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Investigating Bioprocessing Variables That Influence Antibody Stability During Therapeutic IgG Production

Stephanie Davies

Mark Smales; Shahid Uddin[*]

University of Kent, Research School of Biosciences and The Centre for Molecular Processing, Canterbury, Kent, CT2 7NJ UK

[*] MedImmune, Formulation Sciences, AKB Building, Granta Park, Cambridge, CB21 6GH UK

The presence of particles and aggregates in biotherapeutic products is a concern for the manufacturers of such biologics due to the potentially associated immunogenic responses observed in some patients. In addition to this, the presence of particles and aggregates may result in a decrease of the efficacy and potency of the drug formulation. In this work we have investigated the upstream and downstream bioprocess conditions that may induce or reduce/eliminate IgG antibody particle formation when produced in cultured Chinese hamster ovary (CHO) cells.

Initially we have investigated the influence of different cell culture conditions on subsequent antibody particle and aggregate formation. Two variables were investigated, the time of culture harvest and batch vs. fed-batch cultures. Particle analysis results from stability studies on antibody material generated under these different conditions show a higher number of particles in antibody formulations when the antibody material was produced from CHO batch cultures as opposed to fed batch cultures. There was no observable significant difference in the particle numbers observed in antibody samples at different culture days from fed-batch cultures. We further extended the study to investigate the intracellular ‘state’ of the cells throughout culture in order to determine if the intracellular state and stress levels of cultured CHO cells reflected the amount of particles present in the purified material. Using a qRT-PCR approach, we have identified a number of genes whose expression is either up or down-regulated between harvest days and between batch vs. fed-batch cultures. These stress related genes indicate the perception of stress in CHO cells later in culture and appear to relate to the relative particle numbers ultimately observed in the purified antibody material. We are now determining whether these genes can be used as biomarkers to predict the likelihood or relative levels of particle formation from a culture.

We have also investigated downstream bioprocessing steps and the influence on particle formation, specifically washing steps during Protein A purification. We have shown

that the incorporation of an additional wash step during a Protein A purification process significantly reduces the number of particles detected in antibody formulations after a three month stability study. The ‘spiking’ of this wash step back into purified antibody appears to accelerate the formation of particles, suggesting that components present within the wash step actively promote particle and aggregate formation. We also observe different antibody SEC-HPLC profiles between those molecules purified with the wash step compared to those that were not. We are now in the process of analysing this wash step fraction using western blotting and MALDI TOF/TOF mass spectroscopy methods in order to determine what culture components or product impurities are being removed. Interestingly, we have already confirmed that one of the components of this wash step is one of those genes identified as being up-regulated during culture in our qRT-PCR screen described above. Finally, we have also utilised an established microscopy technique; Atomic Force Microscopy (AFM) in order to investigate and determine the most likely mechanism for antibody particle formation.