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Lab on chip for medical and clinical applications

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Lab-on-a-chip (LoC) devices represent systems where microfluidics converges with state-of-the-art technologies, playing an immense role in reshaping clinical and biomedical sciences. This review deeply explores the design principles and diverse applications of LoC devices, ranging from point-of-care diagnostics to entire human-on-a-chip devices. Notably, LoC devices showcase remarkable adaptability and versatility. While LoC devices offer many advantages over conventional laboratory assessment methodologies including small sample size, reduced assay time and cost-effectiveness, the field faces many challenges in terms of designing, standardizing and large-scale production of the devices. In the end, while shedding light on how LoC devices stand at the forefront of the innovative technologies in the field of clinical and biomedical sciences, the review also emphasizes on their applications and integration with state-of-the-art technologies like AI and machine learning, along with their limitations and the further necessary developments for their widespread acceptance.

1. Introduction

1.1 Overview of lab-on-a-chip technology

Lab-on-a-chip (LoC) technology represents a pioneering amalgamation of fluidics, electronics, optics, and biosensors that performs various laboratory functions at a miniaturizing scale on a single chip that ranges from millimeters to a few square centimeters processing small volumes of fluids typically ranging from 100 nL to 10 μL.[1–3] By consolidating multiple laboratory processes such as sampling, sample pretreatment, chemical reactions, product separation and isolation, detection, and data analysis onto a single chip, LoC systems minimize reliance on bulky instrumentation and extensive manual intervention, thereby enhancing automation and operational efficiency.[2,4] Although laboratory techniques are very precise, due to the time-consuming analyses, trained technician demand, and large sample and reagent volume requirements leading to cost escalation, the compact LoC has gained importance in recent years.[5,6] Their miniaturized design reduces reagent and sample volumes, lowering costs while enabling high-throughput analysis. The primary advantage of these systems stems from their compactness, which offers portability, minimal consumption of samples, reagents, and power, and significantly shorter assay times.[2,4] As mentioned in Fig. 1 the concept of LoC technology

developed in a sequential manner. The development of LoC technology dates back to the 1970s, when Terry *et al.* introduced a miniaturized gas chromatography analyzer on a silicon wafer.[7,8] The concept gained prominent recognition since the conceptual work on miniaturized total chemical analysis systems (μTAS) by Manz *et al.* in 1990 while a groundbreaking advancement was accomplished by Harrison and Manz in 1993 with their pioneering discovery of on-chip capillary electrophoresis; since then the field of LoC technology has experienced intensive development in many biotechnological areas spanning from basic theoretical models and academic proof-of-concept studies to commercial applications over the subsequent decades.[2,9–11] In 1998, Whitesides and colleagues introduced soft lithography for microfluidics using PDMS, which significantly advanced the field by enabling high-fidelity replication of microscale features, optical transparency down to 280 nm, and rapid fabrication with low curing temperature and time.[12] In the early 2000s, droplet microfluidics emerged as a promising technique for studying droplets ranging in size from a few microns to several hundred microns.[13] In 2004, Shuler and co-workers applied micromachining techniques to create multi-compartment cell culture systems, where fluid flow between tissue compartments was regulated by microchannels; this "cell-on-a-chip" approach laid the foundation for organ-on-a-chip.[14] The organ-on-a-chip concept began to take form in 2005, when Huh and his team developed a foundational lung-on-a-chip design using PDMS channels above and below a porous membrane to mimic the human lung environment.[15] In 2007, the Whitesides group introduced paper-based microfluidics (μPADs), which use capillary action to move liquids, offering low-cost and

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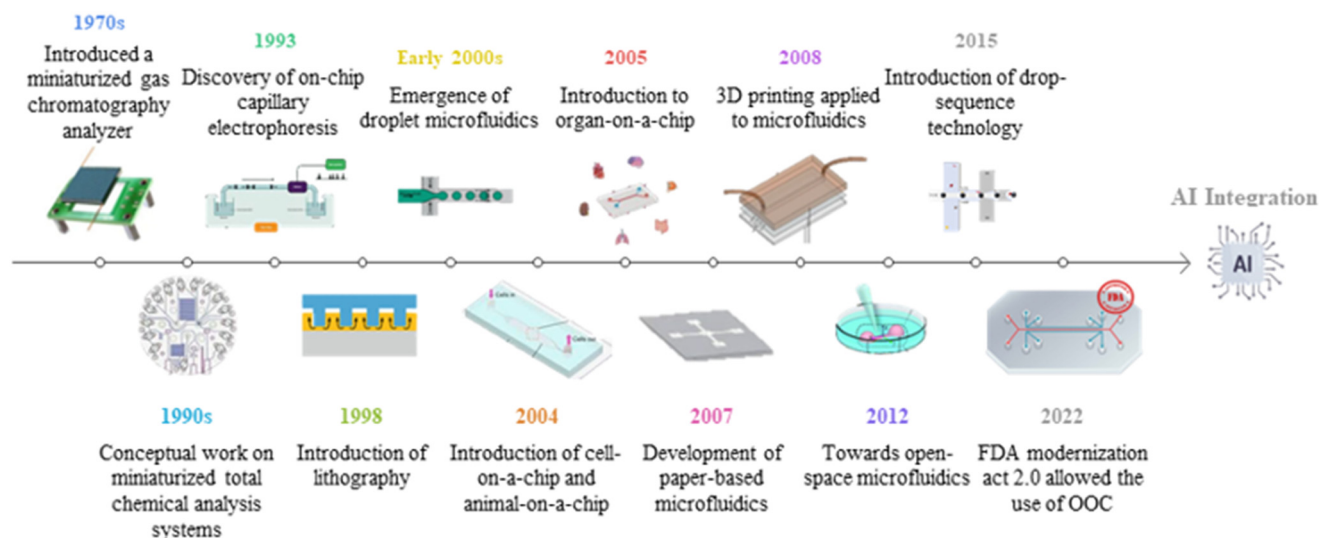
Fig. 1 Schematic illustration of key milestones in LoC technology.

portable diagnostic devices. Since then, µPADs have become a transformative tool for point-of-care diagnostics, food safety, environmental monitoring, and toxicology due to their affordability, portability, and ease of use.[16] In 2012, Kaigala and colleagues presented open-space microfluidics, a new approach that eliminates sealed channels and enables localized chemistry and analysis directly on biological surfaces.[17] In 2015, droplet-based microfluidic technologies such as Drop-seq and inDrop were introduced, facilitating high-throughput single-cell RNA sequencing by encapsulating single cells with barcoded beads in nanoliter droplets.[18] Finally, in December 2022, the FDA Modernization Act 2.0 approved the use of alternative non-animal testing methods, including organ-on-a-chip, for generating drug safety and efficacy data.[19]

Although the early focus of the development of LoC was to develop devices with applications such as DNA analysis, protein separation, and cell sorting, demonstrating the potential of miniaturized laboratory processes, in recent years their application has broadened to include personalized medicine, real-time monitoring of biological processes, and high-throughput screening for drug discovery.[4] Furthermore, in recent years LoC has advanced through integration of biosensing technologies which has enabled the convergence of chemical and biological components into a single platform. It has expanded capabilities to include portability, disposability, real-time detection, unprecedented accuracies, and simultaneous analysis of different analytes in a single device.[20] The integration of artificial intelligence (AI) and machine learning (ML) with LoC systems has enhanced diagnostic accuracy and reliability enabling predictive analytics for disease outbreaks, treatment responses, and complications, while automating workflows from sample handling to data interpretation to reduce human intervention and error.[4]

This tutorial review provides a comprehensive overview of LoC technology—from its developmental origins and engineering principles to its current applications in biomedical research and diagnostics—while highlighting the transformative role of AI integration in expanding performance, accessibility, and clinical relevance. By tracing the trajectory of LoC innovation and discussing future directions, we aim to offer both newcomers and experienced practitioners a consolidated understanding of the field's technological, analytical, and translational landscape.

2. Fundamentals of lab-on-a-technology

2.1 Basics of microfluidics

As briefly described above, LoC systems operate based on microfluidics technology, which is based on the science and engineering of manipulating and processing small volumes of fluids in microchannels measuring between 1 and 1000 micrometers.[2,3,21,22] Fluid flow behaves significantly differently at micro and macro scale levels. Micro scale flows are typically smooth and are commonly referred to as laminar flow. Parameters that are often ignored in macro flows, such as surface force, shear force, diffusion, air dampening, and viscosity, become crucial in micro scale flows.[6,21,23]

In microfluidics, gravitational forces take a backseat whereas surface tension and capillary forces play a dominant role. Microfluidic devices are governed by laminar flow, with surface areas becoming more prominent than volume. Transporting fluid into microchannels involves various methods, with pressure-driven flow and electro-osmotic flow standing out as two crucial approaches.[6] To translate these principles into practical devices, microfluidics relies on a set of integrated components, each fulfilling a distinct role.

3. Microfluidic platforms and components

Fluidic platforms and components of LoC devices are the basic building blocks of these devices.[24] Within a well-defined and cost-effective fabrication technology, the microfluidic platform offers a range of fluidic unit operations that are designed for easy combination. Such unit operations include transport, metering, valving, mixing, and separation as well as amplification of particles. Additionally, these operations involve reagent storage and incubation. These platforms enable the implementation of various application-specific systems including miniaturization, integration, automation, and parallelization of biochemical assays in a convenient and adaptable manner, based on the same fabrication technology.[25,26]

3.1 Microfluidic platforms

The LoC devices are intended to perform very specific functions, hence while selecting materials for the microfluidic platform special attention should be paid to device application, required function, and the degree of integration. The material selection not only influences the intrinsic properties of the devices and the methods of fabrication, but also influences the choice of components.[2,6,24] While selecting these materials in addition to cost, other factors like flexibility, air permeability, electric conductivity, solvent compatibility, optical transparency, and biocompatibility of the material should be carefully considered.[2] Common materials for microfluidic platforms include the following (Table 1).

3.1.1 Silicon and glass. As indicated by Staicu C. E. *et al.*, silicon and glass have emerged as the initial materials used to develop microfluidic platforms.[27] They share certain favorable properties such as chemical resistance, thermostability and solvent compatibility which are crucial for microfluidics. Additionally, glass offers distinctive advantages like being a poor conductor of electricity and inert to various substances. It exhibits good optical transparency and can be easily processed by chemical or physical methods.[2,6,24,27] Glass can also be bonded with polymeric materials like polydimethylsiloxane (PDMS) to form complex 3D structures.[27] However, high fabrication cost and high toughness are their major pitfalls.[6,24]

3.1.2 Polymeric platforms. Polymers have become an intriguing substitute for microfluidics, mainly owing to their cost-effectiveness, capacity for mass production, and adaptability to formulation changes and chemical modification. Based on their physical properties, polymers can be categorized into three groups namely thermosets, elastomers, and thermoplastics.[2] Commonly used polymers in microfluidics include polydimethylsiloxane (PDMS), polymethylmethacrylate (PMMA), fluoropolymers, cyclo-olefin polymers, copolymers (COPs/COCs), and thiolene polymers (TEs).[24,28]

Out of the abovementioned polymers, PDMS, a silicon-based elastomer has gained special attention due to its simple fabrication requirements and compatible characteristics such as chemical, physical, and biological resistance. In addition to being cost effective, PDMS possesses some noteworthy properties such as biocompatibility, transparency, high elasticity, low surface tension, gas and water permeability, rapid fabrication, and ease of implementation. Despite the numerous advantages, PDMS does have certain drawbacks like low pressure resistance and a high absorption capacity, necessitating the need for surface modification.[2,6,24,27]

3.1.3 Epoxy resin. Epoxy resin, which is commonly used in molecular diagnostics such as DNA amplification through point of care technology (POCT), is an oligomer with a three-dimensional crosslinked network. Its increasing prominence in microfluidics is attributed to its vital characteristics including high Young's modulus, hydrophobic nature, high optical transparency, remarkable biocompatibility, resistance to chemicals, thermostability, and toughness along with simple and rapid fabrication requirements.[28]

3.1.4 Paper. Following the introduction of paper, a cellulose-based material, to microfluidics by Martinenz *et al.*, it gained rapid interest due to its natural abundance and renewability making it a cost-effective and biodegradable solution for microfluidic platforms with added benefits of recyclability and easy disposal. Its flexibility, low thickness, light weight, and thermal stability further enhance its suitability in this field. Being chemically and biologically inert, it is also easy to sterilize. The porous and hydrophilic nature of paper enables aqueous transport, filtering, and separation, while its permeability to gases and liquids extends its functional versatility. Moreover, it can be chemically modified and conjugated with biomolecules such as peptides and nucleotides. Another factor contributing to its rapid acceptance is its alignment with the WHO's ASSURED criteria for diagnostics, which emphasize affordability, sensitivity, specificity, user-friendliness, rapidity and robustness, equipment-free operation, and deliverability to end-users.[2,24,29,30]

In addition to the most used materials, various metals, metal-thin films, and hydrogels are trying to pave their path in the realm of microfluidics.[6]

3.1.5 Hybrid platforms. Hybrid microfluidic systems that are composed of multiple different types of substrates have been recognized as a versatile and superior platform, which can draw benefits from different substrates while avoiding their limitations.[40]

3.1.5.1 PDMS–COC hybrid platform. Hybrid platforms combining cyclic olefin copolymer (COC) and PDMS leverage the complementary properties of both materials. COC offers optical transparency and biocompatibility essential for imaging, while PDMS enables better gas permeability to perfusion culture. Such COC–PDMS devices have been applied in diverse microfluidic applications including lipidic mesophase screening, cell analysis, microfluidic mixing, and

Table 1 Comparing LoC materials in terms of advantages and disadvantages along with their application

Material	Pros	Cons	Diagnostic application
Silicon	Well-characterized for surface modifications *via* silanol (–Si) groups. Chemically inert and offers high design flexibility[31]	High production cost and optical opacity in the visible range, limiting imaging and fluorescence-based detection in biological assays[27,32]	Utilized in nucleic acid detection through integrated PCR and hybridization microarrays[34]
		High elasticity complicates fabrication of reliable valves and pumping components[32] Being electrically conductive, it may interfere with electro-osmotic pumping[33]	Applied in organ-on-chip platforms for drug toxicity assessment[31]
Glass	Exhibits low nonspecific adsorption and background fluorescence. Highly compatible with biological samples. Cost-effective, user-friendly, thermally stable (>1400 °C), chemically resistant, and optically transparent[27,32]	Requires high bonding temperatures and voltages, which present challenges during device manufacturing[33]	Applied in point-of-care diagnostics, cell-based assays, nucleic acid analysis, drug delivery studies, and immunoassay/-molecular biology platforms[32]
Polymer (PDMS)	Non-toxic, biocompatible, optically transparent, gas-permeable, flexible, and lightweight. Easy room-temperature bonding with low fabrication cost. Enables cell culture on microchannel surfaces and allows fabrication of intricate structures at both micro- and macro-scales[27,35,36]	Hydrophobic nature, absorption of hydrophobic analytes, scalability issues in mass production, and attenuation of acoustic waves[27] Not ideal for high-pressure or chemically intensive, long-duration experiments[37]	Widely used in organ-on-chip models for studying drug interactions, disease mechanisms, and real-time cellular responses. Applied in development of blood flow models[36]
Epoxy resin	A thermosetting polymer with excellent biocompatibility, mechanical strength, chemical resistance, thermal stability, and toughness. Supports fabrication of microfluidic devices quickly and economically without cleanroom facilities.[28] Highly scalable, compatible with soft and photolithography, and reliable for extended experiments[37]	Direct 3D printing is challenging due to long curing times[38]	Applied in DNA amplification and point-of-care diagnostic chips[28]
Paper	Intrinsic porosity and microstructure enable capillary-driven flow, making it highly suitable for microfluidics. Mechanical and conductive properties can be tuned *via* solvent treatments. Natural white color aids in colorimetric assays. Environmentally friendly—easy to recycle and dispose[32]	Lacks optical transparency, unsuitable for absorbance spectroscopy, and incompatible with open-channel applications. Sample recovery is difficult due to adsorption onto fibers. Natural variability introduces measurement errors. Enzyme and reagent stability is often poor on paper substrates[39]	Employed for detecting infectious and non-infectious diseases, monitoring biomarker levels for health assessment, and identifying antibiotic resistance[39]

microphysiological systems such as aorta-on-a-chip and liver-on-a-chip models. In recent work, a hybrid COC–PDMS microfluidic platform (HCP) was used to culture Huh7 hepatoma cells in a perfusion environment, demonstrating its suitability for proof-of-concept experiments in liver-on-a-chip development, particularly when using immortalized cell lines for reproducibility.[41]

3.1.5.2 Paper–polymer hybrid platform. Paper/polymer hybrid microfluidic devices have emerged as low-cost platforms for biological and biomedical applications, combining the ubiquity, recyclability, and 3D porous structure of paper with the durability of polymers. Paper offers inherent capillary-driven flow without external pumps, compatibility with various biological samples, ease of functionalization for biomolecule binding, and visual contrast for colorimetric assays. Despite challenges such as nonspecific adsorption, evaporation, and limitations in optical detection, integrating paper into polymeric regions enhances performance and stability (Table 2).[40]

3.1.5.3 Emerging unconventional hybrid platforms in LoC devices. In addition to the commonly used and well-established hybrid platforms, recent innovations have explored unconventional material combinations such as paper/tape, paper/glass microcapillary, and thread-based systems, offering new avenues for low-cost, integrated diagnostics. Paper/tape devices enable simple, reconfigurable 3D structures for rapid nucleic acid extraction, amplification, and detection, as demonstrated by an HPV DNA assay completed in under an hour. Paper/glass microcapillary systems integrate nucleic acid capture, purification, and LAMP detection with minimal user intervention, while thread-based hybrids leverage the wicking properties of cotton or polyester threads to enhance sample delivery and enable sensitive or semi-quantitative assays for biomarkers such as CEA, ferritin, BSA, and nitrite. These emerging platforms, though less conventional, highlight the growing diversity of material strategies in LoC development and their potential to expand accessibility in resource-limited settings.[40]

In the above section we learnt about the types of microfluidic platforms based on the materials; now let's dive deep into the classification of platforms according to the main liquid propulsion principle. Based on main liquid

Table 2 Paper/polymer hybrid microfluidic platforms and their biomedical applications

Application	Platform type	Key details	Performance metrics
DNA extraction & PCR integration[42]	Paper/photopolymer resin	One-step DNA extraction from diverse biological samples	LOD: 10^4 copies per mL (HBV nucleic acids), <2 min extraction
	Filter paper/PDMS/PMMA[43]	DNA extraction + PCR amplification from human whole blood	DNA yield: 5.6–21.8 ng (0.25–1 μL blood), <7 min
	Chitosan-modified filter paper[44]	Enhanced DNA capture efficiency	98% (K562 human DNA), 95% (bacteriophage DNA), >30× enrichment of phage DNA
Nucleic acid extraction & LAMP integration	Polysulfone membrane/PMMA[45]	Plasma separation for nucleic acid amplification	275 ± 33.5 μL plasma from 1.8 mL blood in 7 min
	Paper/PDMS[40]	Instrument-free diagnosis of bacterial meningitis and whooping cough-causing bacterium, B. pertussis	LOD: 5 copies per LAMP zone (*B. pertussis*), 45 min
	Paper/PDMS/glass[46]	Multiplexed foodborne pathogen detection[46]	LOD: 21.5 (S. aureus) & 20.9 (V. parahaemolyticus) copies per μL; 100-fold PCR sensitivity[47]
DNA hybridization[40]	Paper/PMMA SpinChip	ssDNA probe-functionalized GO nanosensors for multiplex LAMP	LOD: 6 (N. meningitidis), 12 (S. pneumoniae) DNA copies per assay
Protein analysis – LFAs[40]	Paper/polymer with electro-wetting valves[48]	Colorimetric detection of T7 bacteriophage	LOD: 10^8 PFU mL^{-1}, 40 min
	Paper/plastic Nitrocellulose/PDMS	Sandwich LFA for dengue NS1 antigen Immunoassays without pumps, cavity-induced microstreaming	LOD: 84.66 ng mL^{-1} 18 min assay, smartphone app
	Paper/plastic	Smartphone-based urine analyte detection	Glucose, protein, pH, RBC
Protein analysis – ELISA[40]	Paper/PMMA (56 microwell)	Rapid biomarker detection *via* office scanner	Comparable to commercial ELISA, <1 h
	Paper/plastic	Multiplex detection of protein biomarkers, viruses, nucleic acids	—
	PMMA/paper PnP device	High-sensitivity immunoassays, analyte enrichment	10× sensitivity increase
Whole-cell detection[40]	Paper/PDMS/glass	Aptamer-functionalized GO biosensor for foodborne pathogens	LOD: 61 CFU mL^{-1} & 800 CFU mL^{-1}, 10 min
	Paper/PDMS	AST of uropathogens, species-specific enzymes, chromogenic medium	83.3–100% agreement with conventional
3D cell culture[40]	Paper/glass	3D culture with impedimetric quantification of cancer cells	Up to 3 day non-invasive measurement
	Paper/PMMA	Wax-printed paper microreactors with diffusion channels	Studied cell proliferation, EGFR phosphorylation
	Paper/PDMS/PMMA	3D culture under chemical gradients	HeLa cell viability & protein expression profiling

propulsion principles, the microfluidic platforms are divided into five types namely, capillary flow platform, pressure driven platform, centrifugal platform, electrokinetic platform, and acoustic platform.[24–26]

3.1.6 Capillary flow platform. As the name suggests, these platforms operate on the principle of capillary flow, where liquid movement is regulated by the wettability and feature size of the porous or microstructured substrate. All required reagents are preloaded onto the strip during fabrication. The readout is typically done optically, often manifesting as a color change that can be seen by the naked eye. Cellulose is the preferred material in the case of capillary flow platforms. The potential for automated on-site measurement using an inexpensive, small disposable test strip along with energy-free simple actuation makes this platform highly promising for POCT and patient self-testing applications. The capillary flow platform faces numerous challenges including difficulty in flow control and difficult mixing within the predominantly laminar flow regimes inside the porous structures.[18,19,24]

3.1.7 Pressure-driven platform. In pressure-driven platforms, fluid flow is propelled by mechanical forces, typically generated by active components like pumps and valves. Valves regulate the flow of the fluid, while pumps are responsible for creating the fluid flow. These platforms are particularly advantageous for applications where the reaction to be conducted is not yet fully characterized and the assay parameters are not completely fixed yet. However, the integration of the valves and pumps into the platform adds complexity and cost to the system.[24] As described in Table 3 the pressure-driven platform category encompasses five distinct types of platforms.[26]

3.1.8 Centrifugal platform. Centrifugal platforms use centrifugal forces as the primary driving factor along with addition forces such as Euler force, Coriolis force, and capillary force that contribute to liquid transportation. Here, the microfluidic systems are designed onto a disk-like structure featuring a central orifice for mounting onto a rotating axis.[24,26]

The platform comprises three essential components: a plastic disposable rotor, a cartridge, and an analyzer

Table 3 Types of pressure-driven platforms along with their characteristics and applications, adapted from Daniel Mark 2009 (ref. 26)

Platform	Characteristics	Applications
Linear actuated devices	• Liquid flow is controlled by mechanical displacement of liquid by means of a plunger • Liquid control is confined to a linear, one-dimensional liquid flow • Usually, liquid calibrants and reaction buffers are prestored in pouches	It is used in POCT to determine blood parameters. Additionally, it's used in a lab-in-a-tube analyzer for amplification-based nucleic acid tests
Pressure driven laminar flow	• Liquid transport relies on pressure gradient, resulting in hydrodynamically stable laminar flow • Pressure is applied through diverse internal or external sources like syringes, pumps, gas expansion, pneumatic displacement of membranes, *etc.* • Samples and reagents are introduced onto chip inlets either batch-wise or in a continuous mode	Phase transfer magnetophoresis technology operates through pressure driven laminar flow and is used for continuous DNA extraction. Additionally, it finds its application in the integrated detection of bacteria, DNA purification, PCR, and fluorescence readouts
Microfluidic large-scale integration	• This microfluidic channel circuitry incorporates chip-integrated microvalves, utilizing flexible membranes positioned between a liquid-guiding layer and a pneumatic control-channel layer • The status of microvalves, whether closed or open, is determined by the pneumatic pressure applied to the control-channels • By combining multiple microvalves, more complex units like micropumps, mixers, multiplexers, *etc.* can be assembled with hundreds of units on one single chip	These platforms find application in LoC designed for nucleic acid extraction from minimal cell volume. Furthermore, these platforms are utilized in tasks like protein crystallization, immunoassays, automated cell culturing, DNA synthesis
Segmented flow microfluidics	• This platform operates based on multiphase fluid flow through microchannels • Dispersed liquid phase droplets are immersed in the second continuous phase (usually gas or liquid) forming micro-confinements ranging from a few picolitres to microliters • These droplets can be transported through pressure gradient or can be merged, split, sorted, or processed without any dispersion in the microfluidic channels	These platforms find diverse applications including single cell analysis, single organism analysis, DNA analysis, drug screening, protein crystallization, and chemical synthesis

instrument. The rotor is responsible for specimen processing, the cartridge contains preloaded dried reagents, and the analyzer assists actuation and readout.[25] The channel arrangement allows modulation of microfluidic flow by adjusting the rotation speed of the axis, which allows controlled movement of the fluids without the need for actively controllable components such as pumps and valves. As the rotating disks are difficult to bring into contact with each other mechanically and active components are difficult to set up, the centrifugal platforms are operated with the help of siphon valves, capillary valves, or hydrophobic barrier valves. These valves are triggered by modulating the rotation speed.[24]

Centrifugal microfluidic platforms are applicable in diverse fields including integrated plasma separation, DNA extraction, protein-based and nucleic acid-based assays, clinical chemistry assays, chromatography, and protein crystallization. The modular set up and the disposable and easily exchangeable cartridges are the two major advantages of the centrifugal platforms. However, challenges arise from the need for additional actuation or sensing function during rotation, limited flexibility, and the absence of completely portable systems. These drawbacks restrict the widespread use of this platform.[26]

3.1.9 Electrokinetic platform. Electrokinetic platforms include two types of microfluidic platforms, electrokinetic and electrowetting.

3.1.9.1 Electrokinetic platform. Use of electrokinetic platforms stands as one of the earliest LoC technologies. They work on the fundamental principle that individual components within a sample will move in an electric field as per their charge-to-mass ratio, thereby defining their respective electrokinetic mobility.[24] Fluid propulsion on the electrokinetic platform relies on the movement of the liquid layer right at the interface to the solid phase.[25] In addition to the movement of charged particles through externally applied electric fields, small capillaries such as microfluidic channels also exert a secondary effect on the bulk liquid causing lateral movement known as electroosmotic flow (EOF) and it can be used to separate different types of molecules or particles within the bulk liquid.[24] Depending on buffers and/or sample involved, several electrokinetic effects such as electroosmosis, electrophoresis, dielectrophoresis, and polarization superimpose each other.[26] Silicon or glass is commonly preferred as the material for these platforms. These platforms are typically employed in the analysis of DNA and proteins.[25] Among these electrokinetic effects, dielectrophoresis (DEP) has gained particular prominence due to its versatility and unique ability to manipulate particles regardless of their net charge.

Dielectrophoresis (DEP) is a microfluidic technique that separates dielectric particles by applying a non-uniform electric field (NUEF), inducing polarization forces. Unlike electrophoresis, it does not require particles to be charged.

Its key advantages include being label-free, fast, accurate, efficient, and easily integrated into microfluidic systems, making it valuable for bio-molecular diagnostics and cell separation. However, challenges remain in achieving higher levels of system integration, optimizing efficiency (especially regarding Joule heating in DC-iDEP), and simplifying the fabrication of complex 3D electrode structures. Despite these drawbacks, DEP is widely applied for tasks such as cell separation, plasma separation, and bacteria isolation.[49]

These platforms present two primary benefits: pulse-free pumping without any moving part thanks to the electroosmotic actuation of liquid flows, and prevention of dispersion in EOF flow, thus averting the broadening of the sample plugs during chromatographic separation. However, the drawbacks include the necessity for high-performance detection technologies due to reduced volumes and technical challenges arising from the development of pH-gradients over time or during operation.[25]

3.1.9.2 Electrowetting. The electrowetting effect was first described by Lippmann in 1875. Here, the droplets are immersed in a second immiscible continuous phase, either gas or liquid, as stable microconfinements. These droplets rest on a hydrophobic surface featuring a one- or two-dimensional array of individually addressable electrodes. Application of voltage between the electrode and the liquid droplet induces the increased wettability of a solid surface through polarization and electric fields. This phenomenon, known as "electrowetting-on-dielectric" (EWOD), serves as a tool to control the contact angle of liquids on surfaces. By adjusting voltages between adjacent electrodes, droplets can be generated, transported, split, merged, and processed. These unit operations are freely programmable for each individual droplet allowing real-time control of assay. The electrowetting technique finds application in enzymatic assays crucial for diagnostic purposes and glucose concentration measurement. Additionally, these systems also play a role in automated sample preparation of peptides and proteins intended for matrix-assisted laser desorption/ionization mass spectroscopy.[25,26]

The foremost advantage of the EWOD technique lies in its significant potential to manipulate numerous single droplets in parallel. Notably, this platform excels in handling nanoliter liquid volumes with high precision and offers flexibility in programming the droplet movement. Despite its low sample and reagent requirements, the development of portable systems has been hindered by the bulky electronic instrumentation requirements to operate the platform. Moreover, variation in the wetting abilities of different patient materials influences droplet transport behavior, resulting in differences in volume or movement speed.[26]

3.1.10 Acoustic platform (surface acoustic waves). The acoustic platform microfluidic unit operations are controlled by surface acoustics waves (SAWs). These waves have an amplitude of only a few nanometers. These waves are generated by a piezoelectric transducer chip like quartz fabricated by placing interdigital electrodes on top of a piezoelectric layer. The surface acoustic wave platform uses droplets residing on a hydrophobic surface in a gaseous environment (air). These droplets are moved by the stream on the solid–liquid interface induced by the surface acoustic waves. Most of the unit operations such as droplet generation, transport, mixing, *etc.* are freely programmable and hence it offers high flexibility.[26]

3.1.11 Microfiltration platform. The microfiltration platform is a cross-flow filtration microdevice, composed of two PDMS microchannel layers separated by a porous PCTE membrane, designed for the continuous, real-time extraction of cell-free blood plasma from whole blood. This device is particularly applicable in clinical settings for continuously monitoring a patient's inflammatory response during cardiac surgeries involving cardiopulmonary bypass (CPB) procedures, as it allows for the extraction of diagnostic plasma proteins (*e.g.*, complements and cytokines) using significantly smaller blood volumes compared to traditional methods. The platform demonstrates high protein recovery (over 80%), no hemolysis, and low biofouling, ensuring effective and reliable performance for future clinical applications, especially when pre-coated with anticoagulants like heparin to maintain filtration efficiency. Its robust design and ability to provide a continuous stream of cell-free plasma make it suitable for integration with microimmunoassay systems for point-of-care diagnostics and real-time inflammation tracking during surgery.[50]

Recently in a study conducted by Javanifar *et al.* in 2025, they introduced a green microfiltration approach within a LoC format designed to filter *Escherichia coli* (*E. coli*) by integrating positively charged electrospun polyacrylonitrile (PAN) fibers into a microfluidic chip. In this context, the microfiltration technique involves using the charged PAN nanofibers as a functional filter medium to selectively capture or retain *E. coli* cells as fluid flows through the microstructured channels of the LoC system.[51]

4. Fabrication

The fabrication of LoC devices typically refers to manufacturing and device assembly prior to its use. There is a plethora of fabrication methods available, and the selection of the fabrication method is influenced by factors like functional needs and platform materials as shown in Table 4.[52]

4.1 Molding

Molding is widely used for producing disposable microfluidic devices. Molding-based fabrication methods are divided into three types, replica molding, injection molding, and hot embossing. Replica molding, which is a soft lithography technique that uses PDMS cast over patterned silicon molds bonded to polymeric or glass substrates, offers low-cost, rapid prototyping, but is limited to aqueous applications due to poor solvent compatibility. Injection molding enables high

Table 4 Fabrication methods for various platforms in LoC, adapted from G. D. Kipling et al.[52]

Material	Fabrication method
Glass	Photolithography, chemical etching, micromachining, powder blasting, water jet cutting
Rigid polymer	Micromachining, injection molding, hot embossing, 3D printing
Soft polymer (PDMS)	Photolithography, injection molding, hot embossing, 3D printing[53]
Paper	Wax patterning, alkyl ketene dimer printing, flexographic printing, shaping/cutting

throughput, reproducible production of thermoplastic devices by injecting molten polymer into heated molds followed by cooling below the glass transition temperature before demolding. While it supports mass production with excellent reproducibility, it is restricted to thermoplastics due to involved high-cost.[54]

Hot embossing, which imprints using heat and pressure, provides accurate replication across various thermoplastics but is time-consuming and demands expensive mold fabrication.[54]

4.2 3D printing

3D printing is a layer-by-layer manufacturing technology that uses a 3D digital model which can be interpreted and created by the printer. This approach offers rapid prototyping for microfluidics. Among its methods, fused deposition modeling (FDM) is a cost-effective, compact, fast, and easy to operate fabrication method that supports various biocompatible thermoplastics such as ABS, PLA, polycarbonate, polyamide, polystyrene, and nanocomposites. FDM is used to create PDMS casting molds and has been applied in fabricating capillary valves for centrifugal microfluidic discs, reusable reactionware for chemical synthesis, and microfluidic immunosensors. Stereolithography (SL) uses UV or structured light to polymerize resin layer by layer. It enables rapid fabrication of fine features, though biocompatible resins essential for microfluidics. SL has been applied to create micromixers, cell separation chips, gradient generators, microneedles, GelMa cell-encapsulation structures, and active components like pumps and valves. Multi Jet Modeling (MJM) ejects light-cured photosensitive resin droplets offering high accuracy and multi-material capability for drug transport and cell viability studies, multi-material microfluidic valves with elastic membranes, and non-planar PDMS channels, but at high cost. Two-photon polymerization (2PP) uses a near-infrared laser for nanometer-sized feature fabrication supporting applications such as micromixers, parallel cell counters, and microlens arrays inside a glass substrate though it is slow and costly.[55]

4.3 Nanofabrication

Nanofabrication enables nanoscale features in microfluidics through top-down and bottom-up approaches. Top-down techniques include extreme ultraviolet lithography (13 nm light) for nanopillars, electron beam lithography for sub-10 nm nanochannels, biosensors, and tissue engineering (high precision but slow and costly), and nanoimprint lithography

for low-cost, high-throughput multiscale channels with roll-to-roll potential. Bottom-up methods like anodic aluminum oxidation create uniform porous hexagonal cells (4–200 nm) and ultrathin membranes for cell biology and diagnostics, offering rapid, low-cost production.[55]

5. Simulation

Fundamentally, LoC consists of a system of wells and channels etched onto platforms, coupled with nanostructured, biofunctionalized surfaces targeted towards analysis.[56] Microfluidic channels are pivotal to LoC systems, and the development of LoC particularly depends on the presence and correct functionality of microfluidic channels and other liquid handling components. Given the high cost involved in the production of LoC systems, it's imperative to validate the design before fabrication to prevent unnecessary repeated fabrication.[57] Additionally, understanding the analytical processes during chip operation, along with the chip layout and manufacturing process, is essential to evaluate design trade-offs and constraints. As shown in Fig. 2, Pfeiffer et al. developed a system-level LoC simulator to account the complexities. They combined Kirchoffian network analysis and topological sorting from electrical circuit simulation with the sequential-modular structure of process flowsheet simulation. It employed fast, accurate physiochemical models and allows simulation of complex designs in only seconds.[58] By elucidating the functioning of microfluidic systems, simulation enables theoretical optimization of design, potentially saving time and cost. Simulation is particularly valuable when microfluidic channels are expected to serve additional functions beyond transport, offering insight into their anticipated performance before embarking on expensive and time-consuming production. In essence, simulation is an inexpensive means to ensure whether an LoC will exhibit the envisioned behavior.[57]

The myriad of available simulation software programs available in the market with diverse functionalities need a thoughtful approach before embarking on the simulation process. Setting up a simulation involves a thorough assessment of the problem, identifying the need for simulation, understanding the reason behind its application, and defining the expected results. It helps in making decisions about the choice of simulation software to employ. The currently available simulation software options on the market include finite element analysis (FEA) software, COMSOL Multiphysics, Coventor, ANSYS (fluent), CFD-ACE+,
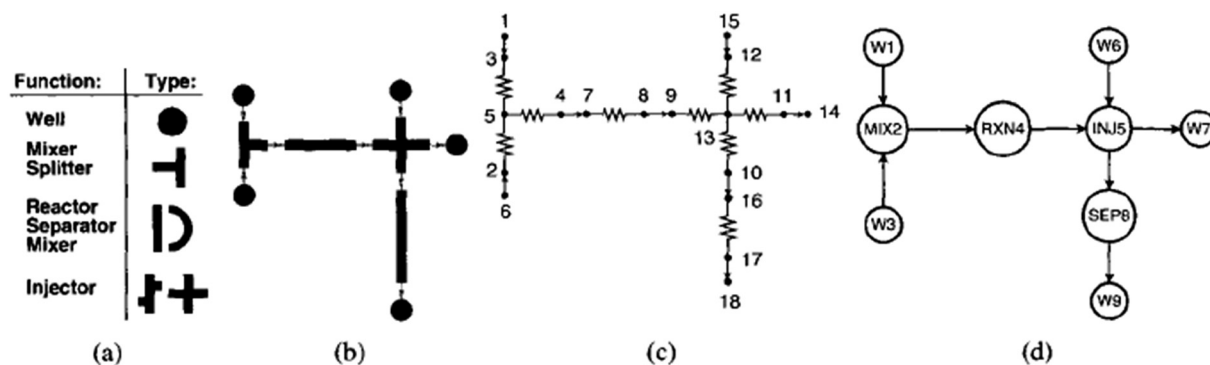
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Fig. 2 The LoC simulation process: a) partial library of LoC units, b) channel topology constructed from the library of unit, c) resistor network representation of the channel topology, and d) directed acyclic graph representation of the channel topology. Reproduced from ref. 58 with permission from Elsevier, copyright 2006.

and Flow 3D; each software is crafted to fulfill distinct roles.[57]

6. Components of lab on chip devices

As discussed earlier, the selection of platforms for LoC systems depends on the intended use of the device. The choice of platform influences the selection of functional building blocks of the LoC system.

Lab-on-a-chip systems are designed to execute a multitude of standard laboratory functions, which include crude sample handling, sample and reagent mixing and reacting, separation, and subsequently detecting analytes. To implement these functions, different standard functional components can be incorporated onto a single chip. As illustrated in Fig. 3, LoC components are classified into eight major groups as follows namely injector, preparator,

transporter, mixer, reactor, separator, detector, controller, and power supply. It also summarizes the function of each component of LoC systems.[59]

As highlighted by Lim *et al.* (2010), following the integration of the core functional components in a LoC system, particular attention must be given to the detector, since it ultimately defines the device's analytical performance. The selection of an appropriate detection mechanism influences not only sensitivity and specificity but also the overall design, integration approach, and applicability of the system, warranting a detailed comparison of the main available methods.[59]

Working principles of detection mechanisms

LoC detectors operate by employing transducers that acquire a physical signal from the analyte and convert it into an
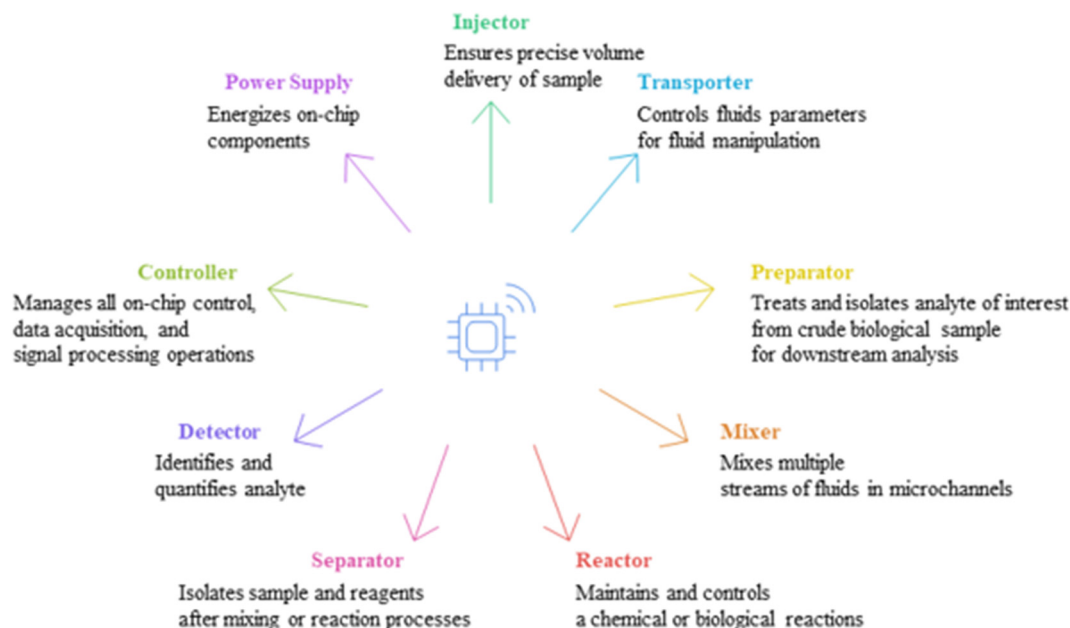

Fig. 3 Overview of LoC components and their functions.[59]

Table 5 Comparison of detection mechanisms in LoC systems

Detection mechanism	Optical detection[60]	Electrochemical detection[61]
Advantages	High sensitivity and specificity, often capable of single-cell analysis	High sensitivity even in small sample volumes due to high surface-to-volume ratio of miniaturized electrodes
	Wide range of label-free and labeled methods (e.g., fluorescence, SPR, interferometry) allowing flexible assay design	Portable, low-cost instrumentation possible (e.g., screen-printed electrodes)
	Enables real-time detection. Low cost and small form factors are the other advantages	Suitable for on-site applications
Limitations	Demand for complex laser and optical systems makes miniaturization and integration challenging	Performance strongly dependent on electrode material, type of modification, and geometry
	Optical interference (e.g., scattering, absorption) from complex samples can reduce performance	
Applications	Biomolecular detection, medical diagnostics, food and environmental monitoring, early detection of cancer	Glucose monitoring (enzymatic and non-enzymatic sensors)
		Detection of pathogens and nucleic acids via electrochemical immunosensors and aptasensors

electrical signal for analysis. Optical detection, which is the most prevalent detection method in LoC, uses light–matter interactions, such as fluorescence emission from dye-labeled targets, absorption changes in a label-free environment, or refractive index shifts in micro-ring resonators.[60] On the other hand, electrochemical biosensors transduce biological element-target detection events into detectable electrochemical signals. In biosensing measurements, the inherent electrochemical properties of the biological system are used to find access to valuable information.[61] Mass spectrometry identifies compounds by determining the mass-to-charge ratio of ionized analytes and is widely used in proteomics analysis.[60] Table 5 explains the advantages and limitations of two primarily used detection mechanisms i.e., optical detection and electrochemical detection, in LoC systems.

7. Advantages and disadvantages of lab on chip technology

While LoC offers a spectrum of advantages over the conventional laboratory techniques, it has faced a myriad of challenges over the developmental years. The challenges include issues such as surface roughness, the influence of capillary forces, chemical interaction between materials, and the necessity for temperature monitoring and control, which subsequently result in experimental complications.[6,62] Furthermore, additional challenges associated with LoC include the application of devices on a large scale, working with substantial volumes, fabrication processes at reduced cost, enhancing the user-friendliness of the devices and their integration with analytical techniques.[63]

7.1 Challenges beyond technological innovation

Bringing the microfluidic LoC devices into a hospital setting or to commercialization presents a complex set of challenges that extend beyond technological innovations. Before the devices enter the market, they face a multitude of hurdles including regulatory issues, commercialization and clinical

adoption. Moreover, seamless integration into the existing clinical laboratory infrastructure demands consideration of pre- and post-analytical workflows—key elements of the total testing process (TTP) that ensure accuracy and reliability.[64]

7.1.1 Device validation and standardization. The clinical translation of LoC platforms—such as shear-based microfluidic devices for platelet function testing and organ-on-a-chip (OoC) systems—remains constrained by challenges in reproducibility, cross-laboratory comparability, and regulatory acceptance. These limitations stem from variability in assay design, material properties, surface treatments, sample handling, hemodynamic settings, and analytical readouts, as well as inconsistent reporting practices.[65,66] In the case of OoC systems, additional diversity in parameters such as cell origin and type, material mechanical properties, biochemical stimuli, and chip design —while driving technological advancement—also impedes the establishment of standardized industrial models, delaying widespread implementation.[67] Addressing these gaps requires rigorous standardization and validation across the total testing process (TTP), encompassing pre-analytical, analytical, and post-analytical phases, with equal emphasis on robust sample processing and assay performance.[64] Standardization efforts should unify device design parameters, harmonize protocols for material testing, surface preparation, and flow control, and adopt common performance benchmarks using reference samples and multi-site reproducibility studies.[65,66] For OoC devices, this includes adherence to good manufacturing practices (GMP) and good laboratory practices (GLP) to enable data comparison across studies, ensure reproducibility of tissue functionality validation in vitro, and facilitate regulatory approval. When OoC platforms model barrier tissues, validation of junction integrity—such as by quantifying junctional protein expression—can be critical to assessing model viability.[67] Integration of Good In Vitro Method Practices (GIVIMP), Good Cell Culture Practice (GCCP), and harmonized reporting frameworks will further strengthen traceability, facilitate regulatory review, and build end-user confidence.[65,66] Moreover, OoC technologies that meet the

U.S. FDA's Drug Development Tools (DDT) "fit-for-purpose" qualification, currently a voluntary program, can provide results accepted by regulatory authorities without the need for additional supporting data, thereby promoting their use in drug discovery and development.[67] Coordinated action between Standards Development Organisations (ISO, ASTM, CEN-CENELEC) and multi-sector stakeholders, alongside adherence to GMP/ISO quality systems, remains essential to ensure these microfluidic technologies are precise, dependable, and seamlessly integrated into clinical laboratory and biomedical research workflows.[64–66]

7.1.2 Sample handling in clinical settings. Sample handling in LoC devices presents significant challenges in clinical settings due to the complexity of biological samples, patient-to-patient variability, and the need for precise manipulation.[64] For RNA viruses such as SARS-CoV-2, Ebola, or hepatitis, preparation must include reverse transcription to generate DNA before amplification, adding substantial complexity.[68] In molecular diagnostics, low-concentration samples require preconcentration and nucleic acid extraction typically *via* centrifugation, lysis, and purification, which are infrastructure-intensive and unsuitable for point-of-care testing (POCT).[69] Even saliva, despite its non-invasive and cost-effective collection, requires lengthy pre-analytical homogenization due to high viscosity and non-Newtonian behavior.[70] Automation emerges as pivotal in overcoming these limitations by streamlining workflows, reducing errors, and enabling precise fluid manipulation for rapid testing with minimal reagents and sample volumes. Integration of automated sample handling, injection systems, and image processors into microfluidic platforms facilitates the transition from research to routine clinical use.[64] Technological advances such as microfluidic chips combining free-flow electrophoretic preconcentration, thermoelectric lysis, and gel-electrophoresis purification enable fast, space-saving sample pretreatment without laboratory facilities, ideal for POCT.[69] For saliva-based diagnostics, magnet-beating within a centrifugal microfluidic cartridge homogenizes samples in four minutes, reducing viscosity from 10.4 to 2.3 mPa s and minimizing manual handling.[70] Additionally, AI can automate the entire diagnostic process from sample handling to data interpretation minimizing human intervention and error risk.[4]

7.1.3 Cost, scalability and user-friendliness. Scaling up microfluidic devices from laboratory prototypes to mass production is challenged by complex cartridge integration requiring high manufacturing precision and multi-material assembly, long development cycles of three to five years, high defect rates from multi-step fabrication without standardized protocols, elevated production costs due to low initial volumes and scrap rates, and the absence of consensus standards for dimensions, interfaces, and testing methods.[71] Additional barriers to adoption include the need to align with total testing process (TTP) workflows, integrate into existing laboratory infrastructure, and meet regulatory requirements while maintaining test consistency across devices, especially in high-throughput environments.[64] Solutions include

incorporating design-for-manufacturing principles early to reduce tooling and replication issues, involving manufacturing experts in the design stage, and selecting commercially viable materials such as thermoplastics from the outset to avoid redevelopment. Further, adopting and contributing to SEMI/ISO standardization efforts can enhance quality consistency, reduce costs, and accelerate commercialization, while designing microfluidic platforms that lower reagent use, minimize waste, and enable smaller, less invasive sample collection can improve both cost-effectiveness and user-friendliness.[64,71] Finally, ensuring integration with laboratory information systems (LIS), onboard quality control, and automation compatibility will facilitate seamless scaling from research to routine clinical use while enhancing usability and operational efficiency.[64]

7.1.4 Regulatory framework. Regulatory adoption of LoC and organ-on-a-chip (OoC) devices in clinical settings requires meeting stringent safety, efficacy, and reproducibility standards set by agencies such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA).[67] In the United States, implementation must comply with Clinical Laboratory Improvement Amendments (CLIA) regulations, which classify tests by complexity as waived, moderate, or high and define personnel qualifications and validation requirements; any microfluidic test without prior FDA clearance, or any modification of an FDA-cleared test, is treated as a high-complexity laboratory-developed test (LDT) requiring rigorous validation. Proposed legislation to align LDT validation with medical device approval standards could slow rapid test deployment, particularly for emerging diseases.[64] Barriers to regulatory acceptance also include the lack of standardized protocols for fabrication, operation, validation, and reporting, as well as limited harmonization of acceptance criteria between regions.[67,72] Addressing these challenges requires early engagement with regulatory agencies to clarify requirements, adoption of standardized fabrication and reporting protocols including material specifications, cell sourcing, and culture conditions as well as implementation of robust quality control and validation studies to confirm physiological and pathological relevance. In the EU, the EU Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) emphasizes robust validation protocols, cross-laboratory reproducibility, and standardized OoC designs to facilitate regulatory acceptance, with efforts to harmonize benchmarks and align with initiatives such as the European Partnership for Alternative Approaches to Animal Testing (EPAA). The EMA supports these efforts under the 3Rs principle and integrates OoC validation into frameworks such as REACH and the Cosmetics Regulation. In the U.S., the FDA advances OoC integration through qualification processes, applied research, and collaborative programs with the NIH, EPA, and industry, including the Tissue Chip Testing Centers and a centralized microphysiological systems (MPS) database to assess robustness and reliability. The FDA Modernization Act 2.0 permits the use of alternatives such as

OoC and *in silico* models in drug development, reducing reliance on animal testing. Both the FDA and EMA aim to ensure safety, efficacy, and scientific rigor while fostering market adoption of these technologies through international collaboration and harmonized validation protocols.[67]

8. Applications of lab on chip technology

The advent of LoC technology represents a paradigm shift in medical diagnostics, enabling the examination of physiological samples at point-of-care facilities or in remote and resource-poor locations instead of the conventional method of sending them to a laboratory resulting in significant reduction in diagnosis time. Consequently, it benefits the patients in early detection and receiving timely treatment, thereby improving the overall healthcare outcomes.[56] Moreover, LoC technology finds extensive applications, primarily focusing on areas such as genomics, biochemical analysis, proteomics and cell research, biosensors, biomedical sciences, and drug development.[6,73] The following section covers a few applications of LoC in detail. Fig. 4 indicates the design of lab on chip systems for multifaceted applications. The following section covers a few applications of LoC in detail.

8.1 Point of care testing

Point of care testing (POCT) refers to a clinical laboratory testing carried out near the patient's location without the need for a permanent, dedicated facility and can be performed outside the clinical laboratories. In contrast to the multistep lab testing, POCT has only 3 primary steps: pre-clinical, analytical, and post-analytical (Fig. 5). resulting in notable reduction in test TAT.[6] The reduction in TAT not only circumvents the delay in receiving treatment, but also plays a crucial role in reducing mortality, morbidity, and the quality of life. Additionally, the other advantages of PCOT include – 1) simplified operation, 2) elimination of the need for trained staff, 3) cost-effectiveness, and 4) easy bulk fabrication.[74–78] In recent years, POCT has gained increasing attention especially in limited resource areas which has led to the development of various methods, devices, and biosensors that can fulfill the need for POC diagnostic tools.[79] Additionally, COVID-19 has fueled the increase in the use of POCT to help control the pandemic.[76]

POCT encompasses a diverse array of testing modalities customized to specific applications. POCT diagnostics are predominantly based on two prevalent technologies, lateral flow assay (LFA) technology and nucleic acid amplification. Lateral flow assay technology can be further divided into nucleic acid-based assays and immune-based assays. Immune-based assays employ antibodies to detect antigens, proteins, and hormones, while nucleic acid-based assays utilize DNA/RNA oligonucleotides or aptamers as biorecognition elements.[78]

LFA usually consists of four components namely, a sample pad, conjugate pad, detection zone and absorption pad (Fig. 6).[78] The diagram below illustrates the typical LFA strip.
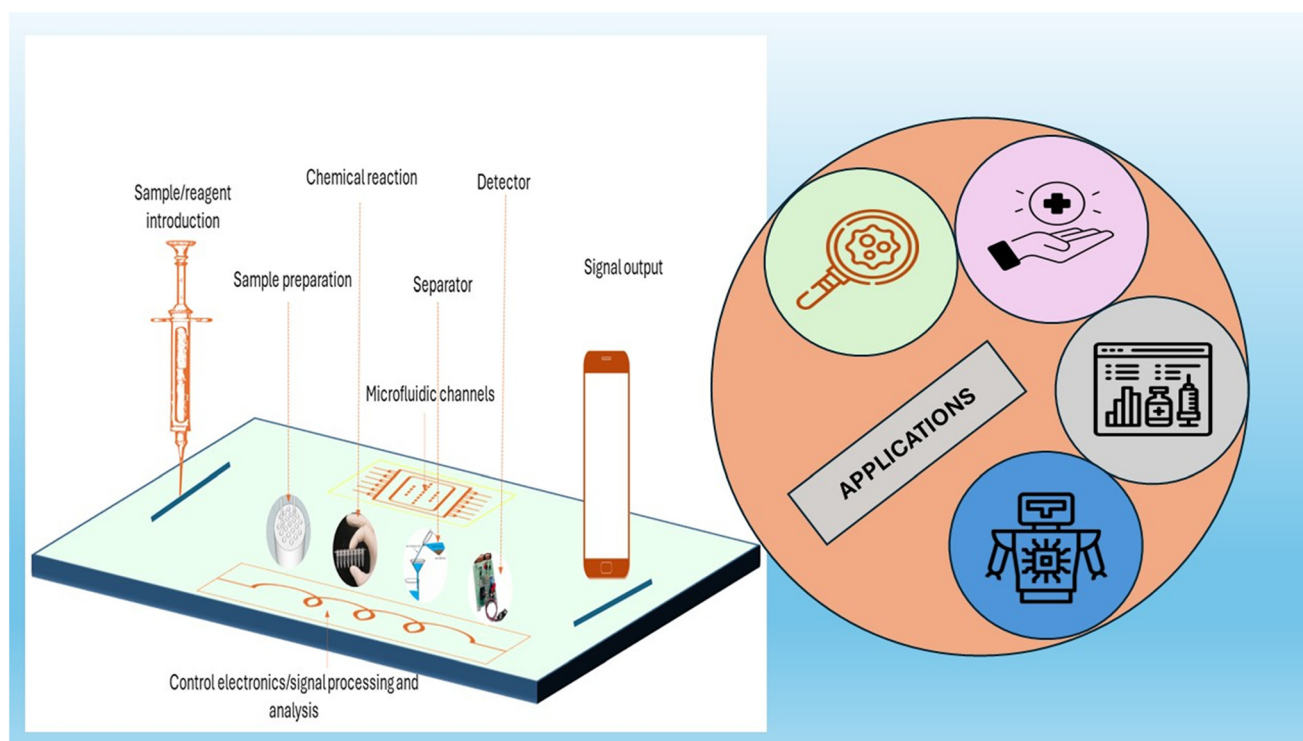

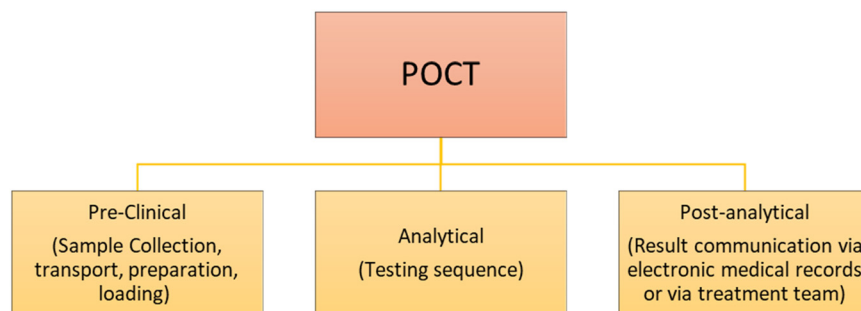Fig. 4   Designing lab on chip systems for multifaceted applications.

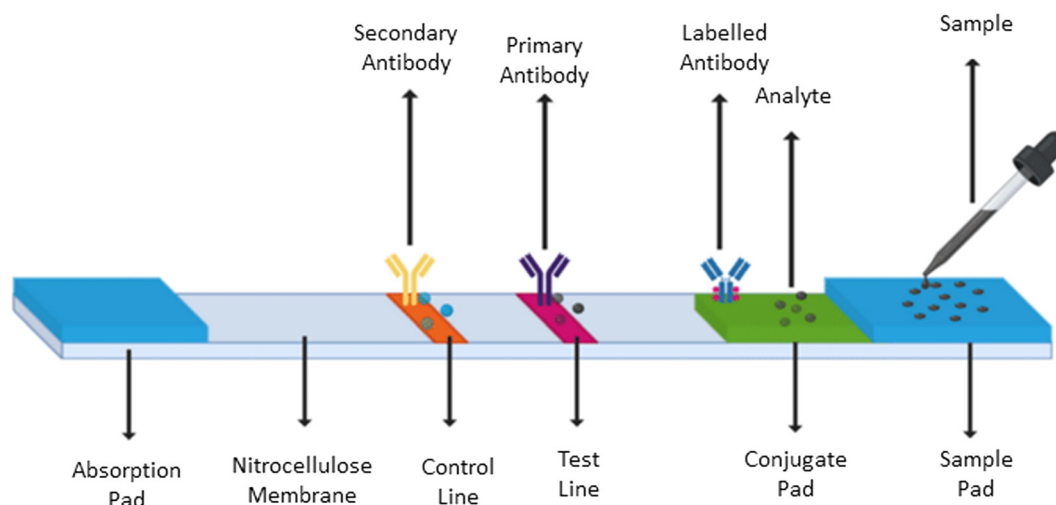**Fig. 5** Steps involved in point-of-care testing.[76]

Fig. 6 Schematic representation of the steps involved in LFAT. Reproduced from ref. 78 with permission from *Frontiers in Bioengineering and Biotechnology*, copyright 2021.

LFAs find application in diagnosis and prognosis of diseases like cancer, rapid detection tests as well as in the diagnosis of certain bloodborne diseases. While LFA is a widely used technique to detect a variety of pathogens and proteins, due to its poor detection techniques, nucleic acid amplification tests (NAATs) have gained popularity in the POCT area. NAATs involve three steps: sample preparation, amplification, and detection (Fig. 7). NAATs are applied in the detection of various infectious diseases like *Mycoplasmas pneumonia*, *Bordetella pertussis*, and *Legionella Pneumonia*,
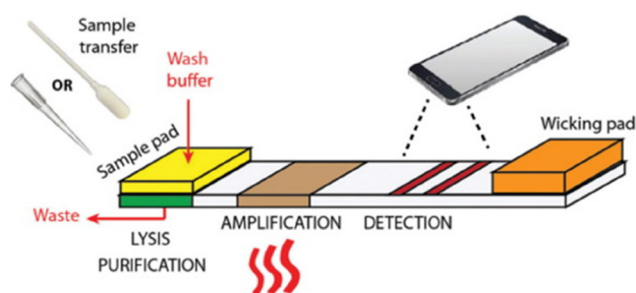

Fig. 7 Schematic representation of the steps involved in NAAT. Reproduced from ref. 80 with permission from *Journal of Biomechanics*, copyright 2021.

various strains of *Influenza* virus, SARS-Cov-2, *etc.* The technique allows rapid detection of *C. difficile*, Strep B, and Streptococcus A in approximately 2 minutes. Moreover, it is also used to determine the viral load in HIV positive patients, and it aids in the diagnosis of STDs like chlamydia and gonorrhea within 30 minutes.[78]

8.2 Organ on a chip

Another innovation of microfluidics is organ-on-a-chip (OoC). OoCs are systems containing engineered or natural miniature tissues grown inside microfluidic chips. OoCs are designed to provide a suitably *in vivo*-like environment to guide a collection of cells to assemble into a 3D tissue capable of replicating one or more organ-level functions or to culture organotypic tissue to retain function.[81] Organ on chip platforms hold significant promise as alternatives to animal models or traditional cell cultures, both of which poorly recapitulate human pathophysiology and human level responses.[82]

Organ-on-a-chip (OoC) technology offers distinct advantages over conventional 2D/3D cell culture and animal models by more accurately replicating human-relevant physiology and disease states.[83] It offers high physiological

Table 6 Examples of organ on chip devices and their applications[81]

Type of OoC	Examples	Applications
Single-organ chips	Gut-on-a-chip, liver-on-a-chip, lung-on-a-chip, skin-on-a-chip, brain/BBB-on-a-chip, heart-on-a-chip	Drug absorption studies, disease modeling, metabolism and toxicity testing, barrier function studies, neurovascular interaction, contractility and electrophysiology analysis
Multi-organ chips (body-on-achip)	Liver–kidney chip, liver–gut–skin–lung chip, cancer multi-OoC systems	Studying systemic drug metabolism, multi-organ toxicity, pharmacokinetics, tumour efficacy/toxicity evaluation
Solid organ chips	Liver chip, tumour chip, pancreas chip, bone chip, cartilage chip	Modeling parenchymal/mesenchymal tissues, drug metabolism, cancer biology, bone and cartilage physiology
Barrier tissue chips	Vascular endothelium chip, gut epithelium chip, corneal epithelium chip, skin epithelium chip	Studying selective transport, barrier integrity, infection models, permeability assays

relevance by closely mimicking human organ structure, mechanics, and biochemistry, while providing a dynamic microenvironment with controlled fluid flow to simulate processes like blood circulation. It supports integration of multiple cell types for complex studies, enables real-time, high-resolution monitoring, and improves the accuracy of drug efficacy and safety predictions. By reducing reliance on animal models, it eliminates interspecies variability and ethical concerns. Its customizable, scalable design suits disease-specific and high-throughput applications, and it delivers human-relevant pharmacokinetic and pharmacodynamic data in hours to days instead of weeks to months.[81] OoC devices are mainly divided into four categories namely single-organ chips, multi-organ chips, solid organ chips and barrier organ chips. Table 6 explains the examples of organ on chips falling under each category and their applications.

In a recent study conducted by Larson *et al.*, the liver-chip model offered several advantages over conventional methods for assessing the hepatotoxicity of cannabinoids, such as cannabidiol (CBD), cannabinol (CBN), cannabichromene (CBC), and cannabigerol (CBG). This model offers a human-relevant alternative to animal models, accurately reproducing known toxicities such as acetaminophen-induced hepatotoxicity and differentiating the toxicity profiles of various cannabinoids. It enables detailed mechanistic insights by revealing distinct modes of action—such as reduced mitochondrial function, oxidative stress, inflammation, and cellular toxicity—and highlighting varying effects on reactive oxygen species and mitochondrial function in hepatocytes and non-parenchymal cells. Serving as a comprehensive evaluation tool, it supports live toxicity screening and allows assessment of cellular morphology alongside effluent-based biomarkers like albumin, LDH, ALT, AST, and cytokines, facilitating a deeper understanding of cannabinoid-induced liver injury.[84]

8.3 Advancement of lab-on-a-chip technology in cancer research and diagnostics

Cancer ranks among the primary contributors to global mortality, and the high mortality rates are likely linked to delayed diagnosis, ultimately resulting in delayed treatment. While methods such as tissue biopsy and various imaging

techniques exist to assess tumor heterogeneity, their inherent limitations constrain their long-term use, particularly in patients who develop resistance to the treatment.[85]

The customizable nature of LoC has been demonstrated as a groundbreaking technology, paving the way for various opportunities in cancer research. It has significantly contributed to the development of preclinical cancer models, identification of cancer biomarkers, screening of anti-cancer drugs, investigating tumor heterogeneity and producing nano-drugs.[86]

8.3.1 Preclinical cancer models: tumor-on-a-chip (TOC). The dramatically increasing significance of metastasis underscores the urgent need to develop sustainable *in vitro* preclinical models to investigate intricate cellular processes.[87] The principal challenges in cancer research include establishing an *in vitro* efficient tumor microenvironment and exploring efficient means for screening anticancer drugs to reveal the nature of cancer and develop treatments.[88] The emerging tumor-on-a-chip platforms are based on soft lithography or three-dimensional bioprinting. To create these models, researchers pattern 3D channels and chambers into polydimethylsiloxane or other flexible polymers using soft lithography or 3D printers (Fig. 8). Hydrogel containing cells or fluid perfusion fill the channels and chambers to replicate
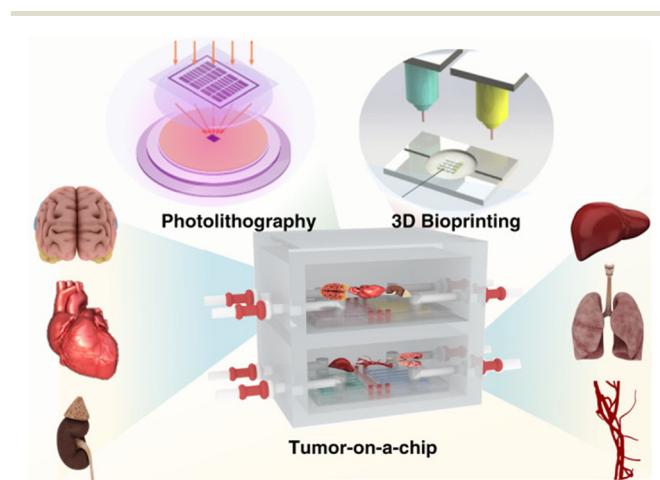
Fig. 8 Schematic representation of tumor-on-a-chip platforms developed based on photolithotrophy and 3D bioprinting. Reproduced from ref. 88 with permission from *Microsystems & Nanoengineering,* copyright 2021.

the architectural and mechanical characteristics of human tissue. These models provide a physical scale that aligns with the tumor microenvironment (TME) and a modular design that enables selection of cells and extracellular matrix (ECM). Additionally, it allows precise control of the physiochemical properties such as interstitial pressure, soluble factor gradients, and oxygen tension. Furthermore, the events in tumor-on-chip platforms can be monitored with high spatiotemporal resolution using microscopy.[89] Such models have potential to address the limitations of traditional 2D and 3D cell culture models and mitigate ethical concerns associated with animal models.[87,88] TOC systems can mimic primary as well as metastatic tumor microenvironments developing single organ or multiorgan TOCs respectively.[86,89,90]

The section below details a few single-organ and multiorgan TOCs.

Ayuso *et al.* pioneered the development of single organ *in vitro* models based on LoC technology, effectively replicating ductal carcinoma *in situ* (DCIS) (Fig. 9a). The design facilitated the generation of a microenvironment characterized by hypoxia and nutrient deprivation, enabling the selective targeting of hypoxic DCIS cells.[87,91] Another example is where Hassell *et al.* established *in vitro* human orthotopic models of non-small-cell lung cancer (NSCLC) (Fig. 9b). These models simulate organ microenvironment-specific cancer growth, tumor dormancy, and responses to tyrosine kinase inhibitor (TKI) therapy. By leveraging the mechanical actuation features of this technology, the

researchers discovered an unknown sensitivity of lung cancer cell growth, invasion, and TKI therapeutic responses to physical indicators associated with breathing motions. These effects seemed to be mediated by changes in signaling through epidermal growth factor receptor (EGFR) and MET protein kinase. These findings may shed light on observed resistance to therapy in cancer patients with minimal residual disease in aerated and mobile lung regions. Additionally, this model serves as an experimental platform for investigating cancer persister cells and mechanisms of tumor dormancy *in vitro*.[90] Shen *et al.* introduced HCC-on-a-chip (Fig. 9c), a model designed to replicate cancer. The hepatic stellate cells are one of the key components in the HCC microenvironments and play an important role in tumor progression and drug resistance. Blood vessels are essential for nutrition supply and metabolite elimination. HCC-on-a-chip is a three-channel microfluidic device developed to culture three distinct cell types in a spatially organized manner to mimic HCC microenvironments. This approach allows us to study the roles of diverse cells in remodeling the TME by altering the secretome, proteome and metabolome. Critical events including tumor cell proliferation, endothelial cell invasion, tumor cell drug resistance, NK cell infiltration and exhaustion were assessed in the HCC-on-a-chip.[92]

In the above section we have discussed about tumor-on-a-chip developed to mimic primary tumors. Moreover, this section discusses multi-organ tumor-on-a-chip models developed to study metastatic cancer. On these chips,
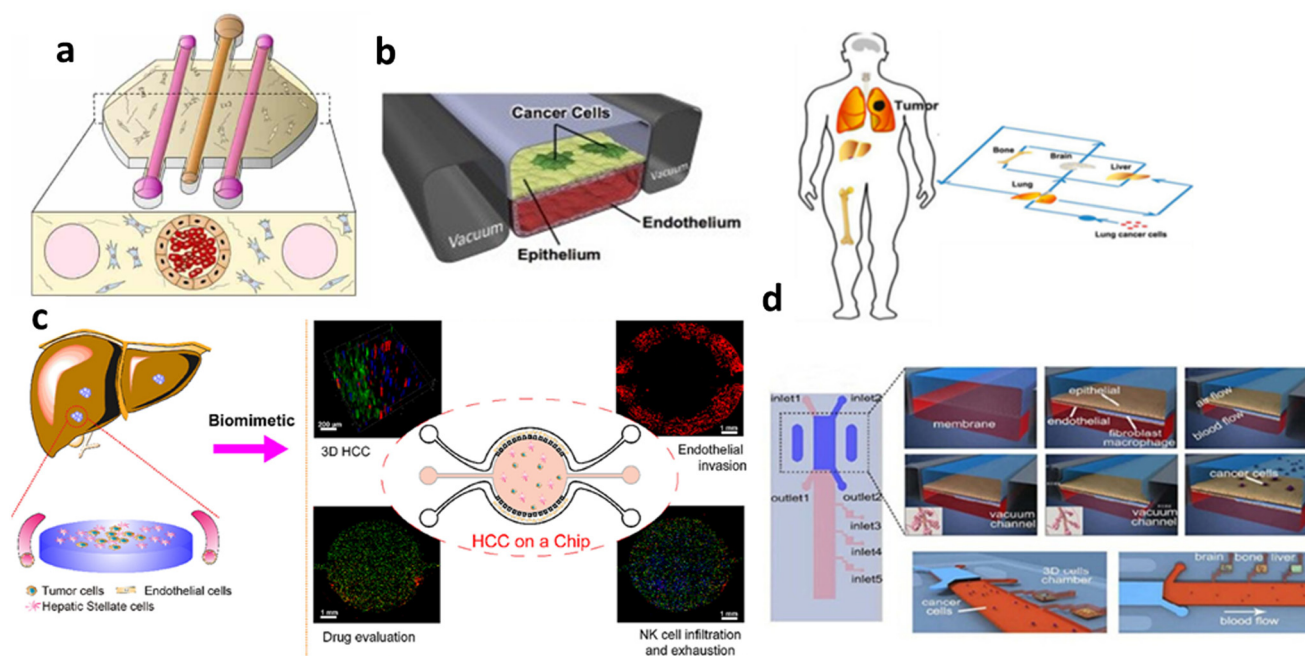

Fig. 9 Schemic representation of single-organ and multiorgan tumor-on-chip. a) Scheme of the microfluidic model for DCIS. Reproduced from ref. 91 with permission from *EBioMedicine*, copyright 2018. b) Scheme for tumor lung chip for NSCLC. Reproduced from ref. 90 with permission from *Cell Reports*, copyright 2017. c) Scheme for tumor liver chip for HCC. Reproduced from ref. 92 with permission from *Acta Pharmaceutica Sinica B*, copyright 2023. d) Scheme for multiorgan tumor on chip for lung cell metastases. Reproduced from ref. 88 with permission from *Microsystems & Nanoengineering*, copyright 2021.

different organoids are separated by specific biomaterials like PDMS, and they relate to each other by channels and controllable fluids. Xu *et al.* designed and constructed a multi-organ microfluidic chip to mimic lung cancer metastasis to the brain, bone, and liver. The system consists of four organs: one upstream lung and three downstream parallel brain, bone, and liver organs (Fig. 9d).[86,88]

8.4 Cancer diagnostics

Cancer can be detected by LoC devices using various biomarkers such as circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), exosomes, non-coding RNA (ncRNA) and various cellular metabolites or proteins.[86]

8.4.1 On-chip CTC detection. CTCs are tumor cells that shed from solid tumors and circulate into peripheral blood. Several studies indicate a close relation between CTCs and metastasis. As CTCs have an ability to reflect the real-time tumor burden and explore tumor heterogenicity, they play a very significant role in cancer detection. Currently, there are various LoC devices, which are used to separate and count CTCs.[86] The section below demonstrates a few LoCs developed for CTC detection.

Deng *et al.* designed and developed an integrated microfluidic system to simplify the separation, purification, and single-cell secretory omics analysis of whole blood CTCs

(Fig. 10a). This system can process 1 ml of whole blood samples in less than 2 hours with a separation efficiency of more than 70%. The platform can also classify CTCs into specific phenotypes based on surface markers and conduct single-cell secretory omics analysis on these subsets.[93] Pahattuge *et al.* created a smart chip that combines modules for CTC sorting, cell counting, and immunofluorescence imaging. This mart chip offers fully automatic operation facilitating seamless separation and detection of CTCs in blood while eliminating the need for human interference (Fig. 10b).[94] In chip developed by Cho H. *et al.* gold nanoparticles were modified with antibodies and Raman signal molecules to label CTCs (Fig. 10c). The CTCs captured on the chip can be characterized and detected *in situ* by surface-enhanced Raman technology. This method has high sensitivity and can distinguish common CTCs and circulating tumor stem cells (CTSCs) according to the difference in Raman signal peaks.[95]

8.4.2 On-chip exosome detection. Exosomes are extracellular vesicles (EV) produced by cells and they range from 30 to 100 nm in size. These exosomes, present in body fluids like serum, ascites, and pleural effusion, facilitate cellular communicators between by transferring diverse cargoes, such as mRNA, ncRNA and proteins.[85,86] Changes of cargo expression in exosomes can indicate tumor status, serving as a valuable tool for cancer diagnosis and prognosis.
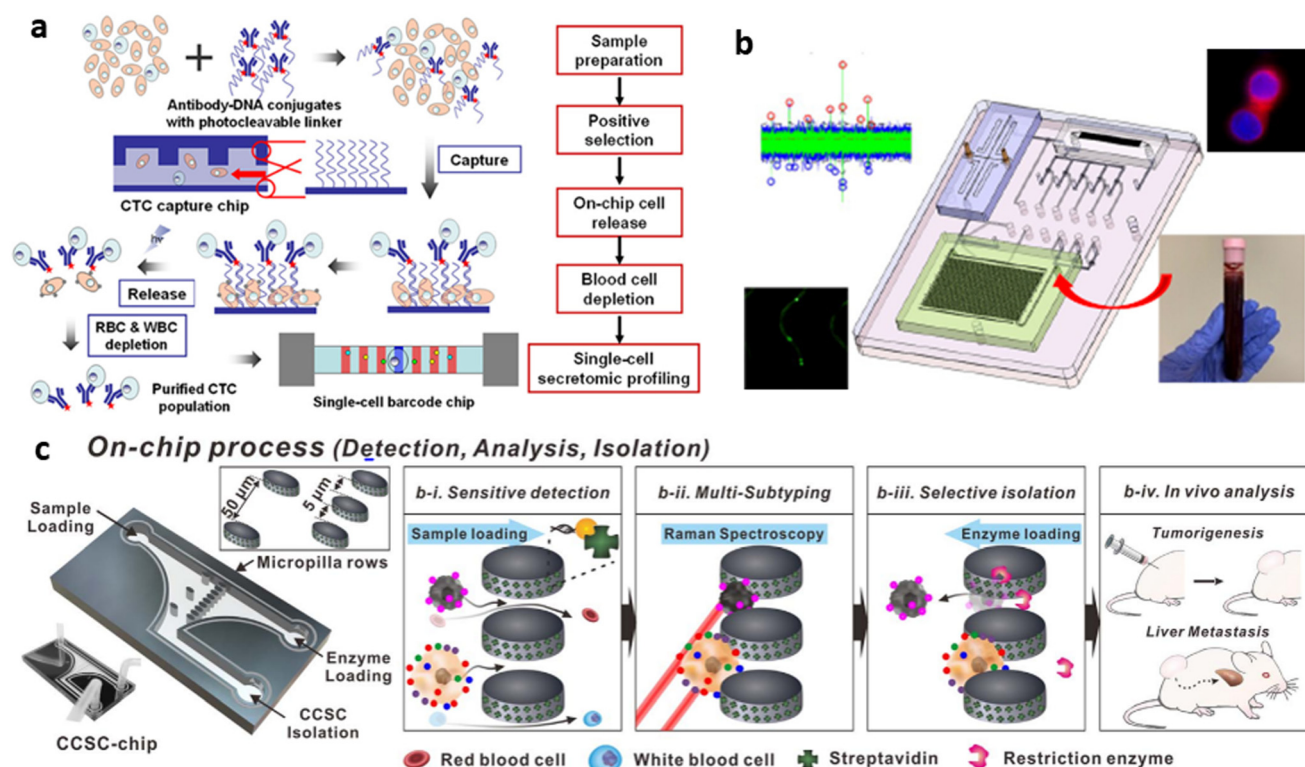

Fig. 10 Schematic representation of on-chip CTC detection. a) Chip designed to simplify the separation, purification, and single-cell secretory omics analysis of whole blood CTCs. b) Chip designed to combine modules for CTC sorting, cell counting, and immunofluorescence imaging. c) A chip with modified gold nanoparticles with antibodies and Raman signal molecules to label CTCs. Reproduced from ref. 94 with permission from *ACS Sensors*, copyright 2021.

The process of cancer detection using exosomes comprises two steps: separation and detection. Various methods are applied for separation, including immunoaffinity-based separation, nanomembrane filter, dielectrophoretic (DEP) separation, lateral displacement, and acoustic fluid separation. The detection method encompasses fluorescence detection, electrochemical detection, and mass spectrometry. Moreover, LoC devices simplify cancer detection by integrating these two steps on a single chip.[86] For example, Fang *et al.* developed a LoC platform specifically designed to detect breast cancer derived exosomes from patient plasma (Fig. 11). This system works based on CD63 antibody conjugated magnetic nanoparticles (Mag-CD63).[87,96] This chip comprises two chambers for immunomagnetic particle collection, two circuitous mixing channels, four inlets, and one outlet. The process involves pre-mixing of the exosome containing sample with magnetic nanoparticles, followed by its introduction onto the chip *via* inlet 1 and simultaneously introducing primary antibody *via* inlet 2. The mixing of these components takes place in channel 1, forming Mag-CD63-Exo-Ab. The immuno-magnetic particles are retained in chamber 1 by a magnetic disc. PBS buffer, introduced through inlet 3, washes the Mag-CD63-Exo-Ab1 complex. Subsequently, a fluorescent/labeled secondary antibody is introduced through inlet 4 and driven to flow and mixed with Mag-CD63-Exo-Ab2 *via* the second mixing channel, then retained in chamber 2. After washing with PBS, the immunocaptured exosomes are examined by an inverted fluorescence microscope.[89]

To better illustrate the similarities and distinctions between these two LoC-based approaches, a comparative summary of CTC and exosome detection is presented in Table 7.

Immunoaffinity. Tajudin *et al.* developed an integrated acoustic immunoaffinity-capture (IAI) platform which is a novel microfluidic system designed for rapid, direct detection of protein biomarkers, such as prostate specific antigen (PSA), from whole blood samples. It seamlessly integrates acoustophoresis-based plasma separation from undiluted whole blood with a miniaturized immunoaffinity-capture system utilizing a porous silicon antibody microarray. This integrated approach significantly reduces assay time, enabling detection of clinically relevant PSA levels within 15

minutes, and improves overall efficiency by minimizing conventional sample preparation steps and automating the process. The platform demonstrates a linear response and high sensitivity, showcasing its potential for advanced point-of-care diagnostics.[101]

8.5 LoC in drug development and discovery

The pharmaceutical industry struggles with a significant hurdle in the form of the limited efficacy of current drug development and discovery approaches. Drug development is a time consuming process with a success rate as low as 10.4% from phase I. Typically, it takes almost 12–15 years for drug development, from discovery to marketing approval. Drug screening is a time consuming process requiring an extensive involvement of animals and humans raising several ethical concerns.[102]

In contrast, LoC devices employ minuscule amounts of reagents, facilitating efficient heat and mass transfer due to the high surface to volume ratio. These devices capitalize on diverse flow regimens with molecular diffusion serving as the predominant mechanism as well as the concept of parallelization. Control in LoC devices is achieved through active and passive mixers, while throughput enhances throughput capacity. Furthermore, these devices offer distinct advantages compared to conventional methods, such as rapid drug development, portability, and user-friendly systems with a high degree of functional integration for drug development.[102] They also enable more precise modelling of physiological conditions, benefiting both fundamental research and drug development, and enable systematic high-volume testing across various aspects of drug discovery. Advancement in tissue-on-a-chip and organ-on-a-chip holds promise in accelerating early stages of drug discovery and mitigating the extensive need for animal testing. The following section outlines notable examples of LoC approaches that play a crucial role in the field of drug discovery.[103]

8.5.1 Drug screening using the droplet microfluidics approach. In the droplet microfluidics approach the droplets are generated using both active and passive approaches. Recently, for high-throughput drug screening droplet-on-demand (DOD) a flow-focusing device was used to produce
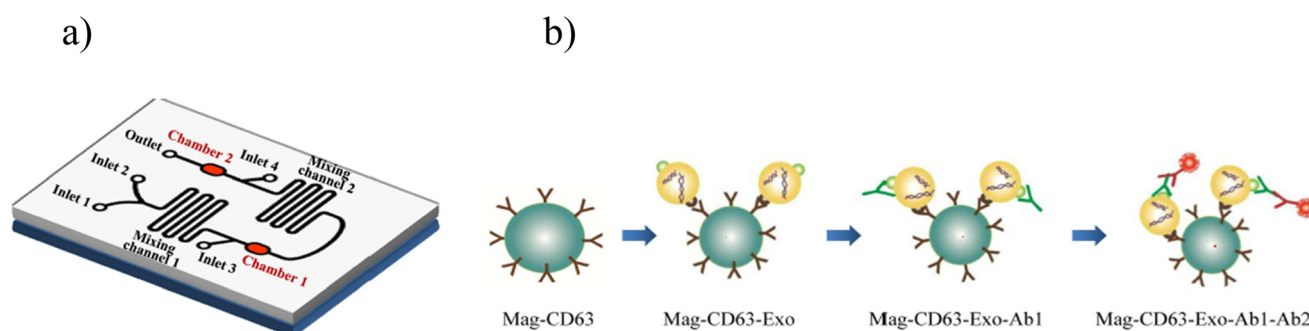

Fig. 11 On-chip exosome detection. a) Schematic representation of the microfluidic chip. b) Workflow for the immunogenetic capture and detection of exosomes. Reproduced from ref. 96 with permission from *PLoS One,* copyright 2017.

Table 7 Comparative summary of CTC and exosome detection using LoC

Parameter	Circulating tumor cells (CTCs)	Exosomes
Target	Whole intact tumor cells[97]	Extracellular nanovesicles carrying a wide range of biomolecules including DNA, RNA, proteins, and lipids[97,98]
Size	~15–25 μm (ref. 99)	~50–100 nm in diameter[98]
Abundance	Rare[97]	High abundance[98]
Separation methods	Size and deformity based, affinity-based (EpCAM)[97]	Viscoelastic flow sorting, acoustic nanofiltration, membrane-based filtration, immunoaffinity, trapping on nanowires, and deterministic lateral displacement (DLD)[98]
Detection technique	Fluorescence-based, flow cytometry based, optical biosensing techniques, photoacoustic cytometry, imaging cytometry[60]	Immunomagnetic beads, electrochemical, 3D self-assembled herringbone nanopatterns, fluorescence of photonic crystals using a filtration system, fluorescence, alternating current electrokinetic (ACE) & fluorescence, microfluidics integrated with surface-enhanced Raman spectroscopy (SERS) and electrohydrodynamic force with SERS using nanomixing fluid flow[98]
Platform materials	PDMS, nanomaterials such as gold nanoparticles and graphene oxide nanosheets[100]	PDMS, paper-based platform[98]
Clinical applications	Cancer prognosis, diagnosis, metastasis monitoring, personalized drug testing[97]	Early and rapid cancer diagnosis, advances understanding of intercellular communication and disease mechanisms, monitors treatment response as well as disease progression along with disease recurrence[98]
Advantages	Enables small-volume handling with integrated sorting and analysis, preserves CTC viability, and processes large blood volumes to improve capture of rare CTCs[97]	Greatly improves the accuracy and sensitivity of exosome analysis at the point of care for early disease detection, exploring personalized medicine, and thereby, improving patient outcomes[98]
Key challenges	Lack of standardization, unvalidated diagnostics and lack of complete understanding of clinical significance, challenges in terms of sensitivity and specificity *e.g.*, rarity, marker heterogeneity, false negatives in EpCAM-only capture[97]	Need for expensive instruments for exosome separation, heterogenicity, sample impurity, reproducibility issues, need for standardization and validation[98]

microdroplets. Gielen *et al.* developed a fully automated DOD platform integrating it with an absorbance detection unit for enzyme kinetics and inhibition. The partitioning of drug molecules in different oil media and at interfaces was investigated using droplet microfluidics assay using only 4.2 nL of reagents and sample with accuracy.[102,104]

8.5.2 Drug screening using tissue-on-a-chip and organ-on-a-chip approaches. The progression in tissue engineering has given rise to the emergence of tissue-on-a-chip or organ-on-a-chip technologies, representing more advanced versions of microfluidic cell cultures. Organs-on-a-chips refer to 3D devices that contain living cells from a tissue or an organ. These devices are structured with layers or channels, facilitating the continuous delivery of substances like air or blood through micropumps. Within these devices, cells can interact with other tissues, mimicking the process in a real body. Mechanical forces are also incorporated to simulate actions like blood flow or breathing. The important organs-on-a-chips that have revolutionized medical science are highlighted below. Some models have already been developed for a variety of organs including liver, kidney, gastrointestinal tract, bone, blood vessels, lungs, muscles, heart, and bone marrow. A notable instance is the heart-on-a-chip developed by Li *et al.* to evaluate the effect of hydralazine hydrochloride on pressure-induced dysfunction of endothelial cells.[102,105]

Li *et al.* developed the heart-on-a-chip using the elastic material PDMS, and microchannel structure that simulated a blood vessel when integrated onto the chip. The cells were cultured from human umbilical vein endothelial cells

(HUVECs) inside the channel, subjected to varying pressures and shear stresses. The generated vessel mimics serve as a valuable platform for assessing the safety and effects of antihypertensive drugs.[102,105]

8.5.3 Drug screening using human-on-a-chip. Huh *et al.* pioneered the concept of human-on-a-chip (Fig. 12), which involves the integration of multiple organs onto a single chip. This system possesses capabilities to replicate the dynamics of drug metabolism entirely *in vitro* before proceeding to the vigorous stages of clinical trials. Human-on-a-chip enhances the authenticity of drug metabolism by testing the toxicity at each organ level and providing a more efficient elucidation of the adverse effects of drug more efficiently in comparison to traditional approaches.[102,106]

9. Integrating artificial intelligence and machine learning with LoC

The integration of artificial intelligence with LoC devices has received significant interest in the last few years due to constantly emerging machine learning and deep learning methods. AI has paved its way in various fields of healthcare including research, clinical diagnostics, precision medicine, and drug discovery and development including clinical trials.[107] By combining the miniaturization and precision of microfluidics with the computational power of AI, these platforms can automate complex processes, enhance analytical accuracy, optimize device performance, and provide real-time decision support. This synergy not only
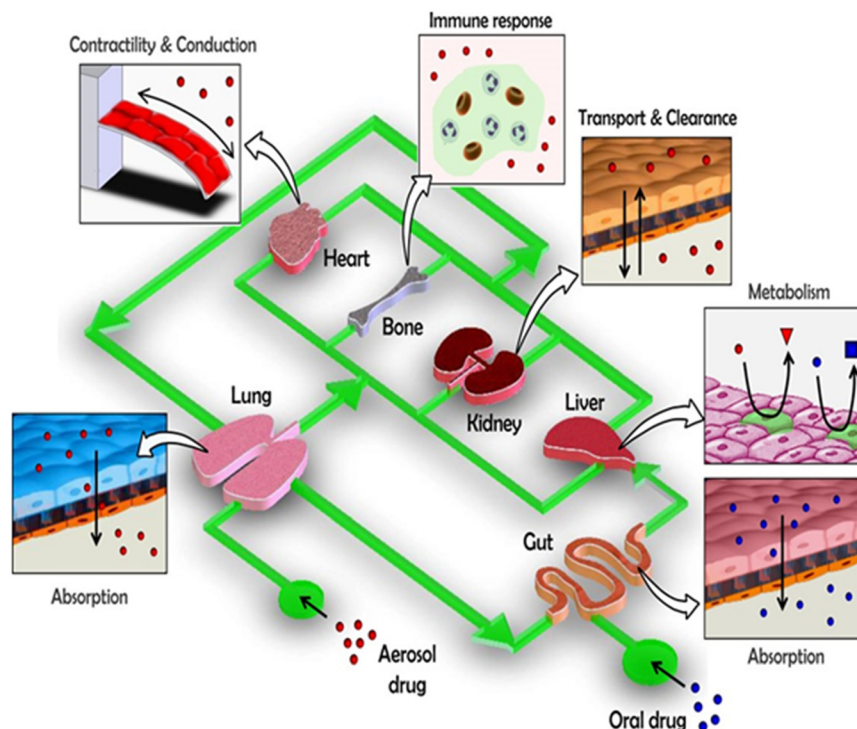
Fig. 12 Drug screening with the human-on-a-chip approach. Reproduced from ref. 106 with permission from *Trends in Cell Biology*, copyright 2011.

improves assay sensitivity, specificity, and throughput but also facilitates scalability, remote accessibility, and seamless integration into clinical and research environments. The following are key roles AI can play in advancing LoC and microfluidic systems.

9.1 AI roles in LoC/microfluidic systems

1. Facilitates design automation & optimizes device performance.[108,109]

2. Enables automated control of microsystem parameters such as flow, thermal, particle movement, and droplet manipulation.[109]

3. Identifies patterns in complex biological datasets and supports advanced cell analysis and personalized medicine approaches.[109]

4. Performs rapid data processing and high-precision image analysis.[110,111]

5. Enables rapid error detection.[111]

6. Supports cloud-based data analytics and evidence-based tele-reporting.[110]

7. Analyzes high-throughput datasets.[72]

8. Automates the calibration process during device fabrication.[109]

9. Enhances sensitivity, specificity and multiplexing in POC diagnostics.[111]

10. Aids in development of personalized drug formulations.[109]

Rizzuto *et al.* designed a microfluidic device integrated with machine learning technology to assess red blood cell

(RBC) deformability by immobilizing them in a planar orientation (Fig. 13). The set up allows the visual inspection of RBCs' ability to regain their original shape after passing through the micro constrictions. The objective in creating this device is to adapt the physiological spleen filtration process for *in vitro* study and monitoring of blood diseases through RBC shape analysis. Subsequently, a microfluidic device replicating the slits of the spleen red pulp area and video data analysis are combined for the characterization of RBCs in rare hereditary hemolytic anemia and it consists of a main channel branched until forming eight parallel microchannels. Each microchannel contains a row of filtering funnel-shaped micro constrictions to mimic the inter endothelial slit (IES) section of the spleen. A healthy RBC deforms its shape and recovers it soon after passing the slits. In contrast, in an RHHA patient the capacity of the RBC to return to the original shape is compromised. Then, two cooperative learning approaches are used for the analysis: the majority voting scheme, in which the most voted label for all the cell images is the class assigned to the entire video; and the maximum sum of scores to decide the maximally scored class to assign. The proposed platform shows the capability to discriminate healthy controls and patients with an average efficiency of 91%, but also to distinguish between RHHA subtypes, with an efficiency of 82%.[112]

Timely and accurate diagnosis is a key to getting treatment on time. AI plays this dual role in diagnosis of minimal residual disease (MRD) which can otherwise lead to relapse or repeat of the cancer. In 2020, Uslu *et al.* designed a biochip that incorporates micron size
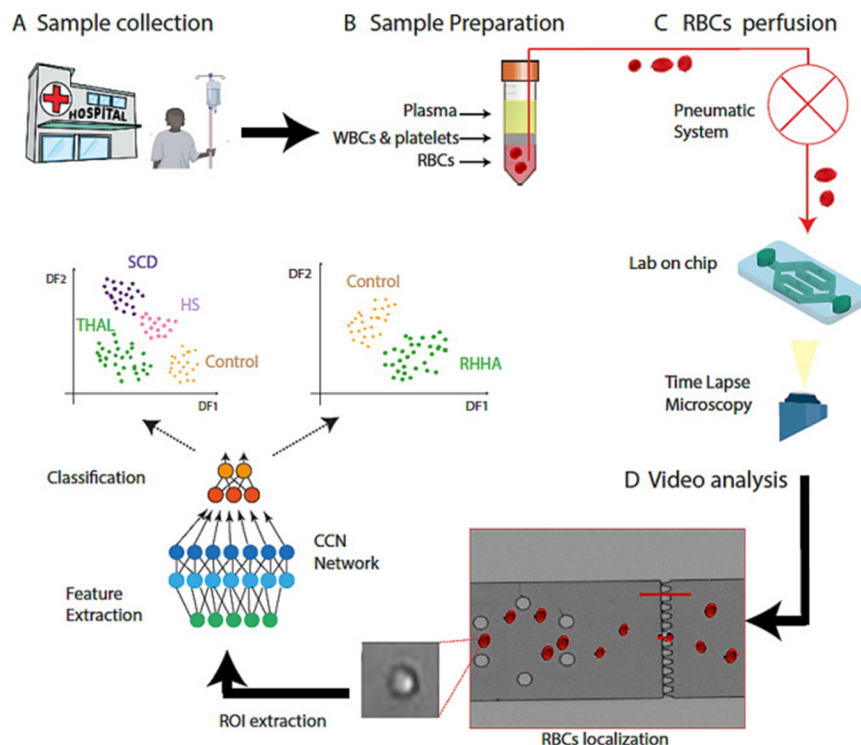
Fig. 13 Schematic representation of RBC shape analysis. A) Sample collection, B) sample preparation, C) RBC perfusion, and D) video analysis. Reproduced from ref. 112 with permission from *Scientific Reports*, copyright 2021.

immunomagnetic beads together with micropad arrays, which thus requires automated detection and quantification of not only cells but also the micropads and the immunomagnetic beads.[113] The main purpose of the biochip is to capture target cells having different antigens simultaneously. In this proposed study, a digital image processing-based method to quantify the leukemia cells, immunomagnetic beads and micropads was developed as a readout method for the biochip. Color, size-based object detection and object segmentation methods were implemented to detect structures in the images acquired from the biochip by a bright field optical microscope. It has been shown that manual counting and flow cytometry results are in good agreement with the developed automated counting. The average precision is 85% and the average error rate is 13% for all images of patient samples, while the average precision is 99% and the average error rate is 1% for cell culture images. With the optimized micropad size, the proposed method can reach up to 95% precision rate for patient samples with an execution time of 90 s per image.[113–116]

Furthermore, Deng *et al.* proposed a model that integrates organ-on-a-chip technology with artificial intelligence in drug evaluation. This innovative approach encompasses a wide range of applications including identification and validation of drug targets, designing of novel drugs, quantification of the structure–activity relationship, drug repurposing, enhancement of research and development (R&D) efficiency, as well as evaluation of absorption, distribution, metabolism,

excretion, and toxicity. Furthermore, the proposed model extends its utilization in aggregating and analyzing biomedicine information and refining the decision-making process to recruit patients for clinical trials. As per their proposed model (Fig. 14), AI plays a crucial role in both data extraction and analysis, as well as playing a vital role in experimental design and control.[107]

OoCs have been extensively employed to replicate nearly all organs in humans for purposes like drug testing, disease modeling, personalized medicine, and others. To enhance the physiological relevance of OoCs, various factors are considered and incorporated such as cell types, stimulations, and materials. Ultimately, the OoCs-AI combination will offer benefits in terms of experimental design and control, as well as data extraction and analysis. This integration holds considerable promise for drug evaluation using OoCs. Abbreviations: iPSCs: induced pluripotent stem cells; ECM: extracellular matrix.[107]

Despite substantial progress of OoCs in the academic realm, where some OoC platforms have been transitioned successfully into commercial products, a host of challenges prevent their widespread integration into industrial settings. OoCs continue to face marginalization within the pharmaceutical industry. To overcome the gap and promote the incorporation of OoCs in the drug development process, it is imperative to foster ongoing engagement, and discussions with OoC developers, end users, and regulatory bodies.[107]

Beyond mimicking human physiology in organ-on-a-chip systems, AI also drives innovations in droplet microfluidics,
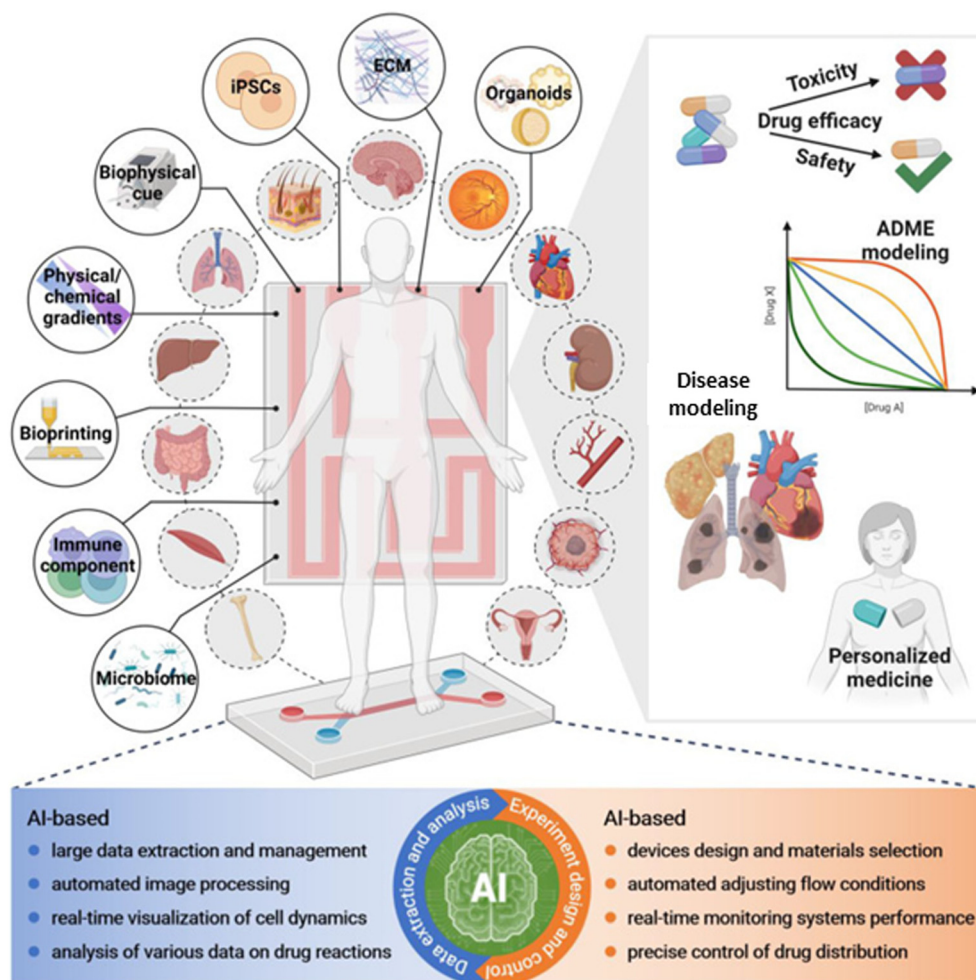
Fig. 14 Schematic representation of organ-on-a-chip integrated with artificial intelligence in drug evaluation. Reproduced from ref. 112 with permission from *Scientific Reports*, copyright 2021.

enabling real-time sorting and high-throughput analysis. Droplet sorting is a pivotal component of droplet microfluidics, enabling the isolation of target droplets for downstream analysis with high precision and throughput.[117] Artificial intelligence (AI) significantly enhances droplet microfluidics by enabling precise control, prediction, and optimization of droplet formation. Machine learning models, such as ANNs, CNNs, Bayesian inference, and XGBoost, can accurately predict droplet size, stability, and formation performance, incorporating factors such as flow rate and surfactant concentration. Tools like DAFD leverage community-fed datasets and federated learning to create adaptable, universal predictive models. AI-driven image analysis, including YOLO-based detectors and deep learning techniques, enables high-speed (up to 100 FPS) droplet detection, classification, and tracking under various microscopy conditions. Neural networks can reverse-predict experimental parameters (*e.g.*, flow rate, concentration) from droplet images with high accuracy, assess mixing efficiency, and detect physical changes such as freezing or nucleation temperatures. Moreover, AI facilitates real-time decision-

making and automated quality control in digital microfluidic platforms, supports intelligent sorting and routing of droplets *via* reinforcement learning or evolutionary algorithms, and improves target isolation and purification when integrated with active sorting components.[109]

Demonstrating this potential, Anagnostidis *et al.* (2022) developed a deep learning-guided, image-based real-time droplet sorting system using convolutional neural networks (CNNs) to classify droplets into empty, single-object, or multiple-object categories, enabling precise, on-demand selection of micro-objects such as single mammalian cells, beads, and 3D cell cultures. Bright-field imaging combined with FPGA-triggered acquisition enabled high-speed image capture, and CNN outputs controlled dielectrophoretic deflection for sorting at rates of up to 40 droplets per second. Training required only a few hundred manually labeled images per class, augmented to thousands *via* rotations and flips, achieving over 90% accuracy for single-cell identification in under 10 minutes on a single GPU. The approach supported complex tasks such as identifying single cells in mixed populations, alternating between

specific droplet types, and enriching proliferating spheroids by four-fold. Unlike traditional fluorescence-based sorting, this AI method enabled robust morphological screening, tolerated droplet polydispersity and varied focal planes, and could be adapted for multiple object types without changing network architecture.[118] Together, these advances highlight AI's transformative role in enabling high-throughput, morphology-driven, and multiplexed droplet sorting for applications in single-cell analysis, organoid screening, and precision microfluidics.[109,118]

In addition to optimizing droplet sorting, AI also plays a pivotal role in enabling advanced image recognition within LoC systems, expanding their analytical and diagnostic potential. The integration of artificial intelligence (AI) into LoC platforms, particularly in smartphone-based mobile health (mHealth) systems, has significantly advanced image recognition capabilities for point-of-care diagnostics. These platforms combine microfluidic chips for automated sample handling, compact optical imaging modules (bright field, fluorescence, lens-free, or lensed systems), and AI algorithms for real-time image analysis. Traditional machine learning models such as support vector machines (SVM), k-nearest neighbor (KNN), random forest, and bootstrap aggregation have been employed for image denoising, region-of-interest detection, and quantitative analyses (e.g., colorimetric assays, fluorescence counting, pH classification), offering robust performance under varying environmental and device conditions. More recently, deep learning approaches, particularly convolutional neural networks (CNNs) and their advanced architectures (MobileNet, U-Net, Inception v3, ResNet, GANs), have enabled automated feature extraction for image enhancement, segmentation, classification, and regression, eliminating the need for manual feature engineering. These methods have been successfully applied to enhance smartphone microscope images to near bench-top quality, identify diseased cells, classify viral detection patterns, and quantify biomarkers. Furthermore, data augmentation using generative adversarial networks (GANs) and unsupervised learning strategies addresses the challenge of limited annotated datasets, improving model generalizability across diverse LoC applications. Collectively, AI integration transforms LoC systems into portable, cost-effective, and highly accurate diagnostic tools, capable of autonomous biomedical image processing and rapid, on-site decision-making across a wide range of clinical and environmental settings.[119]

Point-of-care testing (POCT) has become fundamental to modern healthcare, offering rapid diagnostic results directly at or near the patient and thereby facilitating timely clinical decision-making. The integration of artificial intelligence (AI) into POCT markedly enhances its impact by enabling rapid and accurate diagnostics through real-time synthesis of diverse patient data, which can improve clinical outcomes and increase survival rates, especially during time-sensitive situations such as infectious disease outbreaks. AI-driven predictive analytics optimize device uptime and resource allocation, leading to cost efficiencies and expanded testing capacity, particularly in resource-limited environments. Furthermore, AI-powered portable and mobile health platforms extend access to high-quality diagnostics into underserved regions, empowering frontline healthcare workers to deliver timely, precise care and thus bridging significant gaps in healthcare delivery.[120] Notably, deep learning models such as convolutional neural networks (CNNs) exhibit superior performance over traditional algorithms by effectively modeling complex, nonlinear relationships within high-dimensional datasets typical of multiplexed biomarker assays and image-based tests.[121] These advanced models not only provide increased diagnostic accuracy, robustness to imaging variability, and enhanced feature extraction from high-resolution or noisy data, but also contribute to greater sensitivity in critical applications—including reduced false negatives in myocardial infarction detection and improved classification in complex assessments like COVID-19 immunity profiling.[121] As assay complexity grows, AI-driven approaches ensure the scalability, adaptability, and reliability essential for effective POCT across diverse settings.[120,121]

Despite the transformative advantages that AI brings to point-of-care testing, integration of AI into POCT faces significant bottlenecks spanning regulatory, technical, ethical, and practical domains. The evolving nature of AI/ML models challenges current regulatory frameworks (e.g., FDA SaMD, EU AI Act) that struggle with approvals and post-market oversight of continuously learning algorithms, especially in low- and middle-income countries (LMICs) lacking established pathways and infrastructure.[120] Ensuring high-quality, diverse, and clinically representative datasets is difficult and costly, with issues in ground-truth labeling, sample reliability, and potential bias that undermine model validity and generalizability.[121] Many AI models remain "black-boxes", impeding clinician trust due to limited interpretability, and existing explainable AI (XAI) methods may trade accuracy for transparency or provide inadequate insights, particularly with small datasets. Strict data privacy laws (GDPR, HIPAA) require robust encryption and secure data management; cyber-security concerns and high implementation costs further complicate deployment, especially in resource-limited settings.[120] Effective integration also depends on comprehensive, context-sensitive provider training and seamless interoperability with legacy systems, but high staff turnover and inconsistent connectivity can impede both, particularly in rural environments. Regulatory fragmentation and inconsistent ethical oversight lead to further challenges in bias mitigation and equitable access, and advanced regions enforce strict validation, while frameworks are often lacking in LMICs.[120] Substantial initial investments, ongoing maintenance costs, energy constraints, and limited technical support restrict scalability and sustainability, even when long-term benefits are projected.[121]

In summary, realization of AI's promise in POCT is constrained by regulatory and data-related hurdles, transparency

and explainability limitations, training and infrastructural deficits, and unresolved ethical and equity concerns. Overcoming these challenges will require interdisciplinary collaboration, adaptive policy evolution, inclusive dataset curation, and focus on human-centered innovation.[120,121]

10. Future perspectives and conclusion

LoC technology integrates fluidics, electronics, optics, and biosensors into a miniaturized system, enabling laboratory functions with minimal sample volume, reduced reagent use, faster analysis, improved process control, and cost-effectiveness.[5,6]

Over time, LoC has advanced significantly, with innovations such as OoC models and, more recently, the integration of AI and machine learning. Importantly, these developments are not merely technical milestones but are shaping the clinical and medical utility of LoC platforms. The integration of LoC and AI is set to accelerate drug development and discovery, enable rapid and accurate detection of infectious diseases, and advance transfusion medicine by providing objective assessments of stored red blood cells. Thus, the future of LoC lies in its role as an AI-enabled clinical tool, with the potential to revolutionize diagnostics, therapeutic monitoring, and personalized medicine, offering a clear path from technological innovation to direct healthcare impact.

Author contributions

Bhagyashree Gupte: resources, data curation, investigation, methodology, visualization and writing – original draft. Umesh Jadhav: resources, data curation, investigation, methodology, visualization, conceptualization, supervision, validation, project administration and writing – review. Suresh Gosavi: supervision, validation, review editing. Shweta Jagtap: resources, data curation, investigation, methodology, visualization, conceptualization, supervision, validation, project administration and writing – review and editing.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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