.00987392;
GLUDIF = 6.6E-10;
OXYDIF = 1.5E-09;
ZDIFF = 1.0E-200;
RESIST = 2.45E5;
NSTEPS = 10;
DELTAT = 8640;

#ENDCALC

>>CFX4

>>OPTIONS
   THREE DIMENSIONS
   BODY FITTED GRID
   CARTESIAN COORDINATES
   POROUS FLOW
   LAMINAR FLOW
   ISOTHERMAL FLOW
   INCOMPRESSIBLE FLOW
   TRANSIENT FLOW
   USER SCALAR EQUATIONS 18

>>USER FORTRAN
   USRINT
   USRSRC
   USRTRAN

>>VARIABLE NAMES
   USER SCALAR16 'XY NODAL SHEAR STRESS'
   USER SCALAR17 'YZ NODAL SHEAR STRESS'
   USER SCALAR18 'ZX NODAL SHEAR STRESS'

>>MODEL TOPOLOGY
   >>MODIFY PATCH
   OLD PATCH NAME 'POR1'
NEW PATCH NAME 'POR1'
NEW PATCH TYPE 'POROUS'
NEW PATCH GROUP NUMBER 1

>>MODIFY PATCH
OLD PATCH NAME 'POR2'
NEW PATCH NAME 'POR2'
NEW PATCH TYPE 'POROUS'
NEW PATCH GROUP NUMBER 2

>>MODIFY PATCH
OLD PATCH NAME 'IN1'
NEW PATCH NAME 'IN1'
NEW PATCH TYPE 'INLET'
NEW PATCH GROUP NUMBER 1

>>MODIFY PATCH
OLD PATCH NAME 'IN2'
NEW PATCH NAME 'IN2'
NEW PATCH TYPE 'INLET'
NEW PATCH GROUP NUMBER 2

>>MODIFY PATCH
OLD PATCH NAME 'IN3'
NEW PATCH NAME 'IN3'
NEW PATCH TYPE 'INLET'
NEW PATCH GROUP NUMBER 3

>>MODIFY PATCH
OLD PATCH NAME 'IN4'
NEW PATCH NAME 'IN4'
NEW PATCH TYPE 'INLET'
NEW PATCH GROUP NUMBER 4

>>MODEL DATA
>>DIFFERENCING SCHEME
USER SCALAR3 'NO CONVECTION'
USER SCALAR4 'NO CONVECTION'
USER SCALAR5 'NO CONVECTION'
USER SCALAR6 'NO CONVECTION'
USER SCALAR7 'NO CONVECTION'
USER SCALAR8 'NO CONVECTION'
USER SCALAR9 'NO CONVECTION'
USER SCALAR10 'NO CONVECTION'
USER SCALAR11 'NO CONVECTION'
USER SCALAR12 'NO CONVECTION'
USER SCALAR13 'NO CONVECTION'
USER SCALAR14 'NO CONVECTION'
USER SCALAR15 'NO CONVECTION'

>>PHYSICAL PROPERTIES
>>FLUID PARAMETERS
VISCOOSITY #VISCOS
DENSITY #WATDEN

>>TRANSIENT PARAMETERS
>>FIXED TIME STEPPING
TIME STEPS #NSTEPS * #DELTAT
INITIAL TIME 0.0

>>SCALAR PARAMETERS
>>DIFFUSIVITIES
USER SCALAR1 #GLUDIF
USER SCALAR2 #OXYDIF
USER SCALAR3 #ZDIFF
USER SCALAR4 #ZDIFF
USER SCALAR5 #ZDIFF
USER SCALAR6 #ZDIFF
USER SCALAR7 #ZDIFF
USER SCALAR8 #ZDIFF
USER SCALAR9 #ZDIFF
USER SCALAR10 #ZDIFF
USER SCALAR11 #ZDIFF
USER SCALAR12 #ZDIFF

170
>>POROUS REGION PARAMETERS
PATCH GROUP NUMBER 1
VOLUME POROSITY #PORITY
>>POROUS REGION PARAMETERS
PATCH GROUP NUMBER 2
VOLUME POROSITY #FLDPOR
>>BODY FORCES
PATCH NAME 'POR1'
RESISTANCE CONSTANT #RESIST #RESIST #RESIST
>>SOLVER DATA
>>PROGRAM CONTROL
MAXIMUM NUMBER OF ITERATIONS #ITER
PRESSURE REFERENCE POINT 1 1 1
OUTPUT MONITOR POSITION 1.3E-02 1.3E-02 3.0E-02
MASS SOURCE TOLERANCE #MST
>>ALGEBRAIC MULTIGRID PARAMETERS
WORK SPACE FACTOR 3.0000E+00
>>EQUATION SOLVERS
U VELOCITY 'AMG'
V VELOCITY 'AMG'
W VELOCITY 'AMG'
USER SCALAR1 'AMG'
USER SCALAR2 'AMG'
USER SCALAR3 'AMG'
USER SCALAR4 'AMG'
USER SCALAR5 'AMG'
USER SCALAR6 'AMG'
USER SCALAR7 'AMG'
USER SCALAR8 'AMG'
USER SCALAR9 'AMG'
USER SCALAR10 'AMG'
USER SCALAR11 'AMG'
USER SCALAR12 'AMG'
USER SCALAR13 'AMG'
USER SCALAR14 'AMG'
USER SCALAR15 'AMG'
>>MODEL BOUNDARY CONDITIONS
>>INLET BOUNDARIES
PATCH NAME 'IN1'
NORMAL VELOCITY #NRMVEL
USER SCALAR1 #INGLUCON
USER SCALAR2 #INOXYCON
>>INLET BOUNDARIES
PATCH NAME 'IN2'
NORMAL VELOCITY #NRMVEL
USER SCALAR1 #INGLUCON
USER SCALAR2 #INOXYCON
>>INLET BOUNDARIES
PATCH NAME 'IN3'
NORMAL VELOCITY #NRMVEL
USER SCALAR1 #INGLUCON
USER SCALAR2 #INOXYCON
>>INLET BOUNDARIES
PATCH NAME 'IN4'
NORMAL VELOCITY #NRMVEL
USER SCALAR1 #INGLUCON
USER SCALAR2 #INOXYCON
>>OUTPUT OPTIONS
>>DUMP FILE FORMAT
UNFORMATTED
DOUBLE PRECISION
>>DUMP FILE OPTIONS
ALL USER SCALARS

>>STOP
SUBROUTINE USRINT(U, V, W, P, VFRAC, DEN, VIS, TE, ED, RS, T, H, RF, SCAL, CONV, + X, Y, Z, XP, YP, ZP, VOL, AREA, VPOR, ARPOR, WFACT, + DISWAL, IFT, IBLK, IPVERT, IPNODN, IPFACN, IPNODF, + IPNODB, IPFACB, WORK, IWORK, CWORK)

C
C C UTILITY SUBROUTINE FOR USER-SUPPLIED INITIAL FIELD.
C
C THIS SUBROUTINE IS CALLED BY THE FOLLOWING SUBROUTINE
C CURR INIT

CREATED
13/06/90 ADB

MODIFIED
07/08/91 IRH NEW STRUCTURE
10/09/91 IRH CORRECTION TO IUSED
26/09/91 IRH ALTER ARGUMENT LIST
01/10/91 DSC REDUCE COMMENT LINE GOING OVER COLUMN 72.
03/10/91 IRH CORRECT COMMENTS
28/01/92 PHA UPDATE CALLED BY COMMENT, ADD RF ARGUMENT,
CHANGE LAST DIMENSION OF RS TO 6 AND IVERS TO 2
03/06/92 PHA ADD PRECISION FLAG AND CHANGE IVERS TO 3
02/09/92 NSW REMOVE REDUNDANT COMMENTS
23/11/93 CSH EXPLICITLY DIMENSION IVERS ETC.
03/02/94 PHA CHANGE FLOW3D TO CFDS-FLOW3D, REMOVE COMMA
FROM BEGINNING OF DIMENSION STATEMENT
03/03/94 FHW CORRECTION OF SPELLING MISTAKE
09/08/94 NSW CORRECT SPELLING
MOVE 'IF(IUSED.EQ.0) RETURN' OUT OF USER AREA
19/12/94 NSW CHANGE FOR CFX-F3D
30/01/95 NSW INCLUDE NEW EXAMPLE
02/07/97 NSW UPDATE FOR CFX-4

SUBROUTINE ARGUMENTS
U - U COMPONENT OF VELOCITY
V - V COMPONENT OF VELOCITY
W - W COMPONENT OF VELOCITY
P - PRESSURE
VFRAC - VOLUME FRACTION
DEN - DENSITY OF FLUID
VIS - VISCOSITY OF FLUID
TE - TURBULENT KINETIC ENERGY
ED - EPSILON
RS - REYNOLDS STRESSES
T - TEMPERATURE
H - ENTHALPY
RF - REYNOLD FLUXES
SCAL - SCALARS (THE FIRST 'NCONC' OF THESE ARE MASS FRACTIONS)
CONV - CONVECTION COEFFICIENTS
X - X COORDINATES OF CELL CORNERS
Y - Y COORDINATES OF CELL CORNERS
Z - Z COORDINATES OF CELL CORNERS
XP - X COORDINATES OF CELL CENTRES
YP - Y COORDINATES OF CELL CENTRES
ZP - Z COORDINATES OF CELL CENTRES
VOL - VOLUME OF CELLS
AREA - AREA OF CELLS
VPOR - POROUS VOLUME
ARPOR - POROUS AREA
WFAC'T - WEIGHT FACTORS
DISWAL - DISTANCE OF CELL CENTRE FROM WALL

IPT - 1D POINTER ARRAY
IBLK - BLOCK SIZE INFORMATION
IPVERT - POINTER FROM CELL CENTERS TO 8 NEIGHBOURING VERTICES
IPCDN - POINTER FROM CELL CENTERS TO 6 NEIGHBOURING CELLS
IPFACN - POINTER FROM CELL CENTERS TO 6 NEIGHBOURING FACES
IPNODF - POINTER FROM CELL CENTERS TO 2 NEIGHBOURING CELL CENTERS
IPNODB - POINTER FROM BOUNDARY CENTERS TO CELL CENTERS
IPFACB - POINTER FROM BOUNDARY CENTERS TO BOUNDARY FACES

WORK - REAL WORKSPACE ARRAY
IWORK - INTEGER WORKSPACE ARRAY
CWORK - CHARACTER WORKSPACE ARRAY

SUBROUTINE ARGUMENTS PRECEDED WITH A '*' ARE ARGUMENTS THAT MUST BE SET BY THE USER IN THIS ROUTINE.

LOGICAL VARIABLE LRDISK IN COMMON BLOCK IOLOGC INDICATES WHETHER THE RUN IS A RESTART AND CAN BE USED SO THAT INITIAL INFORMATION IS ONLY SET WHEN STARTING A RUN FROM SCRATCH.

NOTE THAT OTHER DATA MAY BE OBTAINED FROM CFX-4 USING THE ROUTINE GETADD, FOR FURTHER DETAILS SEE THE VERSION 4 USER MANUAL.

*******************************************************************************
DOUBLE PRECISION U
DOUBLE PRECISION V
DOUBLE PRECISION W
DOUBLE PRECISION P
DOUBLE PRECISION VFRAC
DOUBLE PRECISION DEN
DOUBLE PRECISION VIS
DOUBLE PRECISION TE
DOUBLE PRECISION ED
DOUBLE PRECISION RS
DOUBLE PRECISION T
DOUBLE PRECISION H
DOUBLE PRECISION RF
DOUBLE PRECISION SCAL
DOUBLE PRECISION CONV
DOUBLE PRECISION XC
DOUBLE PRECISION YC
DOUBLE PRECISION ZC
DOUBLE PRECISION XP
DOUBLE PRECISION YP
DOUBLE PRECISION ZP
DOUBLE PRECISION VOL
DOUBLE PRECISION AREA
DOUBLE PRECISION VPOR
DOUBLE PRECISION ARPOR
DOUBLE PRECISION WFAC'T
DOUBLE PRECISION DISWAL
DOUBLE PRECISION WORK
DOUBLE PRECISION TIME
DOUBLE PRECISION DT
DOUBLE PRECISION DTINVF
DOUBLE PRECISION TPARN
LOGICAL LDEN, LVIS, LTURB, LTEMP, LBUOY, LSCAL, LCOMP, LRECT, LCYN, LAXIS,
+ LPOROS, LTRANS
LOGICAL LRDISK, LWDISK

C
C CHARACTER(*) CWORK

C+++++++++++++ USER AREA 1 +++++++++++++++++++++++++++++++++++++++++++++
C---- AREA FOR USERS EXPLICITLY DECLARED VARIABLES
C
DOUBLE PRECISION CONVERTO, CONVERTG, C1, C2, INDEN, CELLWEIGHT,
+ BASEUNIT, CONVERC, C3, C7, C9, C11

C+++++++++++++ END OF USER AREA 1 +++++++++++++++++++++++++++++++++++++
C
COMMON /ALL/NBLOCK, NCELL, NBDRY, NNODE, NVERT, NDIM,
+ /ALLWRK/NRWS, NIWS, NCWS, IWPRE, IWPRE, ICMPRE, /ADDMPS/NPHASE,
+ NSCAL, NVAR, NPROP, NDVAR, NDSPROP, NDXXN, NDGEOM, NDCOEFF, NLIST,
+ NRLIST, NTOPO, /CHKUSR/IVERS, IUCALL, IUSED, /DEVICE/NREAD,
+ NWRITE, NRDISK, NWDISK, /IDOM/ILLEN, JLEN, /LOG/LRDISK, LWDISK,
+ /LOG/LEN, LVIS, LTURB, LTEMP, LBUOY, LSCAL, LCOMP, LRECT, LCYN,
+ LAXIS, LPOROS, LTRANS, /MLTGRD/MLEVEL, NLEVEL, ILEVEL,
+ /SGLDBL/IFLGPR, ICHKPR, /TRANSI/NSTEP, KSTEP, MF, INCORE,
+ /TRANSR/TIME, DT, DTINV, TPARM

C
C---- AREA FOR USERS TO DECLARE THEIR OWN COMMON BLOCKS
C THESE SHOULD START WITH THE CHARACTERS 'UC' TO ENSURE
C NO CONFLICT WITH NON-USER COMMON BLOCKS
C
C++++++++++++++++++ END OF USER AREA 2 +++++++++++++++++++++++++++++++++
C
DIMENSION U(NNODE, NPHASE), V(NNODE, NPHASE), W(NNODE, NPHASE),
+ P(NNODE, NPHASE), VFRAC(NNODE, NPHASE), TE(NNODE, NPHASE),
+ ED(NNODE, NPHASE), RS(NNODE, NPHASE), T(NNODE, NPHASE),
+ H(NNODE, NPHASE), XP(NNODE, NPHASE), VIS(NNODE, NPHASE),
+ SCAL(NNODE, NPHASE, NSCAL), DEN(NNODE, NPHASE),
+ V(NNODE, NPHASE), CONV(NFACE, NPHASE)

DIMENSION XC(NVERT), YC(NVERT), ZC(NVERT), XP(NNODE), YP(NNODE),
+ ZP(NNODE), VOL(NCELL), AREA(NFACE, 3), VPOR(NCELL),
+ ARPOR(NFACE, 3), WFACT(NFACE), DISWAL(NCELL),

DIMENSION IPT(*), IBLK(5, NBLOCK), IPVERT(NCELL, 8), IPNODN(NCELL, 6),
+ IPFACN(NCELL, 6), IPNODF(NFACE, 4), IPNODB(NBDRY, 4),
+ IPFACB(NBDRY)

DIMENSION IWORK(NIWS), WORK(NRWS), CWORK(NCWS)

C++++++++++++++++++ USER AREA 3 +++++++++++++++++++++++++++++++++++++++++++
C---- AREA FOR USERS TO DIMENSION THEIR ARRAYS
C---- AREA FOR USERS TO DEFINE DATA STATEMENTS
C
C++++++++++++++++++ END OF USER AREA 3 +++++++++++++++++++++++++++++++++++
C
C---- STATEMENT FUNCTION FOR ADDRESSING
C
IP(I,J,K) = IPT((K-1)*ILEN*JLEN+ (J-1)*ILEN+I)

C---- VERSION NUMBER OF USER ROUTINE AND PRECISION FLAG
C
IVERS = 3
ICHKPR = 2

C++++++++++++++++++ USER AREA 4 +++++++++++++++++++++++++++++++++++++++++
C---- TO USE THIS USER ROUTINE FIRST SET IUSED=1
C
C
C++++++++++++++++++ END OF USER AREA 4 ++++++++++++++++++++++++++++++++++++
C
IF (IUSED.EQ.0) RETURN
C---- FRONTEND CHECKING OF USER ROUTINE
IF (IUCALL.EQ.0) RETURN
C
C ................ USER AREA 5 .........................................

C SCAL1 - Glucose
C SCAL2 - Oxygen
C SCAL3 - PSC 2%
C SCAL4 = SC
C SCAL5 = MYSC
C SCAL6 = LYSC
C SCAL7 = CFUGM 10%
C SCAL8 = CFUMEG
C SCAL9 = BFUE 8%
C SCAL10 = CFUEO
C SCAL11 = Blast Cell 80%
C
C .........................................................................

C GET SCALAR VARIABLE

CALL GETSCA(IUSER SCALAR1', ISC1, CWORK)
CALL GETSCA(IUSER SCALAR2', ISC2, CWORK)
CALL GETSCA(IUSER SCALAR3', ISC3, CWORK)
CALL GETSCA(IUSER SCALAR7', ISC7, CWORK)
CALL GETSCA(IUSER SCALAR9', ISC9, CWORK)
CALL GETSCA(IUSER SCALAR11', ISC11, CWORK)

C INITIALISE SCAL VARIABLE ONLY IF NO RESTART IS USED

IF(. NOT. LRDISK) THEN

C USE IPALL TO FIND 1D ADDRESSES OF ALL CELL CENTRES

CALL IPALL('*', '*', 'BLOCKI', 'CENTRES', IPT, NPT, CWORK, IWORK)
DO 150 IPHASE = 1, NPHASE

C LOOP OVER ALL INTERIOR CELLS

DO 160 I = 1, NPT

C USE ARRAY IPT TO GET ADDRESS

INODE=IPT(I)

C VARIABLE FOR THE PARAMETERS

SCAL1 = Glucose
SCAL2 = Oxygen
SCAL3 = PSC 2%
SCAL7 = CFUGM 10%
SCAL9 = BFUE 8%
SCAL11 = Blast Cell 80%

C-------CONVERSION PARAMETERS
CONVERTO=2.03E-9*32*1000
CONVERTG=0.001

C-------INITIAL CONDITIONS FOR O2 AND GLU
C1=3000*CONVERTG
C2=152*CONVERTO

SCAL(INODE,IPHASE,ISC1)=C1
SCAL(INODE,IPHASE,ISC2)=C2

176
C 160 CONTINUE
150 CONTINUE
C CALL IPALL('POR1', 'POROUS', 'PATCH', 'CENTRES', IPT, NPT, CWORK, IWORK)
C DO 200 IPHASE = 1, NPHASE
C DO 210 I=1,NPT
C----CONVERSION PARAMETERS
INDEN = 2E5
CELLWEIGHT = 2.8E-12
BASEUNIT = 1000000
CONVERTC = CELLWEIGHT*BASEUNIT
C SCALE = CONVERTC1
C------INOCULUM DENSITY FOR DIFFERENT CELLS
C3 = INDEN*CONVERTC*0.02
C7 = INDEN*CONVERTC*0.1
C9 = INDEN*CONVERTC*0.08
C11= INDEN*CONVERTC*0.80
C INODE=IPT(I)
C SCAL(INODE, IPHASE, ISC3)=C3
SCAL(INODE, IPHASE, ISC7)=C7
SCAL(INODE, IPHASE, ISC9)=C9
SCAL(INODE, IPHASE, ISC11)=C11
C 210 CONTINUE
200 CONTINUE
C END IF
C++++++++++++++++++++ END OF USER AREA 5 +++++++++++++++++++++
C RETURN
C END
C SUBROUTINE USRSRC(IEQN, ICALL, CNAME, CALIAS, AM, SP, SU, CONV, U, V, W, P,
+ VFRAC, DEN, TE, ED, RS, T, H, RF, SCAL, XP, YP, ZP, VOL,
+ AREA, VFOR, ARPOR, WFACT, IPT, IBLK, IPVERT, IPNODN,
+ IPFACN, IPNODF, IPNODB, IPFACB, WORK, IWORK, CWORK)
C UTILITY SUBROUTINE FOR USER-SUPPLIED SOURCES
C >>> IMPORTANT <<<
C >>> USERS MAY ONLY ADD OR ALTER PARTS OF THE SUBROUTINE WITHIN <<<
C <<< THE DESIGNATED USER AREAS <<<
C THIS SUBROUTINE IS CALLED BY THE FOLLOWING SUBROUTINES
C USR SRC SCDF SCDS SCED SCENRG SCHF SCMOM SCPCE SCSCAL
SCTE SCVF
C CREATED
C 08/03/90 ADB
C MODIFIED

04/03/91 ADB ALTERED ARGUMENT LIST.
28/08/91 IRH NEW STRUCTURE
28/09/91 IRH CHANGE EXAMPLE + ADD COMMON BLOCKS
10/02/92 PHA UPDATE CALLED BY COMMENT, ADD RF ARGUMENT,
CHANGE LAST DIMENSION OF RS TO 6 AND IVERS TO 2
03/06/92 PHA ADD PRECISION FLAG AND CHANGE IVERS TO 3
23/11/93 CSH EXPLICITLY DIMENSION IPVERT ETC.
07/12/93 NSW INCLUDE CONV IN ARGUMENT LIST AND CHANGE IVERS
TO 4
03/02/94 PHA CHANGE FLOW3D TO CFDs-FLOW3D
03/03/94 FHW CORRECTION OF SPELLING MISTAKE
08/03/94 NSW CORRECT SPELLING
09/08/94 NSW CORRECT SPELLING.
MOVE 'IF(IUSED.EQ.0) RETURN' OUT OF USER AREA.
19/12/94 NSW CHANGE FOR CFX-F3D
02/07/97 NSW UPDATE FOR CFX-4

C SUBROUTINE ARGUMENTS

C IEQN - EQUATION NUMBER
ICALL - SUBROUTINE CALL
CNAME - EQUATION NAME
CALIAS - ALIAS OF EQUATION NAME
AM - OFF DIAGONAL MATRIX COEFFICIENTS
SU - SU IN LINEARISATION OF SOURCE TERM
SP - SP IN LINEARISATION OF SOURCE TERM
CONV - CONVECTION COEFFICIENTS
U - U COMPONENT OF VELOCITY
V - V COMPONENT OF VELOCITY
W - W COMPONENT OF VELOCITY
P - PRESSURE
VFRAC - VOLUME FRACTION
DEN - DENSITY OF FLUID
VIS - VISCOSITY OF FLUID
TE - TURBULENT KINETIC ENERGY
ED - EPSILON
RS - REYNOLD STRESSES
C - TEMPERATURE
H - ENTHALPY
RF - REYNOLD FLUXES
SCAL - SCALARS (THE FIRST 'NCONC' OF THESE ARE MASS FRACTIONS)
XP - X COORDINATES OF CELL CENTRES
YP - Y COORDINATES OF CELL CENTRES
ZP - Z COORDINATES OF CELL CENTRES
VOL - VOLUME OF CELLS
AREA - AREA OF CELLS
VPOR - POROUS VOLUME
APOR - POROUS AREA
WFAC - WEIGHT FACTORS
IPT - 1D POINTER ARRAY
IBLK - BLOCK SIZE INFORMATION
IPVERT - POINTER FROM CELL CENTERS TO 8 NEIGHBOURING VERTICES
IPNCON - POINTER FROM CELL CENTERS TO 6 NEIGHBOURING CELLS
IPFCN - POINTER FROM CELL CENTERS TO 6 NEIGHBOURING FACES
IPFCDF - POINTER FROM CELL FACES TO 2 NEIGHBOURING CELL CENTRES
IPNGBK - POINTER FROM BOUNDARY CENTERS TO CELL CENTRES
IPFACB - POINTER FROM BOUNDARY CENTERS TO BOUNDARY FACES
WORK - REAL WORKSPACE ARRAY
IWORK - INTEGER WORKSPACE ARRAY

178
CWORK - CHARACTER WORKSPACE ARRAY

SUBROUTINE ARGUMENTS PRECEDED WITH A '*' ARE ARGUMENTS THAT MUST
BE SET BY THE USER IN THIS ROUTINE.

NOTE THAT WHEN USING MASS SOURCES, THE FLOWS THROUGH MASS FLOW
BOUNDARIES ARE UNCHANGED. THE USER SHOULD THEREFORE INCLUDE AT
LEAST ONE PRESSURE BOUNDARY FOR SUCH A CALCULATION.

NOTE THAT OTHER DATA MAY BE OBTAINED FROM CFX-4 USING THE
ROUTINE GETADD, FOR FURTHER DETAILS SEE THE VERSION 4
USER MANUAL.

DOUBLE PRECISION AM
DOUBLE PRECISION SP
DOUBLE PRECISION SU
DOUBLE PRECISION CONV
DOUBLE PRECISION U
DOUBLE PRECISION V
DOUBLE PRECISION W
DOUBLE PRECISION P
DOUBLE PRECISION VFRAC
DOUBLE PRECISION DEN
DOUBLE PRECISION VIS
DOUBLE PRECISION TE
DOUBLE PRECISION ED
DOUBLE PRECISION RS
DOUBLE PRECISION T
DOUBLE PRECISION H
DOUBLE PRECISION RF
DOUBLE PRECISION SCAL
DOUBLE PRECISION XP
DOUBLE PRECISION YP
DOUBLE PRECISION ZP
DOUBLE PRECISION VOL
DOUBLE PRECISION AREA
DOUBLE PRECISION VPOR
DOUBLE PRECISION ARPOR
DOUBLE PRECISION WFACT
DOUBLE PRECISION WORK
DOUBLE PRECISION SMALL
DOUBLE PRECISION SORMAX
DOUBLE PRECISION TIME
DOUBLE PRECISION DT
DOUBLE PRECISION DTINVF
DOUBLE PRECISION TPARM

LOGICAL LDEN, LVIS, LTURB, LTEMP, LBUOY, LSCAL, LCOMP, LRECT, LCYN, LAXIS,
+ LPOROS, LTRANS

CHARACTER*(*) CWORK
CHARACTER CNAME*6, CALIAS*24

+++ USER AREA 1 ++++++++ USER AREA 1 ++++++++ USER AREA 1 ++++++++ USER AREA 1
+++ AREA FOR USERS EXPLICITLY DECLARED VARIABLES
+++ Michaelis-Menten Kinetics

DOUBLE PRECISION VMAXGLC, VMAXOXY, GLC, OXY, A1, A2, KMAX, PSC, SC,
+ MYSC, LISC, CFUGM, CFUMEG, BFUE, CFUEO, TOTALCELL, CONVERTC, SKECFUEO,
+ KG, KO, NATURE1, CONVERTDAY, CONVERTV, RECONVERTC, VESSELVOL,
+ ALPHA, SKEFSC, SKESC, SMEYSC, SKELYSC, SKECFUGM, SKECFUMEG, SKEBFUE,
+ SKEKSC, SKEKSC, SMEYSC, SKELYSC, SKECFUGM, SKECFUMEG, SKEBFUE, SKECFUEO,
+ SKEKDC, SKEKYSC, SKEKLYSC, SKEKCFUGM, SKEKCFUMEG, SKBDFUE, SKEKCFUEO, GROWTH,
+ DEATH, EQ3, EQ4, EQ5, EQ6, EQ7, EQ8, EQ9, EQ10, CELLPERM3, MATURE2, MATURE3,
+ MATURE4, NATURES, GROWTH, DEATH

C
C+++++++++++++++++++++ END OF USER AREA 1 ++++++++++++++++++++++++

C

COMMON /ALL/NBLOCK, NCELL, NBDRY, NNODE, NFACE, NVERT, NDIM,
+ /ALLWRK/NRWS, NIWS, NCWS, IWRFRE, IWIFRE, IWCFRE, /ADDIMS/NPRASE,
+ NSCAL, NVAR, NPROP, NDVAR, NPROP, NDXNN, NGEOM, NDGEOM, NILIST,
+ NNLIST, NTUOFOL, /CHKUSR/IVERS, IUCALL, IUSED, /DEVICE/NREAD,
+ NRWRT, NRDISK, NLUMF, /IDOM/ILEN, JLEN, LOGIC/LDEN, LVIS,
+ LTRANS, LTEMP, LBUOY, LCAL, LCOMP, LRECT, LSCAL, LTRANS,
+ /MLTGRD/MLEVEL, NLEVEL, ILEVEL, /SGLDBL/IFLGPR, ICHKPR,
+ /SPARM/SMALL, SORMAX, NITER, INDPRI, MAXIT, NODREF, NODMON,
+ /TRANSI/NSTEP, KSTEP, MF, INCORE, /TRANSR/TIME, DT, DTINV, TPARM

C

C+++++++++++++++++++++ USER AREA 2 ++++++++++++++++++++++++

C ---- AREA FOR USERS TO DECLARE THEIR OWN COMMON BLOCKS
C
C ---- THESE SHOULD START WITH THE CHARACTERS 'UC' TO ENSURE
C ---- NO CONFLICT WITH NON-USER COMMON BLOCKS

C

C+++++++++++++++++++++ END OF USER AREA 2 ++++++++++++++++++++++++

C

DIMENSION AM(NCELL, 6, NPRASE), SP(NCELL, NPHASE), SU(NCELL, NPHASE),
+ CONV(NFACE, NPHASE)

C

DIMENSION U(NNODE, NPHASE), V(NNODE, NPRASE), W(NNODE, NPHASE),
+ P(NNODE, NPRASE), VFRAC(NNODE, NPHASE), DEN(NNODE, NPHASE),
+ VIS(NNODE, NPHASE), ED(NNODE, NPHASE),
+ RS(NNODE, NPHASE, 6), T(NNODE, NPHASE), H(NNODE, NPHASE),
+ RF(NNODE, NPHASE, 4), SCAL(NNODE, NPHASE, NSCAL)

C

DIMENSION XP(NNODE), YP(NNODE), ZP(NNODE), VOL(NCELL), AREA(NFACE, 3),
+ VPOR(NCELL), ARPOR(NFACE, 3), WFACT(NFACE), IPT(*),
+ IBLK(5, NBLOCK), IPVERT(NCELL, 8), IPNODN(NCELL, 6),
+ IPPHAC(NCELL, 6), IPFACD(NFACE, 4), IPNODB(NBDRY, 4),
+ IPPFACD(NBDRY), IWORK(*), WORK(*), CWORK(*)

C

C+++++++++++++++++++++ USER AREA 3 ++++++++++++++++++++++++

C ---- AREA FOR USERS TO DIMENSION THEIR ARRAYS
C
C ---- AREA FOR USERS TO DEFINE DATA STATEMENTS
C

C+++++++++++++++++++++ END OF USER AREA 3 ++++++++++++++++++++++++

C

C----- STATEMENT FUNCTION FOR ADDRESSING
C
C
C IF (IUSED. EQ. 0) RETURN
C
C
C ---- FRONTEND CHECKING OF USER ROUTINE
C
C IF (IUCALL. EQ. 0) RETURN
C
C
C ---- ADD TO SOURCE TERMS
C

180
IF (ICALL.EQ.1) THEN

C++ USER AREA 5 ++++++++-----------------------------------------------

C SOURCES TERM ABBREV.
C
SCALAR1 = Glucose
SCALAR2 = Oxygen
SCALAR3 = PSC (Pluripotent Stem Cell)
SCALAR4 = SC (Stem Cell)
SCALAR5 = MYSC (Myeloid Stem Cell)
SCALAR6 = LYSC (Lymphoid Stem Cell)
SCALAR7 = CFUGM (Colony-Forming Unit - Granulocyte, Macrophage)
SCALAR8 = CFUMEG (Colony-Forming Unit - Megakaryocyte)
SCALAR9 = BFUE (Colony-Forming Unit - Erythroid)
SCALAR10 = CFUEO (Colony-Forming Unit - Eosinophils)

C SCALAR - Glucose Concentration
C OXY = Oxygen Concentration
C
C VMAXGLC = VMax for Glucose
C VMAXOXY = VMax for Oxygen ***ONLY VMAXOXY USED
C *** (FOR COMBINED SOURCES, USE THE LIMITING SOURCE'S VMAX FOR KMAX) ***
C
C KMAX = KMAX (Rate Constant in the M-M expression)
C K1 = KM for GLC (Rate Constant in denominator of M-M for glucose)
C K2 = KM for OXY (Rate Constant in denominator of M-M for oxygen)
C A1 = K1+GLC
C A2 = K2+OXY
C
C KG = 0.9 (Km for Glucose in Separate Source)
C KO = 8.61E-04 (Km for Oxygen in Separate Source)
C K1 = 1/KG
C K2 = 1/KO
C
C TOTALCELL = Total Cell Number
C VESSELVOL = 50.0585 ml
C
C GLC Source = -(KMAX*GLC/(K1+GLC))
C OXY Source = -(KMAX*OXY/(K2+OXY))
C
C------MONOD KINETIC
CONVERTV=32/(1000*3600)
KO=1161
KG=1.1
C------VESSEL VOLUME PER M^3
VESSELVOL = 50.0585/(100*100*100)
C------GROWTH RATE
CONVERTDAY = 86400
SKPS = 3.8/CONVERTDAY
SKESC = 1.3/CONVERTDAY
SKMSC = 2.8E-02/CONVERTDAY
SKLYSC = 1.0E-01/CONVERTDAY
SKECFUGM = 2.1E-01/CONVERTDAY
SKECFUMEG = 1.7E-03/CONVERTDAY
SKEBFUE = 4.2E-03/CONVERTDAY
SKKFKSC = 4.9E-01/CONVERTDAY
SKKFSC = 9.5E-04/CONVERTDAY
SKSFSC = 8.2E-04/CONVERTDAY
SKKMYSC = 1.5E-04/CONVERTDAY
SKKLYSC = 1.0E-03/CONVERTDAY
SKKCFUGM = 1.0E-01/CONVERTDAY
SKKCFUMEG = 2.9E-03/CONVERTDAY
SKKBFUE = 3.2E-01/CONVERTDAY

181
SKKCFUEO = 9.1E-02/CONVERTDAY
SKDSC = 4.2/CONVERTDAY
SKDMYSC = 1.6/CONVERTDAY
SKDLYSC = 3.3E-02/CONVERTDAY
SKDFUGM = 9.1E-01/CONVERTDAY
SKDCFUMEG = 1.4E-01/CONVERTDAY
SKDBFUE = 1.2E-01/CONVERTDAY
SKDCFUEO = 4.4E-02/CONVERTDAY
GROWTH = 0.35/CONVERTDAY
DEATH = 0.5/CONVERTDAY
C----- RE-CONVERT CELL NUMBER FROM KG/M^3 TO CELL/ML
RECONVERTC=1/2.8E-6
C
C*************************************************************
C
C SET PHASE NUMBER

IPHASE=1

C USE IPALL TO FIND 1D ADDRESSES OF ALL CELL CENTRES
CALL IPALL('POR1', 'POROUS', 'PATCH', 'CENTRES', IPT, NPT, CWORK, IWOPK)

CALL GETVAR('USRSRC', 'SCAL ', ISCAL)

C FIND VARIABLE NUMBER FOR SCALAR1 (GLUCOSE)
CALL GETSCA('USER SCALAR1', ISC1, CWORK) ISCA=ISCAL+ISC1-1
C
C FIND VARIABLE NUMBER FOR SCALAR2 (OXYGEN)
CALL GETSCA('USER SCALAR2', ISC2, CWORK) ISCB=ISCAL+ISC2-1
C
C FIND VARIABLE NUMBER FOR SCALAR3 (PSC)
CALL GETSCA('USER SCALAR3', ISC3, CWORK) ISCC=ISCAL+ISC3-1
C
C FIND VARIABLE NUMBER FOR SCALAR4 (SC)
CALL GETSCA('USER SCALAR4', ISC4, CWORK) ISCD=ISCAL+ISC4-1
C
C FIND VARIABLE NUMBER FOR SCALAR5 (MYSC)
CALL GETSCA('USER SCALAR5', ISC5, CWORK) ISCE=ISCAL+ISC5-1
C
C FIND VARIABLE NUMBER FOR SCALAR6 (LYSC)
CALL GETSCA('USER SCALAR6', ISC6, CWORK) ISC=ISCAL+ISC6-1
C
C FIND VARIABLE NUMBER FOR SCALAR7 (CFU-GM)
CALL GETSCA('USER SCALAR7', ISC7, CWORK) ISCG=ISCAL+ISC7-1
C
C FIND VARIABLE NUMBER FOR SCALAR8 (CFU-MEG)
CALL GETSCA('USER SCALAR8', ISC8, CWORK) ISCH=ISCAL+ISC8-1
FIND VARIABLE NUMBER FOR SCALAR9 (BFU-E)

    CALL GETSCA('USER SCALAR9', ISC9, CWORK)
    ISCI = ISCAL + ISC9 - 1

FIND VARIABLE NUMBER FOR SCALAR10 (CFU-E)

    CALL GETSCA('USER SCALAR10', ISC10, CWORK)
    ISCJ = ISCAL + ISC10 - 1

FIND VARIABLE NUMBER FOR SCALAR11 (Mature Compartment1 - Blast)

    CALL GETSCA('USER SCALAR11', ISC11, CWORK)
    ISCK = ISCAL + ISC11 - 1

FIND VARIABLE NUMBER FOR SCALAR12 (Mature Compartment2)

    CALL GETSCA('USER SCALAR12', ISC12, CWORK)
    ISCk = ISCAL + ISC12 - 1

FIND VARIABLE NUMBER FOR SCALAR13 (Mature Compartment3)

    CALL GETSCA('USER SCALAR13', ISC13, CWORK)
    ISCm = ISCAL + ISC13 - 1

FIND VARIABLE NUMBER FOR SCALAR14 (Mature Compartment4)

    CALL GETSCA('USER SCALAR14', ISC14, CWORK)
    ISCn = ISCAL + ISC14 - 1

FIND VARIABLE NUMBER FOR SCALAR15 (Mature Compartment 5 - Death)

    CALL GETSCA('USER SCALAR15', ISC15, CWORK)
    ISCO = ISCAL + ISC15 - 1

******************************************************************************

IF SCALAR1 EQUATION (ISCA) ADD SOURCE TERMS

IF (ISCA .EQ. IEQN) THEN

LOOP OVER ALL INTERIOR CELLS

    DO 300 I=1, NPT

USE ARRAY IPT TO GET ADDRESS

    INODE = IPT(I)

    GLC = MAX (SCAL(INODE, IPHASE, ISC1), SMALL)
    OXY = MAX (SCAL(INODE, IPHASE, ISC2), SMALL)

    TOTALCELL = MAX (SCAL(INODE, IPHASE, ISC3), SMALL) + MAX (SCAL
        + (INODE, IPHASE, ISC4), SMALL) + MAX (SCAL(INODE, IPHASE, ISC5), SMALL) +
        + MAX (SCAL(INODE, IPHASE, ISC6), SMALL) + MAX (SCAL(INODE, IPHASE, ISC
        + 7), SMALL) + MAX (SCAL(INODE, IPHASE, ISC8), SMALL) + MAX (SCAL(INODE,
        + IPHASE, ISC9), SMALL) + MAX (SCAL(INODE, IPHASE, ISC10), SMALL) +
        + MAX (SCAL(INODE, IPHASE, ISC11), SMALL) + MAX (SCAL(INODE,
        + IPHASE, ISC12), SMALL) + MAX (SCAL(INODE, IPHASE, ISC13), SMALL) +
        + MAX (SCAL(INODE, IPHASE, ISC14), SMALL) + MAX (SCAL(INODE,
        + IPHASE, ISC15), SMALL)) * RECONVERT * 0.9

    VMAXOXY = 3.55E-14 * CONVERTV * TOTALCELL * (100 ** 3)

    KMAX = VMAXOXY * KO * KG

300
C

A1 = (1 + GLC * 0.9 * KG)
A2 = (1 + OXY * 0.9 * KG)

C OVERWRITE SOURCE TERMS

SP(INODE, IPHASE) = SP(INODE, IPHASE) - (KMAX * OXY * 0.9 / (A1 * A2)) * (VOL + (INODE))

300 CONTINUE

C IF SCALAR2 EQUATION (ISC2) ADD SOURCE TERMS

ELSE IF (ISC2.EQ.IEQN) THEN

LOOP OVER ALL INTERIOR CELLS

DO 400 I = 1, NPT

USE ARRAY IPT TO GET ADDRESS

INODE = IPT(I)

GLC = MAX (SCAL(INODE, IPHASE, ISC1), SMALL)
OXY = MAX (SCAL(INODE, IPHASE, ISC2), SMALL)
TOTALCELL = (MAX (SCAL(INODE, IPHASE, ISC3), SMALL) + MAX (SCAL(INODE, IPHASE, ISC4), SMALL) + MAX (SCAL(INODE, IPHASE, ISC5), SMALL) + MAX (SCAL(INODE, IPHASE, ISC6), SMALL) + MAX (SCAL(INODE, IPHASE, ISC7), SMALL) + MAX (SCAL(INODE, IPHASE, ISC8), SMALL) + MAX (SCAL(INODE, IPHASE, ISC9), SMALL) + MAX (SCAL(INODE, IPHASE, ISC10), SMALL) + MAX (SCAL(INODE, IPHASE, ISC11), SMALL) + MAX (SCAL(INODE, IPHASE, ISC12), SMALL) + MAX (SCAL(INODE, IPHASE, ISC13), SMALL) + MAX (SCAL(INODE, IPHASE, ISC14), SMALL) + MAX (SCAL(INODE, IPHASE, ISC15), SMALL)) * RECONVERTC * 0.9
VMAXOXY = 33.55E-14 * CONVERTV * TOTALCELL * (100**3)
KMAX = VMAXOXY * KO * KG

Al = (1 + GLC * 0.9 * KG)
A2 = (1 + OXY * 0.9 * KG)

C OVERWRITE SOURCE TERMS

SP(INODE, IPHASE) = SP(INODE, IPHASE) - (KMAX * GLC * 0.9 / (A1 * A2)) * (VOL + (INODE))

400 CONTINUE

C IF SCALAR3 EQUATION ISC3 (ISCC) ADD SOURCE TERMS

ELSE IF (ISC3.EQ.IEQN) THEN

LOOP OVER ALL INTERIOR CELLS

DO 500 I = 1, NPT

USE ARRAY IPT TO GET ADDRESS
\[
\text{INODE} = \text{IPT}(I)
\]

\[
\text{PSC} = \max(\text{SCAL(INODE,IPHASE,ISC3)}, \text{SMALL})
\]

\[
\text{TOTALCELL} = \max(\text{SCAL(INODE,IPHASE,ISC3), SMALL}) + \max(\text{SCAL(INODE,IPHASE,ISC4), SMALL}) + \max(\text{SCAL(INODE,IPHASE,ISC5), SMALL}) + \max(\text{SCAL(INODE,IPHASE,ISC6), SMALL}) + \max(\text{SCAL(INODE,IPHASE,ISC7), SMALL}) + \max(\text{SCAL(INODE,IPHASE,ISC8), SMALL}) + \max(\text{SCAL(INODE,IPHASE,ISC9), SMALL}) + \max(\text{SCAL(INODE,IPHASE,ISC10), SMALL}) + \max(\text{SCAL(INODE,IPHASE,ISC11), SMALL}) + \max(\text{SCAL(INODE,IPHASE,ISC12), SMALL}) + \max(\text{SCAL(INODE,IPHASE,ISC13), SMALL}) + \max(\text{SCAL(INODE,IPHASE,ISC14), SMALL}) + \max(\text{SCAL(INODE,IPHASE,ISC15), SMALL}) \times \text{RECONVERTC} \times 0.9
\]

\[
\text{ALPHA} = \frac{(\text{TOTALCELL} \times 50.0585)}{(-1.996 \times 10^9)} + \frac{(2.0 \times 10^9)}{1.996 \times 10^9}
\]

\[
\text{EQ3I} = \text{ALPHA} \times \text{SKESC} \times \text{PSC}
\]

\[
\text{EQ3II} = -\text{SKDSC} \times \text{SKKSC}
\]

\[
\text{OVERWRITE SOURCE TERMS}
\]

\[
\text{SU(INODE,IPHASE)} = \text{SU(INODE,IPHASE)} + ((\text{EQ3I}) \times \text{VOL(INODE)})
\]

\[
\text{SP(INODE,IPHASE)} = \text{SP(INODE,IPHASE)} + ((\text{EQ3II}) \times \text{VOL(INODE)})
\]

500 CONTINUE

**-----------------------------------------------------------------------------**

**IF SCALAR4 EQUATION SC (ISCD) ADD SOURCE TERMS**

ELSE IF (ISCD.EQ.IEQN) THEN

**LOOP OVER ALL INTERIOR CELLS**

DO 560 I=1,NPT

**USE ARRAY IPT TO GET ADDRESS**

\[
\text{INODE} = \text{IPT}(I)
\]

\[
\text{PSC} = \max(\text{SCAL(INODE,IPHASE,ISC3), SMALL})
\]

\[
\text{SC} = \max(\text{SCAL(INODE,IPHASE,ISC4), SMALL})
\]

\[
\text{TOTALCELL} = \max(\text{SCAL(INODE,IPHASE,ISC3), SMALL}) + \max(\text{SCAL(INODE,IPHASE,ISC4), SMALL}) + \max(\text{SCAL(INODE,IPHASE,ISC5), SMALL}) + \max(\text{SCAL(INODE,IPHASE,ISC6), SMALL}) + \max(\text{SCAL(INODE,IPHASE,ISC7), SMALL}) + \max(\text{SCAL(INODE,IPHASE,ISC8), SMALL}) + \max(\text{SCAL(INODE,IPHASE,ISC9), SMALL}) + \max(\text{SCAL(INODE,IPHASE,ISC10), SMALL}) + \max(\text{SCAL(INODE,IPHASE,ISC11), SMALL}) + \max(\text{SCAL(INODE,IPHASE,ISC12), SMALL}) + \max(\text{SCAL(INODE,IPHASE,ISC13), SMALL}) + \max(\text{SCAL(INODE,IPHASE,ISC14), SMALL}) + \max(\text{SCAL(INODE,IPHASE,ISC15), SMALL}) \times \text{RECONVERTC} \times 0.9
\]

\[
\text{ALPHA} = \frac{(\text{TOTALCELL} \times 50.0585)}{(-1.996 \times 10^9)} + \frac{(2.0 \times 10^9)}{1.996 \times 10^9}
\]

\[
\text{EQ4I} = \text{ALPHA} \times \text{SKESC} \times \text{SC} + \text{SKDSC} \times \text{PSC}
\]

\[
\text{EQ4II} = -\text{SKDSC} \times \text{SKDLYSC} \times \text{SKKSC}
\]

\[
\text{OVERWRITE SOURCE TERMS}
\]

\[
\text{SU(INODE,IPHASE)} = \text{SU(INODE,IPHASE)} + ((\text{EQ4I}) \times \text{VOL(INODE)})
\]

\[
\text{SP(INODE,IPHASE)} = \text{SP(INODE,IPHASE)} + ((\text{EQ4II}) \times \text{VOL(INODE)})
\]
CONTINUE

IF SCALAR5 EQUATION MYSC (ISCE) ADD SOURCE TERMS
ELSE IF (ISCE.EQ.IEQN) THEN
LOOP OVER ALL INTERIOR CELLS
DO 610 I=1,NPT
USE ARRAY IPT TO GET ADDRESS
INODE = IPT(I)
SC = MAX (SCAL(INODE, IPHASE, ISC4), SMALL)
MYSC = MAX (SCAL(INODE, IPRASE, ISC5), SMALL)
TOTALCELL = (MAX (SCAL(INODE, IPHASE, ISC3), SMALL) + MAX (SCAL + (INODE, IPHASE, ISC4), SMALL) + MAX (SCAL(INODE, IPHASE, ISC5), SMALL) + MAX (SCAL(INODE, IPHASE, ISC6), SMALL) + MAX (SCAL(INODE, IPHASE, ISC7), SMALL) + MAX (SCAL(INODE, IPHASE, ISC8), SMALL) + MAX (SCAL(INODE, IPHASE, ISC9), SMALL) + MAX (SCAL(INODE, IPHASE, ISC10), SMALL) + MAX (SCAL(INODE, IPHASE, ISC11), SMALL) + MAX (SCAL(INODE, IPHASE, ISC12), SMALL) + MAX (SCAL(INODE, IPHASE, ISC13), SMALL) + MAX (SCAL(INODE, IPHASE, ISC14), SMALL) + MAX (SCAL(INODE, IPHASE, ISC15), SMALL)) * RECONVERTC * 0.9
ALPHA = (TOTALCELL * 50.0585) / (-1.996E+09) + (2.0E+09 / 1.996E+09)
EQ5I = ALPHA * SKEMYSC * MYSC + SKDMYSC * SC
EQ5II = -SKDCFUGM - SKDCFUMEG - SKDBFUE - SKDCFUEO - SKKMYSC OVERWRITE SOURCE TERMS
SU(INODE, IPHASE) = SU(INODE, IPHASE) + ((EQ5I) * VOL(INODE))
SP(INODE, IPHASE) = SP(INODE, IPHASE) + ((EQ5II) * VOL(INODE))
610 CONTINUE

IF SCALAR6 EQUATION LYSC (ISCF) ADD SOURCE TERMS
ELSE IF (ISCF.EQ.IEQN) THEN
LOOP OVER ALL INTERIOR CELLS
DO 650 I=1,NPT
USE ARRAY IPT TO GET ADDRESS
INODE = IPT(I)
SC = MAX (SCAL(INODE, IPHASE, ISC4), SMALL)
LYSC = MAX (SCAL(INODE, IPHASE, ISC5), SMALL)
TOTALCELL = (MAX (SCAL(INODE, IPHASE, ISC3), SMALL) + MAX (SCAL + (INODE, IPHASE, ISC4), SMALL) + MAX (SCAL(INODE, IPHASE, ISC5), SMALL) + MAX (SCAL(INODE, IPHASE, ISC6), SMALL) + MAX (SCAL(INODE, IPHASE, ISC7), SMALL) + MAX (SCAL(INODE, IPHASE, ISC8), SMALL) + MAX (SCAL(INODE, IPHASE, ISC9), SMALL) + MAX (SCAL(INODE, IPHASE, ISC10), SMALL) + MAX (SCAL(INODE, IPHASE, ISC11), SMALL) + MAX (SCAL(INODE, IPHASE, ISC12), SMALL) + MAX (SCAL(INODE, IPHASE, ISC13), SMALL) + MAX (SCAL(INODE, IPHASE, ISC14), SMALL) + MAX (SCAL(INODE, IPHASE, ISC15), SMALL) + MAX (SCAL(INODE, IPHASE, ISC16), SMALL)) * RECONVERTC * 0.9
ALPHA = (TOTALCELL * 50.0585) / (-1.996E+09) + (2.0E+09 / 1.996E+09)
EQ6I = ALPHA * SKEMYSC * MYSC + SKDMYSC * SC
EQ6II = -SKDCFUGM - SKDCFUMEG - SKDBFUE - SKDCFUEO - SKKMYSC OVERWRITE SOURCE TERMS
SU(INODE, IPHASE) = SU(INODE, IPHASE) + ((EQ6I) * VOL(INODE))
SP(INODE, IPHASE) = SP(INODE, IPHASE) + ((EQ6II) * VOL(INODE))
650 CONTINUE
\[ \text{IPHASE}, \text{ISC12}, \text{SMALL} \] + \[ \text{MAX} (\text{SCAL}(\text{INODE}, \text{IPHASE}, \text{ISC13}), \text{SMALL}) + \]
\[ \text{MAX} (\text{SCAL}(\text{INODE}, \text{IPHASE}, \text{ISC14}), \text{SMALL}) + \text{MAX} (\text{SCAL}(\text{INODE}, \text{IPHASE}, \text{ISC15}), \text{SMALL}) \] * \text{RECONVERTC} * 0.9
\]

\[ \text{ALPHA} = (\text{TOTALCELL} * 50.0585) / (-1.996E+09) + (2.0E+09 / 1.996E+09) \]

\[ \text{EQ6I} = \text{ALPHA} * \text{SKELYSC} * \text{LYSC} + \text{SKDLYSC} * \text{SC} \]
\[ \text{EQ6II} = -\text{SKLYSC} \]

\[ \text{OVERWRITE SOURCE TERMS} \]
\[ \text{SU}(\text{INODE}, \text{IPHASE}) = \text{SU}(\text{INODE}, \text{IPHASE}) + ((\text{EQ6I}) \* \text{VOL}(\text{INODE})) \]
\[ \text{SP}(\text{INODE}, \text{IPHASE}) = \text{SP}(\text{INODE}, \text{IPHASE}) + ((\text{EQ6II}) \* \text{VOL}(\text{INODE})) \]

\[ \text{CONTINUE} \]

\[ \text{IF SCALAR7 EQUATION CFU-GM (ISCG) ADD SOURCE TERMS} \]
\[ \text{ELSE IF (ISCG.EQ.IEQN) THEN} \]
\[ \text{LOOP OVER ALL INTERIOR CELLS} \]
\[ \text{DO 700 I=1,NPT} \]
\[ \text{USE ARRAY IPT TO GET ADDRESS} \]
\[ \text{INODE} = \text{IPT}(\text{I}) \]
\[ \text{MYSC} = \text{MAX} (\text{SCAL}(\text{INODE}, \text{IPHASE}, \text{ISC5}), \text{SMALL}) \]
\[ \text{CFUGM} = \text{MAX} (\text{SCAL}(\text{INODE}, \text{IPHASE}, \text{ISC7}), \text{SMALL}) \]
\[ \text{TOTALCELL} = (\text{MAX} (\text{SCAL}(\text{INODE}, \text{IPHASE}, \text{ISC3}), \text{SMALL}) + \text{MAX} (\text{SCAL}(\text{INODE}, \text{IPHASE}, \text{ISC4}), \text{SMALL}) + \text{MAX} (\text{SCAL}(\text{INODE}, \text{IPHASE}, \text{ISC5}), \text{SMALL}) + \text{MAX} (\text{SCAL}(\text{INODE}, \text{IPHASE}, \text{ISC6}), \text{SMALL}) + \text{MAX} (\text{SCAL}(\text{INODE}, \text{IPHASE}, \text{ISC7}), \text{SMALL}) + \text{MAX} (\text{SCAL}(\text{INODE}, \text{IPHASE}, \text{ISC8}), \text{SMALL}) + \text{MAX} (\text{SCAL}(\text{INODE}, \text{IPHASE}, \text{ISC9}), \text{SMALL}) + \text{MAX} (\text{SCAL}(\text{INODE}, \text{IPHASE}, \text{ISC10}), \text{SMALL}) + \text{MAX} (\text{SCAL}(\text{INODE}, \text{IPHASE}, \text{ISC11}), \text{SMALL}) + \text{MAX} (\text{SCAL}(\text{INODE}, \text{IPHASE}, \text{ISC12}), \text{SMALL}) + \text{MAX} (\text{SCAL}(\text{INODE}, \text{IPHASE}, \text{ISC13}), \text{SMALL}) + \text{MAX} (\text{SCAL}(\text{INODE}, \text{IPHASE}, \text{ISC14}), \text{SMALL}) + \text{MAX} (\text{SCAL}(\text{INODE}, \text{IPHASE}, \text{ISC15}), \text{SMALL}) + \text{MAX} (\text{SCAL}(\text{INODE}, \text{IPHASE}, \text{ISC16}), \text{SMALL}) + \text{MAX} (\text{SCAL}(\text{INODE}, \text{IPHASE}, \text{ISC17}), \text{SMALL})) \* \text{RECONVERTC} * 0.9 \]
\[ \text{ALPHA} = (\text{TOTALCELL} * 50.0585) / (-1.996E+09) + (2.0E+09 / 1.996E+09) \]

\[ \text{EQ7I} = \text{ALPHA} * \text{SKECFUGM} * \text{CFUGM} + \text{SXDCFUGM} * \text{MYSC} \]
\[ \text{EQ7II} = -\text{SKCFUGM} \]

\[ \text{OVERWRITE SOURCE TERMS} \]
\[ \text{SU}(\text{INODE}, \text{IPHASE}) = \text{SU}(\text{INODE}, \text{IPHASE}) + ((\text{EQ7I}) \* \text{VOL}(\text{INODE})) \]
\[ \text{SP}(\text{INODE}, \text{IPHASE}) = \text{SP}(\text{INODE}, \text{IPHASE}) + ((\text{EQ7II}) \* \text{VOL}(\text{INODE})) \]

\[ \text{CONTINUE} \]

\[ \text{IF SCALAR8 EQUATION CFU-MEG (ISCH) ADD SOURCE TERMS} \]
\[ \text{ELSE IF (ISCH.EQ.IEQN) THEN} \]
\[ \text{LOOP OVER ALL INTERIOR CELLS} \]
DO 710 I=1,NPT
C USE ARRAY IPT TO GET ADDRESS
C INODE = IPT(I)
C MYSC = MAX (SCAL(INODE, IPHASE, ISC5), SMALL)
CFUMEG = MAX (SCAL(INODE, IPHASE, ISC8), SMALL)
C TOTALCELL = (MAX (SCAL(INODE, IPHASE, ISC3), SMALL) + MAX (SCAL
+ (INODE, IPHASE, ISC4), SMALL) + MAX (SCAL(INODE, IPHASE, ISC5), SMALL) +
+ MAX (SCAL(INODE, IPHASE, ISC6), SMALL) + MAX (SCAL(INODE, IPHASE, ISC
+ 7), SMALL) + MAX (SCAL(INODE, IPHASE, ISC8), SMALL) + MAX (SCAL(INODE,
+ IPHASE, ISC9), SMALL) + MAX (SCAL(INODE, IPHASE, ISC10), SMALL)
+ + MAX (SCAL(INODE, IPHASE, ISC11), SMALL) + MAX (SCAL(INODE,
+ IPHASE, ISC12), SMALL) + MAX (SCAL(INODE, IPHASE, ISC13), SMALL) +
+ MAX (SCAL(INODE, IPHASE, ISC14), SMALL) + MAX (SCAL(INODE,
+ IPHASE, ISC15), SMALL))*RECONVERTC*0.9
C ALPHA = (TOTALCELL*50.0585)/(-1.996E+09)+(2.0E+09/1.996E+09)
EQ81 = ALPHA*SKECFUMEG*CFUMEG+SKDCFUMEG*MYSC
EQ811 = -SKKCFUMEG
C OVERWRITE SOURCE TERMS
C SU(INODE, IPHASE)=SU(INODE, IPHASE)+((EQ81)*VOL(INODE))
C SP(INODE, IPHASE)=SP(INODE, IPHASE)+((EQ811)*VOL(INODE))
710 CONTINUE
C***************************************************************************
C IF SCALAR9 EQUATION BFU-E (ISCI) ADD SOURCE TERM
C ELSE IF (ISCI.EQ.ISQN) THEN
C LOOP OVER ALL INTERIOR CELLS
C DO 720 I=1,NPT
C USE ARRAY IPT TO GET ADDRESS
C INODE = IPT(I)
C MYSC = MAX (SCAL(INODE, IPHASE, ISC5), SMALL)
BFUE = MAX (SCAL(INODE, IPHASE, ISC9), SMALL)
C TOTALCELL = (MAX (SCAL(INODE, IPHASE, ISC3), SMALL) + MAX (SCAL
+ (INODE, IPHASE, ISC4), SMALL) + MAX (SCAL(INODE, IPHASE, ISC5), SMALL) +
+ MAX (SCAL(INODE, IPHASE, ISC6), SMALL) + MAX (SCAL(INODE, IPHASE, ISC
+ 7), SMALL) + MAX (SCAL(INODE, IPHASE, ISC8), SMALL) + MAX (SCAL(INODE,
+ IPHASE, ISC9), SMALL) + MAX (SCAL(INODE, IPHASE, ISC10), SMALL)
+ + MAX (SCAL(INODE, IPHASE, ISC11), SMALL) + MAX (SCAL(INODE,
+ IPHASE, ISC12), SMALL) + MAX (SCAL(INODE, IPHASE, ISC13), SMALL) +
+ MAX (SCAL(INODE, IPHASE, ISC14), SMALL) + MAX (SCAL(INODE,
+ IPHASE, ISC15), SMALL))*RECONVERTC**0.9
C ALPHA = (TOTALCELL*50.0585)/(-1.996E+09)+(2.0E+09/1.996E+09)
C EQ91 = ALPHA*SKEBFUE*BFUE+SKDBFUE*MYSC
EQ911 = -SKKBFUE
C OVERWRITE SOURCE TERMS
SU(INODE, IPHASE) = SU(INODE, IPHASE) + ((EQ9I) * VOL(INODE))

SP(INODE, IPHASE) = SP(INODE, IPHASE) + ((EQ9II) * VOL(INODE))

720 CONTINUE

-----------------------------------------------------------------------------------

C IF SCALAR10 EQUATION CFU-Eo (ISCJ) ADD SOURCE TERMS
C
ELSE IF (ISCJ.EQ.IEQN) THEN
C
LOOP OVER ALL INTERIOR CELLS
C
DO 740 I=1, NPT
C
USE ARRAY IPT TO GET ADDRESS
C
INODE = IPT(I)
C
MYSC = MAX (SCAL(INODE, IPHASE, ISC5), SMALL)
CFUEO = MAX (SCAL(INODE, IPHASE, ISC10), SMALL)

TOTALCELL = (MAX (SCAL(INODE, IPHASE, ISC3), SMALL) + MAX (SCAL(INODE, IPHASE, ISC4), SMALL) + MAX (SCAL(INODE, IPHASE, ISC5), SMALL) +
+ (SCAL(INODE, IPHASE, ISC6), SMALL) + MAX (SCAL(INODE, IPHASE, ISC7), SMALL) +
+ MAX (SCAL(INODE, IPHASE, ISC8), SMALL) + MAX (SCAL(INODE, IPHASE, ISC9), SMALL) +
+ MAX (SCAL(INODE, IPHASE, ISC10), SMALL) + MAX (SCAL(INODE, IPHASE, ISC11), SMALL) +
+ MAX (SCAL(INODE, IPHASE, ISC12), SMALL) + MAX (SCAL(INODE, IPHASE, ISC13), SMALL) +
+ MAX (SCAL(INODE, IPHASE, ISC14), SMALL) + MAX (SCAL(INODE, IPHASE, ISC15), SMALL)) * RECONVERTC * 0.9

ALPHA = (TOTALCELL * 50.0555) / (-1.996E+09) + (2.0E+09 / 1.996E+09)

EQ10I = ALPHA * SKECFUEO * CFUEO + SKDCFUEO * MYSC
EQ10II = -SKKCFUEO

OVERWRITE SOURCE TERMS
C

SU(INODE, IPHASE) = SU(INODE, IPHASE) + ((EQ9I) * VOL(INODE))
C
SP(INODE, IPHASE) = SP(INODE, IPHASE) + ((EQ9II) * VOL(INODE))
C
740 CONTINUE

-----------------------------------------------------------------------------------

C IF SCALAR11 EQUATION Mature Compartment (ISCK) ADD SOURCE TERMS
C
ELSE IF (ISCX.EQ.IEQN) THEN
C
LOOP OVER ALL INTERIOR CELLS
C
DO 780 I=1, NPT
C
USE ARRAY IPT TO GET ADDRESS
C
INODE = IPT(I)
C
CFUGM = MAX (SCAL(INODE, IPHASE, ISC7), SMALL)
CFUMEG = MAX (SCAL(INODE, IPHASE, ISC8), SMALL)
BFUE = MAX (SCAL(INODE, IPHASE, ISC9), SMALL)
CFUEO = MAX (SCAL(INODE, IPHASE, ISC10), SMALL)

189
MATURE1 = MAX (SCAL(INODE, IPHASE, ISC13), SMALL)

OVERWRITE SOURCE TERMS

SU(INODE, IPHASE)=SU(INODE, IPHASE)+((2*GROWTH) + *(CFUMEG+CFUGM+BFUE+CFUEO))*VOL(INODE)

SP(INODE, IPHASE)=SP(INODE, IPHASE)-((GROWTH)*VOL(INODE))

780 CONTINUE

******************************************************************************

IF SCALAR12 EQUATION Mature Compartment 1 (ISCL) ADD SOURCE TERMS

ELSE IF (ISCL.EQ.IEQN) THEN

LOOP OVER ALL INTERIOR CELLS

DO 790 I=1,NPT

USE ARRAY IPT TO GET ADDRESS

INODE = IPT(I)

MATURE1 = MAX (SCAL(INODE, IPHASE, ISC13), SMALL)
MATURE2 = MAX (SCAL(INODE, IPHASE, ISC14), SMALL)

OVERWRITE SOURCE TERMS

SU(INODE, IPHASE)=SU(INODE, IPHASE)+((2*GROWTH) + *(MATURE1))*VOL(INODE)

SP(INODE, IPHASE)=SP(INODE, IPHASE)-((GROWTH)*VOL(INODE))

790 CONTINUE

******************************************************************************

IF SCALAR13 EQUATION Mature Compartment 2 (ISCM) ADD SOURCE TERMS

ELSE IF (ISCM.EQ.IEQN) THEN

LOOP OVER ALL INTERIOR CELLS

DO 800 I=1,NPT

USE ARRAY IPT TO GET ADDRESS

INODE = IPT(I)

MATURE2 = MAX (SCAL(INODE, IPHASE, ISC14), SMALL)
MATURE3 = MAX (SCAL(INODE, IPHASE, ISC15), SMALL)

OVERWRITE SOURCE TERMS

SU(INODE, IPHASE)=SU(INODE, IPHASE)+((2*GROWTH) + *(MATURE2))*VOL(INODE)

SP(INODE, IPHASE)=SP(INODE, IPHASE)-((GROWTH)*VOL(INODE))

800 CONTINUE

******************************************************************************
C IF SCALAR14 EQUATION Mature Compartment 3 (ISCN) ADD SOURCE TERMS
C ELSE IF (ISCN.EQ.IEQN) THEN
C LOOP OVER ALL INTERIOR CELLS
C DO 810 I=1,NPT
C USE ARRAY IPT TO GET ADDRESS
C INODE = IPT(I)
C MATURE3 = MAX (SCAL(INODE,IPHASE,ISC15),SMALL)
C MATURE4 = MAX (SCAL(INODE,IPHASE,ISC16),SMALL)
C OVERWRITE SOURCE TERMS
C SU(INODE,IPHASE)=SU(INODE,IPHASE)+((2*GROWTH)
+ *(MATURE3))*VOL(INODE)
C SP(INODE,IPHASE)=SP(INODE,IPHASE)-((GROWTH)*VOL(INODE))
C 810 CONTINUE
C IF SCALAR15 EQUATION Mature Compartment 4 (ISCO) ADD SOURCE TERMS
C ELSE IF (ISCO.EQ.IEQN) THEN
C LOOP OVER ALL INTERIOR CELLS
C DO 820 I=1,NPT
C USE ARRAY IPT TO GET ADDRESS
C INODE = IPT(I)
C MATURE4 = MAX (SCAL(INODE,IPHASE,ISC16),SMALL)
C MATURE5 = MAX (SCAL(INODE,IPHASE,ISC17),SMALL)
C OVERWRITE SOURCE TERMS
C SU(INODE,IPHASE)=SU(INODE,IPHASE)+((2*GROWTH)
+ *(MATURE4))*VOL(INODE)
C SP(INODE,IPHASE)=SP(INODE,IPHASE)-((DEATH)*VOL(INODE))
C 820 CONTINUE
C ENDIF
C END OF USER AREA 5 +++++++++++++++++++++++++++++++
C END IF
C OVERWRITE SOURCE TERMS
C IF (ICALL.EQ.2) THEN
C USER AREA 6 +++++++++++++++++++++++++++++++
C END OF USER AREA 6 +++++++++++++++++++++++++++++++
C END IF
C RETURN
C END

SUBROUTINE USRTMN(U, V, W, P, VFRAC, DEN, VIS, TE, ED, RS, RF, SCAL, XP, +
YP, ZP, VOL, AREA, VFOR, ARFOR, WFCT, CONV, IFT, IBLK, +
IPVERT, IPNODN, IPFACN, IPNODB, IPFACB, WORK, +
IWORK, CWORK)

C USER SUBROUTINE TO ALLOW USERS TO MODIFY OR MONITOR THE SOLUTION AT
C THE END OF EACH TIME STEP
C THIS SUBROUTINE IS CALLED BEFORE THE START OF THE RUN AS WELL AS AT
C THE END OF EACH TIME STEP

C >>> IMPORTANT <<<
C >>> USERS MAY ONLY ADD OR ALTER PARTS OF THE SUBROUTINE WITHIN <<<
C >>> THE DESIGNATED USER AREAS <<<

C THIS SUBROUTINE IS CALLED BY THE FOLLOWING SUBROUTINES
C CUSR TRNMOD

C SUBROUTINE ARGUMENTS
C
C U - U COMPONENT OF VELOCITY
C V - V COMPONENT OF VELOCITY
C W - W COMPONENT OF VELOCITY
C P - PRESSURE
C VFRAC - VOLUME FRACTION
C DEN - DENSITY OF FLUID
C VIS - VISCOSITY OF FLUID
C TE - TURBULENT KINETIC ENERGY
C ED - EPSILON
C RS - REYNOLD STRESSES
C T - TEMPERATURE
C H - ENTHALPY
C RF - REYNOLD FLUXES
C SCAL - SCALARS (THE FIRST 'NCONC' OF THESE ARE MASS FRACTIONS)

C_CREATED
C 27/04/90 ADB
C_MODIFIED
C 05/08/91 IRH NEW STRUCTURE
C 01/10/91 DSC REDUCE COMMENT LINE GOING OVER COLUMN 72.
C 29/11/91 PHA UPDATE CALLED BY COMMENT, ADD RF ARGUMENT,
C CHANGE LAST DIMENSION OF RS TO 6 AND IVERS TO 2
C 05/06/92 PHA ADD PRECISION FLAG AND CHANGE IVERS TO 3
C 03/07/92 DSC CORRECT COMMON MLTGRD.
C 23/11/93 CSH EXPLICITLY DIMENSION IPVERT ETC.
C 03/02/94 PHA CHANGE FLOW3D TO CFDS-FLOW3D
C 22/08/94 NSW MOVE 'IF(IUSED.EQ.0) RETURN' OUT OF USER AREA
C 19/12/94 NSW CHANGE FOR CFX-F3D
C 02/07/97 NSW UPDATE FOR CFX-4
C 02/07/99 NSW INCLUDE NEW EXAMPLE FOR CALCULATING FLUX OF A
C SCALAR AT A PRESSURE BOUNDARY
c Xp - X COORDINATES OF CELL CENTRES
C Yp - Y COORDINATES OF CELL CENTRES
C Zp - Z COORDINATES OF CELL CENTRES
C VOL - VOLUME OF CELLS
C AREA - AREA OF CELLS
C VPOR - POROUS VOLUME
C ARPOR - POROUS AREA
C WFACT - WEIGHT FACTORS
C CONV - CONVECTION COEFFICIENTS
C
C IPT - 1D POINTER ARRAY
C IBLK - BLOCK SIZE INFORMATION
C IPVERT - POINTER FROM CELL CENTERS TO 8 NEIGHBOURING VERTICES
C IPNODN - POINTER FROM CELL CENTERS TO 6 NEIGHBOURING CELLS
C IPFACN - POINTER FROM CELL CENTERS TO 6 NEIGHBOURING FACES
C IPFACF - POINTER FROM CELL FACES TO 2 NEIGHBOURING CELL CENTERS
C IPFACB - POINTER FROM BOUNDARY CENTERS TO BOUNDARY FACES
C
C WORK - REAL WORKSPACE ARRAY
C IWORK - INTEGER WORKSPACE ARRAY
C CWORK - CHARACTER WORKSPACE ARRAY
C
SUBROUTINE ARGUMENTS PRECEDED WITH A '*' ARE ARGUMENTS THAT MUST
BE SET BY THE USER IN THIS ROUTINE.
C
NOTE THAT OTHER DATA MAY BE OBTAINED FROM CFX-4 USING THE
ROUTINE GETADD, FOR FURTHER DETAILS SEE THE VERSION 4
USER MANUAL.
C
******************************************************************************
C
DOUBLE PRECISION U
DOUBLE PRECISION V
DOUBLE PRECISION W
DOUBLE PRECISION P
DOUBLE PRECISION VFRAC
DOUBLE PRECISION DEN
DOUBLE PRECISION VIS
DOUBLE PRECISION TE
DOUBLE PRECISION ED
DOUBLE PRECISION RS
DOUBLE PRECISION T
DOUBLE PRECISION H
DOUBLE PRECISION RF
DOUBLE PRECISION SCAL
DOUBLE PRECISION XP
DOUBLE PRECISION YP
DOUBLE PRECISION ZP
DOUBLE PRECISION VOL
DOUBLE PRECISION AREA
DOUBLE PRECISION VPOR
DOUBLE PRECISION ARPOR
DOUBLE PRECISION WFACT
DOUBLE PRECISION CONV
DOUBLE PRECISION WORK
DOUBLE PRECISION SMALL
DOUBLE PRECISION SORMAX
DOUBLE PRECISION DTUSR
DOUBLE PRECISION TIME
DOUBLE PRECISION DT
DOUBLE PRECISION DTINVF
DOUBLE PRECISION TPARM
DOUBLE PRECISION SGNWL
LOGICAL LDEN, LVIS, LTURB, LTEMP, LBUOY, LSCAL, LCOMP, LRECT, LCYN, LAXIS, + LPOROS, LTRANS

C
CHARACTER*(*) CWORK

C+++++++++++++++ USER AREA 1 ++++++++++++++++++++++++++++++++ C
C---- AREA FOR USERS EXPLICITLY DECLARED VARIABLES
C LOGICAL TIME1
C---- Michaelis-Menten Kinetics
C
DOUBLE PRECISION RECONVERTG, RECONVERTO, RECONVERTC, TOTALCELL, G1, + G2, O1, O2, VESSELVOL

C+++++++++++++++ END OF USER AREA 1 ++++++++++++++++++++++++++++ C

C
COMMON /ALL/NBLOCK, NCELL, NBDRY, NNODE, NFACEx, NVERT, NOIM, + /ALLRES/NRES, NWS, NCWS, INRES, INWRES, NCWRES, ADDIMS/NPHASE, + NSCAL, NVAR, NPROP, NSVAR, NVPN Prop, NSVXV, NDEGINT, NCOEF, NILIST, + NREGION, NREGIONP, /CHXS/IRES, IUCALL, IUSED, /CONC/NCONC, + /DEVICE/NREAD, NWRITE, NSVVAR, NSVVARP, /IDUM/ILEN, JLEN, + /LOGIC/LDEN, LVIS, LTURB, LTEMP, LBUOY, LSCAL, LCOMP, LRECT, LCYN, + LAXIS, LPOROS, LTRANS, /MLEVEL/NLEVEL, NLEVEL, ILEVEL,
+ /SGDLBL/IFLGPR, ICHKPR, /SPARM/SMALL, SORMAX, NITER, INPDI, + MAXIT, NODSREF, NDMON, /TRANSI/DTUSR, /TRANSI/NSTEP, KSTEP, MF, + INCORE, /TRANSI/TIME, DT, DTINVF, TPARM

C
C+++++++++++++++ USER AREA 2 ++++++++++++++++++++++++++++++++ C
C---- AREA FOR USERS TO DECLARE THEIR OWN COMMON BLOCKS
C THESE SHOULD START WITH THE CHARACTERS 'UC' TO ENSURE
C NO CONFLICT WITH NON-USER COMMON BLOCKS
C
C+++++++++++++++ END OF USER AREA 2 ++++++++++++++++++++++++++++ C

C
DIMENSION U(NNODE, NPRASE), V(NNODE, NPRASE), W(NNODE, NPHASE), + P(NNODE, NPRASE), VPHAC(NNODE, NPHASE), DEN(NNODE, NPHASE), + VIS(NNODE, NPHASE), TE(NNODE, NPHASE), ED(NNODE, NPHASE), + BS(NNODE, NPHASE, 6), T(NNODE, NPHASE), H(NNODE, NPHASE), + RF(NNODE, NPHASE, 4), SCAL(NNODE, NPHASE, NSCAL)
DIMENSION XP(NNODE), YP(NNODE), ZP(NNODE), VOL(NCELL), AREA(NFACE, 3), + VFOR(NCELL), ARFOR(NFACE, 3), WFAC(NFACE), + CONV(NFACE, NPHASE), IFT(*), IBLK(5, NBLOCK), + IVERT(NCELL, 6), IPNODN(NCELL, 6), IPFACN(NCELL, 6), + IPNODF(NFACE, 4), IPNODB(NBDRY, 4), IPFACB(NBDRY), IWORK(*), + WORK(*), CWORK(*)

DIMENSION SGNWL(6)

C
C+++++++++++++++ USER AREA 3 ++++++++++++++++++++++++++++++++ C
C---- AREA FOR USERS TO DIMENSION THEIR ARRAYS
C
C+++++++++++++++ END OF USER AREA 3 ++++++++++++++++++++++++++++ C

C
DATA SGNWL/1.000, 1.000, 1.000, 1.000, 1.000, 1.000/

C
C

C
DATA SGNWL/1.000, 1.000, 1.000, 1.000, 1.000, 1.000/

C
C---- STATEMENT FUNCTION FOR ADDRESSING
C IF(I, J, K) = IFT((K-1)*ILEN*JLEN+ (J-1)*ILEN+I)
C C---- VERSION NUMBER OF USER ROUTINE AND PRECISION FLAG
C IVERS = 3
ICHKPR = 2

C+++++++++++++++ USER AREA 4 ++++++++++++++++++++++++++++++++ C

194
C---- TO USE THIS USER ROUTINE FIRST SET IUSED=1
C
C IUSED = 1
C
C++++++++++++++++++ END OF USER AREA 4 ++++++++++++++++++++++++++++++++
C
C IF (IUSED.EQ.0) RETURN
C
C---- FRONTEND CHECKING OF USER ROUTINE
C IF (IUCALL.EQ.0) RETURN
C
C++++++++++++++++++ USER AREA 5 ++++++++++++++++++++++++++++++++
C
C VESSELVOL = 50.0585/(100*100*100)
C RECONVERTG = 1000
C RECONVERTO = 1/6.496E-5
C RECONVERTC = 1/2.8E-6
C
C OPEN (500, FILE='..//partial.txt', STATUS='NEW')
C OPEN (501, FILE='..//conc.txt', STATUS='NEW')
C OPEN (502, FILE='..//TotalCell.txt', STATUS='NEW')
C
C TIME1 = .FALSE.
C INQUIRE (FILE='CFX4.STP', EXIST=TIME1)
C
CIPHASE = 1
C
CALL GETSCA('USER SCALAR1', ISC1,CWORK)
CALL GETSCA('USER SCALAR2', ISC2,CWORK)
CALL GETSCA('USER SCALAR3', ISC3,CWORK)
CALL GETSCA('USER SCALAR4', ISC4,CWORK)
CALL GETSCA('USER SCALAR5', ISC5,CWORK)
CALL GETSCA('USER SCALAR6', ISC6,CWORK)
CALL GETSCA('USER SCALAR7', ISC7,CWORK)
CALL GETSCA('USER SCALAR8', ISC8,CWORK)
CALL GETSCA('USER SCALAR9', ISC9,CWORK)
CALL GETSCA('USER SCALAR10', ISC10,CWORK)
CALL GETSCA('USER SCALAR11', ISC11,CWORK)
CALL GETSCA('USER SCALAR12', ISC12,CWORK)
CALL GETSCA('USER SCALAR13', ISC13,CWORK)
CALL GETSCA('USER SCALAR14', ISC14,CWORK)
CALL GETSCA('USER SCALAR15', ISC15,CWORK)
C
CALL IPALL('POR1', 'POROUS', 'PATCH', 'CENTRES', IPT,NPT,CWORK,IWORK)
C
DO 601 I=1,NPT
C 601 INODE = IPT(I)
C
G1 = SCAL(INODE,IPHASE,ISC1)*CONVERTG
G2 = SCAL(INODE,IPHASE,ISC2)*CONVERTG
C
TOTALCELL = (MAX(ST1,SMALL)+MAX(ST2,SMALL)+MAX(ST3,SMALL)+MAX(ST4,SMALL)+
MAX(ST5,SMALL)+MAX(ST6,SMALL)+MAX(ST7,SMALL)+MAX(ST8,SMALL)+MAX(ST9,SMALL)+
MAX(ST10,SMALL)+MAX(ST11,SMALL)+MAX(ST12,SMALL)+MAX(ST13,SMALL)+
MAX(ST14,SMALL)+MAX(ST15,SMALL))*RECONVERTC*VESSELVOL
C

195
WRITE (500, *) G1, G2
WRITE (501, *) G1, G2
WRITE (502, *) TOTALCELL
C
601 CONTINUE
C
END IF
C
C++++++++++++++++++ END OF USER AREA 5 ++++++++++++++++++++++++++++++
C
RETURN
C
END
Bibliography


increased sensitivity to growth factor-induced proliferation and maturation. 
Experimental Hematology. 28, 1401-1412.


Chapman, A. R., M. S. Frankel and M. S. Garfinkel (1999). Stem cell research and applications American Association for the Advancement of Science.


