

**REVIEW**

Intelligent Microfluidics for Smart Bioengineering

Hanxu Chen*  and **Sirawit Tidma** 

Department of Materials Science and Engineering, National University of Singapore, Singapore

*Corresponding Author: Hanxu Chen. Email: hxchen@nus.edu.sg

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ABSTRACT: Bioengineering is evolving towards automated and adaptive systems, creating an increasing demand for technologies that bridge computational intelligence with physical biological processes. Microfluidics provides such an interface by enabling precise spatiotemporal manipulation, high-throughput compartmentalization, and integrated sensing of cells, biomolecules and their microenvironments. Herein, this review focuses on emerging microfluidics for smart bioengineering, from their physical foundations and intelligent bioanalysis to programmable microphysiological systems, synthetic biology, precision cell engineering, and adaptive biomanufacturing. Intelligent microfluidics integrated with biosensing, automated imaging, and artificial intelligence transforms biological responses into machine-readable information while translating computational decisions into controlled interventions. Besides, challenges in scalability, standardization, data interoperability, and biological uncertainty are discussed. Ultimately, the convergence of microfluidics and computational intelligence could shift bioengineering from predefined automation towards closed-loop, adaptive, and autonomous operation.

KEYWORDS: Microfluidics; intelligent; biosensing; adaptive systems; smart bioengineering

1 Introduction

Bioengineering is shifting from empirical, manually operated workflows toward increasingly automated, data-rich, and adaptive systems [1–6]. Advances in single-cell and spatial omics, synthetic biology, engineered tissues, and cell-based therapeutics have increased the complexity of biological experimentation. Together, these developments are driving the emergence of smart bioengineering, in which biological systems can be quantitatively sensed, precisely manipulated, and iteratively optimized [7–13]. Although artificial intelligence (AI) can identify patterns, predict biological behavior, and propose experimental conditions, directly manipulating cells, biomolecules, and their microenvironments remains challenging [14–19]. Moreover, heterogeneous and dynamic biological responses must be translated into standardized, machine-readable data to enable effective integration with computational models [20–24]. Bridging this physical–digital gap therefore requires experimental technologies that combine precise manipulation, scalable data generation, sensing, and programmable control.

Microfluidics is uniquely positioned to address these challenges (Fig. 1) [25–28]. By controlling biological microenvironments at the microscale, microfluidic systems enable precise spatiotemporal regulation of chemical gradients, mass transport, mechanical forces, and cellular interactions while minimizing sample and reagent consumption [29–35]. In addition, droplets, microwells, and microchambers allow thousands to millions of biological reactions to be compartmentalized, making biological heterogeneity accessible to systematic experimental analysis. Integration with automated imaging, biosensors, and computational

analysis further enables simultaneous perturbation and monitoring of biological systems [36–45]. Microfluidics can thus serve as a programmable physical layer for smart bioengineering, translating computational instructions into controlled biological interventions and converting biological responses into structured digital information [46–50]. When coupled with AI, this bidirectional information flow offers a path from predefined automation to adaptive and, ultimately, autonomous experimentation.

Here, we review recent advances in microfluidics for smart bioengineering, spanning its physical foundations, intelligent bioanalysis, and applications. We first introduce the key capabilities of microfluidics, including precise spatiotemporal control, high-throughput parallelization, and integrated sensing and actuation. We then discuss microfluidics-based bioanalysis, covering single-cell phenomics, multimodal analysis, molecular diagnostics, signal acquisition, and computational interpretation. Next, we examine applications in smart bioengineering, including programmable microphysiological systems, synthetic biology and directed evolution, precision cell engineering, and adaptive biomanufacturing. Finally, we assess the current state of the field and discuss outstanding challenges, future perspectives, and development priorities. By connecting technological foundations with analytical capabilities and biological applications, this review aims to provide a comprehensive perspective on microfluidics and to guide researchers across disciplines in advancing smart bioengineering.

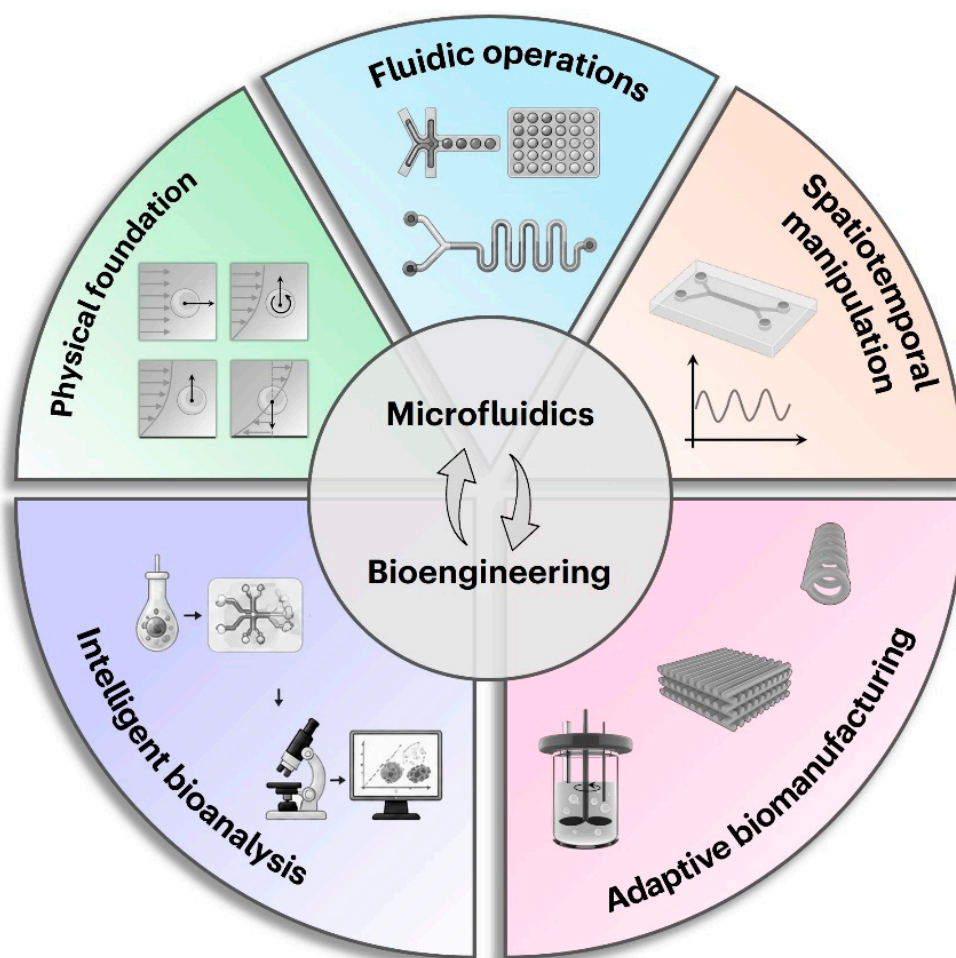


Figure 1: Scheme of the intelligent microfluidics for smart bioengineering.

2 Microfluidics-Based Physical Foundation

The relevance of microfluidics to autonomous bioengineering extends far beyond device miniaturization. Fluids, cells, and biomolecules can be manipulated within highly defined physical environments at the microscale, featured with greater precision, parallelization, and programmability compared with conventional laboratory platforms. More importantly, microfluidics provides a physical interface where biological processes can be coupled with sensors, electronics, and computational algorithms. Recent developments in cyber-biological systems have further illustrated that by integrating engineered biological components with microfluidics and electronics to construct hybrid platforms capable of sensing, processing and responding to biological environments [51–54]. Generally, benefiting from several interconnected characteristics including precise spatiotemporal control, high-throughput parallelization, and integrated sensing and actuation, microfluidics serves as an important physical foundation for smart bioengineering.

2.1 Precise Manipulation of Spatiotemporal Microenvironments

Biological systems continuously respond to dynamic combinations of biochemical and biophysical cues (e.g., nutrients, cytokines, oxygen tension, shear stress, and mechanical forces). Conventional culture plates generally provide spatially averaged and relatively static environments, making it difficult to reproduce the heterogeneous and dynamic conditions experienced by cells *in vivo*. To overcome this limitation, microfluidic systems can exploit predictable laminar flow and microscale mass transport to generate well-defined chemical gradients and dynamically regulated cellular microenvironments (e.g., microbioreactors integrated with multifunctional units) [55–60]. Such controllability has been considered particularly important for development of organs-on-chips. Microfluidic organ-chip systems can reproduce tissue interfaces, vascular perfusion, chemical gradients, and mechanical stimulation within engineered cellular environments. Ingber and colleagues demonstrated how organ-chip platforms can recapitulate aspects of human organ-level physiology and pathophysiology and reproduce clinically relevant responses to drugs, infectious agents and other perturbations (Fig. 2a) [61]. These studies illustrate how precise microenvironmental regulation can transform microfluidics from a passive culture platform into a programmable experimental system. Importantly, microscale control also introduces a temporal dimension into biological experimentation. Cells can be exposed to defined sequences, amplitudes, and durations of stimuli while their responses are continuously recorded. Microfluidics therefore enables biological processes to be interrogated as dynamic input–response relationships, rather than through single static endpoint measurements. Such temporal programmability is particularly valuable for studying cellular adaptation, signalling dynamics, differentiation, and drug responses. In addition, biological conditions can be encoded as controllable physical parameters and subsequently modified according to predefined protocols or computational decisions.

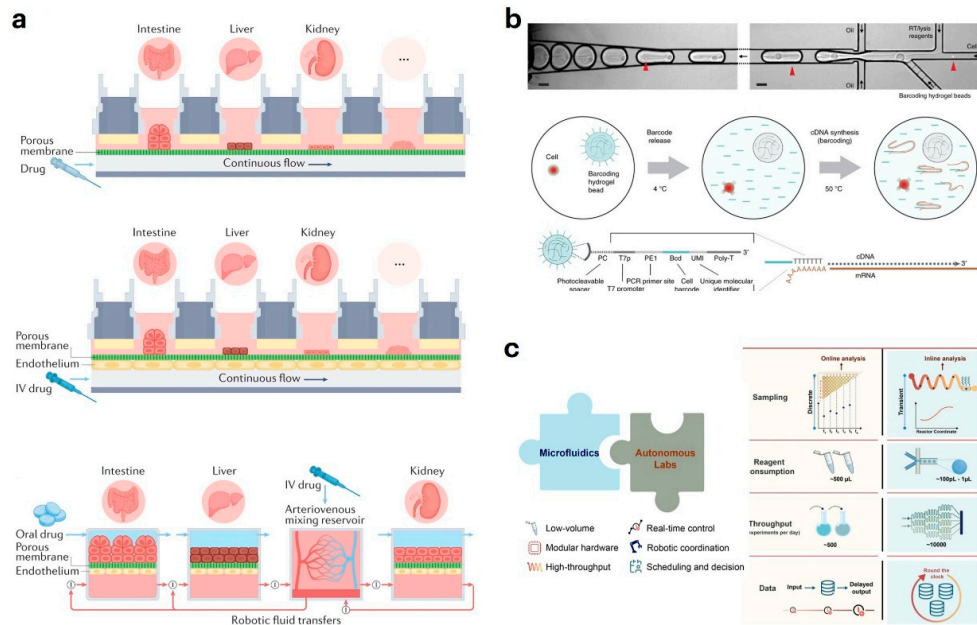


Figure 2: Microfluidics-based physical foundation. (a) Precise manipulation of spatiotemporal microenvironments based on microfluidic organ-on-chips. Reproduced with permission [61]. (b) Droplet microfluidics-based single-cell transcriptome barcoding. Reproduced with permission [62]. (c) Autonomous microfluidic laboratories with self-driving operations and design-test-analyze cycles. Reproduced with permission [63].

2.2 High-Throughput Parallelization

Another defining feature of microfluidics is the ability to partition biological samples into large numbers of microscale compartments. Droplets, microwells, and microchambers can serve as independent reaction vessels containing individual cells, microorganisms, nucleic acids, proteins, or biochemical reaction mixtures [64,65]. Droplet microfluidics has been particularly transformative for single-cell biotechnology. Early droplet-based barcoding strategies enabled thousands of individual cells to be encapsulated and processed in parallel for transcriptomic analysis while substantially reducing reagent consumption (Fig. 2b) [62]. This principle subsequently became an important technological basis for high-throughput single-cell sequencing and contributed to the rapid expansion of single-cell genomics. In addition, microfluidic compartmentalization effectively converts biological heterogeneity from experimental complication into measurable information. Instead of averaging signals across a population, individual cells or biological reactions can be isolated, characterized, and statistically compared. This strategy has progressively expanded from transcriptomics towards genomic, epigenomic, proteomic, and multimodal analyses. Microfabricated platforms based on channels, arrays, and droplets are now being developed to interrogate molecular information at cellular and even subcellular resolution [66–69]. Recent developments have demonstrated that increasing throughput alone is insufficient. For example, spinDrop incorporated additional processing capabilities into droplet-based single-cell sequencing to enrich informative biological material and improve information recovery from challenging samples [70]. Such approaches indicate a transition from simple high-throughput encapsulation towards increasingly selective and information-efficient microfluidic workflows. In brief, the importance of parallelization for smart biotechnology is therefore twofold. First, it enables large biological design spaces to be experimentally explored. Second, it generates sufficiently large and standardized datasets for machine-learning algorithms to identify complex relationships between experimental conditions and biological outcomes.

2.3 Programmable Fluidic Operations

Conventional continuous-flow microfluidic devices typically rely on predefined channel geometries. Although these systems provide precise fluid control, changing experimental workflows may require redesigning and fabricating new devices. Autonomous bioengineering, by contrast, increasingly requires flexible experimental hardware that can respond to variational computational instructions [71,72]. To meet the requirements, digital and reconfigurable microfluidic platforms provide a promising route, where individual droplets can be transported, mixed, divided, and reacted through electronically controlled operations, allowing sequences of fluidic manipulations to be translated into software-defined protocols. This feature is conceptually important because it begins to decouple the experimental procedure from fixed device geometry. Such programmability creates an analogy with computing: whereas conventional microfluidic devices often behave as application-specific hardware, reconfigurable microfluidic systems could increasingly operate as general experimental platforms in which different biological protocols are implemented through software. This transition is particularly relevant when microfluidics is integrated with artificial intelligence. Liu et al. reviewed how AI has already been coupled with droplet microfluidics for droplet generation, materials synthesis, and biological analysis, highlighting the potential of machine learning not only for data interpretation but also for system optimization and control [73]. Thus, microfluidic programmability provides a physical route through which computational decisions can be translated into experimental actions.

2.4 Integration of Sensing and Actuation Modules

An intelligent biological system requires both the ability to manipulate biological processes and the ability to determine how those processes respond. Microfluidic platforms are particularly advantageous in this respect because diverse sensors (e.g., optical, electrochemical, mechanical, and biochemical sensors) can be positioned close to biological samples, substantially reducing the physical separation between experimental perturbation and measurement [74–76]. For example, microscopy integrated with microfluidic devices can continuously record cell morphology, migration, proliferation, and interactions, whereas fluorescence or electrochemical measurements can monitor the molecular and metabolic changes. The resulting datasets may include images, videos, electrical signals, and molecular profiles. AI-assisted microfluidic approaches have increasingly been developed to analyze these heterogeneous datasets, particularly in high-throughput single-cell experiments [77]. Conventionally, measurements of biological experiments are often performed after an intervention and primarily serve as endpoints. In contrast, sensing within smart microfluidic systems can become part of the experimental control architecture, where the whole feedback cycle can therefore be established (biological perturbation; biological response; sensing; data analysis; decision; subsequent perturbation). Once sensing and actuation are connected through computational algorithms, microfluidics can support closed-loop experimentation rather than merely automated sample processing.

2.5 Construction of Physical-Digital Interface for Bioengineering

The convergence of microfluidics, electronics and computational intelligence is beginning to blur the distinction between biological experimentation and engineered control systems. Hybrid systems in which engineered biological components, microfluidics, and electronics are integrated to enable multiple-sensing, actuation, and self-reporting within a unified architecture. Such systems exemplify a broader transition from passive biological devices towards interactive physical-digital platforms, which was further strengthened by AI. Machine-learning algorithms can extract patterns from large microfluidic datasets, identify informative experimental conditions, and potentially determine subsequent experiments. In parallel,

microfluidic systems provide a reproducible physical environment in which these computationally generated decisions can be experimentally implemented [78–80]. Recently, autonomous microfluidic laboratories extend this principle towards self-driving experimentation, where machine learning, laboratory automation, and microfluidic operations are combined within iterative design-test-analyze cycles (Fig. 2c) [63]. Instead of exhaustively exploring experimental parameter spaces, algorithms can select informative experiments, analyze their outcomes, and iteratively refine subsequent experimental conditions. Thus, microfluidics should be increasingly viewed not merely as a miniaturized laboratory, but as a physical-digital interface. It converts computational instructions into precisely defined biological perturbations while simultaneously converting complex biological responses into structured digital information. This bidirectional information flow provides the technological basis for the transition from conventional biotechnology to automated, adaptive and ultimately autonomous biotechnology.

3 Intelligent Microfluidics-Based Bioanalysis

Microfluidic technologies have been widely applied for bioengineering benefiting from the miniaturization of analytical procedures [81–85]. However, its role is now shifting from simply reducing sample consumption and increasing analytical throughput towards generating high-dimensional, dynamic, and machine-readable biological information. By precisely isolating cells and biomolecules, integrating multiple analytical modalities and coupling biological measurements with automated imaging and computational analysis, microfluidics provides an important technological foundation for intelligent bioanalysis, including droplet microfluidics, microbioreactors, organ-on-chips and so on. For instance, conventional population-averaged measurements obscure cellular heterogeneity, whereas microfluidic compartmentalization allows biological information to be resolved at the level of individual cells. Recent developments are further expanding this capability from molecular profiling towards multimodal and functional measurements, establishing a trajectory from single-cell analysis to single-cell phenomics [86–89]. In parallel, microfluidic integration with molecular diagnostics and intelligent sensing increasingly enables automated workflows that connect sample processing, biological recognition, signal acquisition, and computational interpretation.

3.1 From Single-Cell Analysis to Single-Cell Phenomics

Biological tissues consist of heterogeneous populations of cells that differ substantially in their molecular states, functions, and responses to external perturbations. Bulk measurements average these differences and can therefore obscure rare cell populations or transient biological states. Microfluidics provides a natural solution where droplets or microchambers can isolate and manipulate individual cells at dimensions [90–92]. The development of high-throughput droplet-based single-cell RNA sequencing established a scalable approach in which individual cells are co-encapsulated with barcoded beads, allowing transcriptomes from thousands of cells to be analyzed in parallel. For example, Macosko et al. introduced Drop-seq, where nanolitre droplets were used to pair individual cells with barcoded microparticles, enabling massively parallel transcriptomic profiling and demonstrating the power of microfluidic compartmentalization for resolving cellular heterogeneity (Fig. 3a) [93]. The subsequent development of highly scalable commercialized droplet systems further established microfluidics as a core infrastructure for modern single-cell biology. Datlinger et al. developed single-cell combinatorial fluidic indexing by combining transcriptome pre-indexing with droplet microfluidics [94]. This strategy allowed several cells to be loaded into individual droplets and subsequently computationally demultiplexed, increasing sequencing throughput to approximately 150,000 cells per microfluidic channel. More recently, Li et al. combined droplet microfluidics with combinatorial indexing to achieve massive-scale multimodal single-cell sequencing [95]. This approach enabled RNA co-assays

and generated more than 100,000 high-quality single-cell datasets from dozens of frozen clinical biopsy specimens. These results illustrate how microfluidics is evolving from simple cell partitioning towards a programmable platform for large-scale multimodal biological interrogation. Microfluidic single-cell analysis is also expanding beyond mammalian systems. Lan et al. developed droplet targeted amplicon sequencing for high-throughput genetic analysis of diverse microbial populations (Fig. 3b) [96]. By using droplet microfluidics, the method simultaneously tracked multiple antibiotic-resistance genes and their taxonomic associations within complex gut microbial communities. Together, the objective of microfluidic single-cell technologies is no longer simply to process more cells, but to extract more informative biological representations from each cell. Microfluidics becomes not merely a sample-preparation technology but an experimental interface capable of continuously interacting with individual biological systems.

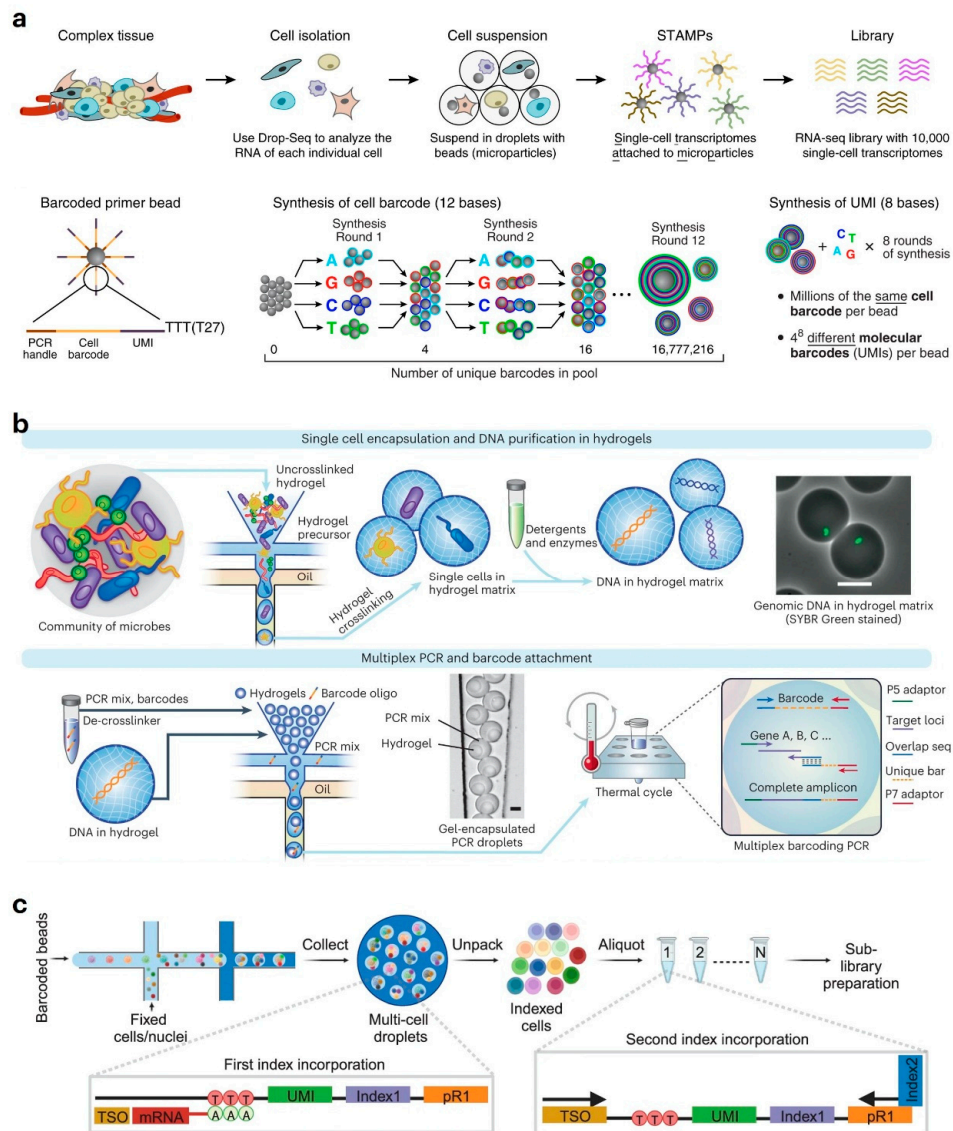


Figure 3: Intelligent microfluidics-based bioanalysis. (a) Molecular barcoding of cellular transcriptomes in microfluidic droplets. Reproduced with permission [93]. (b) Massively parallel single-cell sequencing of diverse microbial populations. Reproduced with permission [96]. (c) Single cell profiling of multiple molecular modalities. Reproduced with permission [97].

3.2 Microfluidics for Multimodal and Spatial Biology

Cellular identity and function emerge from interactions among multiple molecular layers, including the genome, epigenome, transcriptome, proteome, and metabolome. Measuring only one molecular modality therefore provides an incomplete description of cellular states. Single-cell multi-omics addresses this limitation by measuring multiple molecular features from the same cell. Baysoy et al. systematically summarized the rapidly expanding technological landscape of single-cell multi-omics and emphasized that integrating multiple molecular modalities can reveal relationships between molecular regulation and cellular phenotype [98]. Microfluidics plays an important role in this transition because cells and cellular components can be sequentially isolated, manipulated, and processed within highly controlled microscale environments. Microchannel-, microarray-, and droplet-based platforms can support multimodal analysis at cellular and even subcellular resolution. Recent microfluidic strategies have particularly focused on overcoming the throughput limitations of multimodal analysis. For example, droplet overloading methods featured with combinatorial indexing have been developed to increase utilization of droplets and barcodes while maintaining single-cell identity (Fig. 3c) [97]. Such strategies address an important inefficiency in conventional droplet systems, where a large fraction of droplets may contain no cells. Similarly, it was demonstrated that how microfluidic compartmentalization can be integrated with computational indexing to simultaneously increase throughput and the dimensionality of biological measurements [99,100]. It can be concluded that future single-cell platforms will increasingly combine physical compartmentalization with computational deconvolution, allowing experimental throughput to exceed the conventional one cell per droplet paradigm. A limitation of conventional single-cell sequencing is that tissue dissociation destroys information about the spatial organization of cells. However, cellular behavior is strongly influenced by neighboring cells, extracellular matrices, and local biochemical gradients. Spatial multi-omics therefore seeks to preserve the relationship between molecular state and tissue architecture. Microfabricated channels, arrays, and patterned surfaces can provide spatially controlled molecular capture and manipulation, enabling genome, transcriptome, and protein information to be mapped within intact tissues [101–103].

The combination of microfluidics with spatial biology is particularly relevant to autonomous bio-engineering because it transforms biological information from a list of molecular measurements into a spatially organized representation of biological systems. When combined with AI-based image analysis, such datasets could enable computational reconstruction of cellular interactions and tissue microenvironments [104–107]. Ultimately, the integration of single-cell, spatial, and temporal information could generate multidimensional biological representations. Such representations will provide considerably richer inputs for machine-learning models than conventional endpoint measurements.

3.3 Intelligent Molecular Diagnostics

Microfluidics has also substantially influenced molecular diagnostics by integrating sample preparation, reaction, and detection into compact analytical platforms. The small reaction volumes can significantly shorten diffusion distances, reduce reagent consumption, and facilitate rapid thermal/chemical control. For instance, by partitioning samples into thousands of isolated microreactors, individual nucleic-acid molecules can be amplified and quantified using statistical analysis. Microfluidic compartmentalization therefore converts conventional analogue amplification signals into digital molecular measurements. The emergence of CRISPR-based diagnostics has further expanded the potential of microfluidic molecular analysis. CRISPR-associated enzymes provide programmable molecular recognition, whereas microfluidics enables reaction miniaturization, multiplexing, and automated sample handling. Together, these capabilities create opportunities for highly sensitive and portable diagnostic systems. Ackerman et al. developed bead-

based approaches that physically localized CRISPR detection reactions and improved both sensitivity and multiplexing (Fig. 4a) [108]. Such compartmentalized reaction architectures illustrated how microscale engineering can address limitations associated with cross-reactivity and multiplexed molecular detection. Briefly, an intelligent diagnostic platform should integrate four processes, including sample processing, molecular recognition, signal acquisition, and computational interpretation. Rather than producing a binary positive-negative result, these systems could generate multidimensional diagnostic signatures and probabilistic predictions, benefiting from the microfluidics-based natural physical architecture for integrating these steps [109,110].

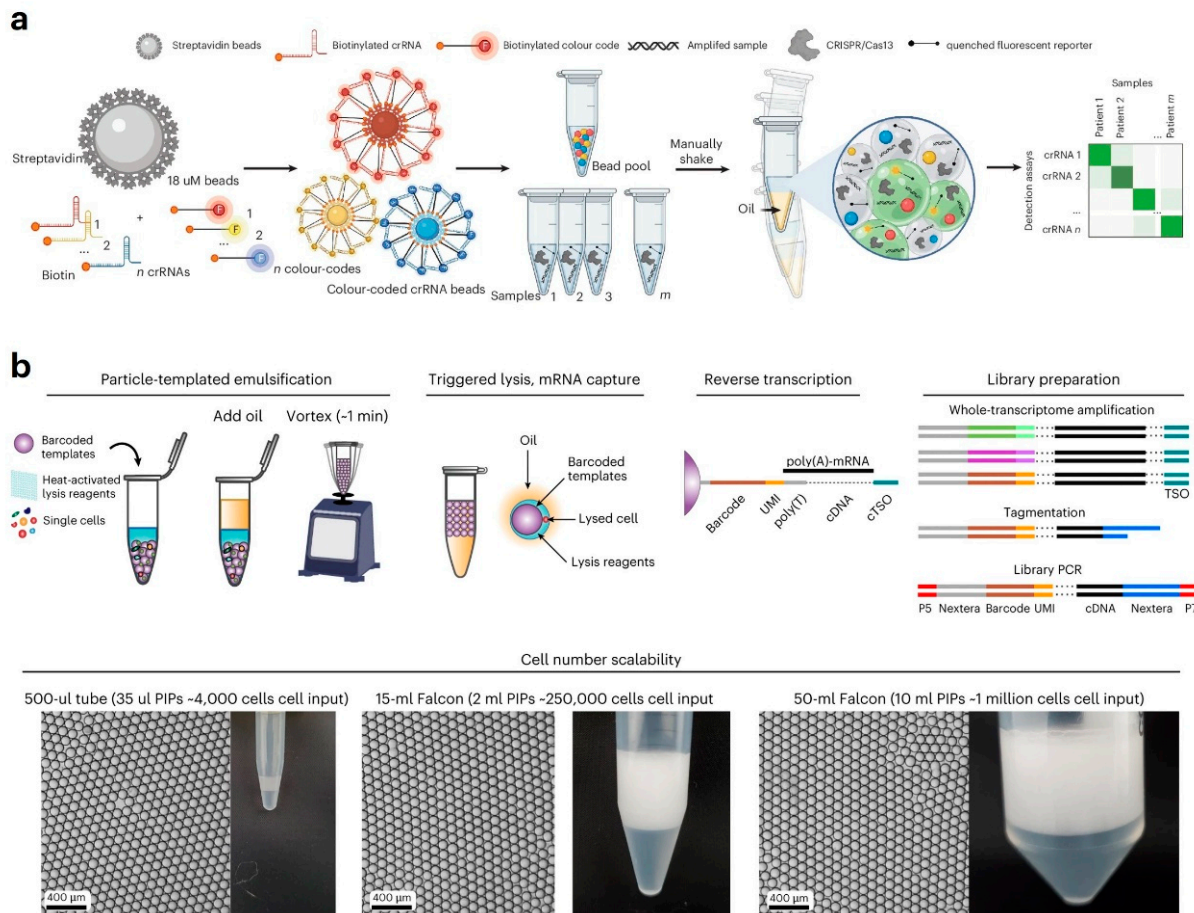


Figure 4: Microfluidics-based molecular diagnosis and high-throughput bioanalysis. (a) Biotinylated beads in equipment-free generated droplets for CRISPR detection reactions. Reproduced with permission [108]. (b) Rapid and scalable templated emulsification for single-cell genomics. Reproduced with permission [111].

3.4 From High-Throughput to Information-Rich Bioanalysis

The evolution of microfluidics-based bioanalysis can be summarized as a transition across three technological stages. The first stage emphasized miniaturization, reducing reaction volumes, and reagent consumption. The second stage emphasized high throughput, using droplets or parallel channels to process thousands or millions of biological samples. The emerging third stage emphasized the information density and biological context. In this stage, the objective is no longer simply to maximize the number of cells for bioanalysis. Instead, microfluidic systems seek to maximize the biological information obtained from each experimental unit by integrating molecular profiles, phenotypes, spatial context, and temporal

responses [112–114]. For example, microfluidics was combined with combinatorial indexing to increase both throughput and multimodal information, whereas next-generation single-cell phenomic technologies aim to link molecular profiles with dynamic functional phenotypes. Interestingly, Clark et al. developed particle-templated instant partition sequencing, which generates uniform emulsions without specialized microfluidic equipment (Fig. 4b) [111]. Such development highlights the need for future microfluidic systems to become simpler, more accessible, and more programmable. Therefore, the future competitive advantage of microfluidics will not derive from miniaturization alone. It depends on the ability to provide capabilities that are difficult to achieve using simpler technologies: dynamic perturbation, longitudinal observation, multimodal sensing, automated selection, and closed-loop experimental control. These capabilities provide a natural bridge between intelligent bioanalysis and autonomous bioengineering platforms.

4 Microfluidics for Smart Applications of Bioengineering

As mentioned above, the transition from intelligent bioanalysis to smart bioengineering represents a fundamental shift in the role of microfluidics: from reading biological systems to actively constructing, perturbing, and optimizing them. Smart biological engineering requires precise control over multicellular organization, extracellular environments, genetic programs, and manufacturing conditions [115–117]. Microfluidics provides a physical framework in which these variables can be manipulated with high spatial and temporal resolution while biological responses are simultaneously monitored. At the tissue level, microfluidic perfusion and microenvironmental regulation enable organoids and microphysiological systems to reproduce increasingly sophisticated aspects of human physiology [118,119]. At the molecular and cellular levels, droplet microfluidics accelerates directed evolution and synthetic biology by enabling large biological design spaces. At the manufacturing level, microfluidic cell processing and miniaturized bioreactors provide opportunities to integrate cell isolation, genetic modification, expansion, and quality monitoring into automated workflows. Briefly, microfluidics transforms biological engineering from a sequence of discrete operations into a controllable, measurable, and increasingly programmable process.

4.1 From Organoids to Programmable Microphysiological Systems

Organoids have emerged as powerful *in vitro* models because stem and progenitor cells can self-organize into three-dimensional structures that reproduce important aspects of tissue architecture and function. However, conventional organoid culture suffers from poorly controlled nutrient transport, heterogeneous morphogen exposure, limited vascularization, and substantial batch-to-batch variability. To overcome these limitations, microfluidics provides a complementary strategy by controlling perfusion, biochemical gradients, and mechanical cues without eliminating the intrinsic self-organization of living tissues. As organoids grow beyond the diffusion limit of oxygen and nutrients, hypoxic and necrotic regions can develop, restricting their maturation and physiological relevance. Quintard et al. developed a microfluidic platform that established functional endothelial networks around spheroids and human pluripotent stem-cell-derived blood-vessel organoids (Fig. 5a) [120]. The vascular networks formed functional connections with the organoids and enabled intravascular perfusion. It has been illustrated that microfluidic flow can serve not only as a transport mechanism but also as an active developmental cue. Microengineering can also extend vascular control towards larger tissue constructs. Arakawa et al. developed three-dimensional soft microfluidic architectures containing capillary-scale synthetic vessels to support perfusion of engineered tissues [121]. Such architectures supported prolonged tissue growth while reducing hypoxia and necrosis, demonstrating how microfluidics can evolve from planar culture devices towards three-dimensional vascular infrastructures.

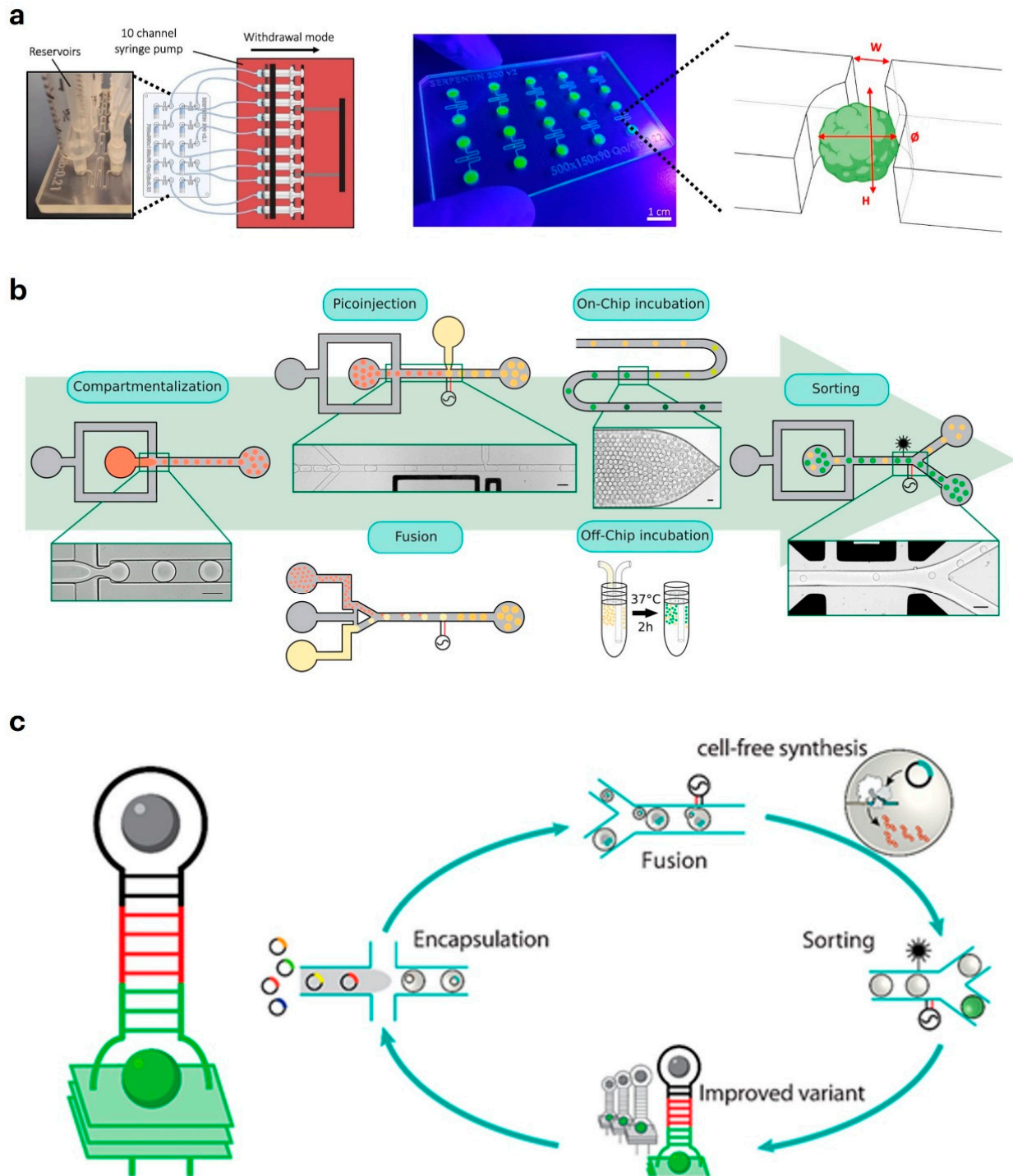


Figure 5: Microfluidics-based programmable microphysiological systems. (a) Lab-on-chip systems for organoid and cell configurations. Reproduced with permission [120]. (b) Typical initial compartmentalization followed by picoinjection, fusion, incubation, and sorting events. (c) Schematic workflow of directed evolution in drops. Reproduced with permission [122].

Rather than attempting to directly specify every cellular interaction, microfluidics defines the boundary conditions, including perfusion, nutrient transport, morphogen gradients, and mechanical stimulation [123–125]. By combining tissue interfaces, vascular perfusion and controlled stimulation, microfluidic devices can reproduce selected physiological processes in experimentally accessible formats. For instance, Goyal et al. engineered an immunocompetent organotypic chip reproducing key features of the human leukaemia bone-marrow niche (Fig. 5b,c) [122]. Such systems demonstrated that how microfluidic tissue models connected patient-relevant biology with quantitative therapeutic evaluation. Future microphysiological systems could therefore move beyond static disease modelling towards programmable preclinical testing. Patient-derived cells could be incorporated into chips, exposed to dynamically controlled therapeutic regimens, and continuously monitored by imaging and integrated sensors. However, increasing physiological complexity introduces an important trade-off. More sophisticated organ-chip systems can improve biological relevance while simultaneously decreasing throughput, reproducibility, and engineering controllability. Smart biotechnology should therefore seek an optimal balance between biological fidelity and experimental programmability, rather than maximizing biological complexity alone.

4.2 Synthetic Biology and Directed Evolution

Synthetic biology aims to engineer proteins, genetic circuits, microorganisms and, cellular behaviors through iterative design-build-test-learn cycles. Although computational methods can rapidly generate biological designs, experimental construction and screening frequently remain rate-limiting steps. Microfluidics is particularly powerful for expanding the test and learn steps [126–129]. Especially, droplet microfluidics compartmentalizes cells, enzymes, or genetic variants into picolitre-scale reactors, maintaining the relationship between genotype and functional phenotype while enabling extremely large libraries to be screened. For example, Agresti et al. developed a droplet-microfluidics platform for ultrahigh-throughput directed evolution of horseradish peroxidase (Fig. 6a) [130]. It was found that approximately 10^8 individual enzyme reactions could be screened within approximately 10 h, allowing variants with substantially enhanced catalytic activity to be identified. Recently, microfluidic platforms have shifted from single-parameter screening towards multidimensional functional characterization. Chou et al. combined droplet microfluidics with automated fluorescence imaging to establish a multiparameter screening platform for genetically encoded fluorescent biosensors (Fig. 6b) [131]. Such system increased screening throughput while allowing several functional parameters to be simultaneously evaluated. Proteins, biosensors, or microorganisms may need to simultaneously balance activity, specificity, stability, and environmental robustness. Interestingly, microfluidics can generate datasets that connect genotype, environmental condition, phenotype, and functional performance [132–134]. These data can subsequently inform computational models that identify promising regions of biological design space.

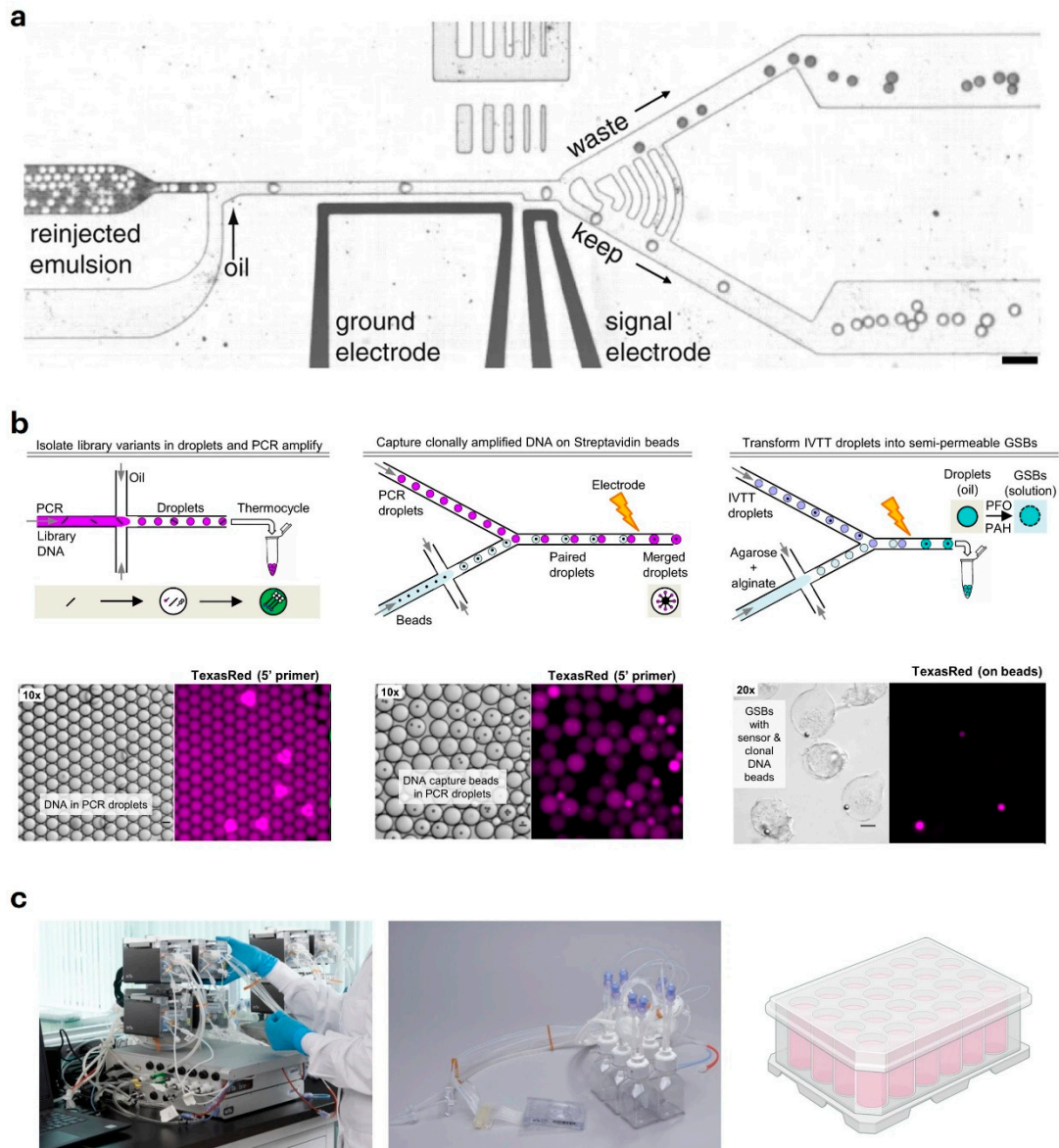


Figure 6: Microfluidics-based smart applications of bioengineering. (a) Modules of the ultrahigh-throughput microfluidic screening platform. Reproduced with permission [130]. (b) Microfluidic workflow to generate a biosensor library for screening of intracellular lactate concentration in cultured mammalian cells and acute brain slice. Reproduced with permission [131]. (c) Precise cell process on the microfluidic platform and gas-permeable well plate. Reproduced with permission [135].

Microfluidics is also increasingly important for bottom-up synthetic biology and artificial cells. Droplet and vesicle microfluidics provide reproducible routes for constructing compartmentalized artificial systems containing biochemical reaction networks. Generally, microfluidics has been considered as an engineering framework that imposes physical boundary conditions on molecular self-assembly, enabling more reproducible construction of cell-like systems. Directed evolution therefore provides a useful prototype for the future architecture of smart bioengineering, including computational design, biological library, microfluidic screening, functional measurement, model updating, and next-generation design [136–138]. Microfluidics thus provides an experimental interface between computational design and biological function,

enabling synthetic biology to progress from manually operated cycles towards increasingly data-driven and potentially autonomous loops.

4.3 Precision Cell Engineering and Therapeutic Manufacturing

Engineering living cells introduces additional challenges because cells are heterogeneous, dynamic, and sensitive to processing conditions. Therapeutic cell engineering may involve a sequence of operations including cell isolation, activation, intracellular delivery, genome editing, expansion, and functional selection [139,140]. Microfluidic technologies can contribute to each stage while reducing reagent consumption and improving control over the cellular environment. Hydrodynamic, inertial, acoustic, magnetic, and affinity-based microfluidic approaches can first isolate specific cell populations according to their physical or molecular properties. Microscale devices can subsequently provide tightly controlled environments for intracellular delivery. For instance, Lissandrello et al. developed a continuous-flow microfluidic electroporation system for delivering mRNA into primary human T cells [141]. The platform enabled high-throughput cell processing while maintaining functional properties relevant to therapeutic manufacturing. It was illustrated that microfluidics can be evolved from an analytical technology towards a cell-processing technology. Cell therapies tend to require harvesting, enrichment, activation, genetic modification, expansion, formulation, and quality assessment. Conventional workflows remain expensive and frequently depend on centralized manufacturing infrastructure. In contrast, microfluidic systems could miniaturize and integrate several of these operations. Wang and Kelley highlighted how fluidic technologies could contribute throughout the adoptive-cell-therapy manufacturing chain, including cell harvesting, isolation, editing, culture, and functional selection [142]. The analysis emphasized that microfluidic control could potentially improve therapeutic potency, process predictability, and manufacturing affordability. Besides, Tantikul et al. reported a high-density microfluidic bioreactor for automated CAR T-cell manufacturing (Fig. 6c) [135]. Primary human T cells were activated, transduced, and expanded within a closed microfluidic system. The platform produced more than 60 million CAR T cells from lymphoma-patient-derived samples and more than 200 million cells from healthy donors while maintaining functional therapeutic properties. These results demonstrated that microfluidic miniaturization does not simply reduce the physical size of manufacturing equipment. Small volumes facilitate rapid mass transport and more precise regulation of nutrients, metabolites and environmental conditions, creating opportunities for process intensification and tighter biological control.

4.4 From Integrated Bioprocessing to Adaptive Biomanufacturing

Conventionally, bioprocesses commonly operate according to predefined protocols and evaluate product quality at selected checkpoints or at the end of production. However, living cells continuously change their metabolic, transcriptional and, phenotypic states during manufacturing [143–146]. A fixed protocol therefore does not necessarily represent the optimal trajectory for every biological target. Microfluidics provides an opportunity to integrate processing and measurement within the same system. Optical imaging, metabolite measurements, secreted biomarkers, and cellular phenotypes could be monitored continuously or at frequent intervals. These measurements could provide real-time estimates of critical process parameters and critical quality attributes. The resulting architecture would transform biomanufacturing into a feedback process, including biological production, sensing, state estimation, process adjustment, and biological production. Unlike conventional pharmaceutical manufacturing, each patient-derived cell population can differ substantially in its initial state and subsequent response to culture conditions. Microfluidic systems

could enable process-aware manufacturing, where environmental parameters are dynamically adjusted according to the measured state of the cells.

Scaling strategies may also differ fundamentally from conventional biomanufacturing [147,148]. Rather than increasing reactor volumes through traditional scale-up, standardized microfluidic modules can potentially be operated in parallel through numbering-up or scale-out. Such an approach is particularly attractive for personalized therapies because each patient represents an independent manufacturing batch. Compact closed systems could ultimately enable decentralized manufacturing closer to hospitals or clinical centers. However, this transition will require standardized cartridges, sterile fluidic interfaces, robust sensors, and regulatory frameworks capable of validating adaptive processes. The integration of microfluidics with process analytical technologies is particularly important. Continuous measurements of cell morphology, metabolism, cytokine secretion, and differentiation state could provide substantially richer descriptions of manufacturing trajectories than conventional endpoint assays [149–151]. Taken together, this progression provides the conceptual bridge between microfluidic bioengineering and the AI-enabled systems. Once computational algorithms can interpret experimental outcomes, predict biological behaviors, and determine subsequent interventions, microfluidic platforms can progress from automated processing towards closed-loop and smart biotechnology.

5 Outlook and Perspectives

Despite substantial advances, translating microfluidics from sophisticated laboratory tools into a broadly deployable infrastructure for smart bioengineering remains challenging. A persistent bottleneck is the physical interface: pumps, tubing, valves, and peripheral instruments often compromise the compactness and operational simplicity promised by microfluidic devices, while differences in chip materials, fabrication methods, surface chemistry, and operating protocols further limit interlaboratory reproducibility. These limitations become particularly important for organ-on-chip systems, cell manufacturing, and clinical diagnostics, where long-term robustness, sterility, and standardized operation are essential. Another fundamental challenge is the scalability. Although microfluidics excels at manipulating small volumes and individual cells, many biomanufacturing processes require substantially larger production capacities; scalable numbering-up strategies must therefore preserve microscale control without introducing excessive system complexity. Besides, smart microfluidic platforms increasingly generate heterogeneous information, molecular profiles, electrochemical signals, and process parameters, yet standardized datasets describing device geometry, flow conditions, and experimental history remain underdeveloped. Biological variability further complicates computational interpretation, where the dynamic evolution of living systems cannot always be represented by deterministic engineering models or trained AI models. Addressing these challenges will require a shift from individually optimized microfluidic devices towards interoperable technological ecosystems encompassing standardized hardware, modular fluidic interfaces, reproducible biological protocols, and machine-readable data infrastructures.

The next development direction of microfluidics should therefore move beyond miniaturization and automation towards adaptive and closed-loop feedbacks. Currently, most systems and protocols are predetermined and microfluidics merely executes a sequence of operations. Future smart platforms can focus on continuously measuring biological states and dynamically modifying experimental conditions. Integrated optical, electrical, mechanical, and biochemical sensors can provide real-time representations of cell phenotype, metabolism and tissue function, while machine-learning algorithms identify patterns, estimate system states, and determine subsequent interventions. Such architectures would transform microfluidics from experimental hardware into an active platform. By active learning, informative experiments can be

prioritized maximally, integrating molecular, imaging, and process data to predict biological responses. In parallel, physical microfluidic experiments can be connected with continuously updated computational calculations, allowing *in silico* predictions to guide measurements and protocols to refine predictive models. The resulting convergence of programmable microfluidics, AI, robotics, and digital biology can provide the foundations for smart bioengineering. Future efforts should prioritize standardized data formats, rigorous validation, and hybrid models that combine physical principles with data-driven learning. Equally important are accessible platforms and interdisciplinary collaboration among engineers, biologists, and clinicians. AI-assisted microfluidics may evolve from a tool for experimental automation into an active partner in hypothesis generation, mechanistic discovery, and the translation of bioengineering innovations into practical biomedical applications. Ultimately, the most important role of microfluidics may not be making bioengineering smaller or faster, but making them programmable, measurable, adaptive, and computationally actionable. It is expected that physical microfluidic operations and computational decision-making become inseparable functional components. Reconfigurable and software-defined microfluidics allows experimental workflows to be modified without redesigning hardware, while standardized modular platforms could enable protocols and data to be transferred. Such integration providing a foundation for more efficient biological discovery, personalized therapeutics, and intelligent biomanufacturing.

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