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# BIOREACTOR PERFUSION VIA SINGLE-USE CENTRIFUGATION HAS FEWER PRODUCT QUALITY IMPLICATIONS COMPARED TO TANGENTIAL FLOW FILTRATION

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During development of a perfusion cell culture process for the production of a therapeutic protein, tangential flow filtration (TFF) technology was evaluated for cell retention in addition to Shire's platform single-use centrifugation technology. Unlike centrifugation TFF is based on a microfiltration membrane and thus has the potential to partially retain many biological compounds, especially when exposed to extracellular matrix proteins and antifoam emulsions (Routledge, 2012; Wu et al., 2011). Retention increases the mean residence time of product at process temperature. In the case of heat-labile molecules longer exposure to bioreactor culture temperature may correlate with changes in quality attributes.

Therapeutic protein was generated from bioreactors equipped with single-use centrifuge and TFF technology for cell retention. Peak viable cell density was slightly higher using TFF, due to the moderate cell loss of centrifugation, but viability by trypan blue exclusion was slightly lower. Metabolic profiles (glucose, lactate, ammonium, glutamate) were not affected by the choice of cell retention technology. Cell specific productivity was similar for both cell retention devices; however, TFF membranes had to be changed periodically to reduce protein retention and thereby achieve the volumetric productivity of the single-use centrifuge. All measured product quality attributes, for both intermediates and drug substance (DS), were comparable between TFF and single-use centrifugation. Nevertheless, drug substance generated from bioreactors equipped with TFF had significantly decreased room-temperature stability compared to DS generated from bioreactors equipped with single-use centrifuges. DS instability of TFF lots, indicated by an increased propensity to generate low molecular weight species (LMW), was more pronounced in later harvests compared to early harvests. Analysis (HPLC-MS & peptide mapping) of degraded drug substance found molecular fragments that corresponded to the subunits of the therapeutic protein. Cleavage occurred at several sites close to the linkage molecules bridging the protein subunits. A literature search for compounds that target the cleavage sites identified metalloproteases and serine proteases as likely agonists for the observed cleavages and serine proteases were detected in a proteomics analysis of both TFF and single-use centrifuge material.

While DS lot stability suffered with TFF the stability of process intermediates was found to be similar between TFF and centrifuge lots. If proteases are responsible for the observed LMW generation said proteases might have greater activity when concentrated and/or when inhibitory compounds are removed during purification. It is plausible that TFF may have contributed to either LMW generation in the cell culture or retention of a protectant moiety. Additional study is necessary to confidently posit a root cause.

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