

Introduction

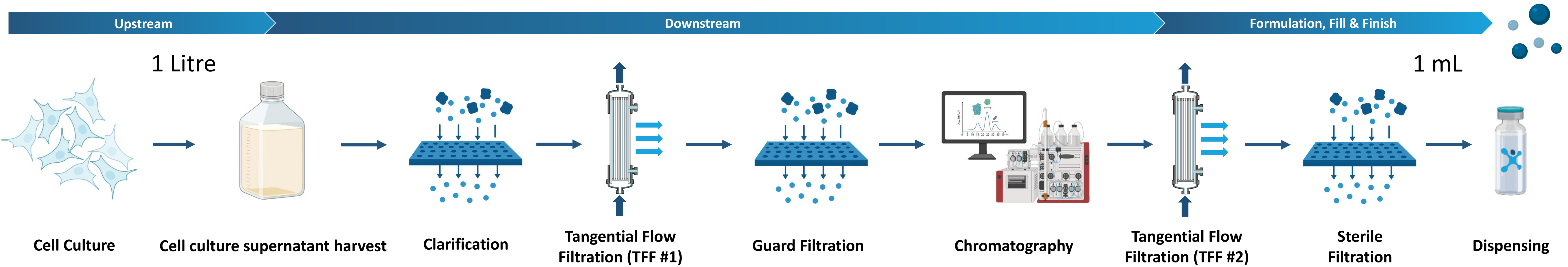
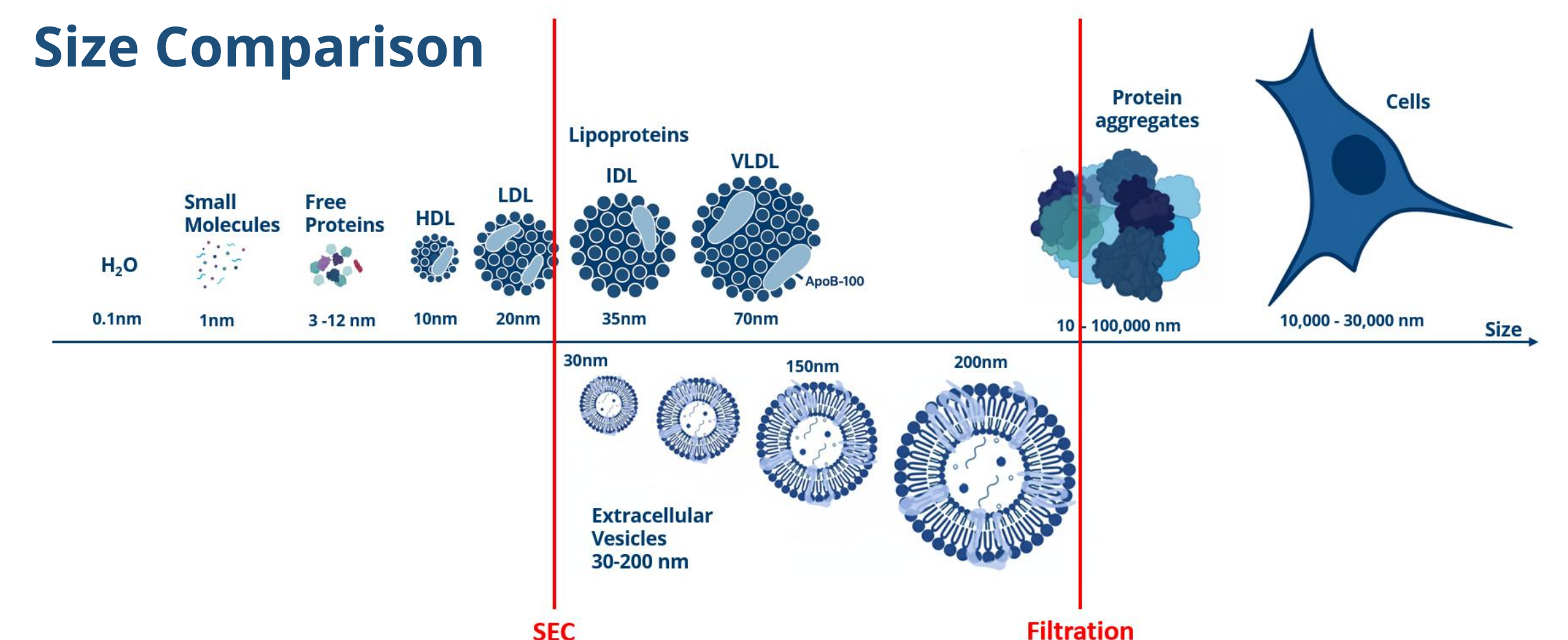
The efficient purification of extracellular vesicles (EVs) from conditioned cell culture supernatant is critical for their therapeutic application. Various scalable purification technologies are employed, including size exclusion chromatography (SEC), ion exchange chromatography (IEX), and affinity chromatography. Among these, SEC has emerged as a popular and widely adopted method for EV isolation due to its gentle nature, which ensures product integrity.

However, SEC purifies all particles above a specific size threshold. Conditioned cell culture supernatant contains a heterogeneous mix of components, including cell debris, protein aggregates, and other particles that are significantly larger than EVs. These larger contaminants can co-elute with EVs or foul chromatography columns, compromising purity and yield. Furthermore, cell fragments and cells that are not removed at the beginning of the process, will be disrupted throughout the process and for EV mimetics (EVMs) which are co-purified with the EVs. Therefore, initial clarification and filtration steps are indispensable in the EV manufacturing process. These upstream unit operations effectively remove bulk cellular material and larger debris, ensuring a cleaner starting material for downstream purification techniques like tangential flow filtration (TFF) and SEC and ultimately improving the overall efficiency and purity of the downstream process.

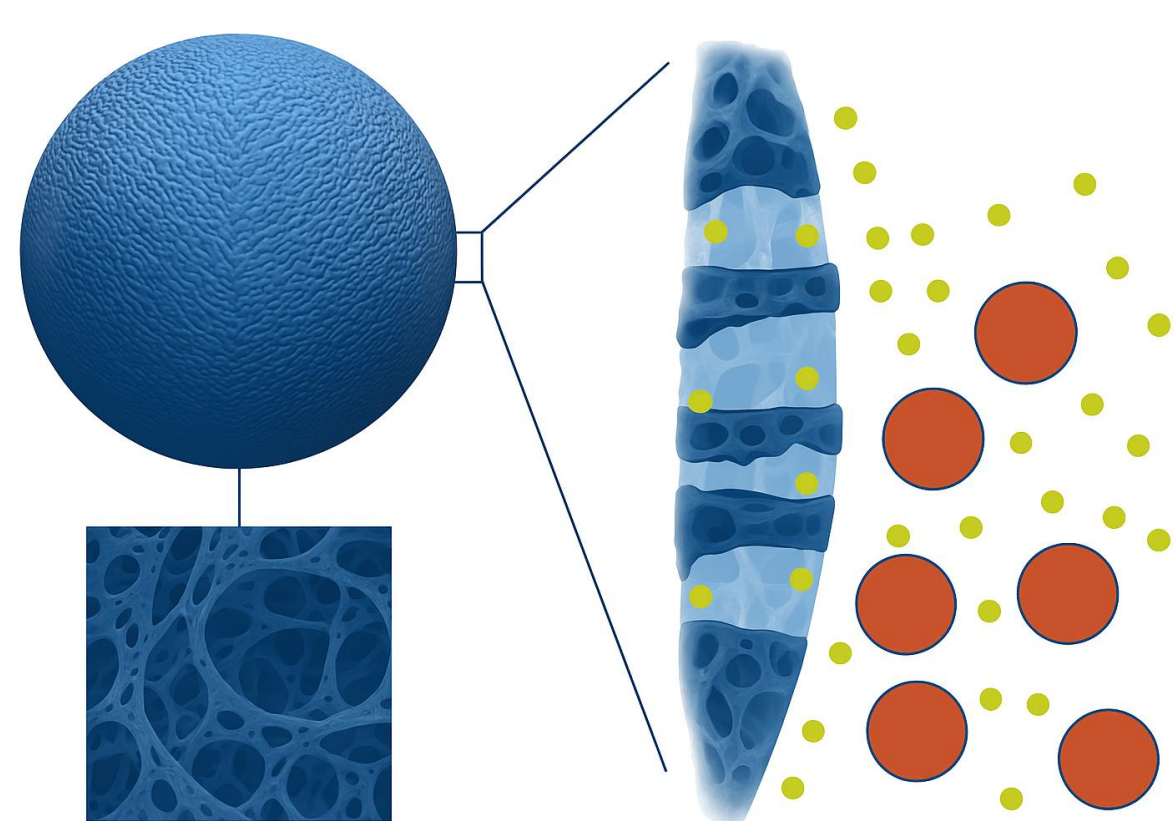
Key process improvements of initial filtration

- Removal of cells, cell fragments, and large protein aggregates
- Prevention of EV mimetic (EVMs) formation, which are particles created through disruption of cells in the downstream process
- Enhanced particle recovery in the subsequent downstream process
- Improved process consistency in the downstream process
- Improved EV product quality (purity, integrity)

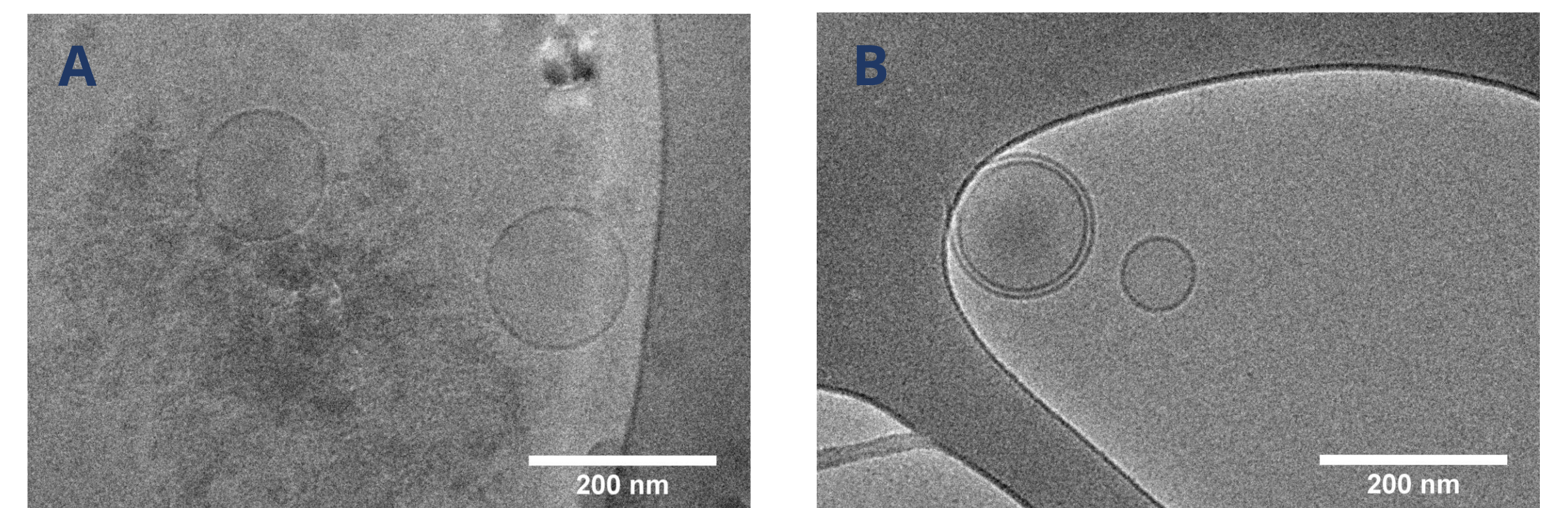
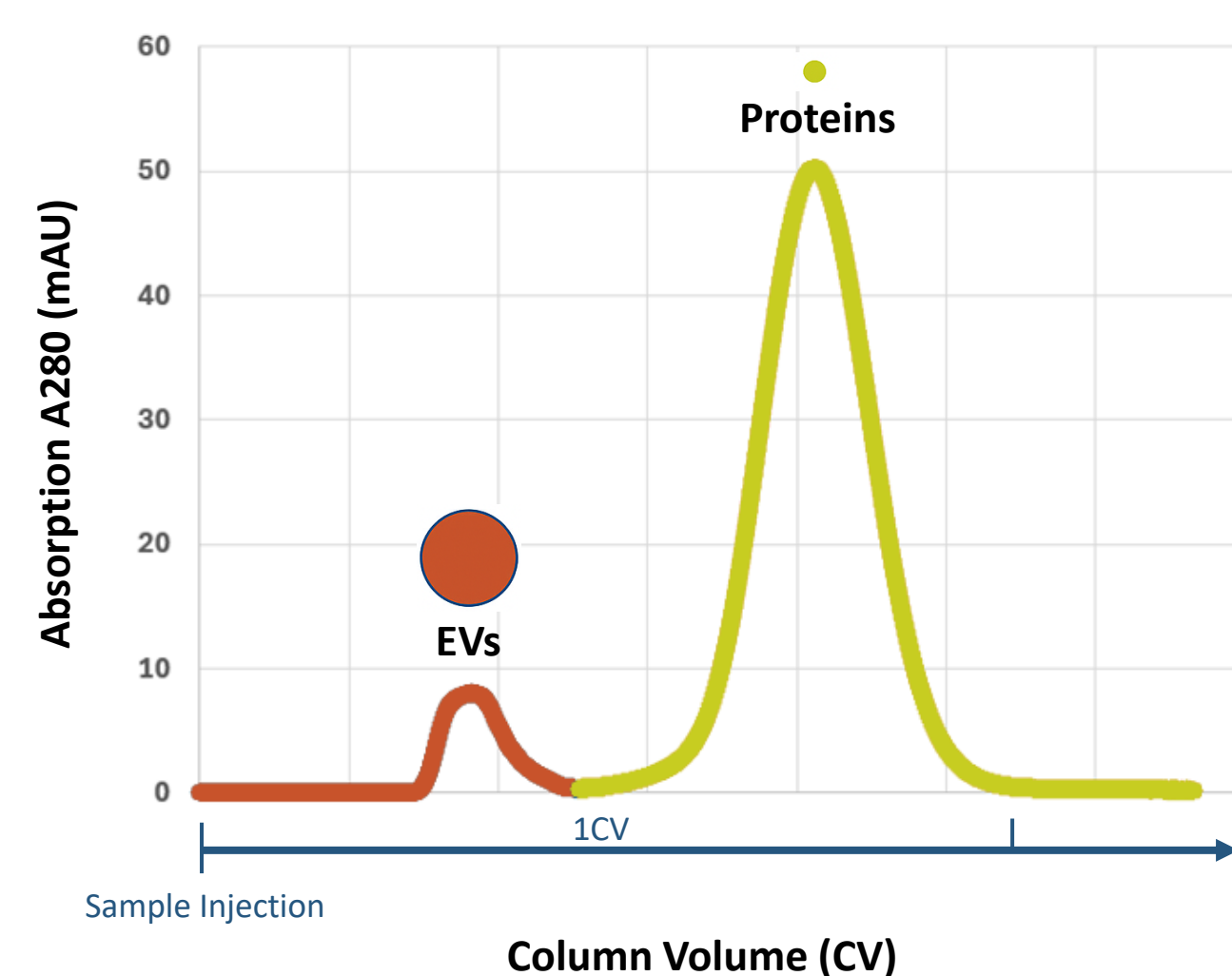
Size Comparison



Principle of SEC



SEC purifies all particles above a specific size threshold!

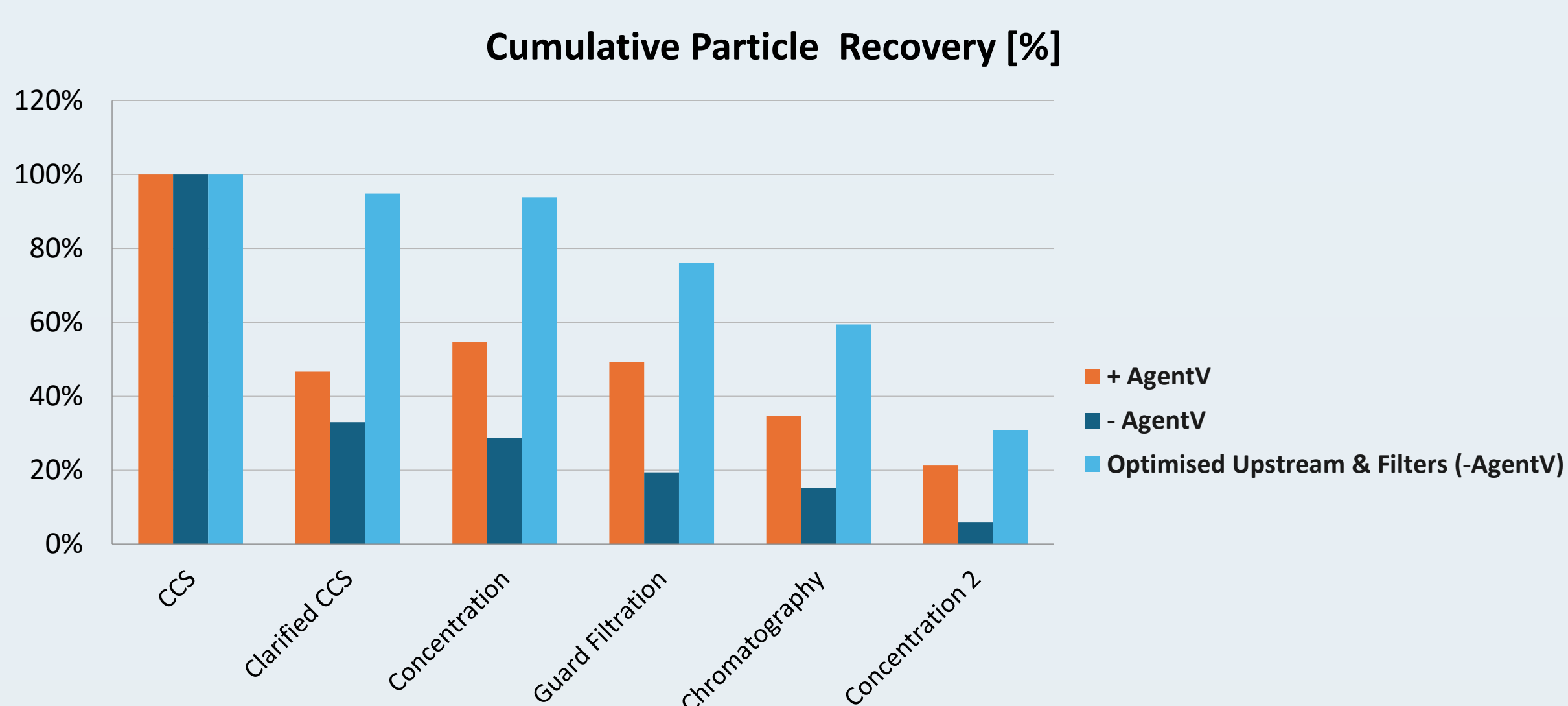


Cryo-TEM images of EV Isolates. [A] Process without filtration but initial centrifugation step (2000g, 10min). [B] Process with clarification filtration and guard filtration. Without filtration steps, chromatin complexes are present in the EV isolate. Centrifugation (2000g, 10min) is insufficient to remove these complexes. With a SEC-based process alone, these complexes can be co-isolated. In contrast to that, a process that incorporates filters with progressively finer pore sizes – such as clarification filter and guard filter – removes chromatin complexes early in the process.

Filtration is an essential step in EV manufacturing, as outlined above, but initial clarification and guard filtration prior to chromatography can result in significant particle loss. At VivaZome, we have evaluated a novel reagent, AgentV (RoosterBio), which is added to conditioned media to prevent filter fouling and improve particle recovery throughout the process.

The graph below demonstrates that cumulative particle recovery increased from only 6% without AgentV to 21% with AgentV treatment. The greatest particle loss occurred during the initial clarification step. To address this, we conducted a filter screening using membranes with retention ratings from 1 µm to 6 µm and optimized the upstream harvest protocol. These improvements increased particle recovery during clarification filtration from 47% (AgentV-treated) and 33% (untreated) to 95% with the optimized protocol and filters.

This highlights that both filter selection (material and retention rating) and upstream harvest conditions are critical. Lower cell viability during EV harvest leads to more contaminating cell fragments, nucleic acids, and chromatin complexes in the conditioned media. These contaminants interfere with filtration, foul membranes, and trap EVs, which leads to reduced particle yield.



Conclusion

At VivaZome Therapeutics, we have conducted filtration studies to identify suitable filter configurations and pore sizes that efficiently remove contaminants without significant EV loss. Depth filters with retention ratings of 1 µm to 6 µm were tested for initial clarification of the starting material. These filters are scalable and suitable for GMP manufacturing. Furthermore, we assessed a novel commercial reagent, which prevents filter fouling and significantly increases EV particle recovery, thereby enhancing the scalability and yield.



Acknowledgements

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