

## Review

# Recent Advances in Magnetically Actuated Droplet Manipulation for Biomedical Applications

Jiaqi Li , Kaixin Su, Hailan Liu and Yuan Zou \* 

Key Laboratory of Clinical Laboratory Diagnostics (Ministry of Education), College of Laboratory Medicine, Chongqing Medical University, Chongqing 400016, China; lijiaqi@stu.cqmu.edu.cn (J.L.); sukaixin@stu.cqmu.edu.cn (K.S.); liuhailan@stu.cqmu.edu.cn (H.L.)

\* Correspondence: zouyuan@cqmu.edu.cn

**Abstract:** The manipulation of droplets plays a vital role in biomedicine, chemistry, and hydromechanics, especially in microfluidics. Magnetic droplet manipulation has emerged as a prominent and advanced technique in comparison to other modes such as dielectric infiltration, optical radiation, and surface acoustic waves. Its notable progress is attributed to several advantages, including excellent biocompatibility, remote and non-contact control, and instantaneous response. This review provides a comprehensive overview of recent developments in magnetic droplet manipulation and its applications within the biomedical field. Firstly, the discussion involves an examination of the distinctive features associated with droplet manipulation based on both permanent magnet and electromagnet principles, along with a thorough exploration of the influencing factors impacting magnetic droplet manipulation. Additionally, an in-depth review of magnetic actuation mechanisms and various droplet manipulation methods is presented. Furthermore, the article elucidates the biomedical applications of magnetic droplet manipulation, particularly its role in diagnostic assays, drug discovery, and cell culture. Finally, the highlights and challenges of magnetic droplet manipulation in biomedical applications are described in detail.

**Keywords:** droplet; magnetic; manipulation; biomedical application



**Citation:** Li, J.; Su, K.; Liu, H.; Zou, Y.

Recent Advances in Magnetically Actuated Droplet Manipulation for Biomedical Applications.

*Magnetochemistry* **2024**, *10*, 28.

<https://doi.org/10.3390/magnetochemistry10040028>

Academic Editor: Adriana Greco

Received: 29 February 2024

Revised: 7 April 2024

Accepted: 12 April 2024

Published: 16 April 2024



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## 1. Introduction

Droplets refer to the fine liquid particles that can settle under static conditions and remain suspended even amid turbulent circumstances [1]. In contemporary times, the manipulation of droplets has become a focal point of interest in the fields of biomedicine [2–5], chemistry [6–8], and hydromechanics [9–11] due to the advantages of droplets' flexibility and independence [12]. Concomitant with the progress in microfluidic technologies, droplet microfluidics realizes the flow control of microdroplets and builds a new platform for biological and medical research. Until now, a variety of droplet control methods such as electrowetting [13,14], optical tweezers [15,16], acoustic manipulation [17], and magnetic manipulation [18–20] have been developed to transport, sort, split, merge and mix droplets.

At present, dielectric electrowetting stands out as the predominant driving method in microfluidic systems [21]. Dielectric electrowetting alters the wettability of droplets on the underlying solid structure by applying a voltage between the upper and lower substrates, moving droplets by altering their surface tension. Their advantages include fast response, high precision, a simplified system, and cost-effectiveness, albeit with the drawback of having a relatively intricate fabrication process. Optical droplet manipulation [22] serves as a flexible and long-lasting method for driving droplets. However, a significant portion of the optical drive forces is counteracted by interfacial resistance forces, resulting in poor performance of droplet manipulations, relatively low droplet velocity, and limited travel distance. Acoustic droplet manipulation [23] utilizing MHz scale surface acoustic waves emerges as a contactless, label-free, and highly biocompatible approach. Nevertheless,

its reliance on an ultrasonic phased array entails a complex hardware configuration. In contrast, the manipulation of droplets through magnetic fields has unique advantages over other methods. Notably, magnetic droplet manipulation boasts good biocompatibility, remote and non-contact control, and instantaneous response, setting it apart as a promising approach in microfluidic systems.

With the development of magnetically manipulated droplet technology in recent years, we have also witnessed its expansion in biomedical applications. Several reviews provide updates and summaries of recent advances in magnetic manipulation technologies in biomedicine. Topics include the applications of magnetic beads in cell sorting, nucleic acid detection, and immunodiagnosis [24], the applications of magnetic particles in DNA amplification and sequencing [25], and the applications of magnetic digital microfluidics in drug screening, protein analysis, and chemical synthesis and analysis [26]. However, other research in the fields of sample diagnostic analysis, drug discovery, and cell culture based on magnetically manipulated droplet technology has not been summarized and discussed. Furthermore, future directions for magnetic manipulation of droplets should also be discussed in terms of their current development prospects and challenges.

In this review, we aim to review the latest developments in magnetic droplet manipulation technology and its diverse applications in the biomedical field. Firstly, we will delve into the distinctive features of droplet manipulation utilizing both permanent magnets and electromagnets. Then, the driving mechanisms of magnetic droplet manipulation are reviewed. Next, we will meticulously detail the biomedical applications of magnetic droplet manipulation, spanning biomedical diagnostic applications, drug discovery, and cell culture. Finally, the highlights and challenges of magnetic droplet manipulation in biomedical applications are introduced.

## 2. Magnetic Manipulations of Droplet

The traditional method of droplet transport is to accurately transport the droplet by fabricating a chemical wetting gradient or anisotropic physical structure on the solid surface, which is a passive moving method, with limitations, inflexibility, slow speed, and other disadvantages [27]. In active methods, droplet generation is initiated or assisted by external forces to exert local actuation and achieve a fast response [28,29]. Active droplet generation can be categorized based on energy sources, including electrical, magnetic, thermal, and mechanical methods. Moreover, these external fields exhibit a relatively low reliance on channel structure and can be easily and flexibly controlled during operation.

The magnetic drive method offers crucial and irreplaceable advantages, allowing for the manipulation of droplets containing magnetic beads through the control of permanent magnets, electromagnets, or hybrid magnetic fields [30]. Magnetically controlled droplets can perform behaviors such as deformation, displacement, rotation, levitation, splitting, fusion, etc. [31]. The movement of the magnetic droplets on a flat surface was primarily determined by the magnetic force [32] and interface resistance [33].

### 2.1. Magnetic Actuation Based on Permanent Magnets or Electromagnets

The magnetic force required for droplet motion or breakup is determined by the intensity and gradient of the magnetic field, as well as by the loading and magnetic susceptibility of the particles. Magnetic force is influenced by multiple factors, and optimal regulation of magnetic force is achieved through the control of the magnetic field. The primary sources of magnetic force include permanent magnets [33–35] or electromagnets [36,37].

Permanent magnets generate a strong homogeneous magnetic field. This is a widely used method for manipulating droplets in biochemical analysis. Permanent magnets can generate high-gradient and stable magnetic fields for the active and reversible manipulation of droplets. Kim et al. [38] described a novel technique that enables active, fast control of a magnetic responsive flexible thin film using a permanent magnet. This technique enables quick, precise, and reversible control over the position and movement of individual droplets. To improve the speed of analysis and the diversity of magnetically controlled droplets in

a two-dimensional (2D) droplet platform, Park et al. [39] presented a three-dimensional (3D) approach for manipulating magnetic droplets, allowing both horizontal and vertical movement of these droplets when subjected to a permanent magnet. This demonstrates the practicality and versatility of employing magnetic manipulation in creating 3D digital microfluidic platforms for numerous biochemical or biomedical applications. However, the volume of permanent magnets is relatively large, making it difficult to finely control droplet manipulation.

Electromagnets represent a more flexible approach to magnetic droplet manipulation compared to permanent magnets, as they can fit into smaller spaces and assume diverse geometries. The strength of the magnetic field of electromagnets is that it can be effectively modulated by adjusting the current flowing through the electromagnet [24]. Yang et al. [20] integrated a superhydrophobic magnetic film (SHMF) with an electromagnetic pillar array (EMPA), a magnetic droplet system platform that is driven by electromagnetic force and can be adjusted in real time [40]. The platform supports various droplet operations, such as path-programmable droplet transfer, parallel transfer of multiple droplets, and merging and mixing of multiple droplets. A digital microfluidics platform that manipulates droplets through electromagnets can be utilized for virus detection. Lu et al. [41] developed a digital microfluidic system designed to detect the influenza A (H1N1) virus using magnetic bead monomers and dual antibodies. The adaptable electromagnetic force provides three operational modes: moving droplets with magnetic beads, merging two droplets, and extracting droplets. The precise control of droplet and magnetic bead movement is achieved by fine-tuning the magnetic flux density. This platform utilizes electromagnetic force to carry out the entire diagnostic process for the H1N1 virus, ensuring a swift and precise diagnosis. However, it should be noted that an increase in current through the electromagnet may lead to Joule heating [42]. This hinders its suitability for deployment in remote regions or areas lacking electricity.

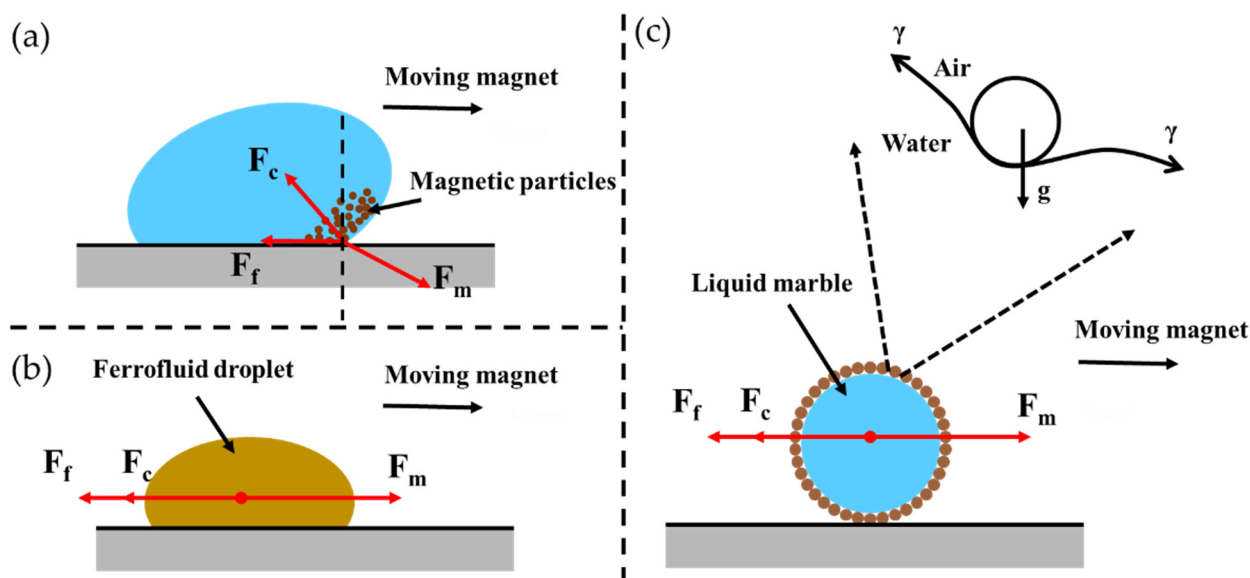
The static magnetic field produced by permanent magnets can furnish a consistent and stable magnetic field [43], while electromagnets can generate adjustable magnetic fields [44]. Through the integration of electromagnets and permanent magnets in a hybrid magnet system [32], the combined magnetic fields from both sources can synergize, creating high-intensity magnetic fields. This configuration enables accurate adjustment of local magnetic fields, providing greater flexibility in the manipulation of magnetic droplets. Based on this theory, Nguyen et al. [45] documented a system designed for the magnetic manipulation of droplets containing ferromagnetic fluid, along with an exploration of its dynamic behavior. The magnetic field is generated by an array of planar coils, and the permanent magnetic moment of a ferromagnetic fluid droplet is generated by the magnetic field of a pair of permanent magnets. The direction of the droplet motion can be controlled by reversing the current in the coils. Next, Sarkhosh et al. [46] studied a new method for manipulating ferromagnetic fluid marbles and droplets using a pair of Helmholtz coils and different structures of permanent magnets. The initial suggestion is to utilize repulsive force to govern the manipulation of both marbles and droplets.

This hybrid system has great potential for biomedical applications. A multi-capsule robot with a helical structure was created [47]. It is controlled by an external electromagnetic field. These swallowable capsule robots navigate within the body cavity, facilitating drug delivery, minimally invasive surgery, and diagnosis. The multi-capsule robot consists of a driving permanent magnet, a joint permanent magnet, and a screw body. Spiral bodies generate propulsion in fluid environments. In addition, robots can form new structures through mutual docking and release to provide use in more active sports.

## 2.2. Magnetic Force and Interface Resistance

Droplet movement or splitting is driven by a magnetic field. It is mainly determined by magnetic forces ( $F_m$ ), capillary forces ( $F_c$ ), and friction ( $F_f$ ) (see Figure 1) [48,49]. The magnitude of capillary force is directly proportional to the surface tension of the droplet and inversely proportional to the capillary radius. A larger contact angle of the droplet

corresponds to a smaller surface tension, resulting in a reduced capillary force acting on the droplet. Magnetic droplets are also hindered from moving by the friction on the liquid surface. The level of friction experienced by a droplet is mainly contingent on the droplet's size and the relative velocity between the droplet and its surface [50]. Long et al. [51] formulated a straightforward force-balance model to elucidate the forces encountered by magnetic droplets. The dynamics and outcomes of droplet manipulation are dictated by a meticulous equilibrium involving the  $F_m$  acting on the bead clusters, the  $F_c$  due to the deformation of the droplets, and the  $F_f$  between the oiled droplets and the substrate surface.



**Figure 1.** Force analysis of the magnetic droplet. (a) Force analysis of magnetic particle droplets. (Reprinted with permission from Ref. [49]. Copyright © 2017, American Chemical Society.) (b) Force analysis of ferrofluid droplets. (Reprinted with permission from Ref. [12]. Copyright © 2022, MDPI.) (c) Force analysis of liquid marbles. (Reprinted with permission from Ref. [52]. Copyright © 2012, Springer Nature.)

Magnetic force,  $F_m$ —

$$F_m = \left( \frac{M}{\rho} \right) \chi \frac{B_m}{\mu_0} \nabla B_m, \quad (1)$$

where  $M$  is the mass of the bead cluster,  $\rho$  is the mass density of the bead material,  $\chi$  is the magnetization of the beads,  $B_m$  is the applied magnetic field, and  $\mu_0$  is the permittivity of a vacuum.

Frictional force,  $F_f$ —

$$F_f \cong K_f R_b \mu_{oil} U, \quad (2)$$

where  $K_f$  is the friction constant,  $R_b$  is the radius of the bottom contact area between the oil ring droplet and the substrate,  $\mu$  is the viscosity of the oil, and  $U$  is the velocity of the droplet.

Capillary force,  $F_c$ —

$$F_{c,max} = 6^{\frac{1}{3}} \pi^{\frac{2}{3}} \gamma_{o-w} \left( \frac{M}{\rho} \right)^{\frac{1}{2}}, \quad (3)$$

where the maximum capillary force is directly proportional to the radius of the dense bead cluster, which, in turn, is proportional to the cube root of the particle volume  $M/\rho$ . This assumption considers that the detached droplets are precisely sized to accommodate the hypothetical particle clusters in a densely stacked spherical structure. Additionally, the maximum capillary force is proportional to the interfacial tension  $\gamma_{o-w}$  between oil and water.

When the magnetic force  $F_m$  is perfectly balanced with the friction force  $F_f$ , it ensures that the droplet and the magnet move at the same speed. When both the frictional force  $F_f$  and the maximum capillary force  $F_c$  exceed  $F_{m,max}$ , the magnet will disengage. When  $F_{m,max}$  is greater than  $F_{c,max}$  but  $F_{c,max}$  is less than the friction force  $F_f$ , this means that the magnetic pull acting on the bead cluster overcomes the interfacial force and a small bead droplet will be split off.

When the magnetic particles in the droplet are uniformly distributed, the ferromagnetic fluid droplet formed mainly includes two motion mechanisms: flow and deformation. Rosensweig believed that the movement of ferrofluids is mainly controlled by a combination of constitutive, static Maxwell and Navier–Stokes (NS) equations [49]:

$$\nabla \cdot v = 0 \quad (4)$$

$$\nabla \cdot B = 0, \nabla \times H = 0 \quad (5)$$

$$\rho \frac{Dv}{Dt} = -\nabla p + \rho g + \mu_0 M \cdot \nabla H + 2\zeta(\nabla \times \omega) + (\lambda + \eta - \zeta)\nabla(\nabla \cdot v) + (\eta + \zeta)\nabla^2 v \quad (6)$$

where  $v$  is the velocity,  $B$  is the magnetic flux density,  $H$  represents the magnetic field,  $M$  is the magnetization,  $p$  is the pressure,  $\rho$  is the density,  $g$  is the gravitational acceleration,  $\mu_0$  is the free space permeability,  $\zeta$  is the eddy viscosity,  $\lambda$  is the bulk viscosity, and  $\eta$  is the shear viscosity. The motion of ferrofluid is mainly determined by the continuity equation and the momentum equation (Equation (6)).

When magnetic particles are encapsulated on the outer surface of droplets, they are liquid marbles, which can transport water-soluble reagents to be used in various biosensors or deliver drugs, and have good biocompatibility. Assuming that the liquid marble is a smooth sphere, the total energy received by the liquid marble is

$$E = \gamma S_{\text{upper}} + (\gamma_{SL} - \gamma_{SA})S_{\text{contact}} + \rho g V H_c \quad (7)$$

In the formula,  $\gamma$  is the surface tension coefficient of the upper free interface, and  $\gamma_{SL}$  and  $\gamma_{SA}$  are the surface tension coefficients of the solid–liquid and solid–air interface, respectively.  $S_{\text{upper}}$  is the upper surface area, while  $S_{\text{contact}}$  is the contact area,  $\rho$  is the liquid density,  $g$  is the acceleration of gravity,  $V$  is the volume of the droplet, and  $H_c$  is the central height [53]. The dynamics of liquid marble are also determined by  $F_m$ ,  $F_c$ , and  $F_f$  [52]. Compared with ordinary droplets, magnetic marbles move much faster [54]. Moreover, the particles on the surface of the liquid marbles can reduce liquid evaporation and better preserve biological reagents.

Shin et al. delved into the fundamental principles of magnetic droplet manipulation and explored diverse actuation modes. Their work explored the equilibrium of forces influencing droplets during transportation and particle extraction [55].

### 2.3. The Driving Mechanisms for Magnetic Droplet Manipulation

Based on the location of magnetic particles, magnetic droplets can be classified into two groups: magnetic particle droplets [56] and liquid marbles [57]. In the case of magnetic particle-based droplets, colloidal suspensions containing magnetic particles, magnetic beads, or nanoscale magnetic particles are introduced into the droplets [58] and then operated on in a closed structure immersed in mineral oil or on a chemically treated hydrophobic plate. Manipulating droplets based on liquid marbles occurs either on solid surfaces or with floating reagents. While both magnetic-driven droplet operation methods mentioned earlier can accomplish fundamental tasks like transferring, merging, and mixing, they still have limitations, including inadequate biocompatibility [26]. The magnetic material in the magnetic substrate will not directly contact the droplets, significantly improving the biocompatibility of the droplets. However, the distribution and splitting of droplets relying on magnetic substrates is a challenge. Here, we introduce four main

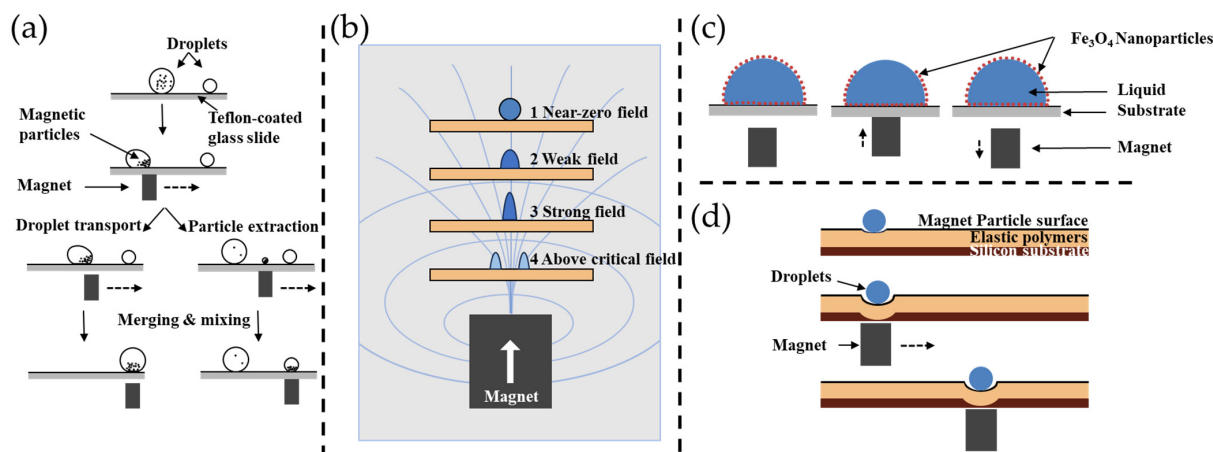


driving mechanisms for magnetic droplet manipulation, which are based on magnetic particles, ferromagnetic fluids, liquid marbles, and magnetic flexible substrates.

### 2.3.1. Magnetic Particles

The most common method of transforming a droplet into a magnetic droplet involves incorporating magnetic particles into the droplet. Magnetic force can be used to induce the excitation of droplets on superhydrophobic surfaces [26]. Typically, magnetic particles consist of a magnetic core and a magnetic shell, or they may be an aggregation of nano-sized magnetic particles embedded in a non-magnetic matrix. The magnetic properties of the beads are determined by the content and distribution of magnetic material inside them, while their biocompatibility and stability in various media depend on the choice of shell/matrix material [25]. There are many types of magnetic particles currently manufactured. The materials used in magnetic cores mainly consist of pure metals (such as Fe, Co, and Ni), oxides ( $\text{Fe}_2\text{O}_3$ ,  $\text{Fe}_3\text{O}_4$ ), or transition-metal-doped oxides and metal alloys (such as CoPt, FeCo, and FePt). Iron oxide has excellent magnetism and low cost, and is the most widely used magnetic material [59]. Magnetic cores without shell protection have hydrophobic surfaces that can cause agglomeration of magnetic particles. The surface of the particles is also prone to oxidation under environmental conditions, which affects the magnetic effect of the particles. To protect magnetic particles, they can be encapsulated with biocompatible materials or surface functionalized [60]. Surface materials fall into two main categories: inorganic molecules, such as silica shells [61,62], carbon [63], and metals [64–66], and organic materials such as surfactants [67] and polymers [68–70]. Magnetic particles have enabled the manipulation (generation, transport, splitting, and mixing) of individual droplets on a 2D surface without the need for a microchannel network or mechanical moving parts (Figure 2a). Sperling et al. [71] combined silica microspheres and  $\text{Fe}_3\text{O}_4$  nanoparticles to prepare millimeter-sized colloidal superparticles that can self-assemble, self-drive, and move on 3D trajectories. In order to improve the targeted delivery ability of magnetic particles. Some scholars [72] have prepared  $\text{Fe}_3\text{O}_4$ @polyvinyl alcohol microspheres by polymerizing polyvinyl alcohol (PVA). The magnetic microspheres have good superparamagnetic properties and driving properties. Due to their high surface-to-volume ratios and the ability to manipulate them through magnetic fields, magnetic particles are well-suited as solid supports for bioassay development. The utilization of magnetic particles is increasingly prevalent in various biological applications.

Due to the enhanced permeability and retention (EPR) effect of drug-loaded nanodroplets, their biomedical applications are reduced. Huang et al. proposed a magnetic particle droplet that encapsulated fluorinated  $\text{Fe}_3\text{O}_4$ - $\text{SiO}_2$  nanoparticles in the nanodroplet. Doxorubicin (DOX) loaded in the shell can locally cause damage to tumor tissue through a static magnetic field [73]. In addition to  $\text{SiO}_2$ -modified magnetic particles, new liquid metal droplet electrodes are also used in electrocardiogram monitoring. By magnetically manipulating the electrodes, ECG signals can be accurately monitored [65]. Magnetic droplets covered with multi-responsive surfactants can precisely control the direction of the droplets, are extremely flexible, and have potential uses in artificial cell research [74].



**Figure 2.** Actuation of the magnetic droplet. (a) Manipulating microdroplets using magnetic particles enables various actions such as droplet transport, merging, mixing, and extraction of magnetic particles. (Reprinted with permission from Ref. [26]. Copyright © 2021, Wiley Online Library.) (b) Trajectory a ferrofluidic droplet under the impact of a coil arranged in a circular spiral pattern. (Adapted with permission from Ref. [75]. Copyright © 2013, Science.) (c) Manipulating liquid marbles using magnetic particles. (Adapted with permission from Ref. [52]. Copyright © 2012, Springer Nature.) (d) Manipulating liquid droplets on a pliable magnetic substrate. (Adapted with permission from Ref. [76]. Copyright © 2017, Springer Nature.). The dotted arrows represent the direction of movement of the magnet.

### 2.3.2. Ferrofluids

The arrangement and interaction of magnetic micro/nanoparticles (NPs) at the interface between two liquids can induce magnetization in structured droplets, resulting in the formation of ferromagnetic droplets (FMLD). In 2021, some scholars [77] conducted fluid dynamics experiments to investigate how the magnetization of FMLDs and their response to external stimuli can be adjusted through chemical, structural, and magnetic modifications. At the liquid–liquid interface, molecular surfactants and NPs are trapped, reducing interfacial energy and endowing the interface with its inherent properties. This functionalization enables novel magnetic materials. Particles lodged at the interface confer high stability to the system, preventing deformation and coalescence. This property finds applications in areas such as drug delivery and encapsulation of fluidic reactors. Liquid–liquid interfaces serve as traps for various substances, including molecular surfactants [78], polyelectrolytes [79], biomaterials [80], and NPs [81], thus minimizing the interfacial energy and giving the interface its inherent properties. This functionalization enables novel magnetic, optical, electrically responsive, and biomimetic materials. Particles plugged at the interface provide the system with high stability against deformation and coalescence, which is relevant for applications such as drug delivery and encapsulation of fluidic reactors [74]. The magnetic properties of a droplet are contingent on the overall quantity of nanoparticles present within it, i.e., a given particle concentration, and also on the droplet volume. The FMLD’s magnetic properties can be further modified by interfering with the mixture of iron oxide and nonmagnetic nanoparticles at the liquid interface. The findings of the study indicate that creating heterogeneous patterns in multi-functional nanoparticle surfactant layers is feasible without sacrificing functionality. This can be accomplished through the selective assembly of nanoparticles in the presence of a magnetic field oriented perpendicular to the interface, akin to domain formation observed in ferrofluids [82].

The distinctive liquid characteristics and robust magnetic responsiveness empower ferrofluid droplets to divide and autonomously form reconfigurable three-dimensional structures. While the manipulation of ferrofluid droplets in static or quasi-static conditions has been thoroughly examined (Figure 2b), the dynamic regimes involving the fragmen-

tation and self-assembly of ferrofluids remain unexplored. Wang et al. [83] reported an unexplored ferrofluid droplet formation and self-assembly triggered by a droplet impact process at low magnetic field strengths, significantly reducing the required magnetic field. The initiation of self-assembly behavior is regulated by the intricate interplay between the kinetic energy of the droplet and the magnetic field, directing the system toward an equilibrium state. The interdependence of magnetic field strength and Weber number allows for the meticulous adjustment of ferrofluid post-impact dynamics, enabling the fine-tuning of self-assembly behavior. Ultimately, it was illustrated that impact-assisted self-assembly of ferrofluids is attainable on surfaces exhibiting varying degrees of wettability.

### 2.3.3. Liquid Marbles

A liquid marble is typically an aqueous solution encapsulated by a coating of hydrophobic powder. The powder forms a coating at the liquid–air interface, isolating the liquid in the marble from the outer surface on which the marble sits [84]. Magnetic liquid marbles can be opened and closed reversibly (Figure 2c). As the magnet ascends towards the liquid marble, the  $\text{Fe}_3\text{O}_4$  nanoparticles migrate in the direction of the magnet. The liquid marble is open at the top. Once the magnet is removed, the  $\text{Fe}_3\text{O}_4$  nanoparticles return to cover the exposed liquid surface [52]. A detailed study of the magnetic actuation of liquid marbles was carried out by Khaw et al. [85]. The horizontal movement of the floating liquid marble is influenced by both magnetic and frictional forces. The authors clarify the interplay between these forces by adjusting parameters such as magnetic flux density, magnetic flux density gradient, concentration of magnetic particles, and the speed of the marble. Generally, optimal sliding conditions involve a low speed of the permanent magnet, high magnetic flux density, and a high concentration of magnetic particles within the floating liquid marble.

Liquid marbles have the capability to encapsulate and transport various materials, exhibiting a unique characteristic of neither adhering to nor wetting surfaces. Additionally, they can responsively react to different stimuli, including changes in pH, electric fields, temperature, or exposure to UV radiation [86]. Magnetic liquid marbles driven by magnetic fields can also be used as mobile phases to transport different kinds of liquids and valuable chemicals on different surfaces (such as water or tilted platforms) [87], which provides a diversity of application areas. Alp et al. [88] combined anionic mixtures with the enhanced diffusion behavior of magnetic liquid marbles and demonstrated their applicability for oil/water separation and manipulation as well as microfluidics. Magnetic liquid marbles containing a mixture of anionic surfactants were prepared using superparamagnetic iron oxide nanoparticles (SPIONs) modified with non-covalently bonded semifluorinated alcohols. In addition to being able to increase the hydrophobicity of the nanoparticles, the self-oscillating behavior of the fluorinated alcohols maintains rapid fracture of the marble and provides convective flow that supports the rapid transformation of the oil phase into the lens form, thus helping to obtain better phase separation. However, magnetic particles on ordinary magnetic liquid marbles may inadvertently escape from the surface of the liquid marble under an external magnetic field, or their shells may open uncontrollably. To solve this problem, Bormashenko et al. [54] for the first time covered ferromagnetic fluid droplets with polyvinylidene fluoride (PVDF) particles and made ferromagnetic fluid-liquid marbles (FLMs). Subsequently, Mohammadrashidi et al. [89] first systematically investigated the manipulation of FLMs and their motion control physics by subjecting them to a DC magnetic field generated by an electromagnet and a pulse-width-modulated (PWM) magnetic field.

### 2.3.4. Droplet Manipulation by Magnetic Flexible Substrates

To manipulate droplets, passive methods such as micro or nanopatterned surfaces enable liquids to wet and spread in specific directions [90]. While these approaches have the ability to govern liquid wetting and spreading without the need for external forces, they are often slow and irreversible. Unlike conventional magnetic droplet manipulation techniques,



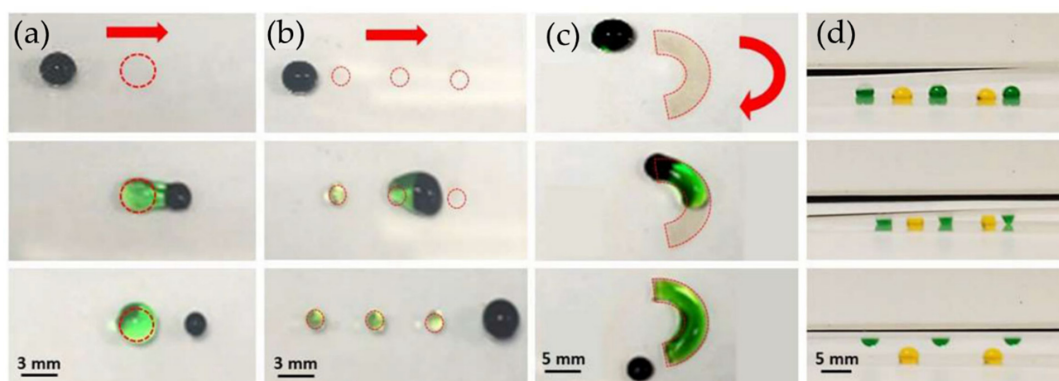
many scholars have prepared various magnetic films, such as a thin film of ferrofluid floating on the surface of a liquid [76], a magnetically responsive flexible film possessing actuating hierarchical pillars on the surface [38], and a micropillar array membrane based on hydrophobic and low-viscosity magnetic functionalized polydimethylsiloxane (MF-PDMS) [91], etc., using only permanent magnets to actively, quickly, precisely and reversibly control the position and motion of purely discrete droplets (Figure 2d).

In dealing with complex liquids characterized by low surface tension, high viscosity, or corrosiveness, Guo [92] described a magnetic digital microfluidic platform (MDMP) capable of manipulating a wide range of droplets. The MDMP was created by integrating a magnet-controlled deformable substrate with a smooth liquid-injected surface. This combination incorporates a magnetic actuation layer and a deformable substrate, facilitating swift indentation under a magnetic field and utilizing the self-gravity of the sample droplet as a driving force for transportation. The remote manipulation mechanism aims to minimize potential damage to delicate samples. Employing a flexible, smooth surface with complete repellent properties facilitates efficient droplet transport. The resulting MDMP allows for rapid, reversible, and precise manipulation of diverse droplet types in air or other immiscible fluids without compromising the properties of the driving fluid.

To manipulate droplets more flexibly and achieve morphological transformation of magnetic substrates, several complex manufacturing processes are required, especially the programming of the local magnetization of the structure. Patterning magnetic materials at the nanoscale has been demonstrated to attain morphological transformation structures at the scale of tens of microns [93]. Besides morphological transformability, the ability to program surface structures is equally crucial for enhanced adaptability to multiphase environments, particularly in biological sample processing and medical diagnostics. Hence, it is imperative to enhance propulsion robustness and compatibility in both wet and dry environments. Hwang et al. [94] designed a magnetically actuated soft, transformable microswimmer for environmentally friendly and sustainable fluidic handling. They are made from a composite blend of magnetic particles and soft UV-curable resin. A custom-developed fabrication system allows programming of the localized magnetization for each part area, along with UV exposure of magnetic-field-induced bending movements and surface microstructures to minimize surface stiction. Demonstrating significant promise as carriers of fluids for biochemical sample analysis or drug delivery.

#### 2.4. Droplet Manipulation

Magnetic control modulation facilitates the processes of droplet generation, deformation, motion, transport, fusion and splitting, and sorting (Figure 3) [95,96]. Both electromagnetic needles and permanent magnets can manipulate the transport, mixing, and dispersion of magnetic droplets.



**Figure 3.** Droplet manipulation on the microfluidic platform. (a) Particle extraction; (b) liquid dispensing; (c) liquid shaping; and (d) cross-platform transfer. The dashed lines in all panels indicate the polydopamine surface energy trap. The movement direction of the permanent magnet is indicated by the red arrows. (Adapted with permission from Ref. [96]. Copyright © 2019, Wiley Online Library).

#### 2.4.1. Droplet Generation

Early methods for generating droplets primarily involved high-speed stirring, layer-by-layer assembly technology, membrane emulsification, interfacial polymerization, etc., all of which can generate micro and nanometer-sized droplets. However, these methods still have shortcomings in terms of droplet stability, uniformity, and monodispersity. Recently, microfluidic chips have served as a platform for the creation of microdroplets. The size of the generated droplets is controllable, with low diffusivity and fast generation, and is not prone to cross-contamination, rendering them suitable for high-throughput analysis [97,98]. Methods for generating droplets utilizing microfluidic chips can generally be divided into active methods [99] and passive methods [100]. The passive approach utilizes the shear force and interfacial tension of fluid flow to generate droplets through the microchannel structure. This is achieved by adjusting the flow rate value and the ratio of the continuous phase to the dispersed phase. It mainly includes the T-shaped channel method, flow focusing method, and coaxial focusing method. The passive method system is relatively simple and easy to operate and is suitable for situations where a large number of droplets simply need to be generated quickly. Commonly used methods of active methods include the electrowetting method, magnetic method, pneumatic method, thermal drive method, etc. The principle is to use external force to generate a pressure difference at both ends of the liquid and use the shear force and surface of the liquid at the intersection of the microfluidic channels. Droplets are formed due to the difference in tension. The active method system is comparatively intricate and demands sophisticated chip processing, leading to heightened experiment complexity and costs. However, the active method can control a single droplet as needed and has great advantages in the controllability of the droplets.

In active droplet generation methods [101], the generation of droplets using magnetic fields can be easily achieved through the use of simple permanent magnets or by incorporating electromagnets into microfluidic devices. Moreover, magnetism is advantageous compared to other methods as it remains unaffected by pH, temperature fluctuations, and surface charge. Amiri Roodan et al. [102] introduced a numerical model that elucidates the microfluidic generation and manipulation of ferrofluid droplets in the presence of external magnetic fields. It shows that the magnetic field has a greater influence at low flow rates, and for a ferrofluid concentration of 50% ( $v/v$ ), the change is more significant. Liu et al. [103] documented the dynamics of the ferrofluid droplet formation process in a flow-focusing configuration. The findings indicate that the size of the droplets escalates with the augmentation of the magnetic field strength. Harischandra et al. [104] first proposed the concept of shaping droplets at the air–liquid interface. Nonmagnetic liquids can be squeezed, rotated, and molded at an air–ferrofluid interface with controllable deformation. They can be used to form thin films of various shapes at the air–ferrofluid interface, with potential use as an active environment for mammalian cells. Through the research conducted by these scholars, we have gained a profound understanding of the generation of magnetic droplets.

#### 2.4.2. Droplet Transportation and Sorting

Magnetically driven droplet manipulation is a low-cost and simple method. The power to move a droplet on a low-friction surface may come from internal superparamagnetic particles or paramagnetic salts. Magnetic elements act as valves and actuators to pump fluids that are otherwise immiscible [12].

For example, permanent magnets were used to transport water droplets encapsulating hydrophilic magnetic beads in a flat-bottomed tray reactor immersed in silicone oil [105] or utilize superhydrophobic electromagnetic needles (EMNs) to perform complex liquid manipulations on open surfaces [37]. Switching or adjusting the EMN power supply allows for a comprehensive array of droplet operations, encompassing droplet transfer, fusion, mixing, magnetic bead (MB) extraction, and droplet dispensing, all executed with simplicity and flexibility.

Apart from droplet transport controlled by magnetic fields, investigations into droplet transport using magnetically controlled substrates have garnered significant attention. Combining the unique properties of superhydrophobic and magnetically responsive surfaces, magnetically responsive superhydrophobic surfaces can be fabricated through a one-step spraying method [106]. Ben et al. [107] developed super wettable magnetic microcilia array surfaces with structures that can be switched by external magnetic fields for droplet manipulation. This innovative surface allows for the continuous transportation of droplets along specific directions and accomplishes lossless directional transport of droplets. Hu et al. [68] fabricated a gradient-shaped magnetic material comprising magnetic particles and shape memory polymer. Superparamagnetic droplets exhibit self-directed transportation on the surface influenced by magnetic gradients. Concurrently, reversible control of surface morphology is attainable through the shape memory effect, facilitating on/off control of droplet sliding. The integration of directional self-propulsion and switchable sliding control has been successfully achieved.

Recently, it was observed that the propulsion generated by microorganisms can be utilized to apply the force necessary for droplet movement. Magnetotactic bacteria (MTB) enable the biological actuation of droplets on superhydrophobic surfaces [50]. This approach in droplet microfluidics proves to be effective for bacterial culture and sorting. Not only that, Yuan et al. [108] produced alginate beads containing bacteria encapsulated with  $\text{Fe}_3\text{O}_4$  magnetic nanoparticles ( $\text{Fe}_3\text{O}_4$  MNP). These capsules can be manipulated using an external magnetic field, enabling effective sorting after the culture process.

#### 2.4.3. Droplet Split

Microparticles with a substantial surface area to volume ratio can host biomolecules and act as carriers for biomolecules when equipped with surface-functionalized receptors. Among these, magnetic particles are particularly effective and widely utilized in separation and detection processes due to their ability to be manipulated solely by magnetism. Wang et al. [109] described a remarkably efficient method for intra-droplet magnetic particle concentration and separation utilizing permanent magnets and EWOD for droplet manipulation. This approach concentrates and segregates magnetic particles into split droplets. Another new open-surface platform for droplet manipulation is based on an array of bendable nozzles that are dynamically controlled by magnetic fields [110] made with  $\text{Fe}_3\text{O}_4$  microparticles embedded in a polydimethylsiloxane matrix. The transport, mixing, and splitting of droplets can be controlled by bringing together and separating the tips of these nozzles under the influence of magnets.

However, there is a lack of easy magnetic actuation strategies to achieve versatile multiscale droplet manipulation. Jiang et al. [111] developed a magnetically driven Janus origami robot designed for versatile cross-scale droplet manipulation, encompassing three-dimensional transport, merging, splitting, dispensing, releasing sub-droplets, stirring, and remote heating. The robot enables untethered manipulation of droplets ranging from approximately 3.2 nL to about 51.14  $\mu\text{L}$ . It excels in splitting droplets and accurately dispensing (minimum ~3.2 nL) and releasing (minimum ~30.2 nL) sub-droplets. The externally applied voltage's magnitude and frequency are easily adjustable, creating an imbalance in surface tension at both ends of the droplet, thus propelling its movement. Magnetic control is straightforward and instantaneous. The combination of magnetic control and electric field control, offering characteristics like non-contact, convenience, controllability, and safety, proves advantageous for controlling droplets. A magnetoelectrically controlled magnetic liquid metal (MLM) approach also enables high-performance multiple manipulations of droplets [112]. The prepared MLM has good active and passive deformation capabilities. Under the action of a magnetic field, controllable transport, splitting, merging, and rotation is achieved. In addition, controllable electric field manipulation in alkaline and acidic electrolytes was achieved.

#### 2.4.4. Droplet Fusion and Mixing

For a variety of uses, including environmental control, medical diagnostics, and biochemical analysis, effective droplet manipulation is essential. While there is much promise for magnetic actuation, more work needs to be carried out to achieve high speed, precise manipulation, and enhanced mixing over a wide range of droplet volumes. A droplet fusion method that does not require a unique microchannel network or other equipment was developed by Gu et al. [113] and is based on variations in droplet interfacial tension. Reagents can be added to droplets sequentially as needed for multi-step reactions.

A simpler method to manipulate free droplets using ferrohydrodynamics is proposed [114]. Regarding the transportation of droplets, a set of periodic lines of ferrofluid on top of a silicon wafer is created by a single bar magnet and dynamically changed by the rotation of a magnetic stirrer beneath it. The findings indicate that the velocity of droplet motion depends on the rotation speed of the magnetic stirrer and the size of the droplets. To achieve better droplet mixing efficiency, smaller bar magnets were added to the above device to create discontinuous patterns at the mixing points.

Compared with closed microfluidic systems, open-surface microfluidic platforms have many advantages, such as easy fabrication, convenient interface, and no bubble clogging [115]. The manipulation of droplets depends on the localized deformation of an elastic membrane under an external magnetic field, facilitating swift droplet mixing by controlling the reciprocal coalescence of droplets to induce internal fluid circulation and enhance transport within the droplet. The study confirms that the reciprocating movement of droplets significantly enhances mixing efficiency, with higher driving frequencies generally resulting in shorter mixing times. Chen et al. [116] showcased that magnetic actuation can be easily attained by adorning a magnetoresponsive membrane with microcilia. When exposed to a magnetic field, the film promptly responds and undergoes localized deformation, collaborating with microcilia to achieve surface superhydrophobicity, accommodating a broad range of volumes from 2 to 100  $\mu\text{L}$  at speeds reaching up to 173 mm/s. This robust system facilitates swift and thorough droplet mixing in just 1.6 s. These new methods make droplet fusion and mixing more efficient.

#### 2.4.5. Other Manipulation

Magnetic levitation has been a prominent research area in recent years. Nevertheless, in most existing configurations, which typically employ two magnets with similar poles facing each other, this manipulation space is very limited. Zhang et al. [117] suggested an innovative magnetic levitation configuration utilizing a single-ring magnet, allowing for object manipulation and density-based measurements. To achieve efficient and rapid extraction of magnetic particles using magnetic levitation, it is imperative to streamline the mechanical equipment. Employing a synergistic interplay of capillary, magnetic, and electrowetting forces for extracting particles from droplets proves instrumental in facilitating effective and swift cleaning solutions on DMF platforms. This integrated approach not only significantly reduces detection time but also enhances detection sensitivity, expressed as a remarkable 90% improvement in the detection limit [118].

Moreover, droplet microfluidics also offers the capability to regulate emulsion formation and has emerged as a valuable tool in fabricating vesicles and protocells. Emulsion-based multicompartment artificial cells, known as ACDC droplets, are produced through microfluidics and acoustic levitation [17]. These droplets can be manipulated in situ to dynamically reconfigure the network of separated compartments, adjust the structure's orientation as needed, and apply thermal and light energy.

### 3. Biomedical Applications

In recent years, there has been significant progress in droplet manipulation technology, with droplet manipulation finding widespread applications in biomedical analysis. Existing droplet manipulation technologies include optical, acoustic manipulation, hydrodynamic manipulation, dielectric manipulation, and magnetic manipulation. Among them,

magnetic control has unique advantages because it can control droplets in a non-contact manner, and magnetic energy is easy to obtain and portable, laying the foundation for POC detection. Magnetic manipulation is becoming a common requirement in the biomedical field, such as for strain/enzyme activity screening [119], cell separation [120–122], magnetic biosensors [123,124], therapy (thermal cancer treatment) [125], drug and gene delivery [126,127], deep brain stimulation and tissue engineering for regenerative medicine [128,129], MRI diagnostics [130], theranostics [131], magnetic microreactors [132], etc. Combining magnetic hyperthermia (MHT) and immunotherapy can be used to ablate primary tumors and simulate metastatic tumor suppression. Pan et al. [133] synthesized monodisperse high-performance superparamagnetic  $\text{CoFe}_2\text{O}_4@\text{MnFe}_2\text{O}_4$  nanoparticles for thermal ablation of primary tumors. This nanoparticle shows great potential against primary and metastatic tumors. An electrochemical sensing system based on magnetic nanoparticles can quantitatively analyze the methylated septin9 (mSEPT9) gene to detect early colorectal cancer (CRC) [134]. In this segment, we will discuss three major applications of magnetic droplet manipulation in biochemical analysis, namely biomedical diagnostic applications, drug discovery, and cell culture.

### 3.1. Biomedical Diagnostic Applications

Magnetic droplet manipulation enables droplet operations including transport, distribution, fusion, and even opening. In bioassays, multi-step processes such as extraction, purification, dilution, mixing, and detection of small volumes of samples can be performed [49]. The application of magnetically manipulated droplets in biomedical diagnostics improves the performance of traditional experiments in terms of sensitivity, reproducibility, testing efficiency, and throughput [55,135,136]. In the subsequent sections, we will delve into and thoroughly discuss biomedical diagnostic applications centered around magnetically manipulated droplets. This will encompass detailed explorations of sample preparation, nucleic acid analysis, protein analysis, microbial analysis, and various other innovative biomedical diagnostic applications.

#### 3.1.1. Sample Preparation

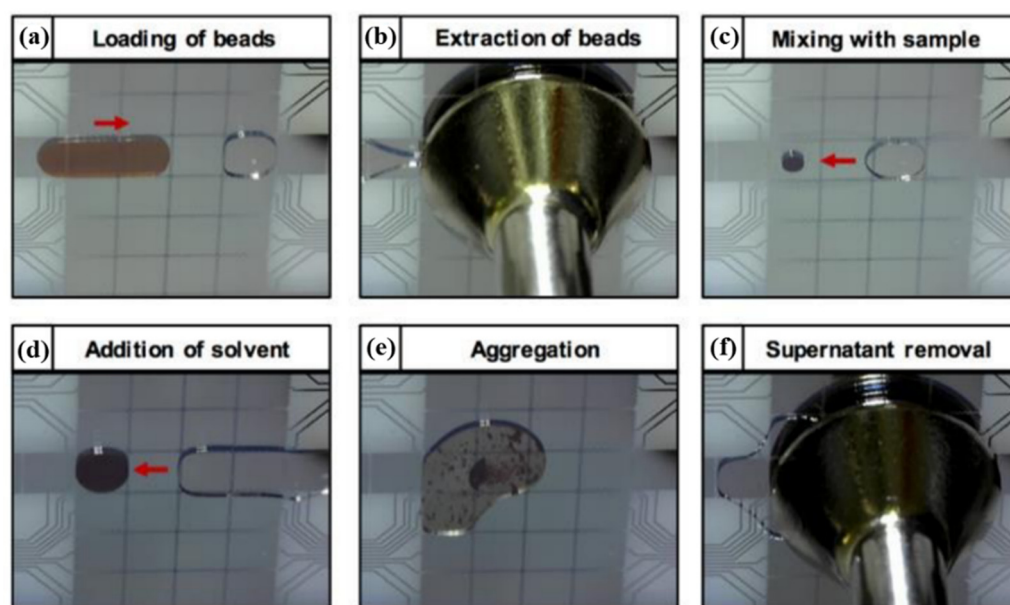
Sample preparation is an initial and critical step in vivo diagnostics and includes lysis, mixing, extraction, dilution, incubation, or other operations before detecting the target [137]. In numerous analytical techniques, particularly those involving high-throughput methods, sample preparation can be intricate, labor-intensive, and time-consuming. DMF, leveraging the capture function and mobility of MB, has the potential to streamline the sample preparation process and significantly decrease the operational time [138].

Encapsulation of single cells or microbeads within droplets is extensively employed in digital detection, single-cell sequencing, and drug screening. However, the arrangement of particles is entirely random, governed by the Poisson distribution. The theoretical likelihood of single-particle encapsulation typically hovers around 10%. In scenarios demanding ultra-high complexity, digital detection or the measurement of a substantial number of particles, the count of partitions can often become exceptionally large, leading to a notable increase in the number of invalid droplet statistics and redundant data for detection. To address this issue, a bead-ordered array droplet (BOAD) system has been introduced to disrupt the Poisson distribution [139]. The BOAD system ingeniously integrates sheath flow, dean vortex, and compression flow channels to achieve the ordered arrangement of particles for the first time. This system is capable of achieving the fastest-ordered arrangement of particles within the shortest structural span. The single-bead packaging efficiency is increased to as high as 86%. The additional use of encapsulated encoding beads and IL-10-targeting magnetic beads showcased the potential of ultrahigh multiplex bead-based digital detection. Consequently, the BOAD system holds significant promise for numerous applications demanding high rates of single-particle encapsulation in limited partitions, including protein preparation, multiplex digital bioassays, single-cell analysis, drug screening, and single-exosome detection.



Magnetic digital microfluidics is being recognized as a promising tool for proteomic sample preparation. Leiper et al. [140] have developed a protocol for bottom-up Liquid Chromatography-Mass Spectrometry (LC-MS)-based proteomics sample preparation on a commercially available digital microfluidic device requiring only 100 mammalian cells. In this context, efficient cell lysis conditions optimized for DMF were developed, along with a detergent buffer system compatible with downstream proteolytic digestion and subsequent LC-MS analysis on the DMF chip. This represents the first reported proteomics sample preparation workflow on a DMF chip device, enabling sensitive analysis of limited biological materials (Figure 4).

Sample preparation mostly adopts complex processes in traditional biomedical experiments, including lysis, affinity MB target capture, washing, and nucleic acid amplification. Employing magnets for droplet manipulation can enhance detection performance by simplifying the sample preparation process, minimizing sample loss, and bolstering detection capabilities [141].



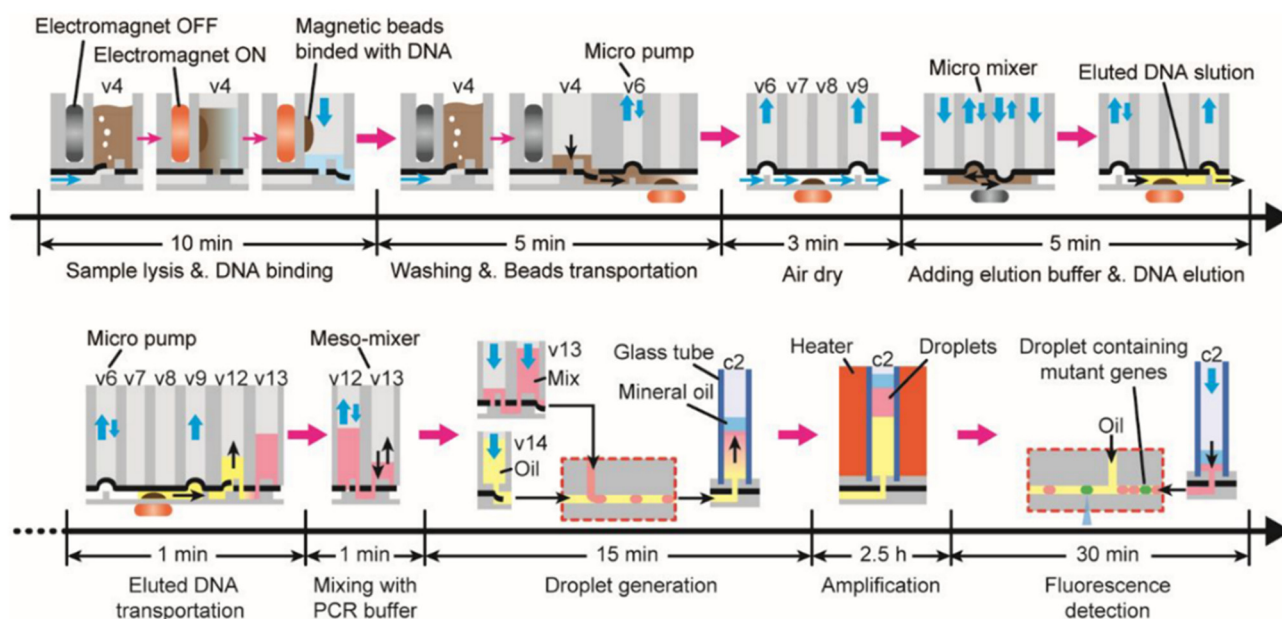
**Figure 4.** Sample clean-up by DMF-SP3 using a permanent magnet. The red arrows represent the direction of droplet movement. (Adapted with permission from Ref. [140]. Copyright © 2019, Royal Society of Chemistry).

### 3.1.2. Nucleic Acid Detection

Nucleic acid testing is primarily used to detect nucleic acid molecules, such as DNA or RNA, in biological samples. Nucleic acid testing plays a crucial role in determining the presence or absence of specific gene sequences in an organism, serving purposes such as pathogen detection, hereditary disease screening, and gene expression analysis. The fundamental principle of nucleic acid detection involves extracting nucleic acids from a biological sample, utilizing specific primers (guide sequences) to bind to the target gene sequence, and ultimately detecting the presence or absence of the target nucleic acid sequence through various reaction steps, including Polymerase Chain Reaction (PCR), Nucleic Acid Hybridization, and more. PCR, in particular, has emerged as a powerful tool for genetic analysis, enabling applications such as genotyping, sequencing, gene expression analysis, diagnosis, etc. Droplet-based microfluidics have garnered attention for constructing more flexible and scalable systems that facilitate the delivery of numerous samples or reagents as individual droplets for chemical and biochemical analysis [142,143]. Nucleic acid extraction is the prerequisite for nucleic acid detection and diagnosis. Obtaining purified nucleic acid in a short time is a key step to ensure accurate and reliable test results. Shen et al. [144] captured nucleic acids in a microwell array in a one-well-one-bead manner.

One-step detection of SARS-CoV-2 can be performed on the chip. The development of integrated “sample-through detection” on-site testing is the future direction of nucleic acid testing. Through magnetic manipulation separation technology based on microfluidics, it is expected to realize portable and low-cost nucleic acid extraction, becoming a new nucleic acid sample processing alternative for testing after collection in the field [145].

The investigation of circulating tumor DNA (ctDNA) released into the peripheral blood during tumor cell death has emerged as a promising alternative to invasive tissue biopsy [146]. Nevertheless, the actual limitation lies not in the amplification reaction but in the complexity of sample preparation. Indeed, due to the extremely low concentration of these molecules (typically a few nanograms per milliliter) and the extensive fragmentation (within the 50–1000 base pair size range), effective isolation of ctDNA from blood remains challenging, particularly in the early stages of cancer [147]. The current study [148] presented a microfluidic approach that integrates a dynamic magnetic extraction procedure with droplet-based digital PCR (ddPCR). This approach maximizes the DNA-binding surface area within the chip to capture short DNA fragments and potentially recover purified samples in picoliter volumes, enabling high-sensitivity mutation detection. The efficiency of capturing circulating cell-free DNA (ccfDNA) is comparable to standard column-based DNA extraction methods. This technology requires smaller amounts of materials and reagents and holds higher potential for automated and multiplexed DNA analysis. Geng et al. [149] took a step further and introduced a “sample-to-answer” detection method for rare ctDNA (Figure 5). Utilizing the “3D scalable” design paradigm, they successfully developed an integrated droplet digital PCR (IddPCR) microdevice that automates the entire liquid biopsy process, from extracting ctDNA using magnetic beads in 2 mL of plasma to generating and amplifying over 30,000 droplets for detection. This work presents a viable solution for the automation of liquid biopsy.



**Figure 5.** Diagram of the workflow of liquid biopsy in the IddPCR microdevice. The red arrows represent the operation process of IddPCR microdevice, the blue arrows represent the direction of movement of magnetic beads, and the black arrows represent the direction of liquid flow. (Adapted with permission from Ref. [149]. Copyright © 2020, American Chemical Society).

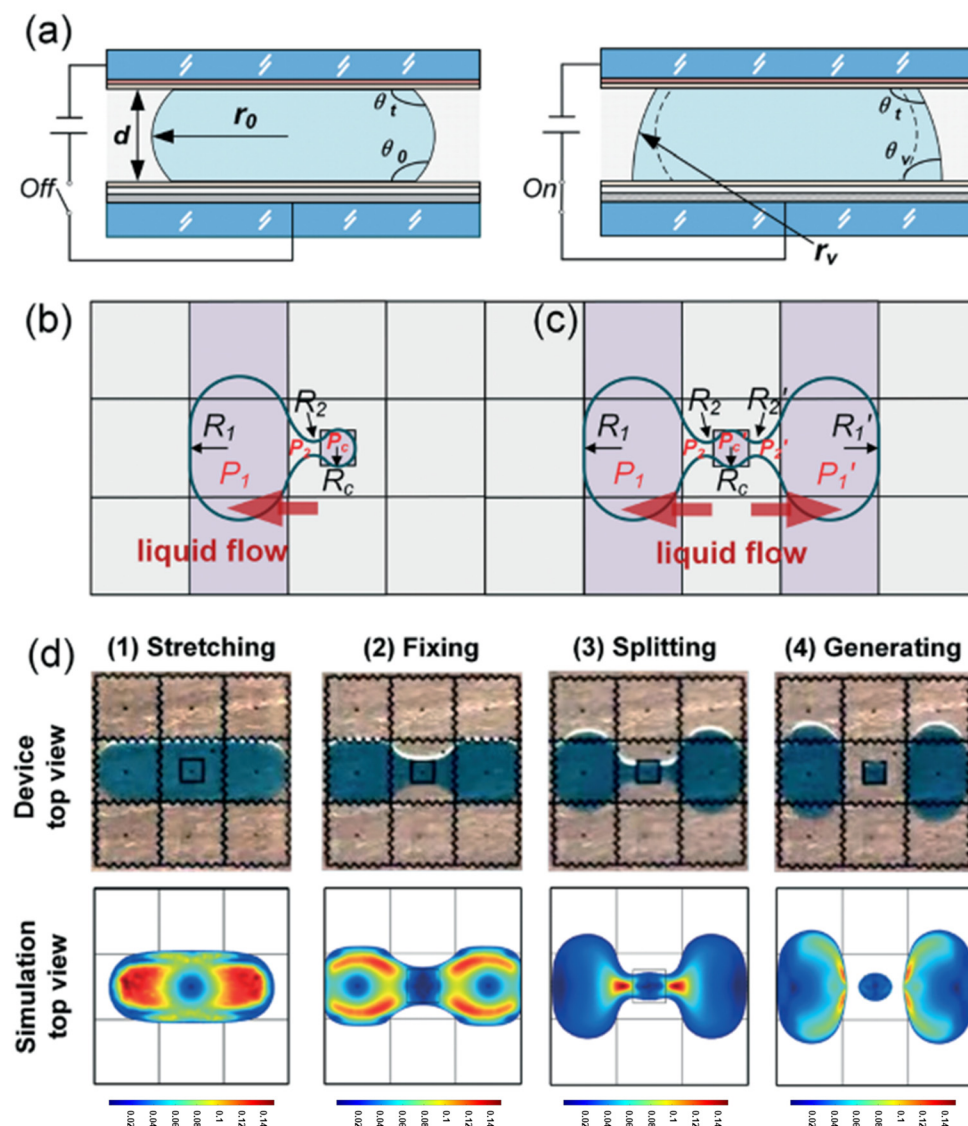
Significant advancements have been achieved in the creation of portable nucleic acid amplification devices designed for on-site patient use. Nanayakkara et al. [150] reported a simplified method for purifying and amplifying DNA from complex samples with a minimum number of steps. Chitosan-coated microparticles were employed to both lyse human cells and capture the liberated DNA in a single mechanical stirring step.

Subsequently, the magnetic microparticles were transferred to the PCR, enabling direct amplification of the bound DNA from the microparticle surface.

### 3.1.3. Protein Detection

Detection and analysis of proteins are of critical importance in the diagnosis and treatment of clinical diseases, especially in dealing with infectious diseases. Immunoassay focuses on the interaction between antibodies and antigens to quantify analytes for various applications, including medical diagnostics, pharmaceutical research, and biological research. Both competitive and non-competitive assay modes were employed for measurements. The utilization of magnetic particles as solid supports presents an appealing approach due to their ability to enhance surface area to volume ratio, improve mixing, and reduce diffusion time and reagent consumption, resulting in accelerated analysis and reduced cost per assay [141,151], which marks a significant milestone as both techniques primarily describe noncompetitive immunoassays. Ng et al. [97] presented the inaugural competitive immunoassay for  $17\beta$ -estradiol ( $E_2$ ) using digital microfluidics. They also introduced an on-chip particle separation and resuspension method capable of eliminating over 90% of unbound reagents in a single step. This approach offers a rapid, low-waste, and cost-effective instrument for the quantitative analysis of proteins and small molecules in small sample volumes. To achieve broad droplet manipulation, a hydrophilic pattern, called a surface energy trap, was introduced onto a polytetrafluoroethylene-coated hydrophobic substrate. Yet, substrates coated with polytetrafluoroethylene are challenging to modify. So, Kanitthamniyom et al. [96] improved the polydopamine coating process and analyzed the surface characteristics of polydopamine surface energy traps. On platforms utilizing polydopamine surface energy traps, they successfully demonstrated various droplet manipulations, including particle extraction, liquid dispensing, liquid shaping, and cross-platform transfer, in both single and two-plate configurations.

To achieve more detection within a limited area, that is, to increase the detection throughput of the system, a “one-to-three” droplet splitting technology has emerged, capable of generating submicroliter droplets that exceed the traditional geometric limits of electrowetting dielectric digital microfluidics (Figure 6) [152]. They implemented an on-chip magnetic bead chemiluminescence immunoassay method using the “one-to-three” technology for parallel detection. The microdroplets generated by this technology allow for significantly reduced magnetic forces while still retaining magnetic beads. In just 10 min, they could detect samples of five B-type natriuretic peptide analytes on a single chip. To enhance sensitivity and enable multiplex detection. In comparison to chemiluminescence methods, the more straightforward Raman scattering effect has also found application in digital microfluidic immunoassays. Wang et al. [153] proposed a digital microfluidic immunoassay based on Surface-Enhanced Raman Scattering (SERS) for rapid, automated, and sensitive detection of disease biomarkers. They synthesized a SERS tag labeled with 4-mercaptobenzoic acid (4-MBA), which had a core-shell nanostructure, displaying a strong signal, good uniformity, and high stability. The designed sandwich immunoassay enabled the sensitive detection of antigens through strong SERS signals by labeling immune complexes with SERS tags functionalized with detection antibodies. In comparison to the standard enzyme-linked immunosorbent assay (ELISA) method, the DMF-SERS method exhibited excellent sensitivity (limit of detection of 74 pg/mL) and selectivity for H5N1 detection, with a shorter detection time (less than 1 h) and lower reagent consumption (approximately 30  $\mu$ L). This method holds great potential for the automatic and sensitive detection of various infectious diseases.



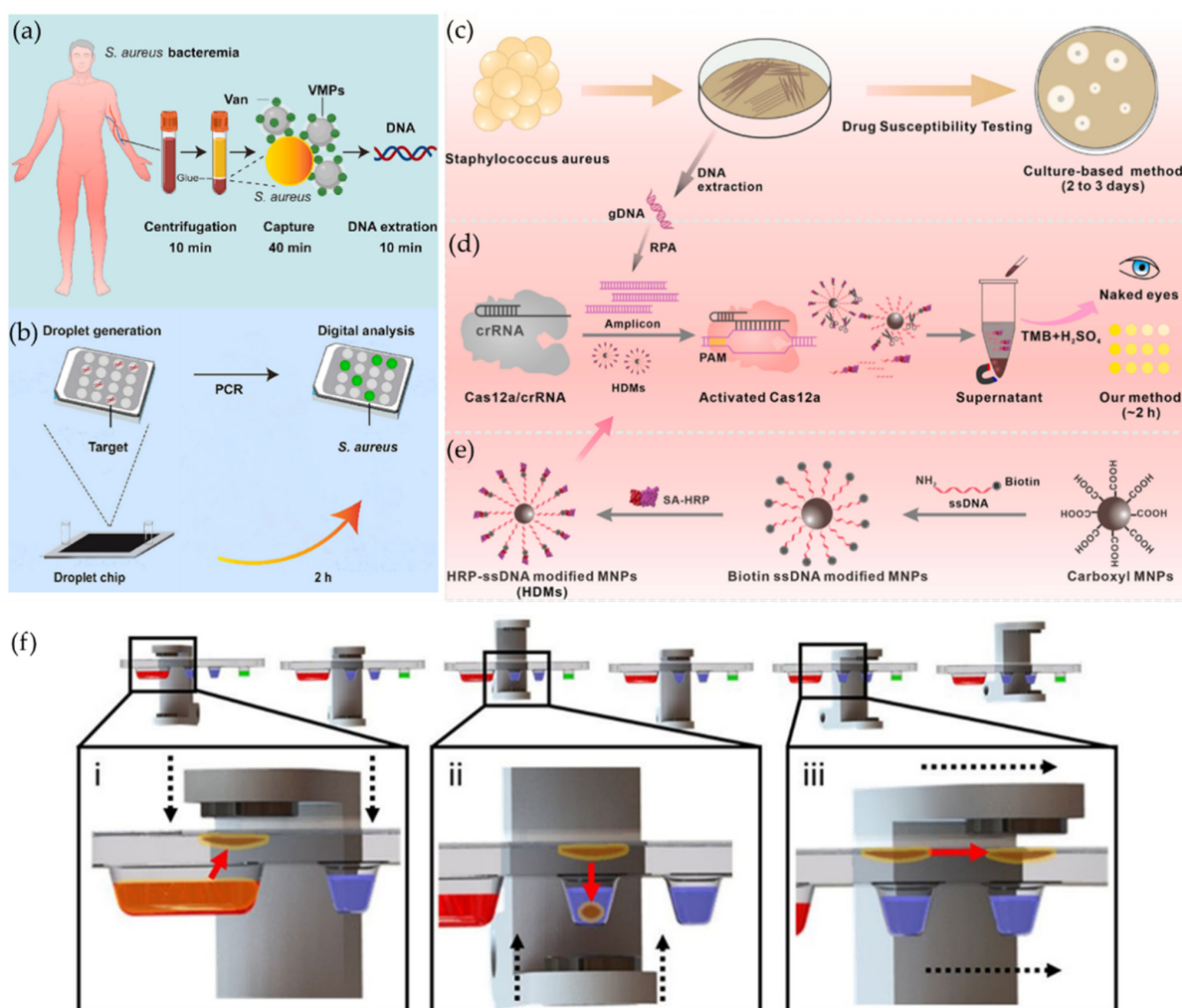
**Figure 6.** “One-to-three” droplet splitting method for microdroplet generation. (a) Cross-sectional representation of the digital microfluidics (DMF) chip before and after applying voltage to the droplet. (b,c) The conventional droplet splitting method and the “one-to-three” droplet splitting method, respectively. P and R represent pressure and radius of curvature, respectively, and the red arrow indicates the direction of liquid flow. (d) Top-down view of the flow in the “one-to-three” droplet splitting method. Device views and simulation views are presented from top to bottom. (Adapted with permission from Ref. [152]. Copyright © 2021, Royal Society of Chemistry).

### 3.1.4. Bacteria and Virus Detection

Microorganisms are ubiquitous in our environment and exert a profound influence on human society. However, there remains a need for efficient tools to screen live bacteria, and droplet microfluidic technology offers a promising solution to address this challenge. Yuan et al. [154] presented an all-water microfluidic workflow facilitated by oil mediation, wherein the cultivation environment comprised entirely of water fosters bacterial growth through enhanced cell–cell and cell–environment interactions. Alginate beads incorporating  $\text{Fe}_3\text{O}_4$  magnetic nanoparticles were employed for on-chip manipulation of microcapsules. This core–shell structure isolates bacteria and magnetic particles in the core and shell, respectively. Droplet microfluidics not only facilitates the screening of live cells but also allows for bacterial enrichment. By combining vancomycin-modified magnetic nanoparticles (VMP) with a separation gel, *Staphylococcus aureus* can be efficiently en-



riched from blood samples [155]. Subsequently, the enriched samples are detected using ddPCR. This culture-free method, based on nanoparticles allows for the concentration and detection of *Staphylococcus aureus* in whole blood within a mere 3 h, representing a substantial reduction in detection time compared to traditional culture methods (3–5 days). With a detection limit as low as 10 CFU/mL, this approach significantly enhances the diagnostic rate of blood bacteremia (Figure 7a,b).



**Figure 7.** Illustration of the *S. aureus* capture and detection process. (a) Involves the collection of blood samples, centrifugation, capture of *S. aureus*, and extraction of genomic DNA. (b) Encompasses the generation of droplets, PCR, and subsequent digital analysis. (Adapted with permission from Ref. [155]. Copyright © 2023, Science Direct.) (c–e) The illustration of CRISPR/Cas12a-based bioassay for MRSA identification. (Adapted with permission from Ref. [156]. Copyright © 2023, Science Direct.) (f) Particle transfer actuation. (i) Lowering the upper magnet to attract the particle plug into the oil for collection on the PTFE surface, (ii) raising the lower magnet to introduce the particle plug into reagent droplets, and (iii) rotating the magnet to facilitate particle transfer between wells. The black arrows represent the direction of movement of the magnet, and the red arrows represent the direction of movement of the magnetic beads. (Adapted with permission from Ref. [157]. Copyright © 2018, Nature).

*Klebsiella pneumoniae* (KP or *K. pneumoniae*) stands as a significant nosocomial pathogen, notably responsible for severe respiratory infections. Such infections, marked by high mortality rates, pose a particular threat to infants and can lead to invasive infections even in healthy adults. Chen et al. [158] developed a quantitative detection platform using an immunochromatographic test strip (ICTS) based on nanofluorescent microspheres (nFM)



for the rapid identification of *Klebsiella pneumoniae*. This platform allows for specific differentiation between *Klebsiella pneumoniae* and non-pneumoniae *Klebsiella* samples. Experimental results demonstrate 100% consistency between the immunochromatographic test strip method and traditional clinical methods in detecting and diagnosing clinical samples. During the purification process, silicon-coated magnetic nanoparticles (Si-MNPs) were employed to effectively eliminate false positive results, showcasing robust screening capabilities. Furthermore, the pertinent literature in this field indicates that magnetic droplet manipulation can also be employed for nucleic acid amplification detection. Wu et al. [156] developed a rapid, specific, and ultrasensitive colorimetric assay utilizing recombinase polymerase amplification (RPA) and magnetic nanoparticle (MNP)-assisted CRISPR/Cas12a for detecting the *mecA* gene in MRSA bacteria (Figure 7c–e). This method demonstrates a strong linear correlation in detecting the *mecA* gene within the range of 1 to  $1 \times 10^6$  aM, with a detection limit as low as 0.2 aM. Additionally, it enables rapid and accurate identification of MRSA through observable color changes within 2 h, making it a promising tool for point-of-care testing (POCT) in the practical application of drug-resistant bacteria identification.

Viral infection constitutes a significant public health challenge, and magnetic droplet manipulation can be applied to detect viruses as well. For example, some scholars [157] proposed a droplet magnetic fluid (DM) platform that utilizes functionalized magnetic particles to miniaturize and automate experiments for use in point-of-care (POC) clinics. Users can inject the biological sample into a cartridge with magnetic particles, and then load the cartridge into the instrument. This system allows for quantifying the Hepatitis C virus (HCV) RNA viral load in serum in approximately 1 h. See Figure 7f for detailed operations.

### 3.1.5. Other Molecules Detection

Nearly all cell types have the capability to produce and release exosomes, membranous vesicles that are released into the extracellular matrix following the fusion of intracellular multivesicular bodies with the cell membrane. These vesicles encapsulate substances like proteins, mRNA, and microRNA. Extracellular vesicles (EVs) are extremely popular as biomarkers of disease because they contain components from the cells that release them. However, the EV content is scarce, and the extraction purity and throughput of traditional methods are low. EV isolation through magnetic bead affinity capture can process a large number of samples in a shorter time and has higher capture efficiency than commercially available methods [159]. Investigating cancer-associated exosomes can offer insights into tumorigenesis and clinical implications. The quantification of specific exosomes present in very small clinical samples at low concentrations holds promise for non-invasive cancer diagnosis and prognosis. Conventional techniques like nanoparticle tracking analysis (NTA), Western blotting, ELISA, and flow cytometry have been extensively utilized for exosome measurement. Nevertheless, these methods necessitate large sample inputs, exhibit limited specificity, and possess restricted sensitivity [160–163]. The droplet digital Exosome-Enzyme-Linked Immunosorbent Assay (ExoELISA) method represents a groundbreaking approach for the precise quantification of cancer-specific exosomes, achieving unparalleled accuracy in absolute counting. This innovative technique leverages droplet technology, facilitating a single ExoELISA. This refined methodology not only enhances precision but also reduces the sophistication of exosome quantification, marking a significant advancement in the field [164]. An innovative system employing Janus magnetic microspheres as barcoded microcarriers enables multiplex analysis of exosomes derived from urinary bladder cancer [165]. These microcarriers are constructed by co-assembly of droplet templates made of colloidal silica nanoparticles and magnetic nanoparticles under a magnetic field. One hemisphere of the microcarrier exhibits a structural color, while the other has a magnetic response. Utilizing this unique structure, Janus microcarriers can act as barcodes and controllably move in sample solutions, enabling highly sensitive multiplex detection of exosomes. The platform is non-invasive, has excellent sensitivity and specificity, requires low sample consumption, and is easy to operate.

In recent years, magnetic beads have expanded their applications beyond exosome detection to include the isolation of exosomes. Shen et al. [166] employed magnetic beads to isolate serum EpCAM-positive exosomes, and the copy numbers of lncRNARP11-77G23.5 and PHEX-AS1 in EpCAM-specific exosomes were quantified using ddPCR. At the moment, significant strides have been achieved in advancing platforms for generating and manipulating uniform magnetic droplets. These developments are accompanied by the integration of various high-throughput fluorescence-based techniques, enabling highly sensitive analysis of droplet contents. This progress has paved the way for the establishment of a specific, reliable, and highly sensitive exosome quantification platform.

Hormones are chemical information substances synthesized by endocrine cells and secreted into the blood. There are many types of hormones but in small amounts. They are neither the energy source nor the structural component of the body. It mainly affects the growth and development of the human body by regulating the metabolic activities of various tissue cells. For example, human chorionic gonadotropin (hCG) is a type of stimulant banned by the World Anti-Doping Agency (WADA). In male athletes, hCG can stimulate testosterone production and affect muscle size and performance. Using antibody-modified magnetic metal–organic framework nanoparticles (MMOF) as capture probes in urine samples, the SERS signal can be detected to detect the hCG content in urine samples. It can also detect human luteinizing hormone (hLH) and human chorionic gonadotropin (hGH). The hormone content can be detected simply and quickly through magnetic particle capture technology [167]. Magnetic molecularly imprinted polymer (MIP) technology has developed rapidly due to its high specificity, stability, and reproducibility. Moura et al. [168] successfully used L-thyroxine pre-concentrated on magnetic MIP for the first time on a magnetically driven electrode. Electrochemical sensing is performed with a detection limit as low as 0.0356 ng/mL. It provides new ideas for the design and application of magnetic molecularly imprinted polymers and opens up a new perspective for molecularly imprinted polymer technology to replace specific antibodies in biosensors and microfluidics.

### 3.2. Drug Discovery

The process of drug development contributes to advancing human health by enhancing the biological activity of pharmaceutical compounds and minimizing potential side effects, ultimately improving treatment efficacy, combating infections, and extending the overall lifespan of individuals [126]. Drug development includes drug synthesis, screening, delivery, and evaluation [169]. Nonetheless, this undertaking is characterized by its high cost, time-intensive nature, complexity, and extensive scope.

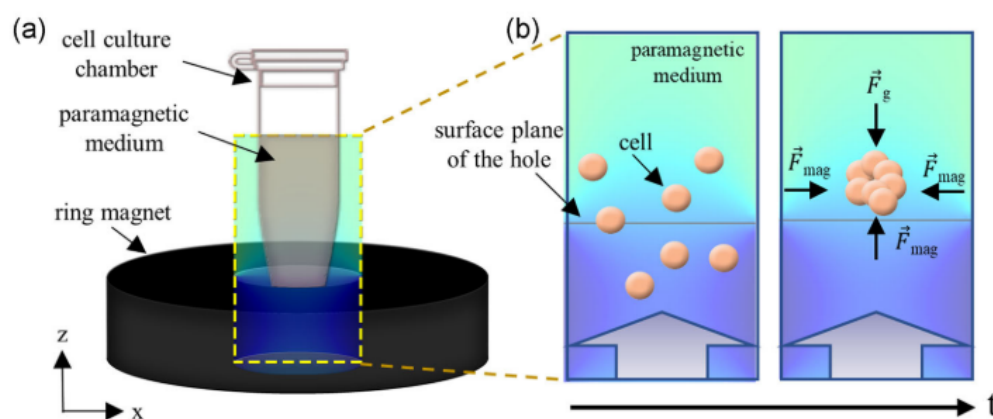
In the exploration of drug–protein binding, the integration of magnetic beads and microfluidic devices capable of generating segmented flows has opened avenues for the development of drug-screening equipment utilizing microfluidic droplets [170]. This approach specifically targets the analysis of drug interactions with specific targets, such as enzymes and cells, marking a pivotal initial stage in drug development. The technology based on microarray and suspension (or microbead) has garnered significant interest due to its widespread application in high-throughput drug screening. However, the throughput of microarrays is consistently constrained by array density and the sluggish molecular diffusion rate. In suspension-based technology, the reaction occurs directly on the surface of the molecular probe-functionalized microcarrier. To enhance microcarrier labeling, anisotropically designed magnetic microtags are employed to establish digital magnetic tags with a stable magnetization direction linked to a digital code [171]. These tags, functionalized with various biomolecule probes, can be suspended in a solution. In comparison to traditional coding technologies, magnetic tags offer several advantages, including minimal background signal, the potential for numerous distinct codes, equipment miniaturization, and the ability to encode in situ. The magnetic chip laboratory method can be applied to label biomolecule probes in drug discovery.

### 3.3. Cell Culture

Live cell manipulation is a widely employed technology in bioengineering, encompassing key fields like cell culture, cell sorting, and single-cell analysis. Cell culture is primarily employed for exploring specific drugs by investigating the sensitivity and resistance of cell lines to various pharmaceuticals. In vitro experiments enable the observation of morphological, structural, physiological, and metabolic changes in cells in response to different drugs, providing deeper insights into the mechanisms of drug action [172]. Traditional cell culture tools, such as culture dishes and well plates, are cost-effective and convenient but are susceptible to contamination. In response to this challenge, magnetic liquid marbles have emerged. Liquid marbles maintain a stable structure with nearly constant internal culture medium volume. The porous surface of liquid marbles not only effectively prevents water evaporation but also facilitates the smooth exchange of oxygen and carbon dioxide. The porous structure minimizes the risk of cross-contamination while providing effective containment. The findings indicated that the liquid marble created a sustained growth environment, thereby enhancing the viability of the tumor cells it contained. This introduces a novel and more reliable method for cell culture that is both efficient and less prone to contamination [173].

Ferrofluids have also exhibited applications in cell engineering beyond liquid marbles. Liu et al. [174] proposed multifunctional droplets coated with a layer of fluorinated magnetic nanoparticles, enabling magnetically driven droplet manipulation. These multifunctional droplets demonstrate excellent biocompatibility for cell culture, prevent molecule leakage, and exhibit high responsiveness to magnetic fields.

Antimagnetic levitation is an emerging technology for remote manipulation of cells in cellular and tissue-level applications. Magnetic levitation system based on a single ring of magnets to overcome the physical constraints of biofabrication (Figure 8) [175]. During the suspension process, the system allows for easy transfer of liquid or solid phases. Through the utilization of magnetic focusing and cell self-assembly, the system facilitates the formation and maintenance of millimeter-scale 3D structures. This enables convenient on-site intervention in cell culture within an open operating space. The suspension protocol can be customized for various cell types, such as stem cells, adipocytes, and cancer cells, representing different cell densities. Modification of the paramagnetic ion concentration and manipulation of medium density provide adaptability to different cell types. The technology permits the manipulation and merging of individually formed 3D biological units, as well as hybrid biofabrication with biopolymers.



**Figure 8.** Magnetic-force-guided levitation and self-assembly. (a) Overview of the magnetic levitation system, where the cell culture chamber is situated on the ring magnet, and its bottom is affixed to the magnet's hole. (b) Schematic depiction of cellular aggregation, with upward magnetic induction indicated by the block arrows. (Adapted with permission from Ref. [175]. Copyright © 2021, Wiley Online Library).

#### 4. Conclusions and Future Prospects

In recent years, there has been a growing interest in the utilization of droplet manipulation. The advancement of microfluidics has led to an expanding scope of research on the biological applications of magnetic droplet manipulation. This article reviews recent advances in magnetic droplet manipulation and its applications in biomedicine. We mainly discuss the characteristics, influencing factors, driving mechanisms, and applications of magnetic droplet manipulation. Magnetic droplet manipulation offers distinct advantages, including cost-effectiveness and the absence of the need for complex instruments merely requiring magnetic field control to handle the droplets. The process is straightforward, eliminating the need for tedious manual operations. Operations can be executed through a microfluidic chip, ensuring efficiency. Rapid detection is facilitated, and simultaneous execution of multiple experiments is achievable by controlling multiple magnets concurrently, thereby reducing reaction time and enhancing detection efficiency.

While magnetic manipulation systems have made significant strides, there are still some challenges that need to be resolved in future research. Firstly, currently, the biggest driving force of magnetic droplet manipulation is the permanent magnet, which still requires a large bulky external mechanical device, limiting further miniaturization and portability. Future devices are expected to be controlled by the micro electromagnet, which can be miniaturized and integrated as a control array increasing the control accuracy. More importantly, the parallel analysis throughput can be easily scaled up by increasing the micro electromagnet control array. Secondly, the droplet volume was still in the microliter range, which also challenged the parallel analysis throughput and the quantity of reagents. Thus, to further expand the scalability, the chip with the micrometer structure needs to be used to generate droplets with nanoliter volume. Thirdly, current methods are primarily limited to optical detection technologies, which need expensive and sophisticated optical devices. Consequently, it is necessary to develop portable signal readout methods to integrate with the magnetic droplet manipulation platform. For example, microelectrode and smartphones with high-quality camera lenses can be used to solve this challenge. Fourthly, current methods are challenging because of the low automation. The integration of micro electromagnet will greatly improve the automation level. Overall, future work about magnetic droplet manipulation is still focused on miniaturization, portability, high throughput, high integration, and high automation, which will promote improvements in the fields of point-of-care testing.

**Author Contributions:** Conceptualization, J.L. and Y.Z.; writing—original draft preparation, J.L.; writing—review and editing, J.L., K.S., H.L. and Y.Z. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was supported by the Natural Science Foundation of Chongqing (2022NSCQ-MSX0784), the Project of Through-Train to Doctor of Chongqing (CSTB2022BSXM-JCX0035), the Youth Project of Science and Technology Research Program of Chongqing Education Commission of China (KJQN202100425), CQMU Program for Youth Innovation in Future Medicine (W0149), the Intelligent Medicine Research Project of Chongqing Medical University (ZHYX202111), and start-up funding from Chongqing Medical University.

**Conflicts of Interest:** The authors declare no conflicts of interest.

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