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Journal article | **Accepted manuscript (Postprint)**

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Herrmann, N., Neubauer, P., & Birkholz, M. (2019). Spiral microfluidic devices for cell separation and sorting in bioprocesses. *Biomicrofluidics*, 13(6), 61501. <https://doi.org/10.1063/1.5125264>

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Spiral microfluidic devices for cell separation and sorting in bioprocesses

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Inertial microfluidic systems have been arousing interest for medical applications due to their simple and cost-efficient use. However, comparably small sample volumes in the μl and ml range have so far prevented efficient applications in continuous bioprocesses. Nevertheless, recent studies suggest that these systems are well suited for cell separation in bioprocesses because of their facile adaptability to various reactor sizes and cell types. This review will discuss potential applications of inertial microfluidic cell separation systems in downstream bioprocesses and depict recent advances on inertial microfluidics for bioprocess intensification. The review thereby focusses on spiral microchannels that separate particles at a moderate Reynolds number in a laminar flow ($\text{Re} < 2300$) according to their size by applying lateral hydrodynamic forces. Spiral microchannels have already been shown to be capable of replacing microfilters, extracting dead cells and debris in perfusion processes and removing contaminant microalgae species. Recent advances in parallelization made it possible to process media on a liter-scale which might pave the way towards industrial applications.

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I. INTRODUCTION

With more continuous bioprocesses being applied industrially, efficient cell separation methods are needed to retain productive cells in the system and thereby increasing process yield. However, currently used techniques like microfiltration and centrifugation show various drawbacks like membrane-clogging, low scalability and challenges in automatization. Since the introduction of first commercial cell-sorting FACS (fluorescence-activated cell sorting) systems, several chip-based microfluidic devices have been developed that offer cost-efficient solutions for separation and sorting of cells. They can be classified into active systems like acoustophoresis, magnetophoresis, dielectrophoresis and deterministic lateral displacement (DLD) that depend on external force fields, and passive systems that include gravitation- and inertia-based techniques. Passive systems are usually favored because of their lower complexity. Microfluidic separation techniques have been reviewed recently¹⁻⁶ with some papers focusing on inertial devices in particular⁷⁻¹¹. Inertial separation is solely based on channel geometry and hydrodynamic forces¹² without requiring cell manipulation by external forces which makes it a robust and easy-to-use method. The most common architectures for inertial separation are straight and spiral microchannels. Straight microchannels are most commonly used for cell separation for medical purposes. Separation of circulating tumor cells^{13,14}, red blood cells^{15,16} and MCF-7 cells¹⁶ could already be shown. Spiral channels stand out because they allow processing at higher flow rates of up to 1 l/min¹⁷ due to their large channel geometry. The here presented channel structures were fabricated in polydimethylsiloxane (PDMS) using soft-lithographic techniques or in poly(methyl methacrylate) (PMMA) using laser cutters. These materials can prospectively be combined with semiconductor manufacturing processes which opens the door for new lab-on-chip applications with elements from both microfluidics and microelectronics¹⁸. Medical applications of spiral microchannels today are manifold and include isolation of

circulating tumor cells^{19,20}, blood cells^{21,22} and sperm cells²³, isolating axons from neuronal cell bodies²⁴, cell-cycle synchronization²⁵, and blood-plasma separation²⁶. As channel dimensions match typical cell sizes, a variety of different cells like mammalian cells, yeast²⁷ and even bacteria²⁸ could be separated. The reproduction of large-scale processes on microfluidic devices, however, is a challenging task.²⁹ Currently, applying microfluidics in microalgae processes shows promising results. A size dependent separation of microalgae cells with different lipid content using dielectrophoresis and platforms for growth and oil production analysis^{30,31} have already been developed. This paper reviews potential applications of microfluidic cell separation and sorting in bioprocesses, thereby focusing on spiral channels as they show the highest potential for implementation in large-scale processes due to their energy efficiency and facile scalability.

A. Separation principle in spiral microchannels

In spiral microchannels with a laminar Poiseuille flow, i.e. Reynolds numbers $Re < 2300$, three forces may cause a size-dependent separation. Shear gradient-induced (i) and wall-induced lift forces (ii) play important roles for separation in both straight and curved microchannels. Introducing curvature to the channel, however, induces a secondary-flow that accelerates the arrangement of particles in the equilibrium position. This is caused by a secondary-flow drag (iii) force, called Dean drag.

The shear gradient-induced lift force is caused by the parabolic velocity profile in the channel that leads to different velocities on either side of the particle. The particle thereby experiences a force pushing it to areas with lesser relative velocity differences which can usually be found in the near-wall region.³² Thus, shear-gradient induced lift forces counteract wall-induced lift forces and particles in that fluid stream therefore arrange at positions in the channel where these forces

are in balance (Fig. 1). In rectangular channels two of these equilibrium positions can be identified. They preferably form close to the center of the channel's side walls. The magnitude of both inertial lift forces depends on particle size and they become stronger at higher Reynolds numbers.⁸ The shear-gradient lift force F_{SG} can be calculated from

$$F_{SG} = \frac{C_{SG}\rho U_{Max}^2 a^3}{D_h}, \quad (1)$$

where C_{SG} is the lift coefficient for the shear gradient lift force, ρ is the fluid density, U_{Max} is the fluid's maximum velocity, a is the particle diameter and D_h is the hydraulic diameter that can be calculated for rectangular channels by $2h \times w / (h + w)$ with h and w being height and width, respectively.³² This shows that F_{SG} becomes larger with decreasing channel dimensions which shows the necessity of microstructures for efficient particle separation.

Wall-induced lift forces result from pressure that is building up in between the particle and the wall. The particle is slowed down by interactions with the wall and a force is induced that directs particles away from the channel wall towards the channel's center.³² The wall-induced lift force F_{WI} can be calculated from

$$F_{WI} = \frac{C_{WI}\rho U_{Max}^2 a^6}{D_h^4}, \quad (2)$$

where C_{WI} is the lift coefficient for the wall interaction force.³²

Dean flows are also a result of velocity differences within the channel. As fluid parcels in the channel center move faster compared to the near-wall region, these parcels are carried towards the outer wall by the fluid's inertia, once a curvature is introduced to the channel. This leads to a recirculation of the parcels and thereby a secondary flow is induced in the shape of two counter-

rotating vortices at the top and bottom surfaces of the channel (Fig. 1).³² The Dean flow is characterized by a dimensionless Dean number that is defined as

$$De = Re \left(\frac{D_h}{2R} \right)^{0.5}, \quad (3)$$

where Re is the Reynolds number and R is the average radius. Thus, smaller radii generate stronger secondary flows.⁸ This secondary flow imparts a drag force on particles that acts differently on particles with different sizes and thereby improves separation efficiency.³³ The terminus “separation efficiency” is hereby used in a purely qualitative way for processes with two or more cell types that differ in size. It takes into account the purity of each outlet fraction and the cellular composition of the original medium as large differences in cellular abundance should also lead to a higher contamination of the smaller fraction at the outlet. Another important consideration is the difference in main cell size. As cell sizes vary a lot, an overlap in cell sizes between two different cell types will reduce the outlet fraction’s purity. Increasing flow rates strongly leads to the Dean-flow becoming the dominant force which rather causes dispersion of the particles than separation.¹² The secondary-flow drag force F_D can be calculated by

$$F_D = 6\pi\mu a U_{SF}, \quad (4)$$

where μ is the fluid viscosity and $U_{SF}=1.8 \times 10^{-4} De^{1.63}$ is the velocity of the secondary-flow.³² Guan *et al.*³⁴ examined spiral microchannels with trapezoidal cross-sections and found that stronger Dean vortices were formed on the channel side with bigger depth which lead to an improved separation.

Additionally to the three mentioned forces, several weak forces also act on the particles. These forces emerge when particles lead, lag or rotate in the fluid stream⁸ and are up to several orders of magnitude weaker than the above mentioned forces, which is why they can usually be

neglected.³² Rotational lift forces only get dominant after an initial equilibrium position is reached and help particles focusing near the channel walls' center.¹² Centrifugal effects play a minor role as the particles' and fluid's densities are too similar.¹⁰

FIG. 1. Major hydrodynamic effects affecting cell positioning in curved, rectangular microchannels. Wall-induced lift forces (F_{wl}) push particles towards the channel's center, whereas shear-induced lift forces (F_{SG}) direct particles towards the side of the microchannel. By introducing curvature to the channel, a secondary flow is induced which applies a Dean drag (F_D) on the particles that supports attaining equilibrium position. As all three hydrodynamic forces are size-dependent, differently sized cells focus at slightly different positions in the channel.

II. APPLICATIONS OF SPIRAL MICROCHANNELS IN BIOPROCESSES

A. Replacement of microfilters

Microfiltration plays an important part in many industrial bioprocesses²⁷ as particles ranging from 10 nm - 10 μ m are difficult to separate from a suspension with other common methods like centrifugation, gravitational settling and adsorption techniques⁹ and biotechnologically relevant organisms typically range in that dimension. However, microfiltration is not flawless as membrane clogging and fouling occur frequently which drastically reduces efficiency of the method through e. g. retention of proteolytic enzymes from dead cells. Substitution of membrane

filters involves interrupting the process which increases risks of contamination and accounts for a major part of operating costs.²⁷

By increasing flow rates in their spiral microchannels slightly from 2 ml/min to 6 ml/min, Warkiani *et al.*²⁷ could show that the device switches from a cell separation mode to a cell retention mode as all cells were focused at the inner wall. This allowed them to incorporate inertial microfluidics into a perfusion bioprocess. Cells were retrieved from the spiral's inner outlet and lead back into the bioreactor for further protein production, whereas cell-free medium containing the produced protein and other small particles like cell debris was collected at the outer outlet and could be used for subsequent protein purification (Fig. 2). Replacing microfilters with spiral microfluidic devices has already been described³⁵ in 2007 but recent advances show that inertial microfluidics can reach throughputs at least comparable to mechanical membrane filters which can process approximately 10^9 cells/ml.³⁴ This is an essential requirement for industrial applications.

Warkiani *et al.*²⁷ could show cell retention for CHO and yeast cells, at the example of *Saccharomyces cerevisiae*, in separate approaches (Table I). CHO cell retention was first conducted with a single spiral at a flow rate of 6 ml/min and a retention efficiency of >95% could be measured for three different cell lines. They then went on to multiplex 84 microchips with four spirals each to retain CHO cells at a flow-rate of 500 ml/min. No change in viability, morphology and proliferation was observed and by measuring the expression of the shear stress biomarker c-Fos, it could be shown that no stress response could be detected in the cell which can probably be explained by the short residence time of only <0.1 s on the average within the spiral. Similarly to this approach, *S. cerevisiae* with a concentration of 10^5 cells/ml was retained at a lower flow rate of 2 ml/min based on smaller channel dimensions and cell sizes (3 -5 μ m for

yeast compared to 10 – 20 μm for CHO cells).²⁷ Yeast cells were retained with >90% efficiency in the single spiral and also in a multiplexed device with 180 spiral microchannels that could process 320 ml/min medium, which represents an improvement compared to commonly used cellulose acetate and teflon filters.²⁷

In a different experiment (Table I), Warkiani and co-workers could show that even cell-cycle synchronization is possible in spiral microchannels based on size differences in the stages of the cell-cycle. Cells in the G_0/G_1 phase were separated from G_2/M -phase cells, which are generally larger in size, at a cell concentration of 10^6 cells/ml and a flow rate of 1 ml/min. After the separation process, smaller cells with diameters $<14 \mu\text{m}$ were enriched more than 2.7 fold at the outer outlet. It would thereby be possible to retain only highly productive growing cells in the perfusion process.

In 2017, Kwon *et al.*³⁶ actually incorporated spiral microfluidic devices in perfusion processes for cell retention over a course of 18 – 25 days with peak CHO cell concentrations of 20 - 30×10^6 cells/ml (Table I). In their first experiment, the process was run in a 350 ml bioreactor for 4 days in a batch mode with subsequent perfusion mode for another 14 days, applying a perfusion rate of two vessel volumes per day. The goal of the process was IgG₁ production. On day 10 the peak cell concentration of 22.7×10^6 cells/ml was reached with a cell viability of $99 \pm 1\%$. For cell concentrations $<15 \times 10^6$ cells/ml, a retention efficiency of $99 \pm 2\%$ could be achieved which dropped to $82 \pm 3\%$ for cell concentrations in the range 20 - 23×10^6 cells/ml. Within 18 days, 263 mg IgG₁ were produced.

Separately from the perfusion processes, Kwon and co-workers investigated cell retention efficiencies for even higher cell concentrations using increased channel dimensions

(1000×260/80 μm).³⁶ At a flow rate of 4 ml/min, retention efficiencies of >84% could be reached for a CHO cell concentration of 43.6×10^6 cells/ml (Table I).

FIG. 2. General setup for cell retention with spiral, trapezoidal microchannels in perfusion bioprocesses. Medium from the bioreactor is pumped through the spiral at a specific flow rate where the dispersed cells (A) get focused at the channel's inner wall by hydrodynamic effects (B). Cells then exit the spiral from the inner outlet and are lead back into the bioreactor while cell-free medium is obtained from the spiral's outer outlet and e. g. used for product recovery (C).

This shows that inertial microfluidic devices can also be used for processes with high cell concentrations although they might probably not be applicable for current high-density processes with cell concentrations $>100 \times 10^6$ cells/ml because of the small channel dimensions. By parallelizing the spirals, high throughputs may be generated which make inertial microfluidics more feasible for up-scaling to industrial processes.

B. Separation of live and dead cells

Removing nonviable cells and debris is often a crucial step in bioprocesses as dead cells can affect product yield by, for instance, releasing large amounts of proteases into the medium.³⁷ They can thereby also downgrade the product's quality. In CHO bioprocesses, it could be shown that dead cells make up for up to 30% of the total produced biomass.³⁸ Cell death in bioreactors is amongst others caused by apoptosis and also by shear stress through stirring and sparging in the reactor.³⁷ Current methods for separation of dead cells include inclined settlers³⁹ and more recently also compact settlers.⁴⁰

TABLE I. Overview of applied process parameters for presented applications of spiral microfluidic devices.

Application	Cell concentration [$\times 10^6$ cells/ml]	Input flow rate [ml/min]	Number of spirals	Loops per spiral	Particle	Dimensions [μ m]	Separation/retention efficiency [%]	Reference
Cell retention	1.0	6.0 ^a	4	n. a.	CHO	80/130 $\times$ 600 ^c	>95	27
Cell retention	10.0	500.0	336 ^c	n. a.	CHO	80/130 $\times$ 600	n. a.	27
Cell retention	0.1	2.0 ^a	8	n. a.	Yeast	30/70 $\times$ 450	90	27
Cell retention	0.1 [g/l]	320.0	180 ^c	n. a.	Yeast	30/70 $\times$ 450	>90	27
Cell cycle synchronization	1.0	1.0	1	n. a.	CHO	80/130 $\times$ 600	n. a.	27
Cell retention	4.8	1.0	1	8	CHO	80/130 $\times$ 600	99	36
Cell retention	43.6	4.0	20 ^c	6	CHO	260/80 $\times$ 1000	>84	36
Live-dead cell separation	3.5	1.5	1	8	CHO	80/130 $\times$ 600	99.7	41
Live-dead cell separation	10.0	8.0	2 ^d	6/8	CHO	80/130 $\times$ 600 200/140 $\times$ 1000	99.9	41
Live-dead cell separation	4.0	6.0	4 ^c	6	CHO	80/130 $\times$ 600	99.9	41
Removal of contaminants	0.3 – 6.3 ^e	1.0	1	8	Micro-algae	80/130 $\times$ 600	>90	12
Parallelization	n. a.	1000.0	20 ^c	2.5	Micro-beads	536 $\times$ 3000	88	17
Cascading	n. a.	22.0	3 ^d	6	Micro-beads	536 $\times$ 3000	~95	17
		12.0				336 $\times$ 1800		
		7.0				236 $\times$ 1200		

^a for a single spiral

^b heights of outer walls $\times$ width of channel's base; microchannels with a trapezoidal cross-section were used except for the last two applications with microbeads where rectangular channels were used

^c parallelized spirals were used.

^d cascaded spirals were used.

^e *P. tricornutum* concentrations from 0.3 – 6 $\times 10^6$ cells/ml and a fixed *T. suecica* concentration of 0.3 $\times 10^6$ cells/ml were used.

Kwon *et al.*⁴¹ applied inertial microfluidics to separation of nonviable cells and cell debris from viable CHO cells. CHO cells are the most frequently used hosts for expression of recombinant proteins, accounting for more than 70% of the total worldwide recombinant proteins. Separation of the generally smaller dead cells in inertial microfluidic systems is difficult, because viable and nonviable cells overlap partly in size which sets a natural limit for the efficiency of the separation process.⁴¹ The size difference is caused by cell-shrinkage in the early stages of apoptosis which is important for regulating the activity of apoptotic nucleases and caspases.⁴² In their experiments⁴¹, Kwon and co-workers focused on maintaining high viable cell concentrations while concurrently removing as many dead cells as possible.

In a first experiment (Table I), their system was tested for separation of cells $<10\ \mu\text{m}$ with 3.5×10^6 cells/min, a flow rate of 1.5 ml/min and a split ratio of 0.36 which is defined as outer outlet flow rate divided by inner outlet flow rate. Dead cells and debris were focused at the outer outlet and a live cell retention efficiency of 99.7% was reached with a dead cell removal efficiency of 6.1%. They then proceeded to characterize their device by examining the correlation of cell concentration, flow rate, cell viability and flow split ratio on live cell retention efficiency, dead cell removal efficiency and dead cell removal purity which they defined as portion of nonviable cells in the outer outlet (Table II). Especially cell concentration seems to have a big impact on the separation process which can be explained by an increase of cell to cell interactions in the small microchannel with increasing cell concentration. In their final experiment, effects of cascading and parallelization on separation efficiency were investigated. For cascading, a wide spiral with six coils was connected to the already characterized narrow microchannel with 8 coils as a first stage of size-dependent separation (Table I). Flow rate could be increased to 8 ml/min and cell concentration was set to 10×10^6 cells/ml. This resulted in a

live cell retention efficiency of 99.9% while the dead cell removal efficiency dropped to 3.4%. In the parallel approach with four spirals (Table I) and inverted fluid flow at 4×10^6 cells/ml cell concentration and 6 ml/min flow through, the same live cell retention efficiency could be reached but dead cell removal efficiency dropped even further to 3%. However, even a small dead cell removal efficiency can have big impacts on bioprocesses as microfluidic systems can be run continuously.

TABLE II. Effect of process parameters on separation measures, measured at cell concentrations from 1 - 10×10^6 cells/ml, flow rates from 0.9 - 1.5 ml/min, viabilities from 30 - 80% and flow split ratios from 0.32 - 0.85 in a spiral microfluidic device with eight loops, an inner depth of 80 μm , an outer depth of 130 μm and a width of 600 μm .⁴¹

	Cell concentration	Input flow rate	Cell viability	Flow split ratio
Live cell retention efficiency ^a	-	0	0	-
Dead cell removal efficiency ^b	++	+	-	++
Dead cell removal purity ^c	--	+	-	- ^d

^afraction of total viable cells in inner outlet.

^bfraction of total dead cells in outer outlet.

^cpurity of nonviable cells in outer outlet

^d0 means no correlation was observed; - means weak negative correlation; -- means strong negative correlation (separation measures change >10 % in measured range); + means weak positive correlation; ++ means strong positive correlation (separation measures change >10 % in measured range)

C. Removal of contaminants in microalgae cell cultures

Microalgae cells are becoming more prominent in biotechnological processes, especially as source of biomass and production hosts for biofuels because of their ability to capture carbon dioxide.¹² However, processes working with microalgae cells are especially susceptible to contaminations, primarily by zooplankton, phytoplankton-lytic bacteria, virus and other algae due to difficult sterilization processes.⁴³ These cross-contaminations with other microalgae species are often inevitable. Resource competition and secretion of harmful secondary metabolites thereby leads to drastic decreases in process yield and product quality. Current methods to treat these contaminations include microfiltration, addition of chemicals acting against the pollutant and changes in environmental conditions like harsh changes in pH.⁴³

In laboratory set-ups, time-consuming and labor-intensive techniques like serial dilutions and selective agar plates are usually applied to reduce contaminations. Syed *et al.*¹² investigated the potential of inertia based microfluidic systems on separating the common invading microalgae *Phaeodactylum tricornutum* from a culture of *Tetraselmis suecica* cells. After tests with 6 μm and 10 μm microbeads and microalgae cells, they found that the best separation was reached at a flow rate of 1 ml/min. The fusiform *P. tricornutum* with approximate diameters of $25.7 \pm 3.5 \mu\text{m}$ was focused at the outer outlet whereas *T. suecica* with a diameter of $10.7 \pm 0.8 \mu\text{m}$ exited through the inner outlet. Both cell types were focused with efficiencies $>90\%$. In a second experiment (Table I), cell concentrations of *P. tricornutum* were gradually increased from 0.3×10^6 cells/ml to 6×10^6 cells/ml while the *T. suecica* concentration was held at 0.3×10^6 cells/ml to investigate the impact of pollutant concentration on the separation process. At all concentrations, *P. tricornutum* focusing efficiencies remained over 90% which demonstrates the insensitivity of the method to the contaminant's concentration. After the separation, no measurable change in *T. suecica* vitality could be detected which distinguishes inertial microfluidic cell separation from other common methods for removal of contaminants. *T. suecica* was then reinoculated to test the sustainability of the purification process and it was found that *P. tricornutum* contamination remained suppressed until day 10 and at the end of the third week, contamination was still fifty times lower than in unpurified samples. By applying the separation process in a, for instance, two-week interval, *P. tricornutum* contamination could be suppressed. However, it was remarked that parallelization of the process is essential for upscaling to an industrial scale.

Li and co-workers⁴⁴ also worked on separating microalgae cells. They used straight microchannels to separate cells of the biodiesel producing alga *Euglena gracilis* based on

different shapes of the same organism to gain a shape-synchronized population. 10^5 cells/min could thereby be separated using five different outlets. In a different experiment by Condina *et al.*⁴⁵, spiral microchannels were used to separate beer spoilage bacteria from yeast for subsequent identification using mass spectrometry. Separation efficiencies of >90% were reached at a flow rate of 1.5 ml/min. They could thereby improve the limit of detection for common contaminating bacteria in the beer industry and reduce the time for detection of contaminations.

D. Bead-linked separation of specific cells and proteins

For spiral microchannels, a confinement ratio of $a/D_h > 0.07$ was shown empirically, below which all particles flow through the outer outlet.⁴⁶ From this follows that the minimal cell size that can be focused in spiral microfluidic devices is limited by the channel dimensions. Sarkar *et al.*⁴⁷ developed a method that can evade this problem by linking cells to microbeads of different size with subsequent microfluidic separation. By binding specific cells to antibody-coated microbeads (10 and 15 μm) with biotin-streptavidin linkages in a single binding step, they were even able to separate CD4+ from CD8+ T cells and T cells from B cells although these cells show only minimal differences in size (6 – 8 μm). For the latter approach, microbeads were coated with anti-CD3 and anti-CD19 antibodies, respectively. Their Dean Flow Fractionation Device (channel height was 115 μm) was thereby able to process around 10^5 beads/s. It could be observed that the focusing position in the channel was determined by the bigger part of the bead-cell pair. In case microbeads and cells had the same size, an additive effect of the two sizes on focusing behavior was observed. In a different approach, Sarkar and co-workers separated three specific HIV-antibodies from total IgG-fractions of serum containing less than 1% antibodies that they targeted.⁴⁷ Smaller microbeads with diameters of 10, 4.5 and 1 μm were used to improve surface to mass ratio which in turn improves antibody binding efficiency.

Approximately 95% of all three antigen-specific antibodies were purified with less than 5% cross-contaminations.

The bead-linked separation approach could be used in the purification process of monoclonal antibodies and for separating small cells like bacteria which has only been done in few cases.^{48,28} A cascaded setup could potentially even enable separation of a large number of different proteins or similar-sized cells.

III. ADVANCES IN THROUGHPUT

For most industrial processes, cell separation with flow rates in milliliter-scale, as shown by the previously mentioned applications, are insufficient. A single microchannel, however, can only process small flow rates while still maintaining efficient particle separation. Increasing flow rates in microfluidic devices can therefore only be done effectively by cascading or parallelization. Efficient upscaling of channel dimensions is restricted by the confinement ratio and as the biotechnologically relevant group of bacteria is very small (about $1 \times 3 \mu\text{m}$ for *E. coli*⁴⁹), channel dimensions have to be kept small, too. Cascading has already been applied, e. g. for separation of cells from blood samples^{50,51} but the focus rather lying on reaching higher separation efficiency compared to single devices than increasing throughput. Parallelization is difficult because in-plane parallelization would occupy too much space and stacked systems suffer from differences in inlet pressure depending on the distance of the microchip to the pump.

Since the flow rate is a constitutive factor for reaching wanted equilibrium positions, Miller *et al.*¹⁷ developed a modular manifold, enabling equal inlet pressure distribution on a stack of 20 spiral microchannels with a toroidal channel design (Table I). This allowed them to separate microbeads with sizes of 45 and 250 μm at a flow rate of 1 l/min which excels previous

parallelization approaches of inertia-based devices significantly.^{27,48} They thereby focused ~88% of the large microbeads in the inner outlet while ~73% of the small particles exited the spiral through the outer outlet.

In a cascaded approach (Table I), described in the same paper, Miller and co-workers used spirals of three different widths (500, 300, 200 μm) to separate microbeads reaching from 1 – 300 μm . Cascading allowed them to successively remove larger particles down to the smallest microbeads. To increase recovery rates, two recirculations were performed per separation step. By using a spiral with a comparably large cross-sectional area first, higher flow rates can be applied. It was also shown empirically in their experiments that for large spirals ($>300 \mu\text{m}$), the minimum focusing size rather correlates with $0.021356 \times H^{1.33623}$ where H is the channel height. In this case, an initial flow rate of 22 ml/min was set which automatically decreased to 12 ml/min in the second spiral and 7 ml/min in the third spiral. In the 500 μm spiral, ~95% of particles $>95 \mu\text{m}$ could be focused whereas in the 200 μm spiral, ~96% of particles $>50 \mu\text{m}$ were separated. Increasing the number of recirculations could eventually increase separation efficiencies even further.

However, it should be taken into account that most industrial bioprocesses are based on cell cultures with densities several orders of magnitude higher compared to the cell suspensions in the described experiments. Cell concentrations can easily exceed $10^7 - 10^8$ cells/ml.⁵² This leads to a severe increase in cell-to-cell interactions and might subsequently lead to clogging of the microchannel. In recent work by Maloudi *et al.*⁵³ particle separation in scaled-up microchannels (500/900x2000 μm) was examined. By increasing channel size at a constant throughput, cell-to-cell interactions could be reduced. It was shown that increased channel dimensions clearly lower the inertia of flow but particle separation was still possible. Additionally it could be shown that

even a single-loop trapezoidal channel is able to separate particles sufficiently. Reducing the channel's length could therefore be another possibility to decrease the risk of clogging without diminishing the device's performance too heavily. The experiments⁵³ were conducted using a suspension of microcarriers (100 μm diameter) and mesenchymal stem cells. As shown by Maloudi *et al.* in 2018⁵⁴, microcarriers can be separated from mesenchymal stem cells at a flow rate of 30 ml/min and with a total yield of 94% using spiral microchannels. This indicates that an increased throughput can not only benefit cell separation for industrial purposes but also medical applications. Different medically relevant cell types like aforesaid mesenchymal stem cells, circulating tumor cells^{19,20} and chondrocytes⁵⁵ could thereby be separated even faster from cell mixtures.

IV. CONCLUSIONS

Inertial microfluidic systems could already be applied for several bioprocessing steps, including replacement of microfilters, live-dead cell separation, removal of contaminating microalgae species and separation of specific cells and proteins by linkage to differently sized beads. As applying inertial systems for bioprocesses was just introduced recently, it is expected that the presented methods will be severely improved in the next years. The flexibility and scalability of these systems also allows for application to entirely different processes. The most relevant issue currently is parallelization, which is essential for upscaling to industry size. As natural limits are set for the flow rate in a microchannel, cascading and parallelization are the only ways to increase throughput beyond 1 l/min while still maintaining efficient particle separation. Although parallelizing microchannels appears easy, pumps are needed, leading to high energy costs with increasing system size which might make these systems unfeasible for industrial scale processes. Risks of system failure are, however, minimized by a parallel setup.

These risks include primarily clogging of the microchannels through cell agglomeration. Inertial microfluidic systems still have to be adapted to some biotechnologically relevant organisms like fungi and bacteria. The bead-linked approach could help here to apply microfluidic separation processes to other protists of different size.

ACKNOWLEDGMENTS

We gratefully acknowledge the funding of the Federal German Ministry of Education and Science (BMBF) for the program "New products for the bioeconomy", project no. 031B0381 (SepaDiElo). We also acknowledge support by the Open Access Publication Funds of TU Berlin.

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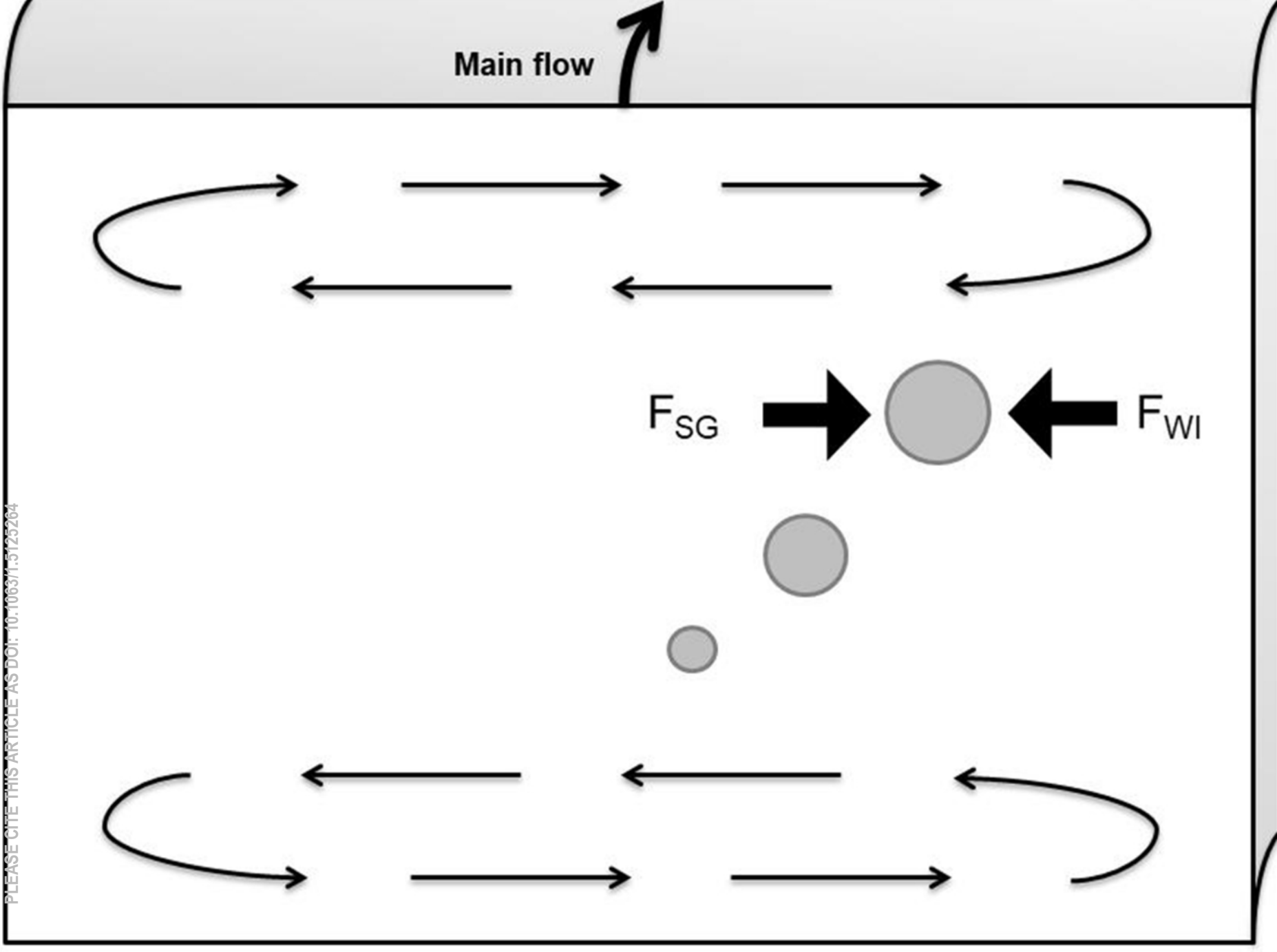
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