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Droplet microfluidics for biomedical applications: emerging trends and future developments

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Abstract

Droplet-based microfluidics has witnessed tremendous progress in the past two decades, and this technique has been demonstrated as one of the most promising technologies to synthesize high-end and versatile materials with advanced functions, which provide great possibilities for numerous applications. Herein, the recent progress in preparing high-value natural microspheres by droplet-based microfluidic techniques is summarized comprehensively. We start with an in-depth articulation of the working principles of droplet-based microfluidics and surface modification for microdevices. Subsequently, droplet-based microspheres' fabrication methods have been discussed and summarized. Furthermore, the emerging representative biomedical applications of the different types of natural microspheres are outlined systematically. After that, we consider the challenges that hinder droplet-based microfluidic improvement in academic and industrial applications. Eventually, we will point out the perspectives of droplet-based microfluidics and aim to advance droplet-based microfluidics and sophisticated applications. The scope of this review is not only to offer an in-depth understanding of droplet-based microfluidics but also to open new pathways for versatile applications.

Introduction

Microfluidic technology facilitates precise control of the fluid flow and manipulation of the droplet at the microscale efficiently and promptly[1]. As an essential branch of microfluidics, droplet-based microfluidics has witnessed significant progress in various fields, including but not limited to chemical reactions[2–4], smart materials fabrication[5,6], cell screening[7], disease diagnosis[8,9], embolization therapies[10,11], and biological sample detection[12]. For example, in recent years, droplet-based microfluidic technology has been demonstrated to be much more effective and superior for biocompatible embolic microsphere preparation than conventional embolic pharmaceutics in terms of stability, structure, efficiency, and safety, since the fluid is easy to control[10,13,14].

Droplet-based microfluidics refers to generating a series of tiny droplets in a microdevice with a 1–1000 μm feature size by active or passive methods[15]. Active methods refer to droplets generated by external forces like magnetic, mechanical, light[16,17], or electric fields[18]. In contrast, droplet formation depends on the geometrical structures of the microdevices in the passive microdevices[19]. The T-shaped[20–22], Y-shaped[23,24], flow-focusing[25–28], and co-axial microchannel[29,30] are the most widely used structures. The specific surface area is significant in microfluidic devices owing to the small characteristic size, which results in high mass and heat transfer performance[31]. In addition, microfluidic technology provides numerous outstanding advantages, such as fast and efficient mixing performance[32], tiny sample consumption, and controllable fluid flow processes[33], overcoming the shortcomings of traditional production[34]. All the above-mentioned unique advantages endow droplet microfluidics with great potential applications in the biomedical field.

Droplet-based microfluidics has played an increasing role in microparticle/microgels fabrication in recent years; as a template for the synthesis of microparticles, droplet-

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based microfluidics enables the generation of microspheres with uniform size, high monodispersity, excellent mechanical strength, controllable structure, and well-controlled composition via manipulating fluid flow accurately at the submillimeter or microscale[35,36]. More interestingly, droplet-based microfluidics enables the fabrication of diverse microparticles/hydrogels according to the different practical applications by designing the geometrical structures of the microdevices. Additionally, droplet-based microfluidics allows the fabrication of microspheres or particle biomaterials in a compatible environment to ensure the safety of the biomaterials[37] so that these microparticles can be further applied in biomedical fields. Among various microspheres, natural polymer materials like chitosan[38,39], chitin[40], dextran, sodium alginate[41], and agarose[42] have attracted significant attention from academia and industry due to their unique advantages, such as intrinsic biocompatibility, excellent biodegradability, non-toxicity, ease of availability, and low cost[33,43]. Microspheres with tens to hundreds of microns in feature size have emerged as versatile and advanced biomaterials for various applications, including but not limited to tumor therapy[44], biological detection[42], medical diagnostics[45], drug delivery[46], and cell encapsulation[47]. Up to now, microspheres can be synthesized by conventional chemical and physical technologies, of which chemical methods include emulsification cross-linking[48–50] and reversed-phase suspension polymerization, and physical methods include mechanical stirring and phase separation[51]. Apart from droplet-based microparticle synthesis, active and passive droplet microfluidics have been integrated to flexibly and efficiently manipulate the droplets to realize transportation, mixing, splitting, merging, trapping, and sorting[46–49]. These droplet manipulation units can handle organic and inorganic liquids, as well as body fluids like blood, urine, and saliva[12], which facilitates biomedical detection and disease diagnostics automatically and flexibly. Controllable droplet manipulation at the microscale not only helps to enhance efficiency compared to the limited labor but also significantly minimizes the manual operation error during the repeat experiments[52,53], which contributes to the development of sophisticated biomedical applications. Despite the tremendous potential of microspheres/microgels in biomedical fields, microfluidic production of biocompatible microspheres/microgels remains a challenge regarding scale-up preparation, solidification strategies. More importantly, well-defined morphology and composites controlled precisely by microfluidic technologies are another vital area for biological and medical applications. Although published reviews discussed the recent advancement with the active and passive droplet-based microfluidic for biomedical applications, a comprehensive review involved droplet manipulation and corresponding biomedical applications is lacking[38,54–59].

Specifically, this review provides a comprehensive overview of the recent development of droplet-based microfluidics and addresses the advancements for biomedical applications. We systematically introduce the droplet generation mechanisms and summarize several typical microdevices for various types of droplet generation; we also summarize the surface modification technologies within microdevices for diverse droplet generation. Subsequently, we elaborately describe the methods for droplet-based microsphere/hydrogel fabrication. After that, we comprehensively summarized the biological and medical applications within droplets or droplet-templated microspheres. Finally, we present the emerging challenges of droplet-based microspheres for various applications and provide perspectives and outlooks for guiding future research directions.

Droplet generation in different microfluidic devices

In this section, we start with the droplet formation mechanism of different emulsion systems at the microscale. Subsequently, several surface modification strategies for microdevice treatment and their corresponding flow regimes are discussed. Then, several typical microdevices like T-junction, coaxial, flow-focusing, and step-structured microchannels are summarized. Finally, integrated microdevices for droplet high-throughput preparation are outlined.

Compared with the dispersion process regulated by the gravity and inertial forces in the conventional devices, at the microscale, the forces between fluid and fluid, fluid and the wall of the microchannel undergo dramatic variations, the specific surface area will increase significantly, and the velocity gradient of the fluid will also increase dramatically. Therefore, the interfacial tension and viscous shearing force will improve progressively and then replace the gravity and inertia, becoming the dominant forces controlling the entire flow and micro-dispersion process. To figure out the intrinsic mechanism of micro-dispersion, dimensionless parameters are utilized to describe the comparison between different forces. As expressed in Eq. (1), the Reynolds number (Re) represents the ratio between inertial and viscous forces. The capillary number (Ca) refers to the ratio between viscous force and interfacial tension, and the Weber number (We) means the ratio between inertial force and interfacial tension; the corresponding expressions are in Eqs. (2) and (3). Two or multiple immiscible fluids are employed to fabricate droplets with the interfacial tension, inertial force, and viscous shearing force working together, as illustrated in

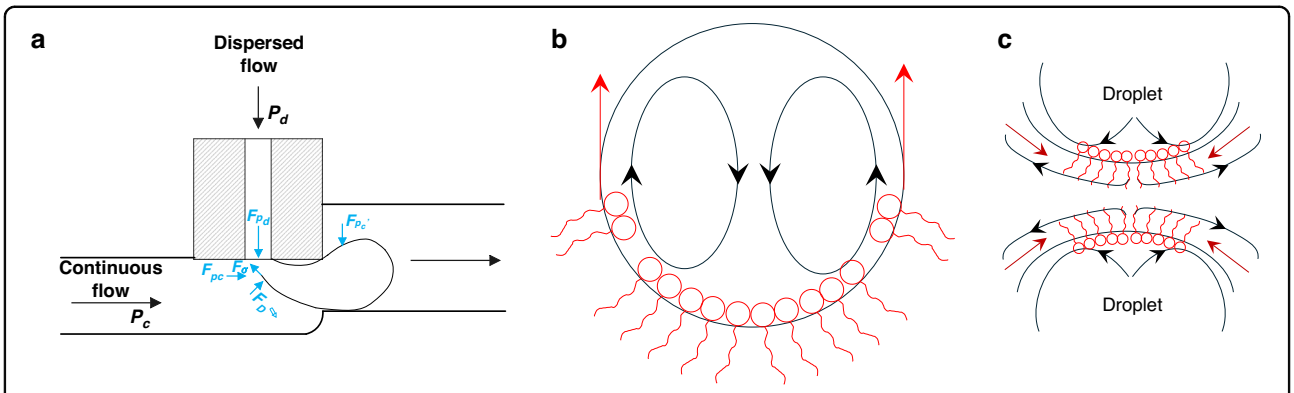

Fig. 1 Droplet formation mechanism in the microchannel. a Working forces in a stepwise junction microchannel for the droplet generation process. **b** Surfactant concentration distribution around the droplet, Marangoni effect. **c** Marangoni stresses exerted between the droplets and form a liquid film to avoid droplet coalescence[69]

Table 1 The frequently used dimensionless parameters at the microscale[2]

Dimensionless numbers	Expression	Physical significance
Capillary number (Ca)	$Ca = u_c\mu_c/\gamma$	The ratio between the viscous shearing force and the interfacial tension
Weber number (We)	$We = Ca \cdot Re = \rho l u^2/\gamma$	The ratio between the inertial force and interfacial tension
Reynolds number (Re)	$Re = du\rho/\mu$	The ratio between the inertial force and the viscous shearing force
Ohnesorge number (Oh)	$Oh = \mu l/(\rho\gamma l)^{1/2}$	The ratio between the viscous shearing force and the inertial force, and the interfacial tension
Viscosity ratio α	$\alpha = \mu_d/\mu_c$	Viscosity ratio of the dispersed phase to the continuous phase
Flow rate ratio β	$\beta = Q_d/Q_c$	Flow rate ratio of the dispersed phase to the continuous phase

l is the characteristic size of the microchannel, d is the feature size of the microchannel, ρ is the density of the fluid, μ is the viscosity of the fluid, and γ is the interfacial tension between two phases, u_c and μ_d are the velocity of the continuous phase fluid and dispersed phase fluid, respectively; Q_c and Q_d are the velocity of the continuous, and dispersed phase, respectively

Fig. 1a[60].

$$\mathrm{Re} = \mathrm{du}\rho/\mu \tag{1}$$

$$\mathrm{Ca} = u\mu/\gamma \tag{2}$$

$$\mathrm{We} = \rho u^2 d/\gamma \tag{3}$$

where d is the characteristic size of the microchannel, u, ρ, and μ are the fluid's velocity, density, and viscosity, respectively. In Eq. (2), γ is the interfacial tension between two phases. Generally, the capillary number values vary from 10^{-3} to 10. Apart from the dimensionless parameter, the flow rate ratio (Q_d/Q_c) and viscosity ratio (μ_d/μ_c) between two phases are always employed to describe the effect of the operating conditions on the micro-dispersion process and droplet size. The frequently used dimensionless parameters at the microscale are summarized in Table 1.

Droplet generation mechanism at the microscale

The geometries of microdevices, dispersion methods, physical properties of the fluids, and operating conditions primarily control the uniformity and monodispersity of droplets generated in microdevices. Additionally, the dispersion mechanism is always determined by the force variation in the microfluidic system, particularly the regulation of interfacial tension and the viscous shearing force. In this section, we systematically summarize four types of droplet formation processes (water-in-oil, oil-in-water, water-in-water, and multiple emulsion droplets) at the microscale.

Water-in-oil droplet generation

The water-in-oil single emulsion is ubiquitous and is significant in applications like particle materials synthesis[61,62], chemical reactions[63,64], the food industry[65], and cosmetics[66,67]. Uniform and monodispersed water-in-oil droplets are commonly generated in microdevices, which are made of hydrophobic materials such as polymethyl methacrylate (PMMA), polydimethylsiloxane (PDMS), copolymers of cycloolefin (COC), etc. The matched materials and fabrication methods for microdevice fabrication have been summarized in our previous review[68]. In hydrophobic microdevices, oil liquid is selected as the

continuous phase, whereas water liquid is utilized as the dispersed phase; two immiscible fluids are transported by pressure pumps or syringe pumps into the corresponding microchannels to generate microdroplets with controllable size and morphology. In the water-in-oil droplet generation process, it is essential to incorporate a suitable amount of oil-soluble surfactants like Span 20, Span 65, and Span 80 into the continuous phase. These surfactants, on the one hand, help to modulate the interfacial tension and prevent droplet coalescence. Among them, non-ionic Span 80 is the most used[69], particularly in combination with hydrocarbon oils like mineral oil, soybean/corn oil. The surfactant's amphiphilic molecular structure, consisting of both hydrophobic and hydrophilic groups[33], and in liquid–liquid systems, the surfactant molecules diffuse in the bulk phase, then absorb at the liquid–liquid interface[69]. On the one hand, the surfactant would significantly lower the interfacial tension of the two immiscible liquid–liquid phases and enable the droplet generation to become more accessible, considerably impacting the droplet size. The decreased degree of the interfacial tension is determined by the absorbed amount of the surfactant molecules at the interface as described by the Gibbs adsorption isotherm in dilute solutions as described in Eq. (4)[70,71].

$$\frac{\Gamma}{\Gamma_{eq}} = \frac{c}{a_L + c} \tag{4}$$

where Γ_{eq} is the surfactant concentration on the surface of the droplet at the thermodynamic equilibrium, c is the concentration of the surfactant in the continuous phase, a_L is determined by the surfactant concentration in the bulk phase, also known as the Langmuir parameter. It is worth noting that the values of the Γ_{eq} significantly vary with the different surfactants according to the reported research[71]. The critical micelle concentration primarily depended on the amount of surfactants added to the microfluidic devices. When the surfactant concentration is below the critical micelle concentration, the interfacial tension generally decreases with the increased surfactant concentration in the system. Typically, reduced interfacial tension is conducive to droplet formation in the microdevices. Moreover, the added surfactants play a critical role in stabilizing generated droplets by stabilizing the liquid–liquid interface further to prevent the coalescence of the droplets[72]. Moreover, the surfactant distribution around the droplets is considerably affected by mass transportation limitations[73], and the surfactant distribution will further hinder the droplet generation and the stability of the generated droplets. Generally, the surfactant will flow unevenly and thereby will form a concentration gradient at the surface of the generated droplets and eventually create a stress gradient in the opposite direction of the flow, this phenomenon is the notable Marangoni effect as displayed in Fig. 1b[69,74]. The droplet coalescence phenomenon can be effectively prevented when the surfactant absorption time at the interface exceeds the droplet formation time (Fig. 1c)[75].

For hydrophobic modification, as displayed in Fig. 2b(i), a facile one-step methodology was found to synthesize superhydrophobic materials by stoichiometrically regulated reaction between water and long organ-silanes, which enabled the creation of nano- or micro-scale siloxane and dispersed those siloxanes in solvents to improve the surface properties[76], the prepared solution was employed to coat the glass, after coating modification, the surface morphology of the glass is illustrated in Fig. 2b(ii), the Scanning electron microscope (SEM) images show that particles aggregated on the surface, leading to the roughness of the surface further enhances the hydrophobicity of the surface. The hydrophobic properties would be improved significantly by simply coating these synthesized materials on different substrates, the water contact angle reaches up to 170°, whereas the sliding angle is lower than 1° (Fig. 1b(iii)), which indicates that these superhydrophobic coatings exhibit excellent properties in terms of synthesis method, cost, applicability, robustness, etc. Apart from the preparation of the superhydrophobic coating, a magnetic-actuated miniature robot (Fig. 2a(i)–(ii))[77], which involved surface-modified steel beads, was developed to manipulate the droplet further to exploit more applications. For water-in-oil droplet manipulation, as demonstrated in Fig. 2a(viii), hydrochloric acid, hydrofluoric acid, and deionized water are mixed in a certain proportion to etch the steel beads to manufacture superhydrophobic steel beads, followed by washing the steel beads with ethanol, acetone, and deionized water. Eventually, the etched steel beads were dried with nitrogen. For oil-in-water droplet or bubble-in-water manipulation, as illustrated in Fig. 2a(ix) and (x), steel beads were modified by 1H,1H,2H,2H-perfluorodecyltrimethoxysilane (PFOTS) by chemical vapor deposition (CVD) at 80 °C for 4 h. After superhydrophobic modification, the water contact angle can reach up to ~158° (Fig. 2a(xi)). To manipulate droplets well and develop more possible applications, PFOTS was utilized to modify the surface properties of the silicon wafers by CVD at 80 °C for 4 h (Fig. 2a(iii)–(vi), (x), and (xii)).

Oil-in-water droplet generation

The oil-in-water single emulsion refers to oil dispersed in an aqueous solution, which is another important emulsion and has always been encountered in practical applications like the cosmetic, pharmaceutical, and food industries. Similar to the water-in-oil droplet formation in microdevices, the oil-in-water droplet is generally fabricated in hydrophilic microdevices, and water-soluble surfactants like sodium dodecyl sulfate (SDS), polyoxymethylene sorbian fatty acid esters (Tween) are usually

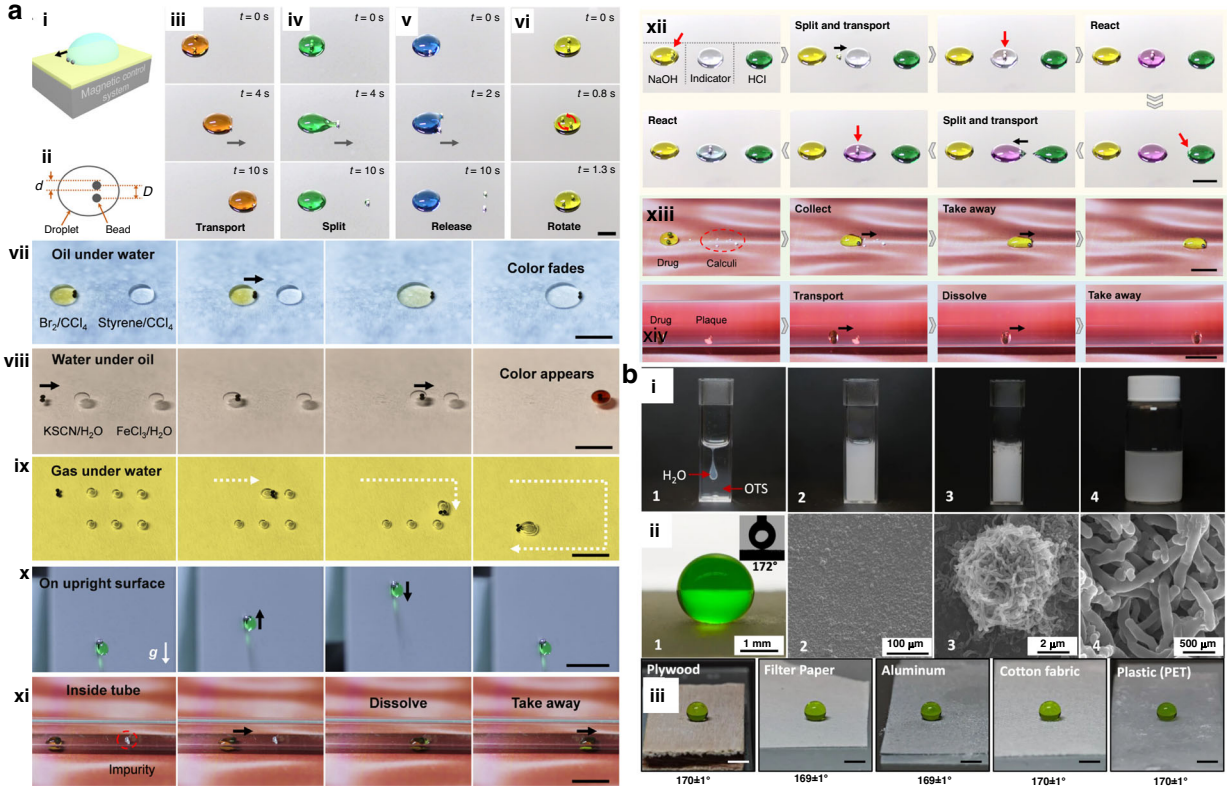

Fig. 2 The hydrophobic and hydrophilic modification of the steel beads or the substrate for droplet manipulation. a The demonstration of the magnetic-actuated miniature robot for droplet manipulation, Copyright (2020), with permission of the Science[77]. **b** hydrophobic coating materials fabrication for substrate modification, Copyright (2021), with permission of the Nature[76]

present in the water phase to coordinate the interfacial tension of the two phases and stabilize the fabricated droplets. The materials used to fabricate microdevices are generally hydrophobic. Therefore, the modification technology of hydrophobic microchannels for oil-in-water droplet preparation is very important. The popular modification methods involved nanoparticle coating, water-soluble surfactant immersion[78,79], quartz capillary embedding[80], CVD, etc. As illustrated in Fig. 6, to improve the throughput, a 3D-printing-integrated hydrophobic microdevice with 40 droplet generators was designed to fabricate both water-in-oil and oil-in-water emulsions on a large scale[81], and hydrophilic surface modification has been successfully confirmed.

Water-in-water droplet generation

With the increased concern for public health and rapid development of biomedical, aqueous two-phase (ATPs), which consist of two immiscible aqueous solutions, are increasingly attracting researchers' attention due to the mild surrounding environment. The aqueous environment enables the handling of certain active biomolecules like proteins and liposomes without denaturation[82], which enables more biomedical applications to become possible. ATPs commonly contain two specific hydrophilic substances, and both can be dissolved in water to form a homogeneity that separates into two water-rich phases when their concentrations are up to a critical value. From the thermodynamic perspective, phase separation will occur when the entropic driving force in favor of mixing is less than the entropic losses[83,84]. Typical ATP systems include two incompatible polymer solutions, or one is a polymer solution, and another is a salt solution[85]. Among them, the most commonly constructed ATPs are the PEG–dextran system, and molecular weight and concentration are pivotal factors in forming ATPs.

Although ATPs have absolute advantages for various biomedical applications, the extremely low interfacial tension makes the formation of the droplets in microdevices under dripping flow extremely difficult; on the contrary, the jetting flow regime is easy to form, and the droplet formation process is predominated by the viscous shearing force and interfacial tension, which cause the poor monodispersity of the droplets. Intensive efforts have been devoted to overcoming this challenge, including designing an asymmetric structure to strengthen the

micro-dispersion process[86], operating at much lower flow rates of two phases to reduce the viscous shearing force and inertial force[87], and introducing the external force like mechanical force[88], acoustic wave[89,90], electric field[91], magnetic field[92], and other forces to intensify the droplet formation process and accelerate the droplet breakup. In summary, the ATP droplets generated in microdevices always combine with some other external forces.

Multiple emulsion droplet formation

Multiple emulsions are regarded as versatile templates of functional microcapsules owing to their abundant geometries and various compartments[93,94]. Coaxial and flow-focusing microdevices are widely used to generate numerous emulsions with controlled structures and regulated components for diverse applications. As demonstrated in Fig. 3a, co-axial microdevices with multiple substructures were designed to fabricate multiple emulsions, a horizontal co-axial microchannel with a transition tube was intended to prepare monodispersed oil-in-water-in-oil emulsions, and then controllably synthesize monodisperse alginate microcapsules with oil cores using emulsions as templates in the presence of acetic acid, the acid environment will induce the calcium ions to release from the complicated calcium solution then occur an ion cross-linking reaction with alginate (Fig. 3a(ii)–(iii)). The thickness of the oil cores (Fig. 3a(vii)–(ix)), the morphology of the oil cores (Fig. 3a(vi)–(xii)), and the number of the cores (Fig. 3a(v)) would be precisely controlled by experimental operating conditions and the concentration of the alginate solution. Researchers found that although the concentration of the alginate solution has a negligible effect on the thickness of the microcapsules, it significantly improves the stability of the emulsion and microcapsules. Microcapsules with a size range from 46.4 to 110.2 μm were prepared successfully. As illustrated in Fig. 3b, multi-stage microdevices (Fig. 3b(ii)–(iv)) were constructed to prepare highly uniform multiple emulsions with multiple components controllably[95]. The results provide a new pathway to precisely encapsulate the drug molecules, biological molecules, biological actives, and chemicals, making drug delivery, biological molecule transportation, dosage injection, and small doses of chemical reaction possible. Furthermore, this versatile platform enables the synthesis of multicompartment materials efficiently and controllably.

Surface modification technologies for microdevices

Surface properties like wettability and adhesion always determine the performance of the microdevices, thereby constraining the related applications[96]. To enable droplet-based products to meet the application and market demands, some surface treatment technologies are proposed to modify the surface of the microchannel into hydrophilic or hydrophobic to address this problem. Chemical composition and roughness of the microchannel surface are the two widely modified aspects to regulate the wettability of the microchannel surface[69,97]. Widely used microdevices made of glass and silicon have inherent hydrophilic surfaces, which result in extremely low flow resistance; these microdevices are suitable for oil-in-water droplet preparation[98]. Apart from glass- and silicon-based microdevices, polymer-based microdevices are attracting considerable attention owing to their outstanding characteristics, such as low cost and versatile fabrication methods. However, most polymer materials are hydrophobic and have intrinsic low surface energies, which makes them unsuitable for oil-in-water droplet generation.

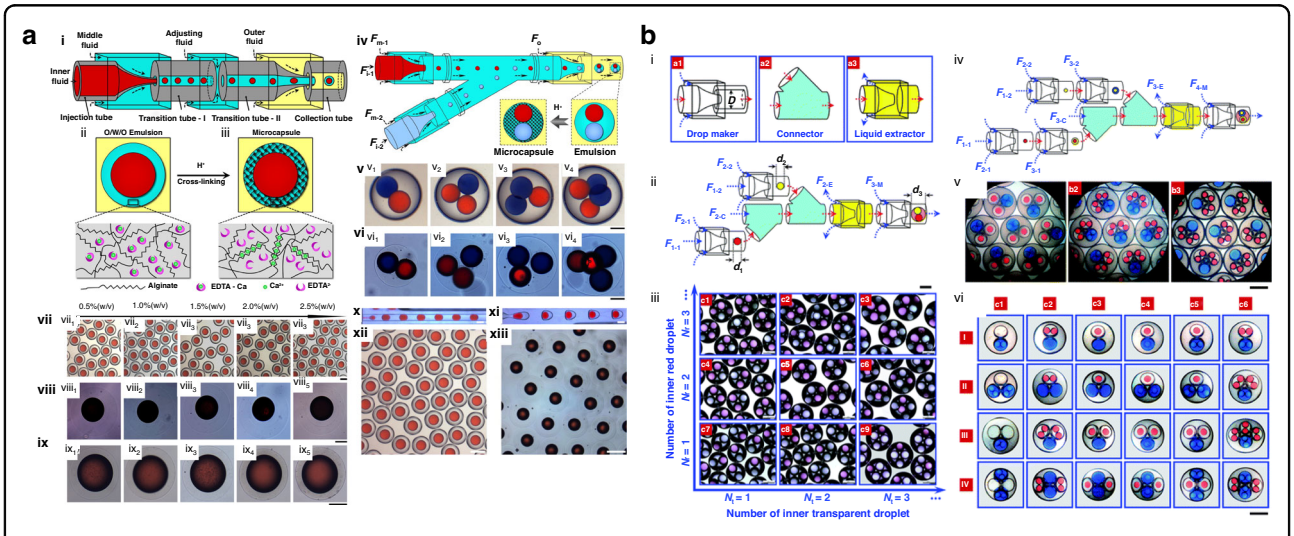

Fig. 3 Multiple emulsions are generated in the co-axial microfluidic device. a Oil-in-water-in-oil emulsion fabrication Copyright (2020), with permission from Elsevier[93]. **b** Copyright (2011), with permission of the Royal Society of Chemistry[95]

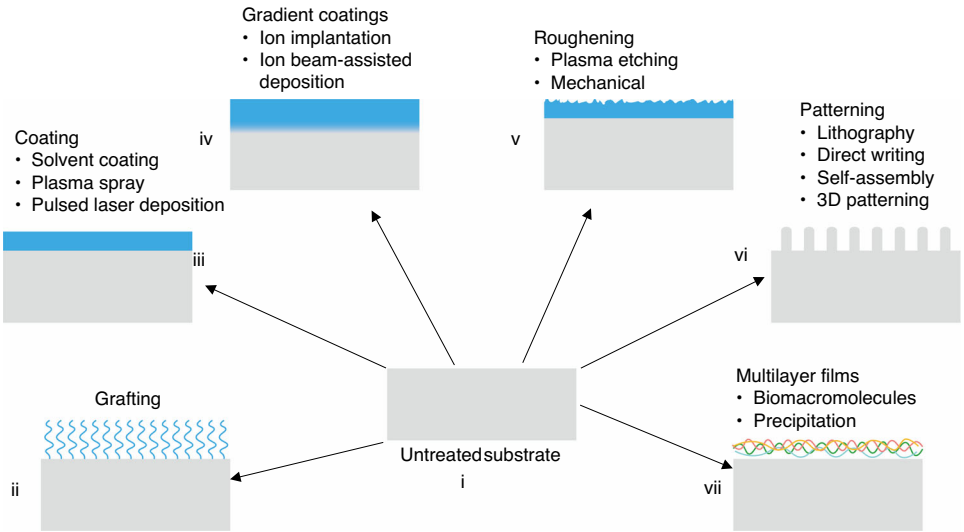

Fig. 4 The methods for the microchannel surface modifications. i. Untreated substrate, **ii**. grafting, **iii**. coating, **iv**. cradient coatings, **v**. roughening, **vi**. pattering, and **vii**. multilayer films. Copyright (2014), with permission of the Oxford Academic[99]

The chemical modifications[99] refer to introducing some functional groups or molecules to activate or passivate the surface of the microchannel. In contrast, physical modification technology generally improves the roughness by exposing the microchannel surface to the laser or plasma[100]. The frequently utilized modification methods are illustrated in Fig. 4 to compensate for their intrinsic disadvantages.

Apart from the surface modification strategies, as previously mentioned, surfactants are also widely added to the continuous phase to pretreat the microchannel and accommodate a wide range of applications. Table 2 summarizes various surfactants employed in microdevices for different applications. Generally, surfactants are involved in the continuous phase to generate droplets[92] and manipulate droplets or nanoparticles flexibly in passive and active microdevices[101].

Additionally, it is worth noting that for biomedical-related applications, biocompatible surfactant Pico-Surf^{TM} is always the top-priority selection due to its easy evaporation for microparticle separation from the continuous fluid, and is biocompatible with biological and medical test samples. However, the only drawback is that the price of the Pico-Surf^{TM} is high and unacceptable for researchers and industrials in low-added chemicals or regular applications. HFE7500 and matching surfactant Pico-Surf^{TM} are selected to achieve sophisticated detection and high-value-added microparticle synthesis goals.

Microfluidic devices for droplet generation

Microdevices are the key components of micro-dispersion; the geometry, surface properties of the materials, operating conditions, and physical properties of the system will significantly affect the micro-dispersion process. Among these factors, the impact of the geometries of microdevices on micro-dispersion is worth paying attention to. The geometry of microdevices has always been an important research direction since the emergence of microfluidics. The typical simple microdevices can be classified into several types: T-junction[21,22,102], Y-junction[103,104], cross-junction[105–107], flow-focusing[26,108–112], co-flowing[19,113], and step structure[114,115]. The structures of these microdevices and corresponding flow regimes are displayed in Fig. 5. Among these structures, the T-junction, flow-focusing[25,116,117], and co-flowing are most commonly used in industry and academic fields[15,118]. The T-junction is most popular due to its simple structure and facile fabrication methods, particularly in liquid-liquid micro-dispersion. In contrast, co-flowing or flow-focusing is a method that is more suitable for microsphere or microcapsule preparation[119–121]. In recent years, some integrated microdevices have also been developed to translate academic results into practical or industrial applications. Moreover, with the rapid development of artificial intelligence (AI), this evolutionary technology is expected to predict and assist in solving numerous complicated issues in droplet microfluidics, nearly without human intervention. Specifically, machine learning algorithms have been developed to assist in predicting the droplet generation performance involving the droplet size and droplet generation frequency in a flow-focusing microfluidic devices[122], which further utilized to optimize the design of the droplet microdevices. Apart from predicting the droplet size and droplet generation frequency, deep learning was also employed to assist in designing the configuration of the droplet generators[123].

Table 2 Surfactants in the continuous phase of the microdevices for different applications

Microdevices	Continuous fluid	Surfactant	Applications	References
PDMS flow-focusing microdevice	Glycerol–Ethanol	Poloxamer 188	Double emulsion droplet-based liposome preparation	192
PDMS flow-focusing microdevice	Fluorinated oil: HFE-7500	Fluoro-surfactant	Water-in-water droplet-based cell-laden microgel production	51
PMMA-based stepwise microdevice	Mineral oil	Span 80	Water-in-oil droplet-based chitosan microsphere preparation	148
PMMA-based stepwise microdevice	Mineral oil	Span 80	Water-in-oil droplet-based dextran microsphere preparation	193
PMMA-based capillary-embedded stepwise microdevice	Mineral oil	Span 80	Microdroplet-based PSQ Microsphere fabrication	194
3D-etched silicon-glass integrated wafer	hexadecane	Span 80	Double emulsion generation	179
Porous glass membrane integrated microdevice	Soybean oil, mineral oil, and tetradecane	Span 80	Emulsification and stabilization of the formed droplets	195
Methacrylate (MA)-based parallelized flow-focusing microdevice	(50:50) mixture of mineral oil and heptane	Span 80	Emulsions and microgels preparation	180
Digital microdevices	Fluorinated oil: Novec 7500 Engineered fluid	Biocompatible surfactant: Pico-Surf 1, Sphere Fluidics	Automatic detection of biomarkers in human plasma	136
PDMS-based flow-focusing microdevice	Fluorinated oil: HFE 7100 and HFE 7500	Self-designed dendronized fluorosurfactants	PCR in droplets	101
PMMA-based capillary-embedded stepwise microdevice	Glycerol–water solution	SDS	Oil-in-water droplet formation	124
PDMS-based integrated step emulsification microdevice	Fluorinated oil: Novec^{TM} 7500 Engineered Fluid	Pico-Surf^{TM}	Scalable production of biomedical microparticles	196
PDMS-based integrated microdevice	Fluorinated oil: HFE7500	Perfluoronated alcohols (PFA)	Cell encapsulation	197
PDMS-based microdevice	Fluorinated oil	Fluorinated surfactant	Single-cell high-throughput mammalian screening	198
PDMS-based microdevice	Mineral oil	Span 80	Core-shell gelatin methacrylate microgel	199
3D-printed microchannel	2 wt% PVP aqueous solution	SDS	Polystyrene microsphere preparation	80

SDS sodium dodecyl sulfate, *PVP* polyvinylpyrrolidone

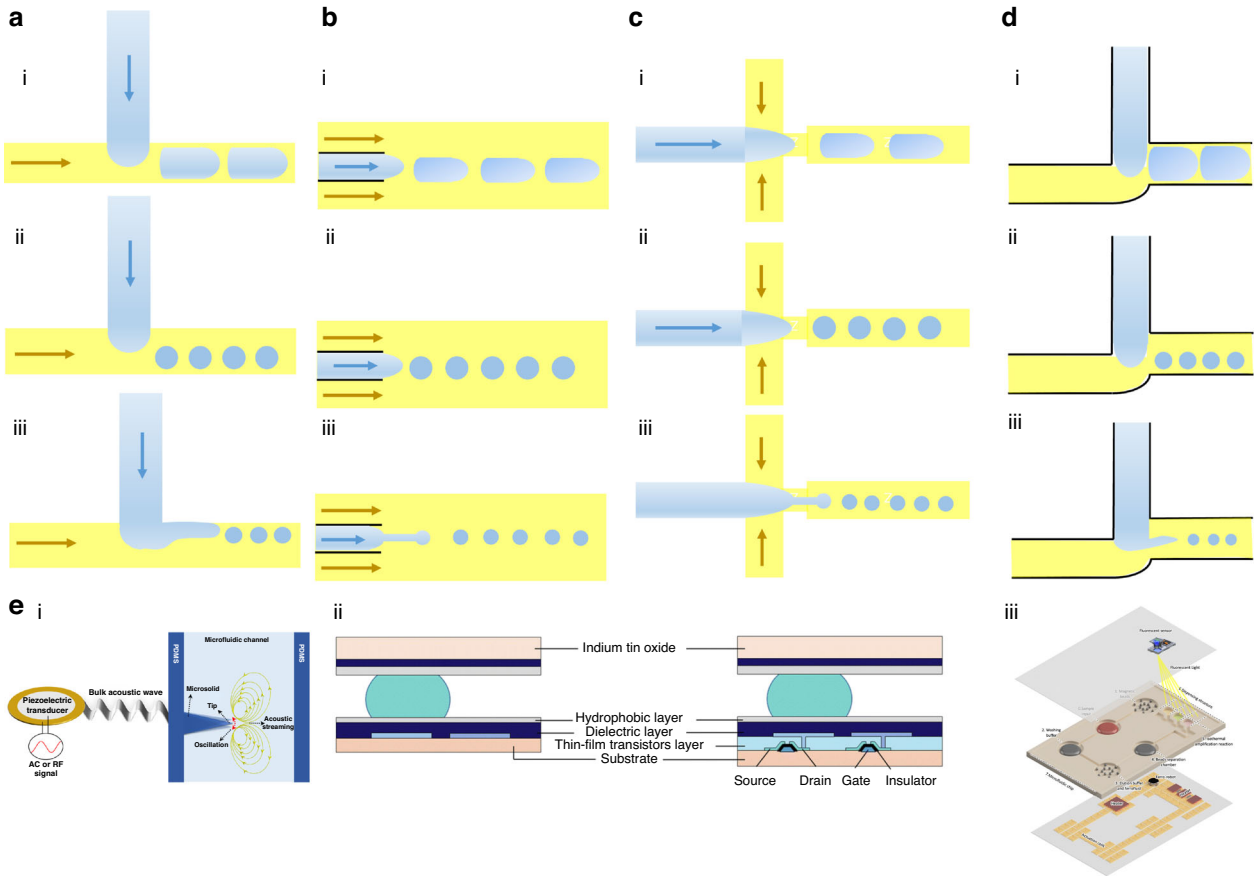

Fig. 5 Passive and active microdevices for droplet generation. a T-junction microfluidic device for droplet generation (i. squeezing flow regime, ii. dripping flow regime, iii. jetting flow regime). **b** Co-axial microfluidic device for droplet formation (i. squeezing flow regime, ii. dripping flow regime, iii. jetting flow regime). **c** Flow-focusing microfluidic device for droplet production (i. squeezing flow regime, ii. dripping flow regime, iii. jetting flow regime). **d** Step T-junction microfluidic device for droplet generation (i. squeezing flow regime, ii. dripping flow regime, iii. jetting flow regime). **e** Active microdevices for droplet generation and manipulation (i. acoustic-based microdevice, ii. electrowetting on dielectric microdevice, iii. magnetic-based microdevice for droplet manipulation)

The methods for droplet generation involve passive and active methods[19]. The active and passive methods for droplet generation at the microscale are illustrated in Fig. 5. For passive methodology, vertical shearing[20,21,124,125], cross-shearing[105], geometrical break-up[28,126], and hydraulic focus shearing are the widely used droplet generation methods; the above-mentioned micro-dispersion processes in T-junction microdevices are displayed in Fig. 5[33,127]. The cross shearing refers to continuous and dispersed phase fluids flowing in the main microchannel and sub-microchannel, respectively; the dispersed phase fluid breaks up and forms droplets periodically with the shearing force exerted by the continuous phase fluid (Fig. 5a). The vertical shearing means that the continuous phase in the sub microchannel shearing the dispersed phase fluid and droplet formation (Fig. 5b). Geometrical break-up refers to the droplet generation controlled by the different geometries, such as steps[115,128], narrow gaps[129], sharp tips[130,131], etc. Except for the passive method, the active droplet generation method has attracted more attention recently due to its flexibility and high integration. Active methods typically involve external forces like magnetic[132,133], electric[134,135], and acoustic[89,90,130,131]. Magnetic-based droplet generation microdevices have been developed for biological sample transportation[136], droplet manipulation[77], and automatic virus testing[137]. Acoustic-assisted microdevices have been developed for nanomaterials synthesis and chemical engineering[90].

Integrated microdevices for droplet high-throughput preparation

To translate laboratory research results into industrialization and commercialization, researchers are dedicated to exploiting the integrated microdevice to improve the droplet generation throughput[81,98,138]. As presented in Fig. 6a, an integrated microdevice equipped with multiple

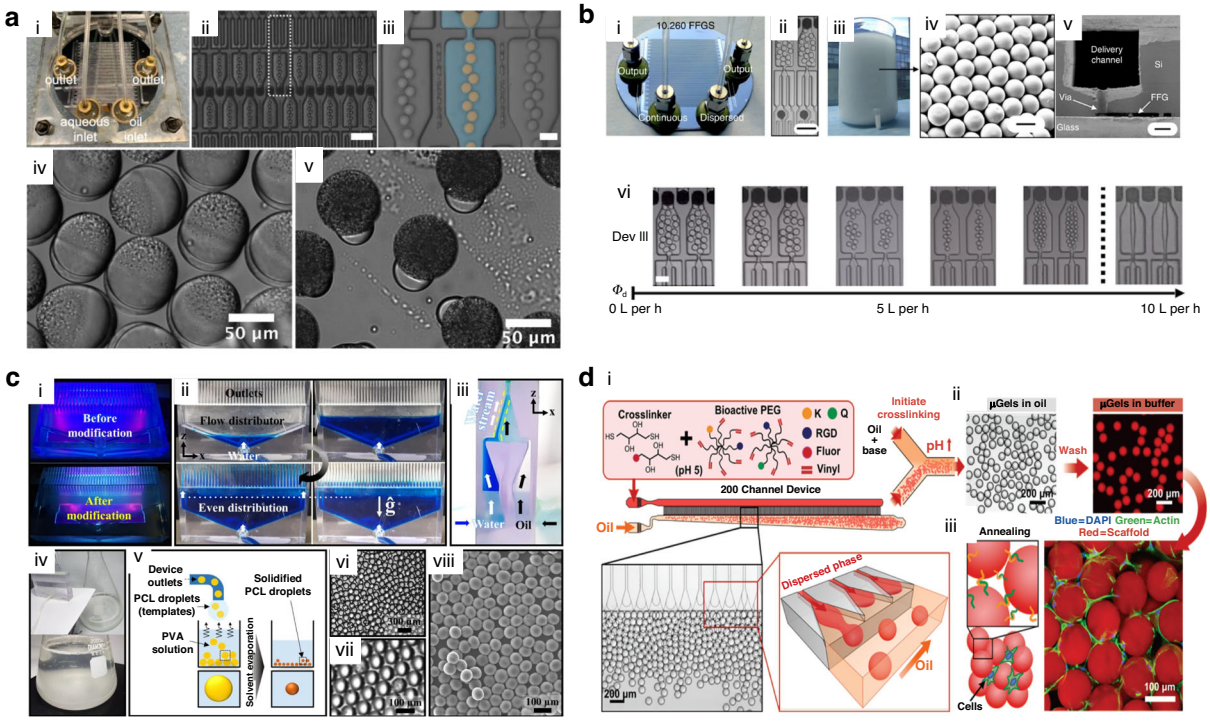

Fig. 6 Scale-up microdevices for microsphere fabrication. a Integrated flow-focusing microdevice for Janus microparticle fabrication. (i) Picture of the silicon-glass microfluidic device for Janus particle scalable production. (ii) The image of droplet formation in the part of the microfluidic device. (iii) The photo of droplets generation a microfluidic generator. (iv) and (v) The microscopy images of Janus particles produced by the integrated microfluidic device under different operating conditions. Copyright 2020, with permission of American Chemical Society[138]. **b** Integrated microdevice incorporating 10,260 droplet generators for polymer microparticles fabrication on a large scale, Copyright 2018, with permission of Nature[98] **c** A parallelized 3D-printed mill-fluidic device for polycaprolactone microparticle preparation in a robust and scalable manner, Copyright 2022, with permission of Elsevier[81]. **d** An overview of microgel formation in situ (i) the bioactive precursors and crosslinkers are transported into a parallelized emulsification microdevice, (ii) organic base is added into the oil phase to increase the pH and therefore induce a cross-linking chemical reaction, (iii) microgels are seeded with cells in solution covalently linked together in situ, Copyright 2019, with permission of Wiley[139]

flow-focusing droplet generators was developed to fabricate amphiphilic soybean oil polymer (SBOP)/ethyl cellulose (EC) Janus particles[138], the fabricated Janus microparticles with uniform size, and controllable structures, which provide a feasible strategy to prepare water-in-oil emulsion for versatile microparticle preparation on a large scale. As displayed in Fig. 6b, an integrated microdevice incorporating 10,260 droplet generators made of silicon and glass was designed to fabricate polymer microparticles on a large scale[98]. The throughput in an integrated microdevice is 4 orders of magnitude greater than the microdevice involving a single droplet generator. It is worth noting that a high aspect ratio flow resistor was designed into this microdevice to alleviate the trade-off effect between the number of droplet generators and the maximum throughput of each droplet generator. The robustness of the microdevice has been demonstrated by the synthesis of the biocompatible microspheres, 8–16 μm polycaprolactone microparticles with a variation coefficient (CV) smaller than 3% have been fabricated in this integrated microdevice successfully[98]. The microparticle production rate in this parallel microdevice can reach up to 277 $g \cdot h^{-1}$ with <5% CV. Water-in-oil droplets or emulsions are the most common templates to fabricate microparticles; however, in practical applications, oil-in-water droplets or emulsions are usually encountered and desired. Unfortunately, the lack of a robust and straightforward microfluidic platform has hindered the development of the mass production of oil-in-water droplets or emulsions, further limiting oil-in-water-based applications. To address this key problem, as illustrated in Fig. 6c, a milli-microfluidic microdevice with 40 droplet generators was invented to produce both water-in-oil and oil-in-water emulsions in a scalable manner[81]. Both the experimental and computational fluid dynamics simulation results demonstrated the feasibility and validity of the droplet generators and the fluid distributor, as well as the hydrophilic surface modification methodology for producing water-in-oil templated poly (ethylene glycol) microgels and oil-in-water templated polycaprolactone microspheres. This research provides a unique strategy to prepare oil-in-water and water-in-oil droplets in a robust and

scalable manner, further offering more possible opportunities in different fields, including versatile microparticle preparation, emulsion production, cosmetic products, and chemical reactions. Additionally, as demonstrated in Fig. 6d, to overcome the trade-off effect between material properties and pore size as well as enhance the microparticle production rate simultaneously[139], a step structure microfluidic device was proposed to manufacture the bioactive microgel flexibly, scalably, and reproducibly.

Droplet-based biomaterials microsphere fabrication (Methods for polymer biomaterials fabrication)

This section elaborates on several approaches for fabricating biomaterials in microdevices, especially natural biomaterials like chitosan, agarose, sodium alginate, dextran, and other biocompatible hydrogen microgels[129,130]. Chemical cross-linking reaction[140,141], UV-induced solidification[141,142], polymerization[143], solvent evaporation[144], and interfacial or self-assembly[145,146] are the most widely used methodologies for fabricating biomaterials in microfluidic devices[58].

Polymerization

Polymerization is one of the most popular approaches to transform droplets or emulsions into versatile microparticles. Polymerization approaches include photopolymerization[91], thermal polymerization, and chemical polymerization. Generally, monomers or oligomers, and initiators are added into the dispersed phase and exhibit uniform concentration in the dispersed phase after stirring or vortex operation. The monomer will undergo the polymerization reaction in the presence of a matched initiator, such as thermal, acid, sodium, or photo-initiator. The generated droplets will solidify into microparticles with heating, ultraviolet light, sodium acid, or polymerization reaction. Among the aforementioned solidification methods, thermal-induced polymerization refers to the process in which a thermal initiator generates radicals at a specific temperature, facilitating polymerization. However, on the one hand, high temperature easily disturbs the interface. Besides, high temperature tends to damage some biological or medical components, which limits its related applications. Ultraviolet is one of the most prevalent approaches owing to its fast response time, which induces solidification within seconds, thereby preventing coalescence. Additionally, ultraviolet polymerization can avoid the clogging of the microchannel because only the initiator is dispersed in the fluid, and UV light is generally at the outlet of the microchannel. For instance, as illustrated in Fig. 7a, a flow-focusing PDMS microdevice was designed to fabricate PEG–Dextran hydrogel microspheres with different structures, specifically, 20 wt% dextran and 40 wt% PEGDA water solution formed ATPs and were injected into the first cross-junction microchannel (Fig. 7a(ii)), and water droplets formed in the second cross-junction microchannel with the fluorocarbon oil shearing. Then, the water droplets were solidified with UV beam curing in situ[82].

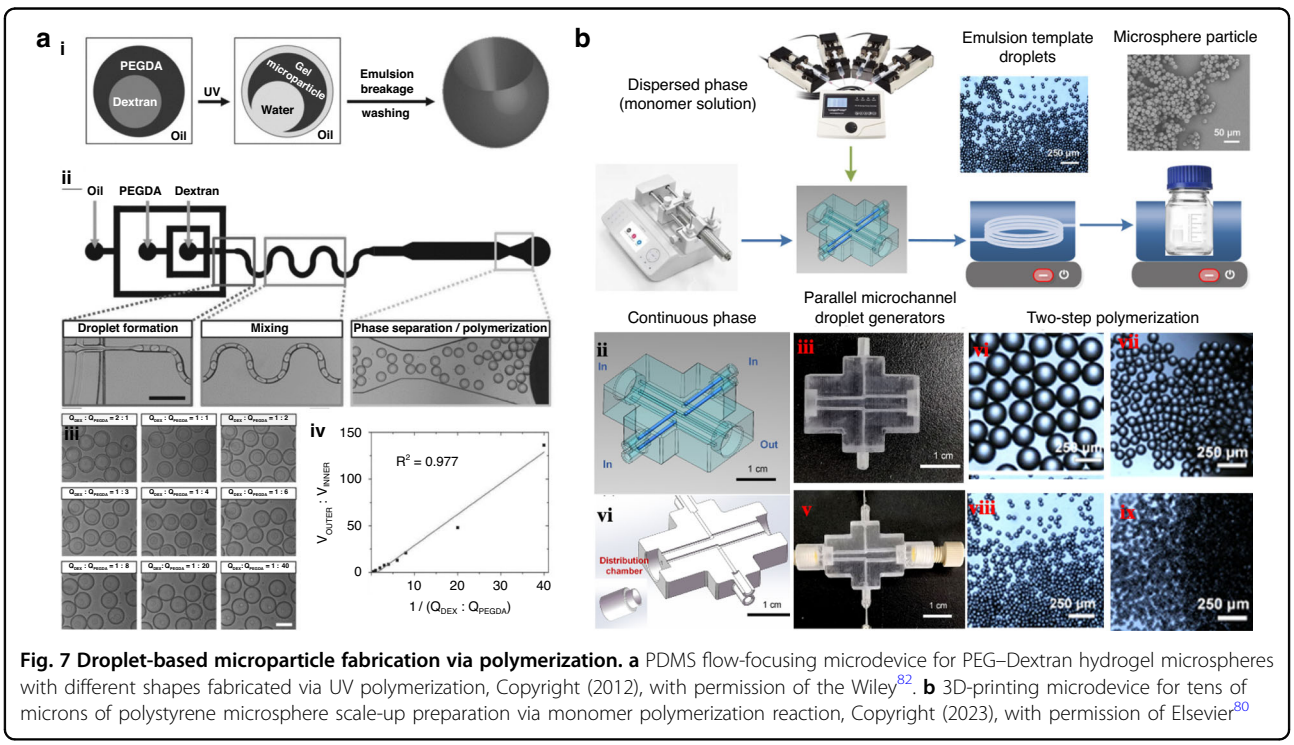

Fig. 7 Droplet-based microparticle fabrication via polymerization. a PDMS flow-focusing microdevice for PEG–Dextran hydrogel microspheres with different shapes fabricated via UV polymerization, Copyright (2012), with permission of the Wiley[82]. **b** 3D-printing microdevice for tens of microns of polystyrene microsphere scale-up preparation via monomer polymerization reaction, Copyright (2023), with permission of Elsevier[80]

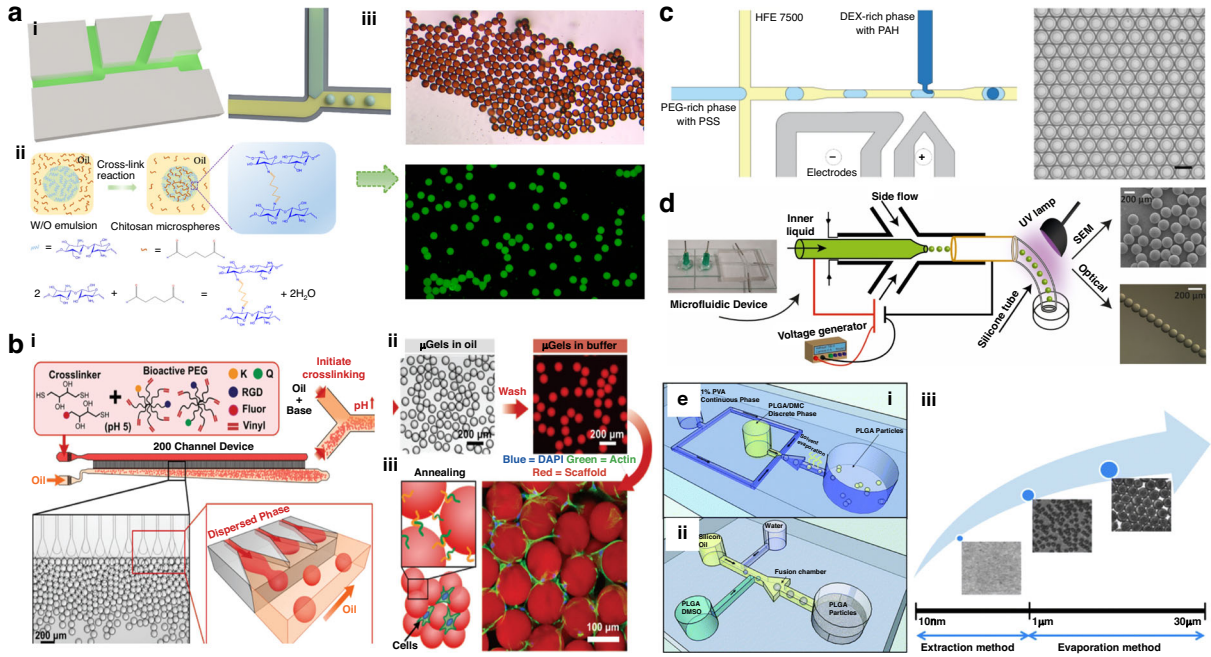

Fig. 8 Microparticle fabrication with different methods in a microfluidic platform. a Chemical cross-linking reaction for chitosan microsphere preparation, Copyright 2020, with permission of American Chemical Society[148]. **b** Ionic cross-linking for PEG microparticle fabrication, Copyright 2019, with permission of Wiley[139]. **c** Phase separation for PEG–Dextran microsphere production, Copyright 2020, with permission of Nature[51]. **d** UV light irradiation for PEGDA microparticle preparation, Copyright 2020, with permission of Elsevier[147]. **e** Solvent evaporation for PLGA microparticle fabrication[149], Copyright 2020, with permission of the Royal Society of Chemistry

Furthermore, as displayed in Fig. 8d, UV-induced solidification was also used to produce uniform PEGDA microparticles and hydrogels in an active-passive integrated co-flow microdevice[147]. As shown in Fig. 7a(iii), the fabricated PEG–Dextran hydrogel microspheres exhibit good uniformity and monodispersity. This work provides a novel route for aqueous droplet-in-oil formation with versatile structures but does not involve the organic solution, which not only helps to simplify the experimental setup but also makes more biological applications possible. The primary weakness of this method is that the price of fluorocarbon oil is high, which may result in the high cost of the raw reagent. Therefore, making the repeated use of the continuous phase fluid is a crucial problem for practical applications. In addition, as illustrated in Fig. 7b, to synthesize polystyrene microspheres, 77 wt% styrene served as a monomer, 20 wt% divinyl benzene (DVB) worked as a cross-linker, and 3 wt% benzoyl peroxide (BPO) served as an initiator, all three reagents constituted an even solution and worked as a dispersed phase solution, and a water solution with 2 wt% SDS and 2 wt% polyvinylpyrrolidone (PVP) addition served as the continuous phase fluid, both the continuous and dispersed phase fluids were pumped into a 3D printing parallel microdevice to prepare polystyrene microspheres with tailored size and good monodispersity[80].

Ionic cross-linking reaction

The ionic cross-linking strategy is widely used to fabricate alginate hydrogel microspheres, where a cross-linking reaction occurs between the gel precursor and divalent ions, such as calcium ions (Ca^{2+}). Unlike UV or thermal polymerization, generally, both the gel precursor and cross-linking reagent are in the same phase, and the reaction is ultra-fast in the microchannel, resulting in the clogging of the microchannel. Apart from the alginate hydrogel microspheres, the ionic cross-linking reaction is popular for chitosan and dextran microsphere fabrication in microfluidic devices. For example, as demonstrated in Fig. 8a, the chitosan microsphere was synthesized in the modified T-junction microreactor with the chemical ionic-linking reaction[148]. As demonstrated in Fig. 8b, the ionic cross-linking reaction has also been utilized to synthesize PEG microparticles[139]. Apart from solid microparticle fabrication, ionic cross-linking is also widely used to prepare hydrogels. The representative scale-up studies for microparticle preparation are summarized in Table 3. However, the water-in-oil system is generally utilized for these chitosan, dextran, agarose, and alginate microsphere preparations in a microfluidic platform. To separate the solid microsphere, laborious operations like repeated washing are always required to remove the organic phase fluid after

Table 3 Scale-up strategies for microdroplet/microparticles/micro-encapsulants fabrication

Microdevice structure	Droplet size and generation frequency	Production rate	CV value Coefficient of variation	References
Y-junction	100–200 μm	Over 1 $kg{\cdot}d^{-1}$	6%	200
Y-junction microdevice	28–43 μm	700 $mL{\cdot}h^{-1}$	5–6%	201
Capillary-embedded step T-junction	30–100 μm 40–80 kHz	N/A	<5%	202
500 nozzles	150–200 μm	150 $mL{\cdot}h^{-1}$	N/A	203
128 cross-junctions	96.4–142.3 μm	128.0 $mL{\cdot}h^{-1}$ 0.3 $kg{\cdot}h^{-1}$	1.3–3.3%	204
128-coaxial microdevice	90 μm	180 $mL{\cdot}h^{-1}$	<3%	205
Geometry controlled	19.6 μm 95 kHz	6 $mL{\cdot}h^{-1}$	<5.61%	116
512 flow-focusing microdevice	86.1 μm	3 $mL{\cdot}h^{-1}$	9%	206
128 ~ 512 Flow-focusing microdevice	100 μm	1.5 $L{\cdot}h^{-1}$	6–20%	181
Coaxial microdevice	598.1–672.6 μm	500 $g{\cdot}d^{-1}$	2.58	207
Porous glass membrane	~40 μm, 25 kHz	3 $mL{\cdot}h^{-1}$	<5%	194
3D-printed milli-fluidic device	32–157 μm	25.6 $mL{\cdot}h^{-1}$	~5%	81
10260 T-junction /flow-focusing droplet generator	8–16 μm	277 $g{\cdot}h^{-1}$	<3%	98
3D printed step-T microdevice	32–52 μm (droplets) 16.9–23.5 (polystyrene particles), 28 kHz	N/A	<10%	80
PDMS 120 flow-focusing/T-junction	240 kHz	10^9 microspheres	<5%	158
A 3D-printed microdevice equipped with 16 identical injection nozzles	2.451 kHz	N/A	~3.4%	160

the preparation of microspheres. In addition, the chemical ionic cross-linking reaction is constrained by the corrosive chemical reagents, which will pose a great challenge for microparticle fabrication in PMMA- and PDMS-made microdevices.

Solvent evaporation

For volatile organic solvents like biocompatible fluorinated oil HFE-7500[18,51] or aqueous solutions serving as the continuous phase, solvent evaporation is an efficient, simple, and economical strategy to dry the microdroplet and obtain the microparticles. As depicted in Fig. 8e, solvent evaporation has been successfully employed to synthesize biodegradable poly-lactic-glycolic acid (PLGA) nano/microspheres[149] with high quality and good monodispersity in a microfluidic platform, which exhibits great potential for drug delivery applications.

Phase separation

Phase separation in immiscible phases is widely used for the Janus or multicompartmental emulsion microparticle preparation within microdevices[150–152]. In addition, phase separation is of great advantage for aqueous systems, for example, for the Dextran–PEG two-phase system, the separation efficiency depends on the molecular weight of the two phases and the mass concentration of the two phases[153]. Phase separation is a facile and efficient approach to realize the separation of the aqueous dextran microdroplets. Generally, solid Janus microspheres can be prepared from Janus droplets of two immiscible components within microfluidic devices; the droplet microfluidic technique has been developed to produce functional hollow Janus micro-encapsulates and micro-hydrogels from double-layered droplets[154], which have a Janus shell as templates (Fig. 9a). In contrast, core-shell microparticles with Janus cores can be fabricated from double emulsions with a Janus core[155] (Fig. 9b). Phase separation is a key technology for most of the above-described Janus microparticle fabrication types. As illustrated in Fig.9c, polymer Janus microparticles were fabricated via UV-directed phase separation using W/O emulsion as the templates (Fig. 9c)[156].

Droplet microfluidics for microparticle scale-up production

When it comes to translating droplet-based microfluidics into practical applications, mass production is a common issue; the production rate of droplets is ~1 mL/h, which

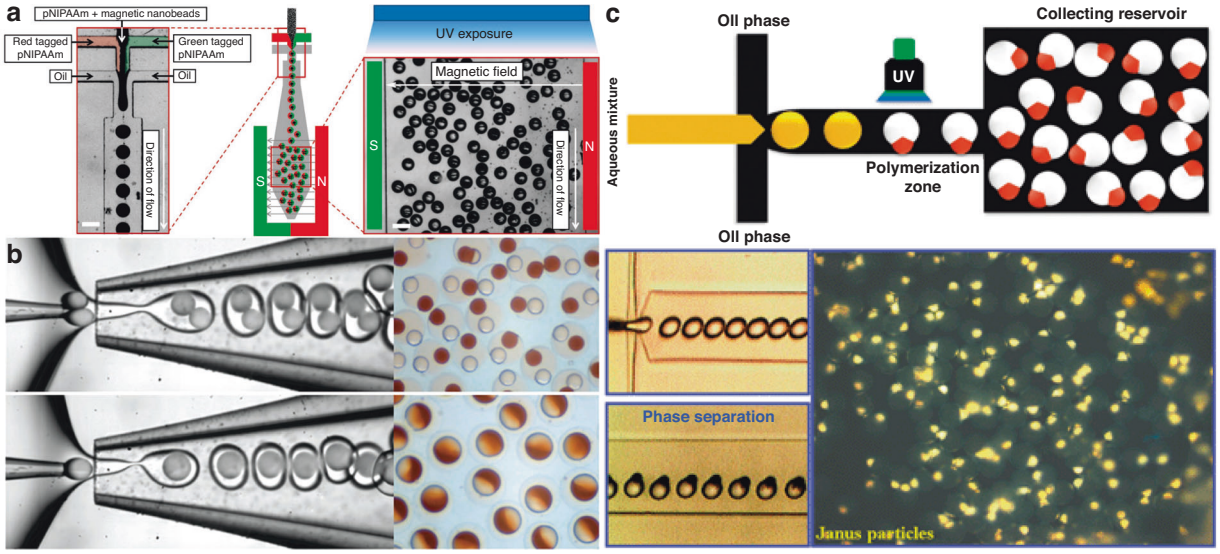

Fig. 9 Janus microparticles/microgels/microencapsulant fabrication via phase separation. a Prepare functional hollow Janus microencapsulates and micro hydrogels from double-layered droplets, Copyright (2010), with permission of the American Chemical Society [154]. **b** Fabrication core–shell microparticles with Janus cores, Copyright (2011), with permission of the American Chemical Society [155]. **c** Produce polymer Janus microparticles via the UV-directed phase separation using W/O emulsion, Copyright (2011), with permission of the Royal Society of Chemistry [156]

dramatically limits the development of droplet-based microfluidics, especially for emulsion or microparticle synthesis for practical applications. Various scale-up strategies have been put forward to address this issue[157]. A uniform fluid distribution network is a significant issue in guaranteeing the high throughput of droplets with equal size and controlled structure. Hundreds of thousands of droplet generators are arranged within a few centimeters of the chip. The typical parallel microdevices for droplet and microparticle mass fabrication are listed in Table 3.

In particular, flow-focusing and T-junction integrated microfluidic platforms are always designed to produce emulsions or microparticles on a large scale. As illustrated in Fig. 10a, an integrated PDMS flow-focusing droplet generator microfluidic chip was designed to produce monodispersed microspheres at a high throughput[158]. The overall design principle is that the two-phase fluid flows through the same distance to reach each droplet generator's inlets, ensuring uniform fluid distribution into the parallel microchannels. Different microspheres, including the SiO_2, ZrO_2, TiO_2, and organic-inorganic hybrid silica microsphere, were successfully manufactured in high throughput with narrow size distribution (coefficient variation CV < 3%), good monodisperse, and superior separation performance, which provides a green and cost-effective way for chromatographic packed microsphere fabrication. Similarly, as demonstrated in Fig. 10b, 10,260 flow-focusing integrated microfluidic chips were respectively developed for 8–16 μm biocompatible polycaprolactone microsphere synthesis with a 277 $g{\cdot}h^{-1}$ production rate[98]. As illustrated in Fig. 10c, a 10,260 T-junction integrated microdevice was developed to prepare water-in-oil and oil-in-water droplets. The flow resistors were incorporated into each droplet generator to break the trade-off effect between the number of droplet formation units and the throughput of each droplet generator. The formation frequency of water-in-oil microdroplets can reach up to more than 1 trillion per hour with CV < 3%. However, the research selected the conventional system to test the performance of the integrated droplet microdevice. Non-Newtonian fluids, viscoelastic fluids, and highly viscous fluids are always encountered in practical applications; therefore, their droplet formation performance is expected to be tested in future research. Another key issue is that the size range in the developed integrated microdevice is narrow; droplets/microspheres in the size range of 50–200 μm are easily fabricated. Whether those smaller than 5 μm biocompatible droplets/microspheres can be manufactured in the integrated microfluidic chip is a decisive factor for biomedical applications. It is worth noting that, apart from the geometrical break-up mechanism, wetting-induced interfacial instability methods were proposed consecutively to prepare microdroplets and microspheres in a 3D printed tip microdevice[159], and to produce both single, double, and multiple emulsions in a 3D printed parallel nozzle microdevice (Fig. 10d)[160]. The advantages of this approach lie in the surfactant-free emulsion

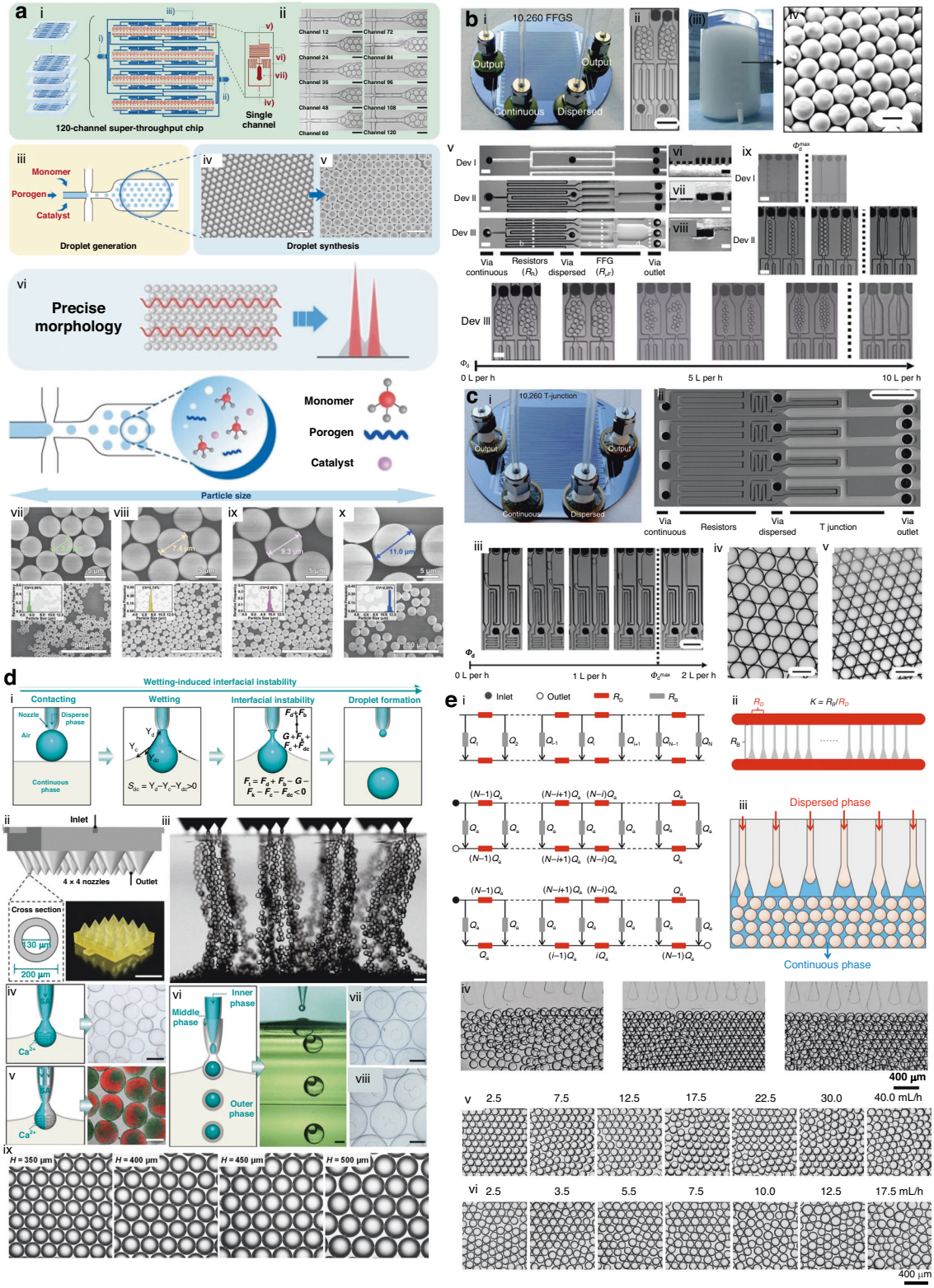

Fig. 10 (See legend on next page.)

(see figure on previous page)
Fig. 10 Typical scale-up strategy for droplet/microsphere fabrication in a high-throughput way. a 120 integrated PDMS droplet generator for versatile functional microsphere fabrication. i. The demonstration of the microdevice; ii. Demonstration of the droplet generation; iii. the microparticle's formation mechanism; vi. morphology and size regulation in the flow-focusing microchannel; vii–x. the SEM image of the fabricated microsphere, Copyright (2024), with permission of Willey[158]. **b** 10,260 integrated flow-focusing microdevice i. the demonstration of the device; ii. The illustration of the droplet generation; iii. The fabricated microspheres; iv. SEM image of the fabricated polycaprolactone microsphere; v. the geometrical structure of the microdevice; vi~viii. the cross-section of the microchannel; ix. the illustration of the droplet generation, Copyright (2018), with permission of Nature[98]. **c** 10,260 integrated T-junction microdevice for oil-in-water droplet fabrication i. the demonstration of the device; ii. the geometrical structure of the device; iii. the droplet generation in the microdevice; iv and v. the optical image of the generated droplets, Copyright (2018), with permission of Nature[98]. **d** A 3D printed microdevice equipped with 16 identical injection nozzles for preparing monodisperse microdroplets via wetting-induced interfacial instability emulsion i. the droplet formation mechanism, ii. the diagram of the parallel integrated microdevice; iii. the representative microscope figure of the droplet formation; vi~v. single and double emulsion formation in the 3D-printed parallel nozzle integrated microdevice; vi. the droplet formation in a single droplet generator; vii and viii. the microscope figure of the generated double and multiple emulsion; ix. the picture of the fabricated microdroplets, Copyright (2025), with permission of the American Association for the Advancement of Science[160]. **e** Microdroplet formation in a ladder network microdevice i. the design strategy via a basic flow resistance model; ii and iii. the schematic illustration of the integrated microdevice for droplet formation; iv–vi. the microscope photos of the prepared microdroplets under different operating conditions, Copyright (2022), with permission of the Royal Society of Chemistry[161]

system, and another key pro is that the droplet formation is independent of the viscosity and the flow rates of the fluids. More importantly, this design concept is easy to scale up and generate versatile emulsions with good robustness. The fabricated droplets exhibit good uniformity and monodispersity as illustrated in Fig. 10d(ix). In summary, this wetting-induced interfacial instability method is beneficial in viscous and viscoelastic droplet preparation in a high-throughput manner, which facilitates producing more smart microspheres and extending the applications. Ladder network design strategy is an effective technique and exhibits great potential in scale-up preparation of monodisperse microdroplets. As demonstrated in Fig. 10e, a ladder network microdevice was designed based on backstepping microflow analysis (BMA) for controllable microdroplet fabrication in a high-throughput way. The flow resistance model of the designed ladder network was described in Fig. 10e(i), the integrated microdevice equipped with multiple droplet generators and the schematic diagram of mass production microdroplets was illustrated in Fig. 10e(iii)[161], the optical micrographs of the generated droplets in different regions of the integrated microdevice was demonstrated in Fig. 10e(iv), it was clear that the formed droplets had good uniformity and mono-dispersity. Figure 10e(v and vi) was the optical microscope graphs of the generated water-in-oil droplets with different flow rates. Although the ladder network design based on BMA achieved uniform flow distribution, the fluid with high viscosity was not demonstrated. Therefore, the universality of the ladder network-designed microdevice should be highlighted in future research.

Droplet manipulation at the microscale

Droplet manipulation at the microscale is of great importance for the related applications. Droplet manipulation operations primarily include transportation (Fig. 11a(iii) and Fig. 11b(viii)), mixing, splitting, merging (Fig. 11a(iii)), sorting, trapping, etc.[162]. All these mentioned droplet operations determine the various practical applications. In this section, we discuss droplet manipulation at the microscale in both passive and active approaches[163–165].

Transportation

Flexible microdroplet/microparticle transportation has been demonstrated to execute automatic chemical reactions, biological detection, and disease diagnosis efficiently and precisely. Magnetism[136,137], electricity, electrowetting-on-dielectric (EWOD)[12], and acoustic waves[164–166] have been successfully applied in microdroplet/microparticle manipulation. Acoustic-based microfluidics can manipulate the droplets or microparticles without contact (Fig. 12b). As demonstrated in Figs. 11a(iii) and 12d, magnetic-based droplet automatic manipulation has been utilized for blood[136] and virus tests[137] in the active droplet microfluidic platform (Fig. 11a and b). Apart from acoustics and magnetism microfluidic platforms, electret-induced polarization on droplets (EPD) was proposed to program the droplets automatically (Fig. 12d); the developed EPD microfluidic system exhibits good generality for handling organic (glycerol, mineral oil, and triacetin) and inorganic liquids (water, HCl, and H_2O_2). Moreover, the EPD microfluidic platform can also automatically actuate various biochemical samples, such as serum, saliva, urine, protein, and living cells. The robustness and generalizability of the EPD microfluidic platform have been demonstrated by automatically regulating different types of droplets. Additionally, light or light responsive materials have been adopted as external driven force to manipulate the droplets efficiently[16], for example, researcher have developed the light-driven charge surface to enable cell suspension droplet via light irradiation[167], more interesting, this strategy have been demonstrated could achieve the fuse

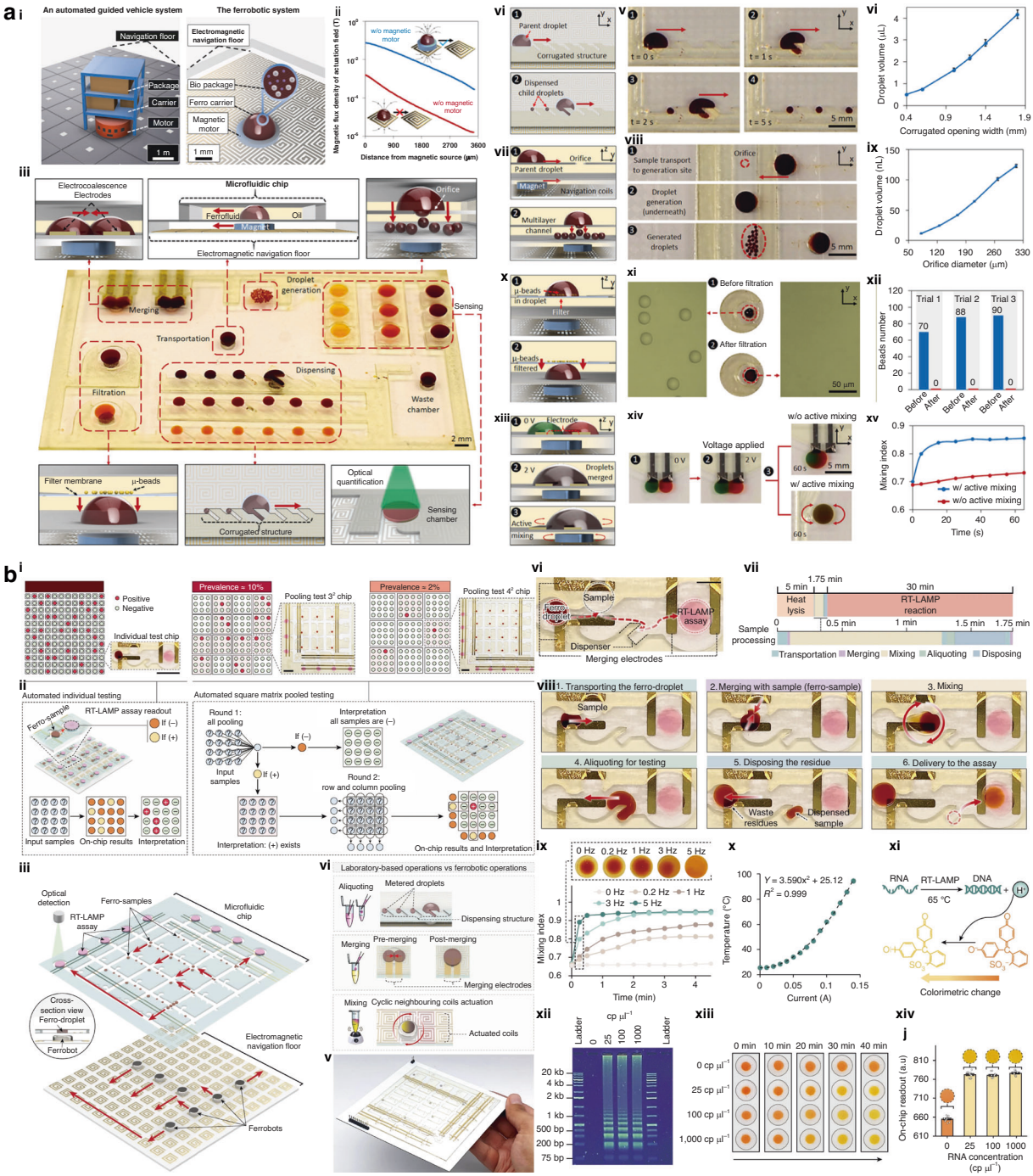

Fig. 11 Digital droplet microfluidics for droplet manipulation and biological sample testing. a Magnetic droplet-based microfluidics for droplet manipulation and biological sample detection (i. the microfluidic design strategy inspired by amazon logistics, ii. the working principles of the magnetic motor, iii. the diagram of the droplet manipulation including merging, transportation, and generation, iv–xv. the performance of the droplet manipulation operation on the magnetic microfluidic platform, copyright (2020), with permission of the Science[136]. **b** Magnetic droplet-based microfluidics for virus automatic test (i and ii. the proposed working principles of the matrix algorithm for virus test, iii. the schematic of the magnetic platform, iv. the working process of the virus test with fer-robot assisted on microfluidic platform, v. the physical diagram of the microfluidic chip, vi–viii. the biological sample manipulated by fer-robot on the microfluidic chip, including merging, mixing, transportation, dispersion, and disposing, ix–xv. the performance of the virus test on the magnetic microfluidic platform, copyright (2022), with permission of Nature[137])

of the viscous sodium alginate droplet and calcium chloride into the biocompatible hydrogen beads, which is conducive to the various biomedical applications. The developed above-mentioned microfluidic platform provides an efficient pathway for biomedical and disease detection.

Mixing

Reagents' mixing performance/efficiency within the microdroplets is critical for reaction, detection response, and mass transfer. Although mixing performance is much more outstanding in microdevices than in conventional devices, the mixing effect is restricted to the straight structure. Special structures like sharp[89] and narrow gap structures, and external agitation such as magnetic[168], surface acoustic waves[90], and electricity are introduced to accelerate the mixing process. In addition, to reduce the flow resistance and improve the mixing efficiency, the microchannel is designed with a round corner rather than a sharp corner.

Splitting and merging

The primary droplet generated in the microdevice can be further split into a series of daughter droplets by passive or active methods to expand the microreactors or detection arrays (Fig. 12a). As illustrated in Fig. 11a(iii), merging operations facilitate the droplets with a series of different concentrations mixing rapidly, thereby achieving multi-step reactions and sequential detection. Inspired by Amazon logistics, the ferrofluid was designed as a robot to execute the blood droplet manipulation in the microfluidic chip (Figs. 11a(iii) and 12c).

Sorting and trapping

Droplet sorting operations are generally used to improve the quality and monodisperse of the microparticles; additionally, droplet sorting plays a critical role in screening. Both the passive and active methods can achieve droplet sorting; the passive method refers to adopting some special structures to sort out the droplets, whereas the active method refers to utilizing external forces to achieve droplet sorting. Trapping operation means fixing the droplet at the assigned location via a designed structure (Fig. 12a(iv)).

Applications

Enormous progress in droplet-based microfluidics has facilitated new pathways to handling and analysis of various samples, the synthesis of materials, disease diagnosis, and disease treatment due to the microscale and precision control of droplet formation and regulation. Droplet-based microfluidics not only enables the manipulation of droplets independently but also realizes some droplet operations, including mixing, splitting, merging, trapping, and extraction, further facilitating the handling and analysis of the samples with high throughput and accuracy. For practical biological and medical applications, selecting a biocompatible and suitable microfluidic system is of significant crucial. In this section, we elaborately elucidate the wide biomedical applications of droplet-based microfluidics.

Drug delivery

When it comes to drug delivery systems, uniform and monodisperse microparticles or microcapsules, especially the natural hydrogel microsphere, have become more and more popular in recent years owing to their biocompatibility, biodegradability, easy accessibility, and low cost. The aqueous two-phase system is of great importance for drug carriers due to the mild water environment. Several microfluidic platforms have been developed for drug encapsulation, delivery, and release. As shown in Fig. 13a, a facile microfluidic system was proposed to prepare compound alginate microcapsules by water-in-water-in-oil templates with controllable structure and size via a one-step route. Bioactive molecules can be encapsulated controllably in the alginate microcapsules' shell and core due to the PEG–Dextran system's intrinsic partitioning effect. The enzyme cascade reaction between the glucose oxidase in the outer PEG layer and horseradish peroxidase in the inner alginate layer has confirmed the biocompatibility and the activity of the PEG–Dextran microcapsule. This controlled compound alginate microcapsule fabrication methodology opens a new green route for manageable multi-drug release[121]. Furthermore, to overcome the slow drug response of the traditional drug delivery system, researchers have made great efforts to address this problem. An aqueous two-phase system in a microfluidic device was presented to controllably fabricate microcapsules that can be employed to release drugs with the assistance of the focused ultrasound successfully[169], further meeting various medical treatment requirements (Fig. 13b). To prepare functional and biocompatible core hydrogel capsules efficiently and flexibly and exploit related applications, a flow-focusing microfluidic platform involved dextran and polyethene two aqueous immiscible phases[170], with the shearing force exerted by the oil phase, the component and the structure of the hydrogel capsule could be regulated easily by adjusting the flow rate of each phase, the number of cores also can be coordinated by the orifice. The fabricated hydrogel capsules were utilized to encapsulate different types of cells, indicating the hydrogel capsules' good biocompatibility and versatility. This developed aqueous-two-phase microfluidic system is facile and convenient, exhibits great potential, and provides more opportunities for functional materials synthesis controllably and multi-drug delivery simultaneously (Fig. 13c).

Embolic therapy

As displayed in Fig. 14a[11], a droplet-based microfluidic program involving a vertical co-axial microdevice has

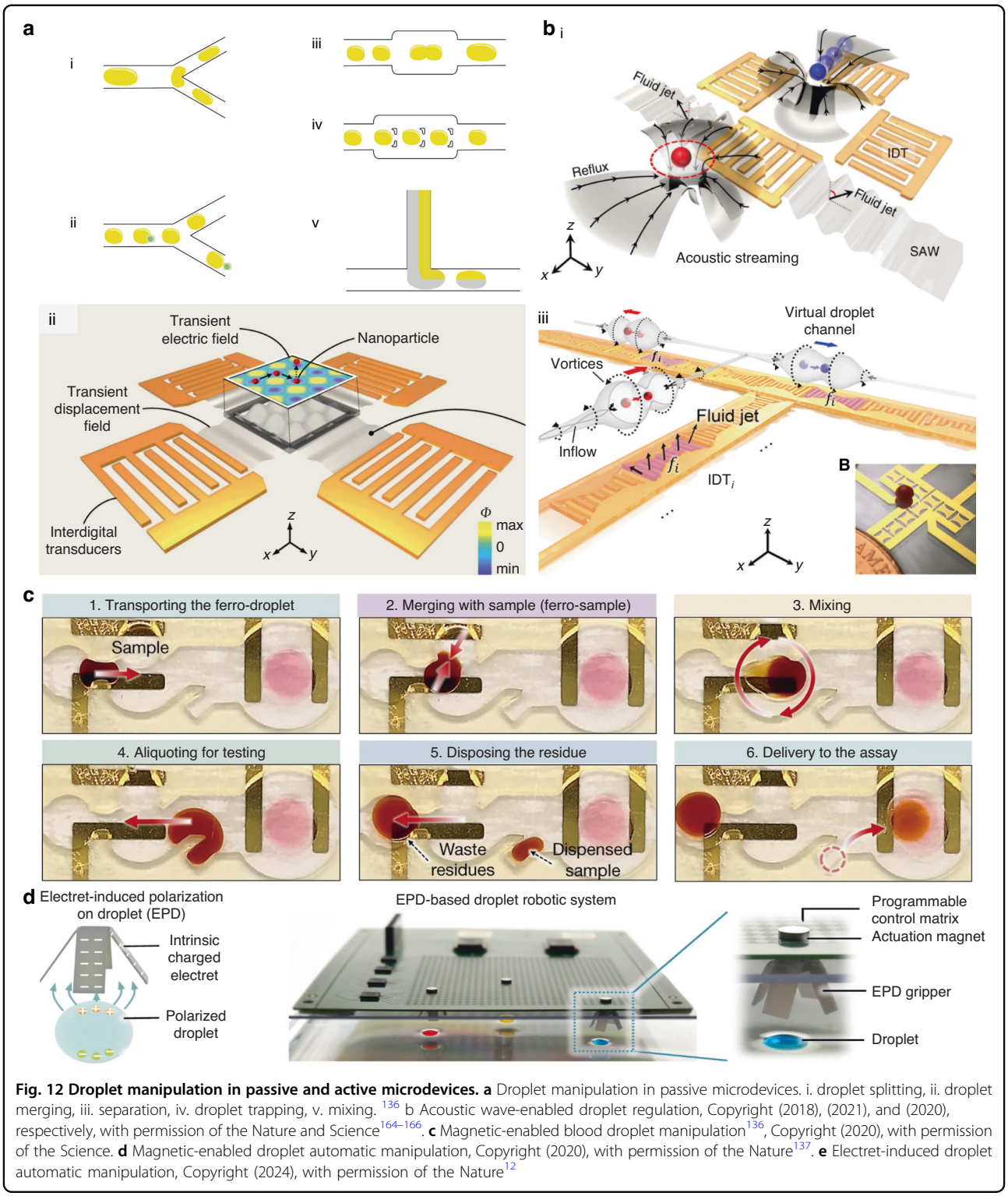

Fig. 12 Droplet manipulation in passive and active microdevices. a Droplet manipulation in passive microdevices. i. droplet splitting, ii. droplet merging, iii. separation, iv. droplet trapping, v. mixing. [136] b Acoustic wave-enabled droplet regulation, Copyright (2018), (2021), and (2020), respectively, with permission of the Nature and Science[164–166]. **c** Magnetic-enabled blood droplet manipulation[136], Copyright (2020), with permission of the Science. **d** Magnetic-enabled droplet automatic manipulation, Copyright (2020), with permission of the Nature[137]. **e** Electret-induced droplet automatic manipulation, Copyright (2024), with permission of the Nature[12]

been proposed for polyvinyl alcohol (PVA) microsphere fabrication with controllable size and physical properties, further applied in the embolization therapy field. The PVA, boric acid mixture solution, and n-octanol are the dispersed and continuous phases, respectively. NaOH solution was added into a solidification bath to solidify the droplet; the mechanism is that the NaOH solution diffuses into the generated droplets promptly; after that, the PVA inside the generated droplets turns into a solid by chemical cross-linking reaction with boric acid in the

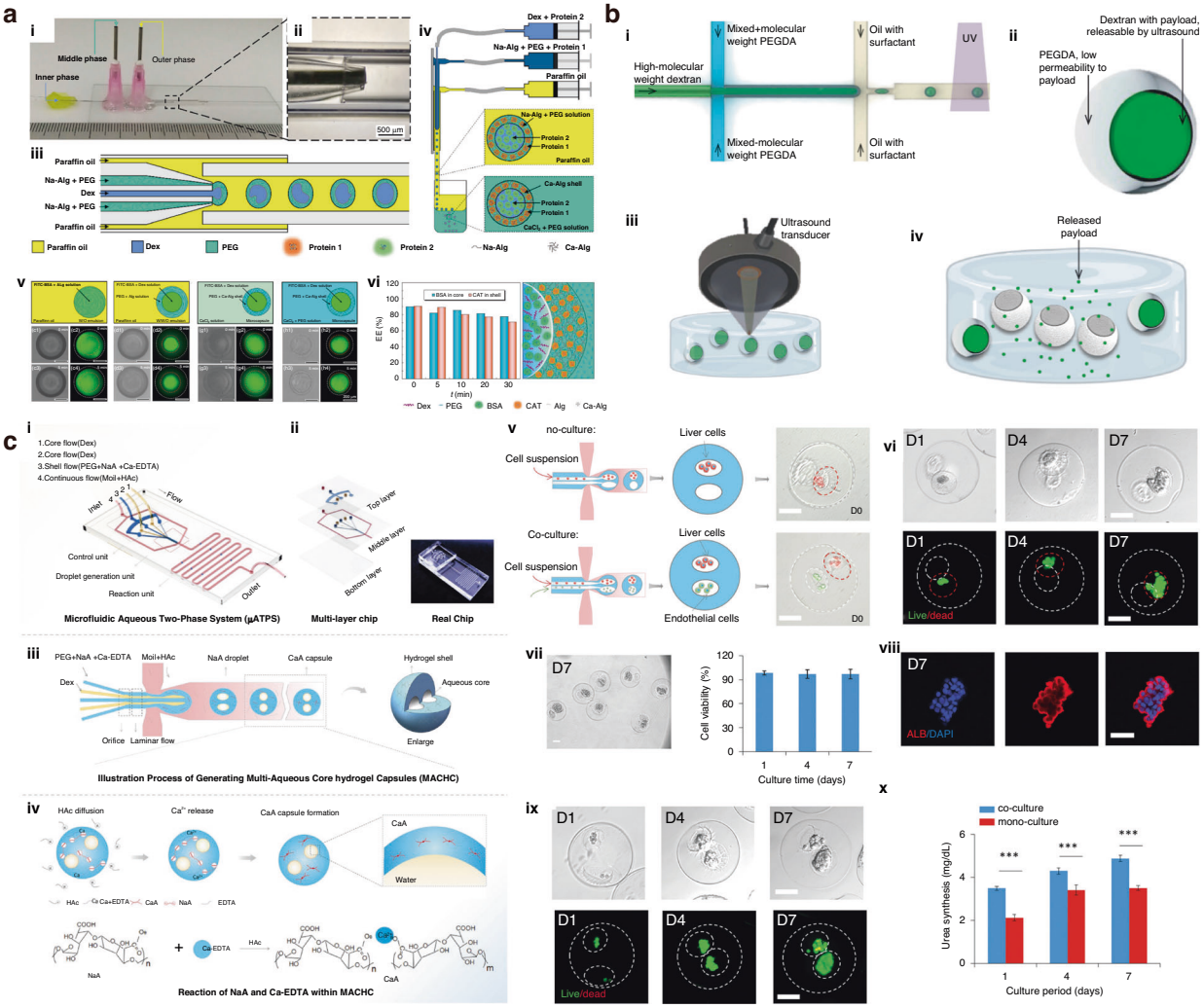

Fig. 13 Droplet microfluidics for biocompatible microcapsule preparation and drug delivery applications. a Schematic diagram of the droplet-based microfluidic platform for alginate microcapsule fabrication and drug release performance evaluation (i. the diagram of the microchannel, ii. the geometrical structure of the microdevice for compound alginate microcapsule preparation, iii. the schematic diagram of the compound alginate microcapsule formation, iv. the experimental setup for compound alginate microcapsule preparation, v. protein distribution in emulsions and microcapsules)[121] Copyright (2019), with permission of the American Chemical Society. **b** A flow-focusing microdevice for biphasic microcapsule manufacturing and drug release performance evaluation (i. the illustration of the designed microfluidic platform for PEG–Dextran microsphere preparation via UV polymerization, ii. the schematic diagram of the aqueous PEG–Dextran microspheres with fluorescein isothiocyanate loading into the dextran phase worked as a drug model, iii. the illustration of the ultrasound transducer triggers the PEG–Dextran drug model, iv. the payload release performance after the ultrasound is exerted on the microcapsule)[170], Copyright (2022), with permission of the American Chemical Society. **c** Schematic diagram of the flow-focusing microfluidic platform for ATPs hydrogel microcapsules manufacture (i and ii. an overview of the integrated microfluidic platform; iii. the illustration of the aqueous hydrogel microspheres formation process, iv. the diagram of calcium disodium ethylene diamine tetraacetate formation mechanism, v. the evaluation process of the encapsulated cells into the fabricated aqueous hydrogel microspheres, vi. the bright-field and fluorescent images of the mono-cultured cells with aqueous hydrogel microspheres at the 1, 4, 7 days, respectively, vii. bright-field graphs of the mono-cultured cells at the 7 days, viii. mono-cultured staining of the cell at 7 days, ix. the bright-field and fluorescent images of the co-cultured cells with aqueous hydrogel microspheres, x. urea synthesis of the mono- and co-cultured cells in the aqueous hydrogel microsphere at 1, 4 and 7 days, respectively), copyright (2020), with permission of Wiley[171]

presence of NaOH. The boric acid and NaOH concentrations primarily determine the chemical cross-link reaction speed and the degree of solidification. The size of the generated droplets in the vertical coaxial can be easily adjusted by the characteristic size of the microdevice and the flow rates of the two phases. In addition, the research results indicated that the elastic and swelling properties of the microsphere can be tuned by changing the concentration of the boric acid in the dispersed phase. Especially when the concentration of the boric acid in the

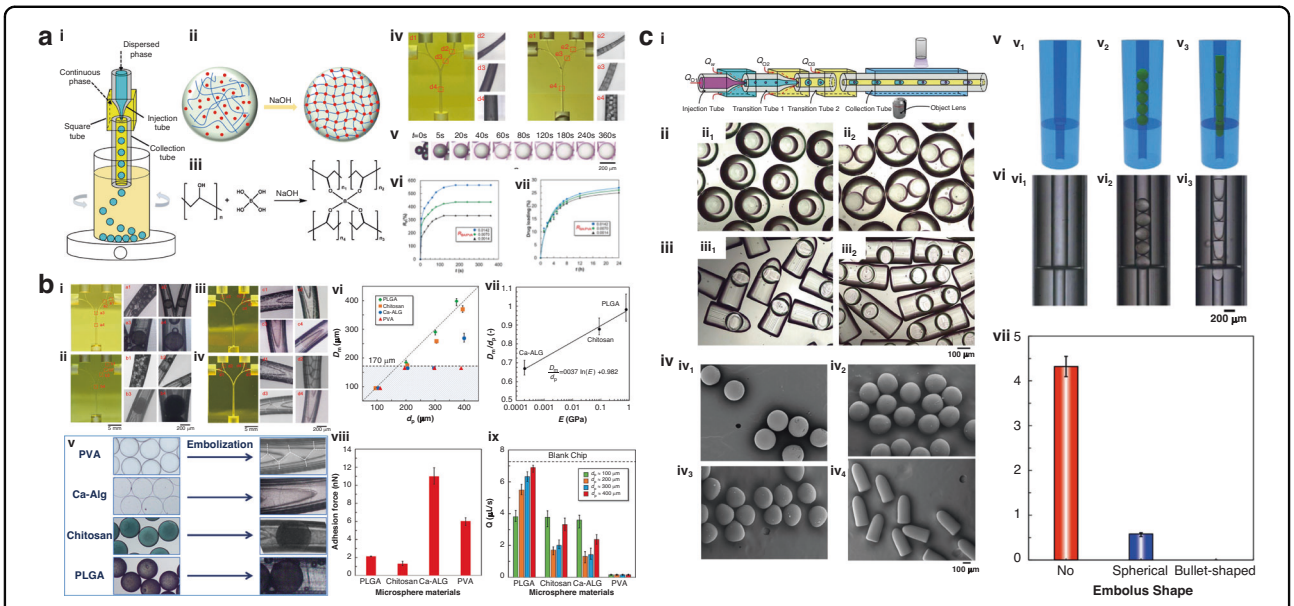

Fig. 14 Microfluidic platform for microsphere fabrication and embolic applications. a Schematic diagram of the microfluidic platform for monodispersed PVA microparticles preparation (i. microfluidic device for PVA microparticle fabrication, ii. the illustration of the droplet generation, iii. the formation mechanism of PVA microspheres via chemical cross-linking reaction, iv. vitro embolization microfluidic device, v. the diagram of the vitro embolization microfluidic after injection PVA microspheres into the microchannels, vi. the optical pictures of the swelling PVA microspheres, vii. the effect of the molar ratios of the boric acid to the PVA on the PVA microsphere swelling ratios, viii. the effect of molar ratios of the boric acid to the PVA on the drug loading), Copyright (2022), with permission of American Chemical Society. **b** Polymer microspheres on the embolization microfluidic chip. (i–iv. the embolization graphs of the PLGA, chitosan, Ca-ALG, and PVA embolic microspheres on the 3D fabricated microdevice, v. the embolization effect of different microspheres into the microchannels, vi. the critical width of the microchannel enables the embolization of the polymer microsphere with different sizes, vii. effect of microspheres' Young's moduli value on the ratio of the diameter of the microchannel to the size of the microsphere, viii. surface adhesion force of different polymer microspheres, ix. the water fluxes variation of the 3D-printing microchannels after injection of different polymer microspheres with different sizes), Copyright (2023), with permission of American Chemical Society. **c** Co-axial microfluidic device for structural microparticle production and its embolic applications (i. the diagram of the microdevice for functional microparticle preparation, ii~iii. the optical graphs of the different-shaped microdroplets and microcapsules, iv. SEM images of structural microparticles fabricated by the spherical- and bullet-shaped droplet templates, v~vi. the experimental embolization performance of the fabricated spherical and bullet-shaped microparticles within the microchannels, vii. the flux of hydroxypropyl methylcellulose for embolization performance evaluation within the microchannel), Copyright (2019), with permission from Elsevier

dispersed phase increases, the elasticity of the microsphere decreases, whereas the swelling degree of the microsphere increases. The microsphere's elasticity and swelling properties are also the key parameters of the practical application field, and the large swelling degree of the microsphere in their research exhibits excellent drug-loading capacity for biological molecules. A specially designed microfluidic chip fabricated by 3D printing with a transparent observe window was utilized to stimulate the blood vessel embolization, specifically, the embolization performance of the microsphere by directly observing the flow situation and water flux of the designed microchannel before and after injection of the microsphere, the results indicated that the prepared PVA microsphere equipped with good embolization performance, which provides valuable guidance for the preparation of the monodisperse microsphere with tunable size, controllable properties further for developing the efficient approach in embolization therapy, drug loading and release and some other clinical applications. Apart from the PVA microsphere, as shown in Fig. 14b[171], researchers also systematically investigated the effects of the physical–chemical–mechanical properties (including elasticity, surface properties, and size) of different microspheres, such as chitosan, calcium alginate, and PLGA, on the embolization performance in the same designed 3D printing microfluidic chip. The evaluation methodology of the embolization performance is the same as the abovementioned PVA microsphere evaluation approach. The results demonstrate that both the physical–chemical–mechanical properties and size of the microsphere significantly affect the embolization efficiency. Microspheres with lower Young's moduli can easily deform in the embolization process, which is advantageous to embolic therapy. In summary, good elasticity, low surface adhesion properties, and proper size of the microsphere are considered as crucial parameters for realizing the desired embolization effect.

To open up droplet-based microfluidics for clinical applications, as depicted in Fig. 14c, a facile and flexible coaxial microfluidic platform integrated with online UV polymerization has been developed to synthesize monodispersed bullet-shaped microparticles with controllable structures and uniform size; the size range of 50 to 700 μm is tunable[10]. Researchers found that the sphericity of the droplet could be manipulated easily by adjusting the flow rates of the continuous phase and viscosity ratio of the dispersed phase to the continuous phase; one important finding is that the curvature of the droplet increases linearly with the continuous phase flow rate increase. To test the practical application, the relationship between the shapes of the microdroplets and the flow velocity has been investigated comprehensively. They found that differently shaped microparticles with the same volume have noticeable velocity differences; the spherical microdroplet flows much faster than the bullet-shaped microdroplet. Additionally, the embolization performance was tested, and the results indicated that the bullet-shaped microparticles exhibit much better embolization efficiency than the spherical microparticles, further proving that the bullet-shaped microdroplets/microparticles can serve as a potential candidate embolic material for embolization/tumor therapy. For embolization therapy or tumor treatment, the size of the microparticle, ranging from 10 to 100, is more proper, which can be fabricated with the proposed microfluidic by adjusting the feature size of the inner and outer microchannel, and the flow rates of the inner and outer phases. In summary, the proposed microfluidic platform provides an efficient and portable scheme for preparing bullet-shaped materials, including microparticles and microcapsules with controllable structure and adjustable size, which not only sheds new light on the development of droplet-based microfluidics but also opens new directions for applications of the droplet-based materials in the medical and clinical fields, like embolization therapy, drug delivery, and release.

Bioactive/cell loading/encapsulation

As shown in Fig. 15a, a microfluidic platform was developed to fabricate self-orienting crescent-shaped ATPs (Dextran–PEG) hydrogel microspheres and utilized these hydrogel microparticles as micro-buckets to transport living cells in vitro. A peptide was employed to modify the hydrogel microparticles' cavities to demonstrate cell loading, cell transportation, proliferation, and release[172]. These research results indicate the great potential of ATP hydrogel microspheres in various bio-related processing and cell therapy. As displayed in Fig. 15b, a water-in-oil system (fluorinated oil-alginate) was developed in a flow-focusing microchannel to prepare alginate hydrogel microparticles for human mesenchymal stem cells[173]; the results demonstrated that the fabricated alginate microspheres have excellent biocompatibility and good stability, which provide a versatile tool in bioactive substance encapsulation and preservation and further applied in tissue engineering.

Biological detection and cell screening

Droplet-based microfluidics, especially digital-based microfluidics, proved to be a promising technique for detecting samples even with ultralow concentration. The whole sample with ultralow concentration can be split into a series of individual droplets by special structures, magnetic fields, and electricity, which creates a higher detection concentration in each droplet. This technique has been demonstrated to be effective for disease diagnostics at the early stage. Each droplet can provide a reactor to encapsulate DNA molecules and enable amplification within the droplet. The droplets in the target molecule can generate fluorescence signals with gene-specific probes after the amplification. The droplet digital polymerase chain reaction (ddPCR) technique has been widely used to detect nucleic acid pathogens. Additionally, ddPCR was successfully applied for H1N1 influenza and COVID-19 detection. Droplet-based biocompatible hydrogel microspheres, which offer mild environments and long mechanical stability, were proven suitable for cell culture and single-cell analysis[51,174,175]. As depicted in Fig. 15a and b, both active and passive flow-focusing microdevices were proposed to prepare water–water–oil emulsions; the oil can be removed simply by evaporation to form uniform aqueous droplets for cancer cell sorting.

Automatic virus test (AVT)

Inspired by Amazon logistics transportation, an active droplet microfluidic platform was developed for biological sample manipulation[101] and COVID-19 virus test[137] automatically and flexibly, as illustrated in Fig. 11a and b. A fer-robotic system as displayed in Fig. 11a(i–iii) was designed to manipulate blood samples. On this developed microfluidic platform, the transportation, splitting, merging, filtering, and mixing of the blood sample can be achieved precisely and efficiently (Fig. 11b(viii–x)). The size of the droplet can be regulated by the width and diameter of the dispersed structure, and orifice, respectively (Fig. 11a(v–ix)). Numerous droplet manipulations can be easily achieved on the developed fer-robotic microfluidic system, which is expected to be applied for biomarker analysis and disease diagnosis. In addition, another fer-robotic platform was proposed. There were 10 fer-robots that worked efficiently in a palm-sized fer-robot microfluidic chip in Fig. 11b(iii–v), carrying biological samples for transportation, splitting, merging, and mixing automatically, as demonstrated in Fig. 11b(vi–viii). Nucleic acid amplification detection reagent was successfully integrated into the microfluidic chip to screen the genetic substance of the COVID-19 virus in clusters, further to diagnose infections. The test results were consistent with the traditional PCR array. More significantly, the developed

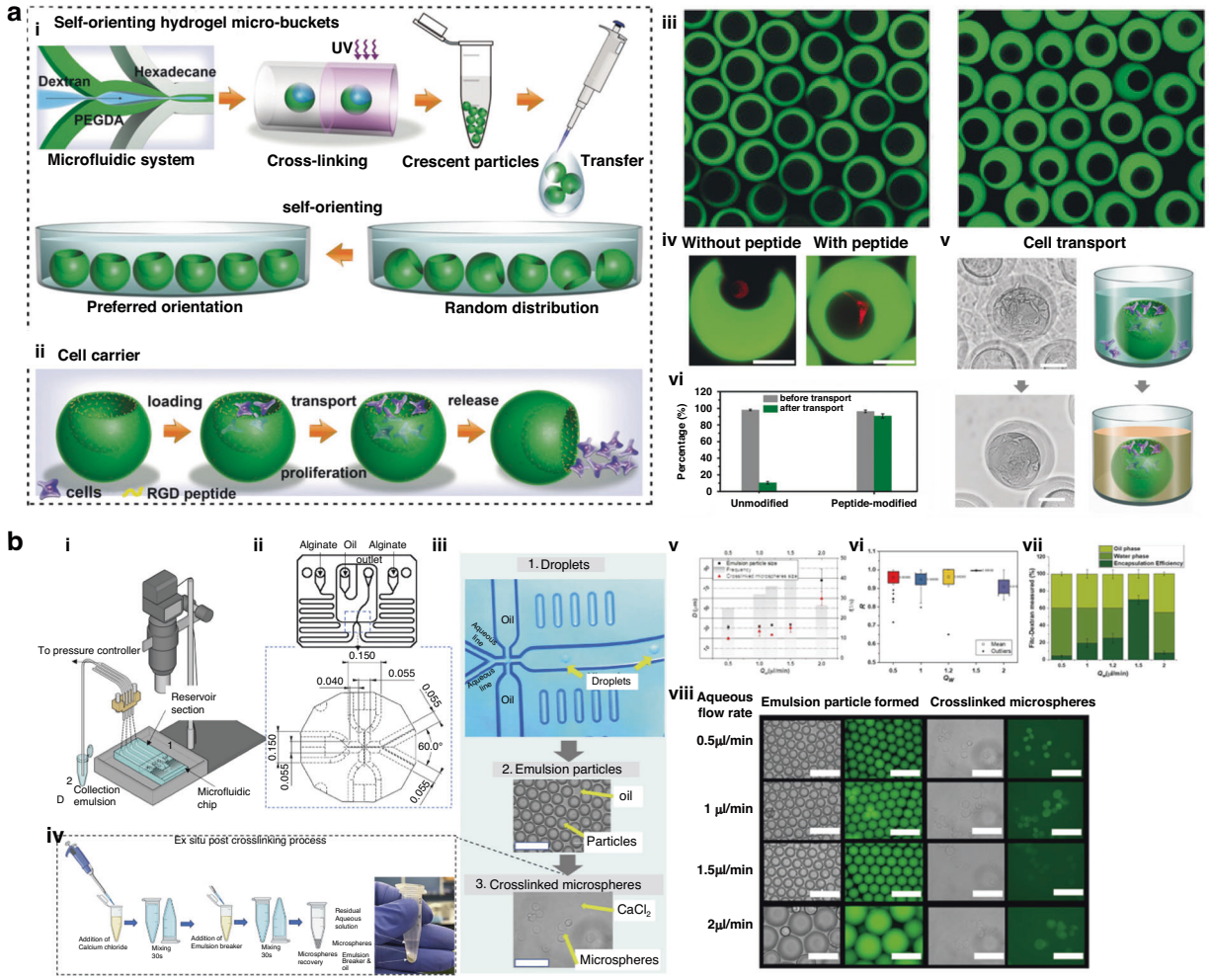

Fig. 15 Microfluidic platform for microsphere fabrication and applications. a Microfluidic preparation of hydrogel microparticles and characterization (i. hydrogel formation mechanism in microchannel, ii. the fabricated microspheres served as a cell carrier, iii. bright-field microscopy images of the hydrogel, iv–viii. the cell transport performance test of the prepared hydrogel, copyright (2019), with permission from Wiey[172]). **b** The schematic of the microfluidic platform for microdroplets and microparticles formation. (i and ii. the microfluidic system and the structure of the microchannel, iii. the microdroplet formation mechanism in microchannel and the microscope images at different times and positions, iv. the post-processing procedures, copyright (2022), with permission from Elsevier[173]

microfluidic platform combined an inexpensive but simple manufacturing process with reliable system operation at the same time. The average matrix algorithm (Fig. 11b(i and ii)) proposed in this work not only reduces reagent consumption significantly but also simplifies the complex test process. The superior performance of this developed microfluidic platform for virus testing was verified, as demonstrated in Fig. 11b(ix–xiv).

Antibiotic susceptibility test (AST)

Droplet-based microfluidics was demonstrated to be a promising platform for drug screening due to microfluidic chips enabling the convenient preparation of a wide range of concentrations on a single chip[176], offering a microenvironment for screening tests. As displayed in Fig. 16a and b, the slip microfluidic chips are specially designed for antimicrobial susceptibility testing via simple manual slide operation. As depicted in Fig. 16c and d, the integrated digital microfluidic system is developed to screen drugs efficiently.

Challenges and perspectives

Challenges

Although droplet-based microfluidics has witnessed tremendous progress in the past three decades, several challenges remain to be addressed in the future. These challenges include but are not limited to the low production rate and high production cost, which exert significant challenges for some chemical fabrication, the efficient integrated microdevices for droplets, multiple

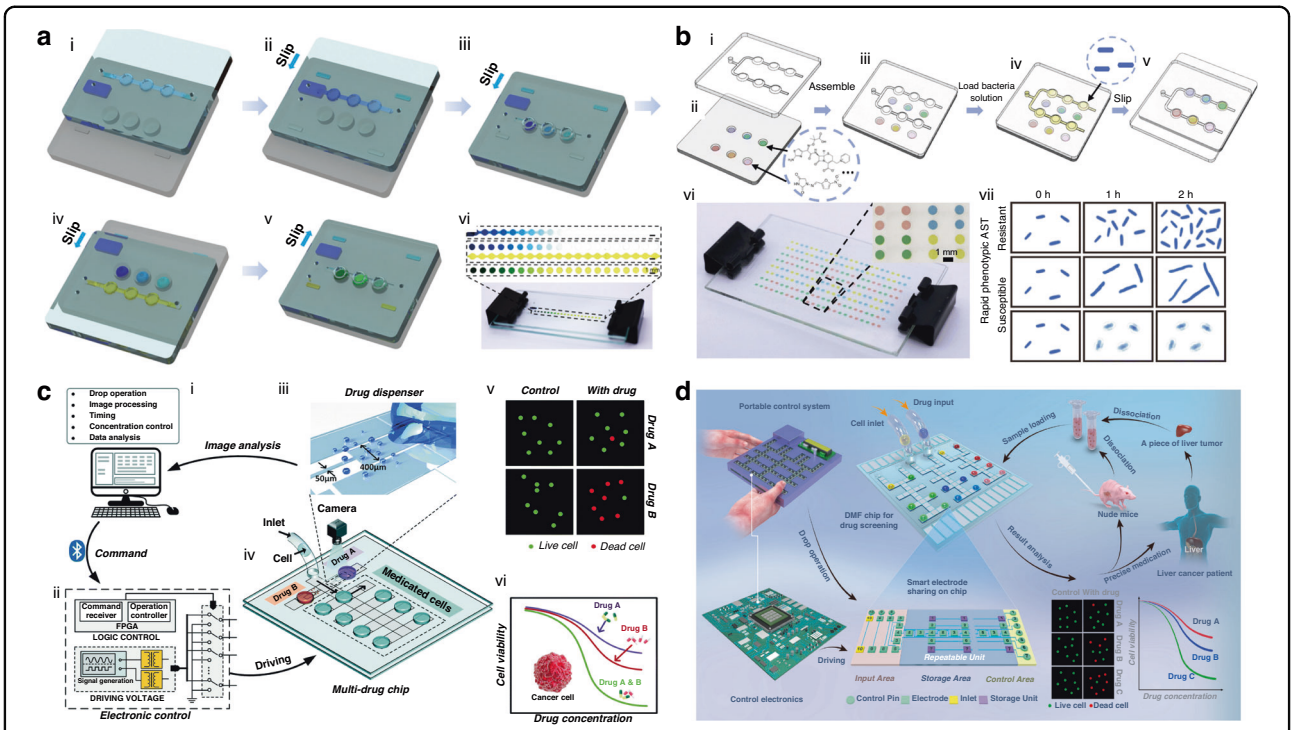

Fig. 16 Microfluidic system for antimicrobial susceptibility testing. a Gradient slip chip for the phenotypic antimicrobial susceptibility test, copyright (2022), with permission of the American Chemical Society. (i. the light and dark blue chamber are the buffer and highly concentrated stock solution, ii. the top plate moves relative to the bottom plate to bring the "chain-of-pearl" channel into contact with the stock chamber and initiate diffusion, iii. the contact with the circular microwells to form gradient droplets, iv. the second aqueous reagent is introduced into the "chain-of-pearl" channel, v. convey the second reagent into the gradient droplets, forming a second series of droplets, vi. bright-field figures showing the gradient slip chip at the diffusion, partitioning-loading, and final partitioning stages.) **b** Integrated microfluidic slip chip for prompt phenotypic antimicrobial susceptibility test, copyright (2022), with permission of the Royal Society of Chemistry. (i. the chain-of-pearls microfluidic chamber, ii. the independent circular microwells preloaded with different antibiotics, iii. the integrated microfluidic slip chip, iv. the bacteria solution was introduced into the chain-of-pearls microfluidic chamber, v. the integrated microfluidic plate enables the "chain-of-pearl" microwell contact into the circular microwells by simple manual operation, the bacterial solution in the "chain-of-pearl" microwells achieved the self-dispersion into the independent droplet and mixed with the preloaded antibiotics, v. the bright-field graphs of the integrated gradient slip chip loaded with colorful dyes, vi. schematic diagram of the antibiotic susceptibility test results). **c** Digital microfluidic platform for single or multi-drug screening. (i. the software control system, ii. the electronic control hardware, iii. the geometry of the droplet dispersion structure, iv. the digital microfluidic chip with drugs preloaded, v. the cell viability picture, vi. the efficiency of the drug with different concentrations). **d** The diagram of the portable digital microfluidic system for drug screening efficiently and precisely, copyright (2024), with permission of Nature[208]

emulsions, and functional microspheres scale-up production. Second, the large phase ratio operating condition is always desired for smaller droplets/microspheres formation, in this way, the continuous phase fluid is severely wasted; therefore, making full use of the continuous phase fluid or realizing the circulation utilization of the continuous phase fluid is an important aspect. Third, exploiting biocompatible new ATPs for biological and medical applications is another challenge. Additionally, designing integrated microdevices for functional materials with tailored structures and properties manufactured on a large scale is also a significant issue. Developing efficient and economical technologies for high-value-added microsphere separation or dissolving fabricated microspheres into stroke-physiological saline solutions or ethanol for biomedical applications is also another significant issue. Eventually, integrating AI with microfluidics is urgently needed to develop to assist the microreactor's optimization, precise design and droplet manipulation automatically.

Perspectives

With the enormous progress of droplet-based microfluidics, significant efforts have been made to translate laboratory results into real applications, such as high-value-added biocompatible particle materials preparation[118] and biomedical detection[177,178]. To facilitate the development of droplet microfluidics, the following research directions should be addressed in the future.

Fabrication of microdroplets/microparticles on a large scale

Droplet-based microfluidics has been demonstrated to be a versatile strategy for high-value-added product fabrication, such as monodispersed and uniform microparticle materials fabrication in the industrial field[81,138] and emulsion preparation[179]. For microparticle materials fabrication, droplet-based microfluidic technology has been successfully utilized to produce emulsions[180,181] and cosmetics[66]. Although various scale-up strategies have been put forward to address the low production rate for microparticle materials preparation, the throughput is still too low to meet the demand of some industrial applications, and the robustness is also poor. The proposed scale-up strategies have several prominent issues. The most protruding issues are complicated and three-dimensional arrangements that are always desired for the parallel production of microdroplets/microparticles, requiring both a uniform fluid distribution strategy and a sophisticated microdevice fabrication strategy. Therefore, in future research, developing more sophisticated and cost-effective strategies to further improve the production rate and lower the manufacturing cost is the key issue to translate the research results into real applications. More importantly, how to execute complicated systems like non-Newtonian fluids, viscous fluids, and viscoelastic fluids in the droplet microfluidic chip is expected to solve some bottleneck problems in the biomedical fields. Another prominent problem is that the published research primarily focused on the traditional system at the ambient temperature and pressure, which is not suitable for some sophisticated hydrogel microsphere preparation. To highlight this problem, the integrated microreactors should consider involving the temperature and pressure modules in the platform to achieve more high-value-added microsphere preparation on a large scale.

Efficient and economic separation techniques for microparticles

Removing the continuous phase fluid from the biocompatible microparticles/hydrogels is desired for biological and medical applications. Contaminating the biocompatible microparticles/hydrogels for disease therapies will induce an immune response, thereby threatening human health. Therefore, highly continuous and efficient separation or drying techniques for continuous phase fluid removal are urgent.

Real-time detection and portable microdevice development

For biomedical applications, the passive droplet-based microfluidic platforms are always restricted by the utilization of complicated fluid transportation instruments like syringes and pumps, which not only result in ease of operations but also induce high costs. Compared with passive droplet-based microfluidic techniques, active digital droplet-based microfluidics is more competitive in both biological and medical fields. With the people's concern for health, automatic and precious integrated droplet-based microfluidic cartridges must be developed for biomarkers, diseases, and antibiotic susceptibility tests efficiently, cost-effectively, and conveniently in the future. Furthermore, conventional antibiotic susceptibility tests have limited labor costs, low sensitivity, and complex sample handling. Therefore, the efficient and automatic digital droplet-based microfluidic technique is a promising strategy to address this problem.

Design and develop new aqueous systems

Aqueous droplets can provide a biocompatible environment, and the subsequent separation is facile and efficient; therefore, to expand the droplet-based microfluidics' applications in diverse biomedical fields, a deep understanding of the aqueous droplet or jet formation mechanism is urgently needed, and the characteristics of the aqueous systems need to be figured out. Additionally, two new aqueous systems need to be explored and designed to open up more biomedical applications.

Integrated artificial intelligence (AI) with microfluidics

The rapid advances in artificial intelligence have boosted the development of microfluidics in these years. On the one hand, facilitating machine learning or artificial intelligence to drive[122,182–184] the automation reaction, material synthesis[185], drug discovery and synthesis[185,186], biomedical detection[177,186], and drug discovery is expected as a promising direction[187] in future research direction; on the other hand, developing the universal robust machine learning model to predict the size of the microdroplets/microspheres is crucial to advance fundamental microfluidic research[122,188]. In addition, AI facilitates detecting, screening, and forecasting abundant data from microfluidic systems, which greatly decreases the cost, labor, and time. For instance, AI algorithms are expected to analyze extensive microscope images of screening cells or biomarkers to further assist in the detection and disease diagnosis[189] in the future. More exciting, the combination of AI with microfluidics is anticipated to provide more opportunities for both droplet-based microfluidic experimental investigations and post-processing efficiency in the biological and disease diagnosis fields[190,191].

Conclusions

In summary, we comprehensively reviewed cutting-edge research on droplet-based microfluidics and its applications in biomedical fields. We start with the versatile

droplet formation in different microdevices and address the aqueous droplet generation process, revealing the working mechanism for droplet formation at the microscale. Following, the surface modification methods for microchannels fabricated via different materials are summarized and discussed. Next, strategies for droplet-based materials fabrication are clarified in detail. Then, we summarized the recent progress of droplet-based microfluidics in biomedical applications. Eventually, the existing challenges and future research directions are proposed to guide future research. This comprehensive review not only provides a deep understanding of droplet-based microfluidics but also guides researchers to conduct significant work in this field to explore more advanced applications.

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Conflict of interest
The authors declare no competing interests.

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