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Research Paper

# Shear contributions to cell culture performance and product recovery in ATF and TFF perfusion systems

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ABSTRACT

Achievement of a robust and scalable cell retention device remains a challenge in perfusion systems. Of the two filtration systems commonly used, tangential flow filtration (TFF) systems often have an inferior product sieving profile compared to alternating tangential flow filtration (ATF) systems, which is typically attributed to the ATF's unique alternating flow. Here, we demonstrate that observed performance differences between the two systems are a function of cell lysis and not the alternating flow as previously thought. The peristaltic pump used in typical TFF perfusion systems is shown to be the single major contributor to shear stress and cell lysis. Replacing the peristaltic pump with a low shear centrifugal pump brought cell growth, cell lysis, particle concentration, and product sieving in a TFF perfusion system to levels comparable with that of an ATF. These results provide a correlation where poor product sieving can be partially explained by high shear in cell retention systems and demonstrate that low shear TFF systems are a feasible alternative to ATF systems.



## 1. Introduction

Batch and fed-batch cell culture technologies currently constitute the majority of recombinant protein manufacturing; however, today's processes are limited by what's achievable in terms of product yields and development times (Chu and Robinson 2001; Kelley 2009; Kantardjieff and Zhou 2014). As the fast growing market of protein-based therapeutics continues to drive the demand for cheaper, higher volume production methods, people have started looking beyond batch processes. Facilitated by recent advances in process monitoring and control, single use equipment, and medium advancements, continuous production options such as perfusion reactors have regained the spotlight as a viable next generation model (Konstantinov et al., 2006; Vogel et al., 2012; Pollock et al., 2013; Walther et al., 2015).

Perfusion, however, comes with its own set of challenges. A more complicated set up increases the risk for contamination and operator error. The higher volumetric media demand places a strain on facility resources, logistics and handling. The correct cell retention device, which lies at the heart of the perfusion system, must be carefully selected to meet one's process demands. Often, there is a tradeoff between the desired criteria. These include, but are not limited to: scalability, reliability, impact on cell culture (i.e. cell viability), and efficiency both in terms of cell clarification at the desired cell densities and recovery of product (Woodside et al., 1998; Voisard et al., 2003). Of the current technologies available, the use of hollow fiber membranes to retain cells based on size is the most common option as it meets many of the above considerations (Altshuler et al., 1986; Zhang et al., 1993; Clincke et al., 2013); however, hollow fiber filtration systems are notoriously susceptible to product sieving and membrane fouling (Mercille et al., 1994; Herrero et al., 1997). This can result in ineffective recovery of product across the membrane and gradual decrease of permeate flow over time which can end a run prematurely.

Tangential flow filtration (TFF) technology relies on use of a recirculation device, most commonly a peristaltic pump, to move cell culture parallel to the membrane surface, generating liquid shear to clear gel layer formation on the filter surface and minimize fouling (Velez et al., 1989; Maiorella et al., 1991; Mercille et al., 1994; van Reis et al. 1997). Higher shear results in better cleaning and improved flux; however, higher shear can also negatively impact cells and cell viability. This often means that one must contend with a certain amount of membrane fouling and product sieving for the sake of keeping cells viable.

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An alternative to the classic unidirectional TFF system is the Alternating Tangential Flow (ATF) system developed by Refine Technology, which has gained popularity in recent years partly for its claims of better product recovery (Voisard et al., 2003; Clincke et al., 2013). Much of the setup, including the hollow fiber unit, remains the same as a TFF. The difference arises from the pump used to drive the filter crossflow. ATF technology utilizes a diaphragm pump to move the cell culture back and forth, instead of unidirectionally, across the hollow fiber. It is hypothesized that this back and forth action may result in some backflushing and regeneration of the hollow fiber, yielding better product recovery (Kelly et al., 2014). Much, however, is unknown about ATFs and indeed even the product sieving phenomenon.

We thus set out to investigate claims of better product recovery in ATF systems by comparing it against classic TFF technology using a peristaltic pump. With our process, the ATF performed better in terms of cell growth, viability and, most importantly, product recovery and membrane fouling. Indeed, hollow fibers operated in TFF mode saw complete plugging on Day 15, leading to early run termination. Because cell growth was also impacted, we then investigated cell death and discovered a disproportionately high amount of cells underwent cell lysis in the TFF setup compared to the ATF. Further investigation into the TFF system revealed that much of the cell death was generated by the peristaltic pump. Switching the high shear peristaltic pump for a low shear centrifugal pump managed to eliminate the previously observed performance differences between TFF and ATF technologies. We therefore concluded that cell lysis generated by the high shear peristaltic pump partly explains the product sieving/membrane fouling phenomenon. To the best of our knowledge, this is the first study that demonstrates that pump shear, and not the alternating flow, is largely responsible for the inferior performance of TFF systems compared to ATF and present a low shear TFF system as a feasible alternative to ATF.

## 2. Materials and methods

### 2.1. Cell culture

The cell retention device used in all perfusion reactors was a polysulfone (PS) hollow fiber module with a 0.2 μm pore size and 1 mm lumen ID. A peristaltic pump (Model 520U, Watson Marlow, Washington, MA) was used as the recirculation device in the TFF system for initial comparison between TFF and ATF systems. A magnetically levitated centrifugal pump from Levitronix (Zurich, Switzerland) was used for studies of a low shear TFF system. Batch reactors were run with the listed peripheral attachments (batch, with peristaltic pump, with pump and hose barbs, with pump, hose barbs, and hollow fiber) using the same tubing and connections as the perfusion systems where applicable. Targeted shear through the hollow fiber was kept below 2000 $s^{-1}$ and matched for all experiments. All studies were conducted in 3L glass stirred tank reactors at a 2L working volume (Applikon Biotechnology, Delft, Netherlands). In-house proprietary recombinant Chinese Hamster Ovary (CHO) cell lines producing monoclonal antibodies were used.

### 2.2. Measurement of cell death

As lysed cells are undetectable by the trypan blue method used by the Vi-CELL Cell Counter (Beckman Coulter Life Sciences, Indianapolis, IN), lactate dehydrogenase (LDH) was instead used as a measure of total cell death. Cell culture was harvested from reactors at the specific time points of cell culture and divided into two batches: untreated and saponin (catalogue #4706, Sigma Aldrich, St Louis, MO) treated (10 μg/L, 15 min at 37 °C). Vi-CELL readings were taken for both sets of samples. The samples were then centrifuged for 5 min at 243 rcf and supernatant taken for LDH measurements. Cell death rate by LDH was calculated using the following equations:

$$LDH/cell = \frac{[LDH]_{saponin} - [LDH]_{untreated}}{VCD_{untreated} - VCD_{saponin}}$$

$$q_{LDH} = \frac{1}{VCD}([LDH] \times X)$$

$$death\ rate = \frac{q_{LDH}}{LDH/cell}$$

where $q_{LDH}$ is the cell specific LDH productivity and X is the perfusion rate (L/day).

Cell lysis was induced by placing cell culture in a sonicator (Model 750D, VWR, Radnor, PA) for 10 min. The percent of lysed cells was calculated using the following equation:

$$\%lysed\ cells = \frac{Total\ cell\ density_{Untreated\ sample} - Total\ cell\ density_{Sonicated\ sample}}{Total\ cell\ density_{Untreated\ sample}}$$

### 2.3. Offline model of product sieving

The perfusion reactor was harvested at day 8 of cell culture and the harvest centrifuged at 243rcf for 15 min to separate cells and larger debris from supernatant. At the same time, the permeate stream through the hollow fiber was collected. These were divided into three separate samples: permeate, supernatant, and cell pellet resuspended in permeate. Each sample was then cycled individually through a 0.2 μm PS hollow fiber with the following set up: both perfusion (permeate flow) and recirculation flow fed back into the original sample reservoir to conserve volume and minimize changes to sample over time. Flow rates were controlled by peristaltic pumps and velocities were set to match shear in the perfusion systems. Samples of 'reactor' and permeate were taken at the indicated time points and IgG concentration measured. Instantaneous product sieving is expressed as the ratio concentration of IgG in permeate to concentration of IgG in reactor. 100% product sieving indicates total product passage across the membrane and 0% indicates no product recovery.

### 2.4. Analytical methods

Viability and cell density were measured by Vi-CELL Cell Counter (Beckman Coulter Life Sciences, Indianapolis, IN). Titer and LDH were measured using a Cedex BioHT (Roche Diagnostics GmbH, Mannheim, Germany). These titer measurements had been previously verified to be in agreement with in house HPLC methods. Particle size was assessed using a Malvern Zetasizer Nano ZS (Malvern, Westborough, MA). Derived count rate is expressed as kilo counts per second (kcps) and is used as a measure of relative number of total particles within the sample.

### 2.5. Statistics

All results where applicable ($n > 3$) are expressed as the mean ± standard deviation. Statistical significance was analyzed using a two tailed Student's $t$ test. All p values below 0.05 were considered significant.

## 3. Results

### 3.1. Comparison between ATF and TFF systems

To assess cell performance and product sieving differences between the two perfusion systems, a TFF and an ATF were run

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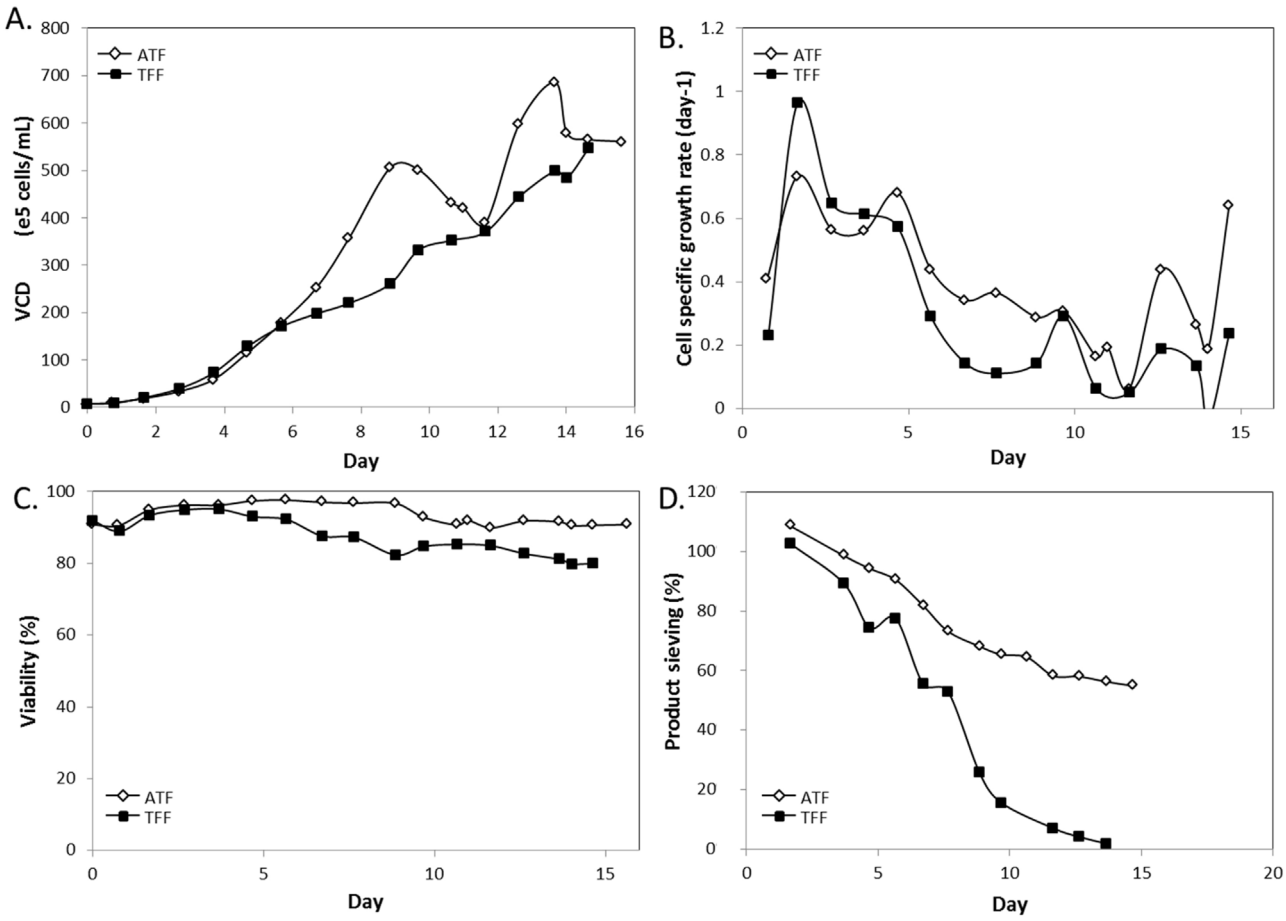


**Fig. 1.** Performance differences between ATF and TFF systems. TFF was demonstrated to be worse in the follow four parameters: A) Viable cell density (VCD), B) Cell specific growth rate, C) Viability, and D) Product sieving. VCD and viability were both measured by a ViCell using the trypan blue method for viable cell detection. At day 8 of culture, a cell bleed was turned on in the ATF reactor in an attempt to maintain a consistent VCD. Fluctuations in VCD readings from day 9 onward reflect this cell bleed. Product sieving was assessed using IgG concentrations of permeate and reactor measured by a Cedex BioHT and represented as the ratio of concentration of IgG in permeate to concentration of IgG in reactor. A ratio of 1 or 100% indicates no product sieving.

in parallel. Cells were inoculated at a target density of $1e^6$ cells/ml and grown in batch mode for two days prior to the start of perfusion. The perfusion strategy consisted of two phases: a ramp up phase during exponential cell growth followed by a constant perfusion rate combined with a cell bleed to maintain the target VCD. Samples were taken daily to monitor cell culture performance. Vi-CELL readings of VCD and viability revealed a slower average growth rate for cells in the TFF reactor (0.32 $days^{-1}$) compared to cells in the ATF (0.49 $days^{-1}$) (Fig. 1b). This contributed to the target VCD being reached 5 days later in the TFF (Fig. 1a). Viability was also negatively impacted in the TFF setup, reaching 81.1% by day 14 compared to 91.5% in the ATF (Fig. 1c), though viabilities for both systems appeared to stabilize over time and were not a huge cause for concern.

Instantaneous product sieving across the hollow fiber was assessed by comparing IgG concentration in the reactor with IgG concentration in the permeate stream. This was expressed as permeate titer over reactor titer (product sieving). Samples were taken daily from the respective streams at the same time point and analyzed using a Cedex BioHT for monoclonal antibody concentration. Fig. 1d reveals the product sieving profiles over time. Product sieving in the TFF decreased at a significantly faster rate compared to the ATF, indicating that the mechanism for product sieving and/or membrane fouling was exacerbated in the TFF setup. Indeed, complete membrane blockage was observed on day 15, and the TFF run prematurely terminated, while the ATF product sieving was maintained at greater than 50% for the entire 20 days of cell culture.

### 3.2. Particle size distribution reveals clues toward product sieving

The distribution and size range of particles within the two systems was measured throughout the course of the run to gain a better understanding of the mechanism behind the product sieving phenomenon. As expected, the profile of particles in the permeate stream was consistent over time with the majority of particles measuring below 10 nm in diameter (data not shown). Analysis of the population within the reactors, however, revealed an accumulation of particles in the 100 nm size range as the perfusion culture progressed. More interestingly, by day 8, there were approximately 4.5 times more total particles in the TFF reactor compared to the ATF (108,760 kcps TFF vs. 24,458 kcps ATF).

Membrane fouling and subsequent product sieving have been linked to cell viability (Karl et al., 1990; Maiorella et al., 1991). Lower cell viability can yield increased DNA (Mercille et al., 1994), proteins (Kelly and Zydney 1994; Kelly and Zydney 1997), and other cellular components (Esclade et al., 1991) in the cell suspension which can act to plug pores in the hollow fiber. This would also theoretically increase the number of particles in the reactor. To determine the correlation between cell death and particle count in our cell line, different modes of cell death were induced. As the trypan blue method used by the ViCell can only detect intact cells, extracellu-

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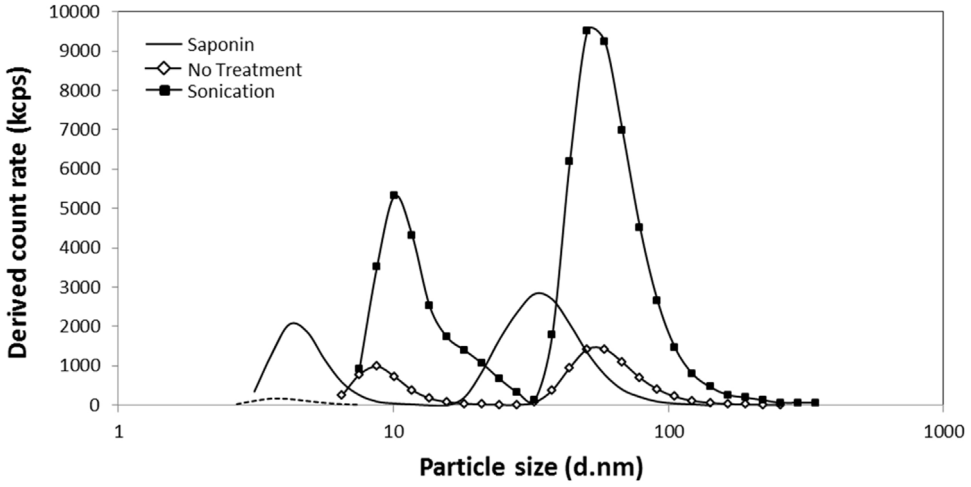


| Treatment | VCD (E5 cell/mL) | % nonviable | % lysed |
|---|---|---|---|
| None | 37.06 | 8% | 7% |
| Saponin | 0.55 | 91.5% | 7% |
| Sonication | 12.63 | 40% | 36% |

**Fig. 2.** Cell lysis results in a significant increase in 100 nm particles. Cell culture was harvested from a perfusion reactor and divided into three samples. One sample (control) underwent no treatment. The second sample received 10 mg/L of saponin and incubated at 37C for 15 min. The third sample was placed in a sonicator for 10 min. The percentage of viable, intact nonviable, and lysed cells after each treatment was calculated using measurements taken from the ViCell and Cedex BioHT (LDH). Particle size distribution was measured using a Malvern Zetasizer Nano ZS.

lar LDH concentration was measured to determine total cell death (Koh and Choi 1987; Lobner, 2000). The difference in cell death rates between the trypan blue method and the LDH method was then attributed to cell lysis.

Cell culture was collected from the early stages of a perfusion reactor when viability was high. Trypan blue and LDH analysis of the sample revealed little cell death in the starting sample (8% nonviable cells and 7% lysed cells). Cell death was then induced by treatment with saponin (loss of viability but not cell lysis) or a prolonged period of sonication (induction of cell lysis). Fig. 2 shows the impact of cell death on particle size distribution. The number of particles in the sample rose with induction of cell death. Interestingly, this change was drastically increased when cells underwent cell lysis compared to apoptosis alone. The predominant peak fell within the 100 nm size range, which is of special interest as these particles are similar in size to the pores of the 0.2 μm hollow fiber.

To determine which particle size range contributes the most to product sieving, cell culture was divided into three groups and assessed separately for product sieving using the offline model as described above. The three groups consisted of: a. the material in the permeate stream (<10 nm in diameter), b. the cell pellet resuspended in permeate (<10 nm and >1 um in diameter), and c. the material in the reactor supernatant (~100 nm in diameter). The particle size distributions as well as the subsequent product sieving profiles are shown in Fig. 3. Groups a (permeate) and b (permeate and the cell pellet) did not result in any significant product sieving for the entire duration of the experiment. When cell culture supernatant containing primarily particles in the 100 nm size range was introduced to a brand new 0.2 μm hollow fiber, product sieving was severe and almost instantaneous (Fig. 3c).

### 3.3. TFF system induces higher rate of cell lysis compared to ATF system

It was thus hypothesized that the higher particle count in the TFF reactor was due to a higher rate of cell lysis and that this increased cell lysis contributes to worse product sieving. The original assessment of cell viability during perfusion was conducted using trypan blue analysis and did not reveal any drastic periods of cell death in either system. The viability never dipped below 80% and, indeed, is even observed to rise in the TFF for certain days (Fig. 1b). The average death rates of both systems are similarly low (Fig. 4a). When cell death was reassessed by LDH quantification, a higher rate of cell death than was originally suggested by the trypan blue method was discovered. The fact that this extra cell death was undetectable by the trypan blue method suggests that a significant portion of cell death within the perfusion system is from cell lysis. Furthermore, while both systems experienced cell lysis, this number was drastically increased in the TFF, which had a significantly higher average death rate (0.22 $day^{-1}$) compared to the ATF (0.09 $day^{-1}$), coinciding with higher particle count and worse product sieving. This finding was reconfirmed using a different cell line, suggesting that this phenomenon is not specific to one cell line (Fig. 4a).

Accounting for this higher death rate also reveals that the growth rates in both systems are similar (Fig. 4b). This is of interest as the other significant difference observed between the TFF and ATF systems was the longer time needed for cells in the TFF to reach the desired VCD. The slower apparent growth rate of the cell culture in the TFF was due solely to the higher rate of cell lysis. Both major differences in system performance can thus be traced to one cause, namely cell lysis. As cell lysis is typically attributed

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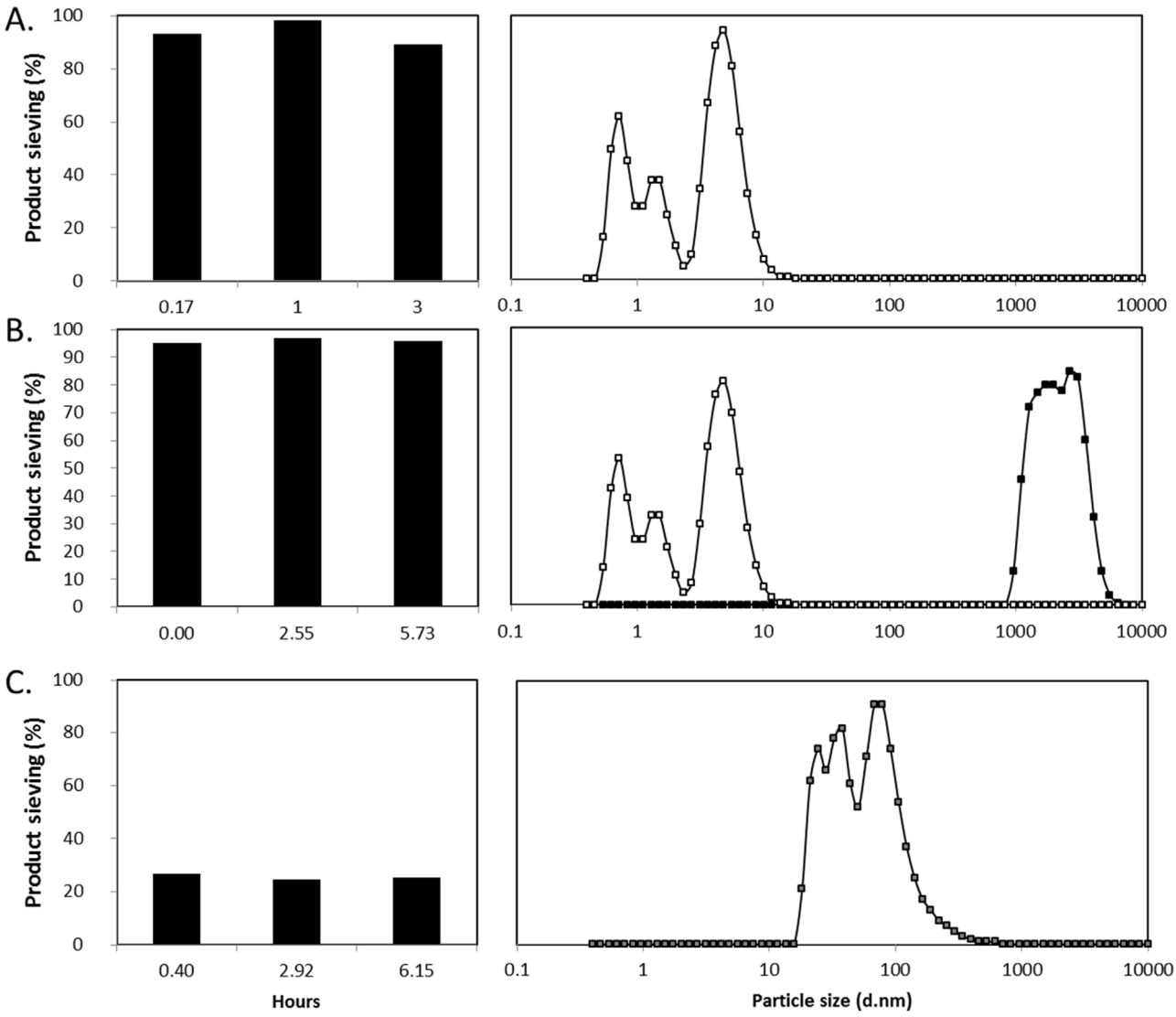


**Fig. 3.** Offline model of product sieving reveal particles in 100 nm size range contribute to product sieving. Permeate and cell culture were collected from a perfusion reactor on Day 8 of culture prior to the start of a cell bleed. The culture was centrifuged to separate cell culture material by size. Three distinct samples were thus obtained: A) permeate, B) cell culture pellet resuspended in permeate, and C) cell culture supernatant. Each separate section was then cycled through an unused 0.2 μm PS hollow fiber mimicking a perfusion setup. Samples from permeate and feed lines were taken at the indicated time points. Concentration of product was measured using the Cedex BioHT and product sieving expressed as concentration IgG in permeate over concentration IgG in feed. Particle size analysis was conducted using a Malvern Zetasizer Nano ZS.

to increased shear, we then turned our attention to investigating sources of shear within the TFF system.

### *3.4. Peristaltic pump is the primary cause of cell lysis in the TFF system*

The recirculation loop with the cell retention device adds extra complexity to the setup along with more potential sources of shear. These sources include the pump used for the movement of cell culture through the recirculation loop, the added connections (hose barbs) needed, and the hollow fiber itself. While the TFF and ATF systems share many commonalities, there are subtle differences in each of these identified areas of concern. For one, the ATF utilizes a diaphragm pump to move cells across the hollow fiber while a traditional TFF setup uses a peristaltic pump. It is conceivable that one type of pump generates more shear than the other. The two ports needed for recirculation in the TFF compared to one in the ATF calls for extra connections which may also be extra areas of cell death. Lastly, the movement of the cells through the hollow fiber is at a constant (TFF) vs a fluctuating (ATF) flow rate. The sustained flux through the fibers may cause excess shear.

Reactors were run in batch mode to isolate the individual contributions these components have on cell death. The conditions are illustrated in Fig. 5a. Briefly, a recirculation loop was added to a batch reactor with a peristaltic pump driving the flow of culture to test the impact of the pump on cell death. Another reactor had the same setup with added hose barbs in the line to test shear generated by the connections. The last condition added a 30 cm or 60 cm long hollow fiber to discern whether or not prolonged exposure to the hollow fiber at a sustained flow rate causes greater cell lysis. A batch reactor without any extra peripherals was run in conjunction to assess the base level of cell death in the culture.

The regular batch reactor without the extra peripherals was able to reach a peak VCD of $12.2e^6$ cells/ml with an average death rate of 0.02 $day^{-1}$ as quantified by LDH (Fig. 5b). In comparison, the batch culture running through a peristaltic pump saw a drastically increased rate of cell death (0.13 $day^{-1}$). Similar to the TFF reactor which had suppressed cell growth due to high cell lysis, the batch reactor with the peristaltic pump peaked at 4.7E6 cell/ml, a nearly

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3-fold decrease, demonstrating that the peristaltic pump is a major source of shear within the TFF system. Interestingly, the other additions (hose barbs, hollow fibers) did not drastically increase cell death beyond the rates generated with the peristaltic pump alone (Fig. 5b). While this does not conclusively eliminate the possibility that these additions generate shear, it does demonstrate that the shear from the peristaltic pump is comparatively so great that it overwhelms and masks the impact of the other sources.

Different modes of operation of the peristaltic pump were then investigated to assess if altering recirculation conditions can reduce cell death. Two factors that were considered were the pump head (or rotations per minute) and the average number of times per hour a cell goes through the pump (controlled by recirculation flow rate). Two separate rates were determined based on previous run conditions. All TFF perfusion data was obtained at a recirculation rate of 400 ml/min at 40 rpm using tubing with a 'large' inner diameter. A 'small' tubing was identified which, at 40 rpm using the same pump head, results in a flow rate of 100 ml/min. A third condition, 10 rpm at 100 ml/min, using the original 'large' tubing was also tested. Average cell death rates showed a trend for increasing death with greater rpm. Increased flow further negatively affected both death and peak VCD despite the larger tubing used (Fig. 5c).

### 3.5. Low shear centrifugal pump reduces cell death and rescues product sieving

To test the theory that product sieving can be rescued by controlling excess cell lysis generated by the pump head, the peristaltic pump was switched out for a centrifugal pump made by Levitronix (Levitronix GmbH, Zurich, Switzerland). This magnetically levitated pump system was first identified in the medical field as a low shear replacement to traditional blood pumps (Morgan et al., 1998; Sakota et al., 2008). Comparison studies between peristaltic and Levitronix pumps in CHO cells demonstrated that the Levitronix pump causes less mechanical stress (Blaschczok et al., 2013). Using this pump allowed for operation at the same recirculation rate as before while decreasing cell lysis for a head to head reassessment against the ATF. The two cultures were again grown to a target VCD at which time a cell bleed was turned on to maintain a steady VCD. Unlike the slowed growth observed with the peristaltic pump, the TFF using a Levitronix pump had the same growth curve as the ATF, reaching the target VCD on the same day (Fig. 6a). More importantly, there was no difference in the specific death rates (Fig. 6b) and no difference in total number of particles within the 100 nm size range (Fig. 6e) between the two systems, indicating that the extra cell lysis in the TFF system observed previously was due to the peristaltic pump as theorized. Switching to a low shear centrifugal pump eliminates the differences between the two systems. Furthermore, lowering cell lysis eliminated the sharp drop in product sieving that ended the previous TFF run prematurely. Indeed, despite a larger initial drop in sieving in the earlier days, the two curves are similar for the majority of the run (Fig. 6d). Less cell lysis allowed TFF perfusion to be extended to the entire 30 days. Final total percent recovery was 73% in the TFF compared to 71% in the ATF.

## 4. Discussion

ATFs have become an increasingly popular alternative to traditional TFF technologies because of claims of better culture performance and product sieving; however, there are disadvantages arising from the increased operational complexity of the ATF control system. Compared to traditional TFF technology, ATF requires more utilities, its controller is less well understood and is thus arguably less reliable than an unidirectional TFF that does not require such control. These are all compelling arguments for being able to operate in TFF mode for perfusion. These benefits become overshadowed, however, if the amount of protein one can produce and recover is drastically reduced as a consequence of using TFF over ATF. Not only is efficiency decreased, but the lack of control over this phenomenon can result in discrepancies during scale up or even batch to batch differences.

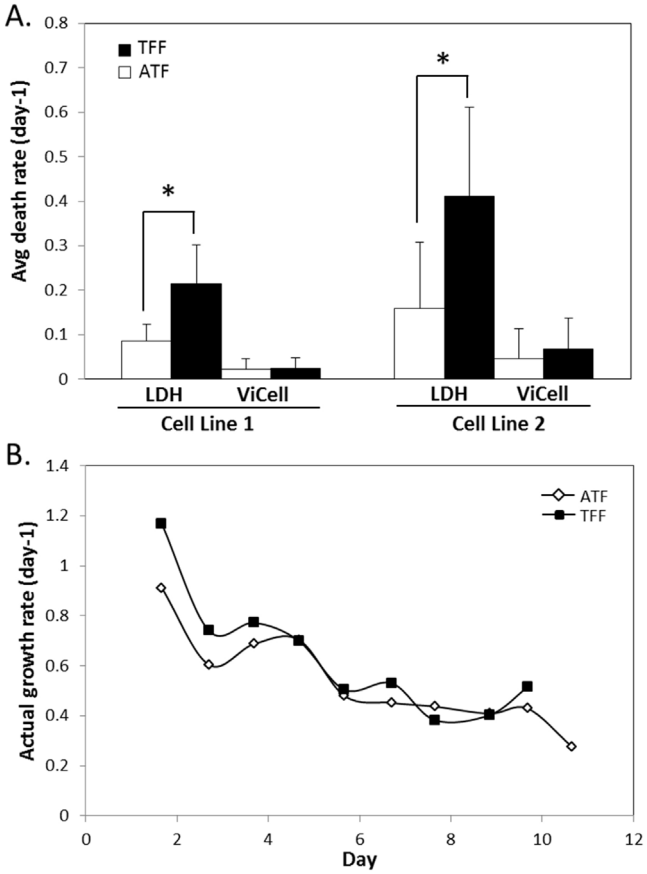


**Fig. 4.** Higher levels of cell lysis found in the TFF reactor A) Comparison of death rates between ATF and TFF reactors using LDH or ViCell as a method of detection. The death rate is expressed as the average across the entire duration of perfusion ± standard deviation; *$p < 0.05$ B) Comparison of growth rates between TFF and ATF reactors over time. Actual cell specific growth rates are similar between the two systems. Actual growth rate is calculated from the specific growth rate calculated from the change in VCD over time plus the death rate as measured by LDH.

Our initial comparison of the two systems revealed slower growth and worse product sieving in the TFF which is in line with previous anecdotal observations. Further investigation into the cause surprisingly revealed that this was not due to the back and forth action of the ATF as originally thought but rather to the ATF's diaphragm pump, which imparts less mechanical stress on the culture. Interestingly, although the perfusion operating conditions had been chosen to fall well below the critical values dictated by previous publications for wall shear stresses (Vickroy et al., 2007) and pump backpressures (Kamaraju et al., 2010), higher cell death was still observed in the TFF system. This was attributed primarily to the peristaltic pump used for recirculation of the cell culture. It is conceivable, though it is outside the scope of this paper, that the drastic differences between observed and reported critical values may be due to the repeated exposure on the order of days and not hours of the cell culture to the pump. The increased death rate was also able to fully explain the growth rate differences observed between the two systems.

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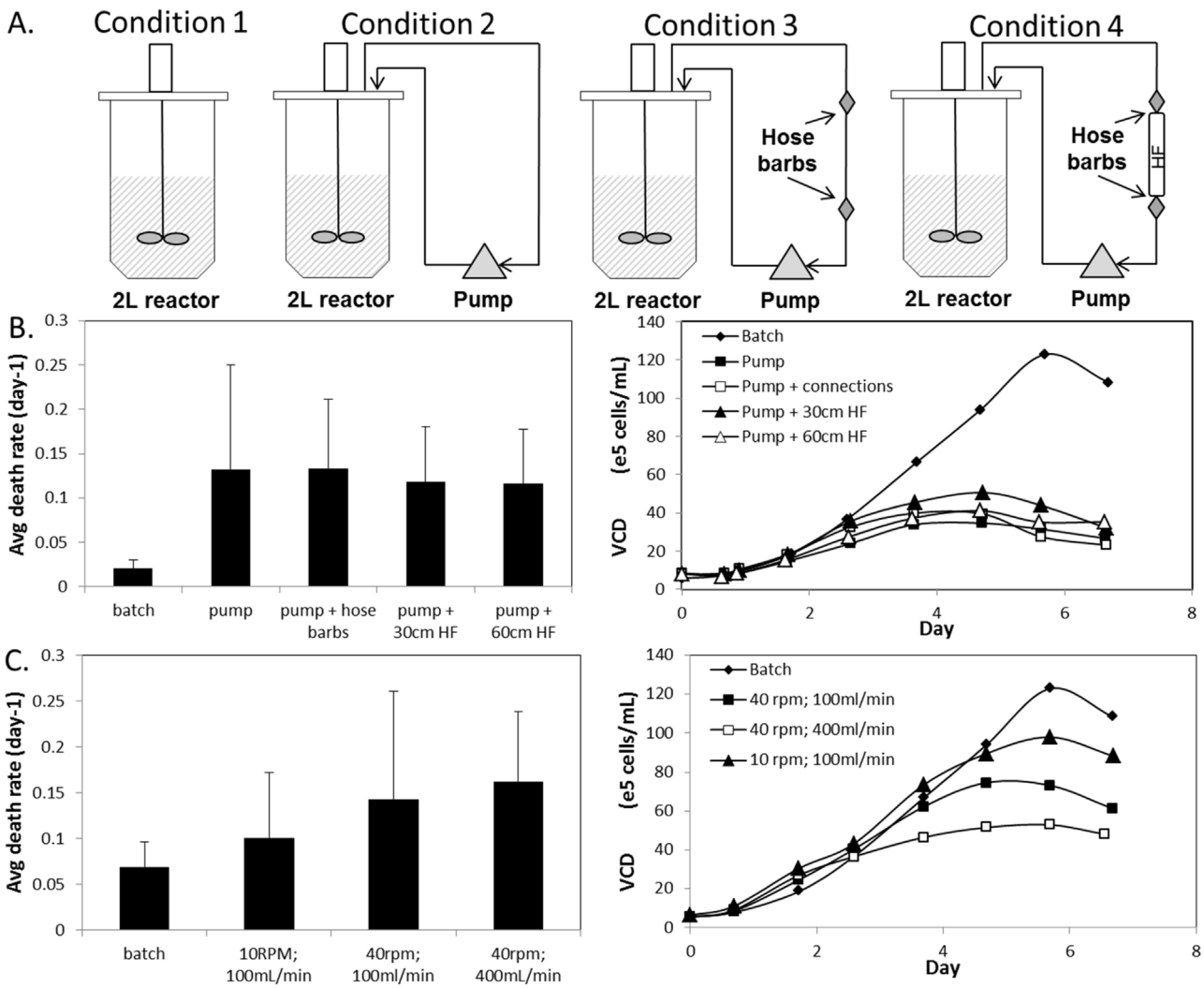


**Fig. 5.** Investigating sources of shear in the TFF system A) Diagram of the experimental setup. Cells were cultured in batch mode with extra peripherals needed for perfusion added on one at a time to separate out the individual contributions to cell death. These reactors were: batch with no peripherals, batch with the recirculation loop and a peristaltic pump, batch with the recirculation loop, peristaltic pump, and hose barb connectors, and batch with the recirculation loop, peristaltic pump, hose barb connectors, and a hollow fiber. Two difference sizes of hollow fibers (30 cm and 60 cm) were used. B) The peristaltic pump is the main contributor to shear. Death rates shown is the death rate averaged across the entire duration of the culture. Error bars is the standard deviation. The peristaltic pump was operated at 40 rpm for an equivalent recirculation rate of 400 ml/min C) Cell lysis is primarily caused by pump rpm. The experiment was rerun using the second setup (batch reactor with recirculation loop and peristaltic pump) and recirculation rates varied. Two different sizes of recirculation tubing was used to achieve the same flow rate at different rpms.

The peristaltic pump shear can be decreased as demonstrated by lowering rpm and flow rate, but, since the recirculation flow rate provides a sweeping action across the hollow fiber that reduces gel layer formation, lowering flow rate is not necessarily desirable in a perfusion system. The freedom to go to higher flow rates is vastly beneficial especially when considering scale up. Thus, switching to a low shear pump while maintaining the same recirculation flow rate is a more viable alternative for TFF perfusion. Indeed, the performance of a TFF system operated with a centrifugal pump was identical to that of an ATF system in terms of cell growth and product sieving. This is in stark contrast to what was previously reported by Karst et al. (2016), who observed worse product sieving in a centrifugal pump TFF system compared to an ATF. This worse product sieving may be related to the reported differences in cell growth and metabolism between the ATF and TFF systems though it is hard to judge without any further data on their process setup and cell death characteristics. We show here that product sieving of a TFF perfusion system can be made similar to that of an ATF system if cell culture characteristics, especially with regards to cell death, are matched.

It is important to note, however, that sieving is alleviated but not solved. Switching to a lower shear pump for the TFF system eliminated product sieving differences between the two systems but substantial product sieving over time was still present. We demonstrated that product sieving in a 0.2 μm PS hollow fiber occurs quickly when exposed to cell culture supernatant. We also demonstrated that the number of particles in the supernatant increases substantially with cell lysis. These particles presumably are cell debris. That both ATF and TFF systems still experienced product sieving suggests several possibilities. First, that the level of cell death in the systems, while lower than with the use of a peristaltic pump, may still be high enough to be an issue. The gentler pumps may impart less mechanical stress but shear is still present in the system and there is still a low level of cell death. It is possible that this level may be enough over time to cause the observed product sieving. As it is near to impossible to avoid all cell death in the culture, this would then suggest that another work around would be needed to address the product sieving issue. While cell debris within the 100 nm size range was demonstrated to contribute to product sieving, the question still remains about which individ-

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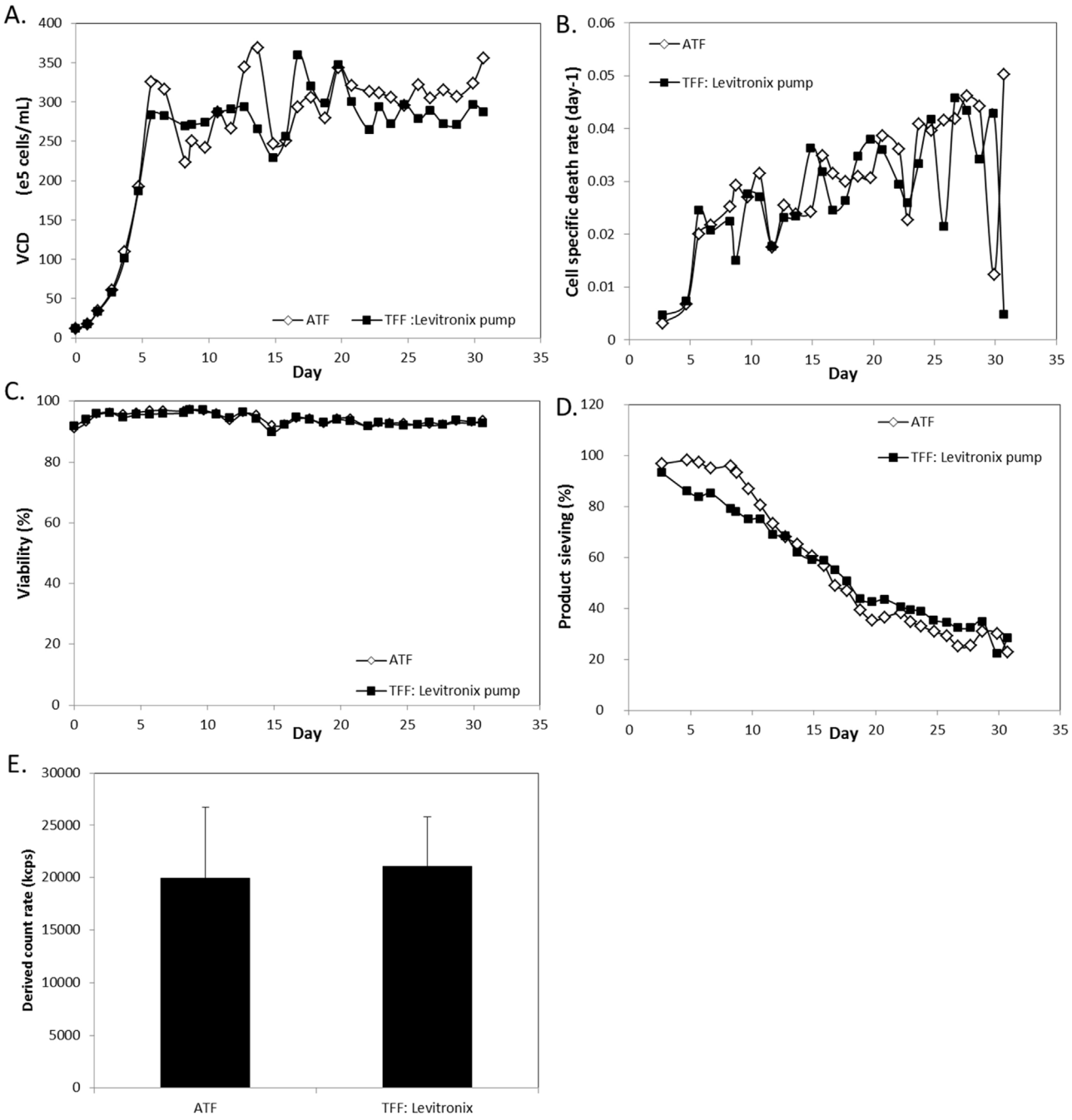


**Fig. 6.** Low shear pump closes the gap between TFF and ATF performance in terms of A) VCD B) specific death rates measured by LDH C) Viability D) Product sieving and E) Derived count rate. The derived count rate expressed is an average over time of the total number of particles within the 100 nm size range measured in the reactor supernatant.

ual component is specifically involved. Most likely, a combination of different components along with certain aspects of cell culture media conspires to produce the product sieving phenomenon. Gaining a better understanding of this phenomenon will help the development of a better solution and ultimately achieve a perfusion process with little to no product sieving.

## 5. Conclusions

Here, we take the first step toward understanding product sieving by demonstrating that cell lysis caused by higher peristaltic pump shear was responsible for the performance differences between the TFF and the ATF. We thus presented a viable TFF alternative to the ATF in the form of a centrifugal rotary pump made by Levitronix. Though no other pumps were investigated in this study, it is conceivable and indeed even expected that any equally low shear pump would yield the same results. To our knowledge, this is the first study demonstrating that poor product sieving can be partly explained by high shear in perfusion systems.

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