ACOUSTIC WAVE SEPARATION – A NON-FILTRATION APPROACH FOR CONTINUOUS CLARIFICATION OF PERFUSION CELL CULTURE PRIOR TO CAPTURE CHROMATOGRAPHY

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Advances in perfusion cell culture have led to cell densities in excess of 100 million cells/mL with product titers similar to those obtained in fed batch (3-5 g/L). This performance has necessitated improvements in the yield and efficiency of the cell harvest and clarification stage to generate a stream of Harvested Cell Culture Fluid (HCCF) for capture chromatography and subsequent downstream processing. This is further driven by the evolution of continuous processes where there is a preference for a continuous feed of HCCF available for direct load to the continuous multicolumn capture chromatography step.

In the present work we report on a novel disruptive and scalable single-use technology for cell retention during perfusion cell culture based on an acoustophoretic separation. Acoustic Wave Separation (AWS) technology exploits the use of low frequency acoustic forces to generate a three-dimensional standing wave across a flow channel. Recirculating cell culture from a perfusion bioreactor enters the flow channel and passes below the acoustic zone. The product-containing stream of HCCF is removed from the recirculating cell culture by passage through the acoustic zone. This yields a well clarified HCCF that can be polished using a small area filter.

We report the continuous cell retention during a perfusion culture of a CHO cell line expressing a mAb. At process development (PD) scale we demonstrate the ability to continuously process CHO cell culture and retain cells at densities of up to 100 million cells/mL, at flow rates of up to 2 bioreactor volumes per day. Since the clarification technology does not involve the use of hollow fiber tangential flow filtration (TFF) we ensure 100% transmission of the mAb through the AWS device. The closed system remains operational for up to 60 days enabling this scalable technology to be suitable for use in clinical manufacture. The post-AWS HCCF is 99% clarified and any residual cellular material can be removed using a small gamma stable membrane filter or directly loaded onto a 0.2 micron filter prior to chromatography. Additionally, no demonstrable adverse effects have been identified for the quality of the HCCF, the product itself, or the viability of the returning perfusion cell culture following cell retention using AWS technology.

AWS technology enables the continuous cell retention from recirculating cell culture withdrawn from perfusion bioreactors in a single-use operation. AWS technology has been shown to perform well at cell densities of up to 100 million cells/mL, so is well positioned to meet the cell retention requirements of emerging higher cell density perfusion processes that are gaining momentum in the biotech space. This novel cell retention approach offers economic benefits in terms of yield improvement as well as eliminating the hollow fiber TFF operation. This offers the advantage of a stable mAb concentration in the HCCF stream during the perfusion process. This facilitates improved process control since the volume of HCCF to load on to the capture columns remains constant which is especially important during continuous multicolumn chromatography. By comparison with hollow fiber TFF, the mAb concentration varies during the cell retention process making an integrated process more complex to control.