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Hydrocyclones for single-use perfusion application

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Why is a hydrocyclone a suitable cell retention device for perfusion applications?

- No clogging
- No product retention
- No moving parts
- Compatibility with single-use technology
- Possibility of 3D printing

Characterization of the hydrocyclone

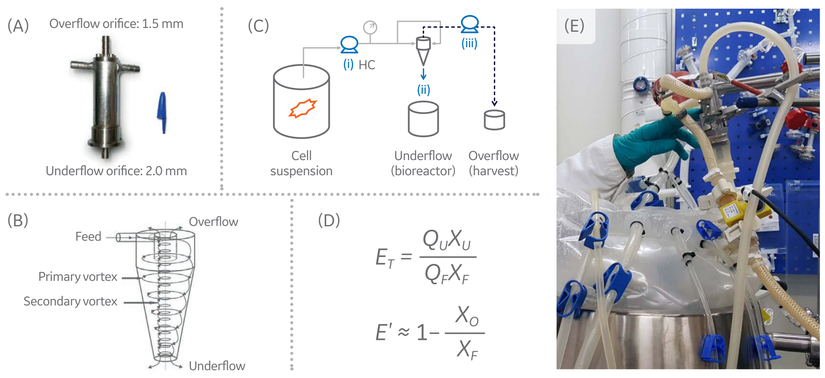

Fig 1. (A) Stainless-steel prototype HC2015; (B) Fluid flow in the HC; (C) Test rig for batch separations to investigate variables relevant to HC attachment (depicted in blue): (i) feed pump, (ii) tubing leaving the underfow, and (iii) overflow pump; (D) Total (E_T) and reduced (E') separation efficiencies calculated to evaluate cell retention performance; (E) HC2015 placed on top of a 50-L single-use bioreactor bag.

(i) Is it important to have a low pulsation pump to feed the HC?

- No, we achieved comparable performance between a low- and high pulse peristaltic pump.

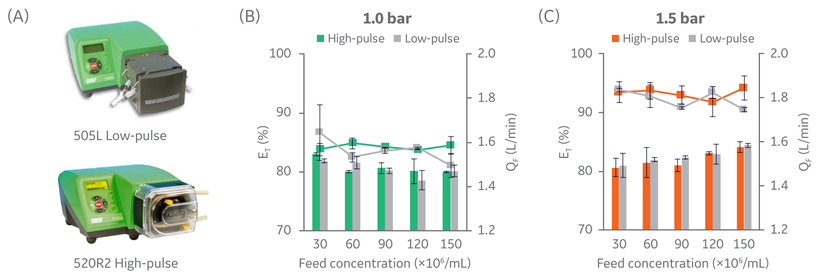

Fig 2. (A) Watson-Marlow pump heads in the feed peristaltic pump. Total separation efficiencies (E_T, bars) and feed flow rates (Q_F, lines) obtained with both pump head models at (B) 1 bar and (C) 1.5 bar.

(ii) How important is the design of the underflow geometry?

- Flow restrictions imposed by means of a 9.5-mm tubing or a 12.7-mm reducer had a negative impact on reduced separation efficiency (Figs 3A–B).
- Recirculation loop should be large enough to enable the umbrella-pattern discharge in the underflow line (Fig 3C).

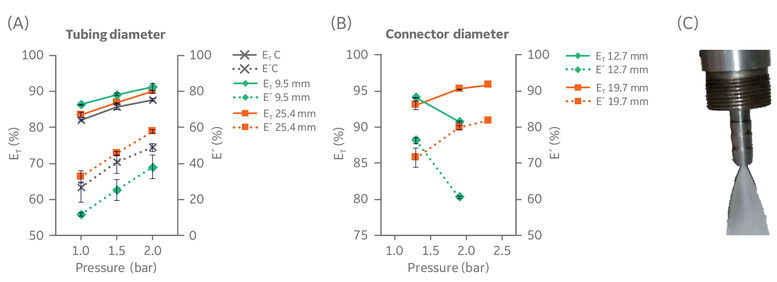

Fig 3. (A) Effect of tubing diameter on separation of Superose™ 6 beads (model for cells) at 5 × 10^6 beads/mL: open underflow discharge (C), 9.5-mm tubing attached to underflow nozzle, or 25.4-mm tubing attached to HC cylindrical body. (B) Effect of presence or absence of 12.7-mm ID reducer in 19.7-mm ReadyMate devices on separation of CHO cells at ~ 20 × 10^6 cells/mL. (C) Typical umbrella-pattern underflow discharge.

(iii) Is it beneficial to use a perfusate pump in the overflow line?

- No. The very high E_T values (Fig 4A) were the result of a very high flow ratio (Q_U/Q_F), and not a result of centrifugal action (lower E´). Therefore, a less clarified harvest was obtained through the overflow (Fig 4B).

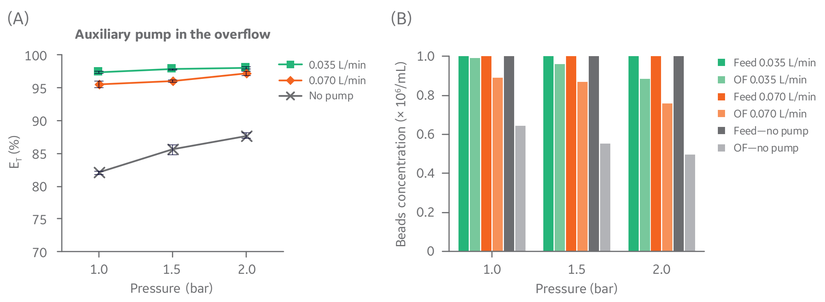

Fig 4. Use of a 520R2 peristaltic pump controlling Q_O at 0.035 (green) or 0.070 L/min (red), or no pump. These Q_O values refer to 1 and 2 RV/d, respectively, for a bioreactor working volume of 50 L. (A) Total separation efficiency. (B) Concentration in the feed and overflow. Superose beads were used as model particles in these tests.

Perfusion runs with the hydrocyclone coupled to a 50-L single-use bioreactor bag

- **Perfusion #1:** HC was installed in a late-stage fed-batch culture, but not with an optimal configuration. Nevertheless, recovery of viability and cell growth were successfully achieved (Fig 5A).
- **Perfusions #2 and #3:** bioreactor bags were customized with a ReadyMate™ TC port on top of the bag wide enough to enable the umbrella-type underflow discharge (Fig 1E). Perfusion runs achieved high cell viabilities with cell-specific perfusion rates of 50 down to 15 pL/cell/d (Figs 5B–C).
- **Perfusion #3:** E_T up to 96% and E' of 79% were achieved at ΔP > 2 bar (Fig 5D). A natural cell bleed with diluted cells occurred through the overflow orifice. Additionally, there was a preferential retention of viable cells, since non-viable cells and debris with smaller sizer were eliminated through the overflow, contributing to a healthier culture environment (Fig 5E).
- 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 correlated with the viability profile (Fig 5F).
- No IgG retention inside the bioreactor was observed (Fig 5F).

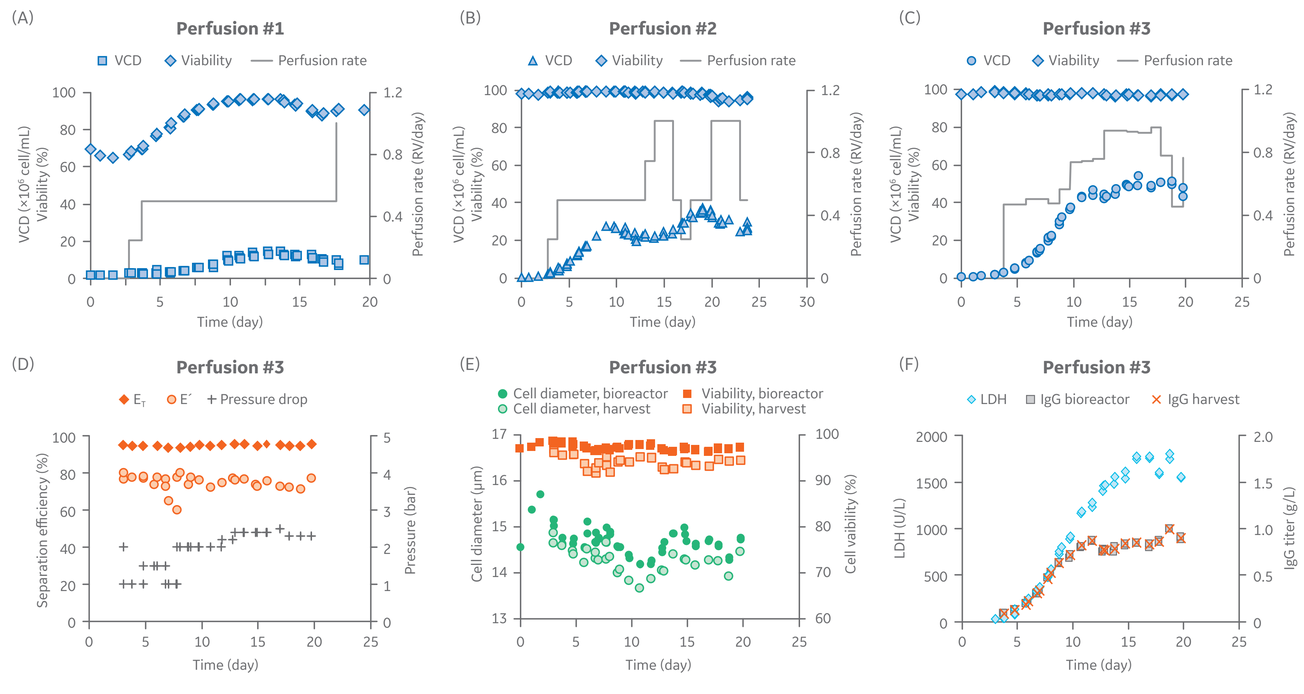

Fig 5. (A–C) Viable cell density (VCD), viability, and perfusion rate in 3 perfusion runs with HC2015. In run #3, optimal operational conditions for the HC separation with no restriction in underflow tubing, coupled to feeding strategy with supplemented ActiPro™ medium resulted in up to 50 × 10^6 cells/mL. (D) Separation efficiencies (E_T and E') obtained in run #3. (E) Viabilities and cell diameter measured in the bioreactor and in the harvest for run #3. (F) Lactate dehydrogenase (LDH) activity and product titer for run #3.

Can hydrocyclones be 3-D printed?

- Yes, preliminary tests with the 3-D printed plastic prototype shown in Fig 6A showed comparable performance, confirming that geometrical proportions inside the hydrocyclone play a key role in separation efficiency.

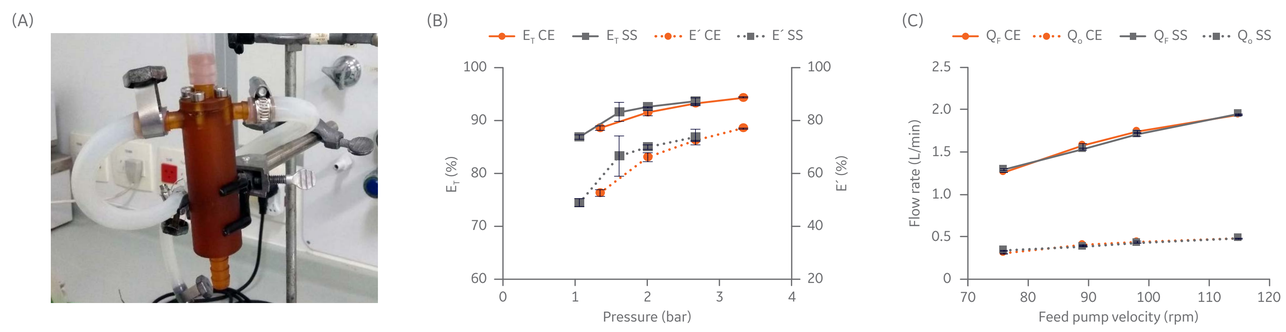

Fig 6. (A) 3-D printed HC2015, made of cyanate ester (CE). Comparison of plastic and stainless steel (SS) prototypes at the same feed pump velocity: (B) separation efficiencies. (C) flow rates in the feed, Q_F, and in the overflow, Q_o.

Conclusion

- For the first time, a hydrocyclone set-up is reported to enable perfusion processes at cell densities in the range of 20 to 50 × 10^6 cells/mL for 20 to 25 days.
- Also for the first time, a HC is operated attached to a single-use bioreactor.
- Pressure drops in the HC higher than 1 bar promoted high separation efficiencies and did not affect cell viability, LDH level, and mAb production.
- An intermittent perfusion at working volume of 40 L bioreactor was successfully operated at perfusion rates up to 1 RV/d. The time intervals of feed pump on and feed pump off can be easily manipulated by means of a timer to increase or decrease the medium exchange per day.
- When continuously operated, this HC can process over 500 L/d of perfusate.

Acknowledgements

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