Hydrocyclone for mAb production in a perfusion single-use bioreactor

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Motivation

Hydrocyclones (HC) are compact and virtually clogging-free devices with potential application in perfusion processes. Cell retention occurs when a centrifugal field is formed from the spiral flow of cell suspension fed at high flow rates (Fig 1B). An underflow stream concentrated in cells returns to the bioreactor, whereas a harvest stream containing few cells is obtained from the overflow orifice. No rotors or moving parts are employed to establish the centrifugal field, simplifying HC manufacturing. Single-use HCs for use in perfusion bioreactors can be made by 3D-printing.

Previous studies have explored HCs as cell retention devices (1), but the application was limited to non-disposable lab-scale bioreactors and relatively low cell densities up to ~ 10 million cells per mL.

The aim of the present work was to evaluate an HC coupled to a 50 L single-use bioreactor in an animal-derived component-free process using Chinese hamster ovary (CHO) cells. Steady-state operation at cell densities in the range of 50 million cells per mL was demonstrated.

Materials and methods

- A stainless-steel HC2015 prototype (3) (Fig 1A) was connected to a 50 L single-use bioreactor bag (Xcellerex™ XDR-50, GE Healthcare).
- Three perfusion runs were performed at 40 L working volume, evaluating different ways to connect the HC underflow port to the bag.
- Due to its high processing capacity, HC operation was intermittent (15 min on, 3 to 6 h off) by connecting a simple timer to the feed peristaltic pump.
- Additional process details are summarized below.

Cell line and medium: CHO-K1 cell line expressing a mAb, cultured in ActiPro™ medium enriched or not with Cell Boost™ 1 and 3 (all GE Healthcare)

Analytics: cell concentration and viability by trypan blue exclusion (Vi-CELL™ XR, Beckmann-Coulter), mAb concentration (CEDEX™ Bio Analyzer, Roche), key metabolites, and LDH level

Product quality: glycosylation, molecular size distribution, charged isoforms heterogeneity

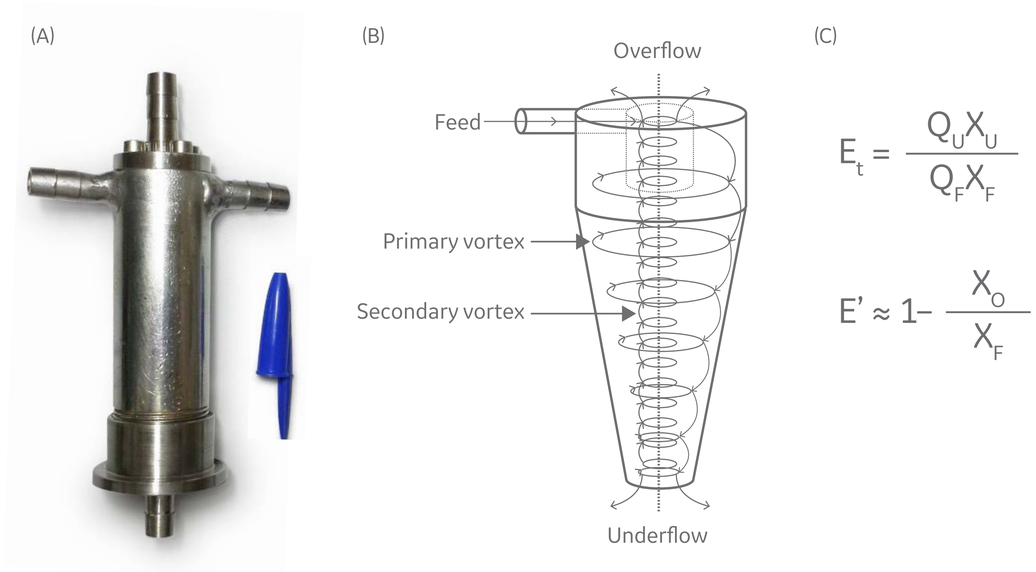

Fig 1. (A) HC2015 stainless-steel prototype (1); (B) Graphic depicting fluid flow inside the HC (2); (C) Total (E_t) and centrifugal (E') separation efficiencies are determined to evaluate cell retention performance.

Results and discussion

For perfusion #1 (Fig 2A), HC was connected to a late-stage fed-batch run for preliminary evaluation of the configuration. Viability was recovered after installing the HC and applying perfusion rates between 0.25 and 1 reactor volume (RV) per day. For perfusions #2 and #3 (Fig 2B and 2C), an extra ReadyMate™ TC port for direct coupling of HC on the top of the bioreactor bag was customized, significantly shortening the recirculation loop. Concentrated cell suspension was freely discharged through the underflow, preserving the typical umbrella-shaped discharge and the gas core inside the HC. The perfusion runs were operated for 20-25 days and achieved high cell viabilities with cell-specific perfusion rates of 50 down to 15 pL/cell/d (Fig 2).

Pressure drops of 2 bar in the HC provided total separation efficiencies up to 96% (Fig 3) and allowed natural cell bleed through the diluted overflow orifice, contributing to a healthier culture environment. Despite the relatively high cell volumetric concentration of the feed, a maximum centrifugal separation efficiency (E´) of 79% was achieved (Fig 3B). An increase in pressure drop up to 2.2 bar for HC operation did not negatively affect cell viability. Increase of LDH levels over time (data not shown) correlated with the viability profile. No mAb retention issues were observed (Fig 4). N-glycoforms presented similar relative abundance as is reported in literature for mAbs, with an increasing proportion of galactosylated species as the culture progressed. Neutral isoforms corresponded to 50% to 60% and high molecular mass aggregates less than 1% (Fig 5).

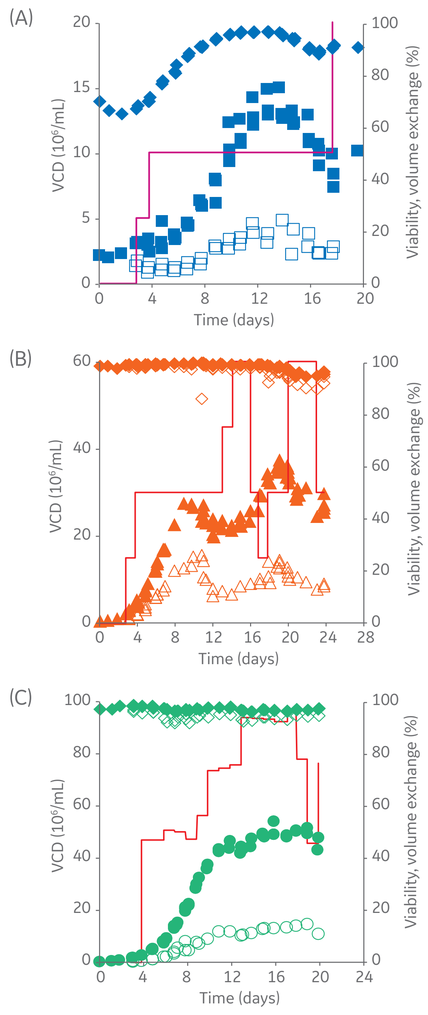

Fig 2. Viable cell densities (VCD) and viability for perfusion runs #1 (A), #2 (B) and #3 (C). Closed symbols refer to bioreactor, and open symbols to overflow (or harvest) stream. Perfusion rates (continuous line) are expressed as bioreactor volume exchanged.

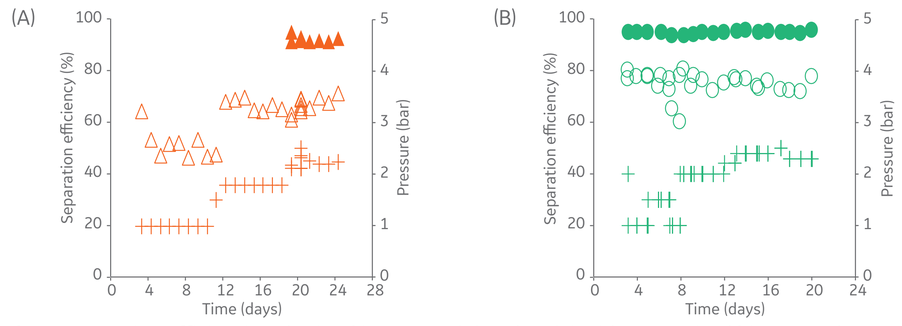

Fig 3. Separation efficiencies obtained in runs #2 (A) and #3 (B) with customized bioreactor bag. Closed symbols refer to E_t and open symbols to E´values. Crosses refer to the pressure values.

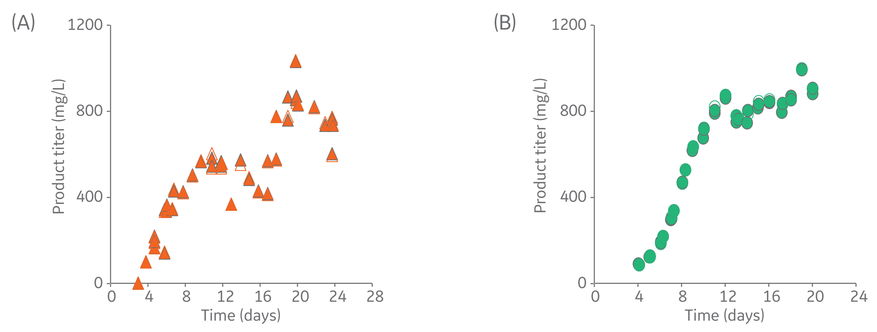

Fig 4. mAb concentration in bioreactor (closed symbols) and harvest (open symbols) for runs #2 (A) and #3 (B).

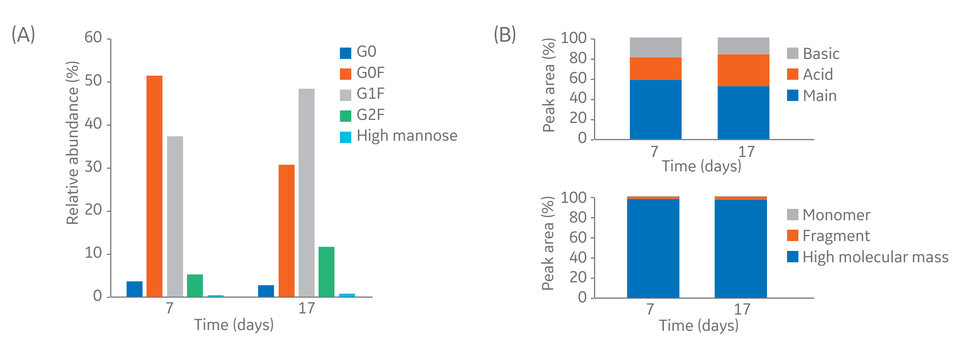

Fig 5. Product quality attributes in an early stage (day 7) and a late stage (day 17) of perfusion #3. (A) N-linked glycoforms; (B) protein charge heterogeneity; (C) molecular size distribution.

Conclusion

For the first time, a hydrocyclone specially designed for mammalian cell separation is reported to enable perfusion processes at cell densities in the range of 20 to 50 million/mL for 20 to 25 days. Also for the first time, a HC is connected to a single-use bioreactor. Pressure drops in the HC higher than 1 bar did not affect cell viability, LDH level, and mAb production. Due to the small dead volume, HC exposes cells to shear stress for a very short residence time. Despite the tiny size, this HC can process over 500 L/d of perfusate when continuously operated, becoming suitable for the bioreactor size range currently adopted for perfusion processes conducted in single-use bioreactors. By using an intermittent medium exchange strategy, a 50 L bioreactor was successfully operated at perfusion rates up to 1 RV/d.

References

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