LEVERAGING A DEEPER UNDERSTANDING OF POLOXAMER188 TO IMPROVE CELL CULTURE PROCESSES

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The industry-wide use of Poloxamer188 (P188) underwent severe scrutiny as a result of lot variability discovered within the past few years. While screening methods have been developed to ensure lot consistency and the root cause of the variability has likely been identified, a fundamental understanding of surfactant-cell interactions has not yet been achieved. As industry continues to push culture densities higher to maximize product yield, higher aeration and agitation are required to supply sufficient oxygen transfer rate. The harsher environment in the bioreactor, depletion of shear protectants, and possible cell physiology change leads to the need of improved shear protection strategies to minimize shear damage in the cell culture process. In this project, novel concentric cylinder mixer (CCM) assay was developed to quantify the relative shear sensitivity of mAb producing Chinese Hamster Ovary (CHO) cell lines in production bioreactors. Compared with other methods to characterize shear sensitivity, the CCM assay requires low sample volume and minimal processing time. Various concentrations of P188 were evaluated using CCM assay to improve shear protection strategies in 3L and 300L bioreactors. Results indicate that cell shear sensitivity dramatically increases upon reaching the cell culture stationary phase, coinciding with viability decline and exponential LDH increase in the bioreactor. With a simple shift in shear protectant concentration, we were able to increase harvest viability resulting in decreased cellular debris, decreased foam stability, and reduction in LDH upon harvest. A strong dose dependent correlation between membrane rigidity and surfactant concentration was also discovered through the studies which provided possible mechanism of how surfactant reinforces cell membrane by decreasing membrane fluidity. The knowledge of cell membrane fluidity combined with the CCM assay contributes to our understanding of cell shear sensitivity and surfactant-cell interactions. These tools can be used to optimize process parameter set points, evaluate media formulation effects on cell sensitivity, and select shear resistant cell lines to improve cell culture process robustness.