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Review

Microfluidics-Based Sensing of Biospecies

Yanlong Xing,[‡] Linlu Zhao,[‡] Ziyi Cheng, Chuanzhu Lv,* Feifei Yu,* and Fabiao Yu*

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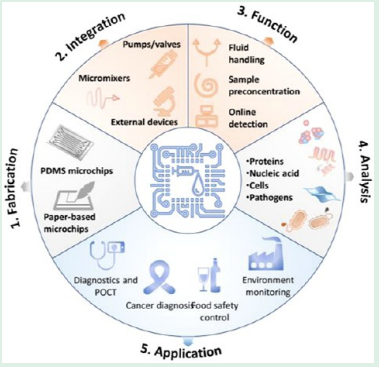
ABSTRACT: Over the past decades, microfluidic devices based on many advanced techniques have aroused widespread attention in the fields of chemical, biological, and analytical applications. Integration of microdevices with a variety of chip designs will facilitate promising functionality. Notably, the combination of microfluidics with functional nanomaterials may provide creative ideas to achieve rapid and sensitive detection of various biospecies. In this review, focused on the microfluids and microdevices in terms of their fabrication, integration, and functions, we summarize the up-to-date developments in microfluidics-based analysis of biospecies, where biomarkers, small molecules, cells, and pathogens as representative biospecies have been explored in-depth. The promising applications of microfluidic biosensors including clinical diagnosis, food safety control, and environmental monitoring are also discussed. This review aims to highlight the importance of microfluidics-based biosensors in achieving high throughput, highly sensitive, and low-cost analysis and to promote microfluidics toward a wider range of applications.



KEYWORDS: *microfluidic chip, functional nanomaterials, disease diagnosis, point-of-care test, analysis of biospecies*

1. INTRODUCTION

Rapid and sensitive analyses of biospecies are of critical importance in the understanding of biological processing in organisms and diagnosis of various diseases.[1] With the development of the economy and increasing focus on human healthcare, food safety control, and environmental monitoring, it is urgently desired to develop advanced tools for the detection of a variety of biomolecules.[2,3] In particular, with the high demand for point-of-care testing (POCT), suitable platforms need to be developed. In pursuit of fabricating on-demand POCT devices, microfluidic-based biosensors have been extensively investigated, in combination with different detection methods, according to the analytes.[4]

The definition of "microfluidics" includes two main aspects: one is the small size of the channel in micrometer scale and the other is the handling of nano- even subnanoliter amounts of liquids.[5] Over the past decades, the miniaturization of analysis systems for chemical and biological applications has attracted increasing research interest. The first miniaturized device was a gas chromatographic air analyzer which was microfabricated in a silicon substrate in the 1970s.[6] Afterward, the concept of micro total analysis systems (μTAS) was introduced to the field of miniaturization, which influenced research efforts and numerous commercial applications.[7]

Compared to conventional systems, microfluidic chip or "lab-on-a-chip" devices offer lots of benefits, for instance, high surface to volume ratio, precise fluid control, low sample consumption, and high integration with functional components.[8,9] Microfluidic systems can enable the manipulation of small volumes of fluids in microchannels with dimensions of a few to several hundred micrometers.[10] The downscaling of fluidic systems is beneficial to analysis of small samples due to their similar dimensions. Additionally, the fluid in microchannels exhibits unusual characteristics compared to macroscopic counterpart. The key parameter to describe the performance of fluids is the Reynolds number (Re), which is influenced by the ratio of viscous forces to inert forces. Typically, $Re > 10^3$ represents macroscopic systems while $Re < 10^2$ stands for microfluidic systems.[11] The low Re number in microfluidic devices describes the nonturbulent or laminar flow, which is an important and typical feature provided by miniaturization. In a laminar flow regime, mixing of fluids is dominated by diffusion at the interface between fluids. Due to the small scales in microfluidics, the diffusion time is shortened and the diffusion-based mixing process has become an efficient process.

In the following sections, we intend to give an overview on the application of microfluidics-based sensing of biospecies including biomarkers, small biomolecules, cells, pathogen, etc. Typical studies have been exemplified to demonstrate the approaches that microfluidics can be exploited for the analysis of these biospecies, followed by the most recent advances in translating the analysis of biospecies into practical applications.

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As illustrated in Figure 1, microfluidic devices can be made of various materials such as inorganic materials, polymers, and paper, according to different applications.[12,13] Integration of microdevices with a variety of chip designs will facilitate the promising function of them, especially when integrating with nanomaterials. For the analysis of biospecies, a series of detection methods including optical techniques, electrochemical, or electrical approaches can be utilized, in combination with microfluidic platforms.[14−16] Therefore, with the rapid and sensitive detection of various biospecies including proteins, nucleic acids, cells, pathogen, etc., microfluidic biosensors exhibit promising applications in different fields, e.g., diagnosis of disease, food safety control, and environmental monitoring.

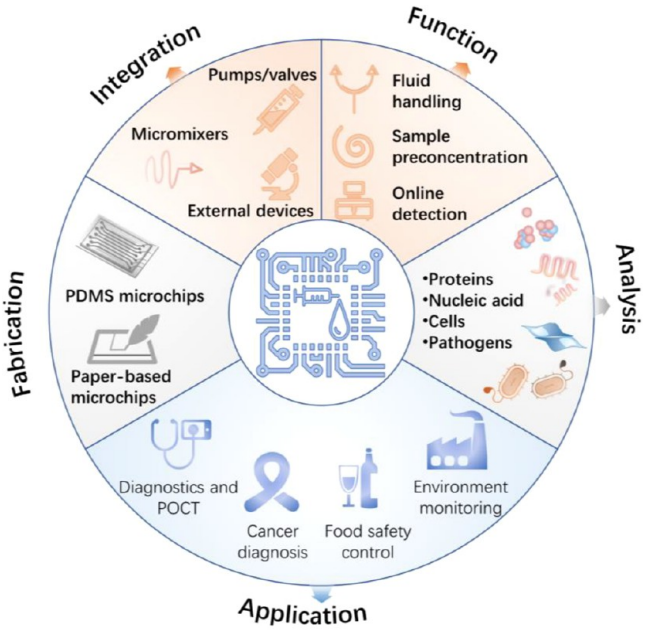

Figure 1. Schematic illustration of the fabrication, integration, and function of microfluidic devices, the microfluidics-based analysis of biospecies, and various applications.

2. MICROFLUIDICS AND MICRODEVICES

2.1. Microdevice Fabrication and Integration. Various materials have been utilized in fabricating microfluidic devices, which can be categorized into three main types: inorganic materials, polymeric materials, and paper. When fabricating a microfluidic device, the desired function, components to integrate, and final applications should be considered to choose a suitable material. Inorganic materials such as silicon, glass, and ceramics have their own advantages and disadvantages. Silicon was the first material used for microfluidic devices, with the silanol groups easily to be modified.[6] However, silicon is transparent to infrared other than visible light, making it difficult to integrate with fluorescence detection. Polymeric materials are organic-based, long-chain materials including elastomers and thermoplastics. Polydimethylsiloxane (PDMS), an elastomer to fabricate soft and flexible microfluidic devices, has become the most commonly used microfluidic substrate. Compared with other materials, PDMS exhibits obvious advantages of low cost, robustness, flexibility, biocompatibility, and gas permeability. PDMS-based microfluidic chips are facile to fabricate using a device mold, which is on the basis of mixing elastomer and curing regents, followed by casting and heating procedures. In addition, using PDMS, either single- or double-layer microchips can be manufactured.[8] Thermoplastics are densely cross-linked polymers that are optically clear and durable. However, it is difficult to integrate valves to seal a microchannel into microdevices made of thermoplastics. Paper-based microfluidic devices have been widely used in fabricating biosensors owing to their high porosity, rich microstructures, and intrinsic capillary effect for fluid transporting. In addition, this material can be obtained from wood or grass, which greatly lowers the cost of fabrication. Additionally, a naked eye-based readout of colorimetric changes could be monitored on paper microfluidic devices, making it promising for facile POCT in different research fields.[13,17]

A typical microfluidic chip is composed of three main components: fluid inlet, microchannel, and fluid outlet. To fabricate microdevices with different functions, integration of functional components is required. Microdevice integration includes three main aspects: (1) integration with microfluidic handling components (pumps, valves, or mixers) into microchannels to generate fluids with different characteristics for reaction, such as continuous flow, stop flow, or microdroplets;[8,9,18] (2) embedding nanomaterials as the functional zone for sample isolation or enrichment;[19] (3) combined with external devices for analyte detection, based on optical, electrochemical, acoustic approaches, etc.[20] Specifically, first, fluid manipulation in microchannels relies on different internally integrated components including micropumps, microvalves, and micromixers to form fluids with continuous flow, stop flow, or microdroplets. The integration of micropumps into microfluidic systems can facilitate pumping of fluid into microchannels, without the need to use external devices.[21] Valves, in particular, pneumatic valves are widely applied in PDMS-based microdevices due to their function and ease of fabrication. By actuating the valves using inert gas (e.g., N_2), the movement and flow direction of fluid inside microchannels can be well manipulated.[22] Micromixer designs have been developed and integrated into microchips in order to achieve the rapid mixing of solutions. Since the laminar flow in microchannels facilitates mixing of solutions by diffusion, diffusive micromixers such as T-mixers are the simplest design for mixing of multiple fluids. In some cases, electrical or magnetic devices can also be applied as external driven forces to accelerate the fluid flow.[21] Second, with the development of nanotechnology, various nanomaterials such as gold nanoparticles (AuNPs), zinc oxide nanowires (ZnO NWs), carbon nanotubes (CNTs), etc., have been integrated with microfluidics for analyte capturing and detection.[23,24] For instance, ZnO nanostructures with different morphologies were grown in situ inside microfluidic channels. After the surface functionalization with capturing antibodies, the dye-labeled analytes were immobilized on the nanostructures and detected by changes in fluorescence response.[19] Third, microfluidic devices could be integrated with external devices for analyte detection, according to the different detection techniques such as optical,[25−27] electrochemical, acoustic approaches, etc.

2.2. Function. Microfluidic platforms offer advantageous functions in fluid transport, sample preparation, analyte isolation and analysis, and material synthesis compared to their macroscale counterparts.[28] To meet the requirements of the desired application, suitable microchip designs and detection techniques should be chosen. Regarding the sample

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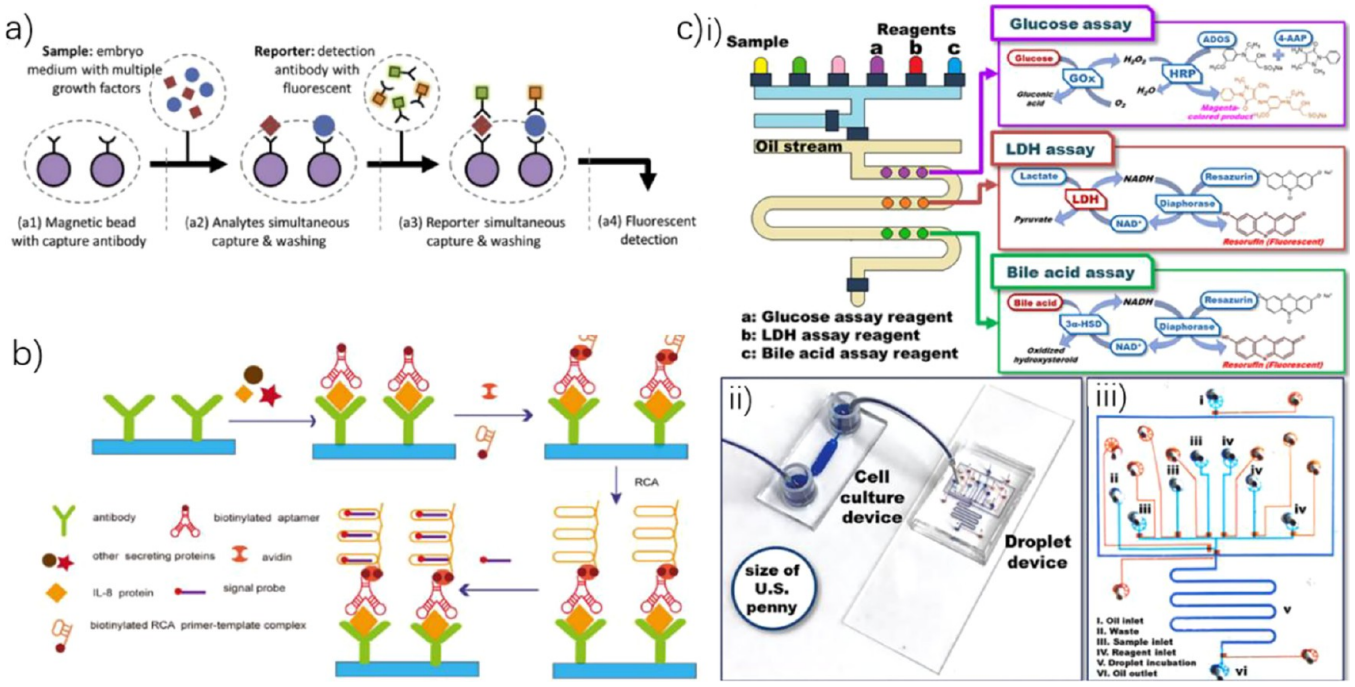

Figure 2. (a) Schematic of simultaneous detection of two single-embryo growth factors (SEGF) from a culture medium. (Reproduced with permission from ref 35. Copyright 2020 Elsevier B.V.) (b) Schematic diagram of the detection of IL-8 by RCA and fluorescence probe. (Reproduced with permission from ref 36. Copyright 2018 Elsevier B.V.) (c) (i) Schematic illustration for the concept of developed droplet-based biochemical assay. In the developed droplet device, three different biochemical assays including glucose, LDH, and bile acid assay could be accomplished at the same time. Since the water-in-oil droplet acts as an isolated biochemical reactor, each assay can be achieved without mutual interferences of samples and reagents. (ii) Photographs of hepatocyte-culturing microfluidic device and droplet device. For the cell injury analysis application, the droplet device was connected with a cell culturing device, and the changes in biochemical metabolites in cell culture media were analyzed in the droplet assay module. (iii) Photograph of droplet device. The microfluidic channels in the flow layer and control layer were filled with red ink and blue ink, respectively. (Reproduced with permission from ref 43. Copyright 2019 American Chemical Society)

preparation, on-chip isolation and preconcentration offers an efficient way to enable the analysis of low-concentration samples. The online preconcentration of samples is normally based on their intrinsic characteristics such as size, charge, affinity, and mobility, in combination with functional components in microchips. For instance, by modifying nanofilters or nanopillars in microchips, extracellular vesicles can be isolated and enriched according to their size differences, which is beneficial for further analysis.[29,30] In pursuit of highly sensitive biosensing approaches, microfluidic-based systems offered promising platforms by integration with functional substrate e.g. nanostructures[31] and various detection methods. In particular, the emerging single-entity studies involving detection of individual cells, vesicles, proteins, or nucleic acids require a suitable platform for analyte trapping and measurement. By confining single analytes in small reaction volumes, the in-depth analysis of individual targets can be performed, which contributes not only to the investigation of the heterogeneities of individual biospecies within a population but also to the development of sensitive biomedical tools for disease diagnostics and related fields.[32,33]

3. MICROFLUIDICS-BASED ANALYSIS OF BIOSPECIES

In this section, in order to demonstrate how microfluidics can be exploited for the analysis of various biomolecules, we mainly focus on reviewing the different approaches integrated with microfluidics that have been utilized for bioanalysis, including optical, electrochemical, electrical techniques, etc.

3.1. Biomarker Analysis. *3.1.1. Protein Assay.* Protein is the basic component of cell, tissue and organ, which plays vital role in biological function of organisms including cell proliferation, apoptosis, intercellular communication, immune response etc. Various proteins function as biomarkers in both physiological and pathological processes. Therefore, detection of proteins is indispensable and effective in clinical application, e.g. disease diagnosis. There are mainly two strategies applied in the detection of proteins: one is immunoassay which is based on the targeted binding of antigen to antibody or aptamer; the other one is enzyme-based analysis which depends on the reaction between enzyme and specific substrate. Furthermore, with the development of microfluidics technology, a variety of microfluidics-based biosensing approaches have been reported for the sensitive assay of proteins using different detection methods.

3.1.1a. Fluorescence Detection. Conventional protein detection methods such as enzyme-linked immunosorbent assays (ELISA) suffer from the drawbacks of laborious process and time-intensive detection. Most reported microfluidic devices utilized patterned capture antibodies in microchannels to target analytes and achieve their detection by fluorescently labeled detection antibodies in solution. However, the solution mixing efficiency dominated by laminar flow in microchannels is limited, and both the analyte capture and detection require certain incubation time. Owing to the high demand of real-time detection of proteins in clinical applications, Konry et al. developed a facile lab-on-a-chip (LOC) approach for the real-time detection of cytokines and antibodies. They applied well

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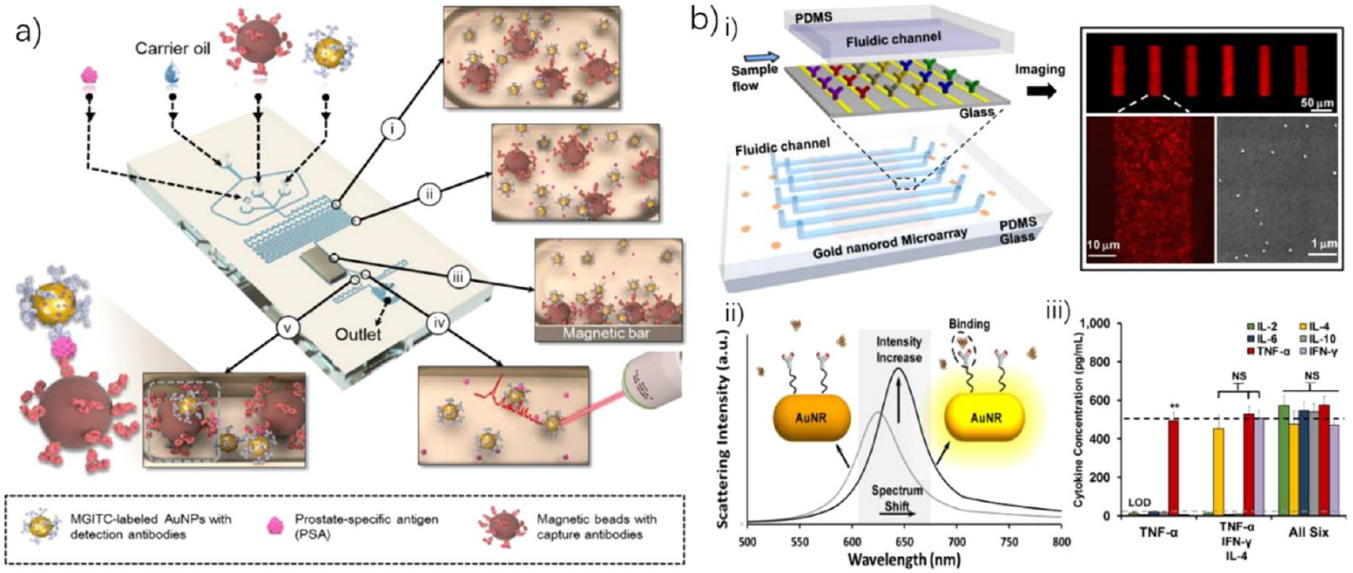

Figure 3. (a) Schematic illustration of the SERS-based microdroplet sensor for wash-free magnetic immunoassay. The sensor is composed of five compartments with the following functions: (i) droplet generation and reagent mixing, (ii) formation of magnetic immunocomplexes, (iii) magnetic barmediated isolation of immunocomplexes, (iv) generation of larger droplets containing the supernatant for SERS detection, and (v) generation of smaller droplets containing magnetic immunocomplexes. (Reproduced with permission from ref 47. Copyright 2016 Royal Society of Chemistry) (b) Schematic and principle of the method. (i) Schematic of the LSPR microarray chip. The nanorod microarray fabrication was performed using a one-step microfluidic patterning technique assisted by electrostatic attractive interactions between the nanorods and the substrate surface within microfluidic channels. Subsequently, these nanorode microarrays were integrated in a microfluidic chip with eight parallel microfluidic detection channels consisting of inlet and outlet ports for reagent loading and washing. Specific antibodies were conjugated to the patterned AuNR microarrays using thiolated cross-linker and EDC/NHC chemistry. The current chip design integrates 480 AuNR microarray sensor spots. The prepared LSPR microarray chip was then imaged under dark-field microscopy and scanning electron microscopy (SEM). (ii) Principle of the LSPR microarray method. Analyte molecules are introduced to an antibody functionalized AuNR LSPR biosensor. Binding of the analyte molecules to the receptors induces a redshift and scattering intensity change of the longitudinal SPR (exaggerated in the illustration). This intensity change is imaged via the characteristic frequency (gray area) using EMCCD coupled dark-field microscopy. (iii) Cytokine concentrations quantified for the samples in part a. Measuring the scattering light intensities of the barcodes and converting them to concentration values using calibration curves allowed for the quantification of the cytokine concentrations. The dashed line in black represents the predetermined value (500 pg/mL) of the analyte concentration. The dashed line in gray represents the limit of detection (LOD) of the LSPR microarray measurement. (Reproduced with permission from ref 52. Copyright 2015 American Chemical Society)

mixed microfluidic device with turbulent flow in reaction region to enhance the solution mixing. By integrating microsphere-based immunoassay to the microdevice, real-time detection of tumor necrosis factor (TNF)-α cytokine and TNF-α inhibitor was achieved by fluorescence readout.[34] In another study, a bead-based digital microfluidic chip was fabricated for simultaneously measuring two growth factors (human TNF-α and human IL-1β) from the same single droplet of embryo culture medium based on a fluorescent immunoassay (Figure 2a). The proposed microchip integrated both bead manipulation and droplet techniques to achieve the control of sample with ultrasmall volume (520 nL), which also allowed the detection of two human embryo growth factors from a single sample within 40 min.[35] Aptamers are single-stranded oligonucleotides that can bind to targets with high specificity and affinity. They are considered as substitutes to antibodies in immunoaffinity assay. Lin et al. reported their work on detecting interleukin 8 (IL-8), a proinflammatory chemokine in neutrophil chemotaxis and activation, based on immunoassay of a sandwich structure consisting of antibodies, IL-8, and aptamers (Figure 2b). The fabricated microfluidic device enabled cell culture and online IL-8 detection. Owing to signal amplification of biotin−streptavidin interaction and rolling circle amplification (RCA), IL-8 could be sensitively detected in tumor cells by fluorescence signal, which exhibited consistent results with commercial ELISA kit.[36] Based on a similar RCA reaction strategy, the same group also developed an open-space PDMS microfluidic chip with fluidic walls, which integrated cell culture and online detection of vascular endothelial growth factor. These microdevice systems provide potential tools for micrototal analysis in future clinical applications.[12] To simplify the cumbersome procedures of reported sandwich immunoassay, an autonomous microfluidic chip which could achieve multiple washing processes and antigen−antibody binding automatically was reported by the Song group. By integration with a fluorescent microscopic counting technique,[37] the self-powered microfluidic device was utilized to quantitatively detect human chorionic gonadotropin (hCG) and carcinoembryonic antigen (CEA) in 10 min.[38]

Regarding the analysis of enzymes, those involved in redox reactions are under investigation. Normally, redox enzymes modulate the redox reaction, using color- or fluorescence-emitting[39−42] compounds to generate detectable optical signals. However, conventional assays face the challenges of time-consuming and large sample consumption. In this case, reactions in microfluidic devices offers a feasible tool to achieve the detection of enzyme in small volume samples. For instance, by combining microfluidic automation and droplet generation, the detection of multiple cell-derived analytes including glucose, lactate dehydrogenase (LDH) and bile acid was achieved by separate enzyme reaction in subnanoliter single droplets (Figure 2c). The computer-controlled microdevice

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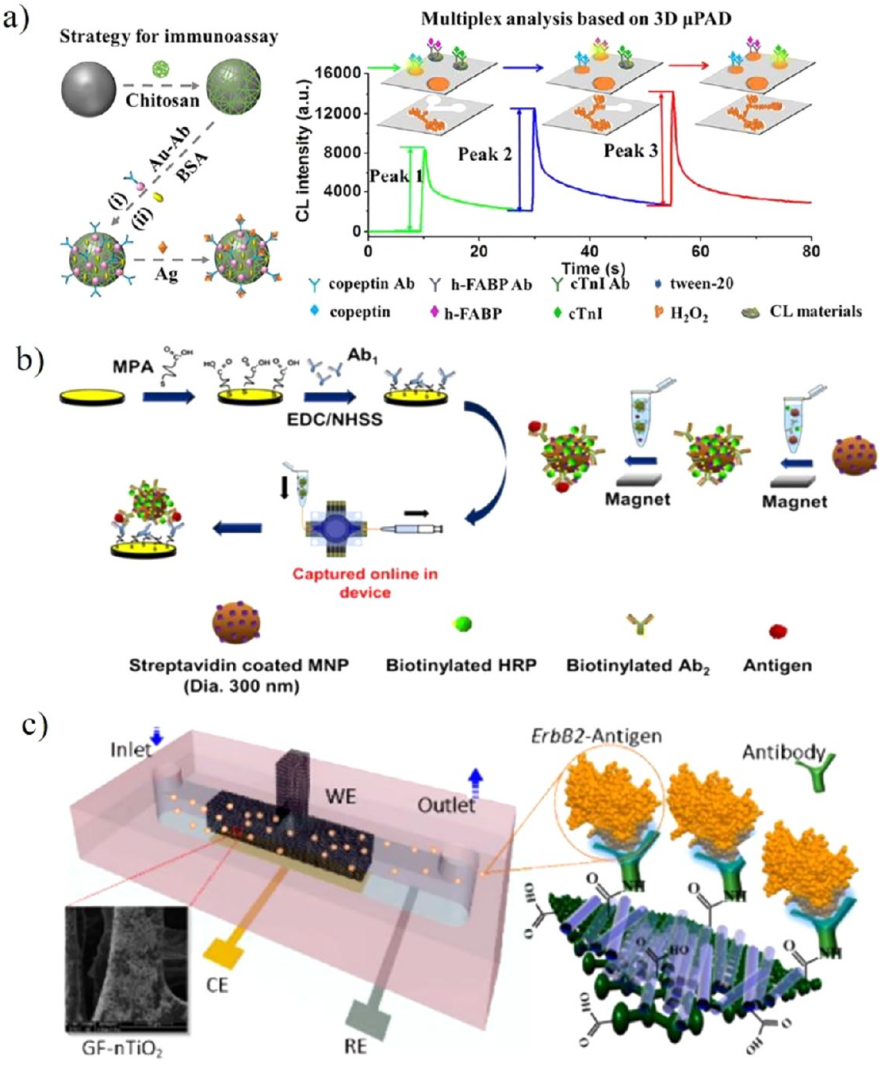

Figure 4. (a) Schematic illustration of proposed immunoassays and fabrication of 3D μPAD. Detection zone no. 1, copeptin detection zone; no. 2, h-FABP detection zone; no. 3, cTnI detection zone. The CL kinetic curve was obtained in the presence of 100 pg/mL of copeptin, h-FABP, and cTnI. (Reproduced with permission from ref 55. Copyright 2019 American Chemical Society) (b) Strategy for multiplexed voltammetric detection with wide dynamic range, showing one sensor with capture antibodies. (Reproduced with permission from ref 58. Copyright 2016 American Chemical Society) (c) Schematic of the microfluidic immunosensor with 3D porous GF electrode modified with carbon doped TiO_2 nanofibers for the detection of breast cancer biomarkers. (Reproduced with permission from ref 65. Copyright 2016 American Chemical Society)

contains microvalves to form laminar flow and a cross-junction to generate sample/reagent water-in-oil droplets. Herein, colorimetric glucose assay and fluorometric assays for LDH and total bile acids were demonstrated, which showed the applicability of this microplatform. Furthermore, by connecting the droplet device to a cell culture microdevice, the continuous monitoring of cell-derived analytes was achieved. This in-droplet microfluidic assay provided a facial and effective tool for multiplexed detection of analytes in small volume samples.[43]

3.1.1b. Raman Detection. Apart from the fluorescent approach,[44] Raman spectroscopy which can provide molecular structure information by measuring the inelastic scattering of photons induced by molecular vibrational events has exhibited its advantages in sensing analytes due to its nondestructive and real-time detection characteristics. Surface-enhanced Raman scattering (SERS), a technique exhibiting enhanced Raman signals of analytes up to 10^{14}–10^{15} when they are in close proximity to plasmonic metallic (e.g., Au or Ag) nanostructures, has attracted widespread research interest the development of biosensors.[45] SERS-based biosensors have been reported to detect various biospecies including proteins, DNAs, microRNAs, and other biomolecules. In particular, when integrated with microfluidic techniques, SERS biosensors offered several advances over conventional macroscale approaches. For instance, Choo et al. reported a SERS-based magnetic solenoid sensor in microdevice for the sensitive detection of anthrax biomarker poly-γ-D-glutamic acid (PGA) in solution. Based on the competitive immunoassay between PGA and PGA-conjugated gold nanoparticles with anti-PGA immobilized magnetic beads (AuNPs), the rapid and sensitive detection of PGA biomarkers was accomplished by the strong SERS signal.[46] However, this experiment was done in continuous flow regime and required a washing step of the unbound reagent from the immunocomplexes. Therefore, the same group developed a SERS-based microdroplet sensor using wash-free magnetic immunoassay technique to detect biomarkers. Their microfluidic device composed of four

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compartments including microdroplet generation, solution, droplet splitting, and SERS nanotag detection (Figure 3a). By embedding a magnetic bar in the third compartment, the immunocomplexes and free SERS nanotags were separated by splitting primary droplets. The Raman signals of the unbound SERS nanotags in the supernatant channel were detected and utilized for the quantitative detection of PSA can biomarkers in serum without a washing procedure. The proposed method offered a limit of detection (LOD) of 0.1 ng/mL, which exhibited great potential for clinical application.[47] Khor et al. reported an integrated SERS-microfluidics device to detect immune checkpoint proteins. By using nano yeast single chain variable fragment (scFv) as an alternative to monoclonal antibodies, the graphene oxide functionalized surface reduces the biofunctionalization steps and integrated alternating current electrohydrodynamics (ac-EHD) which could induce nanomixing to enhance the target scFv binding and minimize the nonspecific interactions, and the multiplexed detection of immune checkpoint biomarkers (PD-1, PD-L1, and LAG-3) from human serum was achieved by SERS readout at a low LOD of 100 fg/mL.[48]

3.1.1c. Surface Plasmon Resonance (SPR) Detection. SPR, which is based on the measurement of refractive index changes, can be applied to sensing of biomolecules in a label-free manner. SPR sensors can sense biomolecular interaction upon binding analyte to capture molecule on the SPR substrate.[49] Metal nanoparticles such as Au and Ag NPs show strong plasmonic properties. Nanoplasmonics stimulated the generation of various optical biosensors. With the development of various metallic nanostructures (e.g., nanoholes) as substrates, SPR sensors exhibit wide application in biosensing, especially when integrated with microfluidics techniques.[50] For instance, to develop a point-of-care system for potential clinical applications, a hand-held microfluidic platform was integrated with a plasmon modality and applied for the detection of hemoglobin. This miniaturized device was inexpensive and could enable label-free and rapid detection.[51] Similar to the SPR technique, LSPR biosensors were also developed based on noble metal nanostructures. Kurabayashi et al. reported a multiplex serum cytokine immunoassay system based on nanoplasmonic biosensor microarrays of AuNRs (Figure 3b). The sensing mechanism is based on the changes in LSPR signal upon capture of target analytes by the antibody conjugated AuNRs. The microdevice consisted of 480 nanoplasmonic sensing spots in microchannels which could facilitate label-free and high-throughput analysis. A mixture solution of six target cytokines (IL-2, IL-4, IL-6, IL-10, TNF-R, and IFN-γ) was applied to the biosensing system. Upon analyte-binding, rapid response was observed and reached an equilibrium within 30 min. The proposed LSPR biosensing platform exhibited faster responding, higher sensitivity, and lower LOD, compared to conventional ELISA technique.[52] Not only the detection of proteins but also the evaluation of protein activity can be accomplished by microfluidic LSPR sensor. In Bhalla et al's work, a microfluidic biosensor platform was fabricated to integrate a nanoplasmonic substrate into microchannels for the LSPR-based detection the DNA-polymerase reaction in real-time. Densely packed nanostructures which are composed of silicon dioxide stems (~40 nm) and gold caps (~22 nm) were utilized as the nanoplasmonic substrate. After modifying a single-stranded DNA (ssDNA) template and spacer molecules on Au nanostructures, the interaction between DNA and DNA polymerase enzyme was monitored. This microfluidic platform offered a superior tool in detecting the activity of a DNA polymerase enzyme in label-free and real-time manner.[53]

3.1.1d. Chemical Luminescence Detection. Chemical luminescence-based biosensors can be used to detect biomolecules by changes in light emission from chemiluminescence (CL), bioluminescence (BL), or electrochemiluminescence (ECL) reaction. The biggest advantage of this kind of biosensor is the high detectability of the light emission in dark without nonspecific signals. Together with the advances in nanotechnology and microfluidics technique, chemical luminescence-based biosensors have been widely developed for detecting various bioanalytes.[54] Cui group developed a CL immunoassay approach for the multiplexed detection of early acute myocardial infarction biomarkers, including copeptin, heart-type fatty acid binding protein (h-FABP), and cardiac troponin I (cTnI). The proposed detection system was based on a three-dimensional microfluidic paper device (3D μPAD) using Co^{2+}/*N*-(aminobutyl)-*N*-(ethylisoluminol) (ABEI) functionalized magnetic carbon composite (Co^{2+}-ABEI-Fe_3O_4@void@C) as the interface (Figure 4a). Magnetic carbon composite first assembled with chitosan and Au NP-bonded antibody, which was subsequently applied for capturing antigens to form immunocomplex. Upon adding H_2O_2, time-resolved CL response could be detected after the reaction between ABEI and H_2O_2. Therefore, the 3D μPAD was designed to have three separate detection spots, enabling the simultaneous measurement of copeptin, h-FABP, and cTnI with a low LOD from human serum samples.[55] In CL assay, the generation of signals depend on external added chemicals. In contrast, in a BL approach, light originates from internal enzyme reaction. Therefore, the bioluminescent strategy is especially suitable for detecting autofluorescent molecules from complex fluidic samples. For example, Dacres et al. developed a bioluminescence resonance energy transfer (BRET) biosensor to detect thrombin activity in human serum. The BRET biosensor is composed of two protein components and a linker of a thrombin recognition site. Upon enzyme reaction, BRET occurs between the two components and both emit light emission. However, linker cleavage by thrombin induced loss of BRET, resulting in increased optical signal from BRET donor component. The detection system was based on a compact microreactor integrating a thermostat and a PDMS microfluidic chip. Using the proposed device, thrombin activity was sensitively measured from human serum samples.[56] ECL biosensing is based on the measurement of ECL signal produced after bimolecular recombination of electrogenerated radicals at the electrode surface. ECL sensing shows increased sensitivity and wider dynamic range than CL approach. After integrated with microfluidic paper-based analytical devices (μPADs), ECL biosensors exhibit as a promising platform for point-of-care testing applications.[57]

3.1.1e. Electrochemical Detection. Electrochemical biosensor is a device which can transduce analyte recognition events into detectable electrochemical signal. Normally, a three-electrode-system including working electrode, counter electrode and reference electrode (Ag/AgCl). The electrochemical biosensor enables the capture of target analyte by immobilized sensing elements on electrode surface. The binding of analyte to biosensing element lead to changes in the electrochemical signal of redox reporter, which can directly reflect the concentration of target biospecies. Electrochemical biosensors have attracted much research focus due to their

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high sensitivity and selectivity, low cost, and multiplexed detection capabilities. In particular, microchip-based electrochemical arrays offer great promise for rapid, high-throughput, and multiplexed analysis of cancer biomarkers.[15] A microfluidic electrochemical sensor was fabricated by wet-etching commercial gold compact discs (CD-R) and patterned insulation procedure, which contains 8 subsets, each is composed of 32 individual microelectrodes. The microelectrode arrays were first decorated by capture antibodies. Then, biotinylated secondary antibodies (Ab2) and biotinylated horseradish peroxidase (HRP) were chemically linked onto 300 nm diameter streptavidin-coated magnetic nanoparticles and applied to capture antigens in solution samples. Injection of a magnetic nanocomplex loaded with target antigens into the fluidic chambers enabled the online capture of nanocomposites on the microelectrode arrays (Figure 4b). Electrochemical analysis was finished by loading hydroquinone and hydrogen peroxide into the microchannels and recording the electrochemical signal changes. The microsystem was utilized to detect prostate specific antigen (PSA), prostate specific membrane antigen (PSMA), interleukin 6 (IL-6), and platelet factor-4 (PF-4) in serum with high sensitivity.[58] In addition, electrochemical microfluidic biosensors can be constructed based on different redox reactions and various microdevices for protein analysis.[59,60] For example, using poly(2,2′:5′,5″-terthiophene-3′-*p*-benzoic) acid and *N*,*S*-doped porous carbon nanocomposite as redox mediators and Nile Blue as a catalytic redox mediator, the detection of hemoglobin and glycated hemoglobin fractions was done on electrochemical channels with a conductive polymer composite sensor.[61] In an electrochemical sensing system, which integrated molecularly imprinted polymers and hybridization chain reaction on a microfluidic paper device, the sensitive detection of target glycoprotein ovalbumin was accomplished by using nanoceria as redox-active catalytic amplifiers when 1-naphthol was presented.[62] In an optically transparent patterned indium tin oxide (ITO) based microfluidic immunosensor, cysteamine capped AuNPs were covalently linked with specific antibody for targeting prostate-specific membrane antigen (PSMA). The sensitive detection of PSMA was based on measuring the changes in differential pulse voltammetry signal of a redox pair ($[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$) upon binding of PSMA on the PSMA antibody immobilized substrate.[63]

Label-free microfluidic electrochemical biosensors were also demonstrated by several research groups, of which the signals depend on the formation of immunocomplex while not external redox species. For instance, a microfluidic paper-based electrochemical immunosensor was fabricated to detect carcinoembryonic antigen (CEA). The working electrode were functionalized with amino functional graphene/thionine/AuNPs nanocomposites to immobilize anti-CEA antibody and improve sensitivity. The highly sensitive detection of CEA of clinical samples was realized by recording the decreased current of thionine which was proportional to the concentrations of analyte upon the formation of antibody−antigen immunocomplex.[64] Dong et al. reported on another label-free immunosensor for the detection of epidermal growth factor receptor 2 proteins on a microfluidic device. This biosensor was constructed based on porous hierarchical graphene foam (GF) decorated with electrospun carbon-doped titanium dioxide nanofibers ($nTiO_2$) as the working electrode. The integration of GF with functional $nTiO_2$ leaded to large surface area, high charge transfer resistance, and porous access to the sensing surface by the analyte, the antibody of ErbB2 (anti-ErbB2) was covalently immobilized on the GF-*n*TiO_2 composite (Figure 4c). Further applying differential pulse voltammetry and electrochemical impedance spectroscopy enables the quantification of breast cancer biomarkers. The two methods exhibited high sensitivities of 0.585 and 43.7 kΩ μM^{-1} cm^{-2} in a wide concentration range of target ErbB2 antigen. By applying various specific recognition element into the porous GF-*n*TiO_2 composite-based microfluidic electrical sensor, many promising applications of biospecies will be accomplished.[65] By integrating a thin layer of manganese-reduced graphene oxide (Mn_3O_4-RGO) nanocomposite on ITO substrate, the working electrode was constructed by functionalizing antibodies on the surface. The uniformly distributed nanocomposite provided large surface area for enhanced loading of antibodies and improved electrochemical reaction at the sensor surface. This microfluidic sensor exhibited excellent sensitivity for cardiac biomarkers.[66]

3.1.1f. Photoelectrochemical (PEC) Detection. A typical PEC biosensor is based on the photocurrent signal changes upon light excitation. PEC immunoassay exhibits high sensitivity merits owing to the separated different energy forms of the excitation source and detected signal, in which photoactive materials are critical element of the PEC biosensor. Recent years, semiconductor nanomaterials have been utilized as promising photoactive material because of their high photocurrent conversion efficiency. Yu et al. reported a PEC microfluidic paper-based immunosensor using CdTe quantum dots (QDs) sensitized 3D ZnO superstructures as photoactive elements. Multibranched hybridization chain reaction was applied to generate double strands DNA with multiple branched arms (mdsDNA) which could form PdAu-mdsDNA conjugate-labeled CEA antibody to bind CEA. Upon the oxidation of hydroquinone by H_2O_2, the concentrations of CEA were determined through the decrease in photocurrent intensity with high sensitivity and prominent specificity.[67] In another study, a magnetic controlled PEC biosensor was fabricated using reduced graphene oxide-functionalized $BiFeO_3$ (rGO-$BiFeO_3$) as the photoelectrode material and target-triggered hybridization chain reaction (HCR) for signal amplification to detect PSA cancer biomarker. The competitive combination of PSA and its aptamer could induce the release of trigger DNA (tDNA) to form long double-stranded DNA which could then integrate glucose oxidase (GOx) onto the surface of the magnetic bead. Therefore, catalytic product H_2O_2 served as the photoelectron acceptor and enhanced the cathodic photocurrent, which then be monitored and used for the quantitative detection of PSA.[68]

3.1.1g. Electrical-Based Detection. Microfluidic biosensors based on electrical signals including current, impedance, etc., offer facile tools for the label-free detection of biomolecules. For example, field-effect transistor (FET) based biosensors have been considered to be rapid, reliable, and sensitive sensing systems for proteins. In an integrated FET microdevice, specific aptamer probes were immobilized on FET sensor arrays for detection of biomarkers for cardiovascular diseases in clinical samples.[69] In an electrical impedance microfluidic sensing device, the detection of protein concentration, as well as protein activity was achieved. The microdevice had a two-chamber structure, with a capture/reaction chamber that had immobilized capture biomolecules for target analytes, and an electrical impedance sensor chamber containing a micropore structure embedded between two gold

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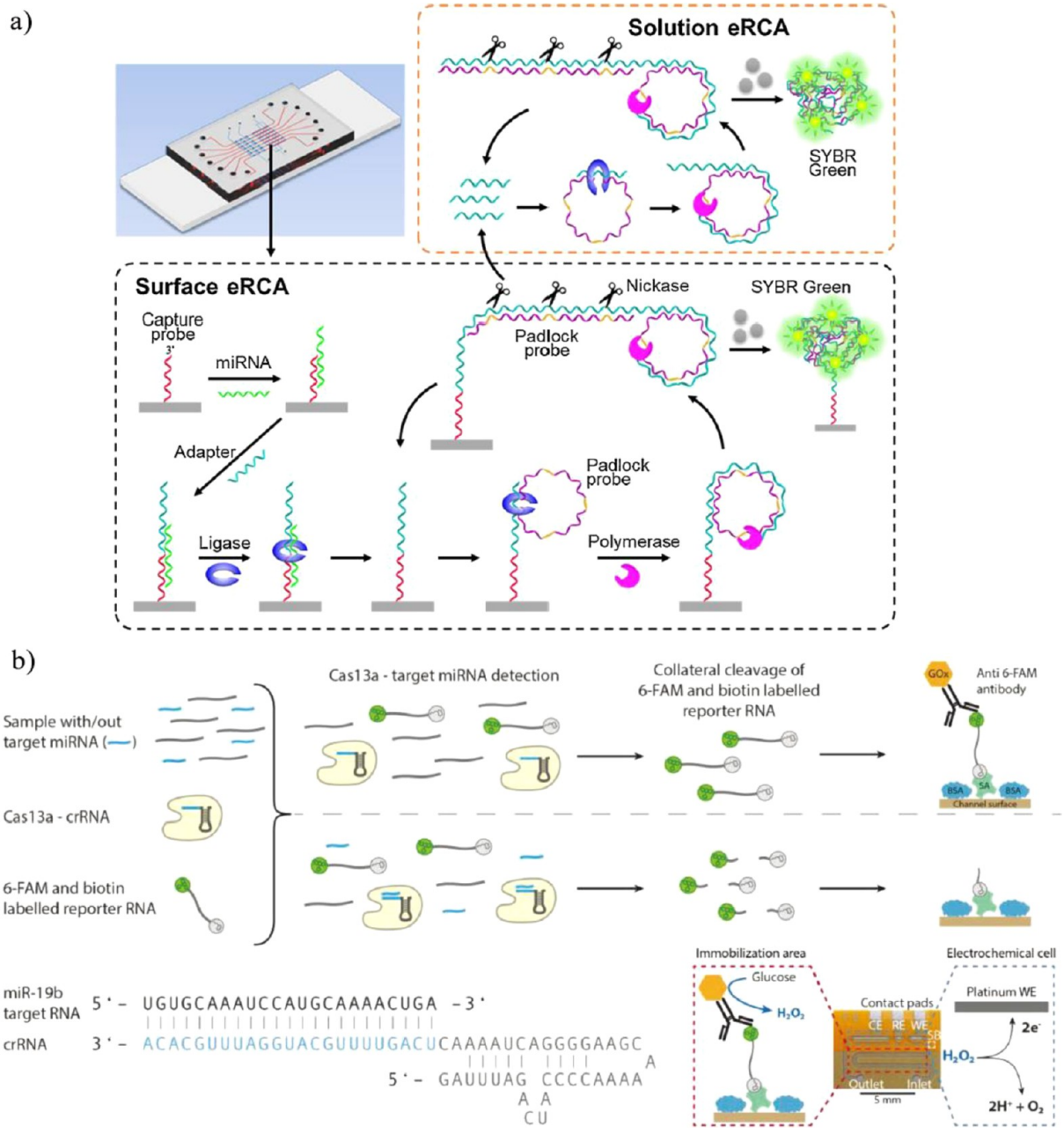

Figure 5. (a) Schematic illustration of the MERCA principle. The on-chip chamber is patterned with probes to capture target miRNAs. An adapter hybridizes with the miRNA and is ligated with the capture probe by T4 DNA ligase. (Reproduced with permission from ref 77. Copyright 2019 Elsevier B.V.) (b) Combination of the CRISPR technology along with an electrochemical microfluidic biosensor for miRNA diagnostics. (Reproduced with permission from ref 82. Copyright 2019 John Wiley and Sons)

electrodes. By recording the electrical impedance signal changes, the protein abundance of the human cytokine interleukin 6 (IL-6) and enzyme activity of the human tyrosine Abelson (Abl) kinase were determined, with sensitivity higher than conventional ELISA method.[70] In addition, by integrating a stretchable, label-free, impedimetric biosensor and a stretchable microfluidic device, a wearable lab-on-a-patch platform was developed for on-body detection of the hormone biomarker, cortisol from human sweat. The integrated microdevice could detect pM levels sweat cortisol quantitatively during exercise. The development of such wearable device offered new possibilities for noninvasive, highly sensitive, and quantitative on-body immunodetection for wearable personal diagnostics.[71] A 3D printed microfluidic device with ~50 μm cross-sectional dimensions on glass slide was fabricated to detect preterm birth biomarkers by the electrophoresis method. By supplying sample solutions carrying analytes, the electropherogram for micro-electrophoresis was measured, showing the separate peaks and different signals of various analytes.[72]

With the development of microfluidic biosensors, various advanced sensing systems have been developed, e.g. dual-modality sensors that integrated both electrochemical and SPR techniques[73] or applied differential pulse voltammetry and electrochemical impedance spectroscopy[65] and many others. These biosensors offer potential approaches for developing multifunctional sensing systems in real applications.

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3.1.2. Nucleic Acid Assay. Nucleic acids including DNA, noncoding small RNAs, such as microRNA (miRNA), etc., have vital physiological functions in the human body. Their dysregulation is closely related to various type of diseases, such as cancer, diabetes, and neurodegenerative diseases. Therefore, they have been considered as important biomarkers for diseases in clinical diagnostics. The vital process for effective treatment relies in accurate diagnosis at an early stage. However, nucleic acids have low abundance in complex biosamples and are highly heterogeneous in nature, which making their detection challenging. In this case, there is a pressing need for the development of sensitive biosensors for rapid and sensitive detection of miRNAs for early diagnostics including liquid biopsies of tumors. Current biosensors for miRNA suffer from laborious sample pretreatment processes and low efficient analysis, which largely restricts their clinical applications.[74] Over the last decades, there is an increasing demand for integrated microfluidic biosensing devices for point-of-care (POC) diagnostics.[20] Combined with different detection methods including optical approaches and electrochemical and electrical based measurement, a variety of microfluidic biosensors for nucleic acids have been developed.

3.1.2a. Fluorescence Detection. Take α-synuclein oligomers as an example; they are toxic to cells and may cause cell death in Parkinson's disease, but have a low concentration on the order of picomolar when generated during fibril formation. Therefore, a fast-flow microfluidics system was integrated with a single-molecule fluorescence technique and applied to monitoring the formation of α-synuclein oligomers and their characterization.[75] Ai et al. reported on a microfluidic biosensor for sensitive fluorescent detection of DNA. Making use of their specific characteristics, single-layered MoS_2 nanosheets were integrated into a PDMS microfluidic device to absorb single strand DNA (ssDNA) other than double strand DNA (dsDNA). Since MoS_2 nanosheets can quench most of the fluorescence in several minutes, the proposed microfluidic system enabled rapid recognition of approximately femtomole levels of ssDNA with high sensitivity and selectivity.[76] Additionally, an RCA strategy was also utilized to detect nucleic acid in microfluidic devices. Zeng et al. constructed an integrated microfluidic exponential rolling circle amplification (MERCA) platform to detect low abundance microRNA in biological samples. The microdevice is composed of programmable microvalves and pumps for precise flow controlling and an assay chamber. Capture probes were first modified in the microchambers. After supplying target miRNA, an adapter and ligase were utilized in sequence. For the analysis of miRNA, a surface enzyme-based exponential RCA (eRCA) method was applied to trigger the solution phase eRNA (Figure 5a), from which the double-stranded amplification product was detected by SYBR Green dye added to the reaction. The proposed system enabled miRNA isolation, fluorescent labeling, and quantification in one workflow without sample pretreatment and further analysis by conventional methods. Using the developed method, the quantitative detection of miRNAs in total RNA, raw cell lysate, and cell-derived exosomes was achieved, with a remarkably low LOD at <10 zeptomole levels, which proved the excellent performance of the developed platform in miRNA analysis in various biological and clinical applications.[77]

3.1.2b. SPR Detection. Bonyár et al. reported on the robust and scalable fabrication of ordered AuNP arrangements on epoxy substrates for label-free plasmonic detection of DNA. The AuNP/polymer nanocomposite showed uniform morphology and superior LSPR characteristics. The label-free DNA detection was proved with this sensor using a parasite derived 20 base pair long specific DNA sequence. After hybridization with 1 μM target DNA, a red shift of 6.6 nm in LSPR absorbance spectrum was detected, which achieved a low LOD of DNA to around 5 nM.[78]

3.1.2c. ECL Detection. Wheeler et al. demonstrated the first integration of ECL with digital microfluidics and applied this platform to the analysis of miRNA. Taking advantage of the droplet manipulation of digital microfluidics and sensitive detection of ECL, the integrated system was applied to an oligonucleotide hybridization assay. It was shown that the proposed approach could selectively detect single nucleotide mismatches with a LOD of 1.5 femtomoles. Utilizing this system to detect miRNA-143 from cancer cell lysates, the discrimination between two different cancer cell lines was accomplished, indicating the potential application of the integrated system in real applications.[79]

3.1.2d. Electrochemical Detection. Recently, an electrochemical biosensor based on Au NR modified μPADs were fabricated by Yu et al. for the sensitive detection of miRNA. The microdevice used in situ grown AuNRs as WE to functionalize the hairpin probe on the μPAD surface and cerium dioxide-Au@glucose oxidase (CeO_2-Au@GOx) for electrochemical signal amplification. GOx could catalyze the oxidation of glucose to gluconic acid, leading to the generation of H_2O_2 and detectable electrochemical signals upon binding of target miRNA. Further by adding 3,3′,5,5′-tetramethylbenzidine substrate and exonuclease I enzyme into the system, the oxidation of substrate by H_2O_2 could be simultaneously observed, indicating an indirect visual detection of miRNA. When applied to the assay of miR-21, both methods provided wide linear concentration ranges and a low LOD at femtomolar levels.[80] In another study, different on-chip miRNA bioassays based on sandwich and competitive formats were applied for the electrochemical measurement of miRNA-197 in undiluted human serum samples. The detection strategy is based on the cross-hybridization of target miRNAs with a complementary single-stranded DNA (ssDNA) or an RNA sequence as a capture probe. In a sandwich miRNA assay, a "capture probe/secondary detection probe/target miRNA" structure was used. By using a fluorescein labeled detection probe and a glucose oxidase-antifluorescein antibody complex, redox reaction between glucose and H_2O_2 was triggered, generating detectable electrochemical signals. Therefore, in a sandwich assay, the detected signal is proportional to the concentration of miRNA-197. However, in a competitive miRNA assay, a fluorescein labeled competitor miRNA was used. The generated signal is inversely proportional to the concentration of miRNA-197. Both techniques presented high sensitivity down to nanomolar levels, while the sandwich assay was even better than the competitive format. The proposed method offered potential tools for developing electrochemical microfluidic biosensors for rapid and sensitive clinical miRNA detection.[81] Recently, Urban et al. reported the first clustered regularly interspaced short palindromic repeats (CRISPR)/Cas13a-powered microfluidic biosensor for the detection of microRNAs, based on an electrochemical method. Using this device, the tumor biomarkers (miR-19b and miR-20a) were detected rapidly (readout time of 9 min and an overall process time of less than 4 h) and sensitively (LOD 10 pM) in a nucleic acid amplification-free manner. (Figure 5b) Real

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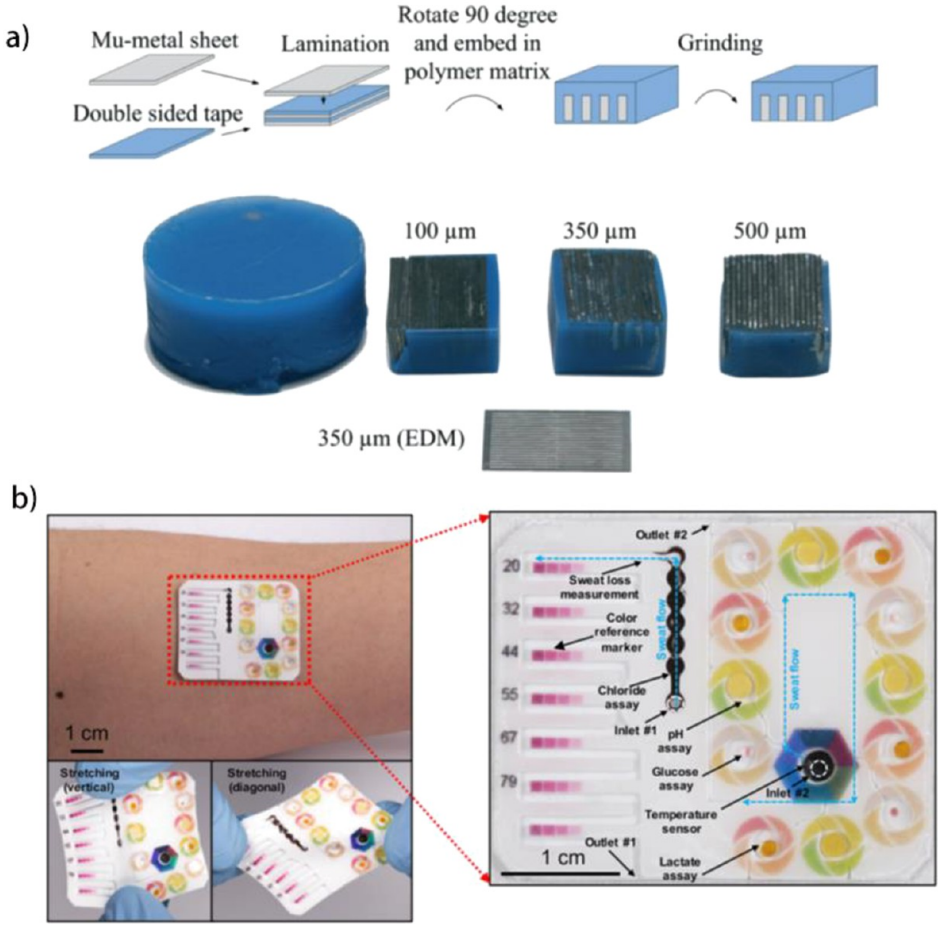

Figure 6. (a) Soft-magnetic lattices used for the manipulation of magnetic microparticles. (Reproduced with permission from ref 90. Copyright 2015 Royal Society of Chemistry) (b) Optical images of soft, flexible microfluidic devices for colorimetric analysis of sweat on the skin where microfluidic channels filled with blue-dyed water (top) and under mechanical deformation with bending (bottom left) and twisting (bottom right). (Reproduced with permission from ref 113. Copyright 2019 American Chemical Society)

application in detecting miR-19b in serum samples of children with brain cancer further proved the feasibility in applying the proposed method in clinical nucleic acid based diagnostics.[82]

3.1.2e. Electrical-Based Detection. FET-based biosensors offer label-free tools for detection of nucleic acid by measuring their intrinsic charges. Bashir et al. fabricated FETs with a deformed monolayer graphene channel, which could generate "electrical hot spots" in the sensing channel and a band gap to enhance changes in the source−drain current. Applications in buffer and human serum samples revealed the high sensitivity of the microdevice toward nucleic acids of 600 zM and 20 aM, respectively.[83] In addition, a microfluidic device with integrated carboxyl-modified multiwalled carbon nanotubes (MWCNT) on patterned ITO glass was fabricated to serve as an impedimetric biosensor for DNA. By modifying DNA probe specific to chronic myelogenous leukemia, the complementary target DNA concentration was detected by recording the decrease in electrical conductivity of the interface rapidly and sensitively.[84]

3.2. Small Biomolecule Analysis. *3.2.1. Glucose.* Diabetes is a worldwide public health problem and causes increasing amount of disability and death every year.[85] Therefore, simple, sensitive, and cost-effective devices for glucose monitoring from body fluid, e.g. blood, is highly desired. In the past decades, various microdevices have been reported for the sensing of glucose, mostly relying on electrochemical detection mechanism,[86] with some studies using other techniques, e.g. optical biosensors measuring transmitted spectrum changes,[87] colorimetric sensors,[88] etc. A research focus in this field is improving the sensing performance and cost of glucose sensing devices. In this case, a variety of promising materials have been applied in construction of microfluidic electrochemical sensors for glucose. For instance, Amornkitbamrung et al. fabricated a PDMS microfluidic electrochemical platform using diamond-like carbon (DLC) thin films as working electrode for the detection of glucose. DLC is regarded as excellent electrode material owing to its electrochemical characteristics such as corrosion resistance and low background current and the inexpensive properties for fabricating microdevices. By immobilizing GOx at the DLC surface, and amperometric flow-injection of glucose, the in-channel glucose sensing was accomplished by monitoring the changes in current response.[89] In another study, self-assembled magnetic bead chains was used in microfluidic platforms to enhance sensitivity in electrochemical detection (Figure 6a). A magnetic field gradient along the microchannel was fabricated by a soft magnetic lattice with a 350 μm interval. The alternating magnetic field lead to the self-assembly of the magnetic beads into chains and thus improved the contact between magnetic beads and the analyte, with more than 5-fold enhanced sensitivity. A proof-of-concept study was achieved by labeling

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GOx in the channel and achieved the rapid and sensitive electrochemical detection of enzymatically generated H_2O_2.[90] Metal−organic frameworks (MOFs) have also been utilized to form enzyme−MOF composites to increase enzyme stability with retained activity. Therefore, surface patterning and integration of a biomineralized enzyme into microfluidic devices can be promising in developing diagnostic devices. Chen et al. fabricated a microdevice using a mussel-inspired polydopamine/polyethylenimine coating to pattern GOx, HRP, and zeolitic imidazole framework-8 (ZIF-8) thin films on a flexible polymeric substrate in microchannels. The device exhibited high selectivity and sensitivity toward glucose. This microdevice showed the potential in developing MOF-based biosensors for bioanalytes.[91] Paper-based analytic devices (μPADs) have also been developed for the sensitive detection of glucose. A hybrid nanocomplex composed of dual enzymes GOx, HRP, and $Cu_3(PO_4)_2$ inorganic nanocrystals in the detection zones retains the activity of the enzymes and helps to transfer analytes between the enzymes. Therefore, the on-chip analysis of glucose was achieved with a high sensitivity.[92]

Despite the glucose in blood samples, glucose from tears, sweat, or saliva can also be detected as important alternatives for the indirect, and less invasive, monitoring of blood glucose levels.[88,93,94] For instance, a tear glucose analysis biosensor was fabricated by integrating a microfluidic thread-based electroanalytical device with an amperometric biosensor composed of poly(toluidine blue O) and GOx. By passive pumping driven microflow injection, the analysis of tear glucose was achieved for nondiabetic human tear samples, which showed a high correlation with blood glucose levels.[93] Additionally, sweat glucose could also be detected by using a microfluidic chip-based wearable colorimetric sensor. The microdevice was composed of several layers for efficient sweat collection and detection. There are five detection microchambers connected by microchannels to one center in the sensing layer. Pre-embedded GOx-*o*-dianisidine reagents in the detection zone were utilized for sensing the glucose in sweat, benefiting from the color change induced by the enzymatic oxidation of *o*-dianisidine. This sensor could facilitate parallel detection simultaneously with high sensitivity, indicating its potential application in rapid sweat glucose sensing.[88]

In recent years, the development of microdevices capable of multiplexed analyses of bioanalytes have aroused high research interest due to their capability in obtaining information regarding several biomolecules. In particular, the detection of glucose and glucose related biomolecules, e.g. lactate which is generated from glucose through anaerobic respiration during intensive exercise,[95] could provide precise monitoring of tissue physiology and pathology.[94,96] For instance, Niu et al. demonstrated a wearable droplet microfluidic device for continuous real-time sampling and detection of glucose and lactate levels in human tissue. The microdevice used an antiphase peristaltic pumping method to facilitate the robust generation of nanoliter-sized droplets. A single-step mix-and-read assay was applied for the measurement of glucose, based on a colorimetric analysis of the GOx-HRP enzymatic reaction. A two-step assay was implemented for detecting lactate, based on the colorimetric analysis of the lactate oxidase−HRP reaction. The developed small, wearable microfluidic biosensor enabled tracking of perturbed glucose and lactate levels in dermal tissue, which showed consistency with changes in peripheral blood levels.[97] In another study, a double-layered 3D microfluidic paper-based analytical device (μPAD) based on CL detection was constructed for the multianalyte sensing of glucose, lactate, cholesterol, and choline. The microdevice had 3D branched microchannel design, which enabled transporting of CL reagent luminol among the detection zones. By modifying the detection chambers with CL catalyst cobalt ion and different oxidases toward the analytes, the signal changes out of luminol and H_2O_2 after enzymatic reactions were measured. The recorded four CL emission peaks in the CL kinetic curve reflected the proportional changes in the concentration of the four analytes, indicating the capability of using the developed microdevice for sensitive multiplexed analysis of glucose and other analytes.[98]

3.2.2. Neurotransmitter. Neurotransmitters including dopamine, norepinephrine, epinephrine, etc., are bioamines that play critical roles in the human nervous system. The imbalance of their concentration can directly lead to neurological disorders, resulting in neurological diseases such as Alzheimer's diseases, Parkinson's disease, etc.[99] Therefore, it is highly desired to develop sensitive detection methods for these catecholamines for the diagnosis of neurological diseases. Dittrich et al. reported a label-free biosensor using on-chip synthesized and functionalized TTF-Au microwires for sensing catecholamines. On a double-layered microchip, the TTF-Au microwires were formed in situ by supplying reaction solutions into the microchannel. Upon further surface modification via Au−S bonds, the microwires were used detect catecholamines such as dopamine, relying on the Raman signal changes of analytes.[100] In addition, an electrochemical microfluidic separation and sensing (EMSS) platform was prepared by Shim et al. for detecting neurotransmitters in human plasma samples. The electrochemical microdevice consisted of an electrochemical microchannel and an amperometric sensor, which could enable separation and detection of neurotransmitters. The sensing probe was constructed by electropolymerization of 2,2′:5′,5″-terthiophene-3′-*p*-benzoic acid with N,S-doped porous carbon to generate a composite layer on carbon electrode, followed by covalently binding of a redox mediator. The developed microfluidic system was successfully applied for the separation detection of various neurotransmitters in clinical human plasma samples with high sensitivity.[101]

3.2.3. Other Small Biomolecules. Despite the above-mentioned biomarkers and important biomolecules, there remain a lot of biorelated analytes that are related to important physiological and pathological processes, e.g. ascorbic acid,[102] human chorionic gonadotropin,[103] nerve agents,[104] cholesterol,[105] etc. Therefore, various sensing techniques have been reported for these types of biomolecules.[34] Herein, representative studies are exemplified. For instance, human chorionic gonadotropin (HCG) is an important biomarker existing in the blood and urine of pregnant women. Recent studies have revealed that elevated levels of HCG have been detected in body fluid of various cancer patients including prostate cancer, choriocarcinoma, etc.[106] Therefore, developing sensitive techniques to detect the level of HCG is of great importance. Chen et al. fabricated a paper-based microfluidic device (111 PADs) for the detection of HCG based on electrochemical immunofiltration analysis. The microdevice consisted of aldehyde-functionalized screen-printed electrodes (SPEs) which were modified with capture antibodies. By applying primary signal antibody decorated AuNPs (GNPs/Ab2) and alkaline phosphatase bound secondary antibody (ALP-IgG) to the system, an immune-based detection was accomplished,

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with electrochemical readout. Using the developed PADs, sensitive analysis of HCG in human serum samples was achieved, indicating the potential of developing point-of-care testing (POCT) devices for healthcare monitoring.[103] A PDMS-based microfluidic electrochemical biosensor was reported for detection of cholesterol which is a vital constituent for normal physiological process and is related to disorders of various diseases. The biosensing chip utilized a two-electrode system, with a platinum as counter electrode and the biocompatible material NiO as a working electrode. By immobilizing cholesterol oxidase enzyme onto the NiO thin film, the specific detection of cholesterol was achieved, which was reflected in the changes of electrochemical signals.[105]

A variety of biomolecules were secreted by the human body and could be directly detected from various body fluids including blood,[107] sweat,[108] tears,[109] and urea.[110] Typically, wearable sensors were widely applied to sensing sweat metabolite and healthcare monitoring.[111] The real-time detection of sweating rate, the total loss of sweat, and the concentration of metabolites in sweat provide useful information on human physiology. However, conventional approaches mainly rely on manual collection procedures and large instruments to analyze the chemical composition, which is laborious and complicated. To address the need for portable devices and rapid sensing, soft, skin-integrated microfluidic systems for sweat analysis have been developed.[112] Rogers et al. reported such a microdevice for the collection and colorimetric detection of sweat. By integrating capillary bursting valves into the device, the flow of sweat to individual microreservoirs was possible for separate assays in a single device (Figure 6b). A colorimetric sensing zone with multiple color reference markers was printed directly on the surfaces of the microplatform for quantitative analysis. With this microfluidic device, rich information on the sweat rate, total loss of sweat, the pH value, and temperature, as well as the chemical composition, was monitored and recorded in real-time.[113] Another flexible microfluidic sweat sensing patch which contains a spiral-patterned component that is integrated with ionselective sensors and an electrical impedance-based sweat rate sensor was utilized to measure the sweat rate and secretion, e.g. ion concentrations.[114] In a recent study, a stretchable impedimetric biosensor was combined with a microfluidic device for the detection of hormone biomarker cortisol in sweat. The microdevice used 3D Au as a working electrode, with an immobilized capture antibody for immunodetection, which enabled picomolar levels of sensing of sweat cortisol. The integrated lab-on-a-patch microfluidic device provided a noninvasive, highly sensitive detection tool for wearable personal diagnostics.[71] With the increasing demand in detecting multianalytes for accurate monitoring of human health, microfluidic-based biosensors which enabled simultaneous detection of several biomolecules have been intensively studied.[115]

3.3. Cell Analysis. Cells, the fundamental units of organisms, play vital roles in building complex living systems. The changes within a cell can transmit to larger systems of cells and promote changes in organs or human body. Therefore, it is of critical importance to understand the cell components and functions via cellular analyses. Microfluidic systems provide an ideal system to study cells, in particular, at single cell levels. Such systems can not only achieve the in situ culturing and capture of cells but also enable the analysis of intact cells, secreted molecules, or lysates of cells, by integrating various functional components.[116] In this section, we will discuss the recent advances on the qualitative or quantitative chemical detection of intact multicells, cell secretions, or lysates, with a particular focus on single cell analysis, based on various techniques including fluorescence,[117−120] electrochemistry, or electrical methods, etc.

3.3.1. Analysis of Intact Cells. Microfluidics systems can enable various measurements of cells and quantitation of intracellular species, owing to the flexibility of such platforms and the ability for both cells and fluid running through the same channels. The characterization of intact cells including their viability, growth rate, etc., can be accomplished on microdevices under culturing or no culturing conditions. For instance, Song et al. developed a microfluidic paper-based biosensor for monitoring of cancer cells. The microdevice integrated graphene oxide (GO) and quantum dots (QDs) as fluorescence quencher and donor, with an aptamer as the molecular recognition element. After adsorbing the aptamers that were labeled with quantum dots coated mesoporous silica nanoparticles on the surface of GO, fluorescence was quenched due to FRET effect. Upon the presence of target cells, the quenched fluorescence was recovered. By employing different colored QDs and recognition elements, three type of cancer cells were simultaneously determined under a single excitation light with high sensitivity.[121] Furthermore, in order to measure the viability, necrosis and apoptosis of cells, a microfluidic integrated impedance cytometer was fabricated for the label-free analysis of cells. Cell sample mixtures after treatment with heat-shock or etoposide to induce necrosis or apoptosis were injected into microchannels and monitored by impedance cytometry. The recorded electrical signals enabled cell discrimination at high throughput (around 200 cell/s), which demonstrated the potential in using microfluidic impedance spectroscopy for cell viability assays.[122] A microfluidics-based hairpin resonator biosensor was developed by Jang et al. for the detection of biological cells in culturing medium. The microsystem composed a microfluidic device and a high-Q dielectric resonator as sensing device. By measuring the scattering parameter (S1 parameter) signals at a resonant frequency of 2.17 GHz, the quantification of cells was accomplished according to the determined linear relationship between S1 parameters and number of cells.[123] In addition to electrical signals, a plasmo-nanomechanical biosensor was reported for monitoring cell dynamic biological processes, e.g. cellular adhesion. The microfluidic device consisted of a hexagonal array of flexible rigid/soft polymeric nanopillars capped with plasmonic gold nanodisks. The polymer nanostructures could mimic soft and rigid tissues to sense the cellular behavior at nanoscale dimension. By measuring the LSPR transmission spectra, the adhesion forces of cells in microchannels were calculated, resulting in studying of the living cell attachment behavior in the developed microfluidic sensing device.[124]

3.3.2. Analysis of Cell Secretion and Lysates. 3.3.2a. Optical Method-Based Sensing. Cells communicate and transmit information between each other through various inter- and intracellular signaling mechanisms, including secretion of functional biomolecules such as proteins and nucleic acid. Therefore, the detection of cell secretion, metabolite or intracellular contents will help to understand cell function and survival mechanisms. Garcia-Cordero et al. reported recently an integrated microfluidic platform for functional secretory immunophenotyping of immune cells. The micro-

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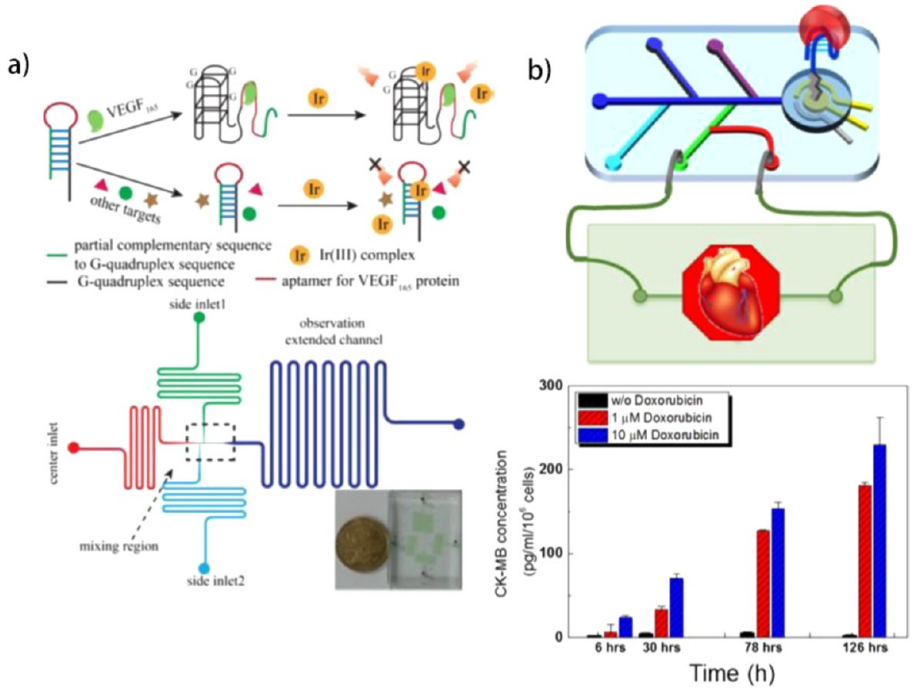

Figure 7. (a) G-quadruplex probe 1 for VEGF165 assay in the microchip. (Reproduced with permission from ref 126. Copyright 2016 Elsevier B.V.) (b) Schematic illustration of microfluidic aptamer-based electrochemical biosensing platform for monitoring damage to cardiac organoids and CK-MB concentrations versus time obtained using the calibration curve. (Reproduced with permission from ref 130. Copyright 2016 American Chemical Society)

device contained cell capture component which had 32 cell culture chambers, each patterned with an array of 492 microwells. The microchannels were functionalized with capture antibodies to capture immune cells and enabled the detection of cells based on fluorescence sandwich immunoassay. A proof-of-concept study was done by measuring the secretion of IL-8 and TNF-α from blood-derived neutrophils and monocytes upon stimulation. The developed microdevice would be useful in diagnosis of diseases with cytokine secretion as biomarkers.[125] Lin et al. reported on the design of a luminescent switch-on probe integrated microfluidic platform for the measurement of cell metabolite and analysis of protein−DNA interaction (Figure 7a). Vascular endothelial growth factor (VEGF165) is a kind of overexpressed protein in tumor cells. Herein, the cell metabolite VEGF165 was measured using a G-quadruplex-selective luminescent iridium-(III) complex and a functional nucleic acid hairpin structure which contained VEGF165 aptamer, G-quadruplex sequence, and its complementary sequence. Upon the presence of VEGF165, the hairpin structure was destroyed, resulting in highly luminescent responses. Additionally, the protein−DNA interaction was also monitored by the changes in luminescence signals.[126] Altug et al. fabricated another microfluidic device for monitoring cell-released VEGF based on nanoplasmonic biosensing. The microdevice consisted of Au nanohole arrays that supported extraordinary optical transmission, which could induce sensitive changes in SPR signals after immunobinding of biomolecules. Additionally, the adjustable microfluidic cell components allowed the analysis of living cells in a controlled culturing medium. Therefore, VEGF secreted from living cancer cells was monitored using the nanoplasmonic microfluidic biosensor, in a label-free manner.[127] Liu et al. reported the use of biomimetic peroxidase mimics instead of natural enzymes in redox reactions and achieved the detection of cell secretions in a microfluidic paper device. In their work, water-soluble molybdenum oxide quantum dots (MoOx QDs) were applied as a biomimetic catalyst due to its peroxidase-like activity. Therefore, the rapid and quantitative detection of H_2O_2 secreted from PC12 cells was achieved based on colorimetric assay.[128]

3.3.2b. Electrical Method-Based Sensing. Transforming growth factor (TGF)-β is a critical fibrogenic molecule secreted by liver cells during liver injury. A high level of TGF-β1 during liver injury is closely related to the activation of stellate cells and epithelial-to-mesenchymal transition of hepatocytes. However, it is challenging to study the intercellular communication between these two types of liver cells. In order to study the influence of alcohol injury on TGF-β signaling between these two liver cell types, a microfluidic electrochemical biosensor was developed for monitoring of cell secretion. The microfluidic platform consisted of five microchambers, two for coculturing hepatocytes with stellate cells, and three with aptamer-modified microelectrodes for monitoring cell-secreted TGF-β. The actuatable design of the walls separating microchambers was an important feature, which could enable the diffusing of cell secretions between different chambers. After functionalizing the microelectrodes with TGF-β aptamer, the TGF-β secreted by liver cells was bound to the microelectrodes and induced changes in electrochemical signals. Using this reconfigurable microdevice integrated with miniature biosensors, alcohol injury induced secretion of TGF-β molecules by hepatocytes was monitored, as well as the additional TGF-β secretion from neighboring stellate cells triggered by the diffused TGF-β between microchambers. Therefore, not only the cell secretion but also the communication between two cell types via the same signaling molecule could be detected, using the proposed microfluidic biosensor.[129] Khademhosseini et al. developed an aptamer-

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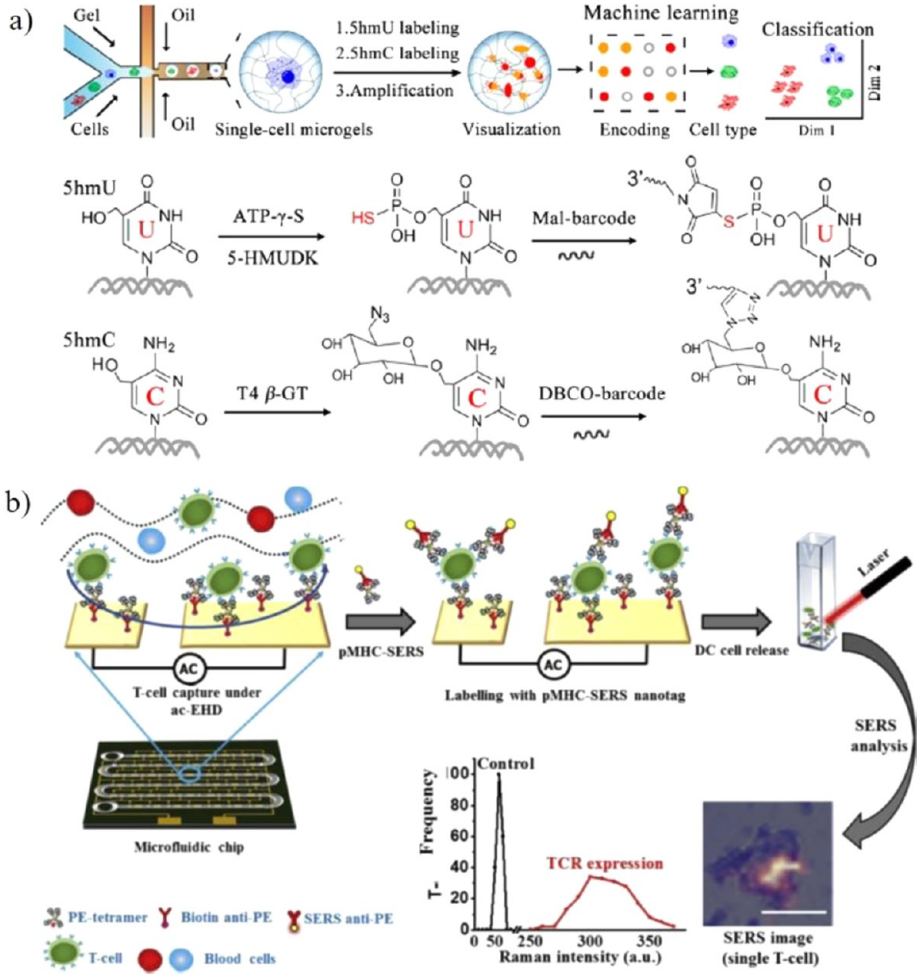

Figure 8. (a) Design and workflow of sc5hmU/5hmC-microgel visualizing single-cell 5hmU and 5hmC. (Reproduced with permission from ref 145. Copyright 2020 American Chemical Society) (b) Schematic representation of isolation and analysis of T-cells using pMHC-TCR interaction enabled by electric field induced T-cell capture, cell release under DC pulse, and TCR expression analysis using designated SERS-nanotags. (Reproduced with permission from ref 149. Copyright 2019 Elsevier B.V.)

based microfluidic electrochemical biosensor to monitor the cell secreted cardiac biomarkers e.g. creatine kinase (CK)-MB (Figure 7b). Herein, the biosensor was equipped with microelectrodes that were functionalized with aptamers specific to CK-MB for electrochemical sensing. By using a microfluidic heart-on-a-chip system built from human embryonic stem cell-derived cardiomyocytes, followed by drug treatment, the slight changes of CK-MB levels secreted by the cardiac organoids were measured in a drug dose-dependent manner.[130] The same group further reported a label-free and regenerative microfluidic electrochemical biosensing device for continual monitoring of cell-secreted soluble biomarkers in a culture medium. On a liver-on-a-chip system, liver cell released biomarkers including human albumin and glutathione-S-transferase-alpha (GST-α) were monitored upon stimulation of liver toxic drug.[131] The above organ-on-a-chip systems could enable label-free electrochemical detection of cell secretions under culturing conditions, with sensitivity comparable to conventional ELISA method.

In another study, a dual-electrode microfluidic biosensor was fabricated to detect acetylcholine (ACh), a classical neurotransmitter released by leukemic T-cells. The screen printed carbon electrodes were coated with AuNPs and a porous gold layer, respectively, followed by electropolymerization of 2,2:5,2-terthiophene-3-(*p*-benzoic acid) (pTTBA). Afterward, the reaction electrode was modified with acetylcholinesterase, while the detection electrode was functionalized with hydrazine and choline oxidase. The H_2O_2 generated after the enzymatic reactions was then reduced by hydrazine to produce detectable amperometric signals. Real application was done by measuring acetylcholine in human plasma samples. Additionally, the authors successfully used the microfluidic sensor to analyze Ca^{2+}-induced acetylcholine released by leukemic T-cells.[132]

3.3.3. Single Cell Analysis. Deep insights into the dynamic behavior of cells and the mechanism of intercellular communication at single cell level is of vital importance to understand important physiological and pathological processes of multicellular organisms.[133,134] In particular, understanding the cellular and functional heterogeneity of tumors at the single-cell perspective will contribute to cancer diagnostics and therapeutics. Therefore, highly efficient cell trapping and analytical approaches are urgently desired. In recent years, the microfluidics technology has emerged to be a promising tool for single cell analysis owing to its advantages in capturing individual cells to facilitate analysis of cells or cell secretions in a facile and high-throughput manner.[135,136]

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A lot of studies reported the detection of single cells in different aspects, e.g. cell secretion,[137] heat emission,[138] cell growth rate,[139] etc., based on microfluidic chips that were integrated with various functional components. Since the quantification of single cell secreted proteins, functional molecules, etc., is of great importance in studying cell heterogeneity, especially in cancer research, herein, the representative research works regarding the analysis of single cell secretion using fluorescence[118,140] and SERS-based approaches were exemplified.

3.3.3a. Fluorescence-Based Sensing. Chen et al. reported on a microfluidic platform for quantification of single tumor cell secreted proteins based on fluorescence immunoassay. The microchip design contained constricted microchannels that could enable the characterization of tumor cells and clinical samples. By modifying fluorescence-labeled antibodies on the microchannels, the cell released target proteins were bound and detected. Proof-of-principle experiment was done for the quantification of multiple intracellular proteins (e.g., β-actin, α-tubulin, and β-tubulin or β-actin, biotin, and RhoA) from different types of tumor cells, with effective cell-type classifications.[137] Barber et al. demonstrated the long-term single cell encapsulation and real-time measurement of cell secretion on a capillary force-driven microfluidic device. About 500 single cells could be captured inside the nanoliter microchannel and stayed around 16 h of culturing for analysis. Here, a FRET biosensor was applied with fluorescent donor and acceptor linked by a matrix metalloproteinases (MMPs)-active peptide linker. Upon the presence of single cancer cell released MMP, the FRET sensor was cleaved, resulting in fluorescence recovery. Therefore, the protease activity of single cancer cell was monitored.[141] In order to study the efficacy of anticancer therapeutics, a microchamber-based microfluidic device was reported by Dittrich e al for the capture, monitoring, and analysis of single tumor cells. By isolating single human breast adenocarcinoma cells in microchambers with ultrasmall volume (less than 100 pL) followed by incubation with anticancer drugs, the released calcein from individual cells after the loss of membrane integrity were monitored and analyzed by fluorescence response. The microplatform is applicable to the analysis of different drug responses of various cell types, enabling the study of heterogeneous drug responses of individual tumor cells.[142]

Droplet microfluidics provided a powerful platform for single cell encapsulation and analysis.[143] In order to detect the cell released H_2O_2, a microdroplet device integrated with Au nanocluster structure was developed. Single cells were first encapsulated in the microdroplets. Afterward, the living cell secreted H_2O_2 would induce redox reaction of Au−S bond between horseradish peroxidase and Au, resulting in fluorescence changes in response to H_2O_2 concentration. With the proposed method, ultrahigh sensitivity for detection of H_2O_2 (200−400 attomole) was achieved at the single-cell level.[144] In another study, single cells' genomic DNA, 5-hydroxymethyluracil (5hmU), and 5-hydroxymethylcytosine (5hmC) were monitored in single cells encapsulated in microfluidic hydrogel droplets (Figure 8a). First, single cells were encapsulated in individual picoliter droplets. After emulsion break and cell lysis, the genomic DNA were retained in the microgels for further analysis. Following labeling of 5hmU and 5hmC with thiophosphate and azide glucose separately, amplification and hybridization of fluorescent probes, the visualized analyses of the two genomic DNAs were achieved, with the different molecular signatures associated with fluorescence intensity. In this case, the single-cell heterogeneity was determined. Based on machine learning algorithms to decode the four-dimensional signatures of 5hmU/5hmC, three types of cells including nontumorigenic, carcinoma, and highly invasive breast cell lines were successfully discriminated.[145]

3.3.3b. Raman/SERS-Based Sensing. Raman-activated cell sorting is an attractive strategy for single cell analysis due to its label-free detection, noninvasiveness, and high sensitivity. Ma et al. developed a microfluidic-based Raman-activated droplet sorting (RADS) system for screening of live single microalgal cells. *Haematococcus pluvialis* cells bearing different levels of antioxidant astaxanthin were supplied into the microfluidic system and underwent Raman analysis of single cells, prior to encapsulating into microdroplets. After the dielectrophoresis procedure in the following channel, the astaxanthin hyper-producing cells were sorted and collected, with high accuracy of 98.3% and high throughput of ∼260 cells/min. In addition, the sorted cells retained a high viability for downstream detection.[146]

SERS-based sensing offered a promising approach for single cell analysis owing to the enhancement of Raman signal.[147] Moskovits developed a SERS-based microfluidic platform for detecting individual mammalian cells in a continuous flow inside microchannel. Normal and cancerous cells were labeled with SERS tags that were composed of Ag NPs, Raman reporter molecules, and cell-specific affinity biomolecules. Labeled cells were supplied into the microfluidic device under a continuous flow regime. Individual cells were measured when passing through the laser focus beam, with cancer cells displaying specific recognizable Raman signatures. Combined with data analysis such as principal component analysis and classical least-squares, the reliable single cell identification was achieved.[148] In order to analyze T cells, a microfluidic-based cell isolation device integrated with SERS-based analysis approach was reported by Trau et al. In this work, an alternating current electrohydrodynamic method was employed in a microfluidic chip for isolating antigen specific T cells from complicated biosamples using T-cell receptor (TCR) and peptide major histocompatibility complex (pMHC) interaction (Figure 8b). After labeling with SERS nanotag-linked pMHC, the T cells were released and applied for T-cell receptor expression analysis in bulk and single cells. The TCR results clearly revealed the heterogeneity in the T cells, which could reflect the activation of these cells, as well as a patient's response toward the immunotherapy process in potential clinical application.[149]

3.3.4. Analysis of Circulating Tumor Cells. Circulating tumor cells (CTCs) have been demonstrated to be an important biomarker for various kinds of cancers.[150] Since the concentration of CTCs is extremely low in human blood (only 1 cell per 10^9),[151] the efficient isolation and precise analysis of CTCs is still challenging. Microfluidic-based systems have been regarded as a promising platform for isolating and analyzing CTCs, even in single cell level.[152,153] With the development of nanotechnology, various microfluidic devices integrated with nanomaterials have been developed for the isolation and detection of CTCs.[154] For instance, to capture and release CTCs with high efficiency and viability, Yu et al. reported a platform utilizing polymer grafted silicon nanowires. The polymer brushes were functionalized to capture CTCs by the anti-EpCAM. Combined ligand−

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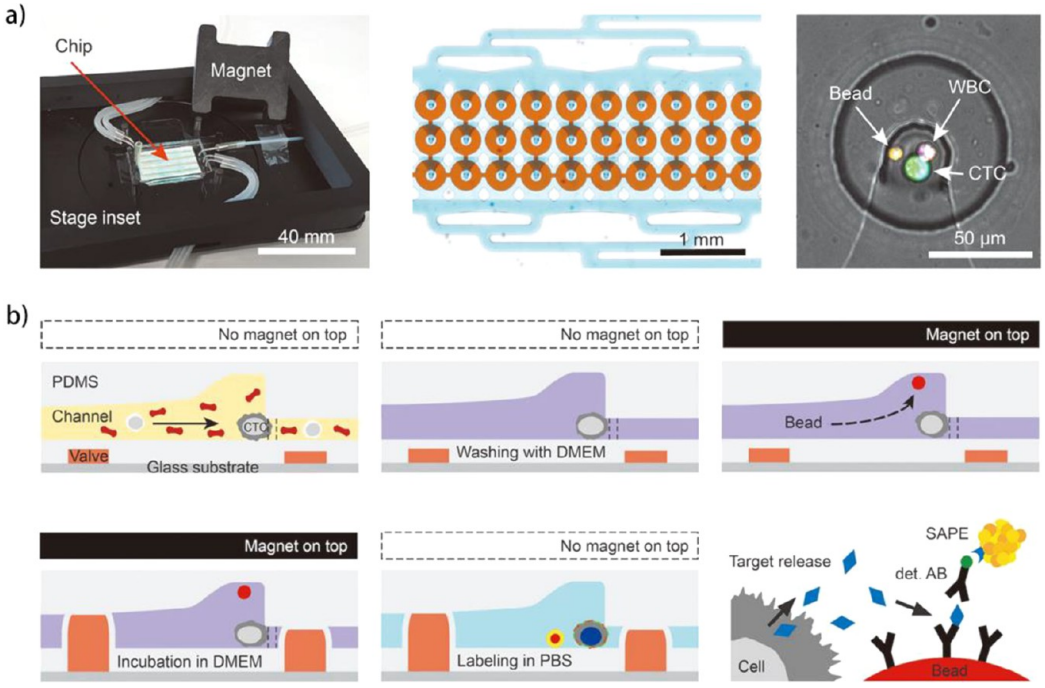

Figure 9. Microfluidic chip design and operation for CTC capture and analysis. (a) The measurement setup consists of the microfluidic chip, the chip holder that can be mounted on the microscope stage, and the lid with a permanent magnet (left). Micrograph of a subset of 30 analysis chambers (middle). Close-up of the center of one analysis chamber, where size-selective trapping of CTCs and magnetic trapping of beads are realized (right). (b) Schematics of the workflow; dashed lines indicate positions of micropillars. Schematics of the sandwich immunoassay employed to detect G-CSF. (Reproduced with permission from ref 163. Copyright 2020 John Wiley and Sons)

receptor interaction, nanostructure amplification, and thermal activation allowed specific and efficient capture of CTCs. Upon temperature stimulation, the cancer cells could be released with high cell viability for further analysis.[155] In another study, by using HER2 recognition peptide-functionalized nanomaterials, isolation of HER2-positive CTCs was accomplished with high selectivity.[156] Immunomagnetic affinity offered promising tools for CTCs isolation due to its specific binding capability. For example, a biomimetic immune-magnetosome was fabricated by decorating a leukocyte membrane on a magnetic nanocluster. The magnetic nanocomposite exhibited magnetic controllability, high stability and specific binding efficiency toward CTCs with low interference from leukocyte.[157] Immunomagnetic beads decorated with a platelet−leukocyte hybrid membrane and specific antibodies was developed for highly efficient and specific isolation of CTCs from human blood samples.[158] Further integrating these immunomagnetic-based isolation methods with microchips would offer advanced tools for developing integrated approaches for CTC enrichment and analysis.[159]

For the detection of CTCs, various approaches including optical, electrochemical, and electrical-based techniques have been applied.[160−162] In Gao et al.'s study, magneto-optical coupled nanoparticles were developed for simultaneous trapping and photoacoustic (PA) detection of rare CTCs. PA imaging could enable deep penetration into human tissue and, therefore, offered advantages over conventional fluorescence techniques. AuNRs were decorated with silica shell, Fe_3O_4 magnetic NPs, and PEG for targeted binding of CTCs, while AuNRs served as the PA reagent for imaging. After the magnetic-based enrichment of CTCs in mimicking the human radial artery system, an ultrasonic transducer was used for collecting PA imaging signals, which successfully obtained the concentration of circulating HeLa cells.[160] Dittrich's group developed an integrated microchamber-based microfluidic system for the capture, isolation, and analysis of membrane markers and protein secretion of single CTCs (Figure 9). After trapping single CTCs derived from clinical samples, fluorescently labeled antibodies toward the membrane biomarkers of CTCs and magnetic bead functionalized with capturing antibodies were supplied into the microchambers. The fluorescent images enabled the identification of CTCs, while the sandwich immunoassay made the quantification of the CTC-secreted granulocyte colony stimulating factor (G-CSF) possible. The bead-based assay exhibited a LOD of 1.5 ng mL^{-1} or less than 3700 molecules per cell, showing the potential application in single CTC profiling.[163]

A ZnO-based microfluidic electrochemical biosensor was reported by Tsuchiya et al. for rapid recognition of CTCs in blood based on pH changes. The microfluidic system composed a three-electrode system, with ZnO as working electrode. Since the intracellular acidity of tumors cells was lower, the developed platform used pH values as useful indicator for detection of CTCs. By recoding the voltage changes between the working and reference electrodes, the changes in pH responses were obtained indirectly and directly related to recognition of CTCs.[164] A microfluidic sensor with embedded electrodes was fabricated by Agah et al. for the detection of CTCs. The biosensing system consisted a constriction channel, which was utilized to generate changes in impedance when cells were passing through. Therefore, the differential impedance profiles were further applied to count and differentiate various CTCs in a label-free manner.[162] The Scoles group reported another kind of label-free method to exploit the abnormal metabolic behavior of CTCs based on the measurement of single-cell metabolism in droplet-based

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microfluidics. Herein, monodisperse droplets that contain individual tumor cells were generated for further analysis. The pH value and secreted molecules of individual cells were monitored without further labeling, which was used to identify and count CTCs. Using this method, as few as 10 tumor cells among 200 000 white blood cells could be detected.[165]

3.4. Pathogen Analysis. Pathogen include bacteria, virus, parasite, fungus, etc., have induced an increasing amount of infectious diseases and become a severe threat to human health. The rapid and efficient detection of pathogens are of vital importance not only for early diagnosis of diseases in clinic practice but also for food safety and ecological balance. Although a conventional plate culturing method is widely used, there is still an urgent need to develop rapid and sensitive detection approaches for emerging POCT. Microfluidic devices have been attractive owing to their advantages in portability, low sample consumption, and flexibility in integrating with various detection methods.[166] The microfluidic devices for pathogen detection can be made of PDMS or paper, using different fluid handling methods such as continuous flow or droplet formats. The detection techniques are mainly based on immunoassays or DNA-based assays. The former method is flexible owing to the generated pathogenic antibodies and epitopes, while the latter is applied when limited antibodies are present in the pathogen-related environment.[167,168] To meet the requirement of POCT, advanced microfluidic platforms should be capable of analyzing pathogens rapidly with high sensitivity and selectivity in various environments without sample pretreatment.[167] In this section, we mainly focus on the recent advances on the analysis of bacteria and virus based on various types of microfluidic platforms and detection strategies.

3.4.1. Bacteria. The sensitive detection of bacteria is of vital importance for early diagnosis and treatment of bacteria-induced diseases. Compared to traditional methods, microfluidics technology can achieve rapid and efficient detection, by integrating functional components and various detection approaches including optical, electrical-based, or acoustic-based sensing methods.[169−171]

First, optical sensors based on fluorescence, Raman, SERS, colorimetric methods, etc., could enable rapid, multiplex and cost-effective detection of pathogens, which is favorable for potential clinical applications.[172,173] For instance, Mahshid et al. reported a nano/microfluidic device with 3D nanostructured for the detection of bacteria. The microdevice was fabricated by uniformly dispersing gold hierarchical nano/microislands (NMI) based 3D structures at the bottom of an analysis well for enhanced capturing and detection of bacteria. Using this microdevice, the probe-free capture of RFP-labeled *Escherichia coli* (*E. coli*) and immunocapture of FITC-labeled methicillin-resistant-*Staphylococcus aureus* (MRSA) were validated (Figure 10). In addition, by measuring the fluorescence response after capturing, the fluorescence-based sensing of these two types of pathogenic bacteria were demonstrated, with a linear range between 50 and 104 CFU mL^{-1} and average efficiency of 93% and 85% for the detection of *E. coli* and MRSA, respectively. By further characterizing the surface structure of the 3D NMIs, it was indicated that the spatial orientation of these nanostructures could result in the quantitative detection of fluorescently labeled bacteria; however, the nanorough protrusions could induce effective probe-free capture of bacteria. Therefore, the proposed nanostructure-based microfluidic sensor could serve as a promising platform for the sensitive fluorescent detection of bacteria.[174]

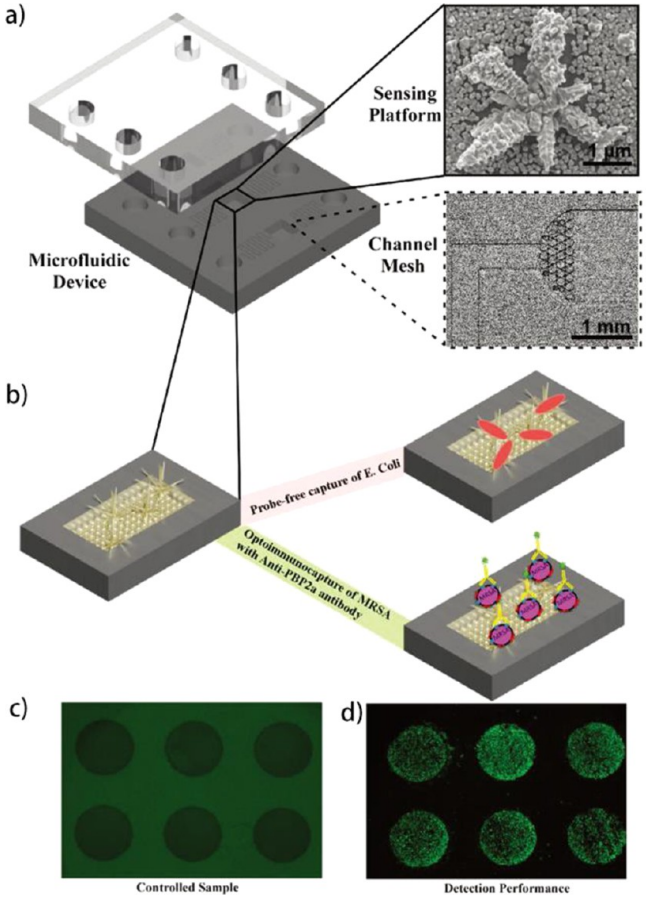

Figure 10. Schematic illustration of an integrated fluidic device for selective capture and analysis of bacteria. (a) The device integrates a hierarchical 3D nano/microisland (NMI) detection platform at the bottom of an analysis well and a fluidic sample delivery system. (upper inset) Top view SEM image of 3D NMI detection platform representing shrublike islands grown electrochemically. (lower inset) Micropillars at the interface of the analysis well and outlet microchannel. (b) Schematic of probe-free capture of *E. coli* and immunocapture of MRSA by shrub-like islands. (c, d) Fluorescence images demonstrate the selectivity of the approach toward MRSA (c) in the absence and (d) presence of anti-PBP2a antibody. (Reproduced with permission from ref 174. Copyright 2018 John Wiley and Sons)

In order to develop a smartphone-based microfluidic sensor for the detection of foodborne pathogen, Lin et al. first isolated Salmonella using immunomagnetic separation, followed by labeling the bacteria with immune fluorescent microspheres. Continuously injecting the fluorescent bacteria into the microfluidic device resulted in detectable fluorescent spots that could be monitored on a smartphone application. Based on a preset algorithm, the quantification of target bacteria was possible.[175] Apart from immunoassay, aptamer-based detection of bacteria was also developed. For instance, in light of the need for rapid detection of *Acinetobacter baumannii* (AB) which was a type of bacteria associated with infectious diseases, Lee et al. reported on a dual aptamer assay integrated microfluidic biosensor. Specifically, AB-specific aptamers were immobilized on magnetic beads for capturing. A second aptamer was conjugated to quantum dots for measuring bacteria based on the fluorescence response. With this

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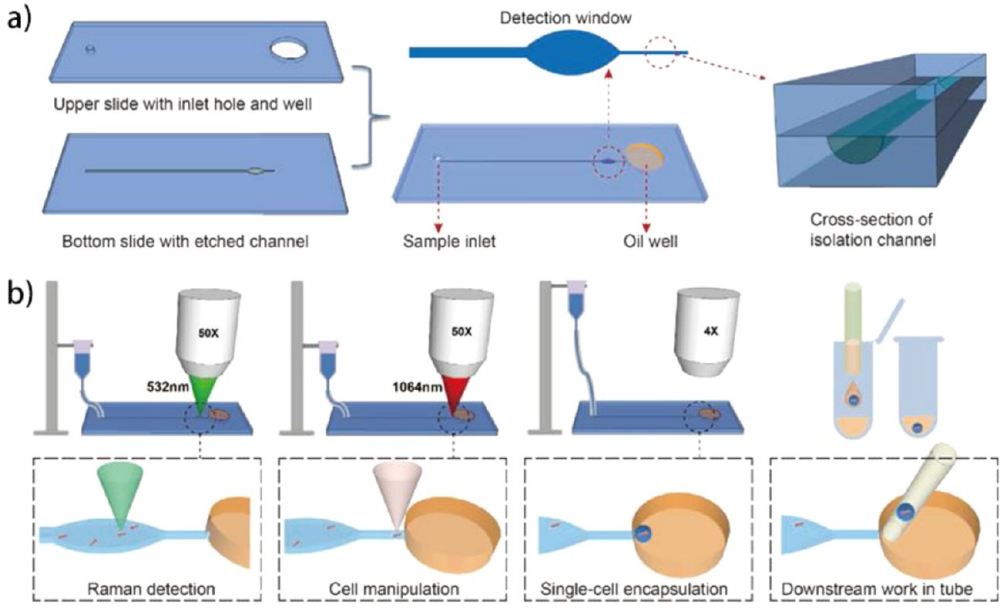

Figure 11. Design of the RAGE chip. (a) The RAGE chip consists of two quartz layers bonded together: with the inlet hole and oil well on the upper layer and the microchannel etched on the bottom layer. The upper surface of the cell channel is smoothened to avoid refraction of the laser that penetrates the upper layer. (b) The RAGE procedure includes four steps: (i) SCRS acquisition in the elliptic window via the 531 nm laser; (ii) moving the target cell to the channel tip via the 1064 nm optical tweezers; (iii) encapsulation of the cell into a microdroplet; (iv) transfer of the one-cell-harboring microdroplet to a PCR tube for cell or for single-cell cultivation. (Reproduced with permission from ref 172. Copyright 2019 John Wiley and Sons)

microdevice, a sensitive detection of AB within 30 min was accomplished.[176] Furthermore, in another study, a microfluidic device with aptamer-anchor polymer bound nanosensor arrays was fabricated for the detection of protein efflux from microorganisms at the single molecule level. By using synthetic DNA aptamers coupled to single-walled carbon nanotubes (SWNTs), the selective detection of protein was achieved, relying on positive changes in optical signal owing to the NIR emissive characteristics of SWNTs. Based on the developed method, the label-free detection of individual protein from *E. coli* was done in real time, demonstrating the wide applicability of this approach in the analysis of various proteins from different type of cells.[177] Wong et al. developed an adaptable microfluidic device for rapid classification pathogen and antimicrobial susceptibility testing (AST) at the single-cell level. The microfluidic chip featured parallel channels on a bottom layer and a top pneumatic control layer for trapping various bacteria. The successful separation of three fluorescently labeled bacterial species (*S. epidermidis*, *M. bacteremicum*, and *E. coli*) were demonstrated according to their shape and size differences. In addition, the antimicrobial susceptibility of the bacteria was determined on the microdevice by monitoring the growth of bacteria in the presence of antibiotics, which showed high concordance.[178]

To screen the genome sequencing of single cells, Ma et al. reported their work on using a Raman-activated gravity-driven single-cell encapsulation and sequencing (RAGE-Seq) strategy in an integrated microfluidic system (Figure 11). Individual cells were first detected by single-cell Raman spectra, followed by cell manipulation and encapsulation into microdroplets. Downstream analysis of the individual microdroplets provided single cell genome sequencing. The proposed Raman-based sorting and microdroplet encapsulation approach was successfully utilized for genome sequencing of single *E. coli* cells in urea samples.[172] SERS nanoprobes were also integrated into microfluidic dielectrophoresis (DEP) device for the rapid online SERS detection of bacteria, e.g. *Salmonella enterica* serotype Choleraesuis and Neisseria lactamica. AuNPs-based nanoaggregate embedded beads served as SERS nanotag for the identification of bacteria at single bacterium level, using a DEP-Raman biosensing strategy.[179] Antibiotic susceptibility test (AST) is vital for the diagnosis and treatment of bacterial infected diseases. However, conventional methods suffered from laborious sample pretreatment procedure and longtime analysis. To address these issues, Huang et al. reported on a microfluidic-based platform which combined an isolation zone with membrane filter and detection zone with SERS substrate for bacteria metabolite separation and measurement. *E. coli* was first injected to membrane filtration to obtain concentrated bacteria metabolites. Afterward, the sample flowed through the microchannel and reached the Ag SERS substrate to facilitate the detection of purine derivatives from bacteria. By using antibiotics pretreated *E. coli*, the detected SERS signal illustrated the AST of this bacteria with a concentration of 10^3 CFU/mL, demonstrating the efficacy in utilizing this integrated microfluidic system for measuring bacterial metabolite and AST determination.[180]

Colorimetric assay is a direct and rapid detection approach which has been applied for measuring bacteria and their metabolites after combined with microfluidic platforms.[181] In the study from Lee's group's, the authors constructed a dual-aptamer paper-based microfluidic chip for detection of various bacteria. By using an immobilized aptamer for capturing bacteria and biotin-labeled second aptamers for measurement, the color changes were directly monitored after a tetramethyl benzidine (TMB) streptavidin (blue) color reaction, therefore permitting the simultaneous detection of multiple bacteria.[182] Lin et al. built an AuNPs-based biosensor integrated with smart phone imaging APP for detection of *E. coli*. Bacteria were captured by a immunomagnetic approach, while they were

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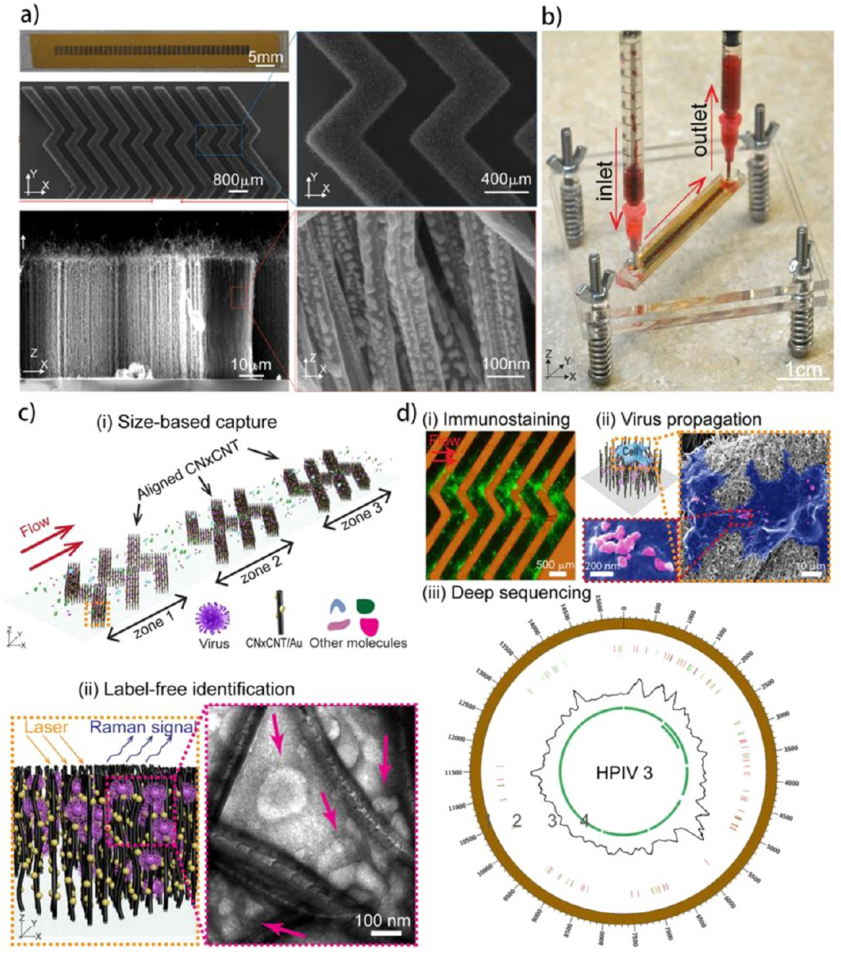

Figure 12. Design and working principle of VIRRION for effective virus capture and identification. (a) Photograph and SEM images of aligned CNTs exhibiting herringbone patterns decorated with gold nanoparticles. (b) Picture showing assembled VIRRION device, processing a blood sample. (c) Illustration of (i) size-based capture and (ii) in situ Raman spectroscopy for label-free optical virus identification. Images of electron microscopy showing captured avian influenza virus H5N2 by CNxCNT arrays. (d) On-chip virus analysis and enrichment for NGS: (i) on-chip immunostaining for captured H5N2, (ii) on-chip viral propagation through cell culture, and (iii) genomic sequencing and analysis of human parainfluenza virus type 3 (HPIV 3). (Reproduced with permission from ref 190. Copyright 2020 The American Association for the Advancement of Science)

detected by color changes of AuNPs owing to aggregation that could reflect the number of bacteria.[183]

Electrochemical biosensors for the detection of bacteria normally employ conducting and semiconducting materials as the electrode. Upon binding of target bacteria on the functionalized electrode, the changes in electrochemical signals could be monitored and associated with the quantification of bacteria.[167] For instance, a microfluidic electrochemical biosensor was fabricated by Uludag et al. for real-time bacteria detection. Based on the standard immunoassay using electrochemical sensing of HRP-TMB induced signal changes, not only the quantification of *E. coli* from real water samples, but also the cross-reactivity studies with other bacteria have been demonstrated. The microdevice was also regenerative by modifying the sensor surface, which was facile and cost-effective in pathogen detection.[184] In another work, an amperometric microfluidic biosensor based on AuNPs was manufactured for detection of bacteria. By coupling specific antibodies and electrochemical active molecules on redox-active AuNPs, the nanocomplex could be bound with bacteria to form an electropolymerized self-assembled layer. Upon applying voltage, a redox-active current could be detected by conventional electrochemical detection, with low LOD of 10 CFU/mL. Clinical application in detecting *P. aeruginosa* and *S. aureus* in human plasma samples demonstrated the diagnostic potential of the proposed method.[185]

3.4.2. Virus. In the past decades, viral infectious pathogens such as the Ebola virus, severe acute respiratory syndrome (SARS) virus, avian influenza virus (AIV), etc., have induced a high death rate and become one of the major public health concerns. Therefore, the rapid and sensitive detection of pathogenic virus or virus metabolites by portable devices can enable early diagnosis and intervention.[186] To sensitively and specifically detect AIV, Zheng et al. designed a microfluidic device based on nanostructured ZnO and fluorescence immunoassay strategy. Specifically, ZnO nanorod was integrated into microdevice to form herringbone structures, with the aim of enhancing mixing and capturing of virus samples on the immunologically functionalized nanostructure surface. Afterward, the detection of AIV was achieved by multiplexed sandwich immunoassay. Due to the 3D morphology of the nanostructure and optical property of ZnO nanorods, the proposed assay exhibited a quite low LOD of H5N2 AIV. Additionally, the captured viruses could be

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enriched by dissolving ZnO nanorods in slightly acidic solution for subsequent off-chip analyses. Thus, the proposed microfluidic platform could serve as a powerful system for rapid and sensitive detection of multiple viruses.[187] In Chang et al.'s study, a microfluidic DEP chip was manufactured to detect dengue virus using fluorescence immunosensing. Antibody modified beads were injected into microfluidic channels and driven by DEP force to capture the fluorescence-labeled virus targets. The fluorescence signal was recorded and utilized to quantify the virus. Using this microfluidic platform, the on-chip detection was accelerated to only 5 min, and the LOD for dengue virus could reach as low as 104 PFU/mL.[188] In addition to immunoassay, the detection of viral nucleic acid based on microfluidic fluorescent assay was also developed. Du et al. reported an automated two-layer microfluidic chip for Ebola RNA detection based on clustered regularly interspaced short palindromic repeats (CRISPR) strategy. The CRISPR associated (Cas) genes (Cas13a) first bound with CRISPR RNA, with the complex then hybridized with Ebola target RNA. The cleavage of random RNA strands by the Cas13a-CRISPR RNA-Ebola RNA complex would release fluorophores for rapid detection. With the proposed rapid and amplification-free method, the Ebola RNA could be detected at a low LOD of ~20 pfu/mL within 5 min, which could be promising POC diagnostic platform.[189]

Based on label-free SERS detection, Terrones demonstrated a microfluidic device with CNT arrays for the capturing and detection of virus (Figure 12). The microdevice was constructed with CNT arrays to enable the separation and enrichment of virus due to the differential filtration porosity. Afterward, the AuNPs that were decorated on CNTs could facilitate the on-chip SERS measurement of virus in a label-free manner. An important feature of the microchip was that the viruses remained viable after on-chip capture and analysis and thus could be isolated and purified for further down flow characterization, e.g. PCR. Proof-of-principle experiments were demonstrated by measuring different subtypes of avian influenza A viruses and human clinical samples with respiratory infections within a few minutes. Within the proposed microfluidic biosensor, an LOD of ~10^2 EID_{50}/mL (50% egg infective dose per microliter) and a virus specificity of 90% were achieved, with sensitivity comparable to conventional RT-qPCR detection.[190]

Not only virus but also virus lysates could be analyzed by other optical detection such colorimetric or luminescent analysis, either by immunoassay or aptasensing strategies.[191−193] For instance, a nanomaterial-based microfluidic platform was reported for the sensitive and selective detection of the avian influenza virus. The 3D nanostructured PDMS surface of the microdevice could enable immobilizing antibodies for capturing virus. Further supplying antibody conjugated AuNPs into the system induced colorimetric reaction that can be monitored by the naked eyes. With the injection of silver enhancer and integration of smart-phone imaging system, the proposed microfluidic biosensor could enable the rapid and sensitive detection of avian influenza virus.[191] Using aptamer−analyte interactions and colorimetric measurement, the multiplex detection of viral envelope proteins on a single microfluidic device was demonstrated by Seal et al. The microfluidic channels were integrated with micropillars that were decorated with aptamers to improve the binding of protein-aptamer for virus capturing. After the subsequent injection of aptamer functionalized AuNPs, the sandwich morphology was formed. The detection mechanism was based on the colorimetrical signal changes induced by the formation of Ag coated Au after supplying silver regents into the system. The developed microfluidic method was successfully utilized to detect Zika and chikungunya envelope proteins at clinical concentrations.[192]

4. APPLICATIONS OF MICROFLUIDIC BIOSENSORS

Based on the various techniques in analyzing biospecies, in this section, we principally put a focus on introducing the most recent advances in translating the analysis of biospecies into practical application in three research fields: clinical applications (mainly disease diagnosis), food safety control, and environmental monitoring.

4.1. Clinical Applications. *4.1.1. Cancer Diagnosis (Point-of-Care Immunodiagnostics).* Microfluidic technologies have made impressive strides in the detection and analysis of CTCs, exosomes, cell-free nucleic acids (ctNAs), and protein biomarkers, in some cases, directly from whole blood.[194,195] Mounting evidence indicates that detecting a combination of different cancer biomarkers can lead to a more sensitive and precise detection of cancer and the determination of tumor type.

The main challenge in separating and detecting CTCs in blood is their extremely low concentration compared to the that of natural blood cells. Chen et al. fabricated a 3D printing microfluidic device to isolate carbon tetrachloride peripheral blood.[196] The inner surface of the microfluidic channel was successfully functionalized with anti-EpCAM antibodies, and the designed fluid flow enhanced the antibody−antigen interactions between CTC-antibody and binding site of tumor cells in the microfluidic channel. The influence of fluid flow rate and channel length were optimized to improve the capture efficiency of EpCAM positive human CTCs (PC3 prostate cancer, MCF-7 breast cancer and SW480 colon cancer). The results showed that the CTC capture efficiency of MCF-7 was as high as 92.42 ± 2.00%, for SW480 was up to 87.74 ± 1.22%, and that of PC3 cells was 89.35 ± 1.21%. In addition, it has been proven that 3D printed microfluidic devices can separate CTCs even from artificial blood samples. Combined with the analysis of DNA released in the blood and the enzymatic lysis of tumor cells, the proposed CTCs isolated technology is the way of the future in early diagnosis of cancer metastasis.

Research on sequence mutations and cfNAs methylation in diagnosis of cancer is a promising research domain. Compared with healthy controls, the levels of cfNAs may be significantly different in plasma or serum samples. In addition, due to cfNA fragmentation, RNA instability and low concentration in body fluids, accurate quantitative analysis of cfNA is still a challenging and active subject in cancer diagnosis. Therefore, it is not difficult to think of implementing simultaneous cfNA test in blood on a single microfluidic chip. Furthermore, microfluidic devices with low cost and sufficient sensitivity can be used as routine analysis platforms for cancer biomarker analysis.[2] A magnetic microfluidic chip was developed for efficient isolation of DNA in raw biological samples (undiluted serum).[197] This strategy relied on two modules: the first one was used to selectively capture all DNA sequences, including mutant and wild-type, and the second was applied to separate the extracted first molecules into millions of picoliter droplets for ddPCR with high-resolution genetic testing. The proposed method provided highly sensitive and specific isolation of

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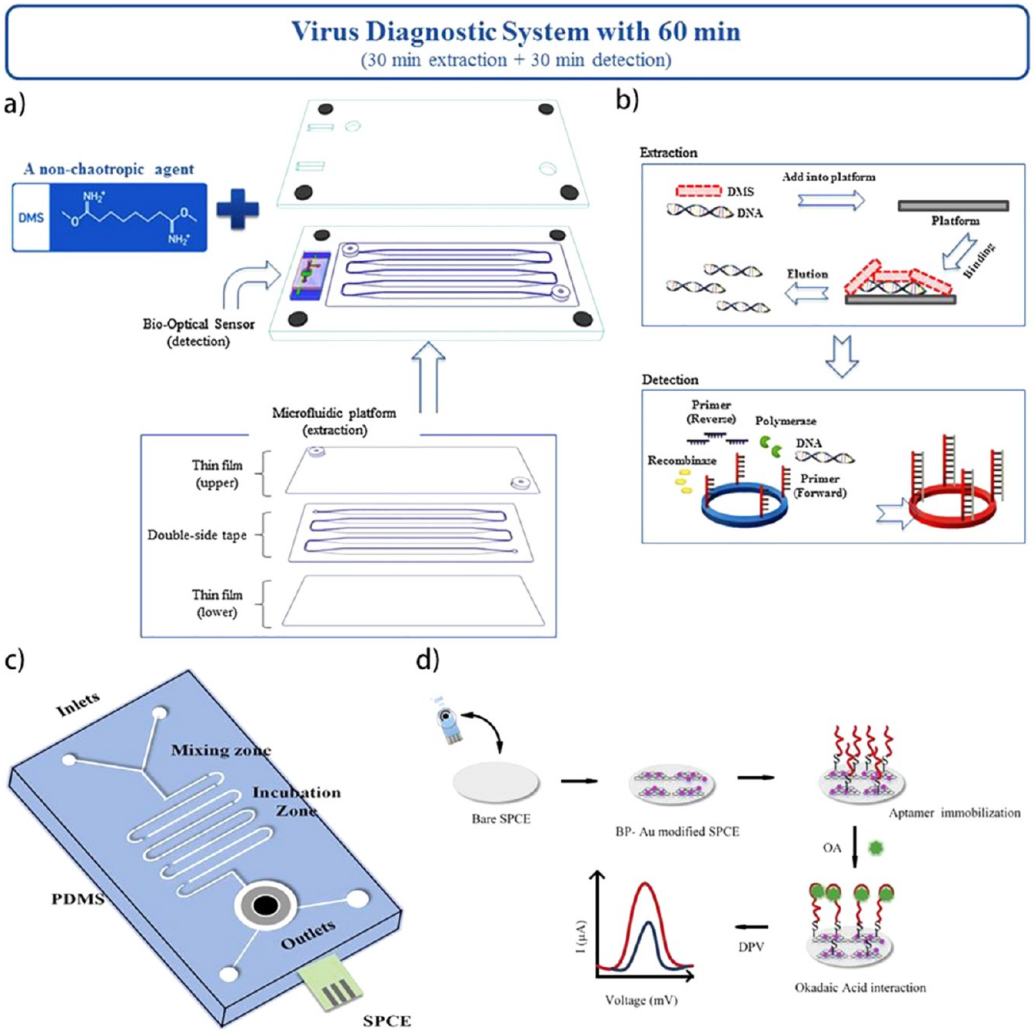

Figure 13. Schematic representation of the principle of the rapid virus diagnostic system for the diagnosis of human adenovirus. (a) Proposed method that combines a DMS-based microfluidic platform for sample processing (extraction) and a bio-optical sensor for detection. (b) Viral DNA extracted from the DMS platform using a one-shot input of all mixture solutions (upper), followed by an ISAD bio-optical sensor for the detection of human adenovirus in a single cartridge (lower). (Reproduced with permission from ref 207. Copyright 2018 Elsevier B.V.) Microfluidic electrochemical aptasensor for the detection of okadaic acid. (c) Graphic of the fabricated PDMS microfluidic chip. (d) Schematic representation of the process of aptamer-based sensing. (Reproduced with permission from ref 219. Copyright 2019 Elsevier B.V.)

mutant ctDNA in cfNAs. By applied these advances in diagnosing breast and colon cancer patients, this method has provided the capacity to perform sample treatment and cfNAs detection simultaneously in a single microdevice. Compared with traditional column-based methods and blind operation, this miniaturized DNA isolation system provided the ability of sensitive DNA amplification methods in cancer diagnostic procedure.

Cancer excretes peptide growth factors, cytokines, and many other protein markers, whose abnormal levels can be used as cancer biomarkers. In most cases, the simultaneous detection of multiple biomarkers at an early stage has additional benefits in reducing unnecessary biopsies and improving diagnostic accuracy.[198,199] The integration of all necessary analytical procedures, including sample-preprocessing, sample-separation, and target detection into a single chip will render many advantages in the quantitative biomarker analysis.[200]

All analysis procedure can be run in a continuous flow state and produce uniform mixing conditions in the microfluidic chip. In addition, the combination of SERS and microfluidic devices can achieve sensitive detection, reproducible measurement, spatially determined assay area, and the required minimum sample volume and low concentration of analyte. Here, the first design and implementation of a droplet-based microfluidic system embedded with rectangular magnetic strips.[47] The magnetic stripe can separate free and bound SERS tags. Therefore, by using this system, a no-wash immunoassay can be achieved, and the SERS intensity can be used for quantitative evaluation of specific biomarkers. Importantly, this method provides convenient immunoassays involving specific biomarkers without washing. This platform has many advantages, including rapid mixing of reagents, controllable reaction time, and the realization of fully automated immunoassays with high reproducibility. In order to further improve the cancer diagnosis accuracy of this droplet device, a microfluidic system based on parallel segmented flow for simultaneous detection of t-PSA and f-PSA biomarkers was designed.[201]

4.1.2. Infectious Disease Diagnosis. Pathogenic microorganisms can induce a variety of infectious diseases that are

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seriously threatening human health worldwide, such as bacterial infection diseases like malaria, sepsis, and viral infectious diseases like corona virus disease, etc.[202,203] In order to achieve effective treatment of pathogenic infection, the accurate and rapid measurement of multiple pathogenic microorganisms is critical. Up to date, different technologies have been utilized in pathogen-diagnostic biosensors, mainly optical approaches including fluorescence, SPR, colorimetry etc., owing to the rapid, sensitive and multiplexed detection capability. In particular, microfluidic optical biosensors have raised extensive research interest.[173,204]

Malaria is a type of infectious disease caused by *Plasmodium* parasites and transmitted by infected mosquitos. It leads to nearly half a million deaths each year and has become a serious threat worldwide. The early detection of malaria can help to reduce the death rate, and POC devices are needed to enable the rapid and facile diagnosis, especially in some developing countries where malaria is highly prevalent. By recognizing the malaria biomarker protein *Plasmodium falciparum* lactate dehydrogenase (PfLDH) enzyme, a microfluidic platform integrating a DNA aptasensor was fabricated by Tanner et al. PfLDH specific aptamers were immobilized onto magnetic microbeads for their capture and detection. After introducing reaction solutions into the detection microchamber, the colorimetric changes induced by the redox reaction would enable the rapid detection of PfLDH. Using both in vitro cultured parasite samples and clinical samples from malaria-infected patients, the performance of the developed microfluidic biosensor toward PfLDH was clearly demonstrated.[205] Recently, an electrochemical microfluidic biosensor was also reported for the detection of PfLDH in whole blood samples. Immunomagnetic beads were utilized for the capture of PfLDH after sample pretreatment. Herein, a disposable paper electrode-based microdevice was used for the electrochemical analysis of the biomarker, which offered a LOD of 2.47 ng mL.[206]

A major cause of death in the intensive care unit is sepsis which is an infectious disease threatening human life. To develop effective approach for early sepsis diagnosis, Kurabayashi reported their work of using a miniaturized device with plasmo-photoelectronic nanostructures to detect citrullinated histone H3 (CitH3), a blood biomarker released by neutrophils. Herein, hemispherical AuNPs arrays were first functionalized by CitH3 capturing antibodies, and then patterned on the MoS_2 as substrate. Upon binding of CitH3, the nanoplasmonic resonance shift under NIR laser-induced signal increase of a few-layer MoS_2 photoconductive channel. The Au nanostructures could also enhance the LSPR signals. Using the microdevice, the authors also demonstrated the label-free and dynamic detection of CitH3 in living sepsis mice models over 12 h at high resolution, offering a potential way for translation to human treatment of sepsis.[203]

Viral infection can induce severe respiratory infection diseases.[207] In particular, after the outbreak of coronavirus disease (COVID-19) worldwide, the development of the reliable diagnosis of such diseases has been regarded as the foremost challenge to overcome. Due to the existing false-positive results detected by conventional RT-PCR, it is highly desired to develop rapid and accurate detection approaches. Wang et al. reported a dual-functional plasmonic biosensor combining LSPR and plasmonic photothermal (PPT) effect for the clinical diagnosis of corona virus. On one hand, by using complementary DNA receptor modified two-dimensional gold nanoislands (AuNIs), the selected sequences from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) could be analyzed via nucleic acid hybridization. On the other hand, the AuNIs could generate thermoplasmonic heat which would enhance the sensing performance.[208] By combining a virus biosensor with microfluidic sample processing module, a facile virus diagnostic system was developed by Shin et al. for the sensitive detection of human respiratory adenovirus (HAdV) (Figure 13a). In the microfluidic channel, a nonchaotropic reagent was integrated with a disposable thin film to facilitate the viral DNA extraction from clinical HAdV samples within 30 min. The detection of viral DNA was accomplished by bio-optical sensor of isothermal solid-phase DNA amplification (Figure 13b). The clinical diagnosis of HAdV in human samples was also validated using the assembled microfluidic biosensing system.[207]

4.1.3. Other Diseases. Apart from the above-mentioned disease-specific microfluidic biosensors, a variety of microfluidic systems have also been developed for the monitoring of other types of diseases such as brain injury, neurodegenerative diseases, diabetes, organ injury, etc.[69,209−213] Alzheimer's disease (AD) is the most common neurodegenerative disease that influences elderly people worldwide. In pursuit of the early diagnosis of AD, Faria et al. reported a simple and sensitive electrochemical-based microfluidic platform for the detection of the biomarker of AD, A disintegrin and metalloprotease 10 (ADAM10). By using immunomagnetic capturing strategy, ADAM10 samples were enriched from human plasma, serum, and cerebrospinal fluid. After supplying the samples, H_2O_2 and HQ into the microchip, the redox reactions on the electrode would induce obvious changes in electrical signal, enabling the rapid and sensitive quantification of ADAM10 in human clinical samples.[212] Blood derived amyloid-β (Aβ), a low abundance peptide, is another important biomarker for the diagnosis of AD at an early stage. In Hwang et al.'s study, an impedance biosensor combining interdigitated microelectrodes and AuNPs sandwich assay was constructed to analyze Aβ. Herein, the employment of AuNPs greatly enhanced the detection sensitivity of the biosensor. Application in plasma samples from mice clearly demonstrated the efficacy of the proposed impedance microfluidic biosensor.[214]

Cardiovascular diseases (CVDs) are one of the leading causes of death worldwide. Detection of cardiac troponin I (cTnI), a cardiac biomarker, would facilitate the early diagnosis of CVDs. An electrochemical microfluidic biosensor targeting human cTnI was reported by John group. The microdevice consisted of chitosan modified mesoporous $Ni_3V_2O_8$ hollow-nanospheres (Ch-$Ni_3V_2O_8$) structure. After immobilizing specific antibody toward cTnI on the nanostructures and inject patient blood samples, the redox reaction on the nanospheres would generate electrochemical readouts for the quantification of cTnI. The microfluidic biosensor exhibited sensitive detection of cTn in a wide range concentrations, with a low LOD of 5 pg/mL. By immobilizing different antibodies, the developed microfluidic system could be applied to detect other CVDs related biomarkers.[215] In another study, a microfluidic device equipped with an FET-based aptasensor was reported for the analyses of four CVDs biomarkers including cTnI, C-reactive protein (CRP), N-terminal pro b-type natriuretic peptide (NTproBNP), and fibrinogen. After immobilizing aptamers on the electrodes, different protein biomarkers were injected separately into the microchambers for incubation. The FET signal recorded before and after the

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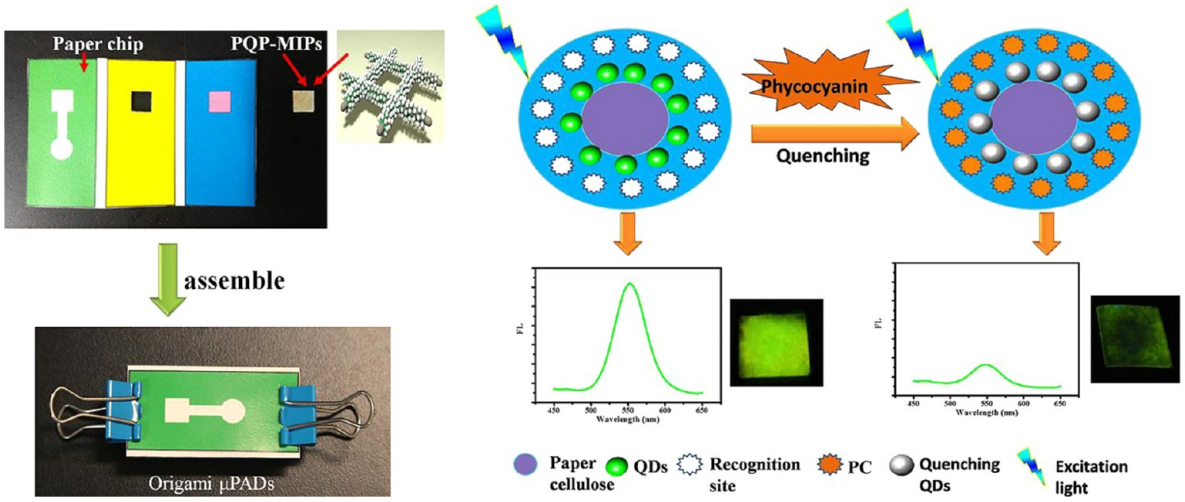

Figure 14. Design, construction, and characterization of 3D origami paper-based microfluidic devices for specific recognition and sensitive detection of phycocyanin using fluorescent QDs combined with molecularly imprinted polymers. (Reproduced with permission from ref 225. Copyright 2017 American Chemical Society)

elution procedure could be correlated to the concentration of proteins.[69]

Chronic kidney disease affects the health of an increasing number of people all over the world. To evaluate kidney damage, urinary albumin levels can be used as a critical indicator. Lin et al. fabricated a microfluidic chip for the sensitive measurement of albumin in urine samples by fluorescent responses. In this microdevice, 16 passive and continuous mixing modules were integrated into one chip, each worked individually for facile fluid loading. Further applying immiscible oil into each module lead to pressure balancing and precise control of a sample-dye mixing ratio. After validating the microchip using an albumin standard solution, samples from 12 CKD patients were detected by recording the fluorescence readouts, with the sensitivity comparable to a traditional well-plate method.[210]

4.2. Food Safety Control. Nowadays, food safety is of high concern for our entire society. Pesticide residues, preservatives, heavy metals, and toxins can influence food quality and may induce foodborne diseases. Although many bulk analytical techniques including chromatography, spectroscopy, and immunoassays have been reported, efficient and portable devices are still in high demand for rapid POCT. Therefore, microfluidic based biosensors integrated with various analytical methods have been developed.[216] CL is one of the optical approaches that have been employed in food analysis, owing to its high sensitivity and selectivity and ease of integration with separation techniques and microfluidic platforms.[217] For instance, a paper-based CL microdevice was fabricated for the detection of dichlorvos (DDV) in vegetables. After sample separation by paper chromatography, the detection zone was cut and patterned into a paper-based microfluidic system. Following by dropping a solution mixture of luminol and H_2O_2 onto the detection zone, the CL signal was generated and recorded for the quantification of DDV, showing a linear response and low LOD. The real application in analysis of dichlorvos residues in vegetables demonstrated the applicability of the proposed method for tracing low levels of pesticides in food samples.[218] Electrochemical based biosensors have also been reported for food quality control. For instance, okadaic acid is a widely prevalent biotoxin in seafood which may induce digestive diseases of human. Therefore, a nanocomposite based electrochemical microfluidic aptasensor was developed by Singh et al. for the detection of okadaic acid. A screen-printed carbon electrode (SPCE) was functionalized with phosphorene−gold nanocomposite, followed by immobilizing aptamers specific to okadaic acid to form the detection area of the microchip (Figure 13c). In this device, potassium ferro-ferri cyanide was utilized as the redox pair to generate electrochemical signals. Upon the interaction of okadaic acid sample extracted from seafood with the developed aptasensor, differential pulse voltammograms were measured, showing a sensitive detection with LOD of 8 pM (Figure 13d).[219]

Since a variety of compounds generated during food contamination or spoilage can be detected by enzyme reactions,[220] over the past decades, electrochemical enzyme-based biosensors have exhibited their capability in efficient and sensitive analysis of food samples. For example, glucose oxidase could be utilized to measure glucose level in fruit products, and galactosidase facilitated the detection of lactose in milk products, etc. Upon using of electrodes functionalized with corresponding enzymes, the relative compounds were determined for food analysis.[221] Mycotoxins, which are severe toxic metabolites of fungus, can induce food contamination and pose a high risk to human health. Recently, PEC biosensors have been regarded as promising techniques for rapid and sensitive detection of mycotoxins due to their inherit advantages. Based on the use of various photoactive materials (e.g., semiconductors) and recognition elements (e.g., antibodies), PEC biosensors were developed for mycotoxin detection by the changes in photocurrent response.[222] Further integrating the above-mentioned biosensors with microfluidic platforms will facilitate the development of rapid, facile, and portable microdevices for food safety control.

4.3. Environmental Monitoring. The deterioration of the environment has become a global concern. Therefore, developing effective and economical approaches for environmental monitoring such as water pollution analysis and soil contamination and air pollution detection are highly desired. Various microfluidic-based biosensors have been reported for detection of environmental-related microbes, heavy metals etc.,

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Table 1. Various Applications of Microfluidic-Based Biosensors

application type		target	biospecies	detection method	ref
clinical applications	cancer diagnosis	prostate/breast/colon cancer	circulating tumor cell	fluorescence and brightfield images	196
		breast/colon cancer	cell free nucleic acid	fluorescence	197
		prostate cancer	protein	Raman spectroscopy	201
	infectious disease diagnosis	sepsis	citrullinated histone H3	photoconduction change assay	203
		gastric diseases	*Helicobacter pylori*	fluorescent assay	202
		malaria	*Plasmodium falciparum* lactate dehydrogenase	aptamer-tethered enzyme capture (APTEC) assay	205
				electrochemical biosensing	206
		respiratory infection diseases	human adenovirus	bio-optical sensing	207
			coronavirus	thermoplasmonic sensing	208
		sexually transmitted infections	*Chlamydia trachomatis* and *Neisseria gonorrheae*	nanoplasmonic biosensing	204
	other disease diagnosis	Alzheimer's disease	A disintegrin and metalloprotease 10	electrochemical immunosensing	212
			amyloid-β	impedance biosensing	214
		brain injury monitoring	glutamate, glucose, and lactate	electrochemical biosensing	211
		diabetes	glucose	bioelectronic sensing	209
		chronic kidney disease	urinary albumin	fluorescent assay	210
		cardiovascular diseases	C-reactive protein, N-terminal pro b-type natriuretic peptide, cardiac troponin I, and fibrinogen	field-effect transistor (FET) based sensor	69
			cardiac troponin I	electrochemical biosensing	215
food safety control		toxicity	mycotoxins	photoelectrochemical (PEC) biosensors	222
		nutrition	vitamin	chemiluminescence	217
		amino acid	histamine tyrosine putrescine	electrochemical enzyme-based biosensor	221
environmental monitoring		toxic protein	phycocyanin	fluorescence	225
		biotoxin	okadaic acid	electrochemical biosensing	219

in combination with different optical and electrical approaches.[216,223,224]

Eutrophication of the water in seas and lakes often leads to algae bloom and deterioration of water quality. Also, the toxic substances generated from blue−green algae threaten marine life and human health. To monitor the levels of cyanobacteria, pycocyanin which is an indicative protein for alga has been analyzed to monitor the marine ecological environment. Chen et al. reported a paper-based microfluidic device integrating fluorescent quantum dots (QDs) and molecularly imprinted polymers (MIPs) for the sensitive detection of phycocyanin (Figure 14). CdTe QDs were first immobilized on paper substrate, and then wrapped with MIPs and integrated into 3D origami paper to form the biosensor. Based on the quenched fluorescence of QDs by pycocyanin, the microdevice was successfully utilized to detect pycocyanin with a LOD of 2 mg/L, showing great potential in environmental monitoring.[225]

In addition, based on the SERS technique, a microfluidic biosensor was fabricated for the detection of uranyl ions (UO_2^{2+}) in natural water samples. UO_2^{2+} is the stable chemical form of radioactive uranium existing in nature, which can cause serious threats to human health. Therefore, the sensitive and recyclable approach for UO_2^{2+} detection is urgently desired. In Wang et al.'s work, UO_2^{2+} specific DNAzyme-functionalized ZnO-Ag hybrids arrays was integrated into microfluidic chip and utilized as SERS sensing device. In the absence of UO_2^{2+}, the Raman reporter rhodamine B bound to DNA exhibited weak Raman signal, while in the presence of UO_2^{2+}, DNAzyme-cleavage happened, resulting in the generation of rhodamine B-modified 5′-single DNA strand and strong SERS signal, which could be used for the sensitive detection of UO_2^{2+} in natural tap water and river water with low LOD and high recovery.[226]

5. CONCLUSION AND FUTURE PERSPECTIVES

The microfluidic chip-based platform has progressed substantially since it possesses many attractive advantages such as low reagent consumption, fast sample processing, high integration, small size, and low cost, which enables a wide range of applications in the fields of liquid biopsy, single cell analysis, nucleic acid detection, drug screening, organ chip, and environmental and biochemical analysis. Focusing on the microfluids and microdevices in terms of their fabrication, integration, and functions, this review has outlined the latest developments in microfluidics-based analysis of biospecies, including biomarkers, small molecules, cells, and pathogens, and has also presented relatively promising applications based on microfluidic biosensors ranging from clinical diagnosis to food safety control and environmental monitoring (Table 1). Up to now, the fabrication of functional microfluidics has been extensively studied and the constructed microdevices have been proven to be promising in promoting the detecting efficacy and accuracy of biospecies.

Despite the great progress which has been made in microfluidics, there still exist many challenges and limitations that hinder the further development of microfluidics beyond academics. For instance, microfluidics-based sensing has limitations in device miniaturization and integration due to the requirements of external instruments, which makes it difficult for application in resource-restricted conditions. Additionally, the accuracy of microfluidic-based sensing of a single analyte need to be verified. In view of this, two further

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directions in microfluidics should be attended to from the perspective of the whole field. (i) Integration of multi-nanotechnologies and novel materials. Many samples in clinical diagnosis and environment monitoring are quite complex, where there exist many kinds of different substances that may interfere with the detection process due to the strong background signal. Integration of advanced nanotechnologies could benefit to sample enrichment, and the selected materials for signal translation together may greatly improve the efficacy of microfluidics-based sensing. (ii) Simultaneous analysis of multiple significant biomarkers. For clinical diagnosis, therapy, and prognosis, it is essential to perform multiple parameter detection to ensure accurate evaluation. Therefore, combining multiple candidates such as proteins, nuclei acids, and other biomarkers into a single platform in a completely automated manner may not only provide valuable information about the disease but also promote the practical application in truly POCT to improve detection credibility. It is anticipated that the rationally designed microfluidic chip combined with advanced techniques and multifunctional materials will facilitate the realization of extensive clinical applications.

AUTHOR INFORMATION

Corresponding Authors

Chuanzhu Lv − *Key Laboratory of Emergency and Trauma, Ministry of Education, Key Laboratory of Hainan Trauma and Disaster Rescue, The First Affiliated Hospital of Hainan Medical University, College of Pharmacy, Institute of Functional Materials and Molecular Imaging, College of Emergency and Trauma, Hainan Medical University, Haikou 571199, China*; Email: lyuchuanzhu@hainmc.edu.cn

Feifei Yu − *Key Laboratory of Emergency and Trauma, Ministry of Education, Key Laboratory of Hainan Trauma and Disaster Rescue, The First Affiliated Hospital of Hainan Medical University, College of Pharmacy, Institute of Functional Materials and Molecular Imaging, College of Emergency and Trauma, Hainan Medical University, Haikou 571199, China*; Email: yufeifei@hainmc.edu.cn

Fabiao Yu − *Key Laboratory of Emergency and Trauma, Ministry of Education, Key Laboratory of Hainan Trauma and Disaster Rescue, The First Affiliated Hospital of Hainan Medical University, College of Pharmacy, Institute of Functional Materials and Molecular Imaging, College of Emergency and Trauma, Hainan Medical University, Haikou 571199, China*; orcid.org/0000-0003-0073-6299; Email: yufabiao@hainmc.edu.cn

Authors

Yanlong Xing − *Key Laboratory of Emergency and Trauma, Ministry of Education, Key Laboratory of Hainan Trauma and Disaster Rescue, The First Affiliated Hospital of Hainan Medical University, College of Pharmacy, Institute of Functional Materials and Molecular Imaging, College of Emergency and Trauma, Hainan Medical University, Haikou 571199, China*

Linlu Zhao − *Key Laboratory of Emergency and Trauma, Ministry of Education, Key Laboratory of Hainan Trauma and Disaster Rescue, The First Affiliated Hospital of Hainan Medical University, College of Pharmacy, Institute of Functional Materials and Molecular Imaging, College of Emergency and Trauma, Hainan Medical University, Haikou 571199, China*

Ziyi Cheng − *Key Laboratory of Emergency and Trauma, Ministry of Education, Key Laboratory of Hainan Trauma and Disaster Rescue, The First Affiliated Hospital of Hainan Medical University, College of Pharmacy, Institute of Functional Materials and Molecular Imaging, College of Emergency and Trauma, Hainan Medical University, Haikou 571199, China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsabm.0c01271

Author Contributions

‡Y.X. and L.Z. contributed equally to this work.

Notes

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REFERENCES

(1) Garcia-Cordero, J. L.; Maerkl, S. J. Microfluidic systems for cancer diagnostics. *Curr. Opin. Biotechnol.* **2020**, *65*, 37−44.

(2) Yang, Y.; Chen, Y.; Tang, H.; Zong, N.; Jiang, X. Microfluidics for Biomedical Analysis. *Small methods* **2020**, *4* (4), 1900451.

(3) Mahato, K.; Kumar, A.; Maurya, P. K.; Chandra, P. Shifting paradigm of cancer diagnoses in clinically relevant samples based on miniaturized electrochemical nanobiosensors and microfluidic devices. *Biosens. Bioelectron.* **2018**, *100*, 411−428.

(4) Nguyen, T. T.; Trinh, K. T. L.; Yoon, W. J.; Lee, N. Y.; Ju, H. Integration of a microfluidic polymerase chain reaction device and surface plasmon resonance fiber sensor into an inline all-in-one platform for pathogenic bacteria detection. *Sens. Actuators, B* **2017**, *242*, 1−8.

(5) Whitesides, G. M. The origins and the future of microfluidics. *Nature* **2006**, *442* (7101), 368−373.

(6) Terry, S. C.; Jerman, J. H.; Angell, J. B. A gas chromatographic air analyzer fabricated on a silicon wafer. *IEEE Trans. Electron Devices* **1979**, *26* (12), 1880−1886.

(7) Manz, A.; Graber, N.; Widmer, H. M. Miniaturized total chemical analysis systems: A novel concept for chemical sensing. *Sens. Actuators, B* **1990**, *1* (1−6), 244−248.

(8) Xing, Y.; Esser, N.; Dittrich, P. S. Conductive single nanowires formed and analysed on microfluidic devices. *J. Mater. Chem. C* **2016**, *4* (39), 9235−9244.

(9) Xing, Y.; Sun, G.; Speiser, E.; Esser, N.; Dittrich, P. S. Localized Synthesis of Conductive Copper−Tetracyanoquinodimethane Nanostructures in Ultrasmall Microchambers for Nanoelectronics. *ACS Appl. Mater. Interfaces* **2017**, *9* (20), 17271−17278.

(10) McNamara, M. C.; Sharifi, F.; Okuzono, J.; Montazami, R.; Hashemi, N. N. Microfluidic Manufacturing of Alginate Fibers with Encapsulated Astrocyte Cells. *ACS Appl. Bio Mater.* **2019**, *2* (4), 1603−1613.

(11) de Mello, A. J. Control and detection of chemical reactions in microfluidic systems. *Nature* **2006**, *442* (7101), 394−402.

(12) Feng, S.; Mao, S.; Dou, J.; Li, W.; Li, H.; Lin, J. An open-space microfluidic chip with fluid walls for online detection of VEGF via rolling circle amplification. *Chem. Sci.* **2019**, *10* (37), 8571−8576.
(13) Ozer, T.; McMahon, C.; Henry, C. S. Advances in Paper-Based Analytical Devices. *Annu. Rev. Anal. Chem.* **2020**, *13* (1), 85.
(14) Chen, W.; Shao, F.; Xianyu, Y. Microfluidics-Implemented Biochemical Assays: From the Perspective of Readout. *Small* **2020**, *16* (9), 1903388.
(15) Jia, X.; Dong, S.; Wang, E. Engineering the bioelectrochemical interface using functional nanomaterials and microchip technique toward sensitive and portable electrochemical biosensors. *Biosens. Bioelectron.* **2016**, *76*, 80−90.
(16) Xing, Y.; Cheng, Z.; Wang, R.; Lv, C.; James, T. D.; Yu, F. Analysis of extracellular vesicles as emerging theranostic nanoplatforms. *Coord. Chem. Rev.* **2020**, *424*, 213506.
(17) Loo, J. F. C.; Ho, A. H. P.; Turner, A. P. F.; Mak, W. C. Integrated Printed Microfluidic Biosensors. *Trends Biotechnol.* **2019**, *37* (10), 1104−1120.
(18) Saucedo-Espinosa, M. A.; Dittrich, P. S. In-Droplet Electrophoretic Separation and Enrichment of Biomolecules. *Anal. Chem.* **2020**, *92* (12), 8414−8421.
(19) Sang, C.; Chou, S.; Pan, F.; Sheu, J. Fluorescence enhancement and multiple protein detection in ZnO nanostructure microfluidic devices. *Biosens. Bioelectron.* **2016**, *75*, 285−292.
(20) Ansari, M. I. H.; Hassan, S.; Qurashi, A.; Khanday, F. A. Microfluidic-integrated DNA nanobiosensors. *Biosens. Bioelectron.* **2016**, *85*, 247−260.
(21) Nge, P. N.; Rogers, C. I.; Woolley, A. T. Advances in Microfluidic Materials, Functions, Integration, and Applications. *Chem. Rev.* **2013**, *113* (4), 2550−2583.
(22) Cvetkovic, B. Z.; Puigmarti-Luis, J.; Schaffhauser, D.; Ryll, T.; Schmid, S.; Dittrich, P. S. Confined Synthesis and Integration of Functional Materials in Sub-nanoliter Volumes. *ACS Nano* **2013**, *7* (1), 183−190.
(23) Pumera, M. Nanomaterials meet microfluidics. *Chem. Commun.* **2011**, *47* (20), 5671−5680.
(24) Xing, Y.; Dittrich, P. S. One-Dimensional Nanostructures: Microfluidic-Based Synthesis, Alignment and Integration towards Functional Sensing Devices. *Sensors* **2018**, *18* (1), 134.
(25) Song, X.; Han, X.; Yu, F.; Zhang, X.; Chen, L.; Lv, C. Polyamine-Targeting Gefitinib Prodrug and its Near-Infrared Fluorescent Theranostic Derivative for Monitoring Drug Delivery and Lung Cancer Therapy. *Theranostics* **2018**, *8* (8), 2217−2228.
(26) Zhang, X.; He, N.; Huang, Y.; Yu, F.; Li, B.; Lv, C.; Chen, L. Mitochondria-targeting near-infrared ratiometric fluorescent probe for selective imaging of cysteine in orthotopic lung cancer mice. *Sens. Actuators, B* **2019**, *282*, 69−77.
(27) Gao, Z.; Sun, J.; Gao, M.; Yu, F.; Chen, L.; Chen, Q. A unique off-on near-infrared cyanine-based probe for imaging of endogenous alkaline phosphatase activity in cells and in vivo. *Sens. Actuators, B* **2018**, *265*, 565−574.
(28) Lopes, R.; Miranda, M. L.; Schuette, H.; Gassmann, S.; Zielinski, O. Microfluidic approach for controlled ultraviolet treatment of colored and fluorescent dissolved organic matter. *Spectrochim. Acta, Part A* **2020**, *239*, 118435.
(29) Wunsch, B.; Smith, J.; Gifford, S.; Wang, C.; Brink, M.; Bruce, R.; Austin, R.; Stolovitzky, G.; Astier, Y. Nanoscale lateral displacement arrays for the separation of exosomes and colloids down to 20 nm. *Nat. Nanotechnol.* **2016**, *11* (11), 936−940.
(30) Jeong, H.; Shin, H.; Yi, J.; Park, Y.; Lee, J.; Gianchandani, Y.; Park, J. Size-based analysis of extracellular vesicles using sequential transfer of an evaporating droplet. *Lab Chip* **2019**, *19* (19), 3326−3336.
(31) Zhao, L.; Xing, Y.; Wang, R.; Yu, F.; Yu, F. Self-Assembled Nanomaterials for Enhanced Phototherapy of Cancer. *ACS Appl. Bio Mater.* **2020**, *3* (1), 86−106.
(32) Ven, K.; Vanspauwen, B.; Pérez-Ruiz, E.; Leirs, K.; Decrop, D.; Gerstmans, H.; Spasic, D.; Lammertyn, J. Target Confinement in Small Reaction Volumes Using Microfluidic Technologies: A Smart Approach for Single-Entity Detection and Analysis. *ACS Sens.* **2018**, *3* (2), 264−284.
(33) Akkilic, N.; Geschwindner, S.; Höök, F. Single-molecule biosensors: Recent advances and applications. *Biosens. Bioelectron.* **2020**, *151*, 111944.
(34) Cohen, N.; Sabhachandani, P.; Golberg, A.; Konry, T. Approaching near real-time biosensing: Microfluidic microsphere based biosensor for real-time analyte detection. *Biosens. Bioelectron.* **2015**, *66*, 454−460.
(35) Lee, M.; Hsu, W.; Huang, H.; Tseng, H.; Lee, C.; Hsu, C.; Shieh, Y.; Wang, S.; Yao, D.; Liu, C. Simultaneous detection of two growth factors from human single-embryo culture medium by a bead-based digital microfluidic chip. *Biosens. Bioelectron.* **2020**, *150*, 111851.
(36) Zhang, W.; He, Z.; Yi, L.; Mao, S.; Li, H.; Lin, J.-M. A dual-functional microfluidic chip for on-line detection of interleukin-8 based on rolling circle amplification. *Biosens. Bioelectron.* **2018**, *102*, 652−660.
(37) Tu, L.; Xu, Y. L.; Ouyang, Q. Y.; Li, X. Y.; Sun, Y. Recent advances on small-molecule fluorophores with emission beyond 1000 nm for better molecular imaging in vivo. *Chin. Chem. Lett.* **2019**, *30* (10), 1731−1737.
(38) Yang, J.; Liu, X.; Pan, Y.; Yang, J.; He, B.; Fu, Y.; Song, Y. A self-powered microfluidic chip integrated with fluorescent microscopic counting for biomarkers assay. *Sens. Actuators, B* **2019**, *291*, 192−199.
(39) Wang, R.; Han, X.; You, J.; Yu, F.; Chen, L. Ratiometric Near-Infrared Fluorescent Probe for Synergistic Detection of Monoamine Oxidase B and Its Contribution to Oxidative Stress in Cell and Mice Aging Models. *Anal. Chem.* **2018**, *90* (6), 4054−4061.
(40) Han, X.; Song, X.; Yu, F.; Chen, L. A ratiometric fluorescent probe for imaging and quantifying anti-apoptotic effects of GSH under temperature stress. *Chem. Sci.* **2017**, *8* (10), 6991−7002.
(41) Luo, X.; Wang, R.; Lv, C.; Chen, G.; You, J.; Yu, F. Detection of Selenocysteine with a Ratiometric near-Infrared Fluorescent Probe in Cells and in Mice Thyroid Diseases Model. *Anal. Chem.* **2020**, *92* (1), 1589−1597.
(42) Han, X.; Song, X.; Yu, F.; Chen, L. A Ratiometric Near-Infrared Fluorescent Probe for Quantification and Evaluation of Selenocysteine-Protective Effects in Acute Inflammation. *Adv. Funct. Mater.* **2017**, *27* (28), 1700769.
(43) Cedillo-Alcantar, D. F.; Han, Y. D.; Choi, J.; Garcia-Cordero, J. L.; Revzin, A. Automated Droplet-Based Microfluidic Platform for Multiplexed Analysis of Biochemical Markers in Small Volumes. *Anal. Chem.* **2019**, *91* (8), 5133−5141.
(44) Chen, Y. S.; Li, L.; Chen, W. J.; Chen, H. Y.; Yin, J. Near-infrared small molecular fluorescent dyes for photothermal therapy. *Chin. Chem. Lett.* **2019**, *30* (7), 1353−1360.
(45) Li, P.; Long, F.; Chen, W.; Chen, J.; Chu, P. K.; Wang, H. Fundamentals and applications of surface-enhanced Raman spectroscopy−based biosensors. *Curr. Opin. Biomed. Eng.* **2020**, *13*, 51−59.
(46) Gao, R.; Ko, J.; Cha, K.; Ho Jeon, J.; Rhie, G.; Choi, J.; de Mello, A. J.; Choo, J. Fast and sensitive detection of an anthrax biomarker using SERS-based solenoid microfluidic sensor. *Biosens. Bioelectron.* **2015**, *72*, 230−236.
(47) Gao, R.; Cheng, Z.; de Mello, A. J.; Choo, J. Wash-free magnetic immunoassay of the PSA cancer marker using SERS and droplet microfluidics. *Lab Chip* **2016**, *16* (6), 1022−1029.
(48) Reza, K. K.; Sina, A. A. I.; Wuethrich, A.; Grewal, Y. S.; Howard, C. B.; Korbie, D.; Trau, M. A SERS microfluidic platform for targeting multiple soluble immune checkpoints. *Biosens. Bioelectron.* **2019**, *126*, 178−186.
(49) Marek Piliarik, H. V.; Homola, J. Surface Plasmon Resonance Biosensing. In *Biosensors and Biodetection*; Rasooly, A. H. K. E., Ed.; Humana Press: 2009; Vol. *503*, pp 65−88.
(50) Singh, P. SPR Biosensors: Historical Perspectives and Current Challenges. *Sens. Actuators, B* **2016**, *229*, 110−130.
(51) Inci, F.; Saylan, Y.; Kojouri, A. M.; Ogut, M. G.; Denizli, A.; Demirci, U. A disposable microfluidic-integrated hand-held plasmonic platform for protein detection. *Appl. Mater. Today* **2020**, *18*, 100478.

https://dx.doi.org/10.1021/acsabm.0c01271
ACS Appl. Bio Mater. 2021, 4, 2160−2191

(52) Chen, P.; Chung, M. T.; McHugh, W.; Nidetz, R.; Li, Y.; Fu, J.; Cornell, T. T.; Shanley, T. P.; Kurabayashi, K. Multiplex Serum Cytokine Immunoassay Using Nanoplasmonic Biosensor Microarrays. *ACS Nano* **2015**, *9* (4), 4173−4181.
(53) Roether, J.; Chu, K.-Y.; Willenbacher, N.; Shen, A. Q.; Bhalla, N. Real-time monitoring of DNA immobilization and detection of DNA polymerase activity by a microfluidic nanoplasmonic platform. *Biosens. Bioelectron.* **2019**, *142*, 111528.
(54) Roda, A.; Mirasoli, M.; Michelini, E.; Di Fusco, M.; Zangheri, M.; Cevenini, L.; Roda, B.; Simoni, P. Progress in chemical luminescence-based biosensors: A critical review. *Biosens. Bioelectron.* **2016**, *76*, 164−179.
(55) Yang, R.; Li, F.; Zhang, W.; Shen, W.; Yang, D.; Bian, Z.; Cui, H. Chemiluminescence Immunoassays for Simultaneous Detection of Three Heart Disease Biomarkers Using Magnetic Carbon Composites and Three-Dimensional Microfluidic Paper-Based Device. *Anal. Chem.* **2019**, *91* (20), 13006−13013.
(56) Weihs, F.; Gel, M.; Wang, J.; Anderson, A.; Trowell, S.; Dacres, H. Development and characterisation of a compact device for rapid real-time-on-chip detection of thrombin activity in human serum using bioluminescence resonance energy transfer (BRET). *Biosens. Bioelectron.* **2020**, *158*, 112162.
(57) Chinnadayyala, S. R.; Park, J.; Le, H. T. N.; Santhosh, M.; Kadam, A. N.; Cho, S. Recent advances in microfluidic paper-based electrochemiluminescence analytical devices for point-of-care testing applications. *Biosens. Bioelectron.* **2019**, *126*, 68−81.
(58) Tang, C. K.; Vaze, A.; Shen, M.; Rusling, J. F. High-Throughput Electrochemical Microfluidic Immunoarray for Multiplexed Detection of Cancer Biomarker Proteins. *ACS Sens.* **2016**, *1* (8), 1036−1043.
(59) Lee, K.; Yoon, T.; Yang, H.; Cha, S.; Cheon, Y.; Kashefi-Kheyrabadi, L.; Jung, H. All-in-one platform for salivary cotinine detection integrated with a microfluidic channel and an electrochemical biosensor. *Lab Chip* **2020**, *20* (2), 320−331.
(60) Yakoh, A.; Chaiyo, S.; Siangproh, W.; Chailapakul, O. 3D Capillary-Driven Paper-Based Sequential Microfluidic Device for Electrochemical Sensing Applications. *ACS Sens.* **2019**, *4* (5), 1211−1221.
(61) Mozammal Hossain, M.; Moon, J.; Gurudatt, N.; Park, D.; Choi, C.; Shim, Y. Separation detection of hemoglobin and glycated hemoglobin fractions in blood using the electrochemical microfluidic channel with a conductive polymer composite sensor. *Biosens. Bioelectron.* **2019**, *142*, 111515.
(62) Sun, X.; Jian, Y.; Wang, H.; Ge, S.; Yan, M.; Yu, J. Ultrasensitive Microfluidic Paper-Based Electrochemical Biosensor Based on Molecularly Imprinted Film and Boronate Affinity Sandwich Assay for Glycoprotein Detection. *ACS Appl. Mater. Interfaces* **2019**, *11* (17), 16198−16206.
(63) Seenivasan, R.; Singh, C. K.; Warrick, J. W.; Ahmad, N.; Gunasekaran, S. Microfluidic-integrated patterned ITO immunosensor for rapid detection of prostate-specific membrane antigen biomarker in prostate cancer. *Biosens. Bioelectron.* **2017**, *95*, 160−167.
(64) Wang, Y.; Xu, H.; Luo, J.; Liu, J.; Wang, L.; Fan, Y.; Yan, S.; Yang, Y.; Cai, X. A novel label-free microfluidic paper-based immunosensor for highly sensitive electrochemical detection of carcinoembryonic antigen. *Biosens. Bioelectron.* **2016**, *83*, 319−326.
(65) Ali, M.; Mondal, K.; Jiao, Y.; Oren, S.; Xu, Z.; Sharma, A.; Dong, L. Microfluidic Immuno-Biochip for Detection of Breast Cancer Biomarkers Using Hierarchical Composite of Porous Graphene and Titanium Dioxide Nanofibers. *ACS Appl. Mater. Interfaces* **2016**, *8* (32), 20570−20582.
(66) Singh, N.; Ali, M. A.; Rai, P.; Sharma, A.; Malhotra, B. D.; John, R. Microporous Nanocomposite Enabled Microfluidic Biochip for Cardiac Biomarker Detection. *ACS Appl. Mater. Interfaces* **2017**, *9* (39), 33576−33588.
(67) Lan, F.; Sun, G.; Liang, L.; Ge, S.; Yan, M.; Yu, J. Microfluidic paper-based analytical device for photoelectrochemical immunoassay with multiplex signal amplification using multibranched hybridization chain reaction and PdAu enzyme mimetics. *Biosens. Bioelectron.* **2016**, *79*, 416−422.
(68) Zhou, Q.; Lin, Y.; Zhang, K.; Li, M.; Tang, D. Reduced graphene oxide/$BiFeO_3$ nanohybrids-based signal-on photoelectrochemical sensing system for prostate-specific antigen detection coupling with magnetic microfluidic device. *Biosens. Bioelectron.* **2018**, *101*, 146−152.
(69) Sinha, A.; Tai, T.; Li, K.; Gopinathan, P.; Chung, Y.; Sarangadharan, I.; Ma, H.; Huang, P.; Shiesh, S.; Wang, Y.; Lee, G. An integrated microfluidic system with field-effect-transistor sensor arrays for detecting multiple cardiovascular biomarkers from clinical samples. *Biosens. Bioelectron.* **2019**, *129*, 155−163.
(70) Mok, J.; Mindrinos, M. N.; Davis, R. W.; Javanmard, M. Digital microfluidic assay for protein detection. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111* (6), 2110.
(71) Lee, H.; Meeseepong, M.; Trung, T.; Kim, B.; Lee, N. A wearable lab-on-a-patch platform with stretchable nanostructured biosensor for non-invasive immunodetection of biomarker in sweat. *Biosens. Bioelectron.* **2020**, *156*, 112133.
(72) Beauchamp, M. J.; Nielsen, A. V.; Gong, H.; Nordin, G. P.; Woolley, A. T. 3D Printed Microfluidic Devices for Microchip Electrophoresis of Preterm Birth Biomarkers. *Anal. Chem.* **2019**, *91* (11), 7418−7425.
(73) Singh, N.; Ali, M. A.; Rai, P.; Ghori, I.; Sharma, A.; Malhotra, B. D.; John, R. Dual-modality microfluidic biosensor based on nanoengineered mesoporous graphene hydrogels. *Lab Chip* **2020**, *20* (4), 760−777.
(74) Schwarzenbach, H.; Hoon, D. S. B.; Pantel, K. Cell-free nucleic acids as biomarkers in cancer patients. *Nat. Rev. Cancer* **2011**, *11* (6), 426−437.
(75) Horrocks, M. H.; Tosatto, L.; Dear, A. J.; Garcia, G. A.; Iljina, M.; Cremades, N.; Dalla Serra, M.; Knowles, T. P. J.; Dobson, C. M.; Klenerman, D. Fast Flow Microfluidics and Single-Molecule Fluorescence for the Rapid Characterization of α-Synuclein Oligomers. *Anal. Chem.* **2015**, *87* (17), 8818−8826.
(76) Huang, Y.; Shi, Y.; Yang, H. Y.; Ai, Y. A novel single-layered MoS_2 nanosheet based microfluidic biosensor for ultrasensitive detection of DNA. *Nanoscale* **2015**, *7* (6), 2245−2249.
(77) Cao, H.; Zhou, X.; Zeng, Y. Microfluidic exponential rolling circle amplification for sensitive microRNA detection directly from biological samples. *Sens. Actuators, B* **2019**, *279*, 447−457.
(78) Lednický, T.; Bonyár, A. Large Scale Fabrication of Ordered Gold Nanoparticle−Epoxy Surface Nanocomposites and Their Application as Label-Free Plasmonic DNA Biosensors. *ACS Appl. Mater. Interfaces* **2020**, *12* (4), 4804−4814.
(79) Shamsi, M. H.; Choi, K.; Ng, A. H. C.; Chamberlain, M. D.; Wheeler, A. R. Electrochemiluminescence on digital microfluidics for microRNA analysis. *Biosens. Bioelectron.* **2016**, *77*, 845−852.
(80) Sun, X.; Wang, H.; Jian, Y.; Lan, F.; Zhang, L.; Liu, H.; Ge, S.; Yu, J. Ultrasensitive microfluidic paper-based electrochemical/visual biosensor based on spherical-like cerium dioxide catalyst for miR-21 detection. *Biosens. Bioelectron.* **2018**, *105*, 218−225.
(81) Kutluk, H.; Bruch, R.; Urban, G. A.; Dincer, C. Impact of assay format on miRNA sensing: Electrochemical microfluidic biosensor for miRNA-197 detection. *Biosens. Bioelectron.* **2020**, *148*, 111824.
(82) Bruch, R.; Baaske, J.; Chatelle, C.; Meirich, M.; Madlener, S.; Weber, W.; Dincer, C.; Urban, G. A. CRISPR/Cas13a-Powered Electrochemical Microfluidic Biosensor for Nucleic Acid Amplification-Free miRNA Diagnostics. *Adv. Mater.* **2019**, *31* (51), 1905311.
(83) Hwang, M. T.; Heiranian, M.; Kim, Y.; You, S.; Leem, J.; Taqieddin, A.; Faramarzi, V.; Jing, Y.; Park, I.; van der Zande, A. M.; Nam, S.; Aluru, N. R.; Bashir, R. Ultrasensitive detection of nucleic acids using deformed graphene channel field effect biosensors. *Nat. Commun.* **2020**, *11* (1), 1543.
(84) Ghrera, A. S.; Pandey, C. M.; Malhotra, B. D. Multiwalled carbon nanotube modified microfluidic-based biosensor chip for nucleic acid detection. *Sens. Actuators, B* **2018**, *266*, 329−336.

https://dx.doi.org/10.1021/acsabm.0c01271
ACS Appl. Bio Mater. 2021, 4, 2160−2191

(85) Dhoot, D. S.; Avery, R. L. Vascular Endothelial Growth Factor Inhibitors for Diabetic Retinopathy. *Curr. Diabetes Rep.* **2016**, *16* (12), 122.
(86) Wu, J.; Wang, R.; Yu, H.; Li, G.; Xu, K.; Tien, N. C.; Roberts, R. C.; Li, D. Inkjet-printed microelectrodes on PDMS as biosensors for functionalized microfluidic systems. *Lab Chip* **2015**, *15* (3), 690−695.
(87) You, K. E.; Uddin, N.; Kim, T. H.; Fan, Q. H.; Yoon, H. J. Highly sensitive detection of biological substances using microfluidic enhanced Fabry-Perot etalon-based optical biosensors. *Sens. Actuators, B* **2018**, *277*, 62−68.
(88) Xiao, J.; Liu, Y.; Su, L.; Zhao, D.; Zhao, L.; Zhang, X. Microfluidic Chip-Based Wearable Colorimetric Sensor for Simple and Facile Detection of Sweat Glucose. *Anal. Chem.* **2019**, *91* (23), 14803−14807.
(89) Triroj, N.; Saensak, R.; Porntheeraphat, S.; Paosawatyanyong, B.; Amornkitbamrung, V. Diamond-Like Carbon Thin Film Electrodes for Microfluidic Bioelectrochemical Sensing Platforms. *Anal. Chem.* **2020**, *92* (5), 3650−3657.
(90) Armbrecht, L.; Dincer, C.; Kling, A.; Horak, J.; Kieninger, J.; Urban, G. Self-assembled magnetic bead chains for sensitivity enhancement of microfluidic electrochemical biosensor platforms. *Lab Chip* **2015**, *15* (22), 4314−4321.
(91) Mohammad, M.; Razmjou, A.; Liang, K.; Asadnia, M.; Chen, V. Metal−Organic-Framework-Based Enzymatic Microfluidic Biosensor via Surface Patterning and Biomineralization. *ACS Appl. Mater. Interfaces* **2019**, *11* (2), 1807−1820.
(92) Zhu, X.; Huang, J.; Liu, J.; Zhang, H.; Jiang, J.; Yu, R. A dual enzyme−inorganic hybrid nanoflower incorporated microfluidic paper-based analytic device (μPAD) biosensor for sensitive visualized detection of glucose. *Nanoscale* **2017**, *9* (17), 5658−5663.
(93) Agustini, D.; Bergamini, M. F.; Marcolino-Junior, L. H. Tear glucose detection combining microfluidic thread based device, amperometric biosensor and microflow injection analysis. *Biosens. Bioelectron.* **2017**, *98*, 161−167.
(94) Ji, X.; Lau, H. Y.; Ren, X.; Peng, B.; Zhai, P.; Feng, S.-P.; Chan, P. K. L. Highly Sensitive Metabolite Biosensor Based on Organic Electrochemical Transistor Integrated with Microfluidic Channel and Poly(N-vinyl-2-pyrrolidone)-Capped Platinum Nanoparticles. *Adv. Mater. Technol.* **2016**, *1* (5), 1600042.
(95) Phypers, B.; Pierce, J. M. T. Lactate physiology in health and disease. *Contin. Educ. Anaesth. Crit. Care Pain* **2006**, *6* (3), 128−132.
(96) Gowers, S. A. N.; Curto, V. F.; Seneci, C. A.; Wang, C.; Anastasova, S.; Vadgama, P.; Yang, G.-Z.; Boutelle, M. G. 3D Printed Microfluidic Device with Integrated Biosensors for Online Analysis of Subcutaneous Human Microdialysate. *Anal. Chem.* **2015**, *87* (15), 7763−7770.
(97) Nightingale, A. M.; Leong, C. L.; Burnish, R. A.; Hassan, S.-u.; Zhang, Y.; Clough, G. F.; Boutelle, M. G.; Voegeli, D.; Niu, X. Monitoring biomolecule concentrations in tissue using a wearable droplet microfluidic-based sensor. *Nat. Commun.* **2019**, *10* (1), 2741.
(98) Li, F.; Liu, J.; Guo, L.; Wang, J.; Zhang, K.; He, J.; Cui, H. High-resolution temporally resolved chemiluminescence based on double-layered 3D microfluidic paper-based device for multiplexed analysis. *Biosens. Bioelectron.* **2019**, *141*, 111472.
(99) Shi, M.; Bradner, J.; Hancock, A. M.; Chung, K. A.; Quinn, J. F.; Peskind, E. R.; Galasko, D.; Jankovic, J.; Zabetian, C. P.; Kim, H. M.; Leverenz, J. B.; Montine, T. J.; Ginghina, C.; Kang, U. J.; Cain, K. C.; Wang, Y.; Aasly, J.; Goldstein, D.; Zhang, J. Cerebrospinal fluid biomarkers for Parkinson disease diagnosis and progression. *Ann. Neurol.* **2011**, *69* (3), 570−580.
(100) Xing, Y.; Wyss, A.; Esser, N.; Dittrich, P. S. Label-free biosensors based on in situ formed and functionalized microwires in microfluidic devices. *Analyst* **2015**, *140* (23), 7896−7901.
(101) Seo, K.-D.; Hossain, M. M. D.; Gurudatt, N. G.; Choi, C. S.; Shiddiky, M. J. A.; Park, D.-S.; Shim, Y.-B. Microfluidic neurotransmitters sensor in blood plasma with mediator-immobilized conducting polymer/N, S-doped porous carbon composite. *Sens. Actuators, B* **2020**, *313*, 128017.
(102) Bi, H.; Duarte, C. M.; Brito, M.; Vilas-Boas, V.; Cardoso, S.; Freitas, P. Performance enhanced UV/vis spectroscopic microfluidic sensor for ascorbic acid quantification in human blood. *Biosens. Bioelectron.* **2016**, *85*, 568−572.
(103) Cao, L.; Fang, C.; Zeng, R.; Zhao, X.; Jiang, Y.; Chen, Z. Paper-based microfluidic devices for electrochemical immunofiltration analysis of human chorionic gonadotropin. *Biosens. Bioelectron.* **2017**, *92*, 87−94.
(104) Cinti, S.; Minotti, C.; Moscone, D.; Palleschi, G.; Arduini, F. Fully integrated ready-to-use paper-based electrochemical biosensor to detect nerve agents. *Biosens. Bioelectron.* **2017**, *93*, 46−51.
(105) Kaur, G.; Tomar, M.; Gupta, V. Development of a microfluidic electrochemical biosensor: Prospect for point-of-care cholesterol monitoring. *Sens. Actuators, B* **2018**, *261*, 460−466.
(106) Roushani, M.; Valipour, A. Using electrochemical oxidation of Rutin in modeling a novel and sensitive immunosensor based on Pt nanoparticle and graphene−ionic liquid−chitosan nanocomposite to detect human chorionic gonadotropin. *Sens. Actuators, B* **2016**, *222*, 1103−1111.
(107) Liu, J.; Jiang, X.; Zhang, R.; Zhang, Y.; Wu, L.; Lu, W.; Li, J.; Li, Y.; Zhang, H. MXene-Enabled Electrochemical Microfluidic Biosensor: Applications toward Multicomponent Continuous Monitoring in Whole Blood. *Adv. Funct. Mater.* **2019**, *29* (6), 1807326.
(108) Koh, A.; Kang, D.; Xue, Y.; Lee, S.; Pielak, R. M.; Kim, J.; Hwang, T.; Min, S.; Banks, A.; Bastien, P.; Manco, M. C.; Wang, L.; Ammann, K. R.; Jang, K. I.; Won, P.; Han, S.; Ghaffari, R.; Paik, U.; Slepian, M. J.; Balooch, G.; Huang, Y.; Rogers, J. A. A soft, wearable microfluidic device for the capture, storage, and colorimetric sensing of sweat. *Sci. Transl. Med.* **2016**, *8* (366), No. 366ra165.
(109) Yetisen, A.; Jiang, N.; Tamayol, A.; Ruiz-Esparza, G.; Zhang, Y.; Medina-Pando, S.; Gupta, A.; Wolffsohn, J.; Butt, H.; Khademhosseini, A.; Yun, S. Paper-based microfluidic system for tear electrolyte analysis. *Lab Chip* **2017**, *17* (6), 1137−1148.
(110) Remiszewska, E.; Malecha, K.; Kruk, J.; Jankowska-Śliwińska, J.; Torbicz, W.; Samluk, A.; Pluta, K. D.; Pijanowska, D. G. Enzymatic method of urea determination in LTCC microfluidic system based on absorption photometry. *Sens. Actuators, B* **2019**, *285*, 375−384.
(111) Li, S.; Ma, Z.; Cao, Z.; Pan, L.; Shi, Y. Advanced Wearable Microfluidic Sensors for Healthcare Monitoring. *Small* **2020**, *16* (9), 1903822.
(112) Martín, A.; Kim, J.; Kurniawan, J. F.; Sempionatto, J. R.; Moreto, J. R.; Tang, G.; Campbell, A. S.; Shin, A.; Lee, M. Y.; Liu, X.; Wang, J. Epidermal Microfluidic Electrochemical Detection System: Enhanced Sweat Sampling and Metabolite Detection. *ACS Sens.* **2017**, *2* (12), 1860−1868.
(113) Choi, J.; Bandodkar, A.; Reeder, J.; Ray, T.; Turnquist, A.; Kim, S.; Nyberg, N.; Hourlier-Fargette, A.; Model, J.; Aranyosi, A.; Xu, S.; Ghaffari, R.; Rogers, J. A. Soft, Skin-Integrated Multifunctional Microfluidic Systems for Accurate Colorimetric Analysis of Sweat Biomarkers and Temperature. *ACS Sens.* **2019**, *4* (2), 379−388.
(114) Nyein, H.; Tai, L.; Ngo, Q.; Chao, M.; Zhang, G.; Gao, W.; Bariya, M.; Bullock, J.; Kim, H.; Fahad, H.; Javey, A. A Wearable Microfluidic Sensing Patch for Dynamic Sweat Secretion Analysis. *ACS Sens.* **2018**, *3* (5), 944−952.
(115) Liao, Z.; Wang, J.; Zhang, P.; Zhang, Y.; Miao, Y.; Gao, S.; Deng, Y.; Geng, L. Recent advances in microfluidic chip integrated electronic biosensors for multiplexed detection. *Biosens. Bioelectron.* **2018**, *121*, 272−280.
(116) Sibbitts, J.; Sellens, K. A.; Jia, S.; Klasner, S. A.; Culbertson, C. T. Cellular Analysis Using Microfluidics. *Anal. Chem.* **2018**, *90* (1), 65−85.
(117) Gao, M.; Yu, F.; Lv, C.; Choo, J.; Chen, L. Fluorescent chemical probes for accurate tumor diagnosis and targeting therapy. *Chem. Soc. Rev.* **2017**, *46* (8), 2237−2271.
(118) Gao, M.; Wang, R.; Yu, F.; Chen, L. Evaluation of sulfane sulfur bioeffects via a mitochondria-targeting selenium-containing near-infrared fluorescent probe. *Biomaterials* **2018**, *160*, 1−14.
(119) Zou, Y.; Li, M.; Xing, Y.; Duan, T.; Zhou, X.; Yu, F. Bioimaging of Glutathione with a Two-Photon Fluorescent Probe and

https://dx.doi.org/10.1021/acsabm.0c01271
ACS Appl. Bio Mater. 2021, 4, 2160−2191

Its Potential Application for Surgery Guide in Laryngeal Cancer. *ACS Sens.* **2020**, *5* (1), 242−249.
(120) Wang, Y.; Yu, F.; Luo, X.; Li, M.; Zhao, L.; Yu, F. Visualization of carboxylesterase 2 with a near-infrared two-photon fluorescent probe and potential evaluation of its anticancer drug effects in an orthotopic colon carcinoma mice model. *Chem. Commun.* **2020**, *56* (32), 4412−4415.
(121) Liang, L.; Su, M.; Li, L.; Lan, F.; Yang, G.; Ge, S.; Yu, J.; Song, X. Aptamer-based fluorescent and visual biosensor for multiplexed monitoring of cancer cells in microfluidic paper-based analytical devices. *Sens. Actuators, B* **2016**, *229*, 347−354.
(122) De Ninno, A.; Reale, R.; Giovinazzo, A.; Bertani, F. R.; Businaro, L.; Bisegna, P.; Matteucci, C.; Caselli, F. High-throughput label-free characterization of viable, necrotic and apoptotic human lymphoma cells in a coplanar-electrode microfluidic impedance chip. *Biosens. Bioelectron.* **2020**, *150*, 111887.
(123) Liu, C.-F.; Wang, M.-H.; Jang, L.-S. Microfluidics-based hairpin resonator biosensor for biological cell detection. *Sens. Actuators, B* **2018**, *263*, 129−136.
(124) Solis-Tinoco, V.; Marquez, S.; Quesada-Lopez, T.; Villarroya, F.; Homs-Corbera, A.; Lechuga, L. M. Building of a flexible microfluidic plasmo-nanomechanical biosensor for live cell analysis. *Sens. Actuators, B* **2019**, *291*, 48−57.
(125) Rodriguez-Moncayo, R.; Jimenez-Valdes, R.; Gonzalez-Suarez, A.; Garcia-Cordero, J. Integrated Microfluidic Device for Functional Secretory Immunophenotyping of Immune Cells. *ACS Sens.* **2020**, *5* (2), 353−361.
(126) Lin, X.; Leung, K.; Lin, L.; Lin, L.; Lin, S.; Leung, C.; Ma, D.; Lin, J. Determination of cell metabolite VEGF165 and dynamic analysis of protein−DNA interactions by combination of microfluidic technique and luminescent switch-on probe. *Biosens. Bioelectron.* **2016**, *79*, 41−47.
(127) Li, X.; Soler, M.; Özdemir, C. I.; Belushkin, A.; Yesilköy, F.; Altug, H. Plasmonic nanohole array biosensor for label-free and real-time analysis of live cell secretion. *Lab Chip* **2017**, *17* (13), 2208−2217.
(128) Liu, M.; Li, S.; Huang, D.; Xu, Z.; Wu, Y.; Lei, Y.; Liu, A. MoOx quantum dots with peroxidase-like activity on microfluidic paper-based analytical device for rapid colorimetric detection of H_2O_2 released from PC12 cells. *Sens. Actuators, B* **2020**, *305*, 127512.
(129) Zhou, Q.; Patel, D.; Kwa, T.; Haque, A.; Matharu, Z.; Stybayeva, G.; Gao, Y.; Diehl, A. M.; Revzin, A. Liver injury-on-a-chip: microfluidic co-cultures with integrated biosensors for monitoring liver cell signaling during injury. *Lab Chip* **2015**, *15* (23), 4467−4478.
(130) Shin, S.; Zhang, Y.; Kim, D.; Manbohi, A.; Avci, H.; Silvestri, A.; Aleman, J.; Hu, N.; Kilic, T.; Keung, W.; Righi, M.; Assawes, P.; Alhadrami, H.; Li, R.; Dokmeci, M.; Khademhosseini, A. Aptamer-Based Microfluidic Electrochemical Biosensor for Monitoring Cell-Secreted Trace Cardiac Biomarkers. *Anal. Chem.* **2016**, *88* (20), 10019−10027.
(131) Shin, S. R.; Kilic, T.; Zhang, Y.; Avci, H.; Hu, N.; Kim, D.; Branco, C.; Aleman, J.; Massa, S.; Silvestri, A.; Kang, J.; Desalvo, A.; Hussaini, M.; Chae, S.; Polini, A.; Bhise, N.; Hussain, M.; Lee, H.; Dokmeci, M.; Khademhosseini, A. Label-Free and Regenerative Electrochemical Microfluidic Biosensors for Continual Monitoring of Cell Secretomes. *Adv. Sci.* **2017**, *4* (5), 1600522.
(132) Akhtar, M.; Hussain, K.; Gurudatt, N. G.; Shim, Y. Detection of Ca^{2+}-induced acetylcholine released from leukemic T-cells using an amperometric microfluidic sensor. *Biosens. Bioelectron.* **2017**, *98*, 364−370.
(133) Tavakoli, H.; Zhou, W.; Ma, L.; Perez, S.; Ibarra, A.; Xu, F.; Zhan, S.; Li, X. Recent advances in microfluidic platforms for single-cell analysis in cancer biology, diagnosis and therapy. *TrAC, Trends Anal. Chem.* **2019**, *117*, 13−26.
(134) Dusny, C.; Grünberger, A. Microfluidic single-cell analysis in biotechnology: from monitoring towards understanding. *Curr. Opin. Biotechnol.* **2020**, *63*, 26−33.
(135) Zhu, J.; Wang, Y.; Chen, P.; Su, H.; Du, W.; Liu, B. Highly efficient microfluidic device for cell trapping and pairing towards cell-cell communication analysis. *Sens. Actuators, B* **2019**, *283*, 685−692.
(136) Lu, S.; Long, Y. Confined Nanopipette-A new microfluidic approach for single cell analysis. *TrAC, Trends Anal. Chem.* **2019**, *117*, 39−46.
(137) Liu, L.; Yang, H.; Men, D.; Wang, M.; Gao, X.; Zhang, T.; Chen, D.; Xue, C.; Wang, Y.; Wang, J.; Chen, J. Development of microfluidic platform capable of high-throughput absolute quantification of single-cell multiple intracellular proteins from tumor cell lines and patient tumor samples. *Biosens. Bioelectron.* **2020**, *155*, 112097.
(138) Inomata, N.; Toda, M.; Ono, T. Highly sensitive thermometer using a vacuum-packed Si resonator in a microfluidic chip for the thermal measurement of single cells. *Lab Chip* **2016**, *16* (18), 3597−3603.
(139) Cermak, N.; Olcum, S.; Delgado, F. F.; Wasserman, S. C.; Payer, K. R.; A Murakami, M.; Knudsen, S. M.; Kimmerling, R. J.; Stevens, M. M.; Kikuchi, Y.; Sandikci, A.; Ogawa, M.; Agache, V.; Baléras, F.; Weinstock, D. M.; Manalis, S. R. High-throughput measurement of single-cell growth rates using serial microfluidic mass sensor arrays. *Nat. Biotechnol.* **2016**, *34* (10), 1052−1059.
(140) Hou, J.; Wang, B.; Zou, Y.; Fan, P.; Chang, X.; Cao, X.; Wang, S.; Yu, F. Molecular Fluorescent Probes for Imaging and Evaluation of Hypochlorite Fluctuations during Diagnosis and Therapy of Osteoarthritis in Cells and in a Mouse Model. *ACS Sens.* **2020**, *5* (7), 1949−1958.
(141) Hassanzadeh-Barforoushi, A.; Warkiani, M.; Gallego-Ortega, D.; Liu, G.; Barber, T. Capillary-assisted microfluidic biosensing platform captures single cell secretion dynamics in nanoliter compartments. *Biosens. Bioelectron.* **2020**, *155*, 112113.
(142) Armbrecht, L.; Gabernet, G.; Kurth, F.; Hiss, J.; Schneider, G.; Dittrich, P. Characterisation of anticancer peptides at the single-cell level. *Lab Chip* **2017**, *17* (17), 2933−2940.
(143) Jing, T.; Ramji, R.; Warkiani, M.; Han, J.; Lim, C.; Chen, C. Jetting microfluidics with size-sorting capability for single-cell protease detection. *Biosens. Bioelectron.* **2015**, *66*, 19−23.
(144) Shen, R.; Liu, P.; Zhang, Y.; Yu, Z.; Chen, X.; Zhou, L.; Nie, B.; Żaczek, A.; Chen, J.; Liu, J. Sensitive Detection of Single-Cell Secreted H_2O_2 by Integrating a Microfluidic Droplet Sensor and Au Nanoclusters. *Anal. Chem.* **2018**, *90* (7), 4478−4484.
(145) Chen, F.; Xue, J.; Zhang, J.; Bai, M.; Yu, X.; Fan, C.; Zhao, Y. Differentiated Visualization of Single-Cell 5-Hydroxymethylpyrimidines with Microfluidic Hydrogel Encoding. *J. Am. Chem. Soc.* **2020**, *142* (6), 2889−2896.
(146) Wang, X.; Ren, L.; Su, Y.; Ji, Y.; Liu, Y.; Li, C.; Li, X.; Zhang, Y.; Wang, W.; Hu, Q.; Han, D.; Xu, J.; Ma, B. Raman-Activated Droplet Sorting (RADS) for Label-Free High-Throughput Screening of Microalgal Single-Cells. *Anal. Chem.* **2017**, *89* (22), 12569−12577.
(147) Yan, S.; Chu, F.; Zhang, H.; Yuan, Y.; Huang, Y.; Liu, A.; Wang, S.; Li, W.; Li, S.; Wen, W. Rapid, one-step preparation of SERS substrate in microfluidic channel for detection of molecules and heavy metal ions. *Spectrochim. Acta, Part A* **2019**, *220*, 117113.
(148) Pallaoro, A.; Hoonejani, M. R.; Braun, G. B.; Meinhart, C. D.; Moskovits, M. Rapid Identification by Surface-Enhanced Raman Spectroscopy of Cancer Cells at Low Concentrations Flowing in a Microfluidic Channel. *ACS Nano* **2015**, *9* (4), 4328−4336.
(149) Dey, S.; Vaidyanathan, R.; Reza, K.; Wang, J.; Wang, Y.; Nel, H.; Law, S.; Tyler, J.; Rossjohn, J.; Reid, H.; Ibn Sina, A.; Thomas, R.; Trau, M. A microfluidic-SERS platform for isolation and immunophenotyping of antigen specific T-cells. *Sens. Actuators, B* **2019**, *284*, 281−288.
(150) Chaffer, C.; Weinberg, R. A perspective on cancer cell metastasis. *Science* **2011**, *331* (6024), 1559−64.
(151) Nagrath, S.; Sequist, L.; Maheswaran, S.; Bell, D.; Irimia, D.; Ulkus, L.; Smith, M.; Kwak, E.; Digumarthy, S.; Muzikansky, A.; Ryan, P.; Balis, U.; Tompkins, R.; Haber, D.; Toner, M. Isolation of rare circulating tumour cells in cancer patients by microchip technology. *Nature* **2007**, *450* (7173), 1235−9.

https://dx.doi.org/10.1021/acsabm.0c01271
ACS Appl. Bio Mater. 2021, 4, 2160−2191

(152) Leung, C.-H.; Wu, K.-J.; Li, G.; Wu, C.; Ko, C.-N.; Ma, D.-L. Application of label-free techniques in microfluidic for biomolecules detection and circulating tumor cells analysis. *TrAC, Trends Anal. Chem.* **2019**, *117*, 78−83.
(153) Ryu, J. Y.; Kim, J.; Shon, M. J.; Sun, J.; Jiang, X.; Lee, W.; Yoon, T.-Y. Profiling protein−protein interactions of single cancer cells with in situ lysis and co-immunoprecipitation. *Lab Chip* **2019**, *19* (11), 1922−1928.
(154) Lin, M.; Chen, J.; Lu, Y.; Zhang, Y.; Song, J.; Hou, S.; Ke, Z.; Tseng, H. Nanostructure embedded microchips for detection, isolation, and characterization of circulating tumor cells. *Acc. Chem. Res.* **2014**, *47* (10), 2941−2950.
(155) Hou, S.; Zhao, H.; Zhao, L.; Shen, Q.; Wei, K. S.; Suh, D. Y.; Nakao, A.; Garcia, M. A.; Song, M.; Lee, T.; Xiong, B.; Luo, S.-C.; Tseng, H.-R.; Yu, H.-h. Capture and Stimulated Release of Circulating Tumor Cells on Polymer-Grafted Silicon Nanostructures. *Adv. Mater.* **2013**, *25* (11), 1547−1551.
(156) Peng, J.; Zhao, Q.; Zheng, W.; Li, W.; Li, P.; Zhu, L.; Liu, X.; Shao, B.; Li, H.; Wang, C.; Yang, Y. Peptide-Functionalized Nanomaterials for the Efficient Isolation of HER2-Positive Circulating Tumor Cells. *ACS Appl. Mater. Interfaces* **2017**, *9* (22), 18423−18428.
(157) Xiong, K.; Wei, W.; Jin, Y.; Wang, S.; Zhao, D.; Wang, S.; Gao, X.; Qiao, C.; Yue, H.; Ma, G.; Xie, H.-Y. Biomimetic Immuno-Magnetosomes for High-Performance Enrichment of Circulating Tumor Cells. *Adv. Mater.* **2016**, *28* (36), 7929−7935.
(158) Rao, L.; Meng, Q.; Huang, Q.; Wang, Z.; Yu, G.; Li, A.; Ma, W.; Zhang, N.; Guo, S.; Zhao, X.; Liu, K.; Yuan, Y.; Liu, W. Platelet−Leukocyte Hybrid Membrane-Coated Immunomagnetic Beads for Highly Efficient and Highly Specific Isolation of Circulating Tumor Cells. *Adv. Funct. Mater.* **2018**, *28* (34), 1803531.
(159) Galanzha, E.; Shashkov, E.; Kelly, T.; Kim, J.; Yang, L.; Zharov, V. In vivo magnetic enrichment and multiplex photoacoustic detection of circulating tumour cells. *Nat. Nanotechnol.* **2009**, *4* (12), 855−60.
(160) Hu, X.; Wei, C.; Xia, J.; Pelivanov, I.; O'Donnell, M.; Gao, X. Trapping and Photoacoustic Detection of CTCs at the Single Cell per Milliliter Level with Magneto-Optical Coupled Nanoparticles. *Small* **2013**, *9* (12), 2046−2052.
(161) Anu Prathap, M. U.; Castro-Pérez, E.; Jiménez-Torres, J. A.; Setaluri, V.; Gunasekaran, S. A flow-through microfluidic system for the detection of circulating melanoma cells. *Biosens. Bioelectron.* **2019**, *142*, 111522.
(162) Ghassemi, P.; Ren, X.; Foster, B. M.; Kerr, B. A.; Agah, M. Post-enrichment circulating tumor cell detection and enumeration via deformability impedance cytometry. *Biosens. Bioelectron.* **2020**, *150*, 111868.
(163) Armbrecht, L.; Rutschmann, O.; Szczerba, B. M.; Nikoloff, J.; Aceto, N.; Dittrich, P. S. Quantification of Protein Secretion from Circulating Tumor Cells in Microfluidic Chambers. *Adv. Sci.* **2020**, *7* (11), 1903237.
(164) Mani, G. K.; Morohoshi, M.; Yasoda, Y.; Yokoyama, S.; Kimura, H.; Tsuchiya, K. ZnO-Based Microfluidic pH Sensor: A Versatile Approach for Quick Recognition of Circulating Tumor Cells in Blood. *ACS Appl. Mater. Interfaces* **2017**, *9* (6), 5193−5203.
(165) Del Ben, F.; Turetta, M.; Celetti, G.; Piruska, A.; Bulfoni, M.; Cesselli, D.; Huck, W. T. S.; Scoles, G. A Method for Detecting Circulating Tumor Cells Based on the Measurement of Single-Cell Metabolism in Droplet-Based Microfluidics. *Angew. Chem., Int. Ed.* **2016**, *55* (30), 8581−8584.
(166) Nasseri, B.; Soleimani, N.; Rabiee, N.; Kalbasi, A.; Karimi, M.; Hamblin, M. Point-of-care microfluidic devices for pathogen detection. *Biosens. Bioelectron.* **2018**, *117*, 112−128.
(167) Cesewski, E.; Johnson, B. Electrochemical biosensors for pathogen detection. *Biosens. Bioelectron.* **2020**, *159*, 112214.
(168) Chen, Y.; Qian, C.; Liu, C.; Shen, H.; Wang, Z.; Ping, J.; Wu, J.; Chen, H. Nucleic acid amplification free biosensors for pathogen detection. *Biosens. Bioelectron.* **2020**, *153*, 112049.
(169) Zhou, W.; Le, J.; Chen, Y.; Cai, Y.; Hong, Z.; Chai, Y. Recent advances in microfluidic devices for bacteria and fungus research. *TrAC, Trends Anal. Chem.* **2019**, *112*, 175−195.
(170) Khobragade, S.; Da Silva Granja, C.; Sandström, N.; Efimov, I.; Ostanin, V. P.; van der Wijngaart, W.; Klenerman, D.; Ghosh, S. K. Direct detection of whole bacteria using a nonlinear acoustic resonator. *Sens. Actuators, B* **2020**, *316*, 128086.
(171) Luo, F.; Li, Z.; Dai, G.; Lu, Y.; He, P.; Wang, Q. Ultrasensitive biosensing pathogenic bacteria by combining aptamer-induced catalysed hairpin assembly circle amplification with microchip electrophoresis. *Sens. Actuators, B* **2020**, *306*, 127577.
(172) Xu, T.; Gong, Y.; Su, X.; Zhu, P.; Dai, J.; Xu, J.; Ma, B. Phenome−Genome Profiling of Single Bacterial Cell by Raman-Activated Gravity-Driven Encapsulation and Sequencing. *Small* **2020**, *16* (30), 2001172.
(173) Yoo, S. M.; Lee, S. Y. Optical Biosensors for the Detection of Pathogenic Microorganisms. *Trends Biotechnol.* **2016**, *34* (1), 7−25.
(174) Jalali, M.; AbdelFatah, T.; Mahshid, S.; Labib, M.; Sudalaiyadum Perumal, A.; Mahshid, S. A Hierarchical 3D Nano-structured Microfluidic Device for Sensitive Detection of Pathogenic Bacteria. *Small* **2018**, *14* (35), 1801893.
(175) Wang, S.; Zheng, L.; Cai, G.; Liu, N.; Liao, M.; Li, Y.; Zhang, X.; Lin, J. A microfluidic biosensor for online and sensitive detection of Salmonella typhimurium using fluorescence labeling and smartphone video processing. *Biosens. Bioelectron.* **2019**, *140*, 111333.
(176) Su, C.; Tsai, M.; Lin, C.; Ma, Y.; Wang, C.; Chung, Y.; Lee, G. Dual aptamer assay for detection of Acinetobacter baumannii on an electromagnetically-driven microfluidic platform. *Biosens. Bioelectron.* **2020**, *159*, 112148.
(177) Landry, M. P.; Ando, H.; Chen, A. Y.; Cao, J.; Kottadiel, V. I.; Chio, L.; Yang, D.; Dong, J.; Lu, T. K.; Strano, M. S. Single-molecule detection of protein efflux from microorganisms using fluorescent single-walled carbon nanotube sensor arrays. *Nat. Nanotechnol.* **2017**, *12* (4), 368−377.
(178) Li, H.; Torab, P.; Mach, K.; Surrette, C.; England, M.; Craft, D.; Thomas, N.; Liao, J.; Puleo, C.; Wong, P. Adaptable microfluidic system for single-cell pathogen classification and antimicrobial susceptibility testing. *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116* (21), 10270.
(179) Lin, H.; Huang, C.; Hsieh, W.; Liu, L.; Lin, Y.; Chu, C.; Wang, S.; Kuo, I.; Chau, L.; Yang, C. On-line SERS Detection of Single Bacterium Using Novel SERS Nanoprobes and A Microfluidic Dielectrophoresis Device. *Small* **2014**, *10* (22), 4700−4710.
(180) Chang, K.; Cheng, H.; Shiue, J.; Wang, J.; Wang, Y.; Huang, N. Antibiotic Susceptibility Test with Surface-Enhanced Raman Scattering in a Microfluidic System. *Anal. Chem.* **2019**, *91* (17), 10988−10995.
(181) Burklund, A.; Saturley-Hall, H.; Franchina, F.; Hill, J.; Zhang, J. Printable QR code paper microfluidic colorimetric assay for screening volatile biomarkers. *Biosens. Bioelectron.* **2019**, *128*, 97−103.
(182) Wang, C.; Wu, J.; Lee, G. Screening of highly-specific aptamers and their applications in paper-based microfluidic chips for rapid diagnosis of multiple bacteria. *Sens. Actuators, B* **2019**, *284*, 395−402.
(183) Zheng, L.; Cai, G.; Wang, S.; Liao, M.; Li, Y.; Lin, J. A microfluidic colorimetric biosensor for rapid detection of Escherichia coli O157:H7 using gold nanoparticle aggregation and smart phone imaging. *Biosens. Bioelectron.* **2019**, *124−125*, 143−149.
(184) Altintas, Z.; Akgun, M.; Kokturk, G.; Uludag, Y. A fully automated microfluidic-based electrochemical sensor for real-time bacteria detection. *Biosens. Bioelectron.* **2018**, *100*, 541−548.
(185) Lee, C.; Chang, H.; Wu, J.; Tseng, F. Ultra-sensitive electrochemical detection of bacteremia enabled by redox-active gold nanoparticles (raGNPs) in a nano-sieving microfluidic system (NS-MFS). *Biosens. Bioelectron.* **2019**, *133*, 215−222.
(186) Ma, Y.; Chen, Y.; Lee, G. An integrated self-driven microfluidic device for rapid detection of the influenza A (H1N1) virus by reverse transcription loop-mediated isothermal amplification. *Sens. Actuators, B* **2019**, *296*, 126647.

https://dx.doi.org/10.1021/acsabm.0c01271
ACS Appl. Bio Mater. 2021, 4, 2160−2191

(187) Yu, X.; Xia, Y.; Tang, Y.; Zhang, W.; Yeh, Y.; Lu, H.; Zheng, S. A Nanostructured Microfluidic Immunoassay Platform for Highly Sensitive Infectious Pathogen Detection. *Small* **2017**, *13* (24), 1700425.
(188) Iswardy, E.; Tsai, T.; Cheng, I.; Ho, T.; Perng, G.; Chang, H. A bead-based immunofluorescence-assay on a microfluidic dielectrophoresis platform for rapid dengue virus detection. *Biosens. Bioelectron.* **2017**, *95*, 174−180.
(189) Qin, P.; Park, M.; Alfson, K. J.; Tamhankar, M.; Carrion, R.; Patterson, J. L.; Griffiths, A.; He, Q.; Yildiz, A.; Mathies, R.; Du, K. Rapid and Fully Microfluidic Ebola Virus Detection with CRISPR-Cas13a. *ACS Sens.* **2019**, *4* (4), 1048−1054.
(190) Yeh, Y. T.; Gulino, K.; Zhang, Y.; Sabestien, A.; Chou, T. W.; Zhou, B.; Lin, Z.; Albert, I.; Lu, H.; Swaminathan, V.; Ghedin, E.; Terrones, M. A rapid and label-free platform for virus capture and identification from clinical samples. *Proc. Natl. Acad. Sci. U. S. A.* **2020**, *117* (2), 895−901.
(191) Xia, Y.; Chen, Y.; Tang, Y.; Cheng, G.; Yu, X.; He, H.; Cao, G.; Lu, H.; Liu, Z.; Zheng, S. Smartphone-Based Point-of-Care Microfluidic Platform Fabricated with a ZnO Nanorod Template for Colorimetric Virus Detection. *ACS Sens.* **2019**, *4* (12), 3298−3307.
(192) Saraf, N.; Villegas, M.; Willenberg, B.; Seal, S. Multiplex Viral Detection Platform Based on a Aptamers-Integrated Microfluidic Channel. *ACS Omega* **2019**, *4* (1), 2234−2240.
(193) Wang, S.; Ai, Z.; Zhang, Z.; Tang, M.; Zhang, N.; Liu, F.; Han, G.; Hong, S.; Liu, K. Simultaneous and automated detection of influenza A virus hemagglutinin H7 and H9 based on magnetism and size mediated microfluidic chip. *Sens. Actuators, B* **2020**, *308*, 127675.
(194) Das, J.; Kelley, S. High-Performance Nucleic Acid Sensors for Liquid Biopsy Applications. *Angew. Chem., Int. Ed.* **2020**, *59* (7), 2554−2564.
(195) Garcia-Cordero, J. L.; Maerkl, S. J. Microfluidic systems for cancer diagnostics. *Curr. Opin. Biotechnol.* **2020**, *65*, 37−44.
(196) Chen, J.; Liu, C.-Y.; Wang, X.; Sweet, E.; Liu, N.; Gong, X.; Lin, L. 3D printed microfluidic devices for circulating tumor cells (CTCs) isolation. *Biosens. Bioelectron.* **2020**, *150*, 111900.
(197) Perez-Toralla, K.; Pereiro, I.; Garrigou, S.; Di Federico, F.; Proudhon, C.; Bidard, F.; Viovy, J.; Taly, V.; Descroix, S. Microfluidic extraction and digital quantification of circulating cell-free DNA from serum. *Sens. Actuators, B* **2019**, *286*, 533−539.
(198) Cheng, Z.; Wang, R.; Xing, Y.; Zhao, L.; Choo, J.; Yu, F. SERS-based immunoassay using gold-patterned array chips for rapid and sensitive detection of dual cardiac biomarkers. *Analyst* **2019**, *144* (22), 6533−6540.
(199) Cheng, Z.; Choi, N.; Wang, R.; Lee, S.; Moon, K. C.; Yoon, S.; Chen, L.; Choo, J. Simultaneous Detection of Dual Prostate Specific Antigens Using Surface-Enhanced Raman Scattering-Based Immunoassay for Accurate Diagnosis of Prostate Cancer. *ACS Nano* **2017**, *11* (5), 4926−4933.
(200) Huang, S.-P.; Chuang, Y.-J.; Lee, W.-B.; Tsai, Y.-C.; Lin, C.-N.; Hsu, K.-F.; Lee, G.-B. An integrated microfluidic system for rapid, automatic and high-throughput staining of clinical tissue samples for diagnosis of ovarian cancer. *Lab Chip* **2020**, *20* (6), 1103−1109.
(201) Gao, R.; Cheng, Z.; Wang, X.; Yu, L.; Guo, Z.; Zhao, G.; Choo, J. Simultaneous immunoassays of dual prostate cancer markers using a SERS-based microdroplet channel. *Biosens. Bioelectron.* **2018**, *119*, 126−133.
(202) Nosrati, R.; Golichenari, B.; Nezami, A.; Taghdisi, S.; Karimi, B.; Ramezani, M.; Abnous, K.; Shaegh, S. Helicobacter pylori point-of-care diagnosis: Nano-scale biosensors and microfluidic systems. *TrAC, Trends Anal. Chem.* **2017**, *97*, 428−444.
(203) Park, Y.; Ryu, B.; Deng, Q.; Pan, B.; Song, Y.; Tian, Y.; Alam, H.; Li, Y.; Liang, X.; Kurabayashi, K. An Integrated Plasmo-Photoelectronic Nanostructure Biosensor Detects an Infection Biomarker Accompanying Cell Death in Neutrophils. *Small* **2020**, *16* (1), 1905611.
(204) Soler, M.; Belushkin, A.; Cavallini, A.; Kebbi-Beghdadi, C.; Greub, G.; Altug, H. Multiplexed nanoplasmonic biosensor for one-step simultaneous detection of Chlamydia trachomatis and Neisseria gonorrhoeae in urine. *Biosens. Bioelectron.* **2017**, *94*, 560−567.
(205) Fraser, L.; Kinghorn, A.; Dirkzwager, R.; Liang, S.; Cheung, Y.; Lim, B.; Shiu, S.; Tang, M.; Andrew, D.; Manitta, J.; Richards, J.; Tanner, J. A portable microfluidic Aptamer-Tethered Enzyme Capture (APTEC) biosensor for malaria diagnosis. *Biosens. Bioelectron.* **2018**, *100*, 591−596.
(206) Ruiz-Vega, G.; Arias-Alpízar, K.; de la Serna, E.; Borgheti-Cardoso, L.; Sulleiro, E.; Molina, I.; Fernàndez-Busquets, X.; Sánchez-Montalvá, A.; del Campo, F.; Baldrich, E. Electrochemical POC device for fast malaria quantitative diagnosis in whole blood by using magnetic beads, Poly-HRP and microfluidic paper electrodes. *Biosens. Bioelectron.* **2020**, *150*, 111925.
(207) Jin, C.; Lee, T.; Koo, B.; Sung, H.; Kim, S.; Shin, Y. Rapid virus diagnostic system using bio-optical sensor and microfluidic sample processing. *Sens. Actuators, B* **2018**, *255*, 2399−2406.
(208) Qiu, G.; Gai, Z.; Tao, Y.; Schmitt, J.; Kullak-Ublick, G.; Wang, J. Dual-Functional Plasmonic Photothermal Biosensors for Highly Accurate Severe Acute Respiratory Syndrome Coronavirus 2 Detection. *ACS Nano* **2020**, *14* (5), 5268−5277.
(209) Perrier, R.; Pirog, A.; Jaffredo, M.; Gaitan, J.; Catargi, B.; Renaud, S.; Raoux, M.; Lang, J. Bioelectronic organ-based sensor for microfluidic real-time analysis of the demand in insulin. *Biosens. Bioelectron.* **2018**, *117*, 253−259.
(210) Wu, J.; Tomsa, D.; Zhang, M.; Komenda, P.; Tangri, N.; Rigatto, C.; Lin, F. A Passive Mixing Microfluidic Urinary Albumin Chip for Chronic Kidney Disease Assessment. *ACS Sens.* **2018**, *3* (10), 2191−2197.
(211) Samper, I.; Gowers, S.; Rogers, M.; Murray, D.-S.; Jewell, S.; Pahl, C.; Strong, A.; Boutelle, M. 3D printed microfluidic device for online detection of neurochemical changes with high temporal resolution in human brain microdialysate. *Lab Chip* **2019**, *19* (11), 2038−2048.
(212) de Oliveira, T.; Erbereli, C.; Manzine, P.; Magalhães, T.; Balthazar, M.; Cominetti, M.; Faria, R. Early Diagnosis of Alzheimer's Disease in Blood Using a Disposable Electrochemical Microfluidic Platform. *ACS Sens.* **2020**, *5* (4), 1010−1019.
(213) Lim, W.; Goh, C.; Thevarajah, T.; Goh, B.; Khor, S. Using SERS-based microfluidic paper-based device (μPAD) for calibration-free quantitative measurement of AMI cardiac biomarkers. *Biosens. Bioelectron.* **2020**, *147*, 111792.
(214) Yoo, Y.; Kim, G.; Park, D.; Kim, J.; Kim, Y.; Yun Kim, H.; Yang, S.; Lee, J.; Hwang, K. Gold nanoparticles assisted sensitivity improvement of interdigitated microelectrodes biosensor for amyloid-β detection in plasma sample. *Sens. Actuators, B* **2020**, *308*, 127710.
(215) Singh, N.; Rai, P.; Ali, M. A.; Kumar, R.; Sharma, A.; Malhotra, B. D.; John, R. A hollow-nanosphere-based microfluidic biosensor for biomonitoring of cardiac troponin I. *J. Mater. Chem. B* **2019**, *7* (24), 3826−3839.
(216) Cate, D.; Adkins, J.; Mettakoonpitak, J.; Henry, C. Recent Developments in Paper-Based Microfluidic Devices. *Anal. Chem.* **2015**, *87* (1), 19−41.
(217) Al Mughairy, B.; Al-Lawati, H. Recent analytical advancements in microfluidics using chemiluminescence detection systems for food analysis. *TrAC, Trends Anal. Chem.* **2020**, *124*, 115802.
(218) Liu, W.; Kou, J.; Xing, H.; Li, B. Paper-based chromatographic chemiluminescence chip for the detection of dichlorvos in vegetables. *Biosens. Bioelectron.* **2014**, *52*, 76−81.
(219) Ramalingam, S.; Chand, R.; Singh, C.; Singh, A. Phosphorene-gold nanocomposite based microfluidic aptasensor for the detection of okadaic acid. *Biosens. Bioelectron.* **2019**, *135*, 14−21.
(220) Chen, D.; Qin, W. J.; Fang, H. X.; Wang, L.; Peng, B.; Li, L.; Huang, W. Recent progress in two-photon small molecule fluorescent probes for enzymes. *Chin. Chem. Lett.* **2019**, *30* (10), 1738−1744.
(221) Kurbanoglu, S.; Erkmen, C.; Uslu, B. Frontiers in electrochemical enzyme based biosensors for food and drug analysis. *TrAC, Trends Anal. Chem.* **2020**, *124*, 115809.

https://dx.doi.org/10.1021/acsabm.0c01271
ACS Appl. Bio Mater. 2021, 4, 2160−2191

(222) Zhou, Q.; Tang, D. Recent advances in photoelectrochemical biosensors for analysis of mycotoxins in food. *TrAC, Trends Anal. Chem.* **2020**, *124*, 115814.
(223) Lim, J.; Ha, D.; Lee, J.; Lee, S.; Kim, T. Review of Micro/Nanotechnologies for Microbial Biosensors. *Front. Bioeng. Biotechnol.* **2015**, DOI: 10.3389/fbioe.2015.00061.
(224) Pol, R.; Céspedes, F.; Gabriel, D.; Baeza, M. Microfluidic lab-on-a-chip platforms for environmental monitoring. *TrAC, Trends Anal. Chem.* **2017**, *95*, 62−68.
(225) Li, B.; Zhang, Z.; Qi, J.; Zhou, N.; Qin, S.; Choo, J.; Chen, L. Quantum Dot-Based Molecularly Imprinted Polymers on Three-Dimensional Origami Paper Microfluidic Chip for Fluorescence Detection of Phycocyanin. *ACS Sens.* **2017**, *2* (2), 243−250.
(226) He, X.; Zhou, X.; Liu, Y.; Wang, X. Ultrasensitive, recyclable and portable microfluidic surface-enhanced raman scattering (SERS) biosensor for uranyl ions detection. *Sens. Actuators, B* **2020**, *311*, 127676.

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