Investigations into Mechanical Stress Caused to CHO Suspension Cells by Single-use Magnetically Levitated, Bearingless Centrifugal Pumps

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1 Introduction

Pumps used in biopharmaceutical processes are crucial to the success of up and downstream processes. For upstream processes in particular, the pump's characteristics and performance must be carefully considered when working with shear sensitive materials such as animal cells (e.g. Chinese hamster ovary cells) [1-3]. Levitronix GmbH has developed a novel single-use pump series which is suitable for applications in the biopharmaceutical industry. Based on their innovative technology, they have created magnetically levitated, pulsation and seal-free pump systems with a pump head made of plastic that is replaced and discarded after a single use. This novel technology avoids the drawbacks of shafts, seals and leaks which are typically observed in traditional pumps and supports the use of single-use pumps in biopharmaceutical processes [4].

This study focused on the evaluation of mechanical stress caused to CHO (Chinese hamster ovary cells) suspension cells by single-use PuraLev® 200SU and PuraLev® 600SU pumps. The comparison criterion was mechanical stress, which was evaluated by the cell death rate, based on cell density. The determination of viable and total cell density was performed using a NucleoCounter NC-100. For these investigations, the PuraLev® single-use pump series from Levitronix GmbH were compared with established systems (peristaltic and 4-piston diaphragm pumps).

2 Materials and methods

Test setup

The setup of the pump circuit is shown in Fig. 1 and comprises a storage vessel (Chemap AG, Switzerland) with a total volume of 12 L and a closed tubing loop. The working volume of the storage vessel was dependent on the investigation conditions and was adjusted to a mean residence time of one minute. This means that the working volume was 3.4 L for a flow rate of 3.4 L/min or 10 L for a flow rate of 10 L/min. For each set of test conditions, three pumps were investigated in parallel over 12 hours (Fig. 2).

In the tubing loop, a single-use pressure sensor (SciLog BioProcessing Systems, USA) and a clamp-on flow sensor (Levitronix GmbH, Switzerland) were installed to control the test conditions and to determine the mechanical stress. Different tube configurations (C-Flex®, Saint Gaubain Performance Plastic) with varied tubing diameters between 1/4" and 1/2" and a hose clamp were used to create a back pressure of 0 - 0.5 bar. The temperature of the double-jacked storage vessel was controlled by a Thermostat (Huber, Switzerland).

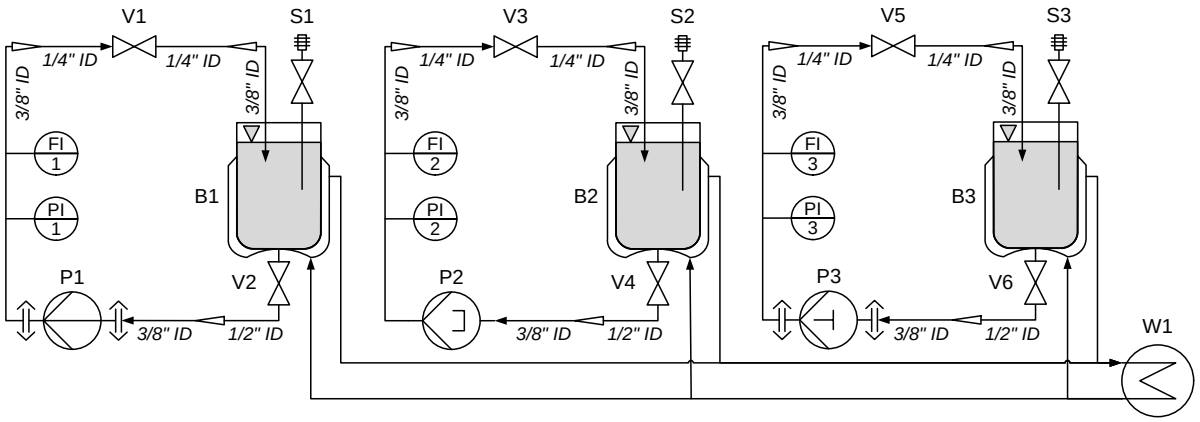

Fig. 1: Setup of the pump circuit for the Levitronix® PuraLev® SU (P1), the peristaltic pump- (P2) and the 4-piston diaphragm pump (P3). (S1) – (S3) Sample valve Clave Connector, (B1) – (B3) storage vessel with bottom outlet valve (V2, V4, V6), (W1) Thermostat, (W1) – (FI3) F, (PI1) – (PI3) pressure sensor.

The storage vessels and the pump loop were autoclaved to ensure sterile and comparable test conditions for the mechanical stress investigations. The 4-piston diaphragm was autoclaved directly in the tubing loop, because no loss of quality was observed. However, the single-use pumps from Levitronix were equipped with ReadyMate connectors (GE Healthcare Life Sciences, Sweden) and gamma-irradiated (Fig. 2, C and D).

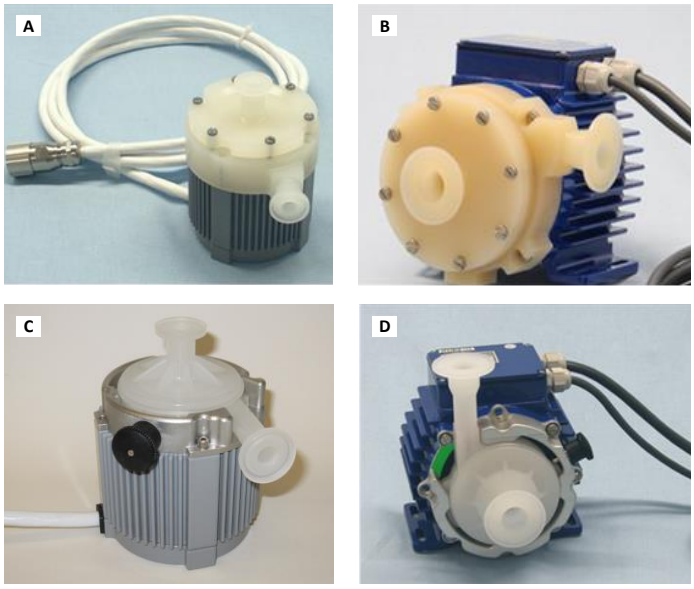

Fig. 2: **Levitronix single-use PuraLev® pump series (A) PuraLev® 200MU, (B) PuraLev® 600MU, (C) PuraLev® 200SU, (D) PuraLev® 600SU.**

Cell expansion

All experiments were performed using CHO XM 111 suspension cells (provided by Prof. Dr. Martin Fussenegger, Swiss Federal Institute of Technology in Zurich, Switzerland). For each experiment (Tab. 1), the CHO suspension cells were thawed from the established working cell bank and cultivated under static conditions in FMX-8 chemically defined minimal medium (Cell Culture Technologies, Switzerland) in 75 cm^2 T-flasks (Corning, Switzerland). Next, the CHO cells were transferred into shake flasks (1 L, Corning, Switzerland) containing ChoMaster HP-1 growth medium (Cell Culture Technologies, Switzerland). In order to ensure that all stress investigations were carried out with cells in similar physiological and metabolic states [1], the CHO cells were transferred into an appropriate scaled wave-mixed single-use bioreactors (BIOSTAT RM 20 L or BIOSTAT RM 50 L; both Sartorius Stedim Biotech, Germany) prior to each pump test (Tab. 1). After reaching a viable cell density of between $3 – 4 \cdot 10^6$ cells mL^{-1} (in mid-exponential growth phase) HP-1 growth medium was added in order to avoid inhibition of cell growth caused by high lactate and low glucose concentrations during sedimentation.

Tab. 1: Cultivation parameter and strategy.

Parameter	T_{75}-Flask	Shake flask 1000 mL	BIOSTAT® RM 20L	BIOSTAT® RM 50L
Temperature:	37 °C	37 °C	37 °C	37 °C
Shaking/rocking frequency:	-	120 rpm	16-30 rpm	16-30 rpm
Amplitude:	-	25 mm	6-7 °	6-7 °
Air:	-	-	0.1 vvm	0.1 vvm
CO_2:	5 %	7.5 %	5-10 %	5-10 %
Cell concentration:	$0.3 \cdot 10^6$ Zellen mL^{-1}	$0.5 \cdot 10^6$ Zellen mL^{-1}	$0.5 \cdot 10^6$ Zellen mL^{-1}	$0.5 \cdot 10^6$ Zellen mL^{-1}
Cultivation volume:	20 mL	30-300 mL	2-10 L	5-25 L
Feeding strategy:	-	-	3 L nach 48 h 5 L nach 72 h	5 L nach 24 h 7 L nach 48 h 8 L nach 72 h
Culture medium:	ChoMaster FMX-8	ChoMaster HP-1	ChoMaster HP-1	ChoMaster HP-1

Pump tests

The CHO suspension cells were transferred under sterile conditions into the three storage vessels. A minimum rotation speed (50 rpm) was set for a few seconds for all pumps in order to fill the pump circuit (tubing loop and pump head) and to calibrate the flow and pressure sensors. Samples were taken periodically, over a 12-hour period, to analyse the mechanical stress on CHO suspension cells.

Analytics

A NucleoCounter® NC-100 (ChemoMetec, Denmark) was used to determine the viable cell density (VCD), total cell density (TCD) and viability. The nutrients (glucose, glutamine) and metabolites (lactate, glutamate und ammonium) in the culture medium were measured using a BioProfile 100 Plus (NOVA Biomedical, USA / Labor-Systeme Flükiger, Switzerland)

Determination of the cell death rate

The cell death rate was determined based on the time-dependent decrease of the viable cell density (Equ. 1). Fig. 3 shows the viable cell density (VCD) as a function of time. The function of curve over time for decreasing viable cell density (VCD) is dependent on the pump type and represents the stress intensity. Based on the results obtained, two subpopulations were defined. During the first two hours, the sensitive population lysed as a result of mechanical stress, leading to an increased percentage of the robust CHO suspension cells. Based on these findings, the cell death rate of the sensitive cells was used as the comparison criterion for all the pumps that were investigated.

$$\frac{dVCD(t)}{dt} = -k_D \cdot VCD(t) \qquad 1$$

To consider the effect of both subpopulations, the sensitive and robust cell death rate ($k_{D,s}$ = 0.6 and $k_{D,r}$ = 0.025) as well as the viable cell density (VCD_s = 0.75, VCD_r = 0.25) were estimated based on previous studies to calculate the temporary viable cell density (VCD(t)). As a result, Equ. 2 and Equ. 3 (shown in Fig. 3) were defined.

$$VCD(t) = VCD_s \cdot \exp^{(-k_{D,s} \cdot t)} \qquad 2$$

$$VCD(t) = VCD_r \cdot \exp^{(-k_{D,r} \cdot t)} \qquad 3$$

The correlation for both populations is given by Equ. 4, which shows a comparable trend for the calculated and experimentally determined values (Fig. 3). Based on these values, the cell death rates $k_{D,s}$ and $k_{D,r}$ were obtained. To ensure the quality of the graphical evaluation, a coefficient of determination ($R^2 > 0.8$) was required. The cell death rate ($k_{D,s}$) of the sensitive cells was used as the comparison criterion for the stress investigations .

$$VCD(t) = VCD_s \cdot \exp^{(-k_{D,s} \cdot t)} + VCD_r \cdot \exp^{(-k_{D,r} \cdot t)} \qquad 4$$

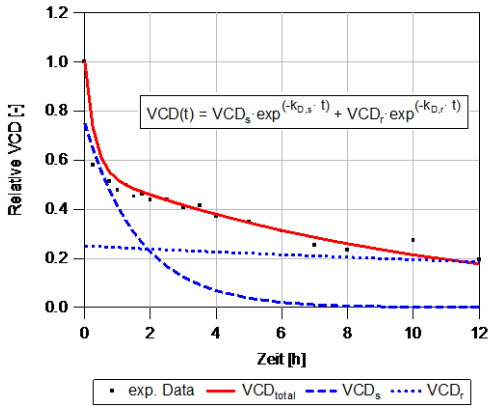

Fig. 3: Determination of the calculated cell death rates for the sensitive $k_{D,s}$ and robust $k_{D,r}$ cells.

3 Results

Comparison of the PuraLev® 200 SU and competitor pumps

The investigations into mechanical stress show a decrease over the process period of 12 h in the viable (VCD) and total (TCD) cell densities as well as the viability of the cells. This indicates that the cell death rate correlates with mechanical stress and is dependent on the pump type. The results for the PuraLev® 200SU and the competitor pumps (peristaltic and 4-piston diaphragm pump) at 3.4 L min^{-1} and 0.5 bar (Fig. 4 A, B, C) show that there was a higher decrease in the viable (VCD) and total (TCD) cell densities for both competitor pumps compared to the PuraLev 200SU.

The comparison of the nutrient and metabolite (lactate and glucose) concentrations shows no differences between the PuraLev® 200SU and the competitor pumps (Fig. 4 D). During the pump tests, glucose consumption was approximately 0.30 g L^{-1} and lactate production increased to between 2.00 g L^{-1} and 2.50 g L^{-1}.

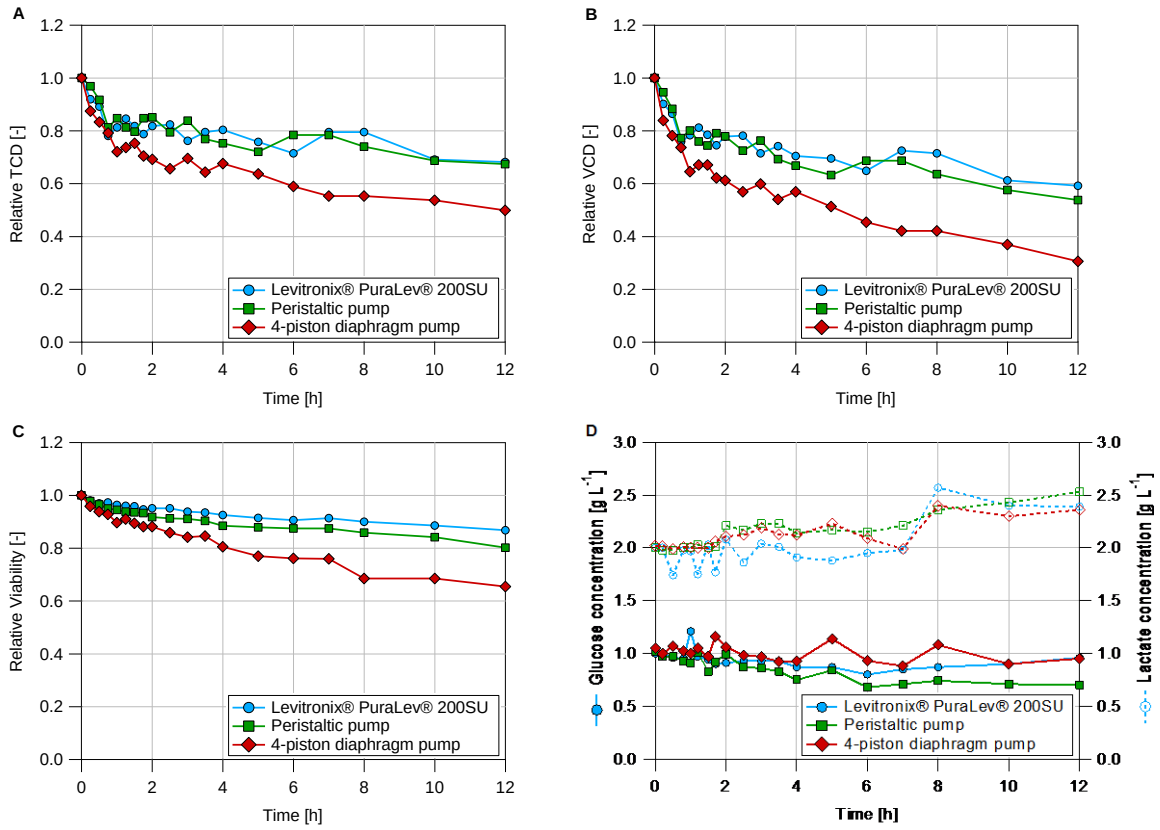

Fig. 4: Graphs of the total cell density (TCD; A), viable cell density (VCD; B), viability (C) and glucose and lactate concentrations (D) against time (12 h) for the PuraLev® 200SU, peristaltic- and 4-piston diaphragm pump.

Fig. 5 shows the cell death rates for the PuraLev® 200SU and the competitor pumps (peristaltic and 4-piston diaphragm pump). The lowest cell death rate was obtained using the PuraLev® 200SU with k_D = 0.023 h^{-1}, indicating that there was less mechanical stress on the CHO sus-

pension cells than in the peristaltic (k_D = 0.03 h^{-1} ≈ 23 %) and the 4-piston diaphragm pump (k_D = 0.036 h^{-1} ≈ 56 %)

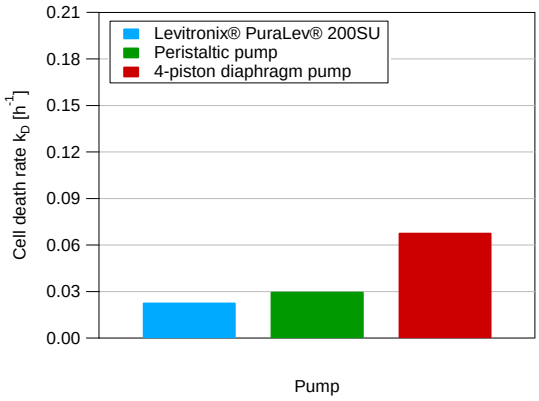

Fig. 5: Cell death rates in the PuraLev® 200SU, peristaltic and 4-piston diaphragm pumps.

Investigations of the PuraLev® 600SU

Further mechanical stress investigations focused on the PuraLev 600SU, the peristaltic and 4-piston diaphragm pump working at 10 L min^{-1} and 0.5 bar. The cell death rates for these conditions are shown in Fig. 6. Here, the lowest mechanical stress was achieved using the PuraLev® 600SU with k_D = 0.014 h^{-1}. Comparing the result with those of the competitor pumps, increasing cell death rates of 1.6 times those of the peristaltic pump and up to 14-times those of the 4-piston diaphragm pump were calculated. This indicates that the PuraLev® 600SU also exerts less mechanical stress on CHO suspension cells than the peristaltic and 4-piston diaphragm pumps.

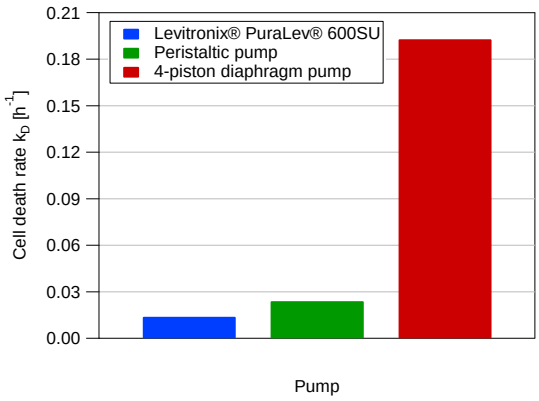

Fig. 6: Cell death rates in the PuraLev® 600SU, peristaltic and 4-piston diaphragm pumps working at 10 L min^{-1} and 0.5 bar.

4 Conclusion

The results of the mechanical stress studies indicate that the single-use bearingless, magnetically levitated centrifugal pumps from Levitronix caused significantly lower cell death rates in CHO suspension cells than the peristaltic and 4-piston diaphragm pumps. Based on the determined viable cell density, viability and cell death rate during the pump investigations, the single-use Levitronix® PuraLev® 200SU und 600SU pumps provided superior pump performance with low mechanical stress. In contrast to the Levitronix pumps, the peristaltic and 4-piston diaphragm pump caused high mechanical stress in CHO suspension cells, based on the cell death rate.

References

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