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Integration of Centrifuges with Depth Filtration for Optimized Cell Culture Fluid Clarification Processes

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One of the biggest challenges in the production of recombinant therapeutic proteins, monoclonal antibodies, and vaccines is the clarification and separation of the product (typically a protein) from the cell culture or fermentation broth. The desired product is present in low concentrations and must be efficiently separated from the other components present in the bioreactor fluid. An overall objective in developing a clarification process is to achieve the highest level of product recovery (yield) and contaminant removal with the fewest number of unit processes. Understanding how each operational step affects the performance of the next step downstream is the challenge at hand.

Centrifugation, in combination with depth filtration, is gaining acceptance as the preferred method for the removal of cells, cell debris, colloids, insoluble precipitants, aggregates, and other materials found in mammalian cell culture and bacterial fermentation fluids. After depth filtration, additional clarification and purification steps including sterilizing-grade filtration, will take place before the product final fill and finish operations.

Cell Culture Fluid Characteristics

In today's bioreactor-based processes, increased cell concentrations and longer culture times result in higher product titers. However, these bioreactor conditions also reduce cell viability, increase cell debris, and raise concentrations of organic constituents in the bioreactor fluid. A larger amount of particles and colloids is generated, especially small sub-micron size entities that will readily plug downstream membrane filters or foul ultrafiltration (UF) systems. A properly designed post-bioreactor clarification process will not only remove the cells, cell debris, and other larger particles, but also these smaller entities.

The characteristics of cell culture fluids vary according to the type of cell expression system and the type of bioreactor used. Fluids in fed-batch and perfusion cultures show lower cell viability, higher cell densities, higher turbidity, and greater concentrations of colloids and polynucleic acids than traditional shorter-lasting batch cultures. These types of bioreactor fluids present new challenges for clarification and secondary filtration that can be addressed by filtration and mechanical separation technologies.

A series of process steps may be necessary to achieve adequate clarified fluid quality before it enters the membrane filter downstream:

- Fed-batch and perfusion culture clarifications typically require two steps (primary and secondary clarification), and sometimes up to four
- Conversely, straight batch cultures can often be processed through a single clarification step, because these fluids are essentially composed of whole cells surrounded by a clear fluid (i.e., no or few colloids)

Primary Clarification with Centrifugation

The primary step of the recovery process should be designed to remove the bulk of large particles, whole cells, and cell debris. This step must also minimize additional cell lysis before and during separation to avoid release of unwanted soluble proteins from whole cells. Primary recovery goals include achieving maximum yields, product consistency, process scalability, adequate product concentration, adequate product stability, cost of production targets, and compliance with current regulatory guidelines.

The primary recovery step is commonly performed by centrifugation due to cost and space advantages over other methods. Centrifugation has proven effective at minimizing shear damage to cells while achieving the primary recovery goals. As shown in the equation, the separation efficiency of solid particles within a centrifugal field is a function of the particle size distribution, the density difference and the suspension viscosity (Fig. 1).

Centrifugation for primary recovery is typically able to handle relatively high concentrations of insoluble material in the feed, but has limitations in its abil-

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ity to remove all the particles below a certain size at a practical flow rate. This means that some form of secondary clarification will be needed to remove the remaining particulates before final purification. Higher feed rates result in higher product turbidity and lower particle capture due to lower retention time within the centrifugal field.

Centrifuges can generally concentrate the cell mass to > 60 percent by volume and offer flexibility because the major optimization parameters, flow rate and rotor speed, can be altered without changing hardware. Recent improvements to the acceleration zone of newer centrifuges have reduced the mechanical stress to the cells and the subsequent release of cell fragments and intercellular release of materials associated with cell lysis.

$$V_g = \frac{d2(\rho_p - \rho_1)}{18\eta}$$

V_g = gravitational settling velocity
d = particle diameter (m)
ρ_p = particle density (kg/m^3)
ρ_1 = liquid phase density (kg/m^3)
η = liquid phase viscosity (kg/ms)

Figure 1. Stokes Law Equation

High-speed disc stack centrifuges are most commonly applied in the removal of cells and cell debris. What makes disc centrifuges unique is the way they combine the benefits of high G-force and surface area to enable high-throughput fine particle separation. Within the field of biotechnology, disc centrifuges are used to separate insoluble solids from liquid in intra- and extra-cellular processes. In the mammalian cell culture, they are ideally suited to clarifying the feed immediately out of the bioreactor.

In a gravitational field, density differential and fluid viscosity combine to determine the settling rate. In a centrifuge, the centrifugal force created by the rotating bowl increases the settling rate by 5,000–15,000 times. This benefit is enhanced by the design of the rotating bowl and the vast surface area provided by the disc stack (Fig. 2).

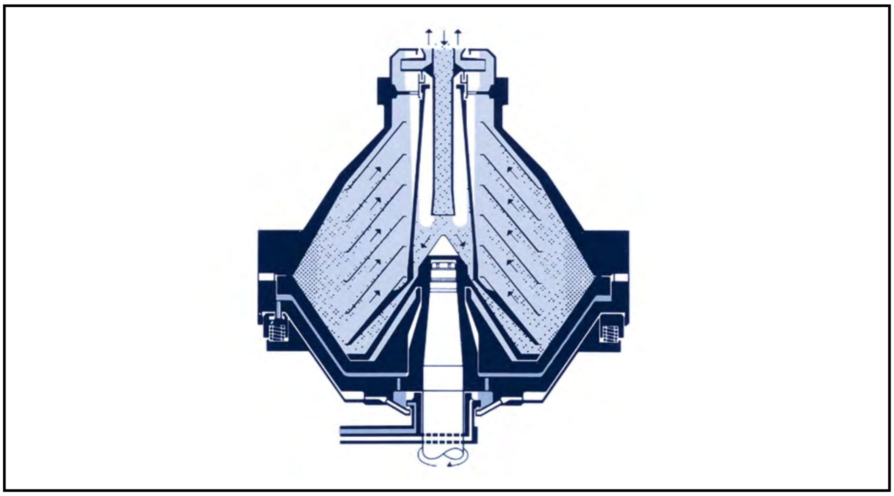

Figure 2. Disc Stack Centrifuge

Disc centrifuges come in many sizes and can satisfy the demands of processes that range from 50–25,000 L. The feed to the centrifuge does not have to be interrupted, making disc centrifuges continuous. They produce two distinct phases, clear centrate and concentrated solids. Disc centrifuges can be cleaned in place, steam sterilized in place, and installed in a manner consistent with bio safety levels 1 and 2 (BL-1 and BL-2) guidelines.

Secondary Clarification with Depth Filtration

Centrifugation effectively removes the bulk of large solids, whole cells, and debris, however the centrate effluent will still contain some amounts of smaller sized contaminants (e.g., aggregates and colloids). Filtration is often required as a secondary clarification or polishing step to remove contaminants, and prevent plugging of downstream processes such as membrane filters and ultrafiltration systems.

Charged depth filters (sometimes called pads) are particularly well suited for this polishing stage, due to their ability to retain a large amount of contaminants using two major types of interactions between filters and contaminant particles:

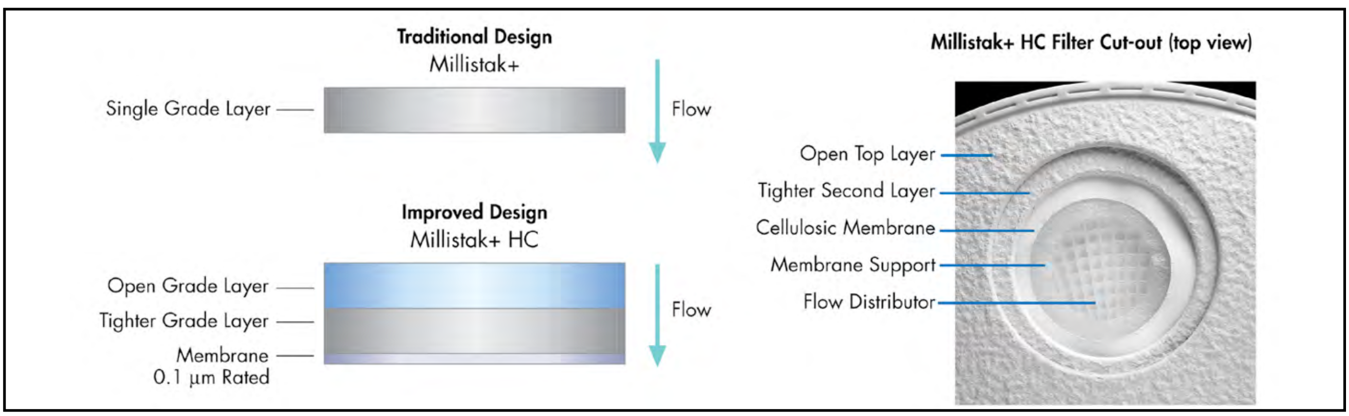

Figure 3. Traditional Single-layer Depth Filter and Millistak+ HC Construction

- Size exclusion—particles are retained if they are too large to penetrate or go through the entire filter thickness.

- Adsorption—molecular and/or electrical forces between the particles and the filter material will cause an attraction and retention of these entities within the filter. This characteristic represents an important benefit in the removal of process contaminants such as DNA/RNA, CHO cell proteins, and lipids, which are not removed by size exclusion and can contribute to significant downstream process fouling (e.g., UF, chromatography media).

Built in a lenticular cartridge format for most biotech applications, the filter material is typically composed of a thick and fibrous cellulosic structure with inorganic filter aids such as diatomaceous earth (DE) particles embedded in the openings of the fibers. This construction results in a large internal surface area, which is key to particle capture and filter capacity based on the two retention mechanisms previously described. The filter's electrostatic (positive) charge also plays a role in the attraction of the contaminants to the filter material, and is carried by the organic resin used to bind the DE particles to the cellulosic fibers.

In the past, cell culture clarification processes have been designed with a succession of two or more depth filter stages either directly post-bioreactor or post-centrifuges, in order to retain the variety of particles and colloids found in those fluids. Recently, higher performing depth filters have been developed to allow the elimination of some of these stages down to only one post centrifugation. These more advanced filters are built with a graded density structure (two layers within one filter, more open upstream, and tighter downstream) to remove a wider range of particle sizes in larger amounts. Millipore's Millistak+® HC products improve filtration by the addition of a 0.1-µm rated cellulosic membrane as a final downstream layer. Figure 3 illustrates the difference in con-

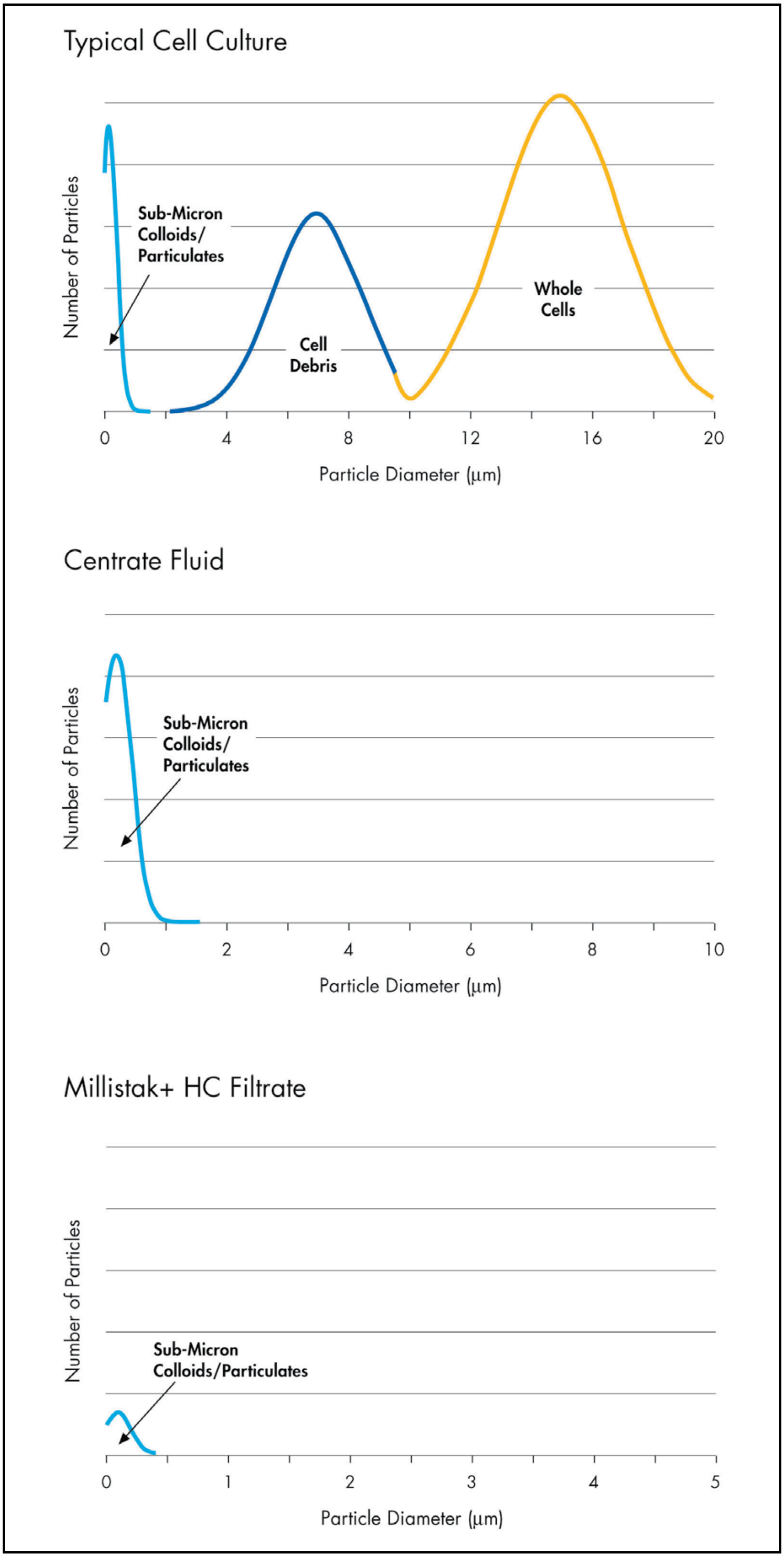

Figure 4. Typical Particle Size Distribution

struction between a traditional single-layer depth filter and the Millistak+HC multi-layer high capacity filter.

Millistak+ HC filters provide the following benefits:

- Thicker filter material with two full-size layers instead of one. More depth available for particle capture
- Better flow distribution and media utilization due to the back pressure provided by the membrane throughout the two top layers
- Higher filtrate quality due to the membrane layer

The multi-media, multilayer design minimizes the number of unit operations while making more efficient use of the filter media through improved flow distribution. Benefits of process compression:

- Process cost savings (20–50%)
- Higher product yield
- Fewer prefilters
- Fewer downstream membrane filters
- Less labor and water for injection (WFI)
- Simpler process
- Fewer filter housings
- 50% smaller footprint

Millistak+ HC depth filters are available in a variety of sizes to meet a wide range of bioreactor capacities from 1 liter to more than 15,000 liters. Typically, small-scale filters used for preclinical and clinical trials are completely disposable, while large-scale process filters utilize reusable, cleanable stainless steel housings.

While developing the secondary clarification process with charged depth filters, particular attention should be paid to product yield. In some cases, the product (therapeutic protein) can get adsorbed by the filters along with the contaminants, and therefore yield can be adversely affected. Proper buffer preconditioning will help prevent product absorption which remains infrequent, but still needs to be taken into consideration. A well-designed secondary clarification step using charged depth filters achieves a very high and consistent filtrate quality, and a simpler, more streamlined process.

Conclusion

High-performance and economical cell culture clarification can be achieved by combining centrifugation and depth filtration technologies. The unique characteristics of these two separation technologies enhanced by recent product improvements provide optimized contaminant removal as illustrated in the particle size distribution graphs (Fig. 4).

The key process benefits of this optimization are:

- Fast processing of large volumes at a minimal cost
- Consistent clarified fluid quality, regardless of bioreactor fluid variability

Combining both depth filtration and centrifugation can help process development engineers optimize their processes and facilitate scale-up. This provides long-term benefits for biopharmaceutical manufacturers including:

- Increased speed-to-market,
- Enhanced product yield,
- More robust manufacturing process, and
- Minimized production costs.