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A computational analysis of the impact of mass transport and shear on three-dimensional stem cell cultures in perfused micro-bioreactors

Himanshu Kaul [1,2], Yiannis Ventikos [3], Zhanfeng Cui [1,*]

[1] Institute of Biomedical Engineering, Department of Engineering Science, University of Oxford, Old Road Campus Research Building, Headington, Oxford OX3 7DQ, UK
[2] Department of Computer Science, University of Sheffield, Regent Court, Sheffield S1 4DP, UK
[3] Department of Mechanical Engineering, University College London, Torrington Place, London WC1E 7JE, UK

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ABSTRACT

In this study, Computational Fluid Dynamics (CFD) is used to investigate and compare the impact of bioreactor parameters (such as its geometry, medium flow-rate, scaffold configuration) on the local transport phenomena and, hence, their impact on human mesenchymal stem cell (hMSC) expansion. The geometric characteristics of the TissueFlex® (Zyoxel Limited, Oxford, UK) microbioreactor were considered to set up a virtual bioreactor containing alginate (in both slab and bead configuration) scaffolds. The bioreactor and scaffolds were seeded with cells that were modelled as glucose consuming entities. The widely used glucose medium, Dulbecco's Modified Eagle Medium (DMEM), supplied at two inlet flow rates of 25 and 100 $\mu l \cdot h^{-1}$, was modelled as the fluid phase inside the bioreactors. The investigation, based on applying dimensional analysis to this problem, as well as on detailed three-dimensional transient CFD results, revealed that the default bioreactor design and boundary conditions led to internal and external glucose transport, as well as shear stresses, that are conducive to hMSC growth and expansion. Furthermore, results indicated that the 'top-inout' design (as opposed to its symmetric counterpart) led to higher shear stress for the same media inlet rate (25 $\mu l \cdot h^{-1}$), a feature that can be easily exploited to induce shear-dependent differentiation. These findings further confirm the suitability of CFD as a robust design tool.

1. Introduction

The principles of tissue engineering can be employed to develop, *ex vivo*, clinically viable, autologous substitutes with remarkably similar properties and functionality as those of the host tissues in order to replace or regenerate human cells, tissues, or organs [1–5]. This, however, requires recapitulation of certain key developmental events *ex vivo* thereby necessitating tight control over the artificial growth environment, as well as robust and cost-effective manufacturing processes compliant with the evolving regulatory framework [6,7]. Bioreactors, based on the premise that certain aspects of the dynamic three-dimensional (3D) environment influence the development of native tissues, are devices that have been successfully utilised towards this end [6,8,9], especially considering the fact that their use makes the entire procedure of cell expansion and development of biologically functional synthetic substitutes less-labour intensive, automated and tractable [6,10].

Despite the technological advances made in the sector of cell therapy and bioreactor technology, bioreactors remain mostly functional 'black boxes' where trial and error eventually leads to the desired outcome. As such, typical bioreactor yields (in terms of desired cellular phenotypes or microbial contamination) are qualitatively poor and the process of cell expansion almost non-reproducible [11]. This stems from the fact that the impact of factors such as transport phenomena, fluid flow, and shear stresses on the expansion, differentiation and growth of cells within a bioreactor, both qualitatively and quantitatively, is poorly understood. Computational Fluid Dynamics (CFD) is a mathematical tool that investigators have recently started utilising to analyse the effect of fluidic forces and stresses on cells [12–16]. The numerical solution of relevant conservation equations results in the evaluation of field quantities of interest, such as hydrodynamic, mechanical, concentration profiles *etc.*; usually defined within a desirable physical configuration of arbitrary shape. In most cases, these equations do not lend themselves to analytical solution; thus numerical techniques become the only non-experimental way to acquire insight on relevant dynamics. While numerical solutions are of course approximations to the real physical system processes, CFD can be employed to characterise the dynamic environment of the native tissue or other synthetic environments, as Song *et al.* [17] reported, with high fidelity. Such techniques can therefore be used to optimise bioreactor design or flow conditions with the aim to obtain desirable cell growth, differentiation, or expansion, as they enable full characterisation of 3D flow fields, concentration gradients, and loads in arbitrary geometries.

* Corresponding author.
E-mail address: zhanfeng.cui@eng.ox.ac.uk (Z. Cui).

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The application of CFD to characterise local fluid dynamics in a variety of bioreactor systems (concentric cylinder, direct-perfusion, rotating hollow-fibre, rotating-wall perfused, wavy-walled) can already be found in the literature [13,18–22]. Song *et al.* [17] used CFD to predict 3D flow fields at the length scale of stem cells. They employed the Microscale Particle Image Velocimetry technique to experimentally measure flow values, and thereby validate the CFD model. Their investigation concluded that CFD predicts flow regimes within 12% of experimentally measured values in the absence of cells (*i.e.* 12% difference between computationally predicted and experimentally measured shear rate values). CFD was also used to model perfusion and the influence of perfusion generated shear stress on 3D cultures [23]. Boschetti *et al.* [24] investigated shear stress generation as a function of parameters, such as scaffold porosity and pore size, medium flow rate, and diameter of the perfused scaffold section. A 3D CFD model [22] was created to investigate mass transfer interactions between the culture medium and micro-carrier attached aggregated hepatocytes seeded within a hollow-fibre bioreactor. In particular, distribution of oxygen within the cellular compartment and cellular consumption of oxygen as an index of cell metabolic activity were analysed. The overall performance of the numerical model in predicting optimal conditions for culturing viable microcarrier-attached aggregated hepatocytes, despite certain limitations that were a by-product of unavoidable simplifications, was reported to be satisfactory.

Yu *et al.* [25] created a numerical model to simulate fluid-flow and oxygen transport in a rotating magnetic bar mixer micro-bioreactor, aiming to determine the operating parameters for animal cell culture. A comparative analysis between a bi-axial bioreactor vessel and its uni-axial counterpart using CFD simulations revealed significant increase in velocity when the bi-axial configuration was used, thereby recommending bi-axial rotation of the vessel as a solution to the problem of inadequate fluid and metabolite transport to and from the cells [26]. CFD simulations were also used to test the hypothesis that geometrical design of micro-pillars in a microfluidic channel will affect fluid flow profiles and, therefore, cell immobilisation efficiency of the micropillar array [27]. According to the CFD analysis, parallelogram shaped micropillars were found to be superior compared with their semi-circular counterparts as the former delivered more optimal flow profiles that minimised the risk of clogging. The results were verified experimentally.

In this study, a multi-physics CFD platform was employed to analyse and compare the impact of bioreactor variables (such as its geometry, medium flow-rate, choice of scaffold encapsulating cells, number of cells encapsulated in the scaffold) on mass transport and fluid flow in the bioreactor and, therefore, on the expansion and growth of metabolising mesenchymal stem cells, encapsulated in a slab-shaped scaffold and in a spherical alginate bead under perfused conditions. This entailed modelling, *in silico*, of the perfusion of cells in a microbioreactor platform, similar to TissueFlex® (Zyoxel Ltd, Oxford, UK), which was developed to conduct cell and tissue culture under almost uniform (in terms of distribution of metabolite/non-metabolite concentrations and shear, as we demonstrate in our results) and precisely controlled environment in a mid-throughput and parallel manner [10]. Model variables included bioreactor configuration, scaffold morphology, media inlet velocity, glucose concentration in culture media, mesenchymal stem cell metabolism (in terms of glucose consumption), cell density, and apoptotic threshold. Human mesenchymal stem cells (hMSCs) were particularly modelled due to their ease of culture, sourcing from patients, plasticity, and potential for autologous therapy. The importance of 3D cell cultures in providing the growing cellular population with a pseudo-native environment has become clearer over the last few years and was, therefore, included in this study. The model provides insight into glucose transport occurring within the bioreactor. The different variables are listed in Table 1 and the two bioreactor configurations tested are shown in Fig. 1.

Table 1
Variables utilised in the model

Variable	Value	
Bioreactor geometry	Symmetrical side inlets, inlet/outlet at the top	
Scaffold morphology	Slab and spherical bead (3D)	
Media inlet velocity	25 $\mu l \cdot h^{-1}$ and 100 $\mu l \cdot h^{-1}$	
Glucose concentration	0.025 $mol \cdot L^{-1}$ (4.5 $g \cdot L^{-1}$) and 0.00625 $mol \cdot L^{-1}$ (1 $g \cdot L^{-1}$)	
hMSC glucose consumption rate	270 $fmol \cdot L^{-1} \cdot h^{-1} \cdot cell^{-1}$ [34]	
Cell density/ml cell suspension	1 million: corresponding to 400 cells in the bead and 35000 in the slab	10 million: corresponding to 4000 cells in the bead and 350000 in the slab
Apoptotic threshold	5% of 1 $g \cdot L^{-1}$ glucose and 20% of 4.5 $g \cdot L^{-1}$ glucose	
$D_{eff,g}$ in water at 37 °C	9.3×10^{-10} $m^2 \cdot s^{-1}$ [28,29]	
$D_{eff,g}$ in alginate at 37 °C (10 $g \cdot L^{-1}$)	6.59×10^{-10} $m^2 \cdot s^{-1}$ [29]	

2. Material and Methods

2.1. Bioreactor dimensions and configuration

In order to design the geometry of the model bioreactor, dimensions of TissueFlex® (Zyoxel Limited, Oxford, UK) microbioreactor were considered. The microbioreactor, constructed with polydimethylsiloxane (PDMS), has the format of a standard 96-well cell culture plate and is perfused using silicone tubing *via* a multi-channel peristaltic pump or multiple syringe pumps [10]. The bioreactor geometry can be modified so that the silicone tubing serving as the inlet and outlet ports can be arranged in different styles — two of which are explored in this study. Fig. 2 shows a schematic diagram, as well as a photograph, of TissueFlex® with 12 microbioreactors. Each microbioreactor has a dimension of 6.6 mm × 11 mm. For the purposes of this study, fluid flow and mass transport in only one microbioreactor was considered.

2.2. Scaffold and cells

In this investigation, we simulated mass transport within two 3D cell culture constructs, modelled as a 1 mm thick porous slab structure and as a porous bead of diameter 1 mm, both synthesised using alginate. Scaffold porosity and permeability are regulated by a multitude of factors, which include concentration of alginate and cross-linking solutions, method of synthesis, and, eventually, the pore size. As such, there is a whole array of case specific porosity and permeability values that can be introduced in the model. Scaffold porosity in excess of 90% can be easily achieved. Furthermore, glucose diffusivity in alginate, proportional to its porosity, can be as high as 90% [28]. As such, both slab and spherical scaffolds were assigned isotropic porosity of 85%. As permeability is rather difficult to characterise there was lack of reasonably consistent values. After carefully reviewing the work conducted by Julian *et al.* we assigned the two scaffolds permeability of 10^{-10} m^2 [29].

Alginate was considered in this analysis due to its wide application [30] in synthesis of micro-carriers employed to immobilise cells due to its inertness, biocompatibility, high porosity, and amenability to various preparation methodologies [28,30-33]. Slab and sphere morphologies were modelled due to their ease of preparation and, as such, pervasive application. Scaffold dimensions were based on ease of synthesis and, therefore, widespread utility. The simplest way to create the spherical shape entails transferring alginate solution through a syringe, which results in beads roughly equal to or greater than 1 mm. As this technique involving syringes is inexpensive, simple, commonly used in cell-culture laboratories, the diameter of the spherical scaffold was set to 1 mm. The height of the slab was limited to 1 mm as the resulting volume provides optimum growing space enabling cell expansion without presenting, in itself, diffusional limitations.