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Review

Magnetic nanoparticles in microfluidic and sensing: From transport to detection

Superparamagnetic nanoparticles are attracting significant attention. Therefore, being explored in microsystems for a wide range of applications. Typical examples include lab-on-a-chip and microfluidics for synthesis, detection, separation, and transportation of different bioanalytes, such as biomolecules, cells, and viruses to develop portable, sensitive, and cost-effective biosensing systems. Particularly, microfluidic systems incorporated with magnetic nanoparticles and, in combination with magnetoresistive sensors, shift diagnostic and analytical methods to a microscale level. In this context, nanotechnology enables the miniaturization and integration of a variety of analytical functions in a single chip for manipulation, detection, and recognition of bioanalytes reliably and flexibly. In consideration of the above, recent development and benefits are elaborated herein to discuss the role of magnetic nanoparticles inside the microchannels to design highly efficient disposable point-of-care applications from transportation to the detection of bioanalytes.

Keywords:
Biosensors / Lab-on-a-chip / Magnetic nanoparticles / Microfluidic / Point-of-care
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1 Introduction

Microfluidics is a growing field of research that provides an excellent opportunity to build analytical devices capable of outperforming classical techniques in many biological, chemical, medical, and engineering fields. Integrated microfluidics biosensors introduce an innovative platform technology for biosensing and offer advantages such as miniaturization, automation, low cost, and high performance in terms of sensitivity and selectivity. Of vital importance is the incorporation of magnetic nanoparticles (MNPs) in microfluidic systems to perform biochemical assays for diagnosis and healthcare. Here, we will briefly discuss an overview and progress made in the field of microfluidics that gave a significant boost of performance and helps this technology live up to its potential.

1.1 A historical overview

The development of microtechnology for the fabrication of sensors leads to the innovation of microelectromechanical systems (MEMS). Of these MEMS, microfluidic systems were emerged as a fundamental part of the microanalysis framework, which has been utilized in different disciplines, for example, clinical diagnostics [1-3], genomics, proteomics [4, 5], drug analysis [6-8], and environmental testing [9]. In comparison with larger analytical laboratory-based machines, the μTAS has an improved potential due to its benefits of disposability, volume efficiency, lower run time, and the ability of in-situ and real-time analysis [10, 11]. Microfluidic systems involve the manipulation of a volume on the scale of microliters that is introduced into the system through a channel from tens to hundreds of micrometers in diameters, with the aim of mixing, transportation, and separation of the introduced analyte [12, 13]. At first, these microfluidic systems were used to develop pressure sensors, airbag sensors, and other mechanically movable structures. This was done through fluid handling devices such as channels in capillary connections, mixers, valves, pumps, and dosing devices.

Further development of these components has led to the creation in 1979 of the first lab-on-a-chip (LOC) analysis sys-

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Abbreviations: **AIV**, avian influenza virus; **CTC**, circulating tumor cell; **DMF**, digital microfluidic; **DMR**, diagnostic magnetic resonance; **EpCAM**, epithelial cell adhesion molecule; **GMR**, giant magneto-resistive; **IL-6**, interleukin-6; **LOC**, lab-on-a-chip; **MEMS**, microelectromechanical systems; **MNPs**, magnetic nanoparticles; **MR**, magnetoresistance; **PDMS**, PDMS; **POC**, point-of-care; **PSA**, prostate-specific antigen; **RBCs**, red blood cells; **RT-PCR**, reverse transcriptase-polymerase chain reaction; **SQUIDs**, superconducting quantum interference devices; **SV**, spin valve structures; **TCID**, tissue-culture infectious dose

Color online: See article online to view Figs. 1–15 in color.

Table 1. Comparison of different types of sensors employed for biosensing [26]

Electrochemical-based sensors	Optical-based biosensors	Mechanical-based biosensors	Microfluidic biosensors
Time consuming	Time consuming	Time consuming	Quick and time saving
Sensitive to the environment	Sensitive to the environment	Sensitive to the environment	Unresponsive to the environment
Require theoretical simulation for data Analysis	Theoretical simulation is not required for data analysis	No complicated simulation is required for data analysis	No theoretical complicated simulation is required for data analysis
Bulky devices required for detection	Bulky optical devices required	Bulky optical devices required	No bulky devices are required

tem by S.C. Terry at Stanford University [14, 15]. However, during the late 1980s and early 1990s, the LOC research started to grow exponentially with the development of micropumps, flow sensors, and concepts for integrated fluid treatment for μTAS [16]. These μTAS concepts integrated lab-scale pretreatment steps, into the microchip design that could extend the sensor's functionality toward a complete laboratory analysis procedure including additional cleaning and separation steps. The development of μTAS for genomics applications and biotechnologies gave an immense boost to research in the mid-1990s. Another boost also came from the military, especially from DARPA (Defense Advanced Research Projects Agency), for their interest in portable biochemical detection systems for warfare use. The added value of μTAS was not only limited to the integration of lab processes for analysis but also the unique possibilities of individual components and the application to other, nonanalysis, lab processes.

1.2 Recent development of integrated microfluidics biosensors

Development of a miniaturized scale framework known as biochips, Bio-MEMS, or LOC [17-19], improves the applications for high-throughput biological screening [20], cell analysis, and clinical diagnostics [21], as well as point-of-care (POC) analysis for biomedical and environmental monitoring [22]. There are many unmatched advantages of microsystems over conventional analytical, chemical, and biomedical tools. It incorporates high sensitivity, high throughput, in-situ observing, less waste, and decreased expense because of constrained liquid example volume, multiplexing, and decrease of the amounts of reactants and the time needed to analyze a sample [23]. The developed microsystems provide an alternate to the diagnostics and enhance the biomedical capacities for fast and reliable results.

Microfluidic systems employing such sensors and their integration with micronanofluidic channels provide a single chip-based platform for investigation in the field of chemistry, biochemistry, engineering physics, biotechnology, and nanotechnology, and life process [24]. These not only simplifies the complexity of biosensing and diagnostic tests, but also minimize the size of analysis equipment, and also reduce the hazard of handling harmful pathogens and chemicals [25]. Advances in microsystems have revolutionized the way for sensing and diagnostic measurement reliably and flexibly, which manages practices, precise control, and control of liquids that are geometrically obliged to a small, ordinarily submillimeter scale. In this context, there are many advances made to develop reliable sensors based on various mechanisms as summarized in Table 1 [26].

Innovation of nanotechnology has allowed miniaturization and incorporation of intricate research laboratory tools and analytical functions on a single platform. It has a promising future in improving detecting strategies and decreasing parallel apparatus needs by embedding internal recognition and handling modules in a single sign chip [27]. Recently microfluidic systems have shown promising potential in various applications that led to superior biological analysis systems including sensing and therapeutic.

Nanoparticle (NP)-based microsystems improve analytical procedures and their demonstration has an incredible effect in research and clinical practice. Figure 1 shows the schematic illustration of nanomaterial-based microfluidic chips using nanopillar, nanowire, gold NP, MNP, graphene oxide, nanofiber, and nanoroughened structure for the capture and detection of biomolecules [28]. Microfluidic systems dealing with NPs in fabrication and analytical processing have a small number of advantages in distinguishing biomaterials and improving signals and their characteristic impact [29]. Integration of diverse inorganic and organic NPs within the microfluidic devices offers new opportunities for future sensing applications including clinical analysis, sustenance quality control, and ecological observing. NPs work as a building block for many microfluidic systems especially in several applications of novel sensing systems. They are used to modify or integrate the transducer materials of microfluidic systems; individually or in the form of matrices to improve different aspects of performance that include detection limit, capability, and response stability. Microfluidic systems incorporated with NPs suggest the solid foundation of robust portable POC chip-based devices [30, 31-37].

Currently, MNPs have been used for the improvement and creation of a microfluidic system for many biosensing applications because of their unique property of superparamagnetism. Because of superparamagnetic behavior, these can be used as actuation handles and detection markers at the same time. MNPs can be used inside the microfluidic channel utilizing well-tuned high gradient fields, applied externally to

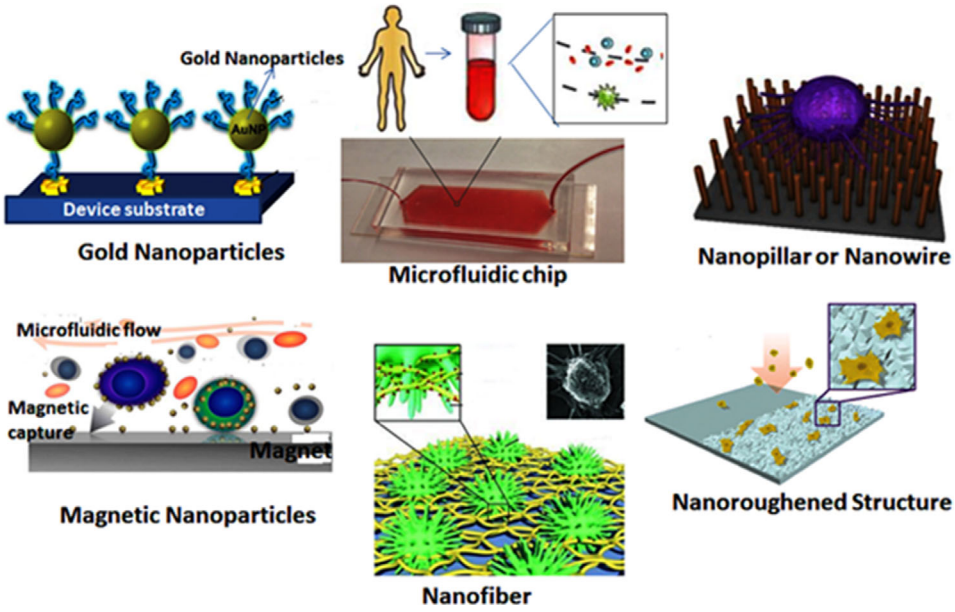

Figure 1. Schematic illustration of nanomaterial-based microfluidic chip. Reprinted with permission from [31, 32], copyright 2019 PubMed Central and Wiley (nanopillar or nanowire reprinted with permission from [33], copyright 2019 PubMed Central, gold nanoparticle reprinted with permission from [34], copyright 2019 American Chemical Society, magnetic nanoparticle reprinted with permission from [35], copyright 2019 American Chemical Society, nanofiber reprinted with permission from [36], copyright 2019 Wiley, and nanoroughened structure reprinted with permission from [37], copyright 2019 American Chemical Society) for the capture and detection of bioanalytes.

Table 2. Types, applications, and advantages of microfluidic integrated biosensors

Types	Applications	Advantages	Ref.
Enzyme-based microfluidics	Rapid detection of biomolecules	High reproducibility, selectivity, and excellent sensitivity of 2.2 mA/mM/cm^2.	[44]
Antibody-based microfluidics	Medical diagnostics, microarray detection, and observing protein-protein interactions.	High selectivity and excellent sensitivity with a detection limit of 1×10^{-4} mg/mL	[45]
Aptamer-based microfluidics	Continuous monitoring of cell-secreted products.	High affinity and selectivity with a detection limit of (5 ng/mL)	[46, 47]
Magnetic digital microfluidics	Molecular diagnostics, immunoassay, cell assay	Reliable performance and better sensitivity of 0.2 ng/mL	[48, 49]
Magnetic nanoparticles-based microfluidics	Biological engineering, biomedical studies, point-of-care diagnostics, environmental monitoring, and precision agriculture.	Higher sensitivity with a detection limit of 10 ng/mL	[50–52]

enable capturing, transportation, marking, and detection of biological molecules from physiological samples [38-40]. Additionally, the flexibility of functionalization with diverse biological agents makes them an ideal candidate as signaling and capturing material in miniaturized on-chip biosensing systems [41, 42]. The growth and characterization of an integrated microfluidic detection system using MNPs as versatile substrates on which the target antigen is captured, as detection labels, or instantaneously as substrates and labels have been regularly used in biosensing. Such magnetic bead-based biochemical detection a system can be applied to ultrasensitive protein detection [43]. Table 2 summarizes useful information about the original state-of-the-art technological advancement in the field of microfluidics [44-52].

Currently, intense efforts have been undertaken to develop innovations in handling and manipulation of MNPs in microfluidic devices for future investigation and analysis applications. The combination of POC assays aided using MNPs to recognize integrated LOC biosensing is elaborated here. All these assay procedures are controlled and boosted by magnetic forces and the existing contests and possible directions for integrated biosensing based on activated magnetic particles in microfluidic devices. Biosensing techniques dependent on MNPs have received significant importance over conventional detection methods. MNPs were used to label biological samples without affecting their biological activities. As biomolecules are not magnetic, MNPs can be utilized to obtain highly precise measurements with reduced sample preparation.

2 Properties of magnetic nanoparticles (MNPs)

NP's physical properties are different from their respective bulk materials (structure, crystallite size, chemical config-

Table 3. Characteristics of magnetic nanoparticles used for biosensing applications

Particles Characteristics	Size (nm)	Analyte	Composition	Sensitivity	Ref.
Cross-linked iron oxide	30	Nucleotide, proteins, viruses	5 nm core with 10 nm dextran coating	Low nM∼pM, 50 viruses/100 μL	[53]
Core/shell	16	Bacteria	Fe core, Iron oxide shell. 2.5 nm shell thickness	20 colony-forming unit CFU/100 μL	[54]
Mn-MNPs	16	Cancer cells	Manganese doped iron oxide	2 cells/1 μL	[55]
Iron oxide	56	Bacteria		1.1×10^5 bacteria/20 μL	[56, 57]
Iron oxide	19.5	Antibody		<1 nM	[58]
Cubic FeCo nanoparticles	12.8	Protein	1.5 nm oxidized shell	2×10^6 proteins	[59]
Antiferromagnetic nanoparticles	100	DNA	Multilayers of ferromagnetic, interlayer of nonmagnetic material	10 pM	[60]

uration, crystal shape and morphology, and various surface effects). MNPs have unique properties, such as high saturation magnetization, the large surface to volume ratio, and can be manipulated by applying a magnetic field. MNPs of different sizes are available with diverse surface coatings and are mostly prepared by the coprecipitation method. MNPs can be iron oxide (maghemite γ-Fe_2O_3 or magnetite Fe_3O_4), alloys (FePt), or pure metals (Fe and Co). They are functionalized with organic or inorganic coatings to attain biocompatibility to target biomolecules. Table 3 summarizes the properties of various types of MNPs [53-60]. As the size of magnetic particles reduces to the nanometer scale, they exhibit superparamagnetism.

Magnetically labeled biomolecules can be isolated from the carrier fluid through externally applied fields. The temperature below which superparamagnetic particles become permanently magnetized is called blocking temperature. Magnetic moments overcome an anisotropic barrier and flip in the opposite direction due to thermal energy above the blocking temperature. In the presence of the magnetic field, diffusion of MNPs into biological tissues perturbed the precession of water protons spins present inside the tissue and changes the magnetic resonance signal measured as a shortening of the longitudinal relaxation time (T1) and transverse relaxation time (T2). Relaxation rates corresponding to T1 and T2 are designated as R1 and R2. NMR and MRI are used to measure T2 for biosensing applications based on diagnostic magnetic resonance (DMR).

Ferrimagnetic and ferroMNPs, are single-domain NPs having the size of about 5-15 nm diameter and can be synthesized in the form of ferrofluid, like 50-500 nm agglomerates encapsulated within biodegradable polymers [61], can be beneficially used in microfluidic systems (MEMS and BioMEMS) [62]. LOC devices can be designed using MNPs because of their unique features. The size and properties of MNPs can easily be tuned according to biological entities (DNA, proteins, viruses, and cells), so they can easily be employed in microfluidic and biological environments [63]. Irreversible aggregation and precipitation are prevented by surfactant in the presence of the field. In microfluidic devices, the property of MNPs to be functionalized is used to achieve specific bioanalytical tasks. MNPs are nontoxic and well tolerated by living organisms, therefore, appropriate for bioapplications [64]. They can be synthesized in nanometer range by the different process making them ideal for probing and manipulating biomolecules with minimum undesirable interactions [65]. Magnetically labeled material can be integrated into microfluidic systems to facilitate targeting, imaging, detecting, delivering, and therapy simultaneously.

3 Magnetic nanoparticles (MNPs) on lab-on-a-chip (LOC)

A LOC is an incorporated device that permits various functions on a single platform for analysis and treatment of the test sample. The fundamental thought is to create innovative LOC tools for POC in-vitro diagnostic testing, which contains sample preparation, biochemical reaction, separation, detection, and hybridization added by statistics acquisition and interpretation. These portable devices are used for many biological applications like biolabeling and detection along with other in-chip applications identified with the specificities of the assembly of nanomaterials (NPs, quantum dots, nanowire, and graphene) with optical, electrical and additionally mechanical properties [66]. This device can be demonstrated as POC testing, in particular, real-time and on-site diagnostic.

The superparamagnetic microNPs are extensively used to tag and detect chemical and biological species and are detected by using different types of sensors [67]. MNPs can be utilized in different devices for biosensing applications with an improvement of sensitivity and stability because of various size, structure, composition, and magnetic properties. Magnetic microbeads have been used to characterize many biomolecular interactions for the detection of nucleic acid (DNA/messenger ribonucleic acid [mRNA]) [68]. Aqueous suspensions of magnetic beads were allowed to settle down onto the substrate to target biomolecules targets including proteins, small molecules/drugs, bacteria, and tumor

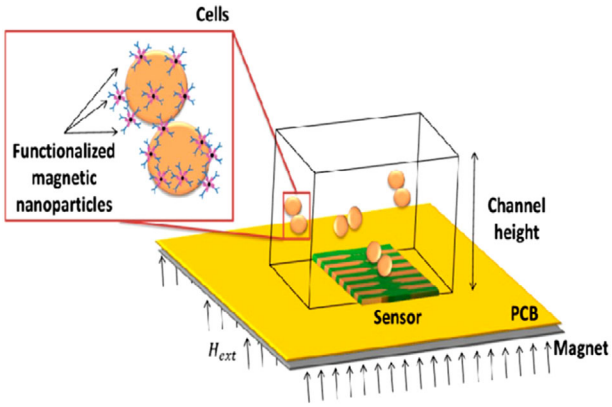

Figure 2. Identification and quantification of cells using functionalized magnetic nanoparticles with the help of sensor on printed circuit board (PCB) in the presence of external magnetic field (H_{ext}). Reprinted with permission from [70], copyright 2019 PubMed Central.

cells [69]. Numerous kinds of MNPs-based chips have been surface functionalized to target biomolecules because of their exclusive magnetic properties, absent in biological systems, as presented in Fig. 2 [70].

Because of the stability of magnetic microNPs, these are useful for measurements that can be repeated without time limitation [71, 72]. Manipulation of MNPs on-chip can be controlled by applying a magnetic field gradient. The microfabricated magnetic sensor makes them well suited for the on-chip biosensing system for the detection of nucleic acid and protein markers because of low cost and small size [73]. The identification of these biomarkers involves a detecting innovation as well as pretreatment steps of the sample that are required to investigate and examine, therefore, appropriate for so-called LOC or μTAS [3]. MNPs have been investigated as a nanotag [74, 75] for analyte quantification, selective binding, extraction, and isolation of DNA and cells [76]. Multipurpose MNPs, including microspheres and microbeads, have been broadly investigated for procedure steps that are essential for LOC diagnostic tests. They are used to mix liquids, to specifically target, concentrate, and label and transfer analytes, such as the biomarkers that should be distinguished from one fluid to other, to perform washing steps, and to test biophysical properties of the analytes. Figure 3 represents the incorporation of various MNPs that leads to biosensing on LOC in which magnetic forces control all diagnostic assay steps [77].

Biomolecules have no magnetic properties and negligible magnetic susceptibility; therefore, the addition of magnetic micronano beads to label them could be used to isolate and measure specific analyte present in a given sample [78]. An external magnetic field then creates forces on all the beads that are measured optically or magnetically. Recent developments in magnetoresistive materials have made possible the fabrication of magnetic field sensors, in which resistance changes in response to the change in a magnetic field. The detector for a magnetic bead can also be produced on a single chip using these materials [79]. The sensitivity of the biosensing system is enhanced by MNPs but also efficiently lessen the requirement of sample preparation. Numerous techniques are available for the detection of biocompatible, physically and chemically stable magnetic labels that are comparatively simple, economical to produce. Several technologies to measure the magnetic field from biological targets labeled with MNPs, such as superconducting quantum interference devices (SQUIDs), giant magnetoresistive (GMR), Hall, and DMR sensors, have been developed [80]. Nuclear magnetic resonance modifying the transverse relaxation of water molecules is the basis of DMR. Magnetometers required magnetically labeled targets to be close to sensors. But DMR is fast because of the generation of signals from the entire sample volume. There are several advantages of chip-based devices that include a requirement of a small amount of sample, rapid handling, high sensitivity, increased accuracy, portability, especially for biomedical applications, for POC diagnostic in the form of a microchip [81]. Magnetic particles bind to a sensor surface magnetoresistive sensors in which resistivity of the material changes with the magnetic field [82]. Magnetoresistance (MR) effect-based sensing mechanism has also been explored for applications in biological sciences. These sensors can sense the magnetic field at room temperature up to nanoTesla. The development of magnetoresistive biochips used for detection and recognition of biomolecules has emerged as an excellent biodetection technique [83], based on the GMR effect and the tunneling magnetoresistance (TMR) effect [84, 85]. GMR is due to the scattering of spin in magnetic multilayers, which is used for the detection of magnetically labeled biological targets with greater sensitivity at low fields [86]. GMR sensors involving ferromagnetic thin layers parted by the nonconducting layer are small in size and ideal for low-cost applications. The magnetic field tends to align magnetic moments concerning each other, and as a result, the resistance of multilayer decreases. For the GMR effect in magnetic layers, magnetic moments must align in the direction of applied magnetic field and antiparallely, when a field is removed. The magnetization ferromagnetic layers can be tuned to align magnetic moments in spin-valve structures (SV). In a GMR-SV structure, the magnetic layers include an antiferromagnetic layer, pinned magnetic layer, and a free magnetic layer with the spacing filled with a conductor (e.g. Cu). The electrons spin get polarized and retain their alignment when potential is applied across the valve. Resistances changes due to scattering of conduction electrons, which depends on the electron spin in an SV sensor [87]. In magnetic tunnel junctions (MTJ), an insulating layer separates two ferromagnetic layers. Electrons pass this barrier that result in a flow of electron perpendicular to plane. Magnetic tunnel junctions sensors have higher MR ratios as compared to GMR and SV sensors at room temperature, therefore, ideal for extremely sensitive biosensing applications [88]. Magnetic biochips are capable to capture and concentrate target molecules in a sample using magnetic interaction with the help of external magnetic field [89]. Figure 4 shows the generation of GMR signals for sensing the specific binding of analyte to the functionalized probe

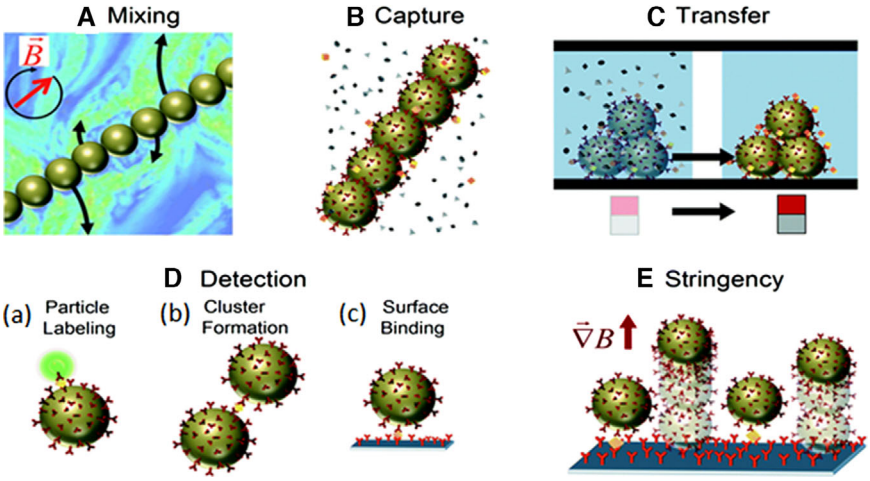

Figure 3. Application of magnetic particles in several process steps of a lab-on-a-chip diagnostic assay. Actuated by applied magnetic fields, magnetic particles have been used (A) to mix fluids, (B) to selectively capture specific analytes, (C) to transfer analytes to another fluid, (D) (a) to label particles for detection, (b) to form clusters for detection, (c) to induce surface binding for detection, and (E) to apply stringency forces to improve the signal-to-background ratio (gradient magnetic field-∇B). Reprinted with permission from [77], copyright 2019 Royal Society of Chemistry.

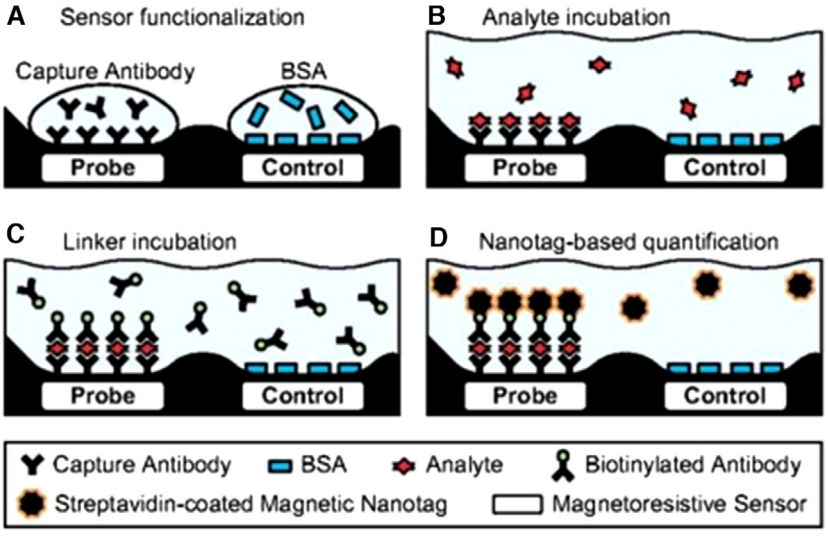

Figure 4. A schematic representation of a GMR sensor for an ELISA)-type protein assay. (A) The probe surface was functionalized with a specific antibody, while the control surface was passivated with BSA. (B) A sample solution was added for the specific binding of analyte proteins to the probe surface; (C) a biotinylated antibody bound to the surface-immobilized analytes. (D) Finally, streptavidin-coated nanoparticles were added for tagging the probe surface by biotin-streptavidin interaction. GMR signals were detected for sensing the presence of analytes on the surface. Reprinted with permission from [90], copyright 2019 Proceedings of the National Academy of Sciences of United States of America.

surface [90]. Probes on the surface of GMR sensors binds to analytes (e.g. nucleic acid, proteins, plasma, cells) for the recognition and evaluation of the biomolecules labeled with MNPs.

The MNPs-based analysis is based on the relaxation of magnetic moments of MNPs. Magnetic relaxation switches are used for MR based assays by exploiting a change in transverse relaxation time (T2). The switching of NPs between dispersed and agglomerated states will change the spin-spin relaxation time that is measured by SQUIDs. Néel relaxation is slower than Brownian relaxation of magnetic particles, which was a basis for a homogeneous immunoassay [91] and a bacterial detection [92]. The Brownian motion of unbound MNPs was undetectable due to a limited range of SQUID (between 1 ms and 1s), restricted by bounding to a bacterium. SQUID is used to determine the Néel relaxation time of the surface of bound particles because it lies in the range of SQUID. Because of this technique, superparamagnetic iron oxide NPs were used as contrast agents for imaging in a one-dimensional scanning system. The system was adjusted with Fe_2O_3 NPs having a diameter of 25 nm and a sensitivity of 10 ng at a distance of 1.7 cm with a three-dimensional resolution of approximately 1 cm. A hypothetical model was created and connected to the deconvolution of scanned images of contrast with two NPs infusion spots for this system. Utilizing the established SQUID system, we had the option to regulate not just the size and quantity of injection, but also their profundities in the phantoms [93]. Brownian motion can be used for biosensing by using alternating current to measure static and dynamic magnetic susceptibility. SQUID is used to detect Brownian relaxation of target analytes bound to NPs in buffer or serum which is slow due to large hydrodynamic size [94, 95].

4 Magnetic nanoparticle-based microfluidics

In the most recent years, MNPs have been utilized in numerous bioanalysis, extending from manual measures for

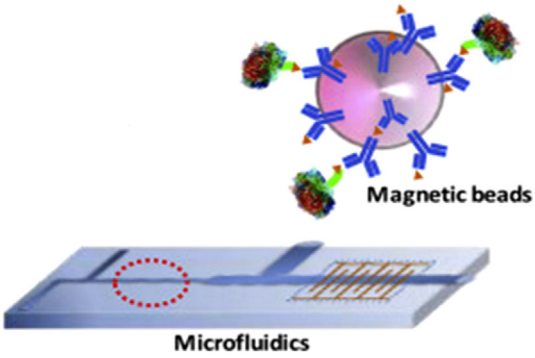

Figure 5. Magnetic beads exploited as immobilization supports and label carriers in microfluidics. They were reprinted with permission from [105], copyright 2019 Elsevier.

essential research to tests in high-throughput instruments for incorporated labs [96-98]. The development of microsystem for the movement of NPs within microchannels is controlled by an external magnetic field that needs microfabricated components perfect with prevailing magnetic-based bioassays and gives an appealing option in contrast to standard techniques for analyte control and infusion [99]. The availability of MNPs and corresponding analyzing reagents along with miniaturization technologies have formed a starting point for magnetic-based μTAS and LOC systems [100]. Magnetic beads are the MNPs with a polymer coating and are available in different sizes functionalized with antibodies, antigens, peptides, nucleic acid, or lectins that confirm binding to specific biomolecules upon interaction. The analyte-bead conjugate can easily be handled inside the microfluidic channel using a magnetic field [101]. Therefore, magnetically inactive cells and other biological entities can be used inside microfluidics after conjugation with MNPs [102-104]. The advancement of the compact systematic analytical device is due to microfluidics systems integrated with magnetic beads. The MNPs are used to label, concentrate, trap, and manipulate analyte and cells in the microchannels, as shown in Fig. 5 [105].

Microfluidic systems with magnetic detectors measure the surface concentration of specifically magnetic bound NPs inside the channel. These devices are designed for small disposable point of use and care application, where the material cost is small. Magnetic labels can be used instead of fluorescent, chemiluminescent, and radioactive labels and replace many electrostatic and optical technologies in microfluidic systems.

Magnetic-based detection method in a combination of microfluidic has received extensive interest in biosensing applications and may become widely used in the future [106,107]. These devices recognize specific molecular targets by measuring the effect of MNPs on water proton relaxation effects. The labeling of bioanalyte with MNPs and the identification of their stray field using incorporated magnetic sensors is essential to determine the relaxation rate of magnetic moments of the magnetic particle or detection of the presence of magnetic biomolecule complex [108]. Microfabricated magnetic particle-based sensors are stable and can be operated by the external field without a time limitation [109]. Recently numerous ultrasensitive MNPs-based biosensors have been reported which are appropriate for diagnostics applications [71, 110-112]. Superparamagnetic particles can be redispersed inside the solution because of zero coercivity and remanence, which is important for target capture or detection. Magnetic NP-based microfluidics is beneficial due to: (a) magnetic fields can be externally applied, or from small conductors that are directly assimilated in the biosensing system, (b) applied current can be adjusted to tune magnetic fields, (c) the labeled biological entity with magnetic markers/NPs can be directed in microfluidic channels employing high-gradient magnetic fields that can be sensed by magnetic sensors, and (d) the adaptability of the magnetic markers to label any biological entity because of the specific binding and surface functionalization. Thus, these multifunctional magnetic markers become ideal and active candidates in miniaturized on-chip biosensors [62]. Magnetic particles have been used in stationary and fluid flow microfluidic systems to mix, wash, and exchange buffer. Due to the high surface to volume ratio and flexibility of surface biofunctionalization of magnetic particles, they are effective to label and capture the target molecules. Besides, optical methods can also be used to detect magnetic particles in complex fluid activated for the magnetic stringency process [77]. Strong magnetic field gradients up to 20 T/mm can be generated by microfabrication technology by using either a miniaturized permanent magnet or electromagnetic coil [113-115]. A combination of macroscale biasing magnet and microscale magnetizable elements is important for the functioning of the magnetic particle-based microfluidic device [116, 117]. A strong magnetic field is required for high-throughput magnetic separators, which are attained by using a permanent magnet, while an arrangement of electromagnets is used in a magnetic manipulator operating on an alternating magnetic field [118]. Sometimes, biasing permanent magnets in conjunction with gradient-producing electromagnets are also used [119]. Soft magnetic materials that include permalloy or nickel, in the form of chip-embedded posts to produce gradient fields are used in most of the designs [120, 121]. Traveling wave fields are also used to achieve a larger domain of influence of the field in some designs [122]. Functionalization of the sensor with specific ligands and samples under examination with MNPs is the most important step before inserting it into the channels of microfluidic devices. Magnetic biosensors have been developed as outstanding pathogen detection devices at room temperature due to small size, less complex instrumentation, and integration adaptability. Cell manipulation using magnetic beads in microfluidic system is simple and reliable to simultaneously separate and purify them [21]. Magnetic biosensors rely upon the identification of either bound functionalized MNPs to a surface of a sensor or the trapped MNPs in the environs of the sensor as depicted in Fig. 6 [123]. It essentially detects the magnetic stray field of bioanalyte labeled with MNPs when it

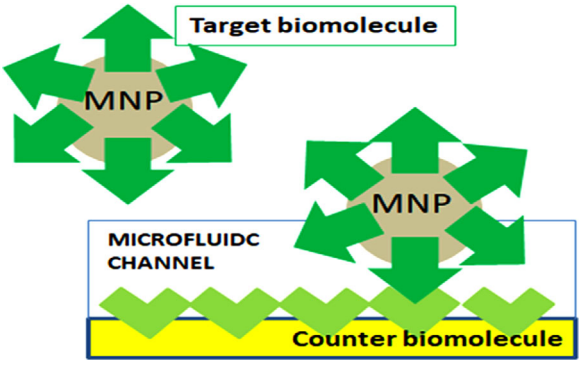

Figure 6. Binding of magnetic particles target molecules in a biological assay.

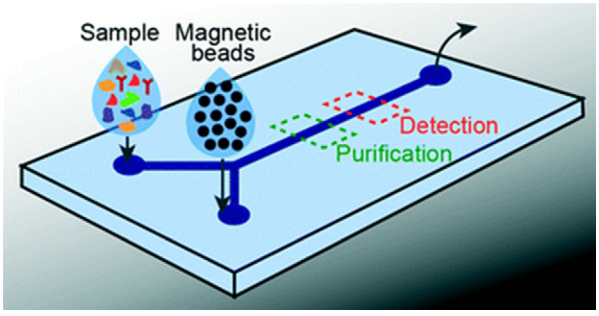

Figure 7. Magnetic beads used in microfluidic assays for detection and purification. Reprinted with permission from [124], copyright 2019 Royal Society of Chemistry.

interacts with complementary bioanalyte bound to the sensor surface.

Magnetic beads have been frequently used in immunoassays, either as mobile substrates on which target is captured or instantaneously as substrates and labels for the detection and purification of antibodies or disease biomarkers as shown in Fig. 7 [124]. Magnetic microchip shown in Fig. 8, has been applied with significant success in clinical applications for the isolation and detection of magnetically labeled biomarkers in unprocessed clinical samples such as blood, sputum with high sensitivity [125].

Dynamics and kinetics of surface-based antigen-antibody in magnetic NP-based microfluidic devices for surface-based bioassay are improved by almost 42% and make these devices 35% more efficient. More target antigens get near to the surface-bound antibody by decreasing the diffusion transport, thus, reducing the detection time. Local concentration of antigen-MNPs complex increases in the existence of the magnetic field in the vicinity of the sensing surface. The target antigen can also be directed to the sensing surface by the optimization of a magnetic field. MNPs-based microfluidic system helps for fast, useful, and effective surface-based bioassays [126]. Microfluidic systems integrated with magnetoresistive sensors devices are used to transport, separate, and detect biomolecules via molecular recognition [127].

5 Applications

5.1 Microfluidic for detection

The microfluidic system has many applications such as quick and precise detection and quantification of biomarkers present inside the tissue [128]. Also, liquid samples are the main challenges in the field of microfluidics. They can be used for measuring target analytes in biological fluids (blood, urine, nasal washes), environmental samples, bioprocessing samples, which often require a combination of biological sample preparation followed by particular detection assays [129]. In these devices, functionalized MNPs are used for detection and signal transduction in biological assay [49]. Particle-based microfluidic systems show the capability to detect biomolecules with high accuracy, identify the small number of molecules and its analyzation on the molecular level, and the measurement of different biomarkers. MNPs enhance the molecular interactions for the quick and precise detection of biological samples labeled with biomarkers [130]. Microfluidic devices integrated with magnetic beads were used for viral sample purification and detection. Sample incubation, a microflow cytometry, and optical detection were integrated on a single chip to purify, count, and collect the target virus conjugated with magnetic beads utilizing a permanent magnet as illustrated in Fig. 9 [131]. Results indicated that the entire diagnosis, including virus incubation and detection with a concentration of 10^3 plaque-forming unit (PFU)/mL, can be done in 40 min by the developed system [131].

A combination of advanced biological detection methods with microfluidics using functionalized magnetic particles has incredible potential for different detection applications. Certain antibodies are restrained on the surface of a sensor according to molecules to be detected in static bead detection. The antibodies present on the surface of the sensor, bound to superparamagnetic NPs, capture antigens present in the analyzed plasma. Unbound molecules were removed by washing. A magnetic field applied perpendicular to sensor surfaces witched on the stray field of superparamagnetic particles [127]. At present, the influenza virus taints a large number of people each year and indicates one of the greatest dangers. Thus, it is essential to build up a diagnostic method that can quickly and accurately detect the virus economically and to competently treat and control seasonal and epidemic strains. Reasonable and profound tests based on the integration of MNPs inside the microfluidic systems have been used to replace standard clinical diagnostic methods.

Figure 10 describe a microdevice integrated with immunofluorescence for avian influenza virus (AIV) detection [132]. AIV was captured specifically by antibodies modified by magnetic beads, and the beads were trapped in a magnetic zone for detection by fluorescence spectroscopy. It is convenient to observe captured bacteria and to visualize fluidic flow in microfluidic devices with fluorescence microscopy. Similarly, an on-chip magnetic immunofluorescence assay was also used for bacterial detection [133].

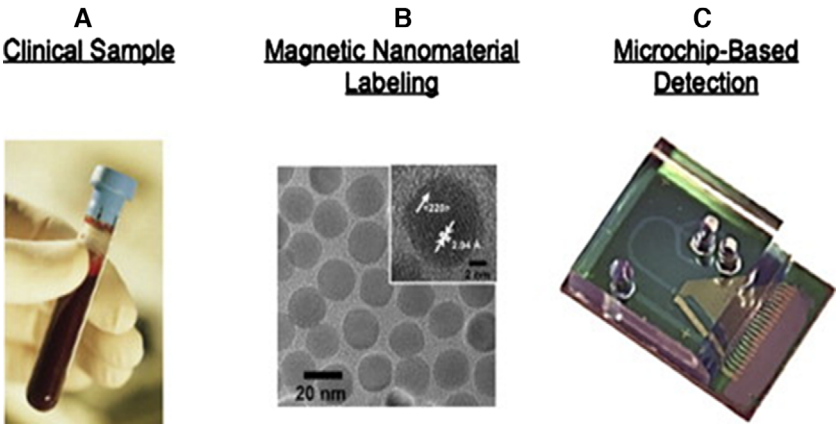

Figure 8. Microchip-based detection of magnetically labeled biomarkers. (A) Unprocessed clinical sample containing cancer biomarkers. (B) A transmission electron micrograph (TEM) images of magnetic nanoparticles. (C) Microfluidic chip for the detection and isolation of magnetically labeled cancer biomarkers. Reprinted with permission from [125], copyright 2019 PubMed Central.

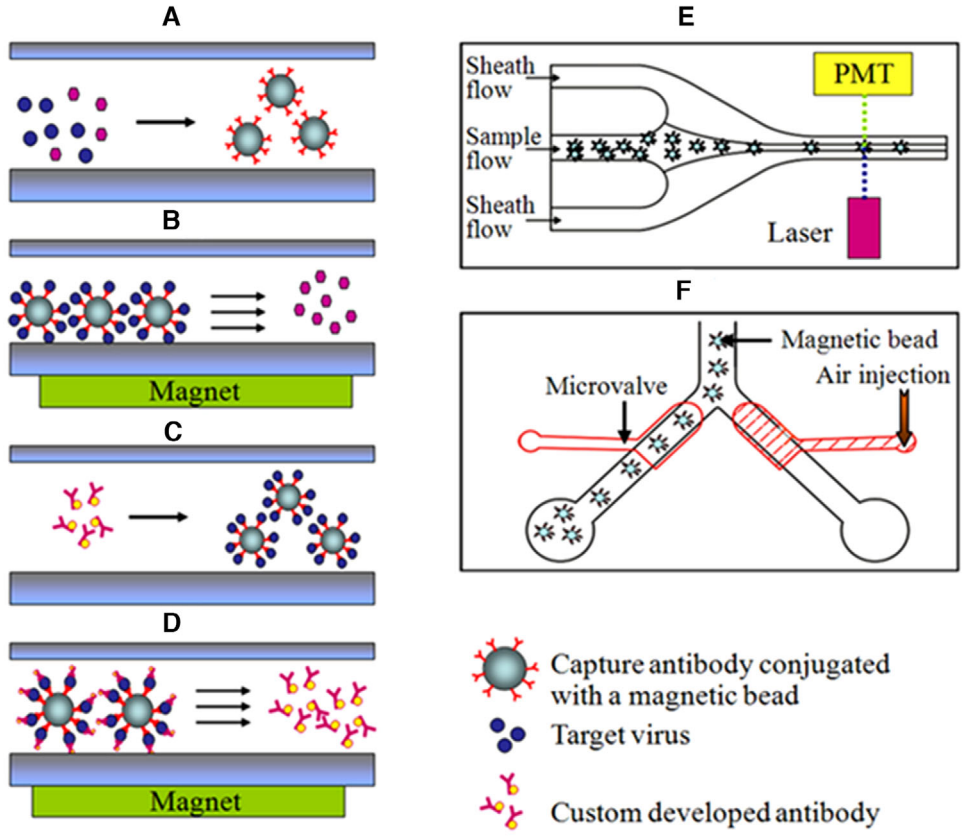

Figure 9. Schematic illustration of the integrated microfluidic chip. (A) Mixing biosamples with magnetic beads; (B) purifying and concentrating the target viruses through a washing process for purification of the target viruses; (C) capture antibodies on the magnetic beads are conjugated with the custom-developed antibodies; (D) washing process for purification of the developed antibodies; (E) fluorescent detection while the magnetic beads pass through optical detection region using a photo-multiplier tube (PMT); (F) sorting of magnetic beads utilizing microvalves. Reprinted with permission from [131], copyright 2019 PubMed Central.

Immunoassay dependent on self-assembled MNPs on-chip was utilized for the recognition of murine monoclonal antibodies with a detection limit of 1 ng/mL in nanoliters of hybridoma cell culture medium. A strong interaction of magnetic chains with the flow rapidly captures target molecules on-chip [134]. Two-step diagnostic processes in a magnetic bead-based microfluidic system have been used for the fast quantification of influenza A virus, consisting of magnetic bead-based fluorescent immunoassay and optical analysis. Fluorescent signal from magnetic complexes consisting of magnetic beads conjugated with an antibody is used to detect virus particles. This magnetic bead microfluidic system was significantly used for the recognition of the influenza virus up to 5×10^{-4} hemagglutinatin units (HAU) which is superior to the traditional systems. The analytical process from target virus purification to optical detection of magnetic complexes optical detection is completed in 15 min with high specificity, which is 8.5% of the time required for manual analysis [135]. Microfluidic passive magnetic separator combined with chip has been used to purify and concentrate human immunodeficiency virus type 1 (HIV-1). Plasma having a virus is first mixed with an antibody attached to superparamagnetic NPs for specific binding of the virus. The virus-NP complexes are isolated from the plasma inside the channels of a microfluidic

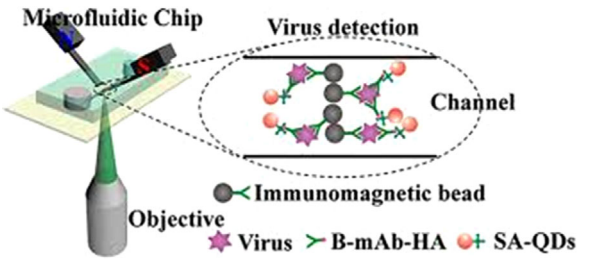

Figure 10. A simple rapid and point-of-care magnetic immunofluorescence assay for avian influenza virus (AIV) and developed a portable experimental setup equipped with an optical fiber spectrometer and a microfluidic device. Reprinted with permission from [132], copyright 2019 American Chemical Society (Influenza B virus (B), monoclonal antibodies (mAbs), hemagglutinin (HA), self-assembled quantum dots [SA-QDs]).

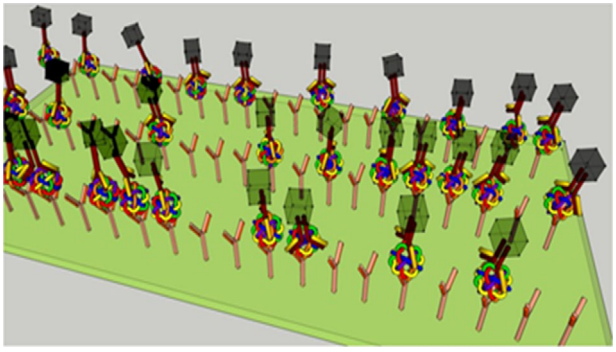

Figure 11. Schematic illustration of influenza A virus detection. Reprinted with permission from [139], copyright 2019 PubMed Central.

device fabricated with micrometer-sized ferromagnetic NPs. Then a viral lysis was streamed through the channels and release human immunodeficiency virus proteins were examined off-chip. Efficiencies of 62% and 45% were achieved for protein extraction of the virus at 10 and 30 μL/min throughputs, respectively. This system is used for the preparation of any viral sample and nucleic acid extraction [136]. Paramagnetic NPs conjugated with glycan in flow injection analysis based biosensor can analyze a broad range of influenza viruses including A/H5N1/Vietnam/1203/2004 protein labeled quantum dots [137]. Reverse transcriptase-polymerase chain reaction (RT-PCR) in the microfluidic system was used to accelerate ribonucleic acid (RNA) viruses, such as dengue or enterovirus, by using antibody-conjugated magnetic beads [71]. The antibody-conjugated magnetic beads captured the virus and reverse transcripted by the μRT-PCR module. This system can identify four serotypes of the dengue virus as well as enterovirus, and also allow mixing, incubation, and reaction of the sample. The incorporation of the conjugated system of antibody and magnetic beads into the microfluidic system is favorable for the rapid diagnosis of microorganisms [138]. A GMR biosensor was established using monoclonal antibodies to viral nucleoprotein with MNPs for simple detection of the influenza virus as can be seen in Fig. 11 [139]. MNPs bind to the GMR sensor in the presence of an influenza virus and change the resistance of the sensor, measured in a real-time electrical readout. Aldehyde groups, covalently attached to biomolecules were used to functionalize the surface of the sensors. Virus extending from 1.5×10^2 tissue-culture infectious dose (TCID) 50/mL to 1.0×10^5 TCID 50/mL (TCID50) concentration in tissue culture is sensed by the developed GMR biosensor. The pathological change was observed in 50% of cell cultures inoculated by this amount of infective dose of pathogenic agents expressed as TCID 50/mL [139].

Active *Escherichia coli (E. coli)* bacteria attached to MNPs were easily separated from the solution having red blood cells (RBCs) with a microdevice integrated with a high magnetic field concentrator. This device was used to sort a large number of beads and cells without losing separation efficiency [140]. A novel LOC using MNPs was used for highly efficient immunoassay to detect pathogenic bacteria during biofunctionalization steps. It is a powerful microfluidic method made up of polydimethylsiloxane (PDMS) channels with a height and width of 50 and 500 μm, respectively, based on a nonpathogenic bacterium *E. coli2, 3*. The primary purpose is to recognize and detect a targeted protein (~10 pg/mL) by guaranteeing better bioactivity (antibodies-ovalbumin) respecting ELISA protocol [141]. The conditions used inside the channels permit rapid biofunctionalization and allow detecting and capturing biological entities integrated into LOC. Fluorescent microscopy was also connected for passivation to characterize grafting of antibodies and BSA. Antibody-functionalized MNPs clusters were used to fabricate 3D microchannel to capture *E-coli* bacteria in milk. The free and bound magnetic nanoclusters were separated based on the size difference. Ultraviolet visible spectroscopy was used to find out the detection limit of *E. coli*, which is found to be 10 cfu (colony forming unit)/mL in a buffer and 100 cfu/mL in milk [142].

5.2 Microfluidic for cell sorting

A vital feature of cell research and major use of LOC devices is the separation of cells. Microfluidic devices used for magnetic separation of cells, consist of integrated magnetic elements below the microfluidic channel, attract magnetic particles within microchannel with a force, offer the merits of biocompatibility, efficiency, and simplicity among various methods [143, 144]. In magnetic separation, magnetic particles get bound to the target molecules, when mixed in a solution containing biomaterial, and released in a suitable medium for further processing. The development of microfluidic devices integrated with miniaturized magnetic cell sorters is an important advancement in the biotechnology area [145]. The channels of microfluidic were designed in such a way that cells flowed through a chamber and were diverted from the direction of flow by an external magnetic field [146]. Figure 12 explains the bioseparation sequence in the microsystem using MNPs in the presence of an externally applied field to capture the target antigens [147].

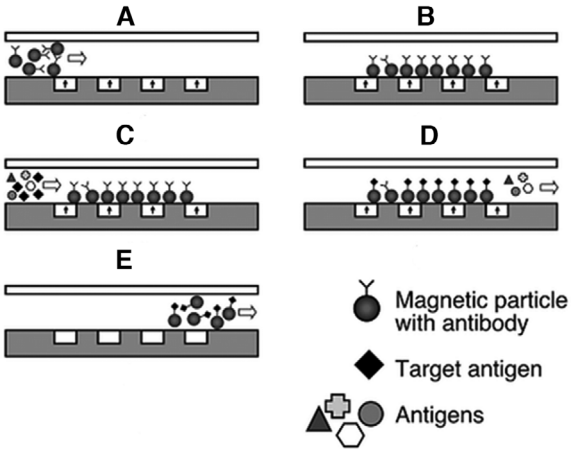

Figure 12. Cross-section of microsystem illustrating bioseparation sequence. (A) Magnetic particles with surface-bound antibodies enter the micro-channel with the bias field applied and the elements magnetized. (B) Magnetized elements capture the particles; (C) target antigens are introduced into the microchannel; (D) target antigens become immobilized on captured magnetic particles; (E) the bias field is removed and the magnetic elements revert to an unmagnetized state releasing the separated material for further processing. Reprinted with permission from [147], copyright 2019 PubMed Central.

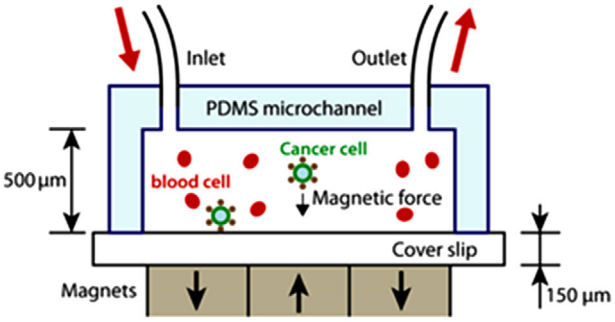

Figure 13. Schematic showing the principle of operation. CTCs in the blood are labeled with EpCAM functionalized Fe_3O_4 magnetic nanoparticles, and captured by the magnetic field as the blood flows through the microchannel. Reprinted with permission from [151], copyright 2019 Royal Society of Chemistry.

Magnetic separation achieved by on-chip free-flow magnetophoresisis, usually used as a substrate for cells, antigens, antibodies, and DNA in bioanalysis. Free-flow magnetophoretic separations could be combined and related to other microfluidic devices to form μTAS for analysis and reaction steps [148]. The magnetic-based microfluidic system has developed as an influential diagnostic tool for cancer. The miniaturization and integration of microfluidic platforms enable the molecular diagnosis of targets tagged with MNPs with high precision. As a result, separation and detection of rare cells have been done by using microchip-based diagnostics [125]. The development of an immunoassay based on hybrid magnetic/plasmonic nanocarriers specifically targets the antibody-conjugated magnetic nanocarriers and is separated from normal blood cells by a magnetic force in a microfluidic chamber [35]. Circulating tumor cells (CTCs) are very significant in cancer investigation over the past decade. It is essential to separate them for analysis because they are very rare in blood (0-10 CTCs/mL of blood) [149]. The design of the microfluidic chips can be structured by controlling the magnetic field strength and the flow rate to capture and detect CTCs [28]. Microfluidic technology along with NMR technology enhances the rate of detection of cancerous cells bound to MNPs with greater sensitivity. Tumor cells were captured by MNPs functionalized with anti-epithelial cell adhesion molecule (EpCAM) antibody in the presence of the magnetic field under the chip. The advancement of miniaturized DMR technology can measure untreated biological samples with the help of MNPs to amplify molecular interactions in real time. The system consisting of microfluidic channels and portable magnets can also identify bacteria, profiling small cancerous cells and quantify sequences of protein biomarkers in multiplexed fashion [150]. Device miniaturization through microfabrication and molecular proximity tests utilizing MNPs will be a high throughput and portable platform for large-scale sensing and can perform specificity and sensitive measurements (up to 1×10^{-12} M). MNPs was also used to label tumor cells in blood samples in the microchip-based CTC capture system as shown in Fig. 13 [151]. A high capture rate with a fast flow rate of 10 mL/h was achieved by creating a strong magnetic field inside the microchannel to detect rare cancer cells (from ~1000 cells down to ~5 cells per mL) with a very low tumor cell to blood cell ratio (about 1:10 (9), including RBCs). Microchip-based CTC detection permits novel diagnostic tools to measure the status of disease activity in patient blood as well as the clonal growth of molecular modifications to detect cancer at an early stage. In this system, 25% MNPs are required to capture tumor cells (at an optimum blood flow rate of 10 mL/hour) which is five times faster than previously reported microchannel based assay [151]. Anti-EpCAM antibody modified Fe_3O_4 NPs using block magnets captured more than 85% spiked tumor cells in blood samples at a flow rate of 10 mL/h. The microfluidic system with immunomagnetic assay functioned in a flip-flop mode successfully detects rare CTCs from clinical blood samples. During the blood screening process, target cancer cells were labeled by functionalized MNPs inside the microchannels.

Magnetic field distribution can be optimized by decreasing the magnetic force in the microchannel, thus, avoiding accumulation near to the inlet. The orientation of the microchip can be changed by a flip-flop system to reduce the immobility of RBCs and nonspecific binding on the surface. After screening, further biological and imaging analysis using rare cells was done. The motion-controlled microchip-based immune-magnetic system is a great tool for SkBr3, PC3, and Colo205 cell lines in spiked screening experimentations and effectively separates CTCs from patient blood [152]. Functionalized superparamagnetic NPs conjugated with anti-EpCAM antibody were used in the microfluidic chamber to capture CTCs from a blood sample [35]. A novel

technique for cell sorting using an array of magnetic traps utilizing biofunctionalized superparamagnetic beads in a channel is developed by microcontact printing [153]. The results revealed the cell capture efficacy and probability to grow captured cells from culture cell lines (leukemia cell line and lymphoid cell line) was 94% better. Magnetic force-based droplet on-chip system using magnetic beads in mineral oil was exploited as an influential tool for numerous biochemical applications. This droplet-based system and RT-PCR were done inside the microchannel for the synthesis of cDNA and WT1 gene amplification that is the analytical factor for acute leukemia from a single cell [154]. A new magnetic bead-based microfluidic system utilizing magnetic bead conjugated with CD 15/45 antibodies is used for the rapid purification of leukocytes, extraction of genomic DNA (gDNA), and fast analysis of gene immediately with a combination of biosensors in 20 min. DNA-specific, surface with switchable, magnetic beads in the lysis solution is used to purify and concentrate leukocytes in human blood followed by extraction of gDNA. This system detects SNP genotyping of methylene-tetra-hydrofolate reductase (MTHFR) C677T related to genetic disease. The extracted DNA is transported to PCR chamber for fast nucleic acid amplification [155].

5.3 Microfluidic for gene/protein analyzation

A microfluidic device capable of complete sample preparation and gene analysis from raw biosamples has been developed using silica-coated superparamagnetic particles. This platform is used for cell lysis, DNA binding, washing, and PCR by SPE in the same chamber [156]. This design is used for genetic detection enabling an analysis of many genes on a single microchip. This device, using the TaqMan probe-based gene-specific PCR assays, is used to isolate DNA from the human blood sample and analyze the Rsf-1 gene [157]. Droplet-based microfluidic chip integrated with MNPs was used to purify DNA by mixing, splitting, and transporting. The genomic material is extracted from the dilute cell sample by MNPs within the droplets through a coil matrix integrated into the chip [158]. Magnetic beads have been used frequently either as mobile substrates to capture target antigen or as labels in microfluidic systems for ultrasensitive protein detection. Detection of proteins in serum or analysis of biotoxins in food samples up to fg per milliliters concentrations was made possible using this microfluidic bead-based assay [124]. A microfluidic chip with a magnetic bead of size 10 nm was also used for immunoassay of adiponectin. The microchip is fabricated using PDMS and nitrocellulose with a hall sensor for sensing magnetic beads. The method is based on enzyme-linked immunoassay, where the antibody is immobilized on nitrocellulose and magnetic beads to label the analyte. The chip takes 2 h 15 min to detect protein followed by fluorescence measurement [159]. Micromachined MNPs were also used as separators in microfluidic systems for magnetic particle-based bioseparation. Biomolecules immobilized on MNPs were separated by an electromagnet in the microfluidic channel for magnetic particle separation for LOC applications that include bioseparation and immunoassay [160]. Molded PDMS network with pump and injector in the microfluidic multiplexed system was used for the detection of cancer biomarkers protein. Superparamagnetic particle-antibody bioconjugates bound to eight electrodes measuring chip were used not only to capture analytes but also to detect cancer biomarker proteins prostate-specific antigen (PSA) and interleukin-6 (IL-6) in serum at sub-pg/mL levels. MNPs conjugated to approximately 90 000 antibodies, and approximately 200 000 horseradish peroxidase (HRP) labels were used to capture antibodies with high sensitivity with detection limits of 0.23 pg/mL for PSA and 0.30 pg/mL for IL-6 in diluted serum mixtures. Biomarkers were measured with standard ELISA in total assay time of 1.15 h. These nanostructures were used for the diagnosis of cancer biomarkers by targeting of paramagnetic NPs [161]. A novel microfluidic immunoassay was utilizing binding of superparamagnetic NPs to beads and deflection of these beads in a magnetic field as the signal was used for measuring the presence of an analyte. The superparamagnetic 50 nm NPs and fluorescent 1 μm polystyrene beads get immobilized with specific antibodies simultaneously when target analytes reacts with them. Only the superparamagnetic NPs get attached to the microbeads by the antigen-antibody complex. In the PDMS microfluidic channel, only the microbeads bound with superparamagnetic NPs by analytes consequently move to the high-gradient magnetic fields under the specific applied magnetic field as shown in Fig. 14 [162]. This magnetic force-based microfluidic immunoassay was used to detect rabbit IgG and mouse IgG as model analytes with a concentration of 244 pg/ mL and 15.6 ng/ mL, respectively. The velocities of the antibody-antigen complex and detection range were measured in microchannel and compared to calculated field gradients [162].

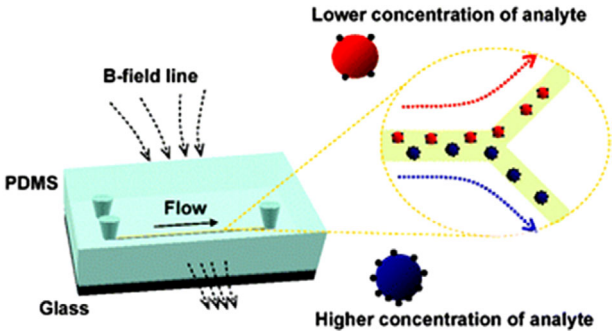

Figure 14. Microfluidic immunoassay utilizing binding of superparamagnetic nanoparticles to beads and deflection of these beads in a magnetic field as the signal for measuring the presence of an analyte. The detection range and lower detection limit can be controlled by the microbeads concentration and the higher magnetic field gradient. Reprinted with permission from [162], copyright 2019 Royal Society of Chemistry.

A digital microfluidic (DMF) platform based on the manipulation of magnetic beads was used to perform heterogeneous sandwich immunoassays [163]. Individual drops of

samples and reagents using electrowetting were free to move without channels and did not get clogged. Attraction, washing, retention, and resuspension of beads were the essential operation in this. Standard curves for heterogeneous sandwich immunoassays were generated by integrating all magnetic-bead droplet operations on human insulin and IL-6 with a total time to result of 7 min for each assay [164]. The antibody-coated paramagnetic particles were isolated and resuspended with the help of particle-based immunoassay with DMFs. Fluids attached to the electrode as tiny discrete droplets are controlled electrostatically that makes them suitable for immunoassay. This technique enables the movement of droplets without the oil carrier. The thyroid-stimulating hormone (TSH) and 17β-estradiol (E2) were models analytes in this particle-based immunoassay that permits noncompetitive and competitive immunoassays in DMFs. This device has excellent potential for qualitative analysis of small molecules including proteins in small reagent volumes [165]. Magnetic separation of probe and PSA followed by oligonucleotides hybridization on the NP probe surface permits the detection of a target protein by recognizing the oligonucleotide sequence released from the NP probe [166]. A developing interest is to incorporate magnetoresistive sensors with small-scale channels and gadgets to manufacture devices that can perform complex examinations. In these sensors, a bead concentrator made up of gold microstructures attracts and moves magnetic particles into a trap to detect target molecules. Less magnetic beads were restrained inside the chamber in case of magnetic beads with a target as compared to bare magnetic beads due to size difference [82]. A biosensing system is developed for the multiamplified detection of DNA. The analysis of DNA, according to configuration, is completed by identifying the target, which is used to capture nucleic acid-functionalized magnetic particles, followed by the self-assembly of magnetic particles and the other two nucleic acids into multicomponents DNA supramolecular structure [167]. Streptavidin-biotin interaction or complementary DNA sequence recognition was used to bind magnetic particles directly to the surface and indirectly by using sandwich type binding [108].

GMR biochip also serves as a molecular diagnostic platform for the reliable detection of magnetically labeled DNA. The DNA targets detected by this chip were PCR products amplified from Human papillomavirus (HPV) plasmids. The concentrations of 10 nM and 10 pM of the target DNA were detectable after PCR. The magnetic labels are a cluster of 10 nm Fe_2O_3 NPs held together by a matrix of dextran, functionalized with streptavidin molecules so it can be attached to the biotin end of the DNA target. Magnetic signals were detected using a double modulation technique in microfluidics in less than 1 h [168].

5.4 Microfluidic for mixing

Rapid, high-throughput, and homogenous mixing of microdroplets inside a microchannel is of great significance for LOC applications [63]. Mixing is an essential and necessary concept for many biological and chemical tests in microfluidics due to small dimensions of microchannel in the device. Inefficient and slow mixing is due to strong viscous forces in fluids. In LOC devices, the rapid mixing of fluids is necessary to confirm homogenous composition for an efficient reaction with low inconsistency [77]. Mixing of fluids can take place in a laminar flow or a static method, but trying on micrometer scale because of small Reynolds number [169]. Magnetic NP scans can be utilized to mix fluids in a LOC system and have also been for microfluidic mixing. In the case of a single chip, MNPs are exploited by applying a rotational gradient magnetic field, in which they form chains due to their dipolar interactions that act as a rotor in fluid, and get aligned in the direction of the applied field due to shape anisotropy. There will be only local mixing in the absence of the field. Rotational chains of particles enhance the mixing by a factor of 4 [170]. Agitation and mixing of fluid are facilitated by magnetic manipulation of superparamagnetic beads in giant vesicles.

The chain breaks up into small fragments by rotating the magnetic field above the critical frequency due to magnetic interaction, which depends on the number of beads and viscosity of the bulk solution for efficient mixing [171]. These vesicles with superparamagnetic microbeads were used on a LOC platform integrated with the magnetic field [172]. By using this method, particles for drug delivery systems or polymer capsules can be efficiently mixed. Adequate mixing enhances the cell-particle collision, which increases the uptake of target cells. This is important for gene transfer experiments of cancer cells [173].

Plugs are the active and flexible self-assembled regularly spaced supraparticle configurations formed by applying a magnetic field to a superparamagnetic particle suspension inside microchannels. The rotation of plugs influences the fluid flow. The dynamic supramolecular micron-scale patterns can be utilized in a broad assortment of on-chip applications and also for unique microfabrication methods without any structural loss [174].

An alternating magnetic field can control the dynamic motion of magnetic beads in microchannel. This produces a rotational motion enhancing the fluid perfusion that results in strong particle liquid interaction, which is the basis of efficient liquid mixing. Microfluidic chip with integrated ferromagnetic plate structures allowed the laminar flow pattern of fluorescein dye and nonfluorescein fluids with 95% mixing proficiency utilizing a mixing length of the only 400 μm and flow rates on the order of 0.5 cm/s [175]. The mixing method is helpful in the LOC system in designing and optimizing system performance. MNPs subjected to oscillating magnetic field retained their structure during their motion, and their position can be detected inside the channel as a function of flow velocity and amplitude of field [176]. Fluids can be mixed after loading into the reaction chamber or can be homogenized to avoid surface depletion of a biochemical reaction at the surface. A time-varying rotational magnetic field was used to agglomerate magnetic particles to form chains from suspension, and such methods were suitable for micromixing

applications in biosensing. Rotating magnetic particles accelerate mixing for biochemical reactions in the presence of a magnetic field and are used in the development of portable microchip biosensors. The mixing process in the microfluidic system based on MNPs also depends on the size of MNPs, inlet velocity of fluid entering the system, and frequency of the oscillating field. Mixing is significantly enhanced on the optimum switching frequency of time-dependent magnetic field [177]. Interaction between a uniform magnetic field and magnetic fluid in a microfluidic chamber can be used for efficient mixing of water-based ferrofluid and mixture of deionized (DI) water and glycerol. Complete mixing can be accomplished with a magnetic flux density of 10 mT and is used to achieve the concentration of MNPs [178].

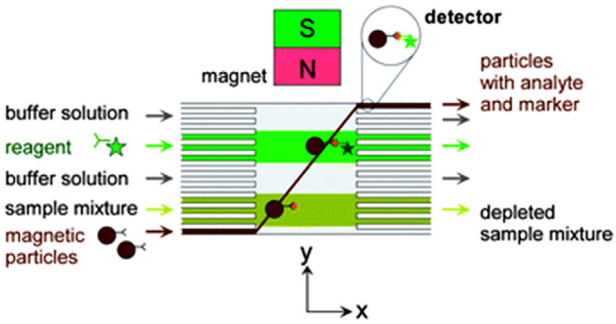

Figure 15. Rapid on-chip multi-step (bio) chemical procedures in a continuous flow—maneuvering particles through colaminar reagent streams. Reprinted with permission from [186], copyright 2019 Royal Society of Chemistry.

5.5 Microfluidic for transport

Colloidal particles are manipulated and guided by applying an external magnetic field to a fluid in magnetophoresis [179]. Many factors govern the movement of particles like a magnetic force, viscous drag, Brownian motion, particle-fluid interaction gravity. The interparticle effects include van der Waal forces, electric double layer interactions, and magnetic dipole-dipole interactions [147]. Microfluidic devices can magnetically control the transport of magnetic particles that will be used to mix and capture analyte [180]. MNPs are utilized to carry and transport biomaterials, such as enzymes, nucleic acid, proteins, and cells for bioapplications including bioseparation, drug delivery, and magnetoreception [147, 181]. MNPs are also used as a substrate to carry, detect, and finally capture specific-labeled analyte. Only bound analytes are labeled and detected after several washing and separation steps by an externally applied magnetic field. Magnetically labeled analytes were separated and immobilized from a carrier with the help of magnetic force experienced by MNPs. Analytes were labeled by fluorescent detection antibodies after being captured by MNPs, which were separated and washed from the magnetic particles and counted during its flow through a capillary after buffer exchange with single-molecule resolution. Brownian motion of magnetic beads and asymmetric magnetic potential facilitates the transport of biomolecules attached to magnetic beads [182]. Chemiluminescent molecules fluorescent dyes have been used as detection labels in microfluidic flow-based assays frequently along with superparamagnetic particles and magnetic plugs. Microchannels manipulating small magnetic particles were used for heterogeneous immunoassay systems. Small-size magnetic particles create a magnetic field of high sensitivity for the detection of small volumes. This immunoassay system was demonstrated with a direct interaction of fluorescein isothiocyanate (FITC) with an immobilized anti-fluorescein isothiocyanate (FITC) conjugate that reaches 90% of maximum signal in 3 min with a small volume of the sample (<1 μL). Parathyroid hormone (PTH) and IL-5 are used in heterogeneous sandwich assays on microscale with relevant sensitivity (∼μg/L) [183]. Functionalized magnetic beads were a promising solution in microfluidic systems and serve as a key for many high-throughput applications including cell separation and biosensing in immunomagnetic based assay. Short strands of DNA (probe oligonucleotide) get bound to the surface of the bead through the streptavidin-biotinbond, promoted by magnetophoretic motion (motion induced by a magnetic field on the magnetic particle) of MNPs. The particles are transported through a coflowing analyte stream comprising biotinylated probe oligonucleotides, labeled with a Cy3-fluorophore using a transverse magnetic field gradient. Fluorescence imaging of magnetic particles present in the third stream of PBS separated magnetophoretically has been used for the quantification of the resulting biotin-streptavidin [184]. Microfluidic devices can be used to perform various biochemical procedures in continuous flow. External magnetic field drag functionalized MNPs from one side to the other side of the chamber. This platform was first demonstrated to bind free biotin in solution to streptavidin-coated magnetic particles with a detection limit of free biotin of 20 ng/mL within 60 s [185]. The principle of the multistep processing system is illustrated in Fig. 15 for rapid on-chip multistep biochemical procedures such as DNA hybridization and intercalation with processing times of <1 min [186]. Particles introduced into the chip with the laminar flow are magnetically deflected by applying magnetic field gradient through streams containing reagents or washing buffer.

Magnetically assisted transport integrated with the microfluidic platform, and waveguide technology was used for detection of multiple samples. Preliminary analysis, optimization, and standardization were accomplished by manipulating magnetic beads using pump as an external magnet for the determination of rabbit IgG, IL-4, a cytokine that enhances production and separation of B cells and to show system reproducibility with RSD estimations of 5% and stated LOD of 10 ng/mL [187]. Proteins in low abundance were detected and captured by beads, functionalized with specific antibodies, and after that marked with a fluorescent product which are immune complexes with enzymes. Fluorescence imaging was used to detect protein molecules. Magnetic particles were loaded in separate microwells of isolated ELISA reaction chambers to reach single target sensitivity [188]. NP-

based bio barcode assay was used to capture analyte targets by magnetic particles, labeled with gold NPs having specific antibodies and DNA fragments. However, the integration into a POC system gets complicated by various fluid handling steps [166]. MNPs used as solid-support microbeads are focused by magnetic susceptibility gradient and magnetic field inside the microchannels to detect the bound analyte. Three types of breast cancer biomarkers were detected by this platform for practical use [189]. The transfer of magnetic particles between liquids is controlled by the balance between the capillary forces, which is due to interfacial tension, and the magnetic forces on the particles [77].

The diagnostic targets can be captured at a controlled time and position inside the channel by dual magnetic and temperature-responsive NPs, which are utilized as soluble reagents. The targets on the poly (ethylene glycol)-modified PDMS channel walls of a microfluidic devices get separated by increasing the temperature and magnetic field of the MNPs associated with biotinylated targets. When the magnetic field is turned off, and the temperature is reversed, the agglomerates captured by MNPs were redisperse into the flow stream in the channel, for further downstream processing. MNPs can be separated after releasing captured molecules, thus, restricting the mobility of NPs.

6 Concluding remarks

There are numerous applications of microfluidic systems using MNPs that respond to an external magnetic field in microchannels, which includes biochips. The magnetic particle-based microfluidic system offers a practical and competent method ranging from mixing of fluids to manipulation, detection, and isolation of molecules ((DNA, proteins, viruses, and cells) from raw samples on a single chip. MNPs are extensively utilized in many biomedical applications (MRI, drug delivery, hyperthermia, etc.) because of their exceptional characteristics like high surface-to-volume ratio and ease of surface biofunctionalization. Magnetic particles play a significant role in capturing and labeling target biomolecules effectively inside the system for investigation. The incorporation of a microfluidic system with MNPs facilitates the fast analysis and performance of biological tests at the POC. The integration of MNPs in channels in fluidic conditions allows us to obtain optimal sensitivity and specificity for biosensing applications. Magnetic-based microfluidic systems enable the development of lightweight, small size, high throughput, and portable devices for the rapid and precise investigation that speed up the chances of treatment is a reliable and straightforward manner. Miniaturization of microfluidic technology and its integration with magnetic particles replace conventional systems and possess many advantages for more improvement and advancement of microfluidic POC-based testing. Magnetic particles with miniaturized microfluidic systems permit measurable in vitro diagnostic testing rapidly in desktop-sized and hand-held instruments with a user-friendly "sample-in result-out" kind of performance. The primary purpose of the microfluidic system is to develop POC, disposable microfluidic devices having the ability of not only adequate mixing but also for the manipulation and isolation of the biomolecules labeled with MNPs that make diagnostic workflows, patient monitoring, and disease management more productive and better. Many challenges still need to be overcome, like agglomeration and aggregation of MNPs, that affect the applications. Effective device operation involves special care to avoid or control accumulation, or even use it more practically by using "smart" MNPs [190]. Clogging and sedimentation is another challenge in microfluidic channels due to larger particle size. Colloidal stability and integrity of ferrofluid are necessary for the flow of fluid inside the microchannels. Using surfactants to improve the biocompatibility of MNPs make the channels dirty and sticky, which affects the movement of magnetic particles. Microfluidic biochips using MNPs with functionalized surfaces offer a reliable solution for POC devices to compete with conventional biosensing systems for manipulation, detection, and isolation of bioanalyte on a single chip.

Exploration and investigation to improve the performance of magnetic particle-based microfluidic devices are under process by refining magnetic particle properties, surface functionalization, and the response of the particle in microfluidic environs. Incorporation of MNPs with a microfluidic system for diversified and efficient manipulation and analysis of biological targets has been a research hotspot in the interdisciplinary field. Future directions include optimization of comprehensive strategies, consistent advancement of novel systems, and innovation of different applications for these devices, which will help to revolutionize biomedical diagnostics and treatment with future benefits to the entire humanity.

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