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Research review paper

Scaling up stem cell production: harnessing the potential of microfluidic devices

Lin Ding [a,*], Steve Oh [c], Jesus Shrestha [b], Alan Lam [c], Yaqing Wang [d,e], Payar Radfar [a], Majid Ebrahimi Warkiani [b,*]

[a] Smart MCs Pty Ltd, Ultimo, Sydney, 2007, Australia
[b] School of Biomedical Engineering, University of Technology Sydney, Sydney, NSW 2007, Australia.
[c] Stem Cell Group, Bioprocessing Technology Institute, Agency for Science, Technology and Research (A*STAR), Singapore, 138668, Singapore
[d] School of Biomedical Engineering, University of Science and Technology of China, Hefei 230026, China
[e] Suzhou Institute for Advanced Research, University of Science and Technology of China, Suzhou 215123, China

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ABSTRACT

Stem cells are specialised cells characterised by their unique ability to both self-renew and transform into a wide array of specialised cell types. The widespread interest in stem cells for regenerative medicine and cultivated meat has led to a significant demand for these cells in both research and practical applications. Despite the growing need for stem cell manufacturing, the industry faces significant obstacles, including high costs for equipment and maintenance, complicated operation, and low product quality and yield. Microfluidic technology presents a promising solution to the abovementioned challenges. As an innovative approach for manipulating liquids and cells within microchannels, microfluidics offers a plethora of advantages at an industrial scale. These benefits encompass low setup costs, ease of operation and multiplexing, minimal energy consumption, and the added advantage of being labour-free. This review presents a thorough examination of the prominent microfluidic technologies employed in stem cell research and explores their promising applications in the burgeoning stem cell industry. It thoroughly examines how microfluidics can enhance cell harvesting from tissue samples, facilitate mixing and cryopreservation, streamline microcarrier production, and efficiently conduct cell separation, purification, washing, and final cell formulation post-culture.

1. Introduction

Stem cells are considered the next frontier in regenerative medicine, with the potential to treat, regenerate, or even replace malfunctioning or unhealthy tissues and organs. Recently, stem cells have also found application in the cultivated meat/lab-grown meat/cellular agriculture industry. This sector is generating significant interest due to the considerable reduction in environmental impact and ethical concerns surrounding animal farming. Multiple reviews have thoroughly examined the application of stem cells in the fields of regenerative medicine (Amabile and Meissner, 2009; Baldari et al., 2017; Squillaro et al., 2016; Wraith et al., 2008) and cultivated meat (Choudhury et al., 2020; Guan et al., 2021). Despite the growing demand for large-scale manufacturing of stem cells, the industry still faces inadequate production capabilities (Jossen et al., 2018; Rodrigues et al., 2018). One major hurdle lies in purifying a uniform population of stem cells, which is crucial for ensuring the safety and efficacy of therapeutic dosages (Shields et al., 2015). Additionally, difficulties in stem cell recovery from tissue samples have been identified, resulting in lower post-transplantation stem cell survival rates and less favourable therapeutic outcomes (Xavier et al., 2016), (Baldari et al., 2017; Choudhury et al., 2020). Compounding the complexity, stem cells are inherently heterogeneous, expressing distinct characteristics (Kucinski and Gottgens, 2020), emphasising the significance of isolating specific stem cell populations to enhance the quality of stem cell products (Sensebe et al., 2013). Stem cells from other donors can potentially trigger the immune rejection of the recipient (Charlesworth et al., 2022). Induced pluripotent stem cells (iPSCs) do not face this challenge, however, there are concerns about their reprogramming and differentiation protocol (Amabile and Meissner, 2009), as well as genome stability (Martins-Taylor and Xu, 2012). Microfluidics has the potential to improve iPSC reprogramming protocols and cell stability through targeted delivery of genes and

* Corresponding authors.
E-mail addresses: lin@smartmcs.com.au (L. Ding), majid.warkiani@uts.edu.au (M.E. Warkiani).

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controlled fluid delivery (Gagliano et al., 2019; Giulitti et al., 2019). In the cultivated meat industry, the cost of culture medium (O'Neill et al., 2021), scale-up production method (Bodiou et al., 2020), differentiation process (Lee et al., 2021), and scaffolding technologies (Bomkamp et al., 2022) remain the leading challenges for bringing cultivated meat to dinner tables. Furthermore, current technologies rely heavily on animal-based products, which conflict with the industry's original purpose.

Microfluidics is a rapidly evolving technology that facilitates the manipulation of small volumes of fluid in micron-scale channels. This technology offers several distinct advantages, including high customizability, precise control of fluid flow and micro-particles, low reagent consumption, and cost-effective production. These features render microfluidics an ideal choice for various applications, such as cell separation (Zhang et al., 2016), single-cell studies (Yin and Marshall, 2012), and investigations into cell interactions and secretions (Qian et al., 2015). As a result, microfluidics can replace traditional cell sorting machines and plate- or well-based methods. Furthermore, microfluidics can enable the development of low-volume, high-throughput media optimization and differentiation protocols, as well as scaffold fabrication.

This review provides an overview of the industrial production process of stem cells and examines various microfluidic devices used in stem cell research and production. We discuss how microfluidic devices can overcome current method limitations, including cell sorters, mechanical stimulation, cell property measurements, droplet-based microfluidics, organ-on-a-chips, and integrated devices. We also explore the future potential of combining multiple microfluidic technologies to accelerate and address complex challenges in industrial stem cell production.

2. Stem cell production in laboratories and industry

The stem cells production processes are divided into upstream production (USP), downstream production (DSP) and formulation and storage (Jossen et al., 2018). The USP stage involves the isolation of tissue samples from donors, followed by purification and the creation of a master cell bank (MCB). These cells are then used to generate a working cell bank (WCB), which can be expanded for downstream applications. In contrast, the DSP stage comprises the collection of the expanded cells, selection of target cells or their secretions, multiple washes, followed by exchange into buffers for final formulation, and storage and transport of the final product (Fig. 1). In this review, the production process of Mesenchymal Stem Cells (MSCs) is outlined as a well-established example in regenerative medicine, with potential application in cultivated meat industry. (See Tables 1–5.)

2.1. Upstream production

2.1.1. Cell source selection and isolation methods

Within the industrial tapestry of regenerative medicine, primary cells from donors remain the dominant source of stem cells. However, this practice raises concerns about the quality and variability of MSC-related products, which can be influenced by donor age and gender (Golpanian et al., 2015; Hass et al., 2011), tissue origin (Hass et al., 2011; Trivanovic et al., 2013), the passages of cells and even intra-batch variations (Lee et al., 2014; Menard et al., 2013; Whitfield et al., 2013). As a result, variations may arise in the number of cells obtained, differentiation capability, secretion profile, therapeutic and proliferation potential, and passage number (Fernandez-Santos et al., 2022; Galipeau and Sensebe, 2018; Golpanian et al., 2015; Levy et al., 2020). Therefore, the need for a stable and consistent cell source is a major challenge in the industry. Recently, iPSCs have been explored as a potential source of MSCs, eliminating the need for biopsy samples from donors (Lian et al., 2010; Villa-Diaz et al., 2012). While this approach has not been commonly adopted for clinical trials due to concerns about the transgenic nature and gene stability, it offers the advantage of reduced heterogeneity between donors and fewer ethical issues. Ongoing research is exploring the feasibility of using iPSCs as a source of MSCs (Toh et al., 2014).

Among the various tissue origins of MSCs, adipose, bone marrow, and umbilical cord tissues are most commonly used due to their accessibility and abundant cell availability (Moll et al., 2016). However, isolating MSCs from these sources requires the elimination of unwanted cell types. This is typically achieved through time-consuming, expensive, and error-prone methods including tissue lysis, gradient centrifugation, membrane-based filtration, and fluorescence or magnetic-activated cell sorting (FACS or MACS)(Sensebe et al., 2013; Xavier

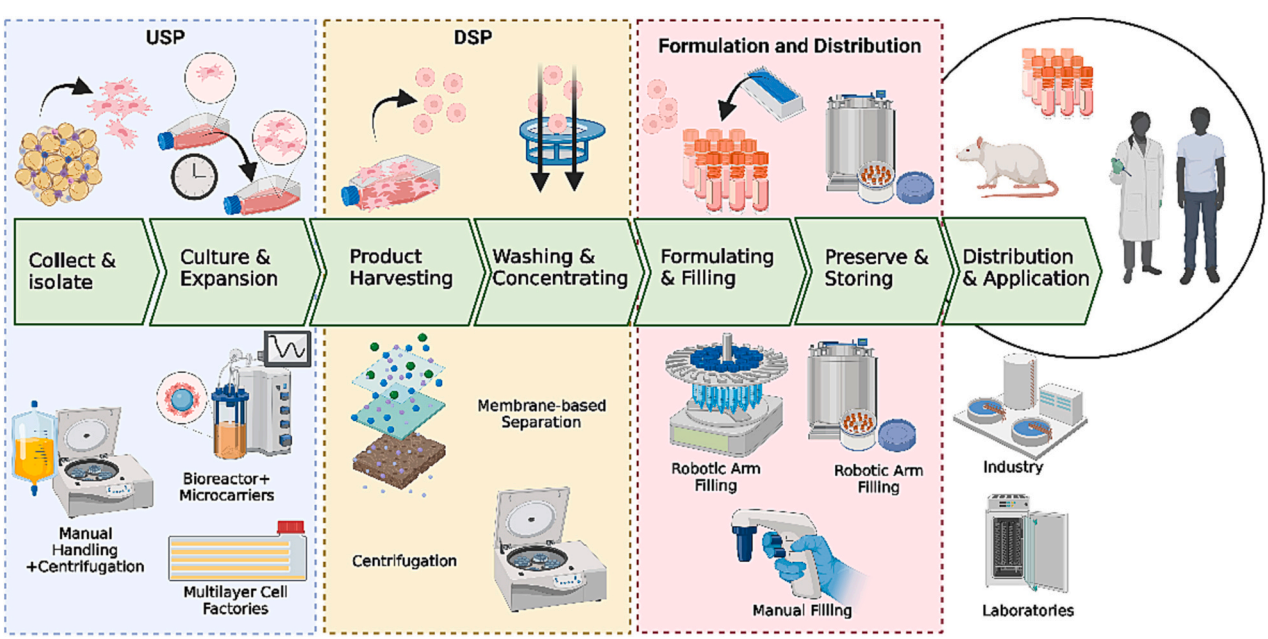

Fig. 1. A general workflow of stem cell production in the industry. This process is normally divided into three parts, upstream production (USP), downstream production (DSP) and formulation and distribution. Remarkably, there have been no radical innovations in cell culture and handling technologies for over 50 years. Current industry practices face significant challenges, including manual handling, cell loss, cell damage, and discontinuous processes.

Table 1
Multiple active and passive microfluidic technologies have been used to isolate stem cells from different sources.

Technologies	Samples	Target cell types	Throughput/flow rate	cells recovery	purity /Enrichment
Active microfluidics					
DEP (Talary et al., 1995)	Bone marrow sample from a donor	PBSC	///	///	5.9-fold enrichment
DEP (Song et al., 2015)	Mixed MSCs and osteoblasts	MSC	0.3 μL/min	92%	84%
DEP (Plouffe et al., 2012)	Whole blood from a donor	HSC, EPC	120 μL/min	96%	81%
DEP (Flanagan et al., 2008)	Cultured mouse neural sphere	NSPC	2 μL/min	>95%	
DEP (Prieto et al., 2012)			2 μL/min	50%	1.4-fold enrichment
DEP (Simon et al., 2014)			3 μL/min		13.2-fold
DEP (Yoshioka et al., 2018)	Cultured MSC and Leukaemia cell lines	MSCs	///	29.1%	83.5%, 2.3-fold
Acoustic (Dykes et al., 2011)	PBMC from multiple donors	PBPC	20 μL/min	98%	89% platelet depleted
Acoustic (Zalis et al., 2016)	Cultured hESCs	Live ESCs	100 μL/min	42.3%	88.1%
Magnetic (Vykoukal et al., 2008)	Adipose samples from donors	MSC	1500 μL/min	///	14-fold enrichment
Magnetic (Zeng et al., 2015)	Digested mouse lung samples	Lung multipotent stem cells	20 μL/min	///	96–99%
Optical Tweezer (Wang et al., 2011)	Mixed culture population	hESC	μm/s	90%	90%
Passive microfluidics					
Rectangular spiral (Nathamgari et al., 2015)	ganglionic eminences of E13 embryos from CD1 mice	Neural stem cells	1 mL/min	84%	67% purity(Yin et al., 2018)
Rectangular spiral (Lee et al., 2014)	Femur bone marrow of adult mice	MSCs	1.6 mL/min	73.2% + −1.5%	6 + −0.4-fold increase from an initial purity of 2.2% + −0.5%
Straight channel (Hur et al., 2012)	Adrenal gland from mice	Adrenal cortical stem cells	60 μL/min	Not given	Not given
Slanted spiral (Song et al., 2017)	Neural induction kit added iPSCs	Neural stem cells	3 mL/min	93%	2.1-fold increase
Rectangular spiral (Guzniczak et al., 2020)	Commercialised umbilical cord blood	Cord blood CD34+ cells	1 mL/min	>90%	>70%
Slanted spiral (Moloudi et al., 2018)	Commercial MSCs with microcarriers	MSCs	30 mL/min	94% in two rounds	Not given
Slanted spiral (Chen et al., 2020b)	Murine MSCs	dead MSCs	5 mL/min	75%	6 times higher senescence marker expression
Labyrinth Channel (Syverud et al., 2018)	Mouse muscle tissue	Mouse muscle stem cells	1.8 mL/min	66.5%	2 folds increase, 84.8% purity
Rectangular spiral + DLD (Lee et al., 2023)	Lysed fat tissue samples	ADSCs	500 μL/min	94.1%	30.4 folds increase, 46.8% purity
TECHNOLOGIES	SAMPLES	THROUGHPUT	APPLICATIONS		
Rectangular spiral (Lee et al., 2011)	hMSCs	2.5 mL/min	cell cycle synchronisation for potential downstream research and applications.		
Straight channel with obstacles (Lillehoj et al., 2010)	mESCs	20 μL/min	Sorting of embryoid bodies with different sizes.		
Slanted spiral (Yin et al., 2018; Yin et al., 2020)	Commercial human bone marrow MSCs	1.5–4 mL/min	Investigate the correlation of physical properties of cells and differentiation potential, therapeutic potential		
Slanted spiral (Poon et al., 2015)	Commercial human bone marrow MSCs	3 mL/min	Promote bone marrow regeneration with bigger size MSCs		

et al., 2016). While stem cell-specific antibodies are commonly utilised in this process, they suffer from high-cost and have the potential to alter the cell surface structure. Furthermore, the heterogeneous composition of stem cells introduces variability in the expression of specific markers, thereby compromising the reliability of antibody-based identification techniques.

In this context, adipose-derived stem cells (ADSCs) and umbilical cord mesenchymal stem cells (UC-MSCs) offer distinct advantages. Their isolation involves culturing lysed adipose/umbilical cord samples in a culture flask for a few passages, eliminating the need for antibody binding (Dykstra et al., 2017; Oberbauer et al., 2015). ADSCs are frequently obtained as by-products of liposuction surgeries and UC-MSCs are from the placenta after childbirth. In contrast, obtaining these cells are less invasive compared to acquiring bone marrow derived MSCs (BMSCs) through aspiration from the hipbone's marrow cavity. The extraction of BMSCs from bone marrow involves a multi-step process and necessitates antibody-based selection methods due to the presence of various adherent cell types (Xavier et al., 2016). The most adopted isolation methods utilise density gradient centrifugation to obtain cell pellets, followed by culture of cells in a flask to eliminate non-adherent cells, and further selection using specific surface biomarkers (Xavier et al., 2016). This procedure is complex and poses risks of cell damage. The use of antibodies and frequent washing can alter the surface protein expression of cells, while not guaranteeing the purity and quality of the isolated cells. Among the three cell sources, UC-MSCs are currently favoured in the industry owing to their robust passage count and elevated secretion of immune-related proteins (Hoang et al., 2020; Jin et al., 2013; Zhu et al., 2023). However, unlike immortalised cell lines, UC-MSCs passages remain limited, thereby constraining their potential for downstream applications. Hence, it is crucial to stabilise the cells in in the fewest passage to setup a reliable master cell bank (MCB) for consistent therapeutic production.

2.1.2. Scale-up approaches

In the field of mesenchymal stem cell (MSC) production, 2D multi-layer flasks remain the predominant culture vessels, meeting basic requirements for cell growth and maintenance (Jossen et al., 2018). These flasks are not without limitations; they present significant scalability challenges and have been shown to induce undesirable changes in key cellular properties such as fate, phenotype, proliferation rate, differentiation capacity, and secretion profiles profile (Yim and Sheetz, 2012; Zhao et al., 2014). To circumvent these limitations, the adoption of bioreactors in conjunction with microcarriers has emerged as an alternative approach for large-scale MSC cultivation. Within the spectrum of bioreactor configurations, stirring bioreactors stand out as the most feasible for cultures exceeding 100 L, surpassing other reactor types in

Table 2
Active and passive devices for mechanical stimulation and properties measurement of cells.

Technologies	Cell source	Purpose of research
DEP (Nourse et al., 2014)	NSCs	Distinguish stem cells with different differentiation potentials by their electrical properties and isolate subpopulations
IFC (Song et al., 2016; Song et al., 2013)	hMSCs	Separate stem cells with osteogenic and adipogenic potentials based on their membrane potential
IFC (Bagnaninchi and Drummond, 2011)	hMSCs	Induce stem cell differentiation by applying different level of electrical stimulation, and the electrical properties of cells can be measured
IFC (Gong et al., 2021)	hMSCs	Measure the electrical properties of cell spheroids to characterise their differentiation potentials
IFC (Crocetti et al., 2014)	hMSCs	Use IFC to detect electrical properties (Cation channel expression level) of cells and correlate them with proliferation potential
Straight channel (Moledina et al., 2012)	mESCs	Observe autocrine and paracrine effects changes of cells under different flow rate
Straight channel (Otto et al., 2015)	hHSCs	Observe deformability of cells and their correlation with cell fate
Hydrophoresis (Choi et al., 2014)	Commercial MSCs	Investigate the correlation between cell adhesion and differentiation states
Slanted spiral (Moloudi et al., 2019)	Commercial MSCs	Recirculating MSCs in the microcarrier-based perfusion culture system
Serpentine channel (Lin et al., 2017)	Cultured iPSCs, NSCs, MSCs	Differentiate subpopulations of stem cells with different physical properties

terms of scalability and efficiency (Ge et al., 2023; Nogueira et al., 2021).

Stirring bioreactor culture provide better control of physiological parameters such as nutrients, pH, and dissolved oxygen levels than 2D flask culture (Bodiou et al., 2020; Chen et al., 2020a). It offers cost-effective and homogeneous conditions for massive cell expansion, resulting in consistent cell product quality at a large scale (Chen et al., 2020a). Despite the numerous advantages mentioned, the use of microcarriers within stirring bioreactors have yet to dominate over 2D flask cultures. Several factors contribute to this: different cell types require specific substrate properties, and existing microcarriers are not universally compatible (Jossen et al., 2018; Perez et al., 2016; Tavassoli et al., 2018). Moreover, microcarriers must mitigate the risk of breakage and the subsequent risk of microplastic contamination in the final products (Gupta et al., 2016).

2.1.3. Culture media

MSCs are typically cultured in α-MEM/DMEM supplemented with 10% foetal bovine serum (FBS) (Martin et al., 2017). The use of serum supplements introduces batch-to-batch variation due to donor-to-donor differences and increases the risk of contamination from pathogens in samples. (Panchalingam et al., 2015; Spees et al., 2004). These issues have prompted the evolution of serum-free and xeno-free media.

Xeno-free media use human blood serum or platelet lysate as culture supplement, diminish the likelihood of allergic reactions but present challenges in terms of cost and limited availability. On the other hand, serum-free media which composited of chemically defined components or animal-free components like algae-produced proteins, has more consistent product quality compared to serum-containing media. Notably, commercial serum-free media have demonstrated enhanced cell proliferation, differentiation, and therapeutic potential relative to traditional serum-based media (Bui et al., 2021; Dam et al., 2021; Mantripragada and Muschler, 2022). However, the simpler composition of serum-free media makes them more susceptible to the effects of cell heterogeneity on culture outcomes (Bui et al., 2021). This has led to a growing consensus that a broader spectrum of serum-free media formulations may be required to cater to the diverse needs of different cell lines. Currently, there is a lack of standard composition of media for MSC culture (Jossen et al., 2018). Research now focuses on creating cost-effective, standardised culture media to ensure consistent MSC growth and differentiation.

Table 3
Droplet-based microfluidic devices for single cell analysis.

Technologies	Throughput	Cell types	Applications
Droplet generator	100 uL/h	mESCs	Developed droplet generator for RNA-sequencing and study the heterogeneity in subpopulations of ESCs (Klein et al., 2015)
	N.A.	Murine HSCs	Use RNA-sequencing to study the differentiation landscape of HSCs, correlate DNA methylation with differentiation (Izzo et al., 2020)
	N.A.	ESCs	Use DNA sequencing to study subpopulations of ESCs classified by different chromatin states (Rotem et al., 2015)
	N.A.	Mouse Muscle stem cells	Perform RNA sequencing the identify cell populations involved in muscle regeneration and key paracrine factors involved in muscle regeneration (De Micheli et al., 2020)
	N.A.	Mouse Muscle stem cells	Identify different muscle stem cells in different state and describe the transcriptional differences in each state (Dell'Orso et al., 2019)
	100 uL/h	MSCs	Single cell encapsulation in hydrogel for bone regeneration(An et al., 2020)
Static droplet array (SDA)	160 chambers	hESCs	Long-term analysis of correlation between cell proliferation and cell marker expression (Sikorski et al., 2015)
	20 chambers	hESCs	On-chip PCR analysing absolute mRNA level in single cell (Zhong et al., 2008)
	48 chambers	Murine ESCs	Precise control of single cell microenvironment for long-term tracking study. (Dettinger et al., 2018)
	1600 chambers	mHSCs	Controlled microenvironment for single cell proliferation study (Lecault et al., 2011)
Microwells	700–6000 traps	mESC	Cell fusion with chemical and electrical methods (Skelley et al., 2009)
	2048 traps	hHSCs	A tool with an algorithm to track cell division and cell phase (Kobel et al., 2012)
	440 traps	hHSCs	Test cancer drug response with patent-derived stem cells (Faley et al., 2009)
DEP array	N.A.	mESCs	Measure the difference of electrical properties of individual cells and their differentiation potentials after time (Zhou et al., 2016)
	8 DEP cages	Oogonial stem cells	Single cell isolation from tissue and observe the relationship between marker expression level and differentiation potential (Silvestris et al., 2018)
Acoustic microfluidics	3 acoustic traps	NSCs	Demonstrate the potential to trap single cell, single spheroid and perfuse different media (Evander et al., 2007)

Table 4
Droplet-based microfluidic devices for microcarriers production.

Type	Material	type	Cells	Material throughput	purpose
Microcarriers	GelMA	Step emulsification	hMSCs	5–30 mL/h (de Rutte et al., 2019)	Large-scale, low-cost microcarriers production for stem cells expansion (Rogers et al., 2021)
	Gelatine crosslinked with genipin	Flow focusing	hMSCs	2.5uL/min	Dissolvable microcarriers for stem cells expansion (Ng et al., 2021)
	PEG	Flow focusing	ESCs	4 uL/min	Produce microcarriers with appropriate ECM that can direct stem cell differentiation (Allazetta et al., 2013)
	PLGA	Co flow	MSCs	5 uL/min	Growth factors integrated microcarriers for differentiation of stem cells (Dashtimoghadam et al., 2020)
Encapsulation	Gelatine crosslinked with PEG	Cross flow	hMSCs (bone)	NA	Injectable encapsulated cells to promote articular cartilage tissue regeneration (Li et al., 2017)
	Alginate Calcium	Cross flow	hMSCs (dental)	200 uL/h	Injectable encapsulated cells to promote articular cartilage tissue regeneration (Moshaverinia et al., 2013)
	Alginate Calcium	Flow focusing	hMSCs	13 uL/min	Control the microenvironment of hMSCs and induce differentiation for research (Chan et al., 2013)
	Alginate Calcium	Co flow	hNSCs, dental MSCs	N.A.	Study stem cell behaviours in a 3D manner (Hidalgo San Jose et al., 2018)
	GelMA	Co-flow with electrostatic field	hMSCs (bone)	100–2000 uL/h	Injectable encapsulated cells for osteogenic tissue construct (Zhao et al., 2016)
	Alginate calcium	Co-flow with electrostatic field	hEndoderm stem cells	NA	Proliferate and differentiate somatic cells from stem cells for transplantation (Feng et al., 2020)
	PEG-based hydrogel	Co-flow	hiPSCs with NHCF-V cells	NA	Make stem cell organoids for potential tissue regeneration purposes (Liu et al., 2020)
	PLGA	Flow focusing and co-flow	mMyoblast	0.1–0.4 ul/min	Produce biocompatible porous microcarriers for better nutrients supply of cells and transplantation (Kankala et al., 2019)
	PLGA	Co flow	C2C12	N.A.	Encapsulate neural cells in hydrogels to produce 3D stem cell niches (Alessandri et al., 2016)
	Alginate Calcium	Co-flow	hNSCs	20 mL/h	Provide new encapsulation methods and scaffold structures for improved tissue engineering outcomes (Kim et al., 2022)
	GelMA	Cross flow	C2C12, hMSCs	50 uL/min	Build encapsulated stem cell fibres for reconstructing neural fibres in vivo (Onoe et al., 2013)
	GelMA	Co-flow	MSCs		Encapsulate MSCs with pancreatic cells to provide a immune-suppressive, favourable environment for pancreatic cells survival and function (Sun et al., 2023).
	Collagen	Cross flow	Pig ADSCs	0.2 mL/h	Encapsulate MSCs for clinical treatment. Cells went through multiple clinical handling process and the cells maintained their potential for therapy (Zuo et al., 2023).
Fibres	Alginate Calcium	Co-flow	Neural stem cells	75 uL/min	Provide potential multifunctional hydrogel fibres for material science, tissue engineering research and regenerative medicine application (Wang et al., 2021)
	Dextran, Alginate Calcium	Flow focusing and co-flow	Pancreatic endocrine progenitor cells	2 uL/min, 40uL/min	A potential source of small-diameter vascular grafts (Liu et al., 2017)
	Alginate Calcium	Co-flow	mMSCs	5–40 uL/min	Make stem cell organoids for potential tissue regeneration purposes (Liu et al., 2020)

2.2. Downstream production

2.2.1. Cell harvesting, washing and concentrating

DSP consists of three key stages, commencing with cell harvesting from culture flasks or microcarriers. Enzymatic methods are employed to detach the cells, followed by subsequent centrifugation or membrane-based technologies to remove the enzyme and/or microcarriers.

The main challenges involved with traditional centrifugation is the labour-intensive and time-consuming process which is further constrained by potentially detrimental effect of centrifugal forces on cell viability and recovery. Also, the frequent manual handling of the vessels in the process enhances the contamination risks (Jossen et al., 2018). Addressing the limitations of traditional centrifugation, especially in the context of large-scale manufacturing, has enabled the emergence of alternative technologies such as dead-end filtration, tangential flow filtration (TFF), and continuous flow centrifugation (Chilima et al., 2018; Schnitzler et al., 2016a). Though innovative, these technologies present their unique challenges. Dead-end filtration and Tangential Flow Filtration both encounter challenges with membrane clogging, particularly when handling large volumes (Schnitzler et al., 2016a; Zydney, 2016). Although TFF has been enhanced to reduce clogging issues, it continues to face significant hurdles in efficiently filtering large culture volumes. In both methodologies, membrane clogging frequently leads to alterations in cell viability and fate, thereby diminishing the therapeutic potential of the cells due to unresolved device blockages (Chilima et al., 2018; Rodrigues et al., 2018; Zydney, 2016). Moreover, continuous flow centrifugation is an alternative technology that combines washing and concentration in one step (Schnitzler et al., 2016b), reducing the chance of contamination and the throughput can go up to 2000 L/h (Sartorius Stedim Biotech, 2017). However, these systems are often costly and still may damage the cells due to centrifugal forces applied like the traditional centrifugation (Joseph et al., 2016). Additionally, the continuous flow centrifugation systems are not capable of harvesting cells from microcarriers at large-scale (Schnitzler et al., 2016a).

In fact, harvesting cells from microcarriers is one of the major limiting factors of using microcarriers in the industry. Cells are harder to detach from microcarriers compared to planar flasks and it results in 20–50% cell loss during harvesting. In certain instances, researchers reported the loss of entire cell population during the cell harvesting steps from microcarriers (Derakhti et al., 2019; Mawji et al., 2022; Roberts et al., 2023; Van Beylen et al., 2021). To overcome the harvesting limitations, next generation microcarriers with dissolvable or cell-releasing feature, such as temperature and UV sensitive microcarriers are being

Table 5
Integrated and uncommon microfluidic devices.

Integrated microfluidic systems	Cells	Purposes
Micromixer + magnetic sorter (Wu et al., 2010)	HSCs	Integrated microfluidic system for purification of specific cell types
Organ-on-a-chip microarrays (Kane et al., 2019)	NSCs	Automatic controlled cell culture and differentiation system
Droplet generator + active microfluidics (Sun et al., 2020)	HepaRG	Use a droplet-based sorter as a chemical sensor to detect chemical secretion level after treating the cells with toxicity assay kits.
Active microfluidics + inertial microfluidics (Sakuma et al., 2019)	MSCs	On-chip spheroids sorting and measurement of Young's modulus
Active microfluidics + CTA (Fan et al., 2019)	MSCs	Measurement of single cell electrical properties for differentiation potential study
Active microfluidics + CTA (Valero et al., 2008)	MSCs	Single cell genetic modification
Micromixer+ two types of inertial microfluidics (Ding et al., 2022a)	MSCs	Detach and separate MSCs from microcarriers
Active microfluidics + passive microfluidics (Jiang et al., 2019)	NSCs	high throughput, high purity isolation of stem cells
Simple channels design		
Straight channel as mini bioreactor (Gagliano et al., 2019; Giulitti et al., 2019)	iPSCs	Use microfluidic channels to generate iPSCs.
Straight channel as mini-bioreactor (Singh et al., 2013)	iPSCs and ESCs	Use microfluidic channels to select iPSCs and ESCs based on the adhesive properties.
Gradient generator (Lyu et al., 2020)	MSCs	Find out the appropriate concentration to induce stem cells differentiation for transplantations
Microchannel + image analysis (Kamei et al., 2010)	ESCs	Use microfluidic channels to isolate characterise the ESCs in different chemical environments.
3D micromixers(Tan et al., 2005)	hMSCs	Increase the chance of immune-affinity binding of cells and antibody-coated microbeads.
3D micromixers (Ding et al., 2022b)	hMSCs	Continuous and rapid mixing of cells and cryoprotectant in labour-free manner.
Microfilter (Schirhagl et al., 2011)	HSCs	Use microfilter to perform size-based purification of stem cells.

explored to address this issue for large-scale adherent cell manufacturing (Tavassoli et al., 2018).

2.3. Formulation and distribution

2.3.1. Cell cryopreservation

In clinical scenarios where stem cells are stored post-harvest, they're cryopreserved in liquid nitrogen. Ice crystal formation during this can harm the cells, affecting their post-recovery performance (Levy et al., 2020; Singh et al., 2017). Cryoprotective agents (CPA) are used to prevent this damage. The predominant CPA is 10% Dimethyl sulfoxide (DMSO), which prevents water crystallisation and adjusts cell electrolyte balance. However, excessive DMSO exposure can harm cell viability and differentiation (Chetty et al., 2013; Kita et al., 2015). Hence, for minimal cell damage, it's crucial to mix cells and DMSO swiftly, maintain a low 1:9 ratio, and limit exposure time. Historically, manual mixing, with its downsides including low throughput and contamination risks, was used. Robotic arm systems, which automate mixing and enhance speed, are now employed, but they can harm cells with prolonged mixing and introduce batch inconsistencies (Thirumala et al., 2013). While these robotic systems speed up processing, they're expensive to run and maintain.

2.3.2. Quality control

The stem cell industry faces a deficiency in universal quality control system due to the diversity in various cell types, cell source, cellular heterogeneity and downstream applications. Notwithstanding the inherent cellular discrepancies previously mentioned, the quality of stem cells may vary significantly due to their diverse culture protocols and production processes employed across different companies and laboratories (Burnouf et al., 2016; Yaffe et al., 2016). The lack of quality control and the inherent variability of cells contribute to contradictory experimental results and clinical outcomes (Levy et al., 2020; Shi et al., 2017; Temple and Studer, 2017; Trounson and McDonald, 2015).

In order to address this issue, the International Society for Cellular Therapy (ISCT) released the well-recognised standard for characterising MSCs in 2008 (Dominici et al., 2006), along with regulatory guidelines for cell viability, potency, tumorigenesis, microbial assessment, and long-term stability (Fernandez-Santos et al., 2022). However, these standards lack specific testing guidelines. Most adopted quality control measures for MSCs often entail labour-intensive methods including immunophenotyping and viability tests via flow cytometry, potency assessments via T-cell co-culturing, and differentiation potential tests. While these methods do serve to enhance quality assurance, challenges to standardisation persist, largely due to the inherent heterogeneity of the cells and the complexity of processes like T-cell co-culture. Additional tests, such as tumorigenicity assessment and senescence tests, although not deemed essential, have attracted attention for their potential relevance within the quality control systems (Galipeau et al., 2016; Menard et al., 2013; Wuchter et al., 2015). Thus, the development of easy-to-use, consistent, and universally applicable quality control procedures remains an unmet need across the production, transport, and application of stem cells (Sensebe et al., 2013).

3. Microfluidic devices used in stem cells research

Microfluidic devices have been proposed as a key technology for addressing many of the abovementioned challenges in the stem cell industry. It provides high precision and low-cost fluid manipulation in a small footprint device (Mark et al., 2010; Neethirajan et al., 2011). Microfluidic devices offer increased efficiency and reduced costs, while requiring fewer reagents and less expertise. Additionally, they enable groundbreaking applications previously unattainable, such as large-scale single-cell sequencing and the development of small-scale tissue or organ models. Herein, we have categorised microfluidic devices into cell separators, cell stimulators/manipulators, droplet-based microfluidics, and organ-on-a-chip, integrated or other microfluidics, highlighting their potential to significantly improve the stem cell production process.

3.1. Cell separators

Stem cells constitute a small subpopulation of cells in tissues. For instance, they account for 2% (Bora and Majumdar, 2017) of cells in adipose tissue and 0.02% in bone (Alvarez-Viejo et al., 2013), while the cell tissues themselves may contain over 15 various cell types (Xavier et al., 2016). To isolate specific stem cell populations, researchers rely heavily on techniques such as FACS (Borchin et al., 2013; Buesen et al., 2009; Fong et al., 2009), MACS (Fong et al., 2009; Freund et al., 2006), or manual selection using microscopy (Colter et al., 2001). These methods select the stem cells based on surface-specific markers and morphology, which have high specificity, but are suffering from low recovery rate of stem cells, costly reagents and equipment (Xavier et al., 2016). They are also limited by low scalability, limited selectivity, and high risk of contamination (Diogo et al., 2012). Moreover, certain stem cells lack definite surface markers that can identify them (Galipeau et al., 2016), limiting the effectiveness of marker-based selection. To overcome these challenges, novel approaches are being developed to isolate stem cells based on their functional characteristics or genetic

profiles. These efforts are critical to advance the study and application of stem cells in regenerative medicine.

In recent years, microfluidic devices have been deployed to isolate cells based on size, deformability, and surface charges. Microfluidic cell separations can be classified into two categories: active and passive methods. Active microfluidics integrate external energy sources such as electric fields, acoustic forces, and magnetism with microchannels to control microparticles, while passive microfluidics rely mainly on channel designs, fluid forces and properties of the liquid flow to manipulate the particle movement. Active microfluidic devices offer monitoring of particles in real time (Yan et al., 2017) and precise control of individual particle and different fluid types (Zhu and Wang, 2016). However, the throughput of active microfluidic devices is generally low and there is a potential risk of damaging cells due to the external energies applied. Passive microfluidics on the contrary, generally have higher throughput and can be easily paralleled to further increase the throughput (Yan et al., 2017).

3.1.1. Active microfluidics

3.1.1.1. Dielectrophoretic devices (DEP) for cell sorting. Dielectrophoretic (DEP) based microfluidic devices represent a cornerstone of active microfluidic systems, offering a versatile platform for cell separation predicated on size, density, and electronic characteristics. The earliest application of DEP on stem cells dates back to 1995, when Talary et al. (1995) used a DEP device to purify peripheral blood stem cells (PBSC) from bone marrow samples. Adjusting the voltage of DEP device allows separation of cells based on different physical attributes while washing away the unwanted cells and collection the desired cell subpopulation (Fig. 2A, Prieto et al., 2012; Simon et al., 2014). This potentially allows perfusion of different reagents or solution to perform multiple functions in one system. More recently, due to the development of microfabrication technologies, the purity and recovery rate of cells increase drastically in DEP-based systems (Song et al., 2015). Despite the advancements, still the DEP systems suffer from low throughput (μL/min scale) for industrial applications.

3.1.1.2. Magnetic, acoustic, and optic-based microfluidic cell sorters. In addition to DEP devices, other types of microfluidic systems, such as magnetic, acoustic, and optic-based, have been employed in stem cell separation. While magnetic microfluidic devices enhance the efficiency of antibody-based separation and recovery, the necessity of using

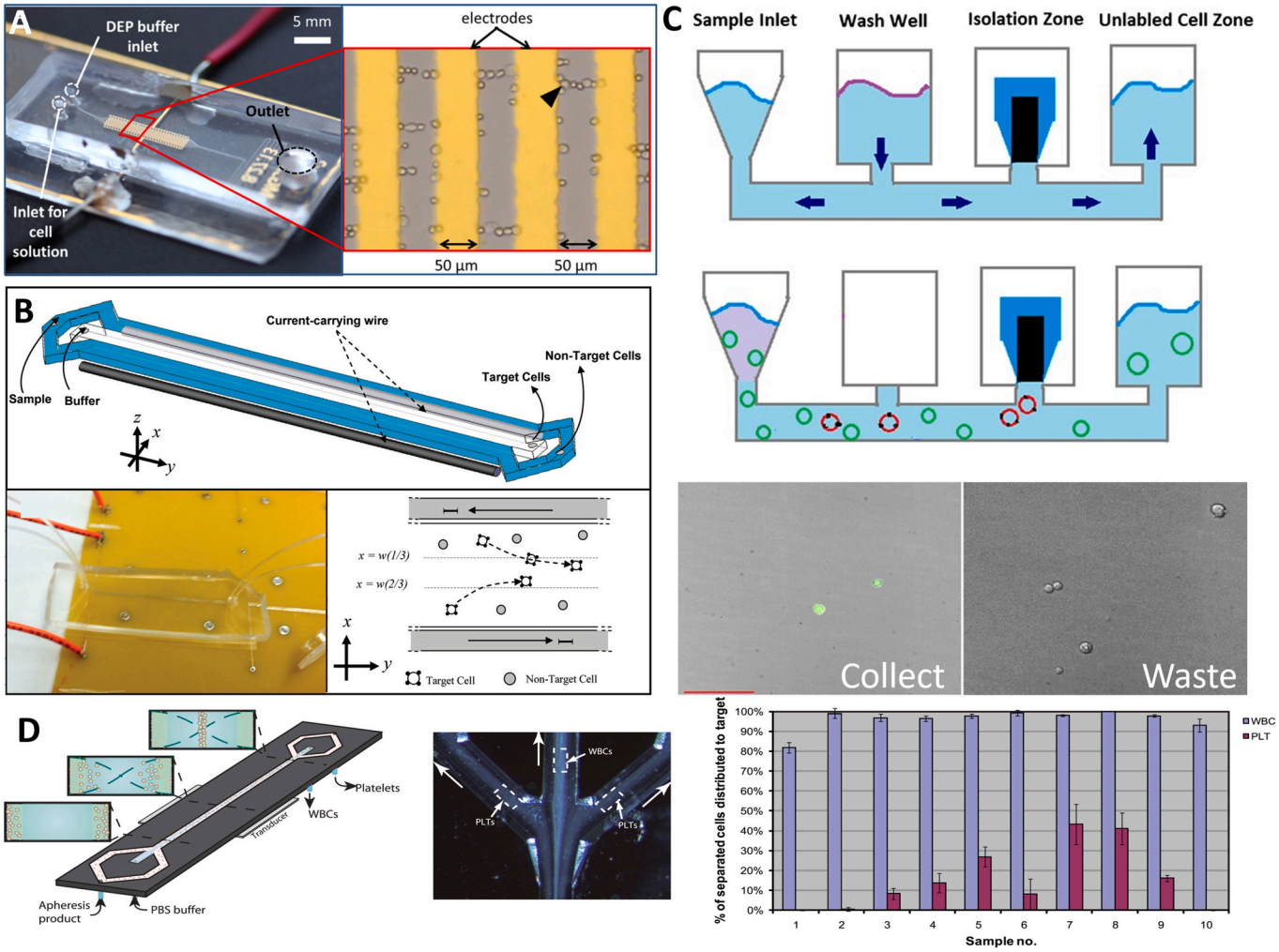

Fig. 2. Active microfluidic devices used for stem cell isolations. **A)** The Dielectrophoretic microfluidic chip (left) used to trap (right), wash and release NSPCs. Reproduced with permission (Simon et al., 2014) Copyright 2014, AIP Publishing. **B)** Magnetic microfluidic device can isolate target cell types with high recovery rate, but cell modification is needed. Reproduced with permission (Plouffe et al., 2012) Copyright 2012, American Chemical Society. **C)** Similar to DEP-based microfluidic devices, magnetic-based microfluidic devices can be operated in different ways to retain the cells in the device and perform washing together with isolation. Reproduced with permission (Zeng et al., 2015) Copyright 2015, John Wiley and Sons. **D)** Acoustic microfluidic devices perform cell size-based separation due to the different reactions of particles responding to the same wave strength (left) with 805% overall recovery rate of white blood cells (WBC, right). Creative Commons CC-BY license (Dykes et al., 2011). Copyright 2011, the Authors. Published by PLOS.

antibodies presents a disadvantage when compared to alternative microfluidic platforms (Fig. 2B, Plouffe et al., 2012). Acoustic microfluidic devices have already been commercialised (IsoFlux™ cell sorter), and separate stem cells based on the size differences compared to other somatic cells (Fig. 2C, Zeng et al., 2015). This demonstrate the capability of microfluidic devices to be used in industrial scale for stem cell isolation from raw blood and tissue samples with minimum target cells loss. The results also showed better preserved cell viability and cell functions. Acoustic microfluidic devices have been demonstrated to separate stem cells from blood (Fig. 2D, Dykes et al., 2011), including separation of live and dead stem cells (Zalis et al., 2016). As for optical microfluidics, optical tweezer is a technology that precisely manipulates individual particles with high-energy laser beam. Wang et al. (2011) have combined optical tweezers in a microfluidic channel to sort fluorescence-tagged hESC from non-tagged cells with a 90% recovery rate and 90% purity. Optic tweezer has amazing accuracy and control, but the throughput is very limited.

Active microfluidic devices have become a promising technology for isolating target cells from clinical samples with high separation resolution and precise control. This technology is particularly useful in the stem cell therapy and cultivated meat industry, where target cells (e.g., MSCs) lack universal markers for purification. While active microfluidic devices typically have a relatively lower throughput, while offering a higher separation efficiency which is especially beneficial when separating stem cells from small tissue samples. The geometries and designs of active microfluidic devices are simple, they can be configured in series or parallel to enhance system throughput or minimise target cell loss. In the future, active microfluidic devices are likely to be developed for high volume processing in stem cell industry applications while maintaining high resolution separation from clinical samples.

3.1.2. Passive microfluidics

Passive microfluidics can be divided into 4 types: hydrophoresis, pinch flow filtration, deterministic lateral displacement (DLD) and

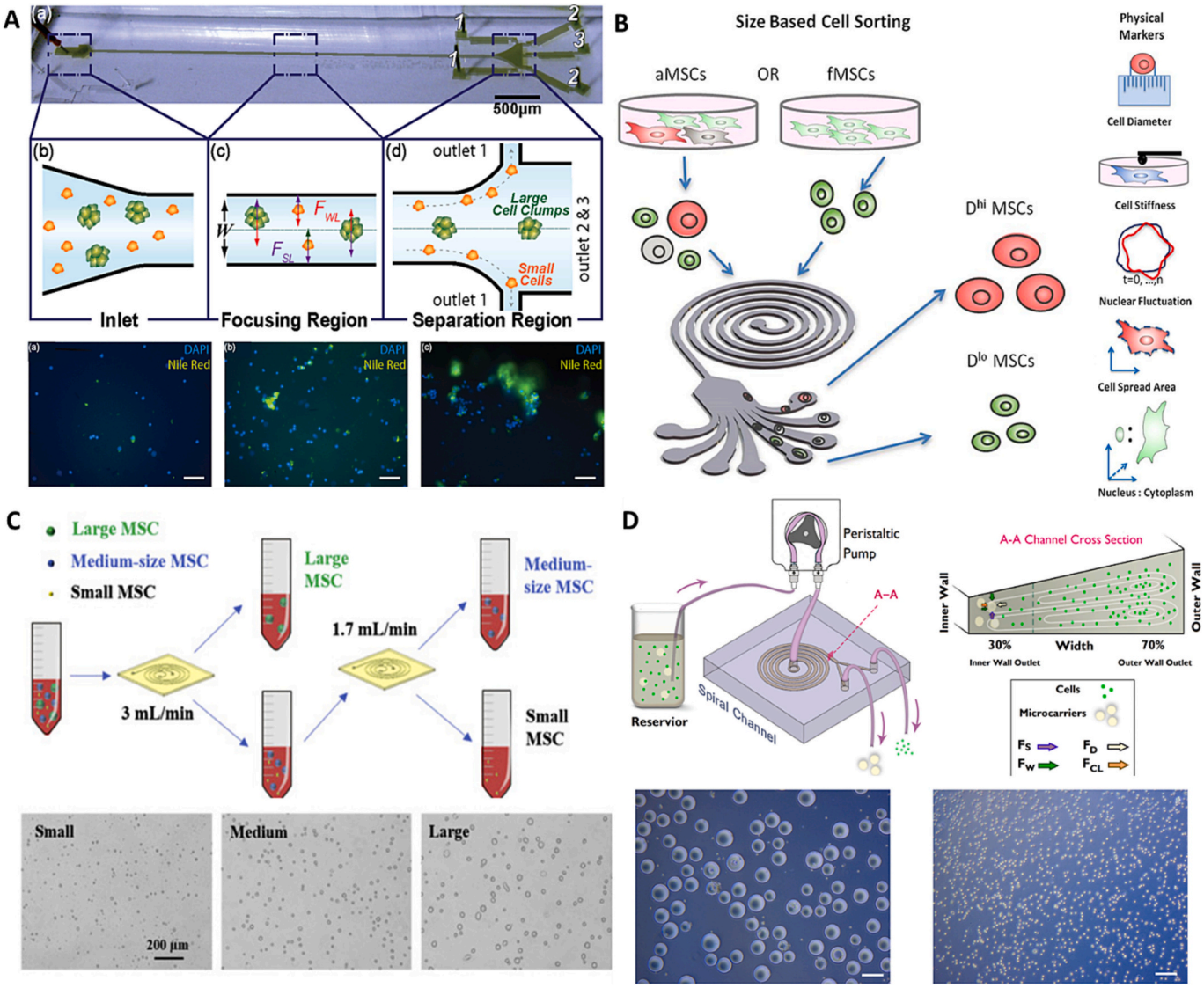

Fig. 3. Different types of inertial microfluidic devices used for stem cell separation. **A)** straight channel inertial microfluidic devices (a) used to separate single adrenal cortical progenitor cells from surrounding cell clusters. The cells collected from different outlet are showed in (b) outlet 1, (c) outlet 2 and (d) outlet 3. Reproduced with permission (Hur et al., 2012). Copyright 2012, the Authors. Published by PLOS. Spiral microfluidic devices used to separate stem cells with different physical properties in one step with multi-exits **B)** and two sequential steps on the same device design **C)**. Reproduced with permission (Lee et al., 2014; Yin et al., 2020) Copyright 2014, National Academy of Sciences. Copyright 2020, Elsevier. **D)** Scaled-up inertial microfluidic devices used for separation of MSCs from microcarriers. 97% of the beads can be collected from the inner outlet (bottom left) and 70% of cells collected in the outer outlet (bottom right). Creative Commons CC-BY license (Moloudi et al., 2019). Copyright 2018, the Authors Published by Springer Nature.

inertial microfluidics (Yan et al., 2017). Among the four types of devices under consideration, hydrophoresis operates within a low flow rate range, pinch flow filtration necessitates sheath flow for effective operation, and deterministic lateral displacement (DLD) encounters issues of high shear stress and frequent clogging (Liu et al., 2013). In contrast, inertial microfluidic devices can be designed in a sheathless setting and operate at highest throughput among the four device types. It does not apply large shear stresses to the cells and is not prone to blockage due to the larger channel dimensions while providing a high separation efficiency. Given the benefits and potentials for industrial applications, this chapter primarily focuses on inertial microfluidic devices.

3.1.2.1. Inertial microfluidics. Inertial microfluidics exploits the phenomena of microparticles moving towards their equilibrium positions inside a microchannel to separate particles with their properties such as size, morphology or density (Zhang et al., 2016). Inertial microfluidics apply gentle forces to the cells, which have been shown to preserve the sensitive stem cell properties during the separation process (Lee et al., 2018; Lee et al., 2014; Moloudi et al., 2018). This is appealing in stem cell extraction from the tissue of origin. Indeed, Purifying stem cells from their tissue origin with inertial microfluidic devices is label-free, high-throughput, clogging-free, requires less manual handling, and preserves high cell viability along with a high recovery rate (Fig. 3A, Hur et al., 2012; Lee et al., 2018). Similar to active microfluidic devices, the throughput of inertial microfluidic devices is within the "mL/min" scale and can be further improved by parallelising these devices together without drastically increase the footprint of the system.

Stem cells can develop into various subpopulations with different physical properties and potential cell fate, even under the similar culture condition (Li et al., 2015). Inertial microfluidics have been used to distinguish different subpopulations of cells with their physical properties and study the cell secretion, gene expression and cell fate in each subpopulation (Fig. 3B, C, (Lee et al., 2014; Yin et al., 2020)). Besides that, they can be potentially used to reduce the batch-to-batch variability of the therapeutic products. Having a pure stem cell population is critical for the safety of regenerative medicine due to the direct administration of undifferentiated stem cells and its potential risks of tumorigenesis (Trounson and McDonald, 2015). This can be achieved by depleting differentiated stem cells from undifferentiated cells. Song et al. (2017) used a multi-outlet spiral chip with the shear flow to isolate neuronal stem cells from other non-neuronal cells in iPSC differentiation process. Multiple outlets of the device allow precise isolation of middle size neuronal cells from large cells & clusters from other smaller sized cells. It's noteworthy that in microfluidic devices, sheath flow—surrounding liquid streams which focus and guide the sample stream—enhances particle migration within the channel by augmenting the force (Hou et al., 2013), but also can act as a washing buffer, facilitating concurrent washing and separation processes. As a result, sheath flow-equipped devices generally have a smaller footprint than their sheathless counterparts, given that the former require fewer loops for focusing.

Inertial focusing have also been applied for separating particles larger than single cells such as cell aggregates and microcarriers. Moloudi et al. employed scaled-up, slanted two-outlet microfluidic devices for two distinct purposes: one for the separation of detached mesenchymal stem cells (MSCs) from microcarriers (as shown in Fig. 3D), and another for perfusion culture of microcarriers. These devices demonstrated high recovery rates and preservation of key stem cell properties, including surface marker expression and differentiation potential. (Moloudi et al., 2018; Moloudi et al., 2019).

Inertial microfluidic devices have been developed as one of the advanced methods for the separation of rare cells from blood samples and multiple commercial devices have been developed (Clearbridge BioMedics, Labyrinth Biotech Inc.). Despite their lower separation resolution compared to active microfluidic devices, inertial microfluidic systems offer advantages in high throughput and low operational and manufacturing costs. These attributes render them ideal candidates for cell separation, perfusion, and concentration in larger volume applications. Also, integrating multiple inertial microfluidic devices in the system can reduce the cell loss during separation and increase the throughput for larger scale production. For example, Lee et al. (2023) combined spiral and DLD device to separate ADSCs from lysed fat tissue samples with 30 folds enrichment and 94.1% recovery.

3.2. Active and passive mechanical stimulation or cell measurement devices

Active and passive microfluidics are also widely used to provide physical stimulations to the cells, study their behavioural changes and measure their physical properties. These devices are normally combined with signal readers or microscope to analyse the response of cells.

3.2.1. Active microfluidics

Active microfluidic devices can be used to stimulate or measure different properties of the cells using external energy sources including acoustic, magnetic or electric fields. Nourse et al. (2014) employed a dielectrophoretic (DEP) device, specifically a microfluidic impedance flow cytometer (IFC), to assess cell size, surface glycosylation, and resting membrane potential. Their aim was to investigate the relationship between membrane capacitance and neural stem cell (NSC) fate. IFCs hold considerable promise for cell studies and have garnered significant commercial interest, as evidenced by products like the Pollen IFC from Amphasys. IFC has been shown to characterise the differentiation potential (Song et al., 2016; Song et al., 2013) and proliferation states of cells (Crocetti et al., 2014). In a similar study, Gong et al. (2021) utilised a scaled-up IFC to characterise the differentiation potential of MSCs cultured on microcarriers. They took advantage of the fact that adipogenic cells, which have higher lipid content, exhibit lower conductivity, while osteogenic cells display higher conductivity due to an increased concentration of calcium ions on the cell membrane. The electrical properties of cells have been correlated with the expression levels of specific genes, serving as indicators of cellular behaviour (Fig. 4A). In this context, IFCs offer a non-invasive and non-damaging approach for monitoring both cell viability and differentiation potential during culture. This stands in contrast to traditional differentiation assays, which typically require more than two weeks to complete. Similarly, the response of cells to acoustic field can be recorded to measure the size and density of cells (Strohm et al., 2019).

3.2.2. Passive microfluidics

Passive microfluidic technologies harness hydrodynamic forces, dictated by channel geometry, to reposition cells within channels, mimicking the fluid dynamics of biological systems. As cells move through these devices, they experience varying levels of shear forces, enabling in-depth analysis of their consequent behavioural changes. The most fundamental application can be seen in straight channels, where fluid flow imparts shear stress upon the cells. Moledina et al. (2012) developed a microfluidic device equipped with five separate culture chambers, each with different flow rates for media perfusion. They examined the effects of these varying flow conditions on mouse ESCs, specifically comparing changes in signalling and proliferation rates across the different chambers. This technique removes endogenously secreted factors by washing away the paracrine elements at a controlled flow rate, thereby facilitating the determination of the minimum paracrine ligands required to guide cells towards a specific fate (Fig. 4B). Beyond this, the structural alterations of microchannels enable entirely novel parameters to be measured and exploited. A hydrophoresis channel allows stickiness-based characterisation of cells (Choi et al., 2014), and cells' deformation in high pressure channel can be used as another parameter to characterise cell fate specifications (Fig. 4C, (Lin et al., 2017; Otto et al., 2015)).

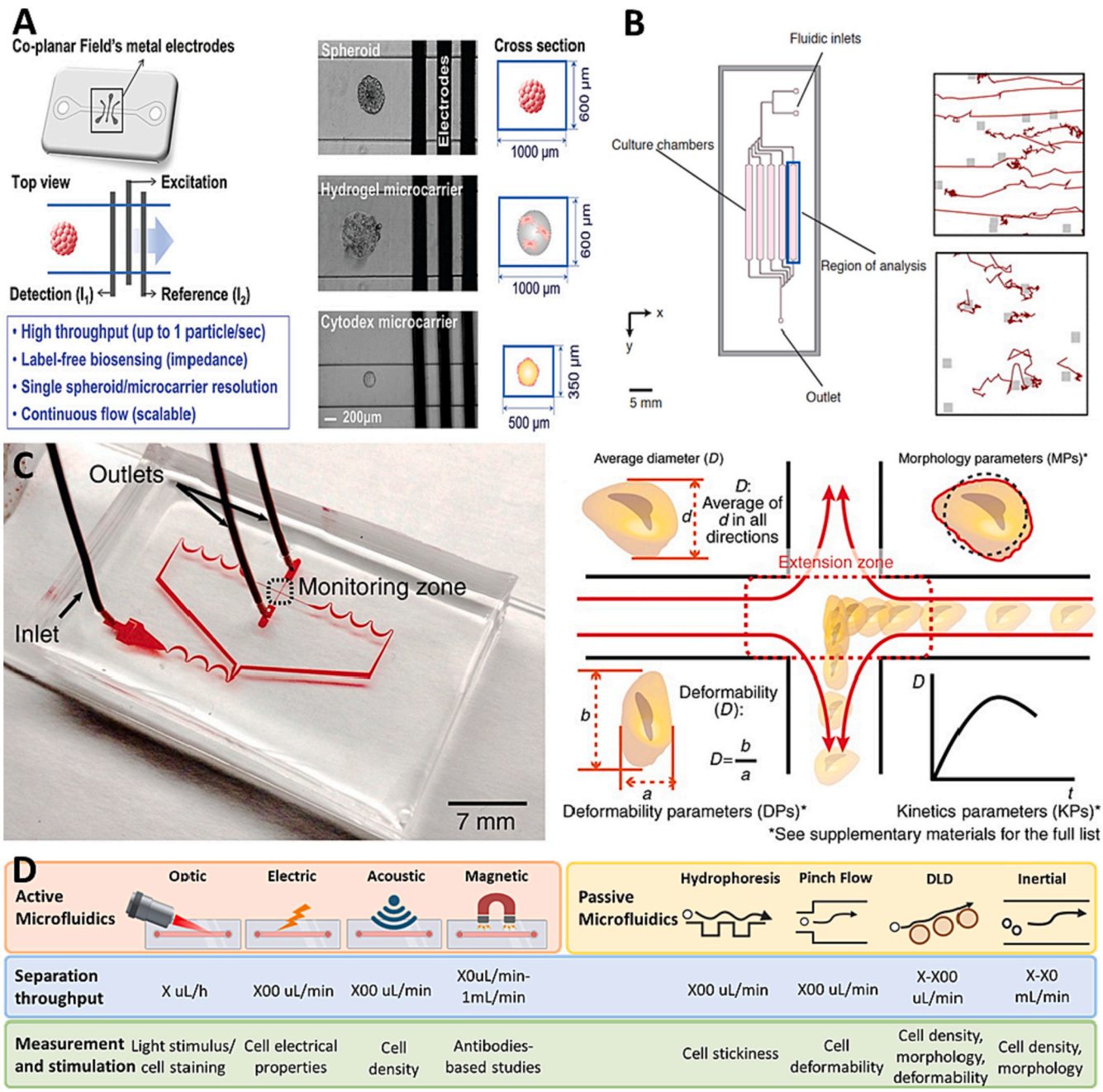

Fig. 4. The active and passive microfluidic devices used for mechanical stimulation and properties analysis of cells. **A)** An impedance flow cytometer used for analysing the drug response and proliferation of microspheres/cells attached microcarriers in a continuous manner. Reproduced with permission (Gong et al., 2021). Copyright 2021, John Wiley and Sons. **B)** A simple microfluidic channel to observe stem cells behaviours under paracrine effect and inertial flow. The cell density and secreted factor trajectories were recorded in lines. Reproduced with permission (Moledina et al., 2012). Copyright 2017, Springer Nature. **C)** Inertial microfluidic devices coupled with high-speed camera to record the deformability of cells under inertial forces. Reproduced with permission (Lin et al., 2017). Copyright 2012, National Academy of Science. **D)** Different research conducted by active and passive microfluidic devices (X < 4, according to the papers reviewed here).

Although originally conceived for research purposes, the high throughput and continuous operation of these microfluidic devices have emerged as potentially valuable assets for the bioprocessing industry. These devices introduce the capability to assess innovative parameters, including cellular deformability, adhesion characteristics, and surface charges. The correlation of these physical characteristics with cell fate or gene expression can offer unprecedented insights (Fig. 4D). These advancements unlock a new realm of possibilities for cell line development and product quality control, signalling transformative potential that could redefine standard practices within the bioprocessing landscape. It exemplifies the extent to which innovative technologies can have broad-reaching implications beyond their initial scope, underscoring the interconnected nature of scientific advancements.

3.3. Droplet microfluidics

3.3.1. Single cell analysis

Historically, cell and molecular biology have relied on bulk sample analysis, which captures average responses from entire cell populations. However, recent developments underscore the importance of single-cell resolution in highlighting cellular diversity and behaviour (Joensson and Andersson Svahn, 2012; Yin and Marshall, 2012). This becomes

particularly crucial in stem cell clinical applications, where cellular heterogeneity can introduce challenges. For example, transplanting even a small population of undifferentiated stem cells in a clinical setting can present the risk of teratoma formation due to inherent variability within the population (Altschuler and Wu, 2010; Yang et al., 2017). This emphasises the need to analyse individual cells, ensuring that critical cellular attributes are not obscured in collective analyses (Qian et al., 2017). Various reviews have delved into different facets of cellular applications, examining the genome (Gawad et al., 2016), epigenome (Schwartzman and Tanay, 2015), transcriptome (Stegle et al., 2015), metabolome (Rubakhin et al., 2011), proteome (Breker and Schuldiner, 2014; Levy and Slavov, 2018), and multi-omics approaches (Kucinski and Gottgens, 2020).

Droplet-based microfluidics has been extensively used to study single cells in recent years. Cells can be conveniently compartmentalised into individual droplets for downstream applications, thanks to the use of sub-nL level fractions and narrowed channels. Microfluidic-based single-cell analysis tools can be divided into three primarily categories of (1) droplet generators, (2) nano-well arrays, (3) hydrodynamic traps and (4) active microfluidics (Radfar et al., 2022). Droplet generators encapsulate individual cells into droplets by alternating liquid and oil phases in micrometre level channel. This method generates millions of droplets/min, with about 10% droplets containing a single cell (Klein et al., 2015). However, droplet generators cannot handle a small sample volume due to the requirement of minimum starting volume, similar to a flow cytometer (Ding et al., 2021). Nano-well arrays and hydrodynamic traps function by introducing cell solutions into the devices, thereby trapping cells into individual chambers or traps. It allows multi-omic studies and monitoring of single cells, while the throughput and scale are limited by the device size. Active microfluidic devices offer meticulous control over individual particles within the fluid or droplets that house a single cell. While these devices excel in terms of accessibility and controllability of individual cells, they suffer from lower throughput and heightened risk of cell damage due to the external forces applied. (Luo et al., 2019).

3.3.1.1. Droplet generators. Droplet generator has become a hot topic in recent years. The high throughput feature attracts great interest in large-

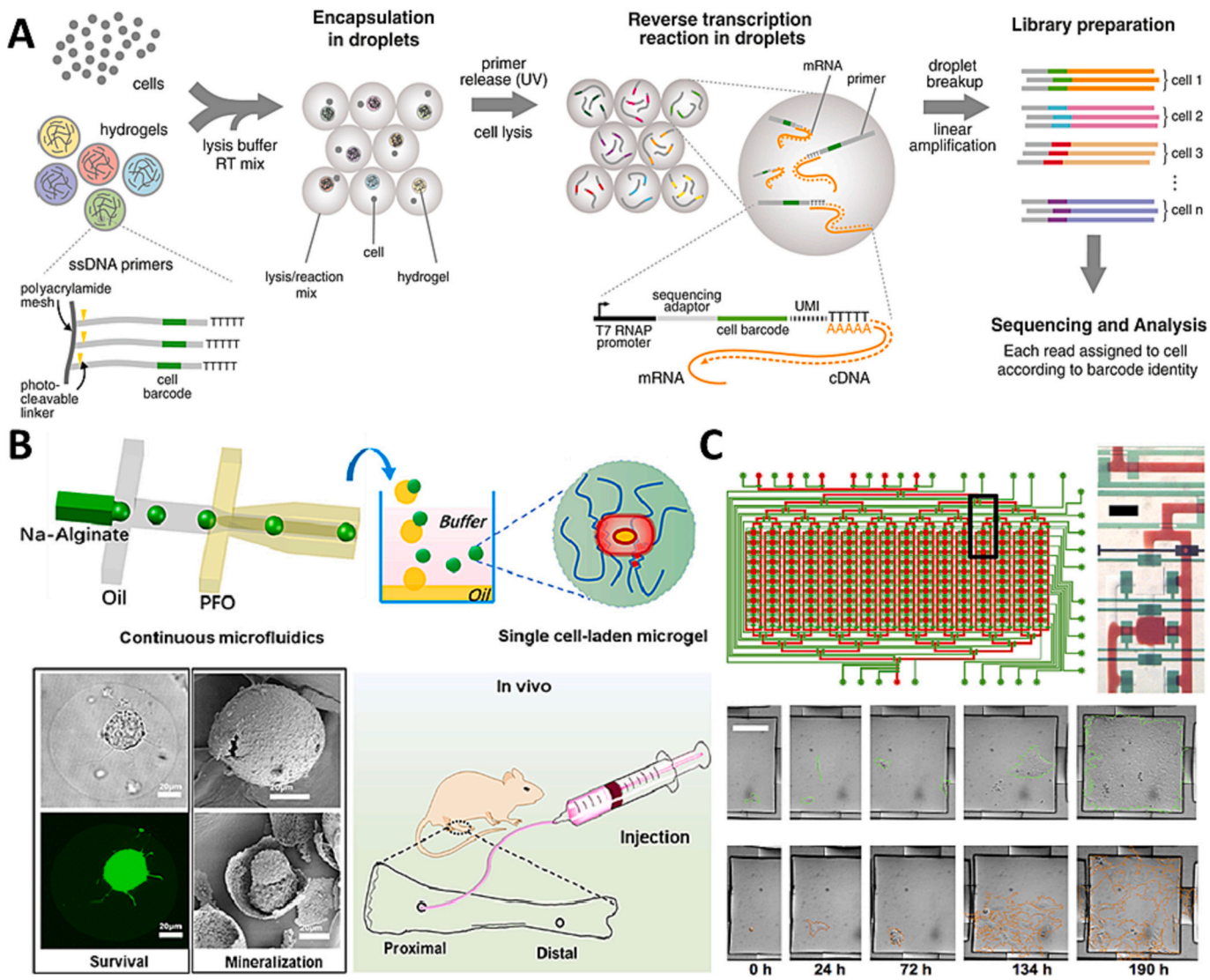

Fig. 5. Droplet-based microfluidic devices used for single stem cell analysis and studies. **A)** Droplet generator-based single cell sequencing reveal pluripotency related gene in ESCs by paring one cell with one barcoded-bead in droplets which captures the cell's genetic material. Reproduced with permission (Klein et al., 2015). Copyright 2015, Elsevier. **B)** Single cell-encapsulated microgel (top) used for regenerative medicine showed a lower clearance rate in the injection site (bottom). Reproduced with permission (An et al., 2020). Copyright 2020, Elsevier. **C)** Static droplet array (top) allows better long-term culture and tracking of single stem cells derived colony (bottom). Reproduced with permission (Sikorski et al., 2015). Copyright 2015, John Wiley and Sons.

scale cell encapsulation for sequencing and molecular studies. One of the pioneering applications of droplet generators in stem cell research was undertaken by Klein et al. (2015) who used the technology to investigate heterogeneity in mESCs. This approach has since gained global acceptance for single cell analysis (Fig. 5A) and was promptly commercialised. De Micheli et al. (2020) used a commercial solution (10× genomics sequencing kit) to sequence the RNA profile of mouse muscle stem cells before and after injury. The populations involved in muscle regeneration and the key paracrine factors involved in muscle regeneration were identified. This finding can be potentially useful not only for muscle regeneration, but also for speeding up the cultivated meat production process. Droplet generators can also be used to perform long-term culture or spheroid development. An et al. (2020) encapsulated single MSC in microgel to generate single MSCs-derived spheroid for bone regeneration. This allows direct intravenous injection due to the small droplet sizes and reduces the chance of clearing out by the host in allogenic injection (Fig. 5B).

3.3.1.2. Droplet arrays. Static Droplet Array (SDA) technology encapsulates cells within confined chambers during the injection of the cell solution. Given that the cells are physically compartmentalised, SDA serves as a useful tool for studies involving cell-cell interactions and cellular secretions (Dettinger et al., 2018; Lecault et al., 2011). To achieve enhanced control over cell trapping, a valving system can be integrated, thereby facilitating multi-modal studies (Fig. 5C, Sikorski et al., 2015).

3.3.1.3. Hydrodynamic traps. Hydrodynamic traps are microfluidic devices equipped with hook-shaped features designed to facilitate single-cell trapping. These devices can house thousands of individual, cell-sized traps, thereby enabling high trap occupancy during cell perfusion. Despite sharing the culture media, the device's compact footprint enables easy staining and large-scale cell screening (Faley et al., 2009). Moreover, it's well-suited for conducting studies on cell-cell interactions (Skelley et al., 2009).

3.3.1.4. Active microfluidics. Active microfluidic devices manipulate single cells via the strong energy field created by the external energy source. Cell properties measurement, such as electrical properties of membrane can be performed at single cell level (Zhou et al., 2016) and can yield more sensitive measurement results (Song et al., 2013). Active microfluidic devices have also been used to perform long-term culture studies. Evander et al. (2007) used an acoustic microfluidic device to trap single NSCs inside the channel, allowing perfusion culture of single cells and observe the proliferation of single cell and compared them to the trapped cell clusters.

As highlighted in the preceding section, the current landscape for standard quality control testing of stem cell products is markedly deficient. Unsuccessful clinical trials can be partially attributed to the heterogeneity of cells. Although there are papers proposing universal standards to quantify the immune-modulatory properties of cGMP-produced MSCs (Menard et al., 2013), insight of stem cell product quality has never been provided at the single cell level. Likewise, a comparison of the stem cells from different cell sources can be beneficial for quality control of products and standardising the cell sources. Stem cell therapy industry requires homogeneous product quality, and a well-characterised single muscle cell profile can be useful as a cell line selection standard. When it comes to cultivated meat products, the quality control process is still in its nascent stages, rendering standardisation premature. However, in light of the recent approval for the sale of cultivated meat products in the USA, the timing is opportune for establishing industrial standards that address both the quality of the cell source and the final product for consumer consumption. Given the fast and low-cost manner of droplet-based microfluidics, new stem cell quality control methods can be developed around it. It can provide a more detailed analysis and a better understanding of the batch-to-batch difference of the products, which would then improve the clinical outcome of stem cell therapy.

3.3.2. Droplet generators for microcarriers and microtubes manufacturing

The high-throughput, customisable and stable nature of droplet generators is also of great interest in the field of chemical synthesis such as microcapsule and microcarrier production. Traditionally, microcarriers are produced by water-in-oil emulsification. This method is simple and effective but results in huge size variation of microcarriers and a huge waste of material (the microcarriers production efficiency can be lower than 50% (Wissemann and Jacobson, 1985)). They also have limited control over the material choices and modification that can be performed on microcarriers. The emergence of droplet generators hugely reduces the waste of materials in a large scale, reduces the size variation and simplifies the post-processing steps. Also, the appliance of droplet generators enables production of combined materials, or microcarriers/microtubes with complicated structures and shapes.

Droplet generators offer significant customisability, capable of fabricating microcarriers of diverse sizes. This enables the culture of cells either in monolayers or in cell aggregates form, with multiple smaller microcarriers embedded inside. These scaffolds can act as the extracellular matrix, thereby enhancing the nutrient and oxygen supply to the cells (Kim et al., 2019). They can also be used to produce fibre-like or sphere structures to encapsulate cells.

3.3.2.1. Microcarriers. One of the main advantages of using a droplet generator is to produce low-cost, mono-sized microcarriers. Rogers et al. (2021) built a step emulsification droplet generator to make mono-size, UV-crosslinked gelatine methacryloyl (GelMA) microcarriers in large-scale. The cost of production was claimed to be 0.01 USD/cm^2 (Fig. 6A). Another advantage of droplet generator is it enables the fabrication of microcarriers capable of handling delicate materials or incorporating multiple components, overcoming the limitations of conventional emulsification technologies. For instance, Allazetta et al. (2013) and Dashtimoghadam et al. (2020) used flow focusing droplet generators to encapsulate growth factors into the microcarriers. These encapsulated factors were subsequently gradually released during cell culture to stimulate stem cell differentiation (Fig. 6B).

3.3.2.2. Cell encapsulation. During the droplet generation process, stem cells are encapsulated in gel-formed droplets. This gel matrix offers structural support to the cells, replicating the *in vivo* stem cell niche and fostering efficient organoid or spheroid development (Alessandri et al., 2016; Chan et al., 2013). The microgel shell can subsequently be dissolved, enabling the spheroid to be released (Chan et al., 2013; Liu et al., 2020). In addition to enhancing cell culture conditions, encapsulating stem cells offers a secure and effective approach for delivering them to injury sites for regenerative therapies. Delivering cells without encapsulation often leads to cell loss and decreased viability post-engraftment (Guerzoni et al., 2019; Zhao et al., 2016). Hence, researchers have demonstrated the utility of droplet generators in encapsulating various types of stem cells, including cardiomyocytes (Guerzoni et al., 2019), MSCs (Li et al., 2017; Moshaverinia et al., 2013), and endoderm stem cells (Feng et al., 2020). These encapsulated cells have been utilised to treat a variety of conditions in animal models. This approach of cell encapsulation also holds promise for addressing challenges related to nutrient supply during large-scale spheroids culture (Kankala et al., 2019).

3.3.2.3. Microfibres. Fibre-like carriers have garnered considerable attention as another type of cell carrier. The process of either encapsulating or cultivating cells inside these elongated fibres has shown promises for generating substantial cell mass with high uniformity. Unlike cell spheroids, where nutrient supply is constrained at the core,

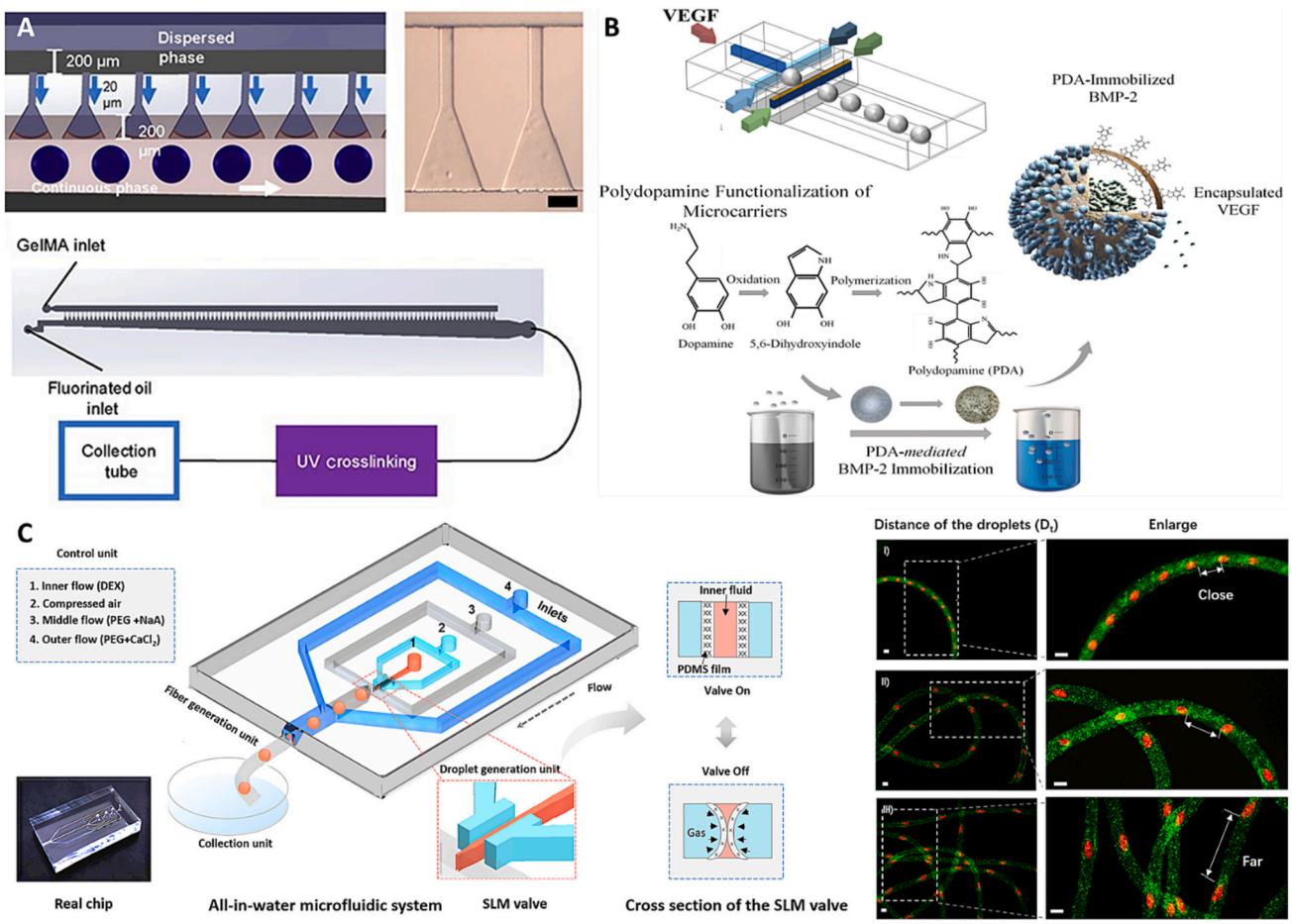

Fig. 6. Scaled-up droplet-based technologies used for microcarriers production/cell encapsulation. **A)** A step-emulsification droplet generator used for large-scale production of gelatine microcarriers. Reproduced with permission (Rogers et al., 2021). Copyright 2021, Oxford University Press. Droplet generator platforms can be customised to add different surface ligands or growth factors **B)** in the microcarriers to promote attachment, proliferation, and differentiation of different cell types. Creative Commons CC-BY license (Dashtimoghadam et al., 2020). Copyright 2020, the Authors, published by Springer Nature. **C)** Cells-ladened microfibres provide different microenvironment compared to microcarriers. Reproduced with permission (Wang et al., 2021). Copyright 2021, American Chemical Society.

fibre-encapsulated cells maintain a constant diameter, thereby ensuring a more even distribution of nutrients.(Liu et al., 2017). Fibre-like structure is also suitable for mimicking a different microenvironment compared to microcarriers (Fig. 6C, (Wang et al., 2021)). For cells that exhibit an extended morphology, such as neural stem cells, microfibre culture has been reported to have enhanced cell morphology and cell-cell interactions compared to other substrates (Onoe et al., 2013). The unique extended shape of the microfibres serves as a superior substrate for the proliferation and differentiation of muscle cells, emulating the structure of muscle fibre tissue in vivo. The potential of using microfluidic devices for producing microfibres and microcarriers has attracted significant attention in the stem cell industry. For instance, CellFiber Ltd. have already commenced utilising a microfluidic technology to manufacture microfibres for cell culture applications.

Currently, harvesting cells from microcarriers remains a significant hurdle in the stem cell bioprocessing sector. To overcome the challenge, it is crucial to develop dissolvable and edible solutions for the stem cell industry, both to minimise the risk of microplastic residues in the final products and to adhere to food and therapeutic goods standards. Moreover, recent research findings are demonstrating enhanced cell expansion rates and improved outcomes in implantation treatments through the use of porous structures in microcarriers (Zhou et al., 2021). The feasibility of this approach has also been supported by advances in microfluidic devices (Wang et al., 2015). While the production of microcarriers using droplet generators at an industrial scale remains an area of ongoing research, the current mL/min scale is not yet sufficient to meet industrial demands. However, similar to active and passive microfluidic separation devices, the challenge could be addressed by parallelising multiple microfluidic devices for microcarrier production.

3.4. Organ-on-Chips (OOC), Others and integrated microfluidic systems

3.4.1. Potential applications of Organ-on-chips (OOCs) in stem cell industry

Organ-on-chips (OOCs) are miniaturised micro-engineered physiological systems that recapitulate the key features of human organs *in vitro*, and they offer advantages such as controlled dynamic conditions, biochemical and mechanical cues, and multi-organ interactions. OOC is most commonly used in research and pharmaceutical industry, for diseases modelling (van Berlo et al., 2021) and drug screening (Dogan et al., 2021). In the stem cell industry, standardised OOC models can potentially provide a comprehensive model for evaluation of treatment outcome and thus a potential scalable standard for cell therapy quality control, or cell line establishment and evaluation. Furthermore, the incorporation of stem cell-derived organoids into the OOC framework has the potential to yield more sophisticated "organoids-on-a-chip" models, characterised by improved maturity and functional capabilities (Park et al., 2019; Wang et al., 2018).

3.4.2. Simple micrometre-level channels

Beyond the technologies previously detailed, emerging microfluidic devices engineered exclusively for specialised applications merit

attention, displaying substantial prospective value. For example, by miniaturising the culture chamber of cells, the delivery of pluripotent mRNA into somatic cells to generate induced pluripotent stem cells (iPSCs) would require significantly fewer reagents (Gagliano et al., 2019; Giulitti et al., 2019). Simple straight-channel microfluidic constructs exhibit markedly enhanced efficiency in cell reprogramming in comparison with conventional well-plate cultures, achieving reprogramming in a mere 12 days as opposed to 28 days. Un-reprogrammed cells can also be selectively eliminated with appropriate design (Singh et al., 2013). The benefits of using microfluidic devices to differentiate and reprogramme iPSCs have been demonstrated across multiple studies (Luni et al., 2022). The precise control of microenvironment, coupled

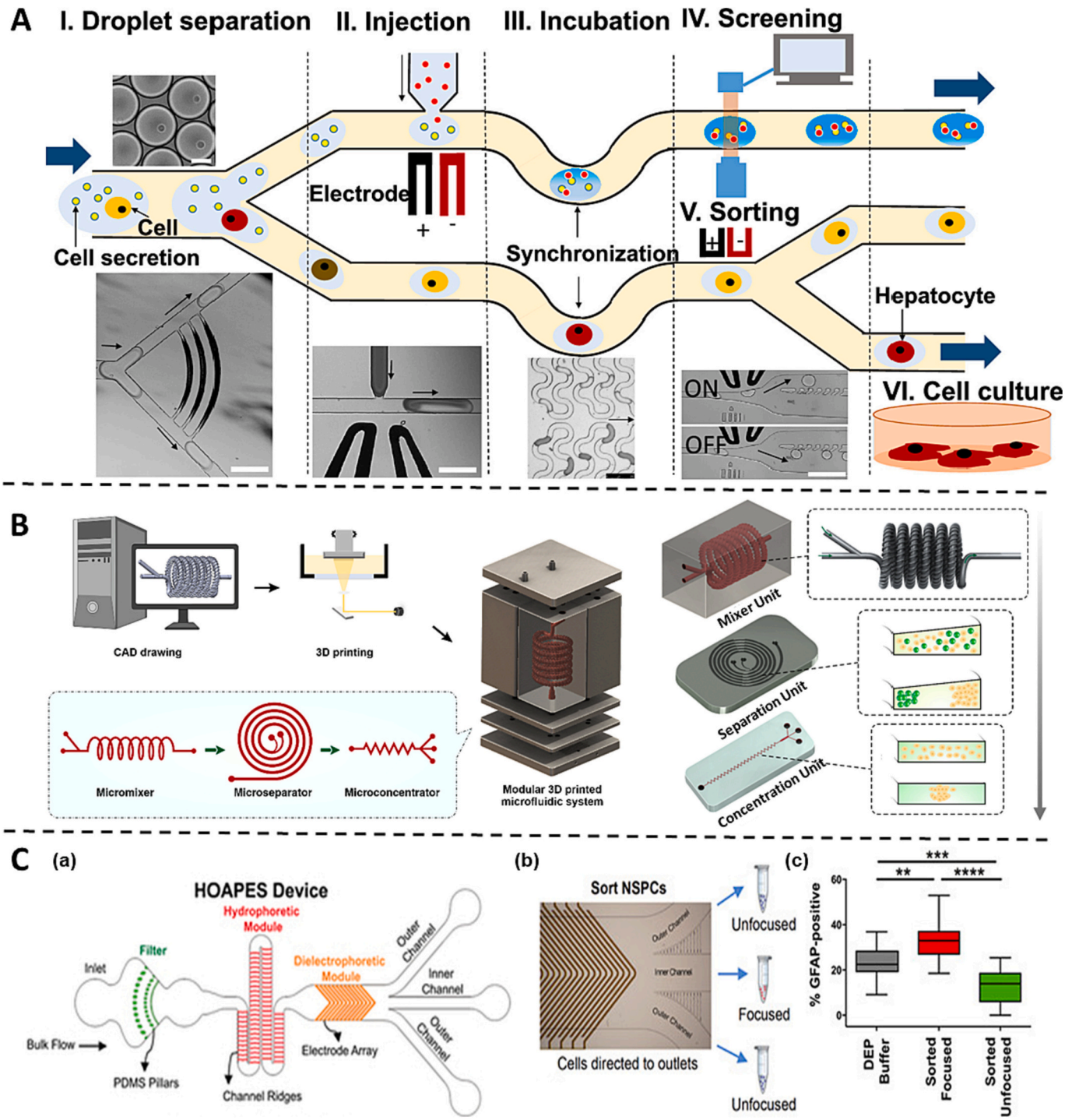

Fig. 7. Microfluidic devices that are hard to categorise and integrated microfluidic devices used for stem cell research. **A)** A droplet generator coupled with chemical sensor to analyse the secreted product while retrieving the cells for cell line establishment. This can be potentially helpful for establishing cell lines with specific secretion profile. Reproduce with permission (Sun et al., 2020). Copyright 2020, American Chemical Society. **B)** Rapid prototyping of a 3D printed modular microfluidic system designed for detaching and harvesting cells from microcarriers culture. Copyright 2022, the authors, published by Springer Nature. **C)** (a) A DEP array + hydrophoresis microfluidic device to improve the throughput of device and purity of isolated NSCs. (b) The outlet design and cells sorting process of the device. (c) The sorted cells are significantly better at differentiating to astrocytes than unsorted cells. Reproduced with permission (Jiang et al., 2019). Copyright 2019, AIP Publishing.

with the ease of manipulation and the potential for tracking conditions, not only optimises the results of existing protocols but also offers opportunities for crafting more efficient standardised methods for industrial applications (Luni et al., 2022). Also, several companies including Cell FE and Indee Labs developed microfluidic systems that disrupt the cell membrane for efficient cell transfection. Such technologies are poised to revolutionise the process of cell line establishment, providing continuous transfection capabilities to inscribe genes within cell lines. Another potentially useful microfluidic design is the branching layouts microfluidic channel which affords the creation of chemical concentration gradients in different dilution factors (Lyu et al., 2020). Concentration of growth factors is a key for cell growth and differentiation at different stages. Gradient generators can be helpful in dosage testing, personalised medicine, and also the cell line development process.

3.4.3. Micromixers

Micromixer has been used in the chemical and cosmetic industries, but the potential is yet to be explored in the stem cell industry. Micromixer is one type of microfluidic device that allows efficient mixing of different solutions in a short time. The remarkable mixing efficiency can be beneficial for mixing cells with immunomagnetic beads for improved recovery of stem cells in tissue isolation processes (Tan et al., 2005). High mixing efficiency is also needed and has been showcased in the stem cell cryopreservation process (Ding et al., 2022b), where high concentration of DMSO causes extra damage to the cells and affect final product quality.

3.4.4. Integrated microfluidic systems

In the active and passive microfluidic sections, the potential of parallelising either active or passive devices to enhance throughput is discussed. Conversely, when integrating diverse types of microfluidic devices into a single system, a multifaceted platform can be established, streamlining intricate applications and optimising efficiency within a closed-loop system. Examples of such systems include one-step immune-based system for purifying stem cells from tissue samples without pre-processing steps (Wu et al., 2010). Multi-omics studies which are hard to perform with traditional technologies can be automated by microfluidic systems now (Fig. 7A, (Sun et al., 2020)). Adding to these advancements, Sakuma et al. (2019) introduced a physical sensor featuring two piezoelectric actuators combined with a sheath-flow in a linear channel. This sensor can read Young's modulus of MSCs spheroids and sort the spheroids with different mechanical characteristics.

Overall, integrating multiple microfluidic devices in one system can perform multiple functions or reinforce the advantages of the

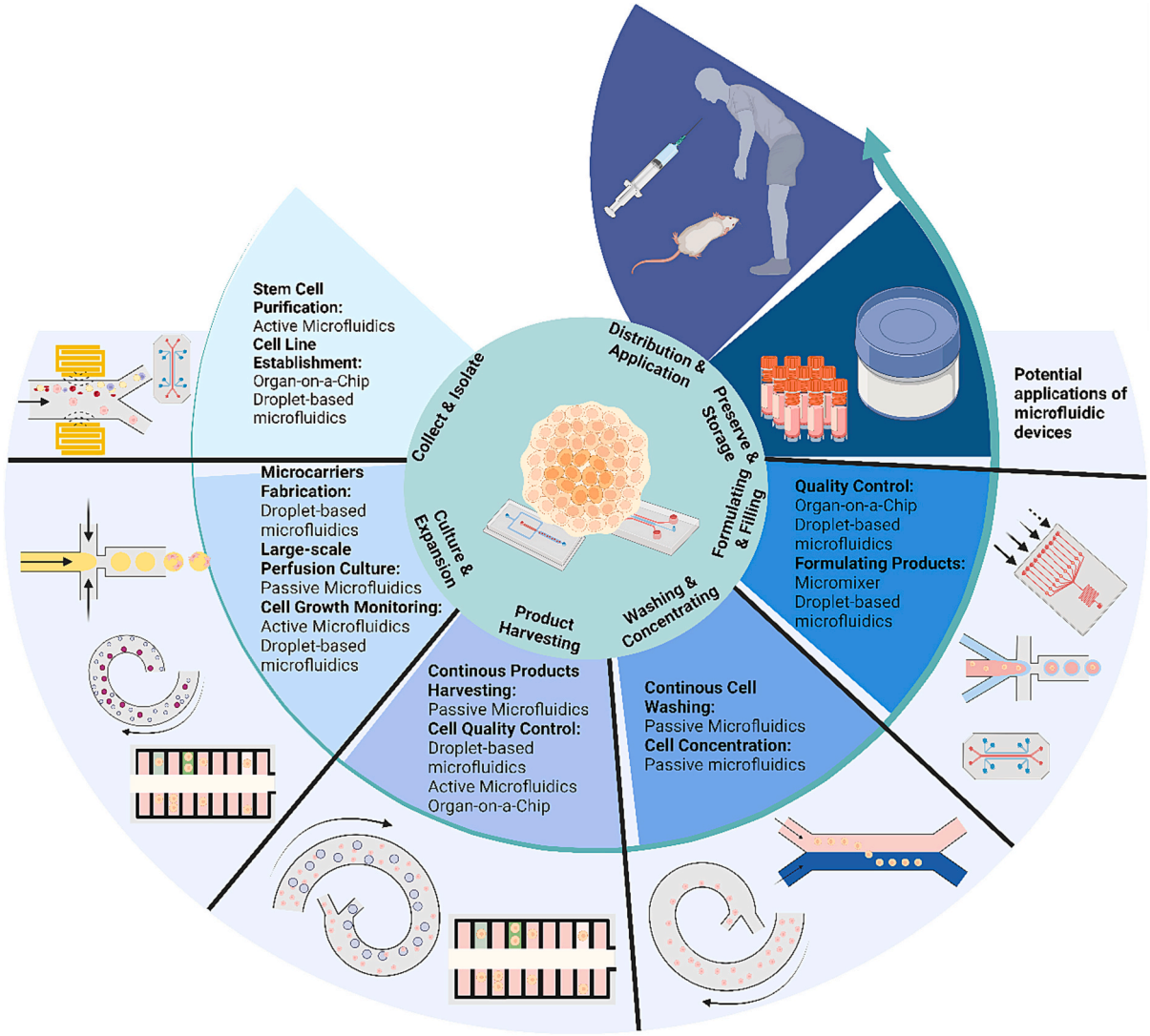

Fig. 8. Potential applications of microfluidic devices in the industrial production processes of stem cells. Highlighting cost-effectiveness, scalability, energy efficiency, automation, and precision, these devices address industry challenges, with potential to reduce costs, standardise assays, and optimise stem cell culture outputs.

microfluidic devices (Yan et al., 2017). Such advancements are particularly beneficial for the stem cell industry, which necessitates a closed production line. Several such integrated systems have demonstrated such potential: the integrated 3D-printed microfluidic system which harvest MSCs from microcarriers (Fig. 7B, (Ding et al., 2022a)) and the active-passive microfluidic system which offers high throughput and purity in stem cell isolation from tissue sources (Fig. 7C, (Jiang et al., 2019)).

4. Conclusion and future perspective

Despite recent breakthroughs in bioprocessing, there remains a significant demand for advanced technologies to optimise workflows and enhance efficiency. However, the emergence of microfluidic technologies offers promising solutions to these challenges. Microfluidics has shown exceptional capabilities in reducing costs, ensuring precise manipulation of fluids, and allowing real-time monitoring of the production process. The transformative potential of microfluidics in both bioprocessing and the stem cell industry is significant. However, its full impact, especially within the realm of stem cell research, remains largely untapped. This highlights an urgent need for more in-depth research and development to fully realise its capabilities (Fig. 8).

Microfluidic devices offer a compelling alternative to traditional processing methods like mixing, separating, dewatering, and centrifuging, which are not only complex and costly but also pose a risk of cell damage. With their capacity for precise control and efficient operation, microfluidics is showing considerable promise for enhancing stem cell processing workflows, particularly at the laboratory scale. For instance, inertial devices coupled with sheath flow and sheathless spiral channels can simultaneously wash and concentrate cells (Hawkes et al., 2004; Zhao and Fu, 2017). Droplet-based microfluidic devices offer high-resolution, single-cell level monitoring of cell quality or cellular products during the production process (Radfar et al., 2023). These devices can even provide insights into previously unmeasurable parameters, such as co-cultures with PBMCs (Xie et al., 2020). Additionally, the incorporation of artificial intelligence (AI) into advanced microfluidic systems holds the potential to significantly improve automation (Annabestani et al., 2020; Lai et al., 2015). Sensors enhanced by AI algorithms can be deployed for more accurate cell quality control, while intelligent pumps can regulate or even predict the flow rate of feed (Abe et al., 2021; Stoecklein et al., 2017).

In conclusion, microfluidic devices offer a significant opportunity to optimise stem cell production at an industrial scale by addressing the existing challenges in the stem cell production process. With the potential to enhance current technologies, microfluidic devices can provide a more efficient, cost-effective, automated, and standardised approach to stem cell processing and production.

Declaration of Competing Interest

Authors, L.D, P.R., M.E.W and S.O, own equity in the Smart MCs PTY LTD. Some schematics used in this review were designed with Biorender.com.

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