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Advances and enabling technologies for phase-specific cell cycle synchronisation

This work critically reviews the cell cycle synchronisation approaches and discusses their operational principles and performance efficiencies. The review also highlights the advances and technological development trends from conventional methods to the more recent microfluidics-based systems. Furthermore, the article discusses the opportunities and challenges for implementing high throughput cell synchronisation and provides future perspectives on synchronisation platforms, specifically hybrid cell synchrony modalities, to allow the highest level of phase-specific synchrony possible with minimal alterations in diverse types of cell cultures.



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Advances and enabling technologies for phasespecific cell cycle synchronisation

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Cell cycle synchronisation is the process of isolating cell populations at specific phases of the cell cycle from heterogeneous, asynchronous cell cultures. The process has important implications in targeted geneediting and drug efficacy of cells and in studying cell cycle events and regulatory mechanisms involved in the cell cycle progression of multiple cell species. Ideally, cell cycle synchrony techniques should be applicable for all cell types, maintain synchrony across multiple cell cycle events, maintain cell viability and be robust against metabolic and physiological perturbations. In this review, we categorize cell cycle synchronisation approaches and discuss their operational principles and performance efficiencies. We highlight the advances and technological development trends from conventional methods to the more recent microfluidics-based systems. Furthermore, we discuss the opportunities and challenges for implementing high throughput cell synchronisation and provide future perspectives on synchronisation platforms, specifically hybrid cell synchrony modalities, to allow the highest level of phase-specific synchrony possible with minimal alterations in diverse types of cell cultures.

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1 Introduction

Cell cycle synchronisation is a process where asynchronous cell cultures are sorted to isolate cell populations according to their specific cell cycle stages. For 50 years, numerous cell cycle synchronisation methodologies have been explored to

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study the cellular properties and cell cycle mechanisms. Grouping distinct subpopulations at specific cell cycle stages have allowed the discovery of disease-specific biomarkers and the development of drugs.⁵

Cell cycle synchronisation is essential to study cell cyclespecific events (Box 1). Differentiating asynchronous cells into phase-specific cell populations offers a unique advantage in studying the molecular and structural events, specifically cell cycle regulatory mechanisms at the level of gene expression and post-transcriptional modifications.⁶



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inertial microfluidic devices for particle/cell separation, bioprocessing industry, and intracellular delivery.

Critical review

Synchronisation has allowed identifying several oncogenes and tumour suppressor genes and their implication in specific cell cycle stages. They have helped to screen various anticancer drugs that selectively target cancer cells staged at particular phases. The synchronisation is also critical in targeted cell therapies that involve nuclear transfer to host cells, as studies show that cells synchronised to G0/G1 phases had higher nuclear transfer efficiency.^{9,10}

The early approaches to cell cycle synchronisation include both chemical blockade and biophysical fractionation techniques. The chemical blockade is performed by adding/ depleting chemical agents that arrest or stall cell cycle progression at specific stages by targeting metabolic reactions.¹¹ The physical fractionation techniques like centrifugal elutriation and FACS exploit the cell cycle phasespecific variations in properties like cell size and DNA content to sort the sub-populations.^{6,12,13} Recently, microfluidic cell separation systems have been developed for cell cycle synchronisation. These systems allowed high throughput cell synchronisation, particularly for low or medium sample volumes. Certain studies have also implemented microfluidic platforms in combination with conventional chemical/physical separation techniques for effective cell cycle synchronisation.¹⁴

While many cell synchronisation methodologies have been explored, the ideal process would be the one where cell cycle synchrony can be achieved for all types of cells, and be maintained across multiple cycles of the cell cycle and maintain cell viability with minimal metabolic and physiological perturbations.¹⁵ The synchronisation of cell populations has substantial research interest and motivated researchers to further the understanding of various biological systems (Fig. 1). Although multiple studies have investigated specific cell cycle synchronisation techniques, there is a significant gap in the literature for a review article covering the development and performance of the multiple cell cycle synchronisation modalities. In this review, we present a broad comparison of synchronisation strategies and scrutinize their advantages and disadvantages. We discuss the operational principles of various techniques and focus on the performance of cell cycle synchrony in multiple cell types, as evidenced by relevant scientific literature. Additionally, we discuss the future perspectives and emerging opportunities to enable large scale, high throughput cell synchronisation approaches.

2 Conventional cell cycle synchronisation techniques

2.1. Chemical blockade/cell cycle arrest

The most conventional cell synchronisation technique is the chemical blockade or cell cycle arrest method, where cell cultures are treated with drugs that block or delay the progression of the cell cycle at specific phases.² The drugs inhibit critical cell cycle events necessary for the progression of the cell cycle process. The drug dosage and incubation time for blockade vary between different cell lines and the cell cycle phase/checkpoint that the drug targets. The most common type of blockade is arrest and release, which involves treating the cell culture with drugs/blocking agents at specific doses for a particular time duration.¹⁶ After treatment, the cell cultures are rinsed, or a neutralising agent is added to remove the blocking effect of the drugs.¹⁷ Arrested cells are then harvested and reintroduced into a growth medium to continue their progress through the cell cycle. Cell cycle synchrony is assessed by fixing and staining cells with DNA binding dyes, followed by quantitative cell cycle phase analysis by flow cytometry.⁶ Phase synchrony can also be achieved by inducing nutrient/serum deprivation in cell cultures to delay the progress of cell cycle events at specific stages.¹⁴ The chemical agents targeting specific cell cycle phases broadly fall under these categories, listed below.



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The cell cycle process consists of four phases; growth phase 1 (G1), synthesis phase (S), growth phase 2 (G2), and mitosis phase (M).¹ The two growth phases separate the visible events of the S and M phases. While there are no observable cellular changes at G1 and G2 phases, cells at these phases are responding to signals from their cell environments to maintain the correct conditions for cell division events. Cells at the G1 phase prepare for entry into the S phase by activating the genes for DNA and chromosomes replication. Cells at the G2 phase interpret intracellular signals from checkpoint pathways monitoring the fidelity of the duplication event completion and the functionality of the mitotic spindle apparatus. The bulk of cellular events happen in the S and M phases. At the S phase, the synthesis of DNA and duplication of centrosomes occur, and at the M phase, separated chromatids facilitate the segregation of sister chromatids and nuclear division (karyokinesis), followed by cell division (cytokinesis) to form independent daughter cells.

The progression of cell cycle is regulated by various checkpoints, often with the involvement of enzyme kinetics, structural biology, and posttranslational modifications.² The three primary checkpoints include the G1/S checkpoint that checks the cell size, growth factors, and DNA damage, the G2/M checkpoint that checks the DNA integrity and the completion of DNA replication, and the M phase spindle checkpoint that checks the chromosome attachment to spindles at the metaphase plate. The function of checkpoints is regulated by a group of protein and enzyme complexes triggered by molecular signals and internal cues like DNA damage. Cyclins (proteins associated with specific cell cycle phases) and cyclin-dependent kinases (CDKs) (family of enzymes that activate/deactivate target proteins of each cell cycle phase) are the core cell cycle regulators.^{3,4} For example, cyclin D1 is a transcriptional regulator which depends on the cell cyle process. It is highly expressed in the late G1 phase, recruits transcriptional corepressors and coactivators to endoderm genes and neuroectoderm genes, and blocks or promotes the induction of corresponding germ layers.^{7,8} Defects and dysfunction of the cell cycle result in events like cell cycle reprogramming and uncontrolled cell proliferation that are associated with cancers and related diseases.²



Fig. 1 Timeline of the latest and notable studies discussed in the review that have successfully implemented conventional (physical fractionation methods: mitotic detachment, centrifugal elutriation, flow cytometry and chemical blockade techniques) and microfluidics-based (dielectrophoresis, acoustophoresis, hydrophoresis, inertial microfluidics) cell separation methods towards achieving phase-specific cell cycle synchronisation.

1. M-phase targeting agents: nocodazole, CDK inhibitors, roscovitine, and colchicine, induce mitotic arrest at various M-phase points, including the G2/M transition and mitotic exit. Nocodazole arrests cells at the spindle assembly checkpoint between the metaphase and anaphase stages, where it functions by inhibiting microtubule polymerisation. It blocks the formation of mitotic spindles that pull apart sister chromatids towards opposite poles during mitosis (Fig. 2A).¹⁷ Similarly, colchicine is a mitotic poison that depolymerises tubulin in microtubules and arrests cells at the metaphase stage and blocks progression to anaphase.¹⁵ Colchicine has been used to synchronise cells to G2/M phases from asynchronous pig mammary cells and fibroblasts.¹⁸ As an example of CDK inhibitors, RO-3306 can reversibly arrest >95% of cells at the G2/M phase with minimal metabolic perturbations.¹⁹

2. S-phase targeting agents: these agents are S phase (G1/S phase arrest) arresting chemicals that function by inhibiting DNA replication *via* the treatment of cell cultures with agents like hydroxyurea, aphidicolin, and thymidine (double thymidine block). Hydroxyurea functions by disrupting the function of ribonucleotide reductase (enzyme), resulting in

decreased dNTP production.¹³ This disrupts the DNA synthesis by depriving DNA polymerase for the dNTPs at the replication forks (Fig. 2A).²⁰ Apart from mammalian cells, hydroxyurea has also successfully synchronised protozoan cells to G1/S cell phases.^{13,21,22} Thymidine functions by disrupting the DNA metabolism pathway, thereby blocking DNA replication. Several studies have recognized the effectiveness of the double thymidine block process to synchronise multiple human (H1299 and HeLa cell lines) and murine tumour cells (EO771 cell line), with >95% cells synchronised at the S phase of the cell cycle (Fig. 2F).^{23–25}

3. Combined usage of blocking agents: in this technique, two or more blocking agents induce cell cycle arrest and synchronise cells at specific cell cycle phases. Several studies have shown that the use of multiple blockers in combination has improved the efficiency of cell synchronisation. Whitfield *et al.* performed cell cycle arrest by first blocking with thymidine, then releasing and subsequent blocking with nocodazole to arrest cells at the M phase. This allowed better synchrony at the G1–S phases of the subsequent cycles because of the release at the M phase.²⁵ Doida and Okada synchronised L5718Y mouse lymphoma cells by successive treatment with excess thymidine followed by the treatment with colcemid (colchicine), where the method applied in one cell generation yielded a high degree of synchrony.²⁶ However, not all combinations of metabolic blocking agents yield synchronised cell populations.²⁷ Thus, cell

synchronisation techniques and their combinations should be chosen to ensure that the process does not affect the cell cycle progression.

Chemical synchronisation methods are suited for obtaining large numbers of synchronised cells. However, the



Fig. 2 Schematic illustrating the operating principle of conventional cell cycle synchronisation methods. A) Chemical blockade: the schematic represents the mechanism by which the drugs induce cell cycle synchrony. Hydroxyurea or thymidine induces G1/S phase cell cycle arrest by disrupting the DNA metabolism pathway and depriving DNA polymerase, thereby inhibiting the DNA replication process. Roscovitine or nocodazole induce G2/M arrest by inhibiting the function of associated cyclins and CDKs responsible for the cell cycle progress. B) Flow cytometry/FACS: the schematic represents how cells of an asynchronous culture are analysed one at a time by a laser beam and light scatter properties are measured by a detector. The technique exploits the overall correlation between cell size and cell age of proliferating cells. C) Centrifugal elutriation: this technique separates cells into specific cell cycle phases based on their size and sedimentation velocity. Cells are introduced into the elutriation chamber with a continuous buffer flow while centrifuged at a uniform speed. These cells are maintained in the chamber at a low flow rate, creating a size gradient. As the flow rate is sequentially increased, the cells start to elute from the chamber in order of size, with the smallest cells (G1 cells) eluting first. D) Mitotic detachment: the schematic represents how cells at the mitosis phase become spherical, resulting in decreased surface volume and are detached and collected from the asynchronous monolayer culture on gentle shaking or inside a standardised shaker. E) Baby machine: this device is operated under pressure variations. A positive pressure induced at the inlet creates pressure differentials in the vertical direction to capture cells across the holes, and pressure differential in the horizontal direction, which causes a lateral flow to collect the synchronised daughter cells (G1 phased cells). Asynchronous cells are introduced and are captured onto the holes while maintaining a steady availability of cell medium allowing cells to grow within. The newborn cells get unattached and are carried away and eluted into a collection chamber as they proliferate. These are the cells that have just completed cytokinesis and entered the G1 phase. F) Cell cycle synchrony performance by chemical blockade: cell cycle analysis data after treatment with control, thymidine double block 2 mM and colchicine 10 nM. DNA content was measured by flow cytometry post-treatment of EO771 cells.²⁴ The data shows enrichment of the S-phase cell population after treatment with thymidine and G0/G1 enrichment on colchicine treatment. Reproduced from ref. 24 with permission from John Wiley and Sons G) cell cycle synchrony performance by flow cytometry/FACS: size-based sorting of L1210 cells, yielding G1 phase enriched cell populations.³⁴ Cells were gated for pre-sorted and post-sorted cell populations and stained with PI for DNA content quantification. The data shows ~90% synchrony to G1 phase of the cell cycle in both cell populations. Reproduced from ref. 34 with permission under open Creative Commons Attribution License.

treatment with chemical agents alters the metabolism and biochemical balance of cell growth and disrupts the cell cycle progression. They have also been known to promote malignant characteristics in cancer cells.^{28,29} Another limitation with chemical methods, especially with cancer cells, is the risk of generating dormant cancer cells. These cells can survive and be reactivated to give rise to metastatic disease, often after successfully treating the primary tumour. Dormant cells undergo cell cycle arrest at G0/G1 phases, making them resistant to cytotoxic chemotherapeutics targeting mitotically active cells (G2–M phases). Thus, some blocking agents might produce resistant dormant cells, and all factors in selecting these agents should be considered.³⁰

2.2. Physical fractionation

Physical fractionation techniques exploit the physical properties of cells, including cell size, cell density, fluorescence emission of labelled cells, and light scatter analysis, to collect cells at specific stages of the cycle from an asynchronous population. These techniques do not involve treating whole cell cultures with chemicals; therefore, it minimises the risk of inducing unintended alterations in cell functions. These techniques include flow cytometry/FACS, centrifugal elutriation, mitotic detachment, and baby machine. Among these, flow cytometry and centrifugal elutriation are used more frequently.

2.2.1. Flow cytometry/FACS. Flow cytometry is the gold standard method for cell cycle synchronisation due to its mature engineering development, sensitivity, and high throughput. In this technique, cells are identified by fluorescence signals when the cell solution flowing in a stream passes a detector at a relatively high speed. The technique provides a high sensitivity of cell surface markers at the single-cell level. With the advances in fluorescent dye and laser technology, flow cytometry is able to track multiple parameters simultaneously.31 Cells are usually fixed and stained with DNA-binding dyes like propidium iodide, Hoechst, DAPI, 7-aminoactinomysin D (7AAD), which allows the determination of the cell cycle phase based on DNA quantity. Additionally, fluorescent labelling of cells and cytosolic expression of specific proteins conjugated with fluorescent tags like GFP can be used to select a specific cell population. The detector checks for either the presence or absence of fluorescence signals emitted from each cell or the light scattering properties of each cell. The conventional process involves ejecting single cells in liquid droplets (~70 µm). Each droplet is analysed by the detector, and based on gating conditions, is sorted differentially to enrich specific cell populations (Fig. 2B). A typical flow cytometry system can routinely sort ~10000 cells per second while tracking 4-5 different fluorescence wavelengths, thereby tracking multiple cell surface markers or tagged bioanalytes.³² Flow cytometry is also used to investigate the performance of other cell cycle synchronisation techniques like chemical blocking (double

thymidine block, hydroxyurea), centrifugal elutriation, or hybrid systems.

Vecsler et al. demonstrated that cell size approximated by light-scatter parameters in standard flow cytometry systems was able to synchronise proliferating HEK293 cells in the G1 phase with minimal perturbations to the cell cycle or cell viability. Proliferating cells exhibit variation in both rates of cell growth and cell cycle progression. Therefore, average cell size correlates to cell age and thus, proliferating cells can be synchronised to the G1 phase based on the size. In this study, cells were labelled with Hoechst stain to correlate FSC-W intensity (forward scatter) with DNA content, and flow cytometry was used to quantitate their DNA content. To minimise the mechanical perturbations on sorted cells, cell cycle synchronisation process was carried out at optimum flow rate and pressure. Cell viability of the sorted cells was \sim 99%, indicating that the HEK293 cells could tolerate the sorting process. The DNA content distribution of pre-sorted vs. post-sorted cells showed highly synchronised G1 phased cell populations (90-95% G1 cells in post-sorted cells vs. \sim 45% pre-sorted cells) (Fig. 2G). Flow cytometric cell sorting for G1 synchronisation has been successfully implemented in other adherent and non-adherent cells like A549 and L1210 cells, with 95-99% of cells synchronised to G1-phase.³³ While the process demonstrates strong selectivity, the synchronised cells are not guaranteed to remain synchronous throughout the cell cycle as it does not account for the size-to-age correlation in proliferating cells. Thus cell cycle progression of cells needs to be monitored post synchronisation.³⁴

2.2.2. Centrifugal elutriation. Centrifugal elutriation exploits the correlation between cell size at specific cell cycle stages and fractionates asynchronous cell populations based on their sedimentation properties, while inducing minimal perturbations on the cellular functions.³⁵ The major advantage of this technique is that it allows the selection of cells staged at all phases of the cell cycle,³⁶ thus allowing the assortment of phase-specific subpopulations. Counterflow centrifugal elutriation has been implemented to synchronise asynchronous populations of a wide variety of cells, including proliferating tumour cells.^{37,38} In these devices, asynchronous cells are loaded into the elutriation chamber and is centrifuged at a constant speed while maintaining a buffer flow. Upon introduction, the cells are retained in the chamber at a low flow rate, creating a size gradient.³⁹ The size gradient is balanced by the centrifugal force and the chamber fluid, allowing the cells to float inside the chamber. As the flow rate is progressively increased, the cells start to elute from the chamber in the order of size, with the smallest cells (G1 cells) eluting first followed by the larger ones (Fig. 2C).¹⁵

In this technique, physical properties like sedimentation velocity and cell size are determinant factors. The sedimentation rate is primarily dependent on the size of the cells in asynchronous cell populations, while the effect of cell density is negligible. This allows larger cells to remain longer in the chamber, even in homogenous cell cultures, as the

smaller cells are released first. As a result, the centrifugal technique can isolate cells within the same culture based on differential cell sizes.¹⁵ This technique can separate cells into all cell phases by exploiting the consistent growth patterns across the different cell cycle stages based on increasing cell sizes. The early elution product is mainly comprised of G0/G1 staged cells while the S and G2/M stages cells are eluted subsequently.^{10,40} Cell synchronisation through centrifugal elutriation is primarily effective in exponentially growing cells, where most of the cells are in the S phase.¹⁵ Cells in suspension medium that do not adhere to each other and have round, uniform shapes are better suited for centrifugal elutriation. However, some adherent cells can be trypsinised and resuspended to become more spherical/uniform for the elutriation process.41 The size-based segregation of differentially staged cells is also affected by changes in the speed of the rotor and flow rates of the elutriation fluid. The centrifugal elutriation process itself imposes significant mechanical stress on the cells, which consequently affects cell viability.42

Cell cycle synchronisation by centrifugal elutriation has been effective with mammalian cells (\geq 97% G1 cells, \geq 80% S cells, and 70–75% G2 cells of asynchronous 9 L rat brain tumour populations),⁴⁰ yeast cells (97% cells synchronised to G1 in *S. cerevisiae*)⁴³ and also parasitic cells like *T. brucei* where 96–97% cells were synchronised to early G1 stage of the cell cycle.¹² To assess the quality of synchronisation, flow cytometry is used to monitor size distribution and DNA content of the elutriated fractions after staining with DNAbinding dyes like propidium iodide.⁶

2.2.3. Mitotic shake-off/detachment. This method of cell synchronisation exploits the property of mitotic cells in monolayer cell cultures to become spherical and detach from the surface of the vessel on gentle shaking in a standardised shaker/centrifugation (Fig. 2D). The method obtained up to 95% of HeLa cells synchronised to the M phase.^{6,44} In terms of duration, mitosis constitutes 10% of the cell cycle, and for collecting large numbers of synchronised populations, the process needs to be repeated multiple times at specific time intervals. The method is also performed in combination with agents like hydroxyurea and is an effective cell cycle synchronisation technique for collecting relatively pure cells with minimal metabolic perturbations.⁴⁵ However, mitotic detachment is only applicable to anchorage-dependent monolayer cell lines such as HeLa and CHO cells.

2.2.4. Baby machine/membrane elution. Helmstetter *et al.* developed a membrane elution technique for cell cycle synchronisation, also known as the baby machine.⁴⁶ This technique is similar to the mitotic shake-off method; batch synchronisation which involves growing cells bound to a membrane. As they divide, the newer cells remain attached while the older cells are eluted. The process is suited for synchronising cells to early G1 phase. The technique was first used for bacterial cells and later for mammalian cells.⁴⁷ While this method has minimal perturbations on cells, the process entirely depends on the membrane (made of

materials like poly-D-lysine) adhering capacity of proliferating cells.48 Thus, cells with limited binding ability cannot be synchronised using this method. This technique was improved by Shaw et al. where a fabricated microfluidic device was used to modify the synchronisation technique, and a pressure difference was used to allow asynchronous cells to get attached to a surface while maintaining a constant delivery of culture medium and elution of newborn cells (Fig. 2E). The method was validated on L1210 mouse lymphocytic leukemia cells to elute cells synchronised to the G1 phase.⁴⁹ Unlike membrane elution systems that depend on surface chemistry for membrane-cell adherence, the baby machine is a pressure-controlled microfluidic device, which successfully uses a pressure differential across 2 µm diameter trapping holes to capture cells and allows them to proliferate and elute cells that have recently completed cytokinesis and entered the G1 phase. The device can elute up to 1000 cells every 12 hours with ~80% of collected cells synchronised at G1. While the device was functional for a single synchronised batch of cells, it was not suited for a continuous supply. Therefore, there is room to improve the functionality and yield of the baby machine. The yield of this synchronisation device is sufficient for transcriptomic analysis; however, for the investigation of protein expression levels, the yield would have to be significantly increased.⁴⁹ Tian *et al.* developed a microfluidic 'baby machine' synchroniser suitable for rodshaped bacterium and fungi cells.⁵⁰ The device immobilises cells from one end and releases a daughter cell everytime the cell completes cytokinesis. The system is integrated with a cell screener, which allows the cell collection, and it is long term culture compatible with the slit array. The system requires no external driving force for operation and is disposable, minimising the risk of contamination.^{50–52} More recently, Chang et al. developed a microfluidic synchroniser using synthetic nanocapped bacterium. They engineered E. coli cells having a synthetic 'stalk' that adheres to microchannel walls and the cells are capped with a magnetic fluorescent nanoparticle. Cells are immobilised in the chamber by an induced magnetic field, and as the daughter cells are formed without the stalk, they are flushed out of the system, yielding a synchronous group of 'baby' cells. Nanoparticle fluorescence can be tracked in the stalks and the daughter cells. The device is easy to fabricate, and the magnetic capped bacteria (MCB) approach can be applied to synchronising other bacterial species.53

3 Microfluidics based cell cycle synchronisation

Microfluidic systems offer significant advantages over conventional methods for cell separation, including reduced sample volumes, simpler sample preparation procedures, high throughput, and high spatial resolution.⁵⁴ Cell cycle synchronisation by microfluidics allows enhanced detection accuracy with minimal human intervention, reducing the risk of sample contamination. Like other physical methods for cell cycle synchronisation, microfluidic systems exploit the variation in intrinsic properties of diverse cell populations to achieve cell separation. Additionally, precise cell separations based on selective cell affinity to surface biomarkers have been performed on cells using microfluidic technology.⁵⁵ Conventional cell separation approaches like FACS and MACS

implemented in microfluidics have allowed circumventing several limitations like high reagent consumption, the need for expensive equipment pieces, and compromising cell viability and purity.⁵⁶ The first micro-fabricated FACS with microfluidic valves achieved a throughput of 20 cells per second.⁵⁷ Multiple studies have also explored encapsulating



Fig. 3 Schematic illustrating the operating principle of the microfluidic applications in cell cycle synchronisation. A) Dielectrophoresis: dielectrophoretic forces result from the interaction between inhomogeneous, high-frequency electric fields with the induced electrical polarisation of cells. The frequency and conductivity of the cell cytosol and cell volume (size) causes the movement of cells towards regions of high field strength (positive dielectrophoresis) or push cells away from the high field region (negative dielectrophoresis). The technique exploits the phasedependent properties like cell size to sort asynchronous cells. B) Acoustophoresis: acoustophoresis is a non-contact and label-free method of cell separation where ultrasonic acoustic resonance induced by piezoelectric material within a microchannel has been known to produce radiation force to manipulate cells or particles. Physical properties like cell size, density, or compressibility influence the movement of cells towards or away from the pressure node in the channel center. With the piezotransducer actuated, stronger acoustic radiation force on larger cells (G2/M and S phased cells) directs them to the central stream faster than the smaller sized cells (G1 phased cells), which remain closer to the channel walls, thereby allowing efficient cell sorting at the outlets. C) Hydrophoresis: it is a passive cell separation technique that uses steric hindrance and secondary flow to manipulate cell particles. The low resistance provided by grooves in the cross-section allows fluid to fill the grooves and induces a transverse movement within the channel. The anisotropic architecture generates a pressure gradient. The cell-obstacle interaction deflects the larger cells (G2/M phased cells) to be diffused out of the rotational streamlines. At the same time, the smaller cells (G0/G1 phased cells) deviate from and follow a different stream, differentiating them from the larger cells. D) Inertial microfluidics: inertial separation is a passive cell separation technique influenced by channel geometry and hydrodynamic forces. The effect of inertial lift and Dean drag forces are used to size fractionate asynchronous cells to obtain synchronised populations of cells in the G0/G1, S and G2/M phases. Cells in the G2/M phase, due to the larger diameter, equilibrate closest to the microchannel inner wall followed by cells in the S and the G0/G1 phase.

cells within droplets and using dielectrophoretic force to steer target cells to the collection outlet.58-60 Microfluidics based FACS systems suffer from low throughputs, and actuating with other forces like acoustic force and dielectric force increases the system throughput by 10–100 times.^{56,61} MACS is also an immune-affinity based cell separation approach, where target cells are tagged with specific antibodies conjugated to magnetic levels. Similar to FACS, microfluidics based MACS also overcome a number of the limitations of conventional MACS.⁵⁶ Studies have demonstrated the use of microfluidicsbased MACS systems to isolate specific magnetically tagged cancer cells from blood with high sensitivity.^{62,63} Microfluidic systems have also been used to isolate cells by 'panning', where a surface functionalised with specific antibodies bind to target cells by coupling. CTCs have been popularly isolated from heterogeneous blood mixtures based on separation by surface marker properties.^{56,64} While these immune-affinity cell separation approaches achieve high purity and recovery rate, it is difficult to detach the antibodies from the target cells and/or the functionalised surfaces. This compromises the cell viability, system throughput and limits the application of sorted cells for subsequent analysis. Therefore for phase specific cell synchronisation, cell separation based on physical cell properties like size, shape, deformability hold more promise for application in real time and clinical settings.

Like most microfluidic components, cell separation processes by microfluidics can be classified into active and passive separation techniques.⁶⁵ Active approaches employ the use of an external force, while passive strategies guide microparticles through specially designed channels.⁶⁶ Techniques like dielectrophoresis and acoustophoresis have proven to be powerful tools for label-free cell separation. Passive techniques allow for a simpler setup as they depend on the intrinsic fluidic force to facilitate particle separation. Deterministic lateral displacement (DLD) was developed in 2004 (ref. 66) and has been widely used for microparticle separation and detection in the last decade. However, several key limitations with DLD remain, including low throughput, pillar clogging issues, and the need for bulky experimental setup.67 More recently, techniques like hydrophoresis and hydrodynamic filtration have also been explored for label-free cell separation and are suited for low throughput cultures. At the same time, inertial microfluidics is better suited for high throughput sorting. Additionally, techniques like pinched flow fractionation (PFF) in symmetric and asymmetric sidechannel devices have been able to sort particles by hydrodynamic filtration.⁶⁸ In the context of this review, we aim to highlight/discuss studies that have implemented microfluidics-based approaches to achieve high levels of phase specific cell synchronisation in diverse cell populations.

3.1. Dielectrophoresis

Dielectrophoretic forces result from the interaction between a non-uniform electric field with the induced electrical

polarization of cells. As a result, cell cytosol properties like conductivity either hold the cells in place near the high field region (positive dielectrophoresis) or push cells away from the high field region (negative dielectrophoresis) (Fig. 3A).^{69,70} Other membrane properties like permeability, capacitance, conductivity, and size of the cells also influence the magnitude of the dielectrophoretic force. In dielectrophoresis, the particle's motion depends on its polarisation properties and the surrounding medium.⁷¹ Trajectories of particle motion are critical for dielectrophoretic cell separation systems, and precise positioning of outlets depend on the accurate prediction of channels.72,73 cells/particle motion within the Dielectrophoresis also allows for label-free cell sorting without the help of immunochemistry. The concept was first demonstrated by Huang et al. to sort five cell lines using a microelectronic array and further developed to exploit the generation of non-uniform electric fields and the precise positioning of cells within microchannels to filter and concentrate cells.^{74,75} Several studies have also explored the integration of dielectrophoresis with other field flow approaches like the use of magnetic, electrical, thermal forces.76-78 Hydrodynamic forces have also been used in conjunction with dielectrophoresis to sort multiple cells by size or electric permeability, including breast cancer cells and CTCs,^{79,80}

Kim et al. used a dielectrophoretic activated cell synchroniser (DACSync) device, which functions by exploiting the relationship between cell volume and its cell cycle phase. The technique was used to enrich MDA-MB-231 cells in the G1 phase.⁵¹ In the control cell population, the ratio of G1 to G2/M phase cells were \sim 5.2:1, whereas, after a single round of sorting through the DACSync device, this ratio increased to 23:1, with the G1 phase cell synchrony reaching 96%. The DACSync device allowed cell synchronisation in a continuous flow manner. The method involved the use of lower strength electric fields than electroporation, and with the device operating in a mode where cells are repelled from high electric field areas, the process had minimal effect on the cell viability. The throughput of this device was $\sim 10^5$ cells per hour per microchannel, which could be increased by integrating multiple channels.⁵¹

More recently, Valero *et al.* used a dielectrophoretic opacity approach to track and synchronise yeast cells to specific cell cycle stages. They exploited the equilibrium between dielectrophoretic forces produced by electric fields of varying frequencies on either side of the sorting microchannels.⁸¹ The opposition of dielectrophoretic forces increased the sensitivity to changes in cell shape throughout the cell cycle in a continuous operative process, as established in a previous study.⁸² The system quantified the dielectric response to specific cell cycle stages from the equilibrium positions in the channels and successfully synchronised a yeast cell culture batch to late anaphase, which was maintained during subsequent cell divisions.⁸¹ Gel *et al.* also synchronised cell cycle phase in L929 mouse fibroblast cells generated as fusants using dielectrophoretic cell trapping in micro-orifice arrays.⁸³

3.2. Acoustophoresis

Acoustophoresis is a non-contact and label-free method of cell separation that allows the implementation of several separation modalities.^{84,85} Ultrasonic acoustic resonance induced by piezoelectric material within a microchannel has been known to produce radiation force to manipulate cell particles (Fig. 3B).^{86,87} The high-intensity sound waves generate pressure gradients that push cells to specific spatial locations.⁸⁸ The magnitude of the radiation force is impacted by the density and compressibility of the cells, the fluid medium, and the amplitude of acoustic waves.⁸⁹ These acoustic forces allow rapid and precise spatial control of particles in microchannels without impacting cell viability.⁹⁰ The waves produced result from the disturbance of the microchannels surfaces or fluid medium by pressure waves of equal magnitude and frequency, travelling in opposite directions.^{84,91} This causes a single stationary wave that contains fixed regions called nodes that remain unaffected by pressure fluctuations and antinodes exhibiting pressure differences. These contrasting regions allow spatiotemporal manipulation of single cells.^{92,93} Acoustophoresis has been implemented in both label/beads based and label-free cell separation techniques and has scope for integration with other cell separation strategies as hybrid systems.^{94,95}

Cell cycle synchronisation by acoustophoresis has been achieved using ultrasonic standing waves to achieve high throughput synchronisation in mammalian cells without the use of additional reagents. Thévoz et al. developed an acoustophoretic cell synchronisation (ACS) device, which utilised volume-dependent acoustic radiation force within a microchannel to selectively enrich specifically phased cells from asynchronous cell populations based on cell-cycle dependent variation according to the cell size. The technique was used to synchronise MDA-MB-231 cells at ~84% G1 phase at a high throughput of 3×10^6 cells per hour per microchannel. In the ACS device, acoustic standing waves were generated in the separation channels. After a single round of synchronisation, the cell separation process enhanced the G1 phase cell population from 59% in the asynchronous population to 84% post synchronisation.⁵² More recently, Olm et al. demonstrated the use of acoustophoresis to synchronise cell cycle phases in mesenchymal stromal cells (MSCs). Fractions of MSCs in G0/G1 phases were enriched to S/G2/M phases by 1.3-2.8 fold in sorted/synchronised cells. The process also did not compromise the phenotype, proliferation, and viability in the separated cells.96

The acoustophoresis devices are excellent representations of the use of microfluidic channels that allow an accurate and efficient method of a volume-dependent ultrasonic cell separation technique with a multistream laminar-flow designed for high purity separation.^{56,97} This method's cell cycle phase synchrony level is comparable to other cell cycle synchronisation techniques like centrifugal elutriation.⁹⁸ Higher purities and throughput have been achieved by optimising the channel geometry, serial integration of the device, and parallel operation.^{99,100} The technique allows the use of a wide range of cells and suspension media and offers a minimal stress and label-free cell cycle synchronisation process with high throughput and fidelity.^{52,87}

3.3. Hydrophoresis

Hydrophoresis is a passive cell separation technique that involves the induction of a pressure gradient brought on by the resistance on fluid flow along slanted grooves and the steric effect between particles and the grooves of the microchannels (Fig. 3C).^{68,101} The hydrophoretic size-based cell separation technique utilises convective, rotational flows induced from regularly patterned anisotropic microfluidic obstacles.¹⁰² The method exploits the correlation between cell size and its position distribution in the hydrophoretic device as a means to sort cells in different phases of the cell cycle.^{103,104} The technique allows accurate particle ordering in a small device footprint ($\sim 1 \text{ mm}^2$), thus facilitating easy parallelisation for high-throughput applications.^{105,106} For effective particle manipulation by this technique, particle diameter should generally be larger than half of the groove gap.¹⁰⁷ The efficiency of cell separation by this method is also dependent upon key geometric (channel width and oblique angle of grooves) and operational parameters (flow rate).^{108,109} A study done by Song and Choi found that the channel width is a key parameter for hydrophoretic devices, i.e., channel width needs to be three times the diameter of cell particles for cell sorting and be greater than 400 µm for cell focussing; the Reynolds number should be less than 10 (Re \leq 10), for optimum cell separation without perturbation.103,110

Cell synchronisation by microfluidic hydrophoresis was evaluated by Choi et al. They demonstrated the use of sizebased hydrophoretic separation to sort cells into specific cell cycle phases. They exploited the correlation between cell size and its position in the device. The method was used to achieve high G0/G1 and G2/M phase synchrony levels in human leukemic monocyte lymphoma cells.¹¹¹ The hydrophoretic device in the study was composed of slanted groove patterns on the straight channel, powering the transverse motion of particles. The cell-obstacle interaction deflects the larger cells (G2/M phased cells), while the smaller cells (G0/G1 phased cells) drive out of the rotational streamlines differentially. The throughput from this device was $\sim 2.4 \times 10^5$ cells per hour per microchannel. After separation, cells from the range 0–300 μ m and 400–1000 μ m were collected from the outlets for the target cells staged in the G2/M and G0/G1 phases, respectively. Flow cytometric analysis showed that post hydrophoretic separation, there was a significant decrease in the G2/M phased cells. The G0/ G1:G2/M ratio increased from 5.2:1 in the asynchronous population to 22.1:1 in the G0/G1 sorted cells, while the G2/

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M:G0/G1 ratio increased from 0.25:1 in asynchronous cells to 5.8:1 after the separation process (~85.2%).¹¹¹ Song *et al.* also demonstrated the use of a continuous flow hydrophoretic microfluidic syringe filter to achieve cell synchronization. The device enriched G1 phased cell distribution from 58% in asynchronous cells to 83.6% in synchronised populations with a throughput of 1.2×10^5 cells per minute.¹¹² Interestingly Migita *et al.* reported the use of a microfluidic device to achieve cell synchronisation on the principle of hydrodynamic filtration. The device was implemented to achieve cell synchrony in Hep2 and NIH/3T3 cells, where the G0/G1 to G2/M ratio increased from 3.3:1 in asynchronous cells to 21.5:1 in synchronised populations.⁵⁴ The synchrony levels are comparable with other microfluidics-based cell synchronisation approaches.^{51,111} Hydrophoretic microfluidic devices also have the scope for being integrated with other cell separation strategies, like magnetophoresis, dielectrophoresis, or inertial migration. 56,104,113

3.4. Inertial microfluidics

Size based particle separation in microfluidic systems have been developed on the principles of inertial migration.^{114,115} The lateral migration of cells in the straight microchannels happens due to the superposition of the shear-induced lift force and the wall-induced lift force, called inertial lift forces $(F_{\rm L})$.^{116,117} In curvilinear channels, Dean drag forces $(F_{\rm D})$ are also present and affect the equilibrium position of particles. These forces equilibrate cells at distinct positions within the microchannel cross-section based on their size relative to the microchannel dimensions, thus achieving cell separation. (F_1) $\propto d^4$, $F_{\rm D} \propto d$, where d = diameter of particle) (Fig. 3D).¹¹⁸ Di Carlo et al. used asymmetrical serpentine microchannels to perform platelet enrichment in blood (100 fold enrichment);¹¹⁹ Warkiani et al. used spiral microchannels with trapezoidal cross-section to separate cancer cells from white blood cells.¹²⁰ High throughputs of $\sim 10^6$ cells per minute were achieved using this technique, which was

		Synchronisation method	Separation principle	G0/G1 purity	G2/M enrichment	Cell viability post sorting	Flow rate/throughput
Conventional methods	Chemical blockade	Double thymidine block	S phase targeting drug	_	~95% S phased cells ¹³⁸	Low	
		Thymidine + nocodazole block	G2/M phase/checkpoint targeting drug	_	~95% mitotic cells ¹⁹	Low	
	Physical fractionation	FACS/flow cytometry	Fluorescence/size-based light scatter properties	90-95%	$\sim 2.5 \times$	Low-medium	$\sim 10^6$ cells per hour (ref. 34)
		Centrifugal elutriation	Size and sedimentation velocity	~90%	$\sim 2 \times$	Low-medium	$\sim 3 \times 10^9$ cells per hour (ref. 12)
		Baby machine	Membrane elution	$\sim 80\%$		Medium	~80 cells per hour (ref. 49)
Microfluidic based methods	Active techniques	μFACS	Fluorescence labels	Not reported	Not reported	Low-medium	100 cells per second (ref. 139)
		μMACS	Homogeneous/inhomogeneous magnetic field	Not reported	Not reported	Low-medium	10 000 cells per second (ref. 140)
		Dielectrophoresis	Inhomogeneous electric field	96%	$\sim 1 \times$	High	2×10^5 cells per hour (ref. 51)
		Acoustophoresis	Ultrasonic standing waves	84%	23×	High	3×10^6 cells per hour (ref. 52)
		Optic	Optical properties-size, polarizability	Not reported	Not reported	High	1500 cells per minute (ref. 141)
	Passive techniques	DLD	Size-, shape-, deformability-based separation in laminar flow	Not reported	Not reported	High	Low
		PFF	Hydrodynamic profile (parabolic velocity profile)	Not reported	Not reported	High	Low
		Hydrophoresis	Inhomogeneous pressure field	~96%	3.7×	High	2.4×10^5 cells per hour (ref. 111)
		Hydrodynamic filtration	Hydrodynamic force	86% (HepG2 cells)	2.9× (Hep2 cells)	High	3×10^6 cells per hour (ref. 54)
		Inertial migration	Lift forces and Dean drag	96% (KKU-100 cells); 86% (MSCs)	2× (KKU-100 cells); 3.6× (MSCs)	High	High, $\sim 15 \times 10^6$ cells per hour (ref. 10)

Table 1 Comparison of conventional and microfluidic cell separation/synchronisation techniques discussed in the review

significantly higher than other microfluidic-based cell separation techniques. Since this technique depends on the intrinsic hydrodynamic forces, the designing process of these systems is relatively simple and can be integrated with other microfluidic modules.^{121,122} Moreover, the microchannel dimensions in these devices are comparably larger than the cell's size that eliminate the problem of clogging within the channels.^{123–125}

Cell cycle synchronisation by inertial microfluidics has been achieved using a spiral microfluidic device by combining the effects of inertial forces and Dean drag forces. Warkiani and colleagues used inertial and Dean drag forces in spiral microchannels to perform high throughput, large scale cell synchronisation of CHO and hMSCs.¹²⁶ The synchronisation was performed based on size differences between G0/G1 and G2/M phased cells. After one round of sorting, the G0/G1:G2/M ratio increased from 1.82:1 in asynchronous CHO cultures to 5.02:1.¹²⁶

Comparable cell synchrony performance was achieved by Lee et al.127 in multiple mammalian permanent cell lines-CHO-CD36 (Chinese hamster ovarian cells), HeLa and KKU-100 (cancer cells) into G0/G1 (>85%), S and G2/M phase enriched cell populations. The G0/G1:G2/M ratio of 2.8/1 of the asynchronous cell population was enriched to 15.7:1 post-synchronisation. The resulting synchronised cells maintained a significantly high throughput ($\sim 15 \times 10^6$ cells per hour) and cell viability (~95%) comparable to other microfluidic systems.^{10,54} This study also demonstrated the first application of this technique to synchronise human mesenchymal stem cells (hMSCs). The hMSCs differ from the other cell lines in that it is an enriched population of putative stem cells from the human bone marrow, resulting in increased variation in cell diameter, morphology, and functionality.¹²⁸ These cells are highly susceptible to contact inhibition and were thus expanded at optimal seeding densities.¹²⁹ A 4-fold enrichment was achieved in the G2/M population compared to the asynchronous cells collected at the outlets.¹²⁷ The hMSCs subpopulations synchronised by this device were compared with the hMSC cells synchronised to G0/G1 and G2/M phases by serum deprivation and contact inhibition. In both comparisons, the cells synchronised by inertial microfluidic systems had better uniformity in DNA content, cell size, and shape (cell cycle phase synchrony) than the hMSCs synchronised by serum deprivation and contact inhibition. More recently, Bogseth et al. developed a co-flow inertial microfluidic device that allows parameters like flow rate, flow rate ratio, output resistance to be tunable for different applications post-fabrication of the device. The integrated device was used to demonstrate enrichment of G1 phased cells from asynchronous A549 cells with G1:G2 ratio increasing from 2.36 in non-synchronised populations up to 6.30 in synchronised cells.⁴⁵ The cell viability post-separation was >90%, which is comparable to similar studies.¹²⁰ Moreover, high throughput achieved by the system makes it a better option than other conventional cell synchronisation approaches.16,130

Inertial microfluidics enjoys distinct advantages over other microfluidic systems, including continuous operation, higher sample throughput, reduced sample processing time while preserving the integrity and viability of sorted cells.¹³¹⁻¹³³ Thus, it is an ideal candidate for use in cell cycle synchronisation.

3.5. Drawbacks of microfluidics mediated cell synchronisation

While microfluidics offers several advantages for high throughput cell synchronisation, there are several key challenges that prevent the application of these techniques in clinical settings and translation to commercial products. A major challenge is the need for cheap, robust, mass-producible device fabrication material for the devices. The majority of the microfluidic cell separation techniques exploit the phase-specific physical variations in cells. One of the main limitations of the techniques is the over-dependence on the size of cells. Most cell lines exhibit size variations up to $\sim 10\%$, disrupting the sorting and capturing process.⁵⁵ However, to expand the clinical utility of these processes, they need to be adapted for immune-affinity based approaches like FACS and MACS.¹⁰¹

Moreover, techniques like dielectrophoresis are affected by the cell media's conductivity, with the sorting becoming more complex in media/buffers with high conductivity. The high conductivity directly causes cell damage/death, thereby compromising the overall cell viability.55 The conductivity of the media can be modified by a process called isodielectric sorting, where a gradient media conductivity is used to ensure that the cell sorting depends mainly on the dielectric properties and the cell size.¹³⁴ In acoustophoresis, the major limitation is the need for specific materials for the devices that are able to transmit the acoustic power to the fluid.¹⁰⁰ Acoustic based separation systems are also not feasible for operating at low flow rates. To abrogate the various drawbacks of individual microfluidic approaches, studies have attempted to combine external forces with techniques like hydrophoresis.^{113,135} Towards this, Yan et al. demonstrated that particle distribution in groove channels could be influenced magnetic by or electric (dielectrophoresis) fields and fractionate cells based on their magnetic/dielectric properties. The equilibrium positions of the particles within the microchannels were also influenced by inertial effects with variation in flow rate within the grooved channels (Table 1).^{113,135-137}

4 Future directions

The application of cell cycle synchronisation techniques has proven immensely significant in studying cell cycle regulation and progression mechanisms. Researchers have attempted cell cycle synchronisation protocols on asynchronous populations of bacterial, plant, protozoan, yeast, and mammalian cells, each with varying levels of phase-specific synchrony.⁶ Cell cycle synchronisation is vital in the context of targeted gene editing.¹⁴² Studies have shown that cell populations that were synchronised to G2/M phases had improved on-target gene editing using zinc-finger nucleases (ZFNs), transcriptional activator-like effector nucleases (TALENs) and clustered regularly interspaced palindromic repeats (CRISPR) technologies.¹⁴³ Cell cycle synchronisation has also been effective at sensitising cancer cells to specific drug treatments.¹⁴⁴ The relevance of cell cycle synchronisation in these vital processes establishes them as a viable tool for disease modelling, regenerative medicine, and cell-based therapies.

Despite studies on multiple cell cycle synchronisation techniques, there remains significant ambiguity over what the best synchronisation technique/s would be.^{145,146} The perfect synchronisation technique is the one that would induce minimal metabolic alterations to the cells with the highest level of phase-specific synchrony and sample recovery. The research trend on developing these techniques has shifted from initially prioritising conventional techniques like chemical blockade/cell-cycle arrest-inducing agents and physical cell separation (membrane elutriation, centrifugal elutriation, FACS) to more recently utilising microfluidic systems and hybrid techniques. This resulted from the potential risks of inducing unintended metabolic alterations and irreversible cell cycle phase arrests associated with whole culture chemical-based synchronisation techniques.

Moreover, agents that induce G0/G1 arrest risk generating dormant cancer cells that have immune-evasion mechanisms against most conventional chemotherapy drugs.30 The implementation of physical fractionation techniques has circumvented a number of those complexities, and the development of microfluidic technology in cell cycle synchronisation processes has allowed for high throughput, and spatial resolution in cell phase synchrony, and its inherent portability (reduced sample volume) has enhanced the potential for development of point-of-care platforms. However, future research on synchronisation processes must prioritise developing technology that can achieve further enhanced levels of phase synchrony in all/most types of cells from large/continuous batches of asynchronous cell populations while maintaining cell viability.

Recently a number of microfluidics-based single-cell studies have been able to influence cell cycle phase dynamics by inducing specific cell culture conditions. Nakagawa *et al.* studied the effect of culturing yeast cells in droplets under different conditions and identified that cell cycle progression was significantly delayed at G1 and G2 phases.¹⁴⁷ Olofsson *et al.* used an ultrasound-based multicellular tumour spheroid culture platform to correlate nuclear segmentation to biological information at the single-cell level. DNA-content analysis was carried out to establish cell cycle state as a function of position within the spheroids.¹⁴⁸ These studies highlight differential microfluidic culture conditions as a source of inducing bias in cell cycle states, which can be exploited towards phase-specific cell sorting.

Several studies have explored the combination of conventional techniques (multiple chemical blockers or a

blocker and physical fractionation technique) and microfluidic techniques to achieve higher levels of phase synchrony.^{14,149} As a future research direction, hybrid cell synchronisation techniques that exploit multiple cell cyclespecific cellular properties like cell size, shape, electrical and magnetic properties, and surface markers in a single process need to be explored. Hybrid microfluidic cell sorting systems have demonstrated high integrity rare cell isolation capacity, particularly at the single-cell level.¹⁵⁰ Especially with the advancement in microfluidic technology and their integration with traditional methods like FACS and MACS, hybrid cell separation systems have achieved enhanced output.55,151 MACS has been implemented successfully for cell separation in both batch and continuous flow processing operations. The scope of magnetic-based sorting to combine both immune-affinity and phase-specific size variations and its applicability in microfluidic approaches makes it ideal for cell cycle synchronisation.^{55,113,151} Advancements in control engineering have also potentiated the automation of complex biological processes.^{152,153} As discussed earlier, FACS/flow cytometry is the gold standard for cell synchronisation and, combining FACS with high throughput microscopy, cell sorter, and deep learning neural network represents a holistic cell separation process with enhanced throughput and sensitivity. Imaging-based flow cytometry has proven to be effective at evaluating cell cycle complexities and their impact on various biological processes. Image-activated cell sorting (iIACS) has facilitated high-throughput, intelligence imagebased sorting of live single cells from heterogeneous populations.¹⁵³ Nitta et al. demonstrated the versatility of the IACS by performing real-time sorting of microalgal and blood cells based on intracellular protein localization and cell-cell interactions from large populations.¹⁵⁴ Isozaki et al. reported an IACS system with a throughput of ~2000 events per second and sensitivity of ~50 molecules of fluorophores, which is 20 times better than existing IACS systems.¹⁵⁵ IACS technology holds potential for diverse applications in disease studies. They allow the linking of image-based information of cells with their molecular underpinnings, thereby facilitating the high throughput sorting of cells into functionally distinct sub-populations. We believe that these platforms have tremendous potential to be developed into more effective cell synchronisation platforms that can incorporate the advantages of both conventional and microfluidic techniques to achieve high throughput and precise cell cycle synchrony.

Conflicts of interest

The authors declare no conflicts of interest.

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