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Characterisation of stresses on microcarriers in a stirred bioreactor

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ABSTRACT

A computational fluid dynamics model for the flow of culture in a Corning™ spinner-flask stirred bioreactor has been used to characterise stresses experienced by microcarriers immersed in the fluid. Validation of the turbulent flow using experimental Particle Image Velocimetry (PIV) found advanced Large Eddy Simulation (LES) to be superior to Unsteady Reynolds averaging (URANS) modelling as a computational strategy for accurately capturing instantaneous velocity fluctuations of the types observed in the experiments. The simulations demonstrated that stress exposures experienced by microcarriers were highest during impeller start-up. After start-up, microcarriers experienced elevated levels of fluctuating stress of magnitudes known to cause cell differentiation, potentially compromising expansion of a homogeneous population with multi-lineage potential. Decreasing the impeller speed from 70 RPM to 50 RPM in the Corning™ flask was not found to necessarily reduce microcarrier stress exposure, because such a measure does not control the spatio-temporal coincidence of the microcarrier population with high regions of stress within the bioreactor. Modifications to bioreactor geometries and operational protocols are identified that can be pursued if such issues with dynamic stem cell culture arise.

Introduction

The therapeutic potential of stem cells holds great promise for the treatment of many illnesses, including Parkinson's and cardiovascular diseases. The number of cells needed for treatment can range from tens of millions to billions, necessitating the production of large quantities of stem cells *ex-vivo* [1]. Stem cell expansion on these scales presents many biological and engineering challenges [2]. The current preferred method for *ex-vivo* cell expansion in the stem cell research area is a stirred bioreactor, due to its scalability [3–6]. The use of microcarriers for achieving stem-cell suspension in stirred bioreactors is particularly suitable for increased cell expansion due to the significant enhancement of surface area for attachment, and the capacity to culture cells at higher densities.

The mixing within a stirred suspension bioreactor needs to be intense enough to fully suspend the microcarriers and adhered cells in order to optimise nutrient and waste-product transfer between the cells and the surrounding medium. However, the act of stirring can induce flow stresses capable of causing deleterious effects such as cell death, reduction

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in cell growth and in some cases microcarrier damage [7,8]. On the other hand, stirring too slowly can also compromise expansion and may give rise to excessive agglomeration of microcarriers and cells, which can lead to poor transfer of oxygen and media to cells, compromising cell viability [9–12].

As well as cell death and inhibited growth rates, unintended cell differentiation is undesirable in a bioreactor system designed to expand a homogeneous population of stem cells. Exposure to shear stress can induce stem cells to undergo differentiation through a process known as mechanotransduction [13,14]. Human mesenchymal stem cells (hMSCs) exhibit significant morphological response when exposed to shear stresses as low as 0.013 Pa [15]. Murine embryonic stem cells (ESCs) exposed to shear stresses of 0.0016 Pa and above displayed enhanced expression of markers indicative of bias towards lineage commitment as opposed to proliferation [16]. The nature of the applied shear stress is also important when considering stem cell differentiation, with Liu et al. [17] demonstrating that hMSCs in non-osteogenic media exposed to intermittent low and high shear stress levels of 0.034 Pa and 0.42 Pa respectively displayed significantly higher levels of osteogenic markers than hMSCs exposed to only the high level of shear stress. While a few studies report undiminished differentiation capacity for hMSCs in suspension cultures [5,18], providing hope that undifferentiated stem-cell expansion can be achieved in an economical manner, understanding of how to engineer the culture conditions to do this robustly is currently poor.

Outcomes such as stem cell expansion, damage and differentiation are major considerations in the design of protocols for the use of stirred bioreactors, and the dynamic nature of the culture in such bioreactors makes computational fluid dynamics (CFD) an important tool for facilitating the development of improved protocols. CFD simulation has been used in previous studies for better understanding of flow conditions within bioreactors [19–22]; to characterise nutrient and gas concentrations, and shear stresses, on and in tissue scaffolds [23,24]; and to quantify the effects of inhibitory signalling on cell populations [25,26]. Relative to these previous applications of CFD, the characteristic time-scales associated with operating protocols for stem cell bioreactors are much larger. A common protocol used to seed cells onto microcarriers in the bioreactor involves two minutes of stirring for every 30 min of rest for the first period of bioreactor operation, typically 24 h [27–30]. Media exchange is another common protocol used to enhance cell expansion, requiring the impeller to be stationary to allow the microcarriers to settle to the bottom of the bioreactor [28]. Both protocols involve multiple restarts of the impeller, lifting the microcarriers off the bottom of the bioreactor towards the impeller blades and magnets, where the fluid forces are orders of magnitude higher than in the regions away from the impeller [31]. Repeated exposure to the high stresses in these regions have the potential to enhance undesirable differentiation in the stem cell population. As a consequence of the widely fluctuating levels of stress within a bioreactor, the CFD simulation techniques need to be able to accurately resolve the relevant time-scales of stress fluctuation over the duration of key protocol time-scales in the bioreactor flow.

A key motivation for CFD simulation that is transient and high-fidelity in nature is the role of turbulence in stirred bioreactors. Indeed, the distinction between the shear stresses of the mean flow and the fluctuating shear stresses associated with turbulence plays an important role in cell differentiation and death [7,32–34]. In laminar flow, the viscous shear stress is an accurate representation of the local mechanical environment experienced by a cell. Quantification of the local forces experienced by a microcarrier or a cell in a turbulent flow is much more complicated. The exposure of a stem cell population to the broadband stresses associated with turbulent flow has the potential to cause well understood lethal effects, and sub-lethal effects that are poorly understood. Croughan et al. [35] described the death of human FS-4 fibroblast cells in terms of the Kolmogorov length scale l_k, with 100% growth for $l_k > 130\,\mu\text{m}$, and 0% growth for $l_k \lesssim 90\,\mu\text{m}$. The critical quantity below which cell growth was inhibited was found to be $\sim 2/3$ of the average microcarrier diameter [8,35]. However, the increased sensitivity to shear stress exposure of hMSCs in comparison to fibroblasts indicates that this criterion is very likely cell dependent [15], and needs to be factored into bioreactor and protocol design.

In this study, we quantify the motion of microcarriers inside a bioreactor using CFD featuring Lagrangian particle tracking, in order to better understand the exposure of microcarriers and hence adherent stem cells to stress inside a bioreactor. This study is the first to quantify the stress exposure of a microcarrier population within a suspension bioreactor, with significant consequences for microcarrier-based bioreactor operation and design. Previous studies have modelled microcarriers within bioreactors using one-way coupling with rigid-body fluid motion [36,37], and as a solid Eulerian phase [38]. The use of Lagrangian particle tracking in this study allows for the first time the local hydrodynamic environment experienced by the microcarriers to be captured over their entire time history. The CFD model was validated with experimentally measured velocity data obtained using particle image velocimetry (PIV). The bioreactor chosen for the study was a Corning™ flask (Fig. 1(a)), featuring a magnet-driven impeller, and baffles to promote mixing. This type of bioreactor is similar to the one used by Hewitt et al. [8] in their study of hMSC expansion.

Numerical model

The work presented here was completed using the commercially available finite-volume CFD software ANSYS-CFX 14.5 (ANSYS, Canonsburg, PA, USA) to solve the Navier–Stokes equations, and featured a novel blended turbulence approach that was implemented in ANSYS-CFX using UserFORTRAN Junction Box subroutines. The computational domain with dimensions is shown in Fig. 1. The fluid inside the flask is considered to be Newtonian and incompressible. In order to accurately model the flow inside the bioreactor, the computational domain was split into two parts: a cylindrical rotating volume around the impeller (rotor), surrounded by a stationary volume comprising the rest of the bioreactor (stator) (Fig. 1(d)). The transient