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Large-Eddy Simulations of microcarrier exposure to potentially damaging eddies inside mini-bioreactors

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ABSTRACT

Mechanically stirred vessels equipped with rotating impellers generate heterogeneous transitional or turbulent flows. However, some cells as animal or human mesenchymal stem cells (hMSC) adhered on microcarriers, are reputed sensitive to hydromechanical stresses arising from stirring. Many publications, especially using Computational Fluid Dynamics, characterize spatial fields of velocity and turbulence inside bioreactors but the exposure frequency to these stresses is never taken into account in the case of animal cell culture bioreactor description. To fill this gap, this study used both CFD Reynolds-Averaged and Large-Eddy Simulations to characterize the hydrodynamics inside 250 mL mini-bioreactors, which is a relevant volume for hMSC cultures. Five impeller geometries were studied. From the velocity and turbulence fields calculated, an energy dissipation/circulation function, related to both frequency and intensity of potentially damaging hydrodynamic events for the cells, was determined for various operating conditions. Based on the simulation results, the marine propeller operating in up-pumping mode seems to be the most adapted condition, since it exhibits a low frequency of exposure to an acceptable intensity of the turbulent dissipation rate. From a general point of view, the new methodology proposed should be used in the future to screen the most adapted bioreactor geometry to biological constraints.

1. Introduction

Industrial biotechnology widely uses stirred tank bioreactors (STR) to carry out microbial fermentations or animal cell cultures [1]. At first glance, STR designs might seem quite straightforward as mixing and consequently homogeneity (regarding substrates, temperature, or solid phase) is obtained *via* the pendulum rotation of one or several impellers. However, because of their turbulent properties, flow and mixing within an STR are actually more complex. Furthermore, these hydrodynamic stimuli interact with biological material inducing several mechanisms that have been only partially deciphered [2–4]. That is why, process design, especially the successful scale-up from bench to pilot plant, demands for a multidisciplinary approach, which includes engineering skills, to accurately describe the flow and turbulent mixing in a bioreactor, and biological skills to study the cell response to hydrodynamic stimuli. This paper only focuses on the engineering issues encountered in STR cell culture bioreactors.

In most industrial applications, fluid flows in STR are turbulent and may be described as an addition of a time-averaged component (*i.e.*, mean circulating flow) and a turbulent fluctuating component. The mean circulation flow includes several recirculation loops whose position, rotation axis and direction, depend on the impeller and bioreactor designs [5]. The turbulent flow is generally described using Kolmogorov's theory of isotropic turbulence [6] that suggests that the energy generated by impeller rotation is transmitted to the fluid by the generation of large eddies. Under the combined influence of inertial and friction forces, these large eddies are split into smaller ones to which they transfer energy at a constant rate ϵ. When the eddy size reaches a threshold value corresponding to the Kolmogorov scale, the viscous forces become predominant over the

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Notation

CFD	Computational Fluids Dynamics
EDC	Energy dissipation/circulation function
EE	Elephant Ear impeller
hMSC	Human Mesenchymal Stem Cells
LES	Large Eddy Simulation
MP	Marine Propeller
MRF	Multiple Reference Frame
μC	Microcarrier
RANS	Reynolds Averaged Navier Stokes
RT	Rushton Turbine
SM	Sliding Mesh
STR	Stirred tank bioreactor
$C_{\mu C}$	Microcarrier concentration ($g\,L^{-1}$)
$d_{EE,\,RT,\,MP}$	impeller diameter [m]
d_p	microcarrier diameter [m]
D	Bioreactor diameter [m]
$h_{EE,RT,MP}$	impeller height [m]
H_{liquid}	Liquid height in the bioreactor [m]
N	Impeller agitation rate [rpm or s^{-1}]
N_H	Minimal impeller agitation rate from which microcarrier cloud expands through all the liquid height [rpm or s^{-1}]
N_{js}	Minimal impeller agitation rate to keep microcarrier in just-suspension [rpm or s^{-1}]
N_P	Impeller power number [–]
N_{Qp}	Impeller pumping number [–]
P	Global power input [W]
Q_p	Pumping flowrate [$m^3\,s^{-1}$]
r	Radial coordinate [m]
Re	Impeller Reynolds number [–]
S_{ij}	Strain rate magnitude computed from the spatial derivative of the instantaneous resolved velocity fields [s^{-2}]
t_C	Liquid circulation time [s]
$t_{C,\,\mu C}$	Microcarrier circulation time [s]
$t_{C,\,\mu C}(\lambda_K < dp)$	Microcarrier circulation time in zones with $\lambda_K < d_p$ [s]
t_M	Mixing time [s]
$u(t)$	Instantaneous liquid velocity [$m\,s^{-1}$]
$\bar{u}$	Time averaged liquid velocity [$m\,s^{-1}$]
v_{tip}	Impeller tip speed [$m\,s^{-1}$]
$V_{\mu c}$	Liquid volume visited by the microcarrier cloud [m^3]
$V_{\mu C}\,(\lambda_K < d_p)$	Liquid volume of zones visited by microcarriers with $\lambda_K < d_p$ [m^3]
ϵ	Turbulent dissipation rate [$m^2\,s^{-3}$]
$\epsilon_{99-\mu C}$	Percentile 99 of the turbulent dissipation rate in zones visited by microcarriers [$m^2\,s^{-3}$]
$\langle\epsilon\rangle$	Volume average turbulent dissipation rate [$W\,kg^{-1}$]
λ_K	Kolmogorov scale [m]
μ	Fluid dynamic viscosity [Pa s]
υ	Fluid kinematic viscosity [$m^2\,s^{-1}$]
υ_T	Additional viscosity calculated by the subgrid scale model [$m^2\,s^{-1}$]
ρ	Fluid density [$kg\,m^{-3}$]

inertial forces. Energy is then entirely dispersed as thermal energy. With a kinematic viscosity υ, the Kolmogorov scale, λ_K is given by:

$$\lambda_K = \left(\frac{\upsilon^3}{\epsilon}\right)^{1/4} \quad (1)$$

Turbulent flow in a stirred tank bioreactor can thus be described, within each elementary volume of the fluid, as a superposition of recirculation loops with a wide range of eddies from large to small size. Computational fluid dynamics (CFD) tools allow the solving of Navier–Stokes equations, which govern flows, and thus allows the simulation of the complex flow for any bioreactor design [7–9]. Previous studies using this methodology have notably pointed out the heterogeneity of the spatial distribution of the turbulent dissipation rate ϵ, from high value areas around the impeller(s), to low value areas, for instance close to the free liquid surface [10,11]. Microbial or animal cells that follow the local flow in the bioreactor are hence submitted to a physical environment displaying rapid fluctuations.

Using CFD, our study applied such an engineering approach to the particular case of an animal cell culture bioreactor with liquid-solid suspension. The solid phase was composed of polymer microbeads, called microcarriers, while the liquid phase was the cell culture medium. This culture system is starting to be used for the cultivation of human mesenchymal stem cell (hMSC) attached to 3D surfaces as microcarriers [12–14]. Today, hMSC attract interest of the biopharmaceutical industry because of their abilities to differentiate *in vitro* into osteocytes, chondrocytes and adipocytes, and to act as “multi-drug vehicles” responding to a body given injury [15,16]. However, cell numbers required for treatments (10^5–10^9 hMSC/dose, [17,18]) are much higher than the quantities available from biopsies (1 hMSC among 10^4 marrow cells [17]). So, the *in vitro* hMSC expansion is required to provide sufficient amounts of biological material for therapeutic applications. However, the influence of mechanical stresses, induced by the fluid flow in STR bioreactors, on growth or multipotency of hMSC is still to be studied. To our knowledge, no extensive study focusing on the impact of mixing on hMSC growth has been undertaken, despite the fact that this impact might be most likely crucial for hMSC culture process development. Firstly, mechanical stresses are well-known to induce hMSC differentiation [19]. Secondly, impact of mechanical stresses on growth of continuous cell lines adhered on microcarriers, such as cells used for vaccine production, has already been proven [20–23]. According to Kolmogorov’s theory of isotropic turbulence, these mechanical stresses comes from energy transmitted on microcarrier surface by eddies whose size were similar to microcarrier diameter. At usual agitation rates, the existence of this kind of eddies in multiple areas of the bioreactor can be demonstrated if local values of Kolmogorov scales are computed using Eq. (1) with local values of ϵ. Croughan et al. [24] also established a cell growth kinetic model for FS-4 cells cultured on Cytodex 1™ microcarriers in spinner flasks:

$$\frac{dC}{dt} = \mu C \lambda_K > L_C \quad (2)$$

$$\frac{dC}{dt} = \mu C - q_1 C \lambda_K \le L_C \quad (3)$$

$$q_1 = K_e\left(\frac{\epsilon}{\upsilon^3}\right)^{3/4} \quad (4)$$

where C is the viable cell density, μ is the specific cell growth rate and $q1$ is the specific cell death rate due to mechanical stresses. According to their model, the specific death rate is proportional to the volume of Kolmogorov eddies λ_k^3. As no other flow characterization tools were available at that time, this volume was only based on the spatial-averaged turbulent dissipation rate $\langle\epsilon\rangle$ resulting from the global power dissipation equation $P = \langle\epsilon\rangle/(\rho V)$. The parameters K_e and L_c are determined by fitting with the cell growth curves obtained at different agitation rates. The former, K_e, is a constant dependent on the reactor geometry and the second, L_c, is a threshold value of the mean Kolmogorov scale (Eq. (1)) under which a decrease of the relative specific growth rate can be