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On the fluid dynamics of a laboratory scale single-use stirred bioreactor

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HIGHLIGHTS

- PIV is used to quantify fluid dynamics in a small scale rigid single-use bioreactor.
- The influence of the hydrodynamic environment upon CHO cells was studied.
- Modest impact upon cell viability and viable cell density is observed.
- Distinct shifts in metabolic behaviour along with IgG_4 productivity is noted.

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ABSTRACT

The commercial success of mammalian cell-derived recombinant proteins has fostered an increase in demand for novel single-use bioreactor (SUB) systems that facilitate greater productivity, increased flexibility and reduced costs (Zhang et al., 2010). These systems exhibit fluid flow regimes unlike those encountered in traditional glass/stainless steel bioreactors because of the way in which they are designed. With such disparate hydrodynamic environments between SUBs currently on the market, traditional scale-up approaches applied to stirred tanks should be revised. One such SUB is the Mobius® 3 L CellReady, which consists of an upward-pumping marine scoping impeller. This work represents the first experimental study of the flow within the CellReady using a Particle Image Velocimetry (PIV) approach, combined with a biological study into the impact of these fluid dynamic characteristics on cell culture performance. The PIV study was conducted within the actual vessel, rather than using a purpose-built mimic. PIV measurements conveyed a degree of fluid compartmentalisation resulting from the up-pumping impeller. Both impeller tip speed and fluid working volume had an impact upon the fluid velocities and spatial distribution of turbulence within the vessel. Cell cultures were conducted using the GS-CHO cell-line (Lonza) producing an IgG_4 antibody. Disparity in cellular growth and viability throughout the range of operating conditions used (80–350 rpm and 1–2.4 L working volume) was not substantial, although a significant reduction in recombinant protein productivity was found at 350 rpm and 1 L working volume (corresponding to the highest Reynolds number tested in this work). The study shows promise in the use of PIV to improve understanding of the hydrodynamic environment within individual SUBs and allows identification of the critical hydrodynamic parameters under the different flow regimes for compatibility and scalability across the range of bioreactor platforms.

1. Introduction

In the last 15 years the biopharmaceutical industry has experienced a significant increase in the uptake and utilisation of single-use technologies, primarily driven by the need for a reduced time to market, an enhanced degree of flexibility and attenuated risk of cross-contamination (Business Insights, 2007). Since the release of the first rocked bag by Wave Biotech US in 1996 (GE Healthcare Life Sciences), several single-use bioreactor (SUB) products have become available for upstream processing, and in particular, for cell culture applications. Such systems typically consist of a multilayer polymer bag mounted on a metal skid, where polypropylene is often used as the contact layer (Barbaroux and Sette, 2006), or of a rigid plastic case moulded to the desired shape and

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geometry. SUBs can be distinguished by their mixing mechanism and/or bioreactor geometry. There are bag-based, stirred tank and hollow-fibre bioreactors (Eibl et al., 2009), mechanically (i.e. tipping, stirring or vibrating) or pneumatically driven devices such as airlift or slug bubble devices (Terrier et al., 2006). The 3 L CellReady was one of the first rigid stirred tank SUBs to be released on the market and has been widely employed for process development as its geometrical configuration facilitates process translation to larger scales of operation. It is part of EMD Millipore's family of Mobius® CellReady SUBs, including 50 L and 200 L vessels suitable for pilot-scale and clinical-scale applications. Recently, a few single-use equipment manufacturers have invested in larger scale systems (examples include the 500 L Pneumatic Bioreactor System and 500 L GE WAVE Bioreactor™). However, greater process predictability and robust scale translation methods must be ensured if laboratory scale single-use bioreactor systems are to be used as scale-down models at the development stage.

Mammalian cells, due to their capacity for assembly, correct protein folding and post-translational modifications, have become the dominant host cell type for the production of therapeutic proteins for clinical use in humans. Monoclonal antibodies in particular represent an important class of therapeutics whose benefits to patients have been recognised in the fields of oncology and immunology (Pavlou and Reichert, 2004; Reichert et al., 2005). Commercial production of these antibodies relies on the development of a robust large scale cultivation process step. While SUBs represent the cost-effective choice for cell cultivation, to date the ability to optimise and translate the process to larger scales has been rather limited. Rigorous fluid dynamics studies and the definition of appropriate scaling parameters in novel SUB systems are crucial to improve understanding of the effect of the hydrodynamic environment on cellular performance, and to ensure the same process and product characteristics are achieved at different scales in line with regulatory demands and Quality by Design approaches.

The importance of a well-mixed environment and the effect of operating conditions on cell growth and productivity have been widely documented (Abu-Reesh and Kargi, 1991). High agitation rates in a stirred tank bioreactor were found to significantly impact upon cell viability, glucose consumption rates and MAb production of hybridoma cells grown in 15% serum medium (Abu-Reesh and Kargi, 1991). A few studies have indicated that cells which are acclimated to high agitation rates are more resistant to the hydrodynamic stresses associated with the impeller rotation than those that are suddenly exposed to an increase in turbulence levels (Petersen et al., 1988; Schmid et al., 1992). In addition, cell physiology plays an important role as cells were found to respond differently to an increase in impeller agitation according to their growth stage (Petersen et al., 1990, 1988). The combination of air flow rate and increasing impeller rates of up to 300 rpm have been found to cause a decrease in stationary phase cell viability of TB/C3 hybridoma from over 95% to approximately 75% (Oh et al., 1989). In addition, Sorg et al. (2011) have demonstrated that the environmental heterogeneity of shear stress is as important as the mean stress that cells experience along their path. In their work, a Lobed Taylor-Couette bioreactor was used to simulate the hydrodynamic stress conditions occurring in the impeller zone of a stirred tank reactor, with estimated mean shear stress values of up to 0.4 Pa. An increase in lactate production and a decrease in antibody titre was observed, whilst consumption of the primary nutrients remained largely unchanged.

Although one of the major operational issues with regards to mammalian cell culture is the cellular response to shear forces (Abu-Reesh and Kargi, 1991; Cherry, 1993; Kretzmer and Schiigerl, 1991), it is generally noted that mammalian cells can physically tolerate the typical hydrodynamic stresses induced by the impeller within stirred tank bioreactors (Oh et al., 1989). The primary cause of damage has been attributed to interfacial shear and therefore to air bubble breakage and coalescence during culture (Ma et al., 2002). The aim for many of the novel mixing regimes designed into SUBs is to provide an environment that further enhances cellular productivity, whilst maintaining mixing performance for optimal cell growth.

The measurement of fluid dynamic characteristics in three-dimensional (3-D) turbulent flows is highly challenging, due to the space–time variation of turbulence levels and energy dissipation rates. The presence of a gas phase enhances the system complexity (Aubin et al., 2004). There have been a number of studies using laser-based techniques to obtain velocity field information within stirred tank reactors (Baldi et al., 2002; Deen and Hjertager, 2002; Ducci and Yianneskis, 2005; Gabriele et al., 2009; Hill et al., 2008; Pan et al., 2008 to name a few). Using both time-resolved and phase-resolved measurements, characteristics such as turbulent kinetic energy (TKE), shear stress and estimates of the rate of viscous dissipation of turbulent kinetic energy (ε) can be determined. However, two-dimensional (2-D) imaging techniques have inevitable resolution limitations depending on the camera field of view and lens properties, as well as dimensional restrictions, making assumptions necessary (Khan, 2005). Spatial fluctuating velocity gradients can be used to obtain an estimate of the rate of viscous dissipation of TKE (Hinze, 1975), however, consideration must be given to inaccuracies that arise from calculating such rates at length scales below the actual measurement resolution (Gabriele et al., 2009). It has been demonstrated that techniques such as the Smagorinsky Closure Method can be useful in estimating ε at such scales with a reasonable degree of accuracy (Meyers and Sagaut, 2006).

Despite the need for detailed information on velocity and mixing characteristics in single-use bioreactors, few studies have focused on the engineering characterisation of these novel devices (Löffelholz et al., 2013; Nienow et al., 2013a). Kaiser et al. (2011) used Computational Fluid Dynamic (CFD) approaches to determine engineering characteristics such as mixing time, power input and oxygen transfer within the 3 L Mobius® CellReady bioreactor. The marine impeller fitted within this bioreactor was found to produce an up-pumping circulation loop directed 25° above the horizontal plane, with the fluid velocity dominated by its radial component. In addition, gas distribution was found to be significantly heterogeneous, with flooding of the impeller observed up to an impeller rate of $N=200$ rpm ($Re=21{,}747$). A stagnant zone characterised by negligible average velocity values was also observed at the drain inlet, leading to cell sedimentation and accumulation in the region (Kaiser et al., 2011). The Euler–Euler approach was employed to simulate multiphase flow in the work of Kaiser et al. (2011). However, as a result of assuming uniform bubble size, a variation of approximately 40% was noted between CFD predictions and measured values of the gas liquid mass transfer coefficient in the bioreactor. While several fluid dynamic investigations have utilised Particle Image Velocimetry (PIV) approaches to obtain flow pattern information, velocity field and local energy dissipation rates in a stirred tank reactor geometry, in most cases results were obtained using a reactor mimic with a standard geometry configuration as opposed to an actual commercially available bioreactor. Furthermore, the impact of these experimentally obtained whole-field flow characteristics upon cellular behaviour has rarely been investigated at working conditions. This work aims to carry out a rigorous fluid dynamic study of the flow within a novel single-use bioreactor (3 L Mobius® CellReady) in order to improve understanding of its flow pattern, mixing efficiency and velocity characteristics. PIV experimentation was conducted using a model fluid made of water and 10 μm particles under un-gassed conditions, to prevent laser refraction caused by the gas phase within the liquid. Although this is not ideal, PIV studies have shown the