Hydrocyclones for single-use perfusion application

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Characterization of the hydrocyclone

(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.

(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 1C).

(iii) Is it beneficial to use a peristaltic pump in the overflow line?
- No. The very high E values (Fig 4A) were the result of a very high flow ratio (QU/QF), and not a result of centrifugal action (lower E'). Therefore, a less clarified harvest had a negative impact on reduced separation efficiency (Figs 3A–B).
- Pressure drops in the HC higher than 1 bar promoted high separation efficiencies and did not affect cell viability, LDH level, and mAb production.

Perfusion runs with the hydrocyclone coupled to a 50-L single-use bioreactor bag
- Perfusion #1: HC was installed in a stage-fed-batch culture, but not with an optimal configuration. Nevertheless, recovery of viability and cell growth were successful (Fig 6A).
- Perusions #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/day (Figs 6C–E).
- Perfusion #3: ET, up to 96% and E' of 79% were achieved at 0.5 to 2 bar (Fig 6D). 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 size 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).

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.

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/day. 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.

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