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Single-cell technology for cell-based drug delivery and pharmaceutical research

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ABSTRACT

Leveraging the capacity to precisely manipulate and analyze individual cells, single-cell technology has rapidly become an indispensable tool in the advancement of cell-based drug delivery systems and innovative cell therapies. This technology offers powerful means to address cellular heterogeneity and significantly enhance therapeutic efficacy. Recent breakthroughs in techniques such as single-cell electroporation, mechanical perforation, and encapsulation, particularly when integrated with microfluidics and bioelectronics, have led to remarkable improvements in drug delivery efficiency, reductions in cytotoxicity, and more precise targeting of therapeutic effects. Moreover, single-cell analyses, including advanced sequencing and high-resolution sensing, offer profound insights into complex disease mechanisms, the development of drug resistance, and the intricate processes of stem cell differentiation. This review summarizes the most significant applications of these single-cell technologies, highlighting their impact on the landscape of modern biomedicine. Furthermore, it provides a forward-looking perspective on future research directions aimed at further optimizing drug delivery strategies and enhancing therapeutic outcomes in the treatment of various diseases.

1. Introduction

The convergence of gene editing, bioengineering, and immunology has propelled cell-based drug delivery systems and cell therapies to the forefront of biomedical research and therapeutic innovation. Living cells, with their exceptional biocompatibility, low immunogenicity, intrinsic tissue-homing capabilities, and ability to traverse biological barriers, represent a promising platform for the development of drug delivery systems [1–4]. Cell-based drug delivery systems harness the capacity of living cells to transport and release therapeutic agents in vivo, whereas cell therapies involve the transplantation or infusion of either healthy or genetically engineered human cells into patients. These introduced cells can replace damaged or diseased cells or augment immune functions to combat disease. Cell therapies encompass a range of modalities like stem cell therapy [5] and immune cell therapy. Notably, immune cell therapies, particularly chimeric antigen receptor T-cell (CAR-T) therapy, have demonstrated remarkable clinical success in the treatment of hematologic malignancies [6,7]. Concurrently, mesenchymal stem cells (MSCs) have shown significant promise in regenerative medicine and the management of inflammatory diseases [8,9]. The recent regulatory approvals of stem cell formulations, such as Ryoncil (remestemcel-L) by the U.S. FDA on December 18, 2024, and Amimestrocel by China's NMPA on January 2, 2025, underscore the growing clinical significance of this field [10,11].

Despite the substantial promise of these advancements, several challenges persist. Some current methods for delivering molecules into cells or utilizing cells as therapeutics are often hampered by limitations such as low efficiency, cytotoxicity, and a lack of precision. Traditional bulk analytical techniques, while effective in providing population-level insights, often mask critical heterogeneities at the single-cell level,

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leading to suboptimal research outcomes and potentially hindering therapeutic development [12–14]. Conventional multi-cell research approaches increasingly fall short of meeting the rigorous demands of cell-related studies, especially in the context of pharmacokinetics and pharmacology [15]. These limitations have catalyzed a significant shift towards single-cell technologies, which offer unprecedented resolution and precision in overcoming these challenges [16,17].

Single-cell technologies have emerged as powerful solutions in

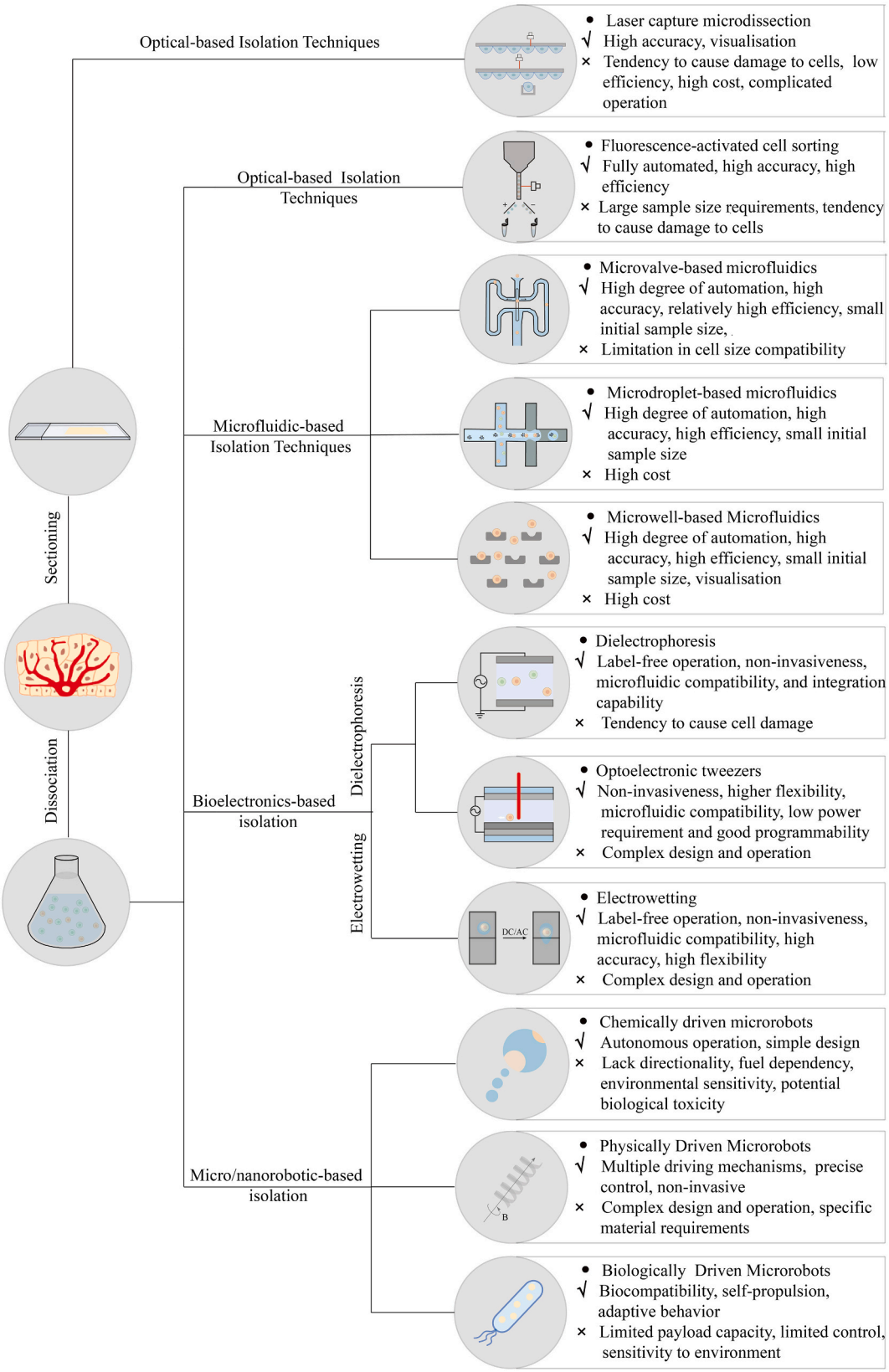

Fig. 1. Schematics of different types of single-cell separation technology.

several key areas of cell-based drug delivery systems and cell therapies. For instance, in the realm of intracellular molecule delivery, techniques such as single-cell electroporation and mechanical perforation, particularly when coupled with engineering tools like microfluidics, enable efficient, high-throughput delivery with low cytotoxicity [18–22]. Furthermore, single-cell encapsulation techniques are being employed to enhance the protection and functional longevity of therapeutic cells, while single-cell electrical stimulation has opened new avenues for improving the efficacy of stem cell therapy by promoting directed differentiation and paracrine secretion [23–27].

Beyond physical manipulation, single-cell analyses, including sequencing and sensing, have become increasingly integral to pharmaceutical development. The past decade has witnessed significant progress in single-cell sequencing, enabling the isolation and detailed analysis of individual cell types with exceptional precision. This technology has been widely adopted in disease pathology research, enhancing our understanding of disease mechanisms, facilitating the identification and evaluation of novel drug targets, and providing crucial insights into the evolution of therapeutic resistance [28–30]. However, single-cell sequencing is limited in its ability to perform real-time, in situ analysis of cells, potentially leading to the loss of critical temporal information. In contrast, bioelectronics-based single-cell sensing offers the advantage of real-time, in situ monitoring, employing technologies such as nanopores and patch clamp to probe the intracellular environment [31]. Moreover, leveraging advanced electronic manufacturing processes, bioelectronic devices with high sensitivity, high spatial resolution, and high throughput have been fabricated at cellular dimensions, enabling manipulation and detection at the single-cell level [32].

This review provides an overview of the applications of single-cell technologies in cell-based drug delivery and cell therapies, emphasizing their critical roles in enhancing therapeutic efficacy and precision. We focus particularly on the application of single-cell analyses in elucidating disease pathogenesis, identifying novel drug targets, optimizing stem cell therapies, and monitoring intracellular delivery processes. By discussing these aspects, we aim to underscore the vast potential of single-cell technologies in advancing modern biomedicine and provide valuable insights into future research directions that will further harness the power of these innovative tools.

2. Single-cell isolation techniques

The first step in single-cell analysis is the isolation of individual cells. Continuous gradient dilution is the most cost-effective method with low technical expertise and equipment requirement. However, it is limited by low efficiency, susceptibility to separation errors, and cell loss due to manual handling [33]. With the increasing demand, significant progress has been made in single-cell isolation technologies based on optics, microfluidics, bioelectronics, and micro/nanorobots (Fig. 1). This section provides a review of these technologies.

2.1. Optical-based isolation techniques

Isolation techniques for single cells with specific attributes—such as morphology, spatial location, and protein expression—are essential for studying targeted cells within tissues. Common methods include laser capture microdissection (LCM) and fluorescence-activated cell sorting (FACS), which are classified as optical-based single-cell isolation technologies due to their reliance on lasers for single-cell isolation or sorting. LCM employs laser to precisely excise target cells from tissue sections under a microscope. This method enables accurate cell isolation, making it ideal for obtaining cell populations or individual cells with defined spatial or morphological characteristics from heterogeneous tissue samples [34]. Furthermore, combining LCM with immunohistochemistry facilitates the isolation of cells with specific protein markers [35,36]. Despite its advantages, LCM has several limitations, including low efficiency, complex operation, and high cost [37], which restrict its broader application in single-cell research. Additionally, the laser cutting process can induce damage to cells. Prolonged exposure or excessive laser interaction can cause irreversible damage, compromising subsequent analyses [38,39].

FACS involves labeling cells with fluorescein and using laser-induced fluorescence to selectively sort cells with high specificity. Target cells are deflected into a collector based on their fluorescence signals [40]. Compared to LCM, FACS is more user-friendly, fully automated, efficient, and highly specific. It is particularly effective for isolating target cells with specific markers in single-cell analysis [41–44]. For example, Lawson et al. used FACS to isolate metastatic and primary tumor cells from mice, followed by single-cell analysis [45]. However, because FACS-based single-cell isolation and analysis are typically performed in 96-well or 384-well plates, multiple plates must be processed simultaneously when large numbers of cells with specific markers need to be analyzed. Therefore, FACS is often combined with microfluidics to enable higher-throughput single-cell analysis of cells with defined markers [46]. For instance, Azizi et al. employed droplet-based microfluidic platforms to sequence CD45+ immune cells from breast cancer patients, which were initially sorted by FACS [47]. Similar to LCM, FACS-based cell manipulation methods usually require laser, which may cause unavoidable and irreversible damage to cells. Additionally, fluid shear forces within the pipelines may also contribute to cell injury. Fortunately, milder thermal and mechanical injuries can be effectively prevented and repaired through the use of heat shock protein inducers, antioxidants, calcium channel antagonists, or cell membrane-stabilizing agents [48,49]. And by adjusting parameters such as flux, cell concentration, flow rate, and light intensity, optimization to reduce cell damage can be achieved [50,51].

2.2. Microfluidic-based isolation techniques

Microfluidic technology is widely used for single-cell separation due to its high automation, which minimizes cell loss, separation errors, and damage from manual handling, significantly enhancing efficiency [33]. It also requires a small sample size, offers high resolution and sensitivity, and is particularly suitable for isolating rare cells [52]. As a result, microfluidics has rapidly become the preferred method for single-cell separation. The three primary microfluidic platforms include microvalve-based, microdroplet-based, and microwell-based systems. For example, Fluidigm's C1 single-cell automation system, developed in 2012, is based on valve technology and offers ease of use and high sensitivity, although it has lower cell throughput compared to droplet-based microfluidics [53,54]. In droplet-based systems, droplet generation is controlled by microchannel geometry and flow rate ratios. Each droplet encapsulates individual cells, enzymes, and molecular barcodes for downstream single-cell analysis. The high throughput, simplicity, and speed of this method make it widely used for single-cell separation and sequencing. Currently, three commonly used droplet-based microfluidic platforms include 10× Genomics Chromium, inDrop, and Drop-seq. These platforms form microdroplets similarly but differ in bead design, retrotransposon location, and cDNA amplification. Among them, 10× Genomics Chromium offers better sensitivity, lower noise and high precision, making it a preferred choice in some studies [55,56]. For example, Zeisel et al. used this system to sequence nervous system cells of mice, creating a single-cell transcriptional profiling map to aid in understanding molecular architecture of the mammalian nervous system [57]. Additionally, droplet-based microfluidics is also widely applied in single-cell proteomics [58]. Microwell-based microfluidics relies on size matching between single cells and micropores. After introducing a cell suspension into the microfluidic channel, cells settle into the micropores while excess cells are washed away. Advances in micro/nano fabrication have expanded the use of microwell-based microfluidics for single-cell separation and sequencing. Key platforms include Microwell-seq and BD Rhapsody. Microwell-seq captures

approximately 5-10 K cells using agarose microporous plates with 10^5 micropores, enabling high-throughput, relatively low-cost single-cell RNA sequencing (scRNA-seq) for cellular heterogeneity studies [59]. The BD Rhapsody platform, which utilizes a microwell plate with approximately $2*10^5$ micropores, offers lower cell viability requirements and visualization [60]. It is widely used in single-cell sequencing and protein detection, alongside 10× Genomics Chromium [61,62]. Microfluidic technology utilizing microdrops and micropores has significantly advanced high-throughput single-cell analysis. However, it faces limitations, including high costs, challenges in specific cell separation, and potential mechanical damage to cells, mainly attributed to the shear forces generated by fluid flow in microfluidic systems [63]. Fortunately, under mild microfluidic conditions, cell damage induced by low shear forces is largely reversible and can be further mitigated through the use of antioxidants, calcium channel antagonists, or cell membrane-stabilizing agents [64,65].

2.3. Bioelectronics-based isolation techniques

In addition to the previously discussed single-cell isolation techniques, researchers have also explored the use of bioelectronic devices, such as microfluidic platforms based on dielectrophoresis (DEP) and electrowetting, as well as optoelectronic tweezers (OET) that integrate DEP.

DEP is a technique that manipulates particles, such as cells and microorganisms, using non-uniform electric fields. When dielectric particles are placed in such a field, the induced dipole moments interact with the field, generating a net force that causes the particles to move along the field gradient. Due to its label-free operation, non-invasive nature, compatibility with microfluidic systems, and potential for integration, DEP is widely used for single-cell isolation. For instance, Kodama et al. developed dielectrophoretic tweezers capable of capturing, transporting, and positioning liposomes with diameters ranging from 5 to 23 μm, facilitating the separation of target cells [66]. Godino et al. combined DEP with open-source computer vision software to fully automate the selection, isolation, and arrangement of individual cells within microfluidic devices [67]. Menze et al. integrated DEP with microfluidic techniques utilizing micropores, achieving high-throughput and precise single-cell separation and sorting [68]. However, excessively high electric field intensities can damage cells and may even result in cell death [69]. To minimize such effects, it is crucial to optimize the electric field intensity and consider the use of antioxidants or calcium ion antagonists.

OET combines optical tweezers with DEP. For OET, applied light can induce non-uniform electric fields through the photoconductive effect. These non-uniform electric fields then manipulate the particles through DEP. Compared to traditional DEP, OET offers greater flexibility and operates with a low light power density, providing good biocompatibility [70]. Consequently, OET holds great promise for single-cell isolation. For example, Devin Keck et al. developed an OET platform that uses a commercial projector and an a-Si photoconductive electrode to manipulate yeast and Candida cells. The study demonstrated that the system could control yeast and Candida cells by illuminating the photoconductive material with visible light in specific patterns. By adjusting the shape, color, and distance between the cells and the light pattern, the system could also regulate the cells' movement speed [71]. To improve throughput, researchers have integrated OET with microfluidics. For instance, Gan et al. combined OET with a micropore-based microfluidic platform, achieving efficient and stable single-cell separation with a 94.1 % micropore filling rate within 120 s [72].

Electrowetting involves modifying the wettability of a droplet on a hydrophobic surface by applying a voltage, which changes the droplet's contact angle and allows for its deformation and movement. In 2000, Michael G. Pollack and colleagues developed a micro actuator for rapidly manipulating droplets, demonstrating the transfer of KCl droplets ranging from 0.7 to 1 μL between electrodes using electrowetting. This validated the feasibility of a digital microfluidic platform based on electrowetting [73]. For instance, Ruan et al. developed a digital microfluidics platform that utilizes fluid dynamics and surface wettability to isolate single cells with 100 % efficiency via droplet manipulation, independent of cell type or input [74]. This platform offers exceptional flexibility, precision, and simplicity in handling minute samples, making it ideal for single-cell isolation. However, electrowetting-based single-cell isolation requires precisely designed electrodes to achieve accurate droplet manipulation, which limits its broader applications. Recently, Tan et al. discovered a phenomenon called orbital electrowetting, which enables faster droplet manipulation without the need for complex electrode arrays or precise circuitry, further enhancing the potential of electrowetting-based microfluidics for single-cell isolation [75].

2.4. Micro/nanorobotic-based isolation techniques

Micro/nanorobots are untethered devices designed at the micron or nanometer scale, capable of performing tasks at the cellular or subcellular level. These robots are non-invasive, highly automated, and easily integrable with instruments such as microscopes, enabling visualized single-cell isolation. As a result, they have gained significant traction in recent years for applications in single-cell separation and other related operations. Due to their small size, these robots cannot carry onboard power sources and instead rely on external energy sources, such as chemical, physical, or biological energy, for propulsion [76].

Chemically driven robots convert chemical and biochemical energy into mechanical energy by utilizing spatially asymmetric reactions of the surrounding fuel. And they are typically driven by bubble propulsion or self-electrophoresis [76,77]. For example, Yoshizumi et al. fabricated polystyrene beads with two segments, made of zinc and platinum, respectively. By modifying a self-assembled monolayer on the platinum segment, these beads successfully captured and transported *Escherichia coli* [78]. To enhance the biocompatibility of chemically driven micro/nanorobots, researchers have explored using biological enzymes as substitutes for metals, which may cause biotoxicity or immune responses. Guo et al. developed a bio-catalytic buoyancy-driven nanorobot by incorporating the catalase into zeolitic imidazolate framework-8 via biomineralization. Functionalized with monoclonal anti-carcinoembryonic antigen fragments, the nanorobot achieved targeted cell capture and efficient long-distance vertical transport [79].

Physically driven robots harness external physical fields, such as magnetic fields, light, sound waves, electric fields, and heat, to enable precise control and manipulation of individual cells [77]. For instance, Lin et al. developed a magnetically actuated, peanut-shaped hematite colloid motor capable of capturing and transporting cells by integrating rolling and swinging motions [80]. Similarly, Li et al. fabricated light-driven micro/nanorobots with diverse structures by combining bulk heterojunction organic semiconductor solar cells with spin-coating technology, enabling stable yeast cell transport [81].

Bio-driven micro/nanorobots typically leverage the response of living cells or microorganisms to stimuli, either moving towards or away from the stimulating sources to propel the robots [82]. For example, Zhang et al. developed autonomous micro/nanorobots by coupling algae with neutrophil membrane-coated polymer nanoparticles via click chemistry. These robots demonstrated movement in simulated lung fluid, with potential applications in treating lung infections [83]. Xie et al. harnessed the phototaxis of Chlamydomonas to create algae-guided micro/nanorobots capable of adhering to, transporting, and releasing microstructures, laying the foundation for their application in single-cell isolation [84]. Despite their potential, micro/nanorobots for single-cell isolation face several challenges, including low separation efficiency, difficulties in controlling chemically driven robots, complex equipment requirements for physically driven robots, and weak driving forces in bio-driven robots. Addressing these limitations is essential to further advancing their application in single-cell isolation.

3. Cells as delivery vehicles or therapeutic contents

With rapid advancements in biotechnology and medical sciences, cell-based drug delivery and cell therapy have emerged as key research areas in next-generation biomedicine. This section provides a brief overview of the applications of single-cell technologies in these fields, focusing on three primary aspects: intracellular molecule delivery, cell encapsulation, and electrical stimulation for enhancing stem cell therapy, and concludes with existing clinical progress on living cells as therapeutics.

3.1. Intracellular molecule delivery

Efficient and safe delivery of drugs, nucleic acids, or proteins into cells is a critical challenge for cell-based drug delivery systems and immune cell therapies. Currently, commonly used single-cell intracellular delivery methods primarily rely on membrane disruption mechanisms to create transient pores for the delivery of target molecules. These methods include, mechanical perforation, chemical perforation, photoporation, and electroporation [85]. Among these, mechanical perforation can be achieved through microinjection, micro/nanoneedle

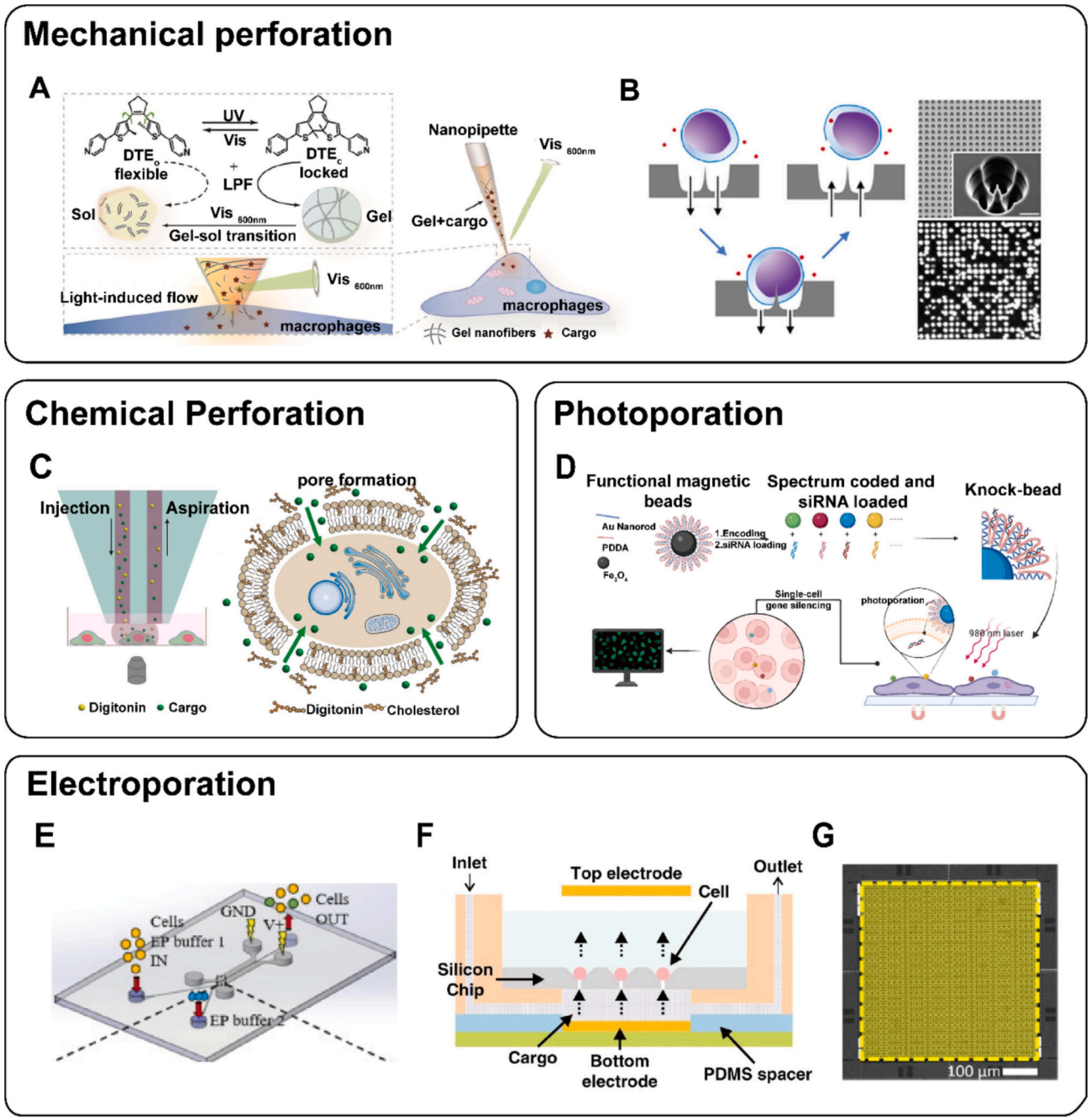

Fig. 2. (A) Photo-triggered cell injection through a hydrogel-nanopipette system [87]. Copyright 2021, John Wiley and Sons. (B) Concept, illustrated for a single capture site of the microfluidic device [88]. Copyright 2020, American Chemical Society. (C) Intracellular delivery to target single cell within the microregion generated by the microfluidic probe [91]. Copyright 2024, American Chemical Society. (D) Single-cell encoded photoporation for high-throughput combinatorial siRNA screening [92]. Copyright 2024, Springer Nature. (E) 3D schematics of the microfluidic chip [94]. Copyright 2020, Elsevier. (F) On-chip multiplexed single-cell patterning and controllable intracellular delivery [97]. Copyright 2020, Springer Nature. (G) The HD-EP chip for the spatially resolved transfection of cells [98]. Copyright 2023, Elsevier.

arrays, or mechanically constrained or fluidic-based cell squeezing [86]. Compared to conventional macroscopic intracellular delivery techniques, single-cell intracellular delivery methods offer higher efficiency, reduced cytotoxicity, more uniform outcomes and more precise dose control. For example, Tian et al. developed a hybrid system combining photoresponsive hydrogels with nanotubes, achieving dose-controllable single-cell drug delivery while maintaining cell viability and reducing the lethal dose of drugs (Fig. 2A) [87]. Rao et al. devised a microfluidic platform that induces transient membrane pores by colliding cells, achieving high throughput, high cell viability, and an 88 % intracellular delivery efficiency (Fig. 2B) [88]. Chung et al. developed a microfluidic cell stretching (μ-cell stretcher) platform that incorporates a viscoelastic methylcellulose solution to achieve high levels of cell deformation, leading to membrane discontinuities for intracellular delivery. This platform demonstrated high throughput (approximately 3.5×10^5 cells/min) and 97 % efficiency for single-cell intracellular delivery [89]. Similarly, Toner et al. utilized extensional flow generated by contraction microchannels to stretch cells, achieving high-throughput intracellular molecule delivery (over 250 million cells per minute), compatible with a wide range of molecules, including proteins, RNA, and CRISPR-Cas9 ribonucleoprotein complexes [90]. Lin et al. developed a single-cell intracellular delivery platform based on an open microfluidic probe, which uses chemical reagents to perforate individual cells and delivers exogenous molecules up to 150 kDa in molecular weight into cells (Fig. 2C) [91]. Guo et al. utilized hybrid microcarriers responsive to near-infrared light and magnetic fields to induce cell photoporation, enabling high-throughput and efficient single-cell siRNA delivery with minimal cellular damage (Fig. 2D) [92].

Electroporation has also become a widely employed technique for single-cell intracellular delivery, owing to its rapidity, high efficiency, ease of operation, and controllable electrical parameters. Single-cell electroporation techniques include in-flow, nanopore, and microelectrode array (MEA) electroporation. In-flow electroporation, based on microfluidic devices, involves exposing cells suspended in a microchannel to an electric field generated by electrodes, enabling single-cell electroporation [93]. For instance, Han et al. developed a microfluidic electroporation platform with tightly spaced electrodes, which can generate a high-intensity electric field at a low operating voltage to achieve cell electroporation. Studies have shown that under optimal parameters, the platform achieves a delivery efficiency of 63 % for HEK-293 cells and 58 % for CHO-K1 cells (Fig. 2E) [94]. Folch et al. and colleagues designed a continuous-flow microfluidic device integrated with polyaniline hydrogel. This system roduces the intense pulsed electric field necessary for electroporation while operating at comparatively low voltages (15-60 V) by optimizing the geometry of the microfluidic channels. And the inclusion of polyaniline hydrogel can further protect the cells from the electrodes. Studies demonstrated that this design significantly improved CHO-K1 cell transfection efficiency to 78 ± 10.4 % with a survival rate of 77 ± 6.1 %, surpassing previous continuous-flow microfluidic systems [95]. In-flow electroporation also offers a more efficient and labor-saving method for preparing cell membrane-coated biomimetic nanoparticles compared to traditional approaches like ultrasound and mechanical extrusion. For example, Liu's team developed a microfluidic electroporation technique to efficiently prepare cell membrane-coated biomimetic nanoparticles. Their research indicated that these nanoparticles exhibited superior colloidal stability, enhancing in vivo imaging and photothermal therapy effects [96]. Nanopore electroporation involves positioning cells in a device with nanoscale channels connected to chambers containing target substances. Electrodes are placed above the cells and below the chamber, and electrical pulses are applied to create localized electroporation at the cell membrane. This technique offers low cytotoxicity and high transfection efficiency. For example, Chang et al. utilized silicon wet etching to create a pyramid-shaped micropore array chip, which employs a vacuum system for single-cell capture, enabling efficient electroporation at low voltage and achieving over 90 % transfection efficiency (Fig. 2F) [97]. Microelectrode arrays (MEAs) allow precise electrical stimulation and monitoring of individual cells with high versatility and single-cell resolution. Zhang et al. developed an MEA chip for single-cell electroporation, optimizing perforation efficiency and cell viability through real-time impedance monitoring. By fine-tuning electroporation conditions for HeLa, MCF-7, and 293 T cells, they achieved efficient EGFP plasmid transfection, demonstrating its potential for personalized therapy [32]. Additionally, Ducker et al. achieved a transfection efficiency of up to 98 % by utilizing a high-density MEA and optimizing electrical stimulation parameters. Moreover, due to the addressability of individual electrodes within the array, this system enables spatially resolved, high-efficiency, and sequential mRNA transfection (Fig. 2G) [98].

3.2. Single-cell encapsulation

Cell encapsulation is a technique that involves enclosing cells within a specific material to protect them from immune system attacks, mechanical damage, and other external stimuli. This process preserves the biological activity of cells, allowing them to perform specific functions both in vitro and in vivo. Cell encapsulation has found wide applications in fields such as cell therapy, gene therapy, drug delivery, and biosensors. For instance, researchers developed artificial islets based on cell encapsulation that can be implanted into diabetic rats. Studies have shown that these artificial islets enabled diabetic rats to maintain normal blood glucose levels for nearly three weeks, significantly exceeding the lifespan of unencapsulated islet groups, which lasted only 6–8 days [99]. Most current techniques focus on multicellular encapsulation, which can mimic natural tissue and create intricate, highly functional cell systems. As a result, multicellular encapsulation is extensively used in tissue engineering and drug screening. While single-cell encapsulation has been less explored, it holds substantial research potential due to its ability to precisely control and study individual cell behavior, as well as its enhanced diffusion rates and low material-to-cell volume proportions.

Single-cell encapsulation techniques are generally classified into three categories: droplet-based encapsulation, microgel-based encapsulation, and nanoencapsulation. [100]. Droplet-based single-cell encapsulation usually employs microfluidic technology to encapsulate individual cells within microscopic droplets. These droplets can precisely isolate and protect each cell, providing an ideal microenvironment for various biological experiments and analyses. For example, combining single-cell microfluidic droplet encapsulation with surface-enhanced Raman scattering (Fig. 3A) or fluorescence imaging (Fig. 3B) enables the construction of single-cell extracellular factor detection platforms. Such platforms provide experimental evidence for using single-cell extracellular factors as biomarkers in early cancer diagnosis [101,102]. Microgel-based encapsulation uses microgels to enclose individual cells within microscale gel particles, providing a protective microenvironment that maintains cellular activity and functionality. Consequently, microgel-encapsulated single cells can be used in cell analysis, tissue regeneration, and cell therapy, offering greater versatility compared to droplet-based methods. For instance, researchers employed microfluidic techniques to achieve microgel encapsulation of individual MSCs. Studies have shown that microgel encapsulation enhances cell survival and extends functional durations without compromising stem cell functionality, making it highly suitable for cell delivery in therapeutic applications (Fig. 3C) [23–25,103]. Nanoencapsulation, inspired by the natural process of spore formation, creates shell-like structures called artificial spores. These structures protect cells from potential damage under harsh conditions, significantly improving cell survival rates [104]. More importantly, these nanoshells can impart novel properties to cells that are not inherent in their natural form, enhancing targeting capabilities or therapeutic effects (Fig. 3D) [105]. For example, Chen et al. combined traditional layer-by-layer assembly with thiol-maleimide chemistry to construct a

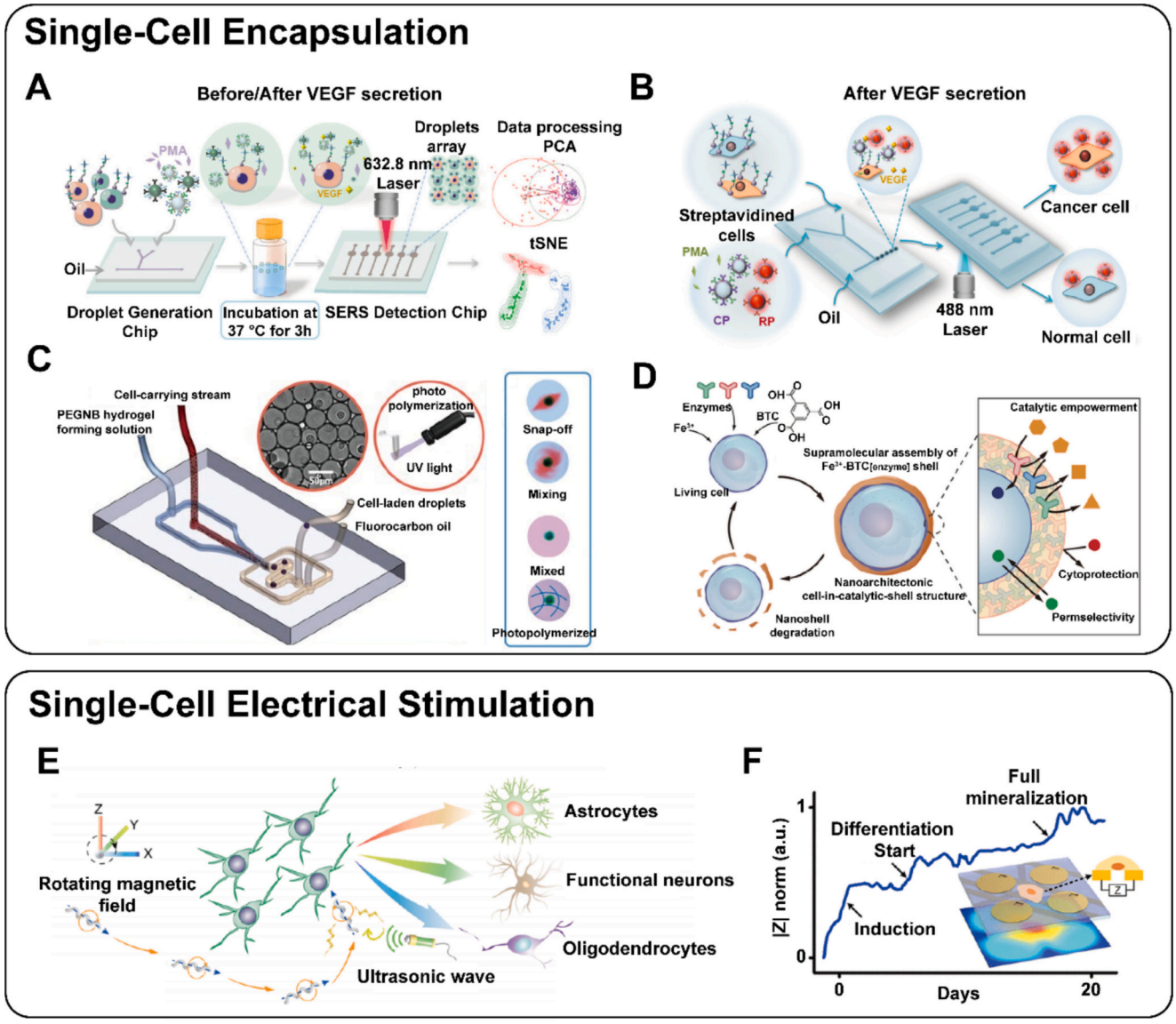

Fig. 3. (A) Workflow of the droplet-based microfluidics for single-cell encapsulation and the surface-enhanced Raman scattering analysis of VEGF secreted by one cell [101]. Copyright 2022, American Chemical Society. (B) Workflow of the droplet-based microfluidics for single-cell encapsulation and the fluorescence imaging analysis of VEGF secreted by one cell [102]. Copyright 2022, American Chemical Society. (C) Schematic of microfluidic chip design for single-cell photo-encapsulation within PEGNB microgels [103]. Copyright 2024, John Wiley and Sons. (D) Schematic for catalytic empowerment of individual living cells by in situ enzyme embedment into Fe^3-BTC shells [105]. Copyright 2022, John Wiley and Sons. (E) Control the neural stem cell fate with biohybrid piezoelectrical magnetite micromotors [27]. Copyright 2021, American Chemical Society. (F) Electrical stimulation and single-cell impedance monitoring of mesenchymal stem cells with microchips [114]. Copyright 2020, American Chemical Society.

matrix metalloproteinase-7 responsive nanoencapsulation layer on single-cell surfaces, showing promise for cell-based therapies, particularly in tumor treatment [106].

3.3. Electrical stimulation of single cells

Stem cells are primitive cells capable of self-renewal and multilineage differentiation. They possess distinctive biological characteristics, including inflammation and tumor-homing abilities, low immunogenicity, immune and inflammatory regulation, and paracrine effects [107–111]. Stem cell therapy exploits these properties by transplanting healthy stem cells into the patient's body to promote the repair and regeneration of damaged tissues. However, direct stem cell injection faces challenges such as low cell survival and suboptimal phenotype, resulting in a limited number of functional cells [23]. Therefore, developing effective strategies to regulate stem cell differentiation and paracrine signaling is essential. Electrical stimulation has emerged as a promising physical approach to induce directed stem cell differentiation and enhance paracrine secretion. For example, Yang et al. developed an injectable cellulose/MXene conductive hydrogel as a carrier for stem cells, facilitating the integration of stem cell therapy with electrical stimulation. Their study demonstrated that electrical stimulation promoted the differentiation of neural stem cells (NSCs) into neurons while inhibiting their differentiation into astrocytes, increasing axonal growth density by 290 % and axonal length by 320 %, leading to significant motor function restoration in rats [112]. Similarly, Mallapragada et al. used 3D printing to construct a nerve regeneration conduit/scaffold based on gelatin and graphene. Their research showed that electrical stimulation using conductive graphene enhanced the secretion of nerve growth factors by MSCs and promoted their differentiation into Schwann cells [113].

The electrical stimulation and monitoring of individual stem cells

allow for in-depth analysis of electrical stimulation parameters and the heterogeneity of stem cell differentiation, further enhancing therapeutic efficacy. Liu et al. developed a micromotor for precise electrical stimulation of individual neurons using magnetic Fe_3O_4 and piezoelectric $BaTiO_3$ nanoparticles. This technology enables detailed studies of electrical stimulation parameters and stem cell differentiation heterogeneity, improving therapeutic outcomes. By optimizing ultrasound parameters, they directed NSCs to differentiate into astrocytes, dopamine neurons, cholinergic neurons, and oligodendrocytes, offering potential treatments for neurodegenerative diseases (Fig. 3E) [26,27]. Zhu et al. fabricated a microelectrode chip capable of both stem cell stimulation and single-cell impedance detection (Fig. 3F). This device allows monitoring of the dynamic process of single-cell development and facilitates the study of stem cell differentiation heterogeneity at the single-cell level. However, the device failed to provide precise electrical stimulation to individual cells, which could compromise the consistency of electrical stimuli and potentially affect the experimental outcomes [114].

3.4. Existing clinical progresses on cells as therapeutics

Cell therapies, particularly immune and stem cell approaches, have witnessed remarkable progress, transitioning from benchside innovation to impactful clinical interventions. As of August 2024, a substantial portfolio of 14 immune cell therapies and 24 stem cell therapies have secured regulatory approvals, targeting a wide range of diseases across neurological, immunological, cardiovascular, and oncological domains [115]. In November 2024, the U.S. FDA granted approval to Aucatzyl, a CAR-T cell therapy, for adult patients with relapsed or refractory B-cell acute lymphoblastic leukemia. Significantly, this marked the first FDA approval of a CAR-T therapy without the requirement of a Risk Evaluation and Mitigation Strategy (REMS) program [116]. December 2024 saw the U.S. FDA approval of Ryoncil, a MSC therapy, for steroid-refractory acute graft-versus-host disease in pediatric patients aged two months and older, representing the first FDA-approved MSC-based therapeutic [117]. Shortly thereafter, the National Medical Products Administration (NMPA) of China approved the clinical use of Amimestrocel Injection in January 2025, marking the first approved MSC-based therapeutic in China [118].

Despite these milestones, widespread clinical translation of living cells still faces substantial hurdles. CAR-T cell therapy, while demonstrating impressive clinical responses, mostly relies on lentiviruses for gene delivery. The inherent risks of random transgene integration, leading to variable expression levels and potentially impacting therapeutic efficacy, remain a concern. While non-viral delivery methods, such as electroporation, offer an alternative, their limited transfection efficiency and associated cytotoxicity pose significant challenges [119]. Emerging single-cell intracellular delivery techniques offer a promising avenue to enhance gene delivery efficiency while mitigating cytotoxicity, potentially revolutionizing CAR-T cell engineering. Similarly, stem cell therapies encounter limitations, including suboptimal phenotype, rapid clearance by the host, and the risk of immune rejection [120].

4. Applications of single-cell analyses in medicine

Single-cell analysis technologies facilitate the precise isolation and examination of specific cell types, thereby enhancing our understanding of disease mechanisms, identifying potential drug targets, and offering insights into the development of therapeutic resistance. This section provides an overview of the applications of widely used single-cell analysis techniques, including single-cell sequencing and single-cell sensing, in the study of pathogenesis, the development of therapies, and the monitoring of intracellular delivery, as well as recent advancements in these fields.

4.1. Research on pathogenesis

Single-cell analysis technologies, such as scRNA-seq, offer the ability to dissect the cellular and molecular landscape at single-cell resolution, thereby revealing the heterogeneity in gene expression, surface biomarkers, and pathophysiological functions [121–125]. Furthermore, these technologies can interrogate cell lineage and reconstruct developmental trajectories [126,127]. By analyzing changes in cell-specific gene expression at various stages of disease, single-cell analysis enables the identification of biomarkers for disease progression and signaling pathways that can inform early interventions, benefiting both disease diagnosis and treatment.

In oncology, researchers leverage single-cell technologies to investigate cancer cells across various features, including proliferation, migration, metabolism, and immune evasion, and correlate them with tumorigenesis, progression, metastasis, and drug resistance [128–136]. Neftel et al. employed scRNA-seq on 28 glioblastoma samples, alongside bulk data from 401 patients, to study transcriptional and genetic heterogeneity. Their findings mapped malignant cell programs, plasticity, and regulatory genetic factors, contributing to the development of glioblastoma treatments [131]. In studies on tumor metastasis, early events of cancer cell dissemination in breast cancer metastasis have been highlighted by scRNA-seq and spatial transcriptomics, providing a foundation for early therapeutic strategies (Fig. 4A) [137].

The microenvironment plays a critical role in disease progression, either supporting or inhibiting it. In addition to revealing heterogeneity, single-cell analysis has characterized the microenvironment landscape, uncovering interactions between structural cells, immune cells, and diseased cells throughout disease progression. This approach has also facilitated the reconstruction of injured cell niches or microenvironments [138–140]. Elyada et al. identified cancer-associated fibroblasts (CAFs) that express MHC class II as a key factor in the resistance of pancreatic ductal adenocarcinoma. These CAFs present antigens to CD4+ T cells and regulate the immune response within pancreatic tumors [140].

Moreover, the secretome of cells plays a pivotal role in their biological functions. A recent study developed hydrogel-based, chemically functionalized micro-containers to isolate single viable cells based on their secreted products at high throughput [141]. Kennedy et al. introduced a non-invasive microfluidic nanopore method for detecting and dynamically analyzing individual cancer cell secretions, offering an efficient tool for investigating the pathological mechanisms of cancer (Fig. 4B) [31]. Another study linked the secretion of IgG by plasma cells to the expression of surface markers, utilizing plasma cell collection and single-cell sequencing technology. This method may help to decipher immune cell subpopulations and their protein secretion abilities in the tumor immune microenvironment [142]. Such analyses focusing on the secretome could advance cell sorting techniques for cell therapy.

Monoclonal antibody, proteasome inhibition, and immune checkpoint inhibition have revolutionized cancer treatments over the past decade [143–145]. However, many patients exhibit limited sensitivity to specific therapies, and therapeutic resistance remains a significant challenge, particularly in metastatic cancers or with emerging treatments [146,147]. Single-cell technologies enable the identification of rare cell subtypes and states, providing a detailed view of the tumor microenvironment. This approach helps to uncover mechanisms of therapeutic resistance and identifies potential targets for overcoming it. It is now widely accepted that cancer therapeutic resistance involves both intrinsic resistance due to pre-existing clones and subsequent Darwinian selection, as well as acquired resistance induced by treatments through non-genetic mechanisms [148]. Not surprisingly, single-cell technology is a powerful tool for understanding these resistance mechanisms. Pre-existing and heritable differences in gene expression can lead to the persistence of tolerant clones, which enrich during Darwinian selection under therapeutic pressure. Using single-cell assays for transposase-accessible chromatin sequencing (scATAC-seq), Fan

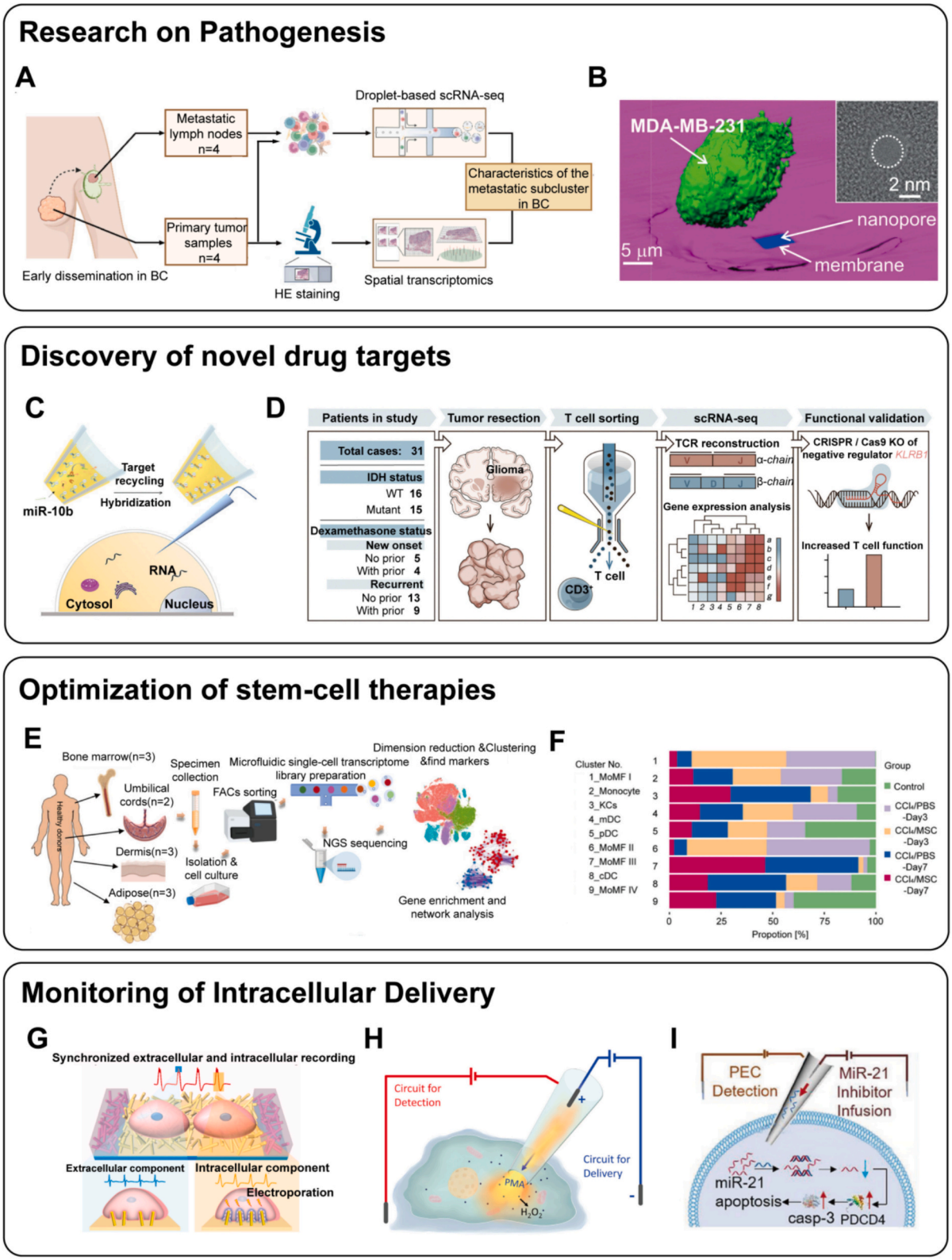

Fig. 4. (A) Expression profiling of 65,968 single cells in paired primary breast tumors and metastatic lymph nodes [137]. Copyright 2023, John Wiley and Sons. (B) Detecting secretions from a single cell with Nanopore [31]. Copyright 2018, American Chemical Society. (C) Diagram of the nanopipette for probing miRNA-10b [159]. Copyright 2021, John Wiley and Sons. (D) Charting the gene expression and clonal landscape of tumor-infiltrating T cells across 31 patients with IDH wild-type glioblastoma and IDH mutant glioma using scRNA-seq [28]. Copyright 2021, Elsevier. (E) The single-cell RNA sequencing analysis on tissue-specific heterogeneity of mesenchymal stem cells [185]. Copyright 2021, John Wiley and Sons. (F) ScRNA-seq data of the mononuclear phagocyte system from the liver [191]. Copyright 2022, Elsevier. (G) Schematic of 3D nanoroded multielectrode array (NRMEA) for synchronized intracellular and extracellular recording by electroporation [195]. Copyright 2021, Elsevier. (H) An integrated photocathodic nanotool was fabricated for dual-functional intracellular drug delivery and evaluation of cellular oxidative stress in single live cells [196]. Copyright 2021, John Wiley and Sons. (I) Schematic illustration of the PEC nanotool for single-cell miR-21 inhibitor delivery and caspase-3 detection [197]. Copyright 2023, John Wiley and Sons.

et al. examined the heterogeneous lineage composition of acute myeloid leukemia cells and profiled a multilineage state acquired during therapeutic interventions, illustrating the development of augmented tolerance [149]. However, resistant clones evolve in response to treatments rather than remaining static. Longitudinal single-cell DNA and RNA sequencing of clinical samples from patients with triple-negative breast cancer (TNBC) revealed pre-existing clones that were selected and reprogrammed by neoadjuvant chemotherapy [150]. This finding underscores the importance of detecting stage-specific markers for diagnosing the development of resistance.

For tumor prognosis research, single-cell analysis can identify unique gene expression patterns or molecular features associated with tumor outcomes, which can serve as prognostic biomarkers. By utilizing these biomarkers, clinicians can more accurately predict disease progression and patient survival, optimizing treatment strategies. For instance, scRNA-seq analysis of primary TNBC samples revealed specific clustering of malignant subpopulations linked to poor clinical outcomes and identified a gene signature in glycosphingolipid metabolism as a predictive biomarker for prognosis [151].

In addition to tumor pathology research, single-cell analysis has been widely applied to study the pathological mechanisms of lung diseases, kidney diseases, cardiac disorders, and neurological conditions. For lung diseases, single-cell sequencing enables high-resolution identification and characterization of lung tissue cell types, thereby enhancing the understanding of disease mechanisms. For instance, scRNA-seq classified mesenchymal and fibroblast subsets in both uninjured and bleomycin-treated mouse lungs, revealing differentiation trajectories [123]. Similarly, single-cell genomics compared pulmonary fibrotic and healthy tissues, uncovering cell state changes following COVID-19 infection [124]. Spatial transcriptomics identified novel pro- and anti-fibrotic markers in influenza-infected aged lungs, shedding light on complex pro-fibrotic networks [125]. Leveraging abundant single-cell data, researchers have constructed unified and accessible datasets to facilitate pathological research applications [152]. For kidney diseases, single-nucleus RNA sequencing (snRNA-seq) of human diabetic nephropathy samples classified epithelial cells and lymphocytes, with the data compared to healthy kidney samples, revealing changes in cell-specific gene expression during early-stage diabetes nephropathy, including alterations in potassium secretion and angiogenesis [153]. In cardiac disease research, Yao et al. developed a silicon nanowire-based transistor to monitor electromechanical coupling signals of individual cardiomyocytes in real time, providing deeper insights into the pathology and pharmacology of cardiac disorders [154]. For neurological disorders, Fu et al. achieved stable long-term recordings of local field potentials and single units in mouse brains for up to eight months, enabling studies on learning, aging, and neurodegenerative diseases [155]. Similarly, Cadwell et al. combined patch-clamp recording, immunohistochemistry, and scRNA-seq to achieve comprehensive multimodal profiling of individual neurons in mouse brain slices [156].

4.2. Discovery of new drug targets

Due to its superiority in reconstructing the evolutionary processes of diseased cells and elucidating their temporal and molecular characteristics, single-cell technology serves as a powerful tool for identifying and validating novel drug targets for complex diseases, particularly metastatic or drug-resistant tumors. It also plays a key role in the development of targeted therapeutic strategies.

Numerous single-cell studies have clarified the genetic heterogeneity of diseased tissues. However, the connections between specific subpopulations, gene expression changes, and disease progression remain poorly understood. Single-cell analysis of clinical samples has enabled the identification of drug targets across various disease stages with unparalleled precision. For instance, the transcriptional landscape of acral melanoma mapped by scRNA-seq revealed immune checkpoints as potential immunotherapeutic targets [157]. Similarly, a comprehensive single-cell analysis of human glioma samples identified S100A4 as a novel immunotherapy target in glioblastoma, where its deletion in non-cancer cells reprogrammed the immune landscape and significantly improved survival [158]. Nanoscale sensors, which integrate a nanotube reactor with duplex-specific nuclease-assisted hybridization chain reaction, have successfully detected intracellular RNA in single cells while preserving the cellular environment. This approach enabled the detection of miR-10b expression levels in normal, metastatic, and non-metastatic breast cancer cells, highlighting miR-10b as a potential therapeutic target for breast cancer (Fig. 4C) [159]. Mathewson et al. utilized scRNA-seq to create a gene expression landscape of infiltrating T cells in isocitrate dehydrogenase (IDH) mutant glioma and IDH-wild-type glioblastoma and identify the CD161-CLEC2D pathway as a potential target for immunotherapy in gliomas and other human cancers (Fig. 4D) [28].

In therapeutic-resistant tumors, single-cell technology has been employed to identify abnormally expressed genes or activated signaling pathways, facilitating the identification of new targets to overcome drug resistance or to prevent resistance formation through multi-target combination therapies [160]. For example, a study tracing cancer progression and metastasis at the subclonal resolution revealed stark heterogeneity in metastatic capacity, along with the identification of KRT17 as a suppressor of tumor invasiveness [29]. Aissa et al. demonstrated distinct gene expression profiles in EGFR inhibitor-tolerant versus sensitive cell types, suggesting that combining crizotinib with EGFR inhibitors could reduce the emergence of tolerant cell populations [161]. Zhang et al. utilized scRNA-seq to investigate chemoresistance in metastatic ovarian cancer, finding that stress-related pathways, such as those involved in cell survival and inflammation, play a pivotal role in chemoresistance, offering potential targets for overcoming it [162].

Differential expressions of biomarkers obtained through single-cell sequencing across patients contribute to the stratification of individuals with the same disease, thereby facilitating more precise, personalized treatment. Leader and colleagues used single-cell sequencing of cells from 35 early-stage non-small cell lung cancer (NSCLC) patients to identify a lung cancer activation module (LCAMhi), composed of PDCD1 + CXCL13 + T cells, IgG + plasma cells, and SPP1 + macrophages. Patients stratified by LCAM scores demonstrated that LCAMhi was associated with better immunotherapy outcomes [163].

Monitoring therapeutic responses is critical for assessing treatment effectiveness, understanding underlying mechanisms, and optimizing interventions—objectives that bulk analysis methods cannot fully address. Fortunately, single-cell technology provides valuable insights into heterogeneous responses, cell states, behaviors, and key molecules and pathways during treatment [164,165]. Samuel Aparicio and colleagues combined single-cell genome sequencing with machine learning to track tumor evolution during treatment. This approach allows the monitoring of genomic changes and the prediction of potential drug resistance, facilitating timely adjustments to treatment strategies and improving patient outcomes [130]. However, the integration of deep learning to enhance the construction of drug response and target prediction models still faces a significant technological gap in single-cell drug sensitivity analysis [166,167].

The significant advances in single-cell technology have expanded its applications across various research domains, driving a revolution in biological studies. Since the advent of scRNA-seq in 2009, numerous single-cell analysis techniques have emerged, especially with the integration of microfluidic technology. Today, various commercial platforms for single-cell analysis are available, including 10× Genomics Chromium [57], BD Rhapsody™ Single-Cell Analysis System [61,62], Illumina® Bio-Rad® Single-Cell Sequencing Solution [168], Singleron Matrix® [169], and MobiChIP™ [170]. These platforms offer high throughput, efficiency, convenience, and automation, significantly advancing the application of single-cell analysis in drug development and other biological research fields. For example, researchers employed single-cell sequencing technology to isolate neutralizing monoclonal

antibodies against SARS-CoV-2 from peripheral blood mononuclear cells of infected patients and then developed JS016, an antibody with potent neutralizing activity and blocking efficacy, for the prevention and treatment of COVID-19 [171]. Danxue Biotech used high-throughput single-cell transcriptome and VDJ sequencing technologies to identify two neutralizing antibodies against SARS-CoV-2, DXP-593 and DXP-604. Studies have shown that DXP-593 exhibits strong neutralizing activity against both pseudovirus and SARS-CoV-2, while DXP-604 is particularly effective against the Indian variant [172,173]. The combination of DXP-593 and DXP-604 may provide a promising cocktail therapy to overcome resistance caused by viral mutations. Furthermore, single-cell analyses have also played a crucial role in drug repurposing [174]. For example, Wang et al. utilized single-cell technology to perform deep sequencing on erythroid progenitor cells from untreated, glucocorticoid-responsive, and non-responsive Diamond-Blackfan anemia patients and found interferon α could serve as a potential therapeutic agent for congenital pure red cell aplasia. Since interferon has been widely used in the treatment of chronic hepatitis B, its safety in pediatric therapy has been well established, and a corresponding clinical trial has been registered [175].

4.3. Optimization of stem-cell therapies

Stem cell therapies have been extensively investigated over the past few decades for their potential in treating autoimmune, inflammatory, neurological, and orthopedic diseases, particularly in tissue healing and regenerative medicine [176–178]. MSCs, first isolated from bone marrow by Friedenstein in 1970, are considered an ideal source [179]. Currently, the majority of MSC research focuses on cells derived from a single source, treating them as functionally homogeneous units for therapeutic applications [180,181]. However, recent studies have revealed the presence of diverse MSC subpopulations, each with distinct biological and functional properties, as well as differing risks, such as embolic potential, in the host (Fig. 4E) [182–185]. As a result, there is an urgent need for precise categorization of MSC subpopulations to distinguish their genotypic and differentiation preferences, verify their regulatory and regenerative differences, and guide the selection of suitable therapeutic cell sources or subtypes. Fortunately, single-cell technology offers a promising solution to address this gap.

In terms of MSC origin, single-cell analyses comparing the effects of MSCs from different sources in the treatment of osteoarthritis have indicated that adipose-derived MSCs are a preferable choice, owing to their lower transcriptomic heterogeneity and higher immunosuppressive capacity [180]. On the other hand, Sun et al. recently isolated distinct subpopulations of human Wharton's jelly-derived MSCs, confirming their heterogeneity. These populations exhibited differences in proliferation, development, and inflammatory response [186]. However, MSCs from various sources shared some highly variable genes, which could serve as potential biomarkers for clinical studies. Similarly, several studies have observed variations in the immunoregulatory capacities of MSCs from different tissues [187,188]. Cai et al. identified four subsets of foreskin and human umbilical cord MSCs using single-cell scRNA-seq, highlighting immune-related MSC subsets as potential candidates for immunoregulation [189]. Moreover, given the inherent heterogeneity of MSCs, researchers have proposed single-cell sorting methods focusing on functional discrepancies, such as differences in secretion, proliferation, and migration, to achieve more uniform MSC populations and improved therapeutic outcomes [190].

Beyond identifying the optimal source of MSCs for specific diseases, single-cell technologies can also elucidate the mechanisms of MSC-based therapies by tracking the evolution of diseased cells after treatment. Flow cytometry and scRNA-seq have been used to precisely distinguish macrophage populations and elucidate the mechanisms of MSC treatment at different stages of acute liver injury. MSCs promoted the transition from proinflammatory Ly6Chi monocytes to Ly6Clo monocyte-derived macrophages (MoMFs) and reduced neutrophil recruitment during the acute injury phase. During the recovery stage, MSCs increased the expression of arginase 1 on MoMFs, promoting liver repair (Fig. 4F) [191]. Various mononuclear phagocytes are also regulated by MSCs in acute lung injury, with scRNA-seq revealing the mechanisms involved, including the regulation of macrophage phenotype, cytokine and chemokine secretion, and antigen presentation [192].

4.4. Monitoring of intracellular delivery

As the demand for cell reprogramming delivery tools and biosensing platforms for tracking intracellular information increases, there is growing anticipation for more sophisticated methods capable of effectively monitoring and controlling cells [193]. One of the key advantages of bioelectronics-based single-cell sensing in drug delivery is the ability to monitor single-cell drug injection or transfection in real time, enabling synchronized responses for cell status analysis.

Xie et al. demonstrated a nanopillar technology that integrates electroporation with intracellular/extracellular action potential recording. However, this approach is unable to simultaneously record both intracellular and extracellular signals [194]. Cerea et al. introduced a novel multifunctional multi-electrode array biosensor that combines network-level electrical recording with single-cell intracellular delivery, offering a comprehensive, ready-to-use platform for extracellular and intracellular recording of cardiomyocytes and enabling non-invasive, selective molecule delivery [22]. Xu et al. developed a nanorod device capable of detecting both intracellular and extracellular potentials simultaneously, achieving synchronized recording during the electroporation transfection process [195]. The intracellular and extracellular data demonstrated good spatial and temporal synchronization, as validated in neonatal rat cardiomyocytes (Fig. 4G). From a photoelectrochemical (PEC) perspective, Ruan et al. designed a bifunctional photoelectrode single-cell nanomaterial [196]. By incorporating a specific target organic molecule/NiO/Ni thin film at the nanopipette tip (Fig. 4H), this device enables direct electroporation-mediated intracellular drug delivery and the evaluation of oxidative stress, highlighting the potential of PEC applications in single-cell nanomaterials. To overcome the challenge of feedback signals in precise single-cell miRNA therapy, Zheng et al. proposed a nanopipette driven by PEC feedback for high-precision miR-21 targeted therapy and conducted tests (Fig. 4I) [197].

5. Conclusion and outlook

The relentless development of micro- and nanofabrication technologies, integration techniques, molecular biology methods, and bioinformatics has significantly broadened the application of single-cell research in drug delivery and pharmaceutical studies. These advancements have demonstrably enhanced the efficiency of cell-based drug delivery systems and improved the therapeutic efficacy of cell therapies. Concurrently, single-cell analysis and sensing have provided profound insights into human development and disease pathology, facilitated the identification of novel drug targets, and accelerated the pace of new drug development. In the current era of rapid artificial intelligence (AI) advancement, the integration of single-cell research with AI has further augmented the efficiency and accuracy of single-cell data analysis. This synergy allows for a deeper exploration of the complexities of cell biology and promotes the expanded application of these technologies in pharmacology. This review has discussed key single-cell isolation techniques, along with intracellular molecule delivery methods, single-cell encapsulation, and electrical stimulation strategies, all aimed at improving the efficiency of cell-based drug delivery systems and enhancing the therapeutic efficacy of cell therapies. It has also explored commonly used single-cell sequencing techniques and bioelectronics-based single-cell sensing in the context of pharmacology, with the objective of guiding researchers in selecting appropriate techniques tailored to their specific research needs and objectives.

Despite the significant progress in single-cell research, several limitations persist. For example, although single-cell sequencing and bioelectronics-based single-cell sensing offer high resolution, the sensitivity and resolution of detection signals at the single-cell level remain constrained, particularly when detecting weak biological signals. Furthermore, while AI has undoubtedly improved the efficiency and accuracy of single-cell data analysis, its performance is still impacted by the presence of low-quality data and necessitates substantial computational resources. Addressing these challenges requires continuous technological and methodological innovation, coupled with interdisciplinary collaborations. Such concerted efforts are essential to achieving further breakthroughs in single-cell research and fully realizing its transformative potential in biomedicine and pharmaceutical sciences. Future directions may involve the development of more sensitive and robust detection platforms, the refinement of AI algorithms to handle noisy or incomplete data more effectively, and the integration of multi-omics approaches to provide a more holistic understanding of cellular function and behavior at the single-cell level. Ultimately, continued progress in these areas will pave the way for more precise and personalized therapeutic strategies, leading to improved patient outcomes.

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Declaration of competing interest

All authors declared that there are no conflicts of interest.

Data availability

No data was used for the research described in the article.

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