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Adaptation for survival: Phenotype and transcriptome response of CHO cells to elevated stress induced by agitation and sparging

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ABSTRACT

In this work, the response and adaption of CHO cells to hydrodynamic stress in laboratory scale bioreactors originating from agitation, sparging and their combination is studied experimentally. First, the maximum hydrodynamic stress, τ_{max}, is characterized over a broad range of operating conditions using a shear sensitive particulate system. Separate stress regimes are determined, where τ_{max} is controlled either by sparging, agitation, or their combination. Such conditions are consequently applied during cultivations of an industrial CHO cell line to determine the cellular responses to corresponding stresses. Our results suggest that the studied CHO cell line has different threshold values and response mechanisms for hydrodynamic stress resulting from agitation or sparging, respectively. For agitation, a characteristic local minimum in viability was found after stress induction followed by viability recovery, while at highest sparging stress a monotonic decrease in viability was observed. If both stresses were combined, also both characteristic stress responses could be observed, amplifying each other. On the other hand, cellular metabolism, productivity and product quality did not change significantly. Transcriptome analysis using mRNA microarrays confirmed that separate adaptation mechanisms are activated in the different stress situations studied, allowing identification of these stresses using a transcriptome fingerprinting approach. Functional analysis of the transcripts was consequently used to improve our understanding of the molecular mechanisms of shear stress response and adaptation.

1. Introduction

Both sparging and stirring are commonly used in bioreactors during the cultivation of mammalian cells (Chalmers, 2000; Nienow, 2006). While stirring is used to provide homogenization and mass transfer, sparging is crucial for oxygenation of the cultivation broth. Despite a large effort of researchers to study the effect of hydrodynamic stress on cell cultures (Chisti, 2000, 2001; Hu et al., 2011), no generally accepted method for hydrodynamic stress quantification in complex turbulent multiphase flows occurring during cell cultivation has been proposed (Hu et al., 2011; Nienow, 2010; Nienow et al., 2013). Due to this lack of proper characterization, mostly ad hoc studies of the hydrodynamic stress originating from combination of sparging and stirring on cell behavior are present in the literature (Kunas and Papoutsakis, 1990; Oh et al., 1989, 1992).

To avoid the problem of characterizing the maximum hydrodynamic stress, τ_{max}, in turbulent mammalian cell culture bioreactors, most studies were performed in laminar flow model systems without the presence of bubbles (Abu-Reesh and Kargi, 1989; Augenstein et al., 1971; Godoy-Silva et al., 2009a,b; Gregoriades et al., 2000; Keane et al., 2002; Ma et al., 2002; Mollet et al., 2007; Tanzeglock et al., 2009; Trinh et al., 1994). Depending whether lethal or sub-lethal responses are considered, reported threshold values for mammalian cells growing in suspension cover a wide range from 8 to 450 Pa varying substantially in between various authors (Hu et al., 2011). Furthermore, as shown by (Tanzeglock et al., 2009), the flow type has a major impact on the measured threshold value indicating limited predictive power of such devices. In this respect, when attempting to analyze the cellular response to the hydrodynamic stress, devices operated under turbulent conditions which are comparable to those found in bioreactors during cell cultivation should be applied (Li et al., 2006;

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Nienow et al., 2013; Sieck et al., 2013; Sorg et al., 2011). Depending on the cell line used and the applied level of hydrodynamic stress, different results are reported in the literature. It seems that if stresses lower that the cell line specific threshold value are applied, no effect on cellular metabolism or product quality are observed (Nienow et al., 2013). On the other hand, when exceeding the cell specific threshold value several negative responses have been reported in the literature including slower growth, change of metabolism, reduced specific productivity up to modification of the mAb glycosylation (Godoy-Silva et al., 2009a,b; Sieck et al., 2013). Furthermore, this effect could become even stronger when applying oscillating stress values simulating large scale bioreactors (Sieck et al., 2013). However, in all these studies only stirring was considered.

The presence of bubbles required to provide oxygen for the cells and to remove carbon dioxide constitutes an additional source of the hydrodynamic stress (Chisti, 2000). In particular, as reported by Boulton-Stone and Blake (1993); Dey and Emery (1999); Garcia-Briones et al. (1994), bubble burst was identified as one possible mechanism where cells are exposed to hydrodynamic stress of very high magnitudes. Furthermore, as reported by Kioukia et al., 1996; Kunas and Papoutsakis (1990); Oh et al. (1989, 1992), bubble breakup and rise through the culture broth are additional phenomena which can impact cell growth and productivity. Even though the addition of Pluronic F-68 is commonly used to prevent cell damage by bubble burst (Ma et al., 2004; Trinh et al., 1994), it remains unclear by which mechanism Pluronic protects the cells (Gigout et al., 2008). Recent work by Liu et al. (2014) as well as Villiger et al. (2014) suggest that the gas jet at the sparger exit may have been underestimated to date and could be potential source of high stresses leading to cell damage in sparged bioreactors operated under turbulent conditions.

Furthermore, there is lack of understanding how mammalian cells respond to hydrodynamic stress of various kinds in terms of the underlying molecular mechanisms of stress response and cells' adaptation (Hu et al., 2011). Transcriptome microarrays can be used to detect gene-phenotype relationships (Jayapal et al., 2007) or as analytic tools to detect fingerprints, e.g. for scale-down model validation (Jayapal and Goudar, 2014). In our previous work, we found that for relatively weak macroscopic stress responses, we could identify a set of differentially regulated genes (Sieck et al., 2013), indicating that transcriptome analysis is a highly sensitive tool to detect and analyze stress responses and clarify adaptation mechanisms in mammalian cells (Griffin et al., 2007).

To further test this methodology, in this work we investigate the effect of hydrodynamic stress originating from sparging, stirring and their combination, on a CHO cell line. In the presented work the maximum local hydrodynamic stress, $\tau_{\max}$ present under a wide range of cultivation conditions was characterized by a novel shear sensitive system (Sieck, 2012; Villiger et al., 2014), taking into account the heterogeneous distribution of energy dissipation rate, and therefore also the corresponding hydrodynamic stress $\tau_{\max}$, in stirred tanks (Derksen and Van den Akker, 1999; Geisler, 1991; Pilz and Hempel, 2005; Sieck, 2012; Soos et al., 2013). Applying this characterization system, two regimes were identified where $\tau_{\max}$ was dominated either by agitation or sparging. Consequently, different values of $\tau_{\max}$ covering both identified regimes were used to investigate the effect of hydrodynamic stress on an industrial CHO cell line in terms of viable cell density, viability, characteristic metabolic rates, specific antibody productivity and product quality. Furthermore, the cells' stress adaptation mechanisms were analyzed using mRNA microarrays to identify characteristic transcriptome fingerprints. These were consequently used to connect the transcripts to known genes and possible related molecular functions.

2. Material and methods

2.1. Cell culture

The CHO cell line used in this work is stably transfected to produce monoclonal antibodies. Prior to bioreactor cultivation, cells were thawed from cell bank vials, transferred to shake flasks and expanded in chemically defined expansion medium. The expansion consisted of at least 4 sub-cultivation steps. Bioreactors were inoculated to a cell density of $0.5 \pm 0.1 \times 10^6$ cells/mL by adding the appropriate amount of expansion culture directly to the chemically defined, proprietary production medium, which contained 1 g/L of Pluronic F-68.

2.2. Cell culture bioreactors

Glass bioreactors of 4 L working volume connected to ez-Controllers (Applikon Biotechnology, Schiedam/NL) and instrumented with dissolved oxygen (DO), pH and temperature sensors were used in this work. A single standard down pumping Applikon elephant ear (DPEE) impeller with a diameter D of 60 mm was attached to the central shaft and located one impeller diameter above the bottom of the bioreactor. This setup was chosen because it allows high agitation rates without vortex formation, compared to a 2 impeller setup. DO was controlled by sparging a constant flow of air coupled with addition of pure oxygen on demand. The air flow rate was adjusted to generate certain values of sparging stress during cultivation; low flow rates were applied when the stress magnitude was dominated by agitation and high ones when $\tau_{\max}$ was controlled by sparging. Since the increase of air flow has a relatively small impact on oxygen transfer rate (OTR), compared to pure oxygen sparging, the required oxygen flow was comparable in all sparging stress cultivations. Examples of total gas flow rate profiles for several cultivations are presented in Figure SM 1 of the Supplementary Material. The pH was controlled by adding carbon dioxide to the gas flow or adding 0.5N NaOH by a dip tube. Feeds were dosed by peristaltic pumps and also added via dip tube.

The volume averaged energy dissipation rate introduced into the bioreactor by the impeller was characterized according to Nienow (1998):

$$\langle \varepsilon \rangle = \frac{P}{\rho_L V_L} = \frac{2\pi M N}{\rho_L V_L} \qquad (1)$$

where P refers to power input, N the applied stirring speed, ρ_L the fluid density and V_L is the liquid volume of the reactor. The torque M applied on the impeller shaft was measured using a Burster 8661 torquemeter (MTS, Switzerland). From the measured power input values, the Power number $Po = P/\rho_L N^3 D^5$ could be obtained. For a single DPEE impeller, Po was equal to 1.6 which is similar to the value of 1.7 reported by Simmons et al. (2007). It is worth noting that, in the range of applied gas flow and agitation rates, no influence of the sparging rate on the measured torque was observed (see Figure SM 2 in Supplementary Information). Consequently, the volume averaged shear rate, $\left\langle \gamma \right\rangle$, and the corresponding average hydrodynamic stress, $\langle \tau \rangle$, introduced into the bioreactor by agitation were evaluated as follows:

$$\begin{aligned} \left\langle \gamma \right\rangle &= \sqrt{\langle \varepsilon \rangle \rho_L / \mu_L} \\ \langle \tau \rangle &= \left\langle \gamma \right\rangle \mu_L \end{aligned} \qquad (2)$$

where μ_L is the fluid dynamic viscosity. For all investigated conditions the impeller Reynolds number, $Re_{\mathrm{imp}} = \rho_L N D^2/\mu_L$, was larger than 10^4, indicating turbulent conditions.

To quantify the maximum hydrodynamic stress present in the bioreactor under various cultivation conditions in the presence of a surfactant, we applied a shear sensitive particle system developed