ANNUAL REVIEWS

Annual Review of Biomedical Engineering

Recent Advances in Aptamer-Based Biosensors for Global Health Applications

Lia A. Stanciu,[1,2] Qingshan Wei,[3] Amit K. Barui,[1,2] and Noor Mohammad[3]

[1]School of Materials Engineering, Purdue University, West Lafayette, Indiana 47907-2045, USA; email: lstanciu@purdue.edu

[2]Birck Nanotechnology Center, Purdue University, West Lafayette, Indiana 47907, USA

[3]Department of Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, North Carolina 27695, USA

Annu. Rev. Biomed. Eng. 2021. 23:433–59

First published as a Review in Advance on April 19, 2021

The *Annual Review of Biomedical Engineering* is online at bioeng.annualreviews.org

https://doi.org/10.1146/annurev-bioeng-082020-035644

Keywords

aptamer recognition, biosensors, point-of-care diagnostics, global health, optical sensors, electrochemical sensors

Abstract

Since aptamers were first reported in the early 2000s, research on their use for the detection of health-relevant analytical targets has exploded. This review article provides a brief overview of the most recent developments in the field of aptamer-based biosensors for global health applications. The review provides a description of general aptasensing principles and follows up with examples of recent reports of diagnostics-related applications. These applications include detection of proteins and small molecules, circulating cancer cells, whole-cell pathogens, extracellular vesicles, and tissue diagnostics. The review also discusses the main challenges that this growing technology faces in the quest of bringing these new devices from the laboratory to the market.

Contents

1. GLOBAL HEALTH AND APTASENSORS

Health has always taken center stage and been a priority for our society. Despite the rapidly increasing funding in health in the past few decades, many grand challenges still exist. For instance, epidemic infectious diseases, including but not limited to coronavirus disease 2019, are prevalent in every corner of the world. The field of global health emphasizes critical health issues that occur worldwide and transcend national boundaries and investigates interdisciplinary collaborative action from physicians, researchers, funding agencies, policy makers, and the general public to promote systematic solutions, ranging from population-based prevention to individual-level clinical care.

Among the grand challenges faced by the field of global health are well-recognized large-scale problems such as human immunodeficiency virus/acquired immunodeficiency syndrome, vaccination, malnutrition, maternal and infant health, and noncommunicable diseases, as well as desired scientific or technical breakthroughs that would improve access to medical care and overcome socioeconomic barriers for people living in resource-limited regions. Diagnostics technologies are critical for identifying health conditions or diseases at early stages, designating treatment courses, and monitoring the outcome of medical interventions. However, current diagnostic tools are often designed for laboratory use and therefore are inadequate for meeting health needs in developing countries. In 2003, the Bill & Melinda Gates Foundation outlined 14 grand challenges in global health (**https://www.gatesfoundation.org/Media-Center/Press-Releases/2003/10/14-Grand-Challenges-in-Global-Health**). One of them particularly calls on researchers to "develop technologies that allow assessment of individuals for multiple conditions or pathogens at point-of-care."

One of the recent trends in medical diagnostics is to develop point-of-care (POC) methods to enable rapid, self-supported testing in outpatient or remote settings to complement standard clinical diagnostics. The ubiquity of these cost-effective tests and integration with digital devices for remote data transmission and analysis are expected to form a completely new strategy for effective public health interventions on a global scale and in near-real-time fashion. This is an emerging area sometimes referred to as precision global health or mobile health (1, 2). Aptasensors stand out uniquely in the new age of digital and personalized medicine and have become one of the

promising candidates to contribute to the acceleration of the translation of conventional benchtop medical diagnostics into POC tests. Aptamers are 3D-folded single-stranded nucleic acids that can selectively bind to target molecules. Compared with antibodies, aptamers have many unique advantages as alternative binding ligands, including flexible sequence design, comparable binding affinity to monoclonal antibodies [dissociation constant (K_d) = 0.1–50 nM], negligible immunogenicity, diverse binding capability (from small molecules and proteins to nucleic acids), cold-train-free storage, and significantly lower cost. Because of these characteristics, aptamers frequently have been studied for POC diagnostics and global health applications in the past decade. Some of the developments have been successfully transformed into commercial products. For instance, an aptamer-based proteomics assay called SOMAscan® (by SomaLogic) is capable of measuring 1,305 human protein analytes in a volume of 50 mL of serum, plasma, or other biological matrices with high sensitivity and specificity (3).

This review aims to provide a timely summary of the recent progress in utilizing aptamer-based biosensors for POC diagnostics and global health. The review starts with a brief summary of different aptamer synthesis methods and types of transducing mechanisms. Then, the focus is given to recent efforts to miniaturize aptasensors into field-portable and POC formats as well as to the clinical applications of aptasensors for the detection of a wide range of targets, from small molecules, proteins, nucleic acids, whole pathogen and cancer cells, and extracellular vesicles to pathological tumor slices. Finally, remaining challenges and future opportunities for aptamer-based biosensors in global health are discussed.

2. SELEX APTAMER SYNTHESIS

In 1990, within a brief time interval, three different laboratories reported their results on the development of an in vitro selection and amplification technique for the isolation of specific nucleic acid sequences that are able to bind to target molecules with high affinity and specificity (4–6). The method was termed SELEX, standing for systematic evolution of ligands by exponential enrichment (6), and the resulting oligonucleotides were referred to as aptamers. Aptamers are artificial nucleic acid ligands that can be generated against amino acids, drugs, proteins, and other molecules. Aptamers often possess a high affinity for their targets, and this affinity is derived from their capability to fold upon binding with their target molecule (i.e., they can either incorporate small molecules into their nucleic acid structure or be integrated into the structure of macromolecules, e.g., proteins). They bind to their targets with binding affinity typically in the low pico- or nanomolar range. A typical SELEX methodology employs negative selection and counter selection steps to select aptamers with high affinity and specificity. Negative selection and counter selection against structurally nonspecific and analog targets, respectively, help in eliminating the nonspecific nucleotide sequences. These steps also help to steer the aptamer selection process toward a specific epitope of the target molecule. The affinity of aptamers to bind with the target molecule during a SELEX process is dependent on the stringency of the target binding and aptamer elution process. Typically, as SELEX progresses, stringency is increased gradually by varying the target/aptamer concentration, buffer composition, detergent concentration, binding and elution temperature, and washing conditions (volume, temperature, and time).

SELEX involves iterative cycles of binding, recovery of bound DNA/RNA, and amplification (**Figure 1**). It begins with a nucleic acid library, obtained via combinatorial chemistry, typically consisting of at least 10^{14}–10^{15} DNA or RNA molecules containing a random region flanked on both sides with fixed primer sequences for amplification. As most aptamer–target interactions are due to hydrogen bonding, RNA libraries offer greater diversity than DNA, but the resulting aptamers are highly prone to nuclease attack. The library is incubated with the target and the

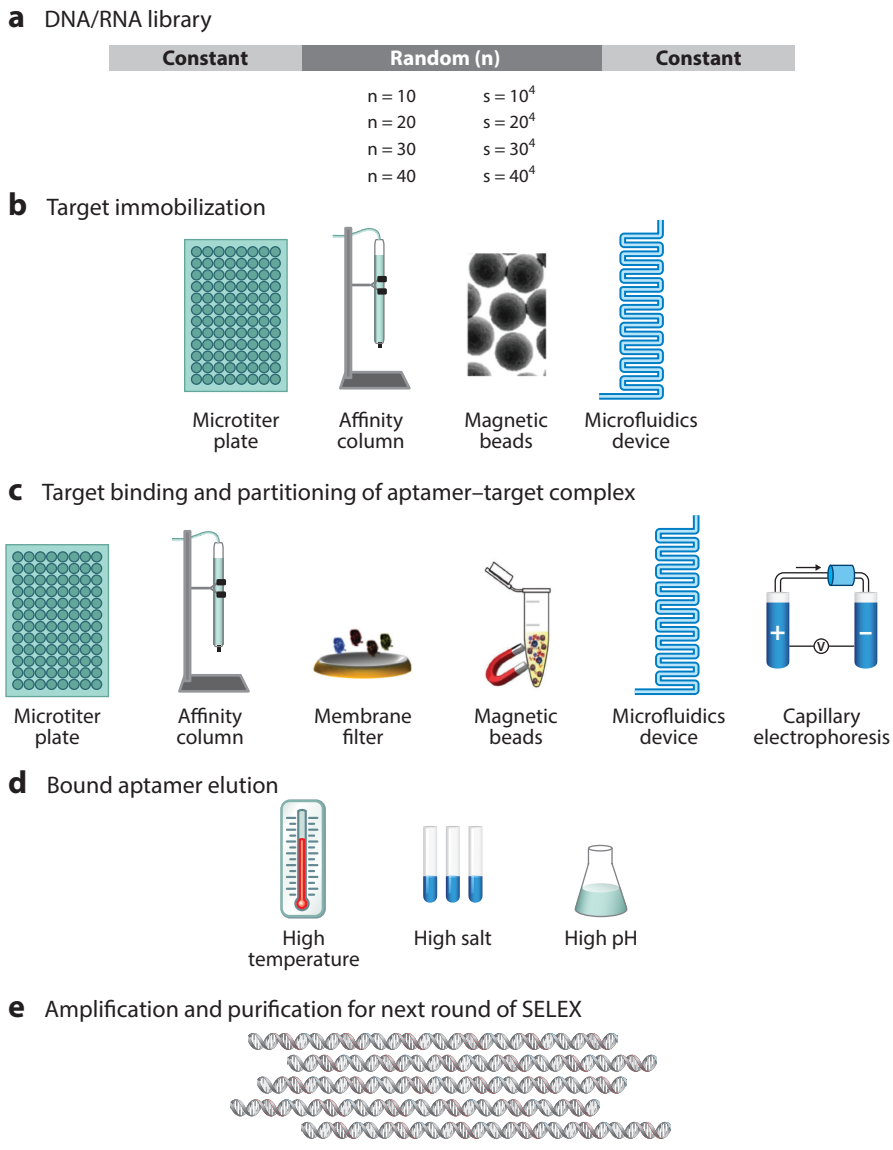


Figure 1

Schematic representation of SELEX process. (*a*) A typical DNA library consists of a random nucleotide region and two constant primer binding regions to help with the amplification required during the subsequent SELEX rounds. (*b*) Examples of various strategies used to immobilize the target during SELEX. (*c*) Techniques used to separate the aptamer–target complex from unbound DNA/RNA pools. (*d*) Elution of aptamers from the aptamer–target complex, typically using high temperature, high salt, and high pH. (*e*) Eluted DNA/RNA aptamers are PCR amplified before being used in the next SELEX round. Abbreviations: PCR, polymerase chain reaction; SELEX, systematic evolution of ligands by exponential enrichment.

first cycle of binding is initiated. The target can be immobilized on magnetic beads (MBs), resin, affinity columns, or microtiter plates or be free in solution. Following incubation, the DNA/RNA not bound to the target must be separated from the bound sequences by magnetic or centrifugal force. Where the target is not immobilized, separation via a membrane filtration

is the most commonly reported technique, although recently capillary electrophoresis has been reported to be an efficient method for separation of bound and unbound DNA/RNA. Elution of target bound DNA/RNA is achieved by washing using an elution buffer usually composed of detergent/chaotropic agent/imidazole at a high temperature (>90°C). To improve the aptamer specificity, it can be useful to employ steps of negative selection (removal of aptamers that bind ligand support) and counter selection (removal of aptamers that bind to structures analogous to that of the target), along with the positive selection rounds. With each cycle the stringency is increased and the concentrations of DNA/RNA and target are decreased. Upon achieving affinity saturation, the enriched library is cloned and sequenced, and individual sequences are investigated for their ability to bind to the target again using various techniques, for example, fluorescent or radioactive binding assays, surface plasmon resonance, and flow cytometry. The analysis of cloned aptamers typically produces tens of sequences with the ability to bind specifically to the target molecules and motif searches, and truncation studies are usually needed to further characterize the aptamer sequences and choose the sequences with the best affinity and specificity. The final aptamer(s) can now be produced in sizable quantities by chemical synthesis. The majority of aptamers reported in the literature have been produced by SELEX using affinity chromatography, nitrocellulose filtering, and magnetic bead technology. However, innovative approaches have appeared to overcome the resolution problem of aptamer target separation during SELEX.

Advances in SELEX for the selection of desired high affinity aptamers have focused on the important and crucial steps of the conventional SELEX methodology, including alternate methods of target/library immobilization, separation and removal of unwanted aptamer molecules, DNA/RNA quantification, and binding efficiency determination.

The details about different variants of SELEX methodologies and the pros and cons of these methodologies are listed in **Table 1**. Once synthesized to bind to a specific target, an aptamer then can be integrated into a sensing configuration to be used for the design of POC platforms, which are the main focus of this review, as well as for aptasensors used in a large variety of other societal sectors (e.g., agriculture, food safety, environment).

3. APTASENSOR TRANSDUCTION MECHANISMS

A typical biosensing system will have a biorecognition element in its structure, which, for the purpose of this review, is an aptamer. In a typical aptasensing system, the recognition element (the aptamer) will specifically bind to the target and generate a signal output. This signal will be either a physical signal or a chemical signal, which will then be transduced to provide a readout that can be analyzed, interpreted, and often amplified for enhanced sensitivity. While **Figure 2** provides an overview of the transduction mechanisms encountered in literature, we describe in some detail only the most commonly encountered ones, namely, colorimetric and electrochemical mechanisms.

3.1. Optical Aptasensors

Optical aptasensors have at least one aptamer in their configuration—sometimes two aptamers or one aptamer and one antibody—serving as highly specific biorecognition elements for analytical targets. Most often, although not exclusively, the signals used to transduce the binding events are fluorescence, colorimetric, or surface-enhanced Raman spectroscopy (SERS) aptasensors.

3.1.1. Fluorescence aptasensors. When a fluorophore material is subjected to light with a higher wavelength than that of the excitation wavelength, photon emission occurs. This signal

Table 1 Advantages and disadvantages of different SELEX variants

SELEX method	Advantages	Disadvantages
Nitrocellulose filter binding	■ Relative ease of selection ■ No special equipment required ■ Equilibrium, in-solution aptamer-target binding	■ Majorly restricted to proteins that can be captured by nitrocellulose filter ■ Large number of selection rounds (8–20) necessary ■ Relative abundance and quick enrichment of filter-binding aptamers
Bead-based	■ Applicable to small molecules, peptides, proteins, and cells ■ Rapid selection of aptamers ■ In-solution binding and equilibrium ■ Ease in controlling selection stringency	■ Target or aptamer immobilization: restricted interaction surface ■ Density-dependent cooperativity for nonspecific interactions
Microfluidic	■ Potential for high-throughput aptamer selections against multiple targets ■ Rapid selection of aptamers	■ Fabrication of microfluidic devices, electronic instruments, and flow pumps required for operation
Microarray-based	■ Equilibrium binding with in-solution target and immobilized aptamer ■ Can be used as a very large-scale binding assay	■ Limited number ($<10^5$) of unique aptamer sequences ■ Aptamer sequences need to be predetermined ■ Fabrication of a microarray with different sequences unique for each target
Microscopic	■ Single-round selection reported	■ Requires expensive and specialized instrument (i.e., atomic force microscopy system)
Cell-SELEX	■ Biomarker discovery ■ Therapeutic potential of selected aptamers	■ Restricted to molecules presented on cell surface ■ Selection of aptamers to an unintended target is very likely
In vivo SELEX	■ Selection of in vivo functional aptamers	■ Selection of aptamers to an unintended target is very likely

is highly sensitive to the environment and can thus be used to measure changes such as analyte binding and make fluorescence biosensors possible. Fluorescence biosensors have the advantages of fast response, high sensitivity, and low cost. The ability of aptamers to change conformation upon target binding can lead to changes in fluorescence properties and thus enable the design of POC devices for a large array of targets, from antibiotics to pathogens. The principle of operation of such aptasensors is driven by the knowledge that some fluorescent dyes are able to change their emission in the presence of the G-quadruplex in aptamers. The G-quadruplex is a nucleic acid structure that contains guanine-rich sequences. For some aptamers, a target binding event leads to a disruption of the G-quadruplex, which in turn results in fluorescence signal changes in the presence of a fluorophore substance. Examples of fluorophores include Alexa Fluor 488, fluorescein, and 6-carboxyfluorescein, as well as quantum dots (QDs) and other semiconducting nanocrystals. A suitable fluorophore material for aptasensor design is expected to be characterized by a high fluorescence lifetime, low photobleaching, and narrow emission bands.

3.1.2. Colorimetric aptasensors. Colorimetric biosensors in general and aptasensors in particular are among the simplest and most often reported types of optical biosensors, whereby the transducing mechanism upon target binding is a color change that can be recognized most of the time by the naked eye and sometimes even quantified by image analysis. Although dyes and signal-enhancing enzymes such as horseradish peroxidase (HRP) have been used to impart or amplify the color change signal in biosensors, it was the discovery of nanotechnology tools that made

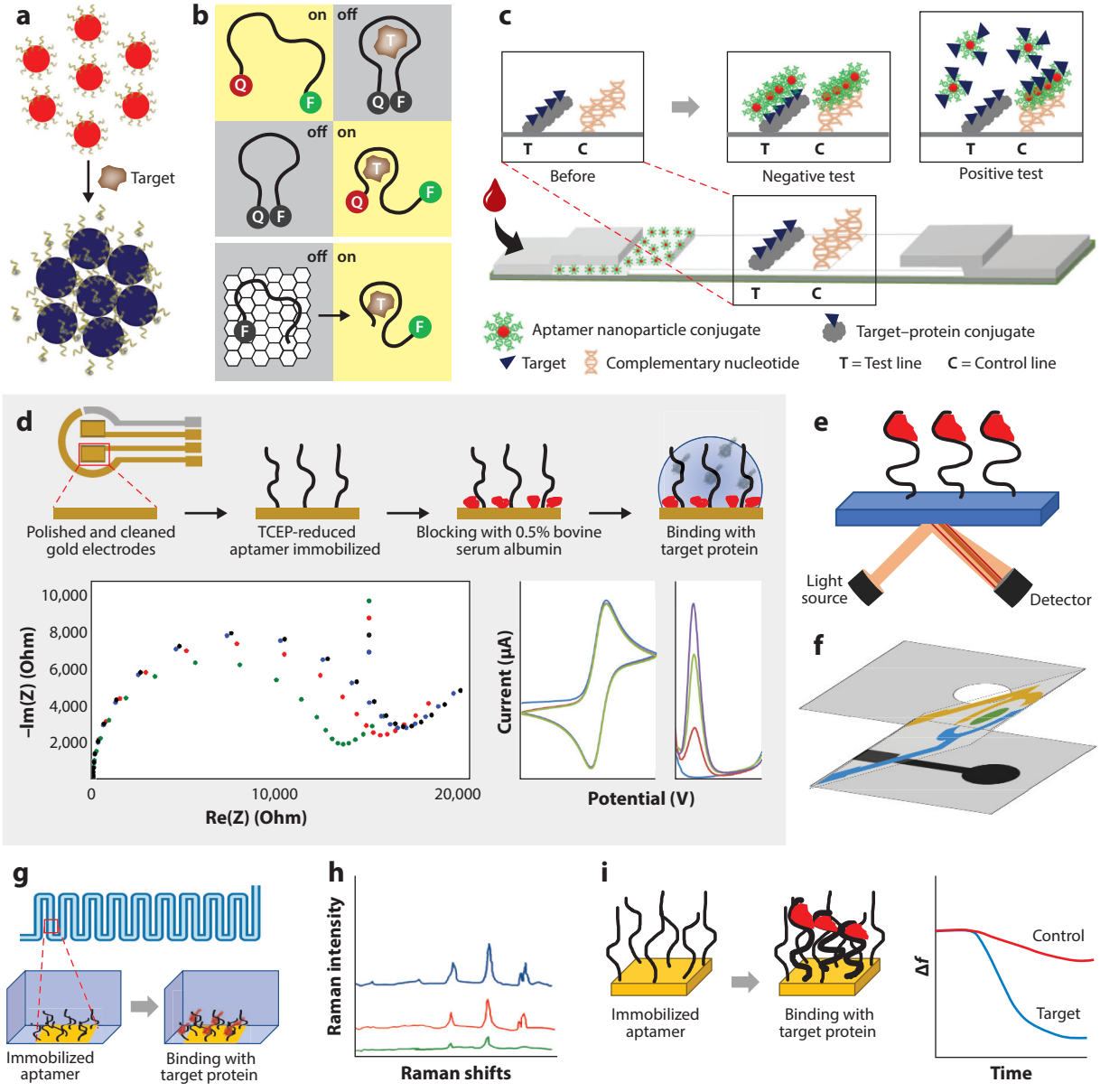


Figure 2

Overview of aptasensing transduction mechanisms employing (*a*) nonenzymatic color change due to particle aggregation, (*b*) fluorescence on or off, (*c*) lateral flow assay, (*d*) electrochemical techniques, (*e*) plasmon resonance, (*f*) paper micropads, (*g*) microfluidic techniques, (*h*) Raman spectroscopy, and (*i*) quartz crystal microbalance techniques. Abbreviations: F, fluorophore; PBS, phosphate buffered saline; Q, quencher; T, target; TCEP, tris(2-carboxyethyl)phosphine.

it possible to use Au and Ag nanoparticles (NPs) for colorimetric biosensing strategies with high signal-to-noise ratio and low detection limits. For Au NPs, for example, the principle of operation is very simple and by now widely known. Due to their surface plasmon resonance, well-dispersed gold NPs emit a pink color, and they have the ability to change color from pink to purple upon

a change of the interparticle distance. Either the color change is observed in solution, such as in a well plate or on a test strip, or it can be visualized in the context of a paper-based assay. In a sandwich-type paper-based lateral flow assay, a colored test line shows up on a previously blank paper-like test strip when the test is positive for the target of interest. Conversely, on a competitive-type lateral flow assay, a test strip initially containing two lines is the negative test, and one of the lines disappears or its color intensity decreases when the target is present in the sample of interest. Antibodies were first used in such assays, in combination with both signal-amplifying enzymes and NPs; however, their drawbacks—including lack of stability at normal operation conditions, high cost, and geometry-related binding difficulties—rendered aptamers as better choices that did not compromise selectivity and sensitivity.

3.1.3. Surface-enhanced Raman spectroscopy aptasensors. The third type of optical aptasensor that has been widely studied is based on SERS principles. SERS has been proven to offer tremendous enhancement of target detection sensitivity in biosensors, reaching single-molecule detection. This is often reported to be due to the large surface enhancement of Raman scattering when target molecules are adsorbed on either plasmonic nanostructures or metallic films with high roughness. That leads to an increase as high as 10^{14} in the Raman signal, which makes very low, even single-molecule, detection limits reachable. Compared with fluorescence techniques, SERS-based biosensors offer the advantages of a much narrower bandwidth of the Raman peaks (a width of less than 1 nm versus up to 50 nm in fluorescence), lack of chromophore signature overlap, and lower background noise in SERS.

SERS-based aptasensors have been reported for the detection of foodborne and other pathogens but also for the detection of proteins (7), toxins (8), and nucleic acids (9) with high sensitivity and specificity.

3.2. Electrochemical Aptasensors

Electrochemical (EC) aptasensors take advantage of the ability of aptamers to specifically bind to a target as well as their superior stability compared with any other biorecognition elements (enzymes, antibodies, etc.) and combine these desirable aptamer characteristics with the high sensitivity, portability, ability for miniaturization, and quantitative detection of EC systems. In such aptasensors, at least one aptamer that can selectively bind to an analytical target is chemically immobilized on a conductive working electrode and then used in an amperometric, impedimetric, or square-wave voltammetry configuration to selectively detect and quantitatively measure the target of interest. For such sensors, the electrical and EC properties of the working electrode materials play a major role in signal sensitivity and thus the detection limit achieved by such aptasensors. Generally, aptamers are bound to working electrode materials via either electrostatic interaction (generally not very favorable for long-term storage stability) or covalent bonding after electrode surface functionalization to enable aptamer binding.

Recent reports on amperometric aptasensors have focused on signal amplification strategies by the use of high surface area and electrical conductivity electrode materials. For thrombin, for example, a recent study (10) used a thiolated thrombin-specific aptamer and a combination of polyaniline, a conductive polymer, and carbon nanotubes, together with a glassy carbon electrode. The limit of detection (LOD) of 80 fM is one of the lowest reported for EC aptasensors. Picomolar or subpicomolar detection limits were claimed by Zhang et al. (11), who reported on an amperometric aptasensor for amyloid beta oligomers.

Several groups used the sandwich structure in the design of a variety of aptasensors. Some of these aptasensors used two different aptamers, while others used one aptamer and one antibody for

the sandwich structure (12). Polymers used on working electrodes for the EC signal transduction not only can have good conductivity for sensitive detection but also can provide readily available and active chemical groups for covalent binding of the aptamers; in addition, they can help provide an antifouling solution for the electrodes (13, 14). A new class of 2D materials, 3D carbides, and nitrides, called MXenes, with extraordinary electrical conductivity, has also been reported for the first time in an EC aptasensor for the detection of the breast cancer marker mucin 1 (15). Research on such novel electrode materials is generally aimed at identifying ways of enhancing the sensing signal, improving the sensitivity, and lowering the detection limit of such devices.

Clearly, the focus of recent research on EC aptasensors is on advancement of the electrode materials design, with a goal of increased detection performance (16) in terms of sensitivity, detection limit, and linear range, coupled with an expansion of targets that can be detected with aptamer-based strategies (13, 17, 18).

4. DIAGNOSTIC APPLICATIONS OF APTAMER-BASED BIOSENSORS

4.1. Detection of Small-Molecule Biomarkers

Small molecules include a large number of biologically active compounds that often carry high significance for human health. They are, however, notoriously difficult to detect because their small size limits the availability of binding sites for the targeting ligand (e.g., aptamer). Over the last decade, however, the use of aptamers for the development of diagnostics for pesticide residues, toxins, antibiotics, drug molecules, and heavy metals has grown exponentially. **Table 2** lists a representative selection of reports that focus on aptasensors for some significant small molecules, as well as their LOD.

Generally, the fluorescent, chemiluminescent, SERS, and EC aptasensors for small-molecule detection tend to display enhanced sensitivity compared with their colorimetric counterparts. However, the simplicity and low cost of colorimetric assays still make them attractive for applications where target detection limits are within their sensitivity range and mostly qualitative detection is being sought.

4.2. Detection of Protein Biomarkers

Aptasensors have been developed for the detection of protein biomarkers in various biological fluids ranging from blood serum to sweat. An aptasensor was developed for the detection of tumor necrosis factor-α (TNF-α), a key inflammatory cytokine in whole blood. The assay was sensitive enough to detect 58-pM TNF-α with a linear range of 6 nM. In another study, an EC aptasensor system was developed for the detection of lung cancer–related protein in blood plasma samples. Silica-coated iron oxide MBs were used to enhance the aptasensor detection limit to 0.023 ng/mL (49). On the basis of similar principles of EC aptasensing, an assay was employed to detect lysozyme (50), C-reactive protein (51, 52), prostate-specific antigen (53), vascular endothelial growth factor (53), and interleukin-6 (54). A recent study reported an aptasensor for the detection of tuberculosis biomarker MPT64, a protein secreted by *Mycobacterium tuberculosis*, in 30 min with a sensitivity of 81 pM in serum (55). In an application that put forward yet another EC aptasensor, Bhardwaj et al. (56) successfully used a DNA aptamer targeting the stem region of hemagglutinin for subtyping the influenza A H1N1 virus. Ren et al. (57) developed an EC sensor using an aptamer-gated zeolitic imidazolate framework-derived porous carbon nanocontainer for enhanced detection of thrombin at 0.57 fM. In yet another development, an aptamer-antibody sandwich assay with methylene blue as an EC indicator was reported for detection of the mucin 16 protein, also known as cancer antigen 125 (58). The LOD of the developed assay is 0.02 units/mL.

Table 2 Aptasensors for the detection of small molecules developed over the last decade

Target compound	Aptasensing strategy	Limit of detection	Reference
Pesticides and insecticides			
Acetamiprid	Electrochemical	0.33 pM	19
Atrazine	Electrochemical	40 pM	20
Carbofuran	Chemiluminescent	88 pM	21
Chloramphenicol	Colorimetric	18.3 pM	22
Chlorpyrifos	Electrochemical	0.35 fM	23
Isocarbophos	Electrochemical	0.01 nM	24
Malathion	Colorimetric	0.06 pM	25
Omethoate	Electrochemical	0.1 nM	24
Phorate	Colorimetric	0.01 nM	26
Profenofos	Electrochemical	0.003 nM	27
Antibiotics and drugs			
Aminoglycosidic antibiotics	Colorimetric	1–100 nM	28
Chloramphenicol	Photoelectrochemical	3.1 nM	29
Kanamycin	Electrochemical	5.8 nM	30
Lincomycin	Chemiluminescent	1.6×10^{-13} mol/L	31
Tetracycline	Colorimetric	45.8 nM	32
Heavy metals			
As^{3+}	Colorimetric	5.3 ppb	33
Cu^{2+}	Electrochemical	0.1 pM	34
Hg^{2+}	Surface plasmon resonance spectroscopy	10 fM	35
Microbial toxins			
Aflatoxin B1	Spectrophotometry	0.1 ng/mL	36
Fumonisin B1	Fluorescence resonance energy transfer	0.1 ng/mL	37
Ochratoxin A	Colorimetric	20 nM	38
Ochratoxin A	Impedimetric	14 pM	39
Staphylococcal enterotoxin B	Surface-enhanced Raman spectroscopy	224 aM	40
Tetracycline	Colorimetric	266 pM	41
Zearalenone	Colorimetric	10 ng/L	42
Other analytes			
ATP	Electrochemical	0.1 pM	43
BPA	Electrochemical	5 nM	44
BPA	Surface-enhanced Raman spectroscopy	3 nM	45
Cocaine	Electrochemical	105 pM	46
Cocaine	Molecular beacons	0.48 nM	47
Mat lysozyme	Colorimetric	1×10^{-4} μg/mL	48

Abbreviations: ATP, adenosine 5′-triphosphate; BPA, bisphenol A.

Table 3 Selected pathogen-detection aptasensing assays and their performance

Pathogen	Aptasensing strategy	Limit of detection	Reference
Bacillus anthracis	Impedimetric	3×10^3 CFU/mL	60
Bacillus thuringiensis	Colorimetric paper chip	3×10^7 CFU/mL	61
Campylobacter jejuni	Fluorometric/luminescence sensors coupled with magnetic separation	10–250 CFU	62
Escherichia coli O157:H7	Fluorescence	100 CFU/mL	63
Hepatitis B	Electrochemical	0.0014 fg/mL	64
Listeria monocytogenes	Aptamer qPCR	5 CFU/mL	65
Norovirus	Colorimetric	200 virus particles/mL	66
Pseudomonas aeruginosa	Colorimetric and electrochemical	60 CFU/mL	67
Salmonella paratyphi A	Fluorescence	10^2 CFU/mL	68
Salmonella typhimurium	Electrochemical graphene composite	5 CFU/mL	69
Staphylococcus aureus	Resonance Rayleigh scattering	Single cell	70
Vibrio parahaemolyticus	Colorimetric	10 CFU/mL	71

Abbreviations: CFU, colony-forming unit; qPCR, quantitative polymerase chain reaction.

Liu et al. (59) reported a SERS assay for the insulin-like growth factor 2 receptor protein with a detection limit of 141.2 fM.

4.3. Detection of Intact Pathogens

Applications of aptamers for the detection of intact pathogens, such as whole-cell bacteria and viruses, have led to the development of rapid and sensitive diagnostics technologies for various pathogens. These aptasensors have compelling advantages over the traditional antibody-based assays, such as low cost, high throughput, ease of use, stability, and minimum batch-to-batch variability. These advantages are likely to contribute to the wide acceptability of aptasensors for diagnostics development in the future. Although the published literature on the topic is much larger, to conform to space limitations, we list in **Table 3** a selection of recently reported aptasensors targeting pathogens.

The detection limits for whole-cell pathogens with aptasensors have reached extremely low levels, down to the single-cell level. However, the great majority of this research is not translating clinically or even reaching the patent stage. Efforts toward improving manufacturing strategies and the discovery of translational technologies are necessary to further harvest these significant successes. The relatively high stability of aptamers versus antibodies offers such an opportunity for integrating aptasensors into mass production and bringing whole-cell pathogen detection from the laboratory to the market.

4.4. Detection of Circulating Tumor Cells

Cancer is the second-leading cause of death worldwide (72). There is always a pressing need to develop novel tools to improve early diagnosis and therapeutic outcomes. Solid tumors can shed circulating tumor cells (CTCs) and other molecular biomarkers (e.g., cell-free DNA) in the bloodstream (73), and these CTCs could serve as a liquid biopsy to analyze various cancers noninvasively for personalized cancer treatment (74). A variety of aptamer-based biosensors or diagnostic assays have been developed recently for CTC analysis, ranging from CTC isolation and targeted imaging to detection.

4.4.1. Aptamers as separation ligands for CTC isolation. Aptamers have been used as recognition ligands to capture and isolate CTCs (75) by targeting different cell membrane proteins (76), epidermal growth factor receptor (EGFR) (77), and epithelial cell adhesion molecules (EpCAM) (78). Nanomaterials and microfluidic devices are two POC-friendly platforms, which are often surface-functionalized with aptamer-based binding ligands to capture CTCs from cultured or clinical samples. For instance, Sun et al. (79) conjugated gold surfaces with aptamers, thus offering a DNA-based nanotetrahedron (NTH) bioscaffold to enhance human hepatocellular carcinoma (HepG2) CTC binding capability by ensuring maximum accessibility of aptamers to the target CTCs. This device captured low-abundance HepG2 cells with a LOD of 3 cells/mL. Shen et al. (80) extended the specific aptamer with an extra 19-nucleotide fragment. This elongated fragment allowed partial hybridization of the aptamer with a complementary probe connected to the gold surface, which captured CTCs with a LOD of 10 cells/mL (80).

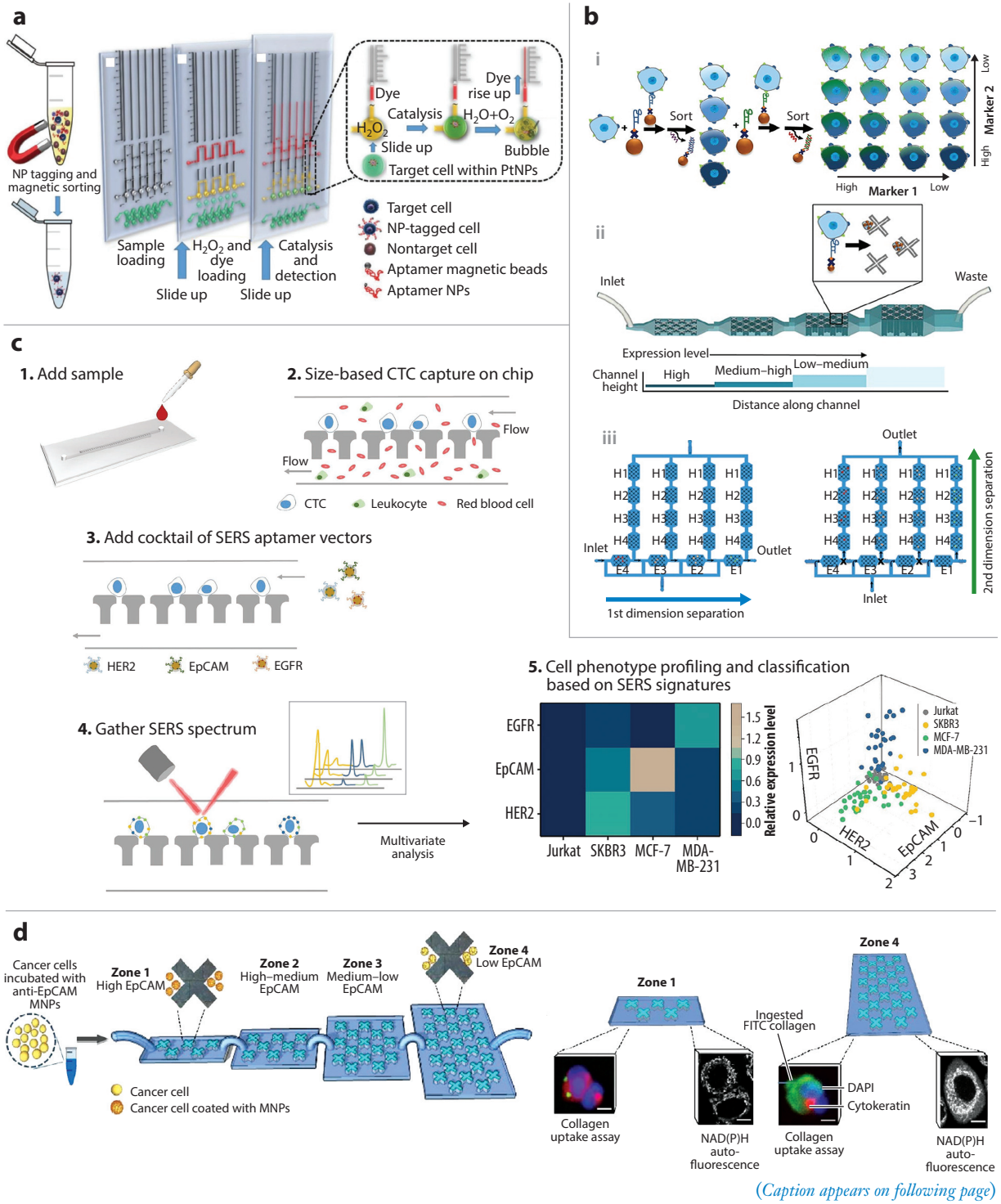
Cell-SELEX–derived aptamers for CTC isolation on microchips are also becoming popular for rapid testing. For example, Xu et al. (81) utilized aptamer-based microfluidic chips to capture cells such as human Burkitt's lymphoma cells (Ramos) and lymphocytes with specific aptamers. This device showed capturing efficiency similar to that of cell-affinity chromatography (82). Several other groups utilized sgc8 aptamers to isolate acute lymphocytic leukemia cells from patients' bloodstreams utilizing various microchip devices, such as a micropillar device (83), a gold NP-herringbone microchip (84), or a 3D DNA network-based microchip (85). To maximize CTC capturing efficiency, the introduction of rough surfaces and multivalent interactions into the microchip devices has become a common strategy. For example, a micropillar array was integrated into a microchip device to increase the probability of interaction between tumor cells and the sgc8 aptamer, which resulted in approximately 95% capturing efficiency and 81% purity (83). The use of a gold NP-herringbone microchip, on the other hand, provided scaffolds to form multivalent aptamer nanospheres to enhance the binding affinity and thus enhance the capture efficiency of CTCs up to 93% (84). A 3D DNA network-based microchip was prepared by rolling circle amplification (RCA), which also provided a multivalent binding network for achieving higher capture efficiency (85). In addition, a NanoVelcro Chip was constructed with an integrated aptamer–silicon nanowire substrate (SiNS) to capture non-small-cell lung cancer (NSCLC) cells from blood samples. The device offered higher than 80% capture efficiency (86). Furthermore, to capture CTCs with variegated phenotypes from patient samples, a modified microfluidic chip was prepared with multiple aptamer combinations. The aptamer cocktail yielded enhanced capture efficiency, which is promising for the rapid characterization of CTC heterogeneity (87).

4.4.2. Aptamers as targeting ligands for CTC imaging. Aptamers have also been conjugated to imaging contrast agents such as NPs and fluorophores as selective optical probes for targeted imaging of CTCs. For example, aptamer-conjugated NPs (ACNPs) have been developed for the targeting and imaging of cells. Here, aptamer-conjugated magnetic NPs (MNPs) were used to extract the target cells from blood samples while aptamer-conjugated fluorescent NPs (FNPs) were used for rapid and sensitive detection through signal amplification (88). Additionally, ACNPs were utilized to selectively capture and identify several CTCs unscathed from complex samples. For example, cells from pseudocomplex samples were captured and imaged simultaneously with the help of MNPs and FNPs conjugated with aptamers (89).

4.4.3. Aptamer-based biosensors or assays for CTC detection and analysis. A number of aptamer-based platforms have been developed for rapid CTC detection and analysis. Optoaptasensors are among the most popular detection methods. Chiu et al. (90) constructed an aptamer functionalized gold nanofilm chip to detect CTCs from breast, gastric, and ovarian cancer cells with a LOD of 10 tumor cells. Visual and quantitative detection of Ramos (leukemia) cells

was achieved by the use of an aptamer accompanied by a volumetric bar-chart chip (V chip) (**Figure 3*a***). This chip quantified the biomarkers by the catalytic reaction between H_2O_2 and ACNPs to convert the number of CTCs into a readily detectable visual signal (91). Labib et al. (92) constructed an aptamer-embedded 2D microfluidic chip to sort different types of CTCs in proportion to the expression levels of HER2 (breast, gastric) and EpCAM (adenocarcinoma) by employing HB5 and SYL3C aptamers, respectively (**Figure 3*b***). Furthermore, aptamers were developed by Zhang et al. (93) to identify CTC subpopulations by profiling membrane proteins of different cell lines (**Figure 3*c***). Here, CTCs were first sieved using a microfluidic chip, and profiling of cell phenotypes was done by SERS (93). Green and colleagues (92, 94) depicted the biochemical and functional phenotypes of CTC subpopulations by using an NP-mediated 2D sorting device (**Figure 3*d***). The microfluidic chip first differentiated the cell subpopulations by the level of EpCAM expression. Then, the isolated tumor cells were assessed by a fluorescent collagen uptake assay and a metabolic NAD(P)H assay (92, 94). Additionally, aptamer-embedded microfluidic chips were utilized to detect genetic mutation of the *TP53* gene, which contributes to the uncontrolled cell growth and therefore the development of cancer. The detection was done through the extraction of genomic DNA from cancer cells (cervical and ovarian) and subsequent Sanger sequencing followed by comparison of the Sanger results with the sequence of the wild-type *TP53* gene (95).

EC aptasensors have also been developed and used for CTC detection by many researchers and groups. For instance, a cell-SELEX–originated EC aptasensor was developed by Shangguan et al. (96) to identify CCRF-CEM (leukemia) cells and used Ramos (human Burkitt's lymphoma) cells as experimental controls. Other groups fabricated EC aptasensors to detect CTCs from serum samples with a LOD of 1 to 100 cells/mL and from clinical samples with a LOD of 3 cells/mL (97–99). In addition, they conjugated a TD05 aptamer derived by the cell-SELEX process with magnetic nanocomposites, which exhibited specific binding capability to Ramos cells in the presence of other CTCs such as A549 (lung), MCF-7 (breast), and SKBR3 (breast) (99). Since nanomaterials can enhance the detection sensitivity through enhancement of the electron transfer activity, a label-free EC impedance spectroscopy cytosensor that incorporated AS1411 aptamer-functionalized graphene was constructed to effectively detect HeLa cells (100). Furthermore, liver cancer–derived CTCs have been detected with a hepatocellular carcinoma–specific aptamer with a LOD of ~3 cells/mL for HepG2 (79) and a LOD of ~3 cells/mL (101) for MEAR. Moreover, SKBR3 CTCs (human breast cancer cells) were detected by an EC cytosensor that engaged mesoporous silica NPs (MSNs). Aptamers were adsorbed on the surfaces of MSNs to increase the EC signals significantly. This mechanism offered a LOD of 13 cells/mL (102). Additionally, a sandwich EC aptasensor (which utilizes two probes: one for capturing and another for signaling) was developed to capture and detect CT26 (colorectal cancer) (103) and HepG2 (liver cancer) (104) CTCs with high sensitivity and selectivity. Zhou et al. (105) combined EC sensors with hybridization chain reaction (HCR) to capture MCF-7 CTCs using a DNA NTH-based aptamer probe. Excellent signal amplification was achieved through the enzymatic catalytic reaction triggered by a large amount of avidin-HRPs conjugated on the biotinylated HCR products. This approach offered an improved LOD of 4 cells/mL (105). A number of other nanomaterials, such as Au-Pd NPs (105, 106) and CuO (107) nanozymes, have been used as signal amplification probes to build EC aptasensors where the EC signals were amplified through the reduction of H_2O_2 by amplification probes. Additionally, Liu et al. (108) constructed a supersandwich EC cytosensor utilizing QDs as electroactive species to amplify signals; this cytosensor detected leukemia CTCs with a LOD of 50 cells/mL. Moreover, Zhou et al. (109) prepared a multiplex EC luminescence cytosensor that incorporated CdS QDs and Au@luminol as EC luminescence nanoprobes for in situ capturing and detection of MUC1-positive MCF-7 cells with a LOD of 20 cells/mL.



(Caption appears on following page)

Figure 3 (*Figure appears on preceding page*)

Aptamer-based devices for CTC capture and detection. (*a*) Working principle of the volumetric bar-chart chip (V chip) involving a PtNP-conjugated aptamer for visual quantification of CTC detection (91). (*b*) Representation of a 2D sorting microfluidic device to capture and sort cells expressing EpCAM and HER2 differently (92). (*c*) Representation of a chip-based device for in situ capture and profiling of CTCs based on SERS detection (93). (*d*) Schematic representation of phenotypic profiling of CTC subpopulations according to their level of EpCAM expression. The phenotyping was performed via the collagen uptake assay and NAD(P)H autofluorescence (94). Abbreviations: CTC, circulating tumor cell; DAPI, 4′,6-diamidino-2-phenylindole; EGFR, epidermal growth factor receptor; FITC, fluorescein isothiocyanate; MNP, magnetic nanoparticle; NP, nanoparticle; PtNP, platinum nanoparticle; SERS, surface-enhanced Raman spectroscopy.

Wang et al. (110) developed a photoelectrochemical (PEC) aptasensor that incorporated an AS1411 aptamer on a film electrode (made of upconversion NPs and TiO_2/CdTe) to identify MCF-7 cells. This PEC aptasensor offered a LOD of 400 cells/mL. Another PEC aptasensor was constructed by Li et al. (111) to capture CTCs. In this sensor, the aptamer was functionalized on the surfaces of $AgInS_2$ NPs, which resulted in a LOD of 16 cells/mL.

Zheng et al. (112) developed a voltammetric cytosensor to detect T47D and MCF-7 cells. In this device, Fe_3O_4@Ag-Pd bimetallic nanocages served as a hybrid nanoprobe that resulted in enhanced EC signals. This sensor yielded a LOD of 42 and 34 cells/mL for T47D and MCF-7 cells, respectively (112). A multiplex voltammetric cytosensor was also constructed to detect and differentiate acute myeloid leukemia cells and acute lymphocytic leukemia cells. Here, two different target cells were captured by two different immobilized aptamers (113). Sun et al. (114) constructed a sandwich-type voltammetric aptasensor to detect HepG2 cells on the basis of co-catalysis of the DNAzyme, hybrid nanozyme, and natural enzyme; this aptasensor provided an improved LOD of 15 cells/mL. The process was improved in a later study by the utilization of Au NPs and MIL-101-type metal–organic frameworks as functionalized hybrid nanoprobes. The improved system provided an excellent LOD of 5 HepG2 cells/mL (115).

Moreover, Li et al. (116) constructed a light addressable potentiometric sensor platform based on porous graphene oxide nanomaterials to enhance the active sensing area, which offered better detection sensitivity. Recently, they also developed a G-quadruplex-forming AS1411 DNA aptamer as an EC aptasensor, which has entered a phase II clinical trial to improve acute myeloid leukemia treatment (116).

4.5. Detection of Extracellular Vesicles

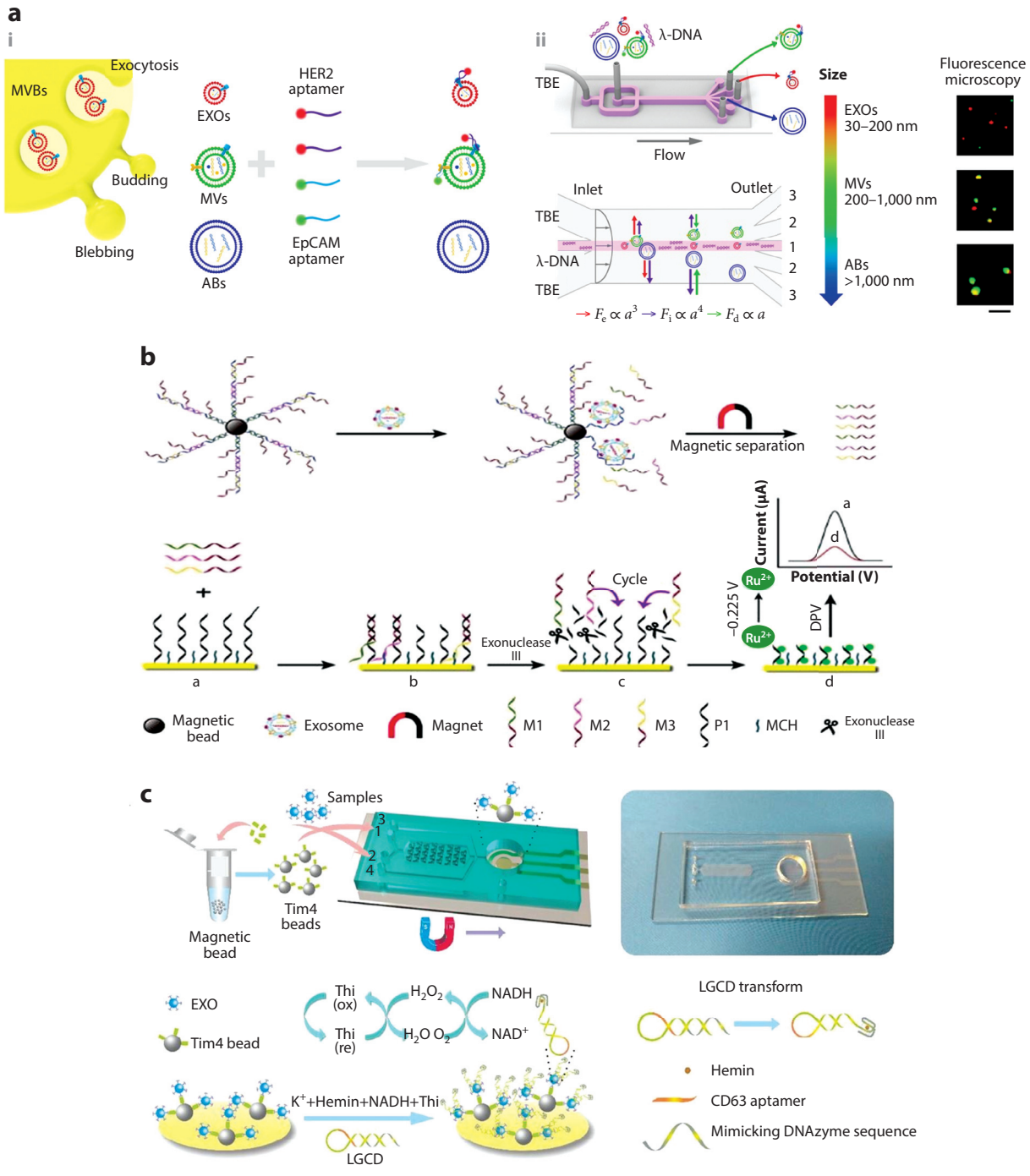
Lipid-bound extracellular vesicles (EVs) found in the extracellular space are secreted by cells (117, 118), and there are three major types of EVs: exosomes, microvesicles, and apoptotic bodies. Of these, the exosome is the most studied and is found in a broad range of biofluids, including synovial fluid, amniotic fluid, bronchial fluid, cerebral spinal fluid, plasma, urine, semen, saliva, breast milk, serum, tears, lymph, bile, and gastric acid (119). Exosomes are secreted by almost all types of cells and the typical diameter of the exosome is 30 to 150 nm (120). On the other hand, microvesicles are 0.1 to 1 μm in diameter and are formed by pinching or direct outward budding of the cell's plasma membrane (120). Apoptotic bodies have greater diameters (0.05 to 5 μm) than the two types of EVs described above. Apoptotic bodies containing intact organelles, chromatin, and small amounts of glycosylated proteins are formed by dead cells and released into the extracellular space. EVs, specifically exosomes, are carriers of rich biomarkers (e.g., surface proteins, microRNA) in extracellular space and have received extensive attention for diagnostic applications in the past few years (119).

4.5.1. Aptamers for EV isolation and capture. To investigate the function and diagnostic potential of EVs, the preanalytical isolation and purification step is crucial for clinical applications

(121). Studies showed that aptamers could bind EVs effectively through exosomal proteins, which can be analyzed subsequently by various assay methods, including the simple colorimetric profiling assays (122). Several groups employed complementary sequences that could disrupt the DNA aptamer-exosomal protein complex structure (85) and then used restriction enzymes to cleave the aptamer structure and release the intact EVs (123). Such nondestructive release mechanisms of captured EVs make it possible to successfully perform downstream molecular analysis on a single platform. A dual-signal amplification aptasensor was developed by Huang et al. (124) to detect leukemia-derived exosomes. They first used MBs conjugated by anti-CD63 antibodies as an effective strategy to isolate exosomes and then employed a nucleolin-recognition DNA aptamer to detect them. The recognition aptamer was connected to an RCA primer for the first layer of signal amplification. Then, the RCA products were detected by a gold NP–quenched fluorescent probe (GNP-DNA-FAM), where the fluorescence signals were amplified for the second time in the presence of a nicking endonuclease (Nb.BbvCl). This aptasensor provided a LOD of 10^5 particles/mL (124). Size-selective separation of EVs is also an important area. HER2 and EpCAM aptamers accompanied by λ-DNA-mediated viscoelastic microfluidics were developed by Liu et al. (125) to sort and analyze EVs derived from breast cancer cells simultaneously (**Figure 4*a***). The particle size-dependent sorting was achieved by the addition of viscoelastic λ-DNA into the EV mixture and the lateral displacement of microvesicles and apoptotic bodies due to the elastic lift force (F_e). The detection of different EV types was realized by targeting EV surface proteins and machine learning–based classification (125). Dong et al. (126) developed an aptamer–magnetic bead bioconjugate to capture LNCaP cell-derived exosomes (**Figure 4*b***). After magnetic separation, the bioconjugate released three messenger DNAs that were hybridized with the probe DNAs attached on a gold electrode. Then, the probe DNAs were cleaved by exonuclease III to release messenger DNAs, which could then diffuse and bind new DNA probes. The cyclic enzymatic amplification (repeated cleavage reactions) caused a turn-off signal in the differential pulse voltammetry, which was correlated to the initial exosome concentration. This platform offered a LOD of 7×10^4 particles/mL (126). A noninvasive ExoPCD microfluidic chip was developed by Xu et al. (127) to isolate EVs with the help of DNA aptamers and was based on a label-free and immobilization-free EC technique (**Figure 4*c***). This diagnostic platform offered a high detection sensitivity toward CD63 proteins on exosomes with a LOD of 4.39×10^3 particles/mL (127). Zhang et al. (128) developed a DNA aptamer-based magnetic isolation system to capture MUC1-positive EVs in breast cancer plasma samples rapidly and release them nondestructively in 90 min with an approximately 78% release efficiency, which is akin to the release efficiency of ultracentrifugation. EVs were captured through the interaction of streptavidin MBs and a biotin-labeled anti-CD63 aptamer. Then, EVs were released nondestructively by magnetic separation (128).

4.5.2. Aptamers for EV detection. Many aptamer-based detection platforms comprising fluorescent aptasensors, EC aptasensors, and colorimetric aptasensors have been developed for exosome analysis and have shown great promise as diagnostic tools to study EVs, especially exosomes.

Fluorescent aptasensors based on fluorescence signal amplification, fluorescence resonance energy transfer, or fluorescence polarization offer a simple operation and high sensitivity in diagnosis. Recently, an RCA aptasensor was developed to detect and amplify the captured exosomes by forming in situ double-stranded DNA products, offering a LOD of 4.27×10^4 particles/mL. In this method, the exosome capture was integrated with a hairpin DNA cascade hybridization reaction (HD-CHR), which converted the extent of exosomes captured to double-stranded DNA products for quantification (129). To improve the exosome purity, magnetic isolation using aptamer-functionalized Fe_3O_4 beads was demonstrated by Li et al. (130). This aptamer probe detected a urinary exosome with a LOD of 10^5 particles/mL. Moreover, for enhanced signal



(*Caption appears on following page*)

Figure 4 (*Figure appears on preceding page*)

(*a*) Schematic illustration of the (*i*) aptamer-based targeting ligands and (*ii*) mechanism of λ-DNA-mediated sorting of extracellular vesicle subtypes (125). (*b*) Representation of a tumor-derived exosome detection mechanism based on differential pulse voltammetry measurement (126). (*c*) Schematic diagram of an ExoPCD chip to isolate and analyze exosomes using a label-free and immobilization-free electrochemical aptasensor (127). Abbreviations: ABs, apoptotic bodies; DPV, differential pulse voltammetry; EXOs, exosomes; M1, messenger DNA 1; M2, messenger DNA 2; M3, messenger DNA 3; MCH, 6-mercapto-1-hexanol; MVs, microvesicles; MVBs, multivesicular bodies; P1, probe DNA; TBE, tris-borate-ethylenediaminetetraacetic acid.

amplification, Zhao et al. (131) incorporated a CD63 aptamer with a CRISPR/Cas12a system. After capturing the exosomal surface protein, the CD63 aptamer released a complementary DNA sequence called a blocker. Then the CRISPR/Cas12a complex recognized the blocker and cleaved a reporter probe, which amplified the fluorescence signals (131). He et al. (132) developed a single-exosome-imaging PTK7-aptamer sensor that was based on HCR amplification, through which diagnosis of early tumor development could be possible. Direct isolation and faster detection of EVs (e.g., exosomes) were made possible by using a Cu-mediated signal amplification aptamer sensor, which offered a LOD of 4.8×10^4 particles/mL. In this system, CD63 aptamer-modified CuO NPs recognized the exosomes to form the MB-exosome-CuO NP complex. Then, the CuO NP was converted to Cu^{2+} by the acidolysis reaction and Cu^{2+} was reduced by sodium ascorbate to produce fluorescent Cu NPs. Finally, the detection was performed by measuring the extent of fluorescent Cu NPs (133). Recently, a novel fluorescence signal amplification aptasensor was prepared by Wang et al. (134) on the basis of liposome-assisted and terminal deoxynucleotidyl transferase–mediated amplification, which offered a better LOD of 3.6×10^5 particles/mL. Two types of fluorescence resonance energy transfer aptasensors have also been demonstrated for exosome detection, namely fluorophore-quencher and fluorophore-NP pairs. The fluorophore-quencher-based aptamer sensors utilize aptamers to form hairpin-like structures to bring together the fluorophores and quencher dyes. Exosomes will open the hairpin structures and cause segregation between the quencher (e.g., Dabcyl) and fluorophore (e.g., TAMRA) to induce turn-on fluorescence signals for simple and fast EV detection (135). In fluorophore-NP aptamer sensors, a Ti_3C_2 MXenes nanocomplex was utilized as a high-surface-area substrate for binding the dye-labeled aptamer (e.g., Cy3-CD63 aptamer). Since there is an intense interaction between the exosomal CD63 protein and the CD63 DNA aptamer, the adsorbed aptamers were easily detached from the nanocomplex and merged with the target exosomes, and this process helped to recover the fluorescence signals when exosomes were present in the sample (136). Finally, Zhang et al. (137) developed a fluorescence polarization aptasensor to detect exosomes derived from a lung cancer cell line, which offered a LOD of 5×10^5 particles/mL. Using this method, sensitive detection and quantification were possible with a sample of approximately 1 μL of human plasma and approximately 30 min of detection time (137).

EC aptasensors offer a low-cost, simple, highly sensitive, and automated solution for POC exosome detection. Many EC aptasensors with different detection strategies, such as direct immobilization, sandwich format, DNA walker, and immobilization-free assay, have been employed to detect EVs. Recently, a direct-immobilized (i.e., DNA aptamer probes immobilized on the electrode surface directly) EC aptasensor was developed by Sun et al. (138) for exosome detection with a low LOD of 100 particles/mL. The sensor was prepared by coating a methylene-blue-labeled aptamer on a thin-film-based indium tin oxide slice. Wang et al. (139) presented an improved EC aptasensor utilizing an aptamer-based NTH structure to detect tumor-derived exosomes. This sandwich EC aptasensor was based on a capture probe–exosome-detection probe format, which is a strategy commonly used to enhance identification and detection. Similarly, a CD63 antibody–gastric cancer exosome–aptamer sandwich sensor for the detection of MUC1 proteins

was developed by Huang et al. (140) by combining hemin/G-quadruplex DNAzyme, H_2O_2 reduction, and RCA amplification. Zhou et al. (141) employed an aptamer as the capturing probe on a microfabricated chip for detecting exosomes, which offered a LOD of 50 exosomes/sensor. In this method, Ag NPs were employed to quantify EpCAM, while Cu NPs were used to report on the prostate-specific membrane antigen (PSMA) (141). To overcome the limitations of immobilizing the aptamer onto the electrode surface, Xu et al. (127) constructed an immobilization-free sandwich aptasensor composed of MB/Tim4 exosomes and a CD63 aptamer, which is based on the specific recognition of the CD63 aptamer and exosomes and nonspecific recognition of Tim4 and phosphatidylserine. This system offered a LOD of $\sim 4 \times 10^3$ particles/mL (127).

Colorimetric aptasensors are appealing for POC detection of EVs with easily readable visual diagnostic results. One of the common colorimetric aptasensor designs is based on Au NP aggregation. In this design, aptamers prevent the aggregation of Au NPs in a high concentration of NaCl. However, in the presence of EVs (e.g., exosomes), the aptamers will be detached from the Au NP surface to bind the protein biomarkers present on the exosomal surface. As a result, the Au NPs start to aggregate and change the solution color from red to light blue within several minutes (122). Nanozymes have also been integrated into aptasensors. For instance, Wang et al. (142) constructed a colorimetric CD63 aptasensor using graphitic carbon nitride nanosheets, which in turn offered a significant increase of peroxidase-like activity. Other novel nanozymes such as Fe_3O_4 NPs with additional separation capability (143) were constructed to detect EVs via the H_2O_2 oxidation reaction and colorimetric readout.

4.6. Tissue Samples Diagnostics

Aptamer-based ligands have also been applied to acquire diagnostic information through in situ immunostaining of tumor tissues. Compared with antibody-based immunostaining, aptamer-assisted staining of formalin-fixed and paraffin-embedded tumor tissues is gaining popularity due to its penetration efficiency and capability of minimizing nonspecific signals coming from the necrotic portion (144). A number of cell/tissue-SELEX–derived aptamers have been developed to stain tissues from prostate, colorectal, breast, and gastric cancers.

A SYL3C-CY3 aptamer was developed by Pu et al. (145) to stain colorectal cancer patients' tissues. This approach utilized the immunofluorescence technique to diagnose EpCAM-positive colorectal cells in frozen tissue samples. Li et al. (146) prepared a detection probe based on W3 aptamer–coated QDs to image metastatic colorectal cancer cells from patients. Also, this probe was used for the immunostaining of tissues from tumor-bearing mice (146). Yuan et al. (147) developed a J3 DNA aptamer labeled with Cy5 dye that showed selective and specific binding to colorectal cancer LoVo cells. This aptamer-based probe generated promising fluorescent images of colorectal cancer tissue sections, which provided valuable immunostaining information for clinical diagnosis of colorectal cancer metastasis. The results showed that the J3-Cy5 probe specifically recognized the metastatic lymph node tissue section with a significant detection rate (~74%). In contrast, it showed an insignificant detection rate for a colorectal cancer tissue section that had no metastasis (147). Recently, an XL-33-1 DNA aptamer labeled with a fluorescein amidite probe was constructed by Li et al. (148) to stain SW620 cells in colorectal patients' tissue samples. This aptamer-based novel probe can be used to detect early metastasis of colorectal cancer.

To selectively identify breast tissue carcinomas, a BC-15 DNA aptamer was developed by Shao and colleagues (149), which targeted heterogeneous nuclear ribonucleoprotein A1 of breast tissue carcinomas over an adjacent healthy breast tissue section.

In summary, although monoclonal antibody conjugated immunostaining is widely used for histopathological diagnosis, aptamer-based tissue staining has emerged as a promising alternative due to its thermostability, penetration efficiency, and easy chemical modification (150).

5. CONCLUSIONS AND FUTURE PERSPECTIVES

Aptasensors have experienced significant growth in the past few decades. Their advantages over conventional antibody-based recognition have been extensively studied and documented. The potentials of aptamer-based biosensors or assay methods in the context of POC diagnostics or global health have also been rapidly explored in the past few years. Their unique properties such as stability as dry reagents, negligible batch-to-batch variation, cost-effectiveness, and high detection sensitivity and specificity are especially appealing for field deployment or implementation in poorly resourced settings. This review article highlights a few representative examples from a plethora of recent studies of aptamer-based sensing technologies for the detection of various clinically relevant biomarkers, such as small molecules, proteins, nucleic acids, whole pathogens, CTCs, EVs, and thin tumor slices. Several notable trends can be summarized, including the following two examples. First, the majority of studies use aptamers as effective binding or targeting ligands for the selective recognition of targeted analytes, although a few studies utilized the 3D structure of aptamers to be a part of signal transduction mechanism (e.g., conformation change-induced signaling). Second, to form a field-deployable POC sensing or diagnostic platform, aptamers are frequently conjugated with microfluidic devices or nanomaterials to enable high-throughput screening or novel signal generation mechanisms.

Currently demonstrated aptasensors still face several major challenges. First, more clinical studies are needed to further validate their performance in analyzing real patient samples. Many existing results were built on simple model systems such as purified cell cultures or spiked samples consisting of standard buffer solutions. The robustness of different aptamer-based methods needs to be further tested in more complex biofluid sample matrices, such as serum, whole blood, saliva, and urine. This will require more cross-disciplinary collaboration between sensor developers (such as chemists or engineers) and healthcare workers (such as clinicians and medical providers) to enable convergent science and further optimize sensing systems. Second, since early diagnosis of diseases plays a significant role in improved medical intervention, timely reporting of health condition changes or disease onset will be a requirement for the new generation of aptasensors. In this regard, aptamer-based biosensors that can provide real-time and continuous-monitoring capabilities are expected to dominate future development. This imposes a significant challenge to develop a completely new set of aptasensors that will not rely on permanent binding events and will rather focus on reversible molecular interactions to enable continuous analyst sampling. Conformation-modulated electron tunneling in aptamer-based EC sensors is one promising example of real-time sensing.

Despite the remaining challenges, the recent development of POC-friendly aptamer-based biosensors has shown tremendous potential for improving personalized diagnostics and global health on a large scale. For example, the integration of aptamer biosensors with emerging wearable technologies could provide an alternative method for long-term health monitoring and disease management. With more clinical validation, scaling-up, and commercialization, aptasensors could provide a more profound impact in the coming era of digital and precision medicine.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

Q.W. and N.M. gratefully acknowledge support from the National Science Foundation (award # 1944167).

LITERATURE CITED

1. Flahault A, Geissbuhler A, Guessous I, Guérin P, Bolon I, et al. 2017. Precision global health in the digital age. *Swiss Med. Wkly.* 147:w14423
2. Steinhubl SR, Muse ED, Topol EJ. 2015. The emerging field of mobile health. *Sci. Transl. Med.* 7:283rv3
3. Candia J, Cheung F, Kotliarov Y, Fantoni G, Sellers B, et al. 2017. Assessment of variability in the SOMAscan assay. *Sci. Rep.* 7:14248
4. Robertson DL, Joyce GF. 1990. Selection in vitro of an RNA enzyme that specifically cleaves single-stranded DNA. *Nature* 344:467–68
5. Ellington AD, Szostak JW. 1990. In vitro selection of RNA molecules that bind specific ligands. *Nature* 346:818–22
6. Tuerk C, Gold L. 1990. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science* 249:505–10
7. Zhu K, Wang ZY, Zong SF, Liu Y, Yang K, et al. 2020. Hydrophobic plasmonic nanoacorn array for a label-free and uniform SERS-based biomolecular assay. *ACS Appl. Mater. Interfaces* 12:29917–27
8. Cheng S, Zheng B, Yao DB, Wang Y, Tian JJ, et al. 2019. Determination of saxitoxin by aptamer-based surface-enhanced Raman scattering. *Anal. Lett.* 52:902–18
9. Bagheri E, Abnous K, Alibolandi M, Ramezani M, Taghdisi SM. 2018. Triple-helix molecular switch-based aptasensors and DNA sensors. *Biosens. Bioelectron.* 111:1–9
10. Su ZH, Xu XL, Xu HT, Zhang Y, Li CR, et al. 2017. Amperometric thrombin aptasensor using a glassy carbon electrode modified with polyaniline and multiwalled carbon nanotubes tethered with a thiolated aptamer. *Microchim. Acta* 184:1677–82
11. Zhang YT, Figueroa-Miranda G, Zafiu C, Willbold D, Offenhausser A, Mayer D. 2019. Amperometric aptasensor for amyloid-β oligomer detection by optimized stem-loop structures with an adjustable detection range. *ACS Sens.* 4:3042–50
12. Ghalehno MH, Mirzaei M, Torkzadeh-Mahani M. 2019. Electrochemical aptasensor for tumor necrosis factor α using aptamer–antibody sandwich structure and cobalt hexacyanoferrate for signal amplification. *J. Iran. Chem. Soc.* 16:1783–91
13. Shandost-Fard F, Roushani M. 2017. The use of a signal amplification strategy for the fabrication of a TNT impedimetric nanoaptasensor based on electrodeposited NiONPs immobilized onto a GCE surface. *Sens. Actuators B Chem.* 246:848–53
14. Wang GX, Han R, Li Q, Han YF, Luo XL. 2020. Electrochemical biosensors capable of detecting biomarkers in human serum with unique long-term antifouling abilities based on designed multifunctional peptides. *Anal. Chem.* 92:7186–93
15. Wang HY, Sun JJ, Lu L, Yang X, Xia JF, et al. 2020. Competitive electrochemical aptasensor based on a cDNA-ferrocene/MXene probe for detection of breast cancer marker Mucin1. *Anal. Chim. Acta* 1094:18–25
16. Yang ZH, Ding XF, Guo Q, Wang Y, Lu ZW, et al. 2017. Second generation of signaling-probe displacement electrochemical aptasensor for detection of picomolar ampicillin and sulfadimethoxine. *Sens. Actuators B Chem.* 253:1129–36
17. Urbanova V, Jayaramulu K, Schneemann A, Kment S, Fischer RA, Zbaril R. 2018. Hierarchical porous fluorinated graphene oxide@metal–organic gel composite: label-free electrochemical aptasensor for selective detection of thrombin. *ACS Appl. Mater. Interfaces* 10:41089–97
18. Wang XY, Gao FX, Gong YY, Liu GT, Zhang Y, Ding CF. 2019. Electrochemical aptasensor based on conductive supramolecular polymer hydrogels for thrombin detection with high selectivity. *Talanta* 205:120140
19. Jiang D, Du X, Liu Q, Zhou L, Dai L, et al. 2015. Silver nanoparticles anchored on nitrogen-doped graphene as a novel electrochemical biosensing platform with enhanced sensitivity for aptamer-based pesticide assay. *Analyst* 140:6404–11
20. Madianos L, Skotadis E, Tsekenis G, Patsiouras L, Tsigkourakos M, Tsoukalas D. 2018. Impedimetric nanoparticle aptasensor for selective and label free pesticide detection. *Microelectron. Eng.* 189:39–45
21. Li S, Wu X, Liu C, Yin G, Luo J, Xu Z. 2016. Application of DNA aptamers as sensing layers for detection of carbofuran by electrogenerated chemiluminescence energy transfer. *Anal. Chim. Acta* 941:94–100

22. Javidi M, Housaindokht MR, Verdian A, Razavizadeh BM. 2018. Detection of chloramphenicol using a novel apta-sensing platform based on aptamer terminal-lock in milk samples. *Anal. Chim. Acta* 1039:116–23
23. Roushani M, Nezhadali A, Jalilian Z. 2018. An electrochemical chlorpyrifos aptasensor based on the use of a glassy carbon electrode modified with an electropolymerized aptamer-imprinted polymer and gold nanorods. *Microchim. Acta* 185:551
24. Fu J, Yao Y, An X, Wang G, Guo Y, et al. 2020. Voltammetric determination of organophosphorus pesticides using a hairpin aptamer immobilized in a graphene oxide-chitosan composite. *Microchim. Acta* 187:36
25. Bala R, Kumar M, Bansal K, Sharma RK, Wangoo N. 2016. Ultrasensitive aptamer biosensor for malathion detection based on cationic polymer and gold nanoparticles. *Biosens. Bioelectron.* 85:445–49
26. Bala R, Sharma RK, Wangoo N. 2016. Development of gold nanoparticles-based aptasensor for the colorimetric detection of organophosphorus pesticide phorate. *Anal. Bioanal. Chem.* 408:333–38
27. Fu J, An X, Yao Y, Guo Y, Sun X. 2019. Electrochemical aptasensor based on one step co-electrodeposition of aptamer and GO-CuNPs nanocomposite for organophosphorus pesticide detection. *Sens. Actuators B Chem.* 287:503–9
28. Derbyshire N, White SJ, Bunka DH, Song L, Stead S, et al. 2012. Toggled RNA aptamers against aminoglycosides allowing facile detection of antibiotics using gold nanoparticle assays. *Anal. Chem.* 84:6595–602
29. Liu Y, Yan K, Okoth OK, Zhang J. 2015. A label-free photoelectrochemical aptasensor based on nitrogen-doped graphene quantum dots for chloramphenicol determination. *Biosens. Bioelectron.* 74:1016–21
30. Sun X, Li F, Shen G, Huang J, Wang X. 2014. Aptasensor based on the synergistic contributions of chitosan–gold nanoparticles, graphene–gold nanoparticles and multi-walled carbon nanotubes-cobalt phthalocyanine nanocomposites for kanamycin detection. *Analyst* 139:299–308
31. Li S, Liu C, Yin G, Zhang Q, Luo J, Wu N. 2017. Aptamer-molecularly imprinted sensor base on electrogenerated chemiluminescence energy transfer for detection of lincomycin. *Biosens. Bioelectron.* 91:687–91
32. He L, Luo Y, Zhi W, Zhou P. 2013. Colorimetric sensing of tetracyclines in milk based on the assembly of cationic conjugated polymer-aggregated gold nanoparticles. *Food Anal. Methods* 6:1704–11
33. Wu Y, Zhan S, Wang F, He L, Zhi W, Zhou P. 2012. Cationic polymers and aptamers mediated aggregation of gold nanoparticles for the colorimetric detection of arsenic (III) in aqueous solution. *Chem. Commun.* 48:4459–61
34. Chen Z, Li L, Mu X, Zhao H, Guo L. 2011. Electrochemical aptasensor for detection of copper based on a reagentless signal-on architecture and amplification by gold nanoparticles. *Talanta* 85:730–35
35. Pelossof G, Tel-Vered R, Liu XQ, Willner I. 2011. Amplified surface plasmon resonance based DNA biosensors, aptasensors, and Hg^{2+} sensors using hemin/G-quadruplexes and Au nanoparticles. *Chem. A Eur. J.* 17:8904–12
36. Seok Y, Byun J-Y, Shim W-B, Kim M-G. 2015. A structure-switchable aptasensor for aflatoxin B1 detection based on assembly of an aptamer/split DNAzyme. *Anal. Chim. Acta* 886:182–87
37. Wu S, Duan N, Ma X, Xia Y, Wang H, et al. 2012. Multiplexed fluorescence resonance energy transfer aptasensor between upconversion nanoparticles and graphene oxide for the simultaneous determination of mycotoxins. *Anal. Chem.* 84:6263–70
38. Yang C, Wang Y, Marty J-L, Yang X. 2011. Aptamer-based colorimetric biosensing of Ochratoxin A using unmodified gold nanoparticles indicator. *Biosens. Bioelectron.* 26:2724–27
39. Rivas L, Mayorga-Martinez CC, Quesada-González D, Zamora-Gálvez A, de la Escosura-Muñiz A, Merkoçi A. 2015. Label-free impedimetric aptasensor for ochratoxin-A detection using iridium oxide nanoparticles. *Anal. Chem.* 87:5167–72
40. Temur E, Zengin A, Boyacı IH, Dudak FC, Torul H, Tamer U. 2012. Attomole sensitivity of staphylococcal enterotoxin B detection using an aptamer-modified surface-enhanced Raman scattering probe. *Anal. Chem.* 84:10600–6
41. Ramezani M, Danesh NM, Lavaee P, Abnous K, Taghdisi SM. 2015. A novel colorimetric triple-helix molecular switch aptasensor for ultrasensitive detection of tetracycline. *Biosens. Bioelectron.* 70:181–87

42. Taghdisi SM, Danesh NM, Ramezani M, Emrani AS, Abnous K. 2018. Novel colorimetric aptasensor for zearalenone detection based on nontarget-induced aptamer walker, gold nanoparticles, and exonuclease-assisted recycling amplification. *ACS Appl. Mater. Interfaces* 10:12504–9
43. Wang G, Su X, Xu Q, Xu G, Lin J, Luo X. 2018. Antifouling aptasensor for the detection of adenosine triphosphate in biological media based on mixed self-assembled aptamer and zwitterionic peptide. *Biosens. Bioelectron.* 101:129–34
44. Zhou L, Wang J, Li D, Li Y. 2014. An electrochemical aptasensor based on gold nanoparticles dotted graphene modified glassy carbon electrode for label-free detection of bisphenol A in milk samples. *Food Chem.* 162:34–40
45. Marks HL, Pishko MV, Jackson GW, Coté GL. 2014. Rational design of a bisphenol A aptamer selective surface-enhanced Raman scattering nanoprobe. *Anal. Chem.* 86:11614–19
46. Taghdisi SM, Danesh NM, Emrani AS, Ramezani M, Abnous K. 2015. A novel electrochemical aptasensor based on single-walled carbon nanotubes, gold electrode and complimentary strand of aptamer for ultrasensitive detection of cocaine. *Biosens. Bioelectron.* 73:245–50
47. Ma C, Wang W, Yang Q, Shi C, Cao L. 2011. Cocaine detection via rolling circle amplification of short DNA strand separated by magnetic beads. *Biosens. Bioelectron.* 26:3309–12
48. Wang W, Wu W-Y, Zhong X, Miao Q, Zhu J-J. 2011. Aptamer-based PDMS–gold nanoparticle composite as a platform for visual detection of biomolecules with silver enhancement. *Biosens. Bioelectron.* 26:3110–14
49. Zamay GS, Zamay TN, Kolovskii VA, Shabanov AV, Glazyrin YE, et al. 2016. Electrochemical aptasensor for lung cancer-related protein detection in crude blood plasma samples. *Sci. Rep.* 6:34350
50. Rodríguez MC, Rivas GA. 2009. Label-free electrochemical aptasensor for the detection of lysozyme. *Talanta* 78:212–16
51. Jarczewska M, Rębiś J, Górski Ł, Malinowska E. 2018. Development of DNA aptamer-based sensor for electrochemical detection of C-reactive protein. *Talanta* 189:45–54
52. Wang J, Guo J, Zhang J, Zhang W, Zhang Y. 2017. RNA aptamer-based electrochemical aptasensor for C-reactive protein detection using functionalized silica microspheres as immunoprobes. *Biosens. Bioelectron.* 95:100–5
53. Crulhas BP, Karpik AE, Delella FK, Castro GR, Pedrosa VA. 2017. Electrochemical aptamer-based biosensor developed to monitor PSA and VEGF released by prostate cancer cells. *Anal. Bioanal. Chem.* 409:6771–80
54. Kumar LS, Wang X, Hagen J, Naik R, Papautsky I, Heikenfeld J. 2016. Label free nano-aptasensor for interleukin-6 in protein-dilute bio fluids such as sweat. *Anal. Methods* 8:3440–44
55. Sypabekova M, Jolly P, Estrela P, Kanayeva D. 2019. Electrochemical aptasensor using optimized surface chemistry for the detection of *Mycobacterium tuberculosis* secreted protein MPT64 in human serum. *Biosens. Bioelectron.* 123:141–51
56. Bhardwaj J, Chaudhary N, Kim H, Jang J. 2019. Subtyping of influenza A H1N1 virus using a label-free electrochemical biosensor based on the DNA aptamer targeting the stem region of HA protein. *Anal. Chim. Acta* 1064:94–103
57. Ren Q, Mou J, Guo Y, Wang H, Cao X, et al. 2020. Simple homogeneous electrochemical target-responsive aptasensor based on aptamer bio-gated and porous carbon nanocontainer derived from ZIF-8. *Biosens. Bioelectron.* 166:112448
58. Lu L, Liu B, Leng J, Ma X, Peng H. 2020. Electrochemical mixed aptamer-antibody sandwich assay for mucin protein 16 detection through hybridization chain reaction amplification. *Anal. Bioanal. Chem.* 412:7169–78
59. Liu Y, Tian H, Chen X, Liu W, Xia K, et al. 2020. Indirect surface-enhanced Raman scattering assay of insulin-like growth factor 2 receptor protein by combining the aptamer modified gold substrate and silver nanoprobes. *Microchim. Acta* 187:160
60. Mazzaracchio V, Neagu D, Porchetta A, Marcoccio E, Pomponi A, et al. 2019. A label-free impedimetric aptasensor for the detection of *Bacillus anthracis* spore simulant. *Biosens. Bioelectron.* 126:640–46
61. Zhou C, You T, Jang H, Ryu H, Lee E-S, et al. 2020. Aptamer-conjugated polydiacetylene colorimetric paper chip for the detection of *Bacillus thuringiensis* spores. *Sensors* 20:3124

62. Bruno JG, Phillips T, Carrillo MP, Crowell R. 2009. Plastic-adherent DNA aptamer-magnetic bead and quantum dot sandwich assay for *Campylobacter* detection. *J. Fluoresc.* 19:427–35
63. Li L, Li Q, Liao Z, Sun Y, Cheng Q, et al. 2018. Magnetism-resolved separation and fluorescence quantification for near-simultaneous detection of multiple pathogens. *Anal. Chem.* 90:9621–28
64. Mohsin DH, Mashkour MS, Fatemi F. 2021. Design of aptamer-based sensing platform using gold nanoparticles functionalized reduced graphene oxide for ultrasensitive detection of Hepatitis B virus. *Chem. Pap.* 75:279–95
65. Suh SH, Choi SJ, Dwivedi HP, Moore MD, Escudero-Abarca BI, Jaykus L-A. 2018. Use of DNA aptamer for sandwich type detection of *Listeria monocytogenes*. *Anal. Biochem.* 557:27–33
66. Weerathunge P, Ramanathan R, Torok VA, Hodgson K, Xu Y, et al. 2019. Ultrasensitive colorimetric detection of murine norovirus using nanozyme aptasensor. *Anal. Chem.* 91:3270–76
67. Das R, Dhiman A, Kapil A, Bansal V, Sharma TK. 2019. Aptamer-mediated colorimetric and electrochemical detection of *Pseudomonas aeruginosa* utilizing peroxidase-mimic activity of gold NanoZyme. *Anal. Bioanal. Chem.* 411:1229–38
68. Liang J, Zhou J, Tan J, Wang Z, Deng L. 2019. Aptamer-based fluorescent determination of *Salmonella paratyphi A* using Phi29-DNA polymerase-assisted cyclic amplification. *Anal. Lett.* 52:919–31
69. Dai G, Li Z, Luo F, Ai S, Chen B, Wang Q. 2019. Electrochemical determination of *Salmonella typhimurium* by using aptamer-loaded gold nanoparticles and a composite prepared from a metal-organic framework (type UiO-67) and graphene. *Microchim. Acta* 186:620
70. Chang Y-C, Yang C-Y, Sun R-L, Cheng Y-F, Kao W-C, Yang P-C. 2013. Rapid single cell detection of *Staphylococcus aureus* by aptamer-conjugated gold nanoparticles. *Sci. Rep.* 3:1863
71. Sun Y, Duan N, Ma P, Liang Y, Zhu X, Wang Z. 2019. Colorimetric aptasensor based on truncated aptamer and trivalent DNAzyme for *Vibrio parahemolyticus* determination. *J. Agric. Food Chem.* 67:2313–20
72. Fitzmaurice C, Allen C, Barber RM, Barregard L, Bhutta ZA, et al. 2017. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: a systematic analysis for the Global Burden of Disease study. *JAMA Oncol.* 3:524–48
73. Williams SCP. 2013. Circulating tumor cells. *PNAS* 110:4861
74. Yu M, Bardia A, Aceto N, Bersani F, Madden MW, et al. 2014. Ex vivo culture of circulating breast tumor cells for individualized testing of drug susceptibility. *Science* 345:216–20
75. Fang X, Tan W. 2010. Aptamers generated from cell-SELEX for molecular medicine: a chemical biology approach. *Acc. Chem. Res.* 43:48–57
76. Ferreira CS, Matthews CS, Missailidis S. 2006. DNA aptamers that bind to MUC1 tumour marker: design and characterization of MUC1-binding single-stranded DNA aptamers. *Tumour Biol.* 27:289–301
77. Wan Y, Kim YT, Li N, Cho SK, Bachoo R, et al. 2010. Surface-immobilized aptamers for cancer cell isolation and microscopic cytology. *Cancer Res.* 70:9371–80
78. Song Y, Zhu Z, An Y, Zhang W, Zhang H, et al. 2013. Selection of DNA aptamers against epithelial cell adhesion molecule for cancer cell imaging and circulating tumor cell capture. *Anal. Chem.* 85:4141–49
79. Sun D, Lu J, Luo Z, Zhang L, Liu P, Chen Z. 2018. Competitive electrochemical platform for ultrasensitive cytosensing of liver cancer cells by using nanotetrahedra structure with rolling circle amplification. *Biosens. Bioelectron.* 120:8–14
80. Shen H, Yang J, Chen Z, Chen X, Wang L, et al. 2016. A novel label-free and reusable electrochemical cytosensor for highly sensitive detection and specific collection of CTCs. *Biosens. Bioelectron.* 81:495–502
81. Xu Y, Phillips JA, Yan J, Li Q, Fan ZH, Tan W. 2009. Aptamer-based microfluidic device for enrichment, sorting, and detection of multiple cancer cells. *Anal. Chem.* 81:7436–42
82. Nagrath S, Sequist LV, Maheswaran S, Bell DW, Irimia D, et al. 2007. Isolation of rare circulating tumour cells in cancer patients by microchip technology. *Nature* 450:1235–39
83. Sheng W, Chen T, Kamath R, Xiong X, Tan W, Fan ZH. 2012. Aptamer-enabled efficient isolation of cancer cells from whole blood using a microfluidic device. *Anal. Chem.* 84:4199–206
84. Sheng W, Chen T, Tan W, Fan ZH. 2013. Multivalent DNA nanospheres for enhanced capture of cancer cells in microfluidic devices. *ACS Nano* 7:7067–76

85. Zhao W, Cui CH, Bose S, Guo D, Shen C, et al. 2012. Bioinspired multivalent DNA network for capture and release of cells. *PNAS* 109:19626–31
86. Shen Q, Xu L, Zhao L, Wu D, Fan Y, et al. 2013. Specific capture and release of circulating tumor cells using aptamer-modified nanosubstrates. *Adv. Mater.* 25:2368–73
87. Zhao L, Tang C, Xu L, Zhang Z, Li X, et al. 2016. Enhanced and differential capture of circulating tumor cells from lung cancer patients by microfluidic assays using aptamer cocktail. *Small* 12:1072–81
88. Herr JK, Smith JE, Medley CD, Shangguan D, Tan W. 2006. Aptamer-conjugated nanoparticles for selective collection and detection of cancer cells. *Anal. Chem.* 78:2918–24
89. Smith JE, Medley CD, Tang Z, Shangguan D, Lofton C, Tan W. 2007. Aptamer-conjugated nanoparticles for the collection and detection of multiple cancer cells. *Anal. Chem.* 79:3075–82
90. Chiu W-J, Ling T-K, Chiang H-P, Lin H-J, Huang C-C. 2015. Monitoring cluster ions derived from aptamer-modified gold nanofilms under laser desorption/ionization for the detection of circulating tumor cells. *ACS Appl. Mater. Interfaces* 7:8622–30
91. Abate MF, Jia S, Ahmed MG, Li X, Lin L, et al. 2019. Visual quantitative detection of circulating tumor cells with single-cell sensitivity using a portable microfluidic device. *Small* 15:1804890
92. Labib M, Green B, Mohamadi RM, Mepham A, Ahmed SU, et al. 2016. Aptamer and antisense-mediated two-dimensional isolation of specific cancer cell subpopulations. *J. Am. Chem. Soc.* 138:2476–79
93. Zhang Y, Wang Z, Wu L, Zong S, Yun B, Cui Y. 2018. Combining multiplex SERS nanovectors and multivariate analysis for in situ profiling of circulating tumor cell phenotype using a microfluidic chip. *Small* 14:1704433
94. Green BJ, Kermanshah L, Labib M, Ahmed SU, Silva PN, et al. 2017. Isolation of phenotypically distinct cancer cells using nanoparticle-mediated sorting. *ACS Appl. Mater. Interfaces* 9:20435–43
95. Reinholt SJ, Craighead HG. 2018. Microfluidic device for aptamer-based cancer cell capture and genetic mutation detection. *Anal. Chem.* 90:2601–8
96. Shangguan D, Li Y, Tang Z, Cao ZC, Chen HW, et al. 2006. Aptamers evolved from live cells as effective molecular probes for cancer study. *PNAS* 103:11838–43
97. Miao P, Tang Y. 2019. Gold nanoparticles-based multipedal DNA walker for ratiometric detection of circulating tumor cell. *Anal. Chem.* 91:15187–92
98. Cao J, Zhao XP, Younis MR, Li ZQ, Xia XH, Wang C. 2017. Ultrasensitive capture, detection, and release of circulating tumor cells using a nanochannel-ion channel hybrid coupled with electrochemical detection technique. *Anal. Chem.* 89:10957–64
99. Dou B, Xu L, Jiang B, Yuan R, Xiang Y. 2019. Aptamer-functionalized and gold nanoparticle array-decorated magnetic graphene nanosheets enable multiplexed and sensitive electrochemical detection of rare circulating tumor cells in whole blood. *Anal. Chem.* 91:10792–99
100. Feng L, Chen Y, Ren J, Qu X. 2011. A graphene functionalized electrochemical aptasensor for selective label-free detection of cancer cells. *Biomaterials* 32:2930–37
101. Qu L, Xu J, Tan X, Liu Z, Xu L, Peng R. 2014. Dual-aptamer modification generates a unique interface for highly sensitive and specific electrochemical detection of tumor cells. *ACS Appl. Mater. Interfaces* 6:7309–15
102. Gu C, Gai P, Liu X, Liu J, Li F. 2018. Ultrasensitive and versatile homogeneous electrochemical cytosensing platform based on target-induced displacement reaction for "signal-on" bioassay. *Sens. Actuators B Chem.* 270:1–8
103. Hashkavayi AB, Raoof JB, Ojani R, Kavoosian S. 2017. Ultrasensitive electrochemical aptasensor based on sandwich architecture for selective label-free detection of colorectal cancer (CT26) cells. *Biosens. Bioelectron.* 92:630–37
104. Kashefi-Kheyrabadi L, Mehrgardi MA, Wiechec E, Turner APF, Tiwari A. 2014. Ultrasensitive detection of human liver hepatocellular carcinoma cells using a label-free aptasensor. *Anal. Chem.* 86:4956–60
105. Zhou G, Lin M, Song P, Chen X, Chao J, et al. 2014. Multivalent capture and detection of cancer cells with DNA nanostructured biosensors and multibranched hybridization chain reaction amplification. *Anal. Chem.* 86:7843–48
106. Liu J-X, Bao N, Luo X, Ding S-N. 2018. Nonenzymatic amperometric aptamer cytosensor for ultrasensitive detection of circulating tumor cells and dynamic evaluation of cell surface N-glycan expression. *ACS Omega* 3:8595–604

107. Tian L, Qi J, Qian K, Oderinde O, Liu Q, et al. 2017. Copper (II) oxide nanozyme based electrochemical cytosensor for high sensitive detection of circulating tumor cells in breast cancer. *J. Electroanal. Chem.* 812:1–9
108. Liu H, Xu S, He Z, Deng A, Zhu J-J. 2013. Supersandwich cytosensor for selective and ultrasensitive detection of cancer cells using aptamer-DNA concatamer-quantum dots probes. *Anal. Chem.* 85:3385–92
109. Zhou B, Qiu Y, Wen Q, Zhu M, Yang P. 2017. Dual electrochemiluminescence signal system for in situ and simultaneous evaluation of multiple cell-surface receptors. *ACS Appl. Mater. Interfaces* 9:2074–82
110. Wang K, Zhang R, Sun N, Li X, Wang J, et al. 2016. Near-infrared light-driven photoelectrochemical aptasensor based on the upconversion nanoparticles and TiO_2/CdTe heterostructure for detection of cancer cells. *ACS Appl. Mater. Interfaces* 8:25834–39
111. Li J, Lin X, Zhang Z, Tu W, Dai Z. 2019. Red light-driven photoelectrochemical biosensing for ultrasensitive and scatheless assay of tumor cells based on hypotoxic $AgInS_2$ nanoparticles. *Biosens. Bioelectron.* 126:332–38
112. Zheng T, Zhang Q, Feng S, Zhu J-J, Wang Q, Wang H. 2014. Robust nonenzymatic hybrid nanoelectrocatalysts for signal amplification toward ultrasensitive electrochemical cytosensing. *J. Am. Chem. Soc.* 136:2288–91
113. Zheng T, Tan T, Zhang Q, Fu JJ, Wu JJ, et al. 2013. Multiplex acute leukemia cytosensing using multifunctional hybrid electrochemical nanoprobes at a hierarchically nanoarchitectured electrode interface. *Nanoscale* 5:10360–68
114. Sun D, Lu J, Zhong Y, Yu Y, Wang Y, et al. 2016. Sensitive electrochemical aptamer cytosensor for highly specific detection of cancer cells based on the hybrid nanoelectrocatalysts and enzyme for signal amplification. *Biosens. Bioelectron.* 75:301–7
115. Chen D, Sun D, Wang Z, Qin W, Chen L, et al. 2018. A DNA nanostructured aptasensor for the sensitive electrochemical detection of HepG2 cells based on multibranched hybridization chain reaction amplification strategy. *Biosens. Bioelectron.* 117:416–21
116. Li F, Hu S, Zhang R, Gu Y, Li Y, Jia Y. 2019. Porous graphene oxide enhanced aptamer specific circulating-tumor-cell sensing interface on light addressable potentiometric sensor: clinical application and simulation. *ACS Appl. Mater. Interfaces* 11:8704–9
117. Zaborowski MP, Balaj L, Breakefield XO, Lai CP. 2015. Extracellular vesicles: composition, biological relevance, and methods of study. *Bioscience* 65:783–97
118. Yáñez-Mó M, Siljander PRM, Andreu Z, Bedina Zavec A, Borràs FE, et al. 2015. Biological properties of extracellular vesicles and their physiological functions. *J. Extracell. Vesicles* 4:27066
119. Doyle LM, Wang MZ. 2019. Overview of extracellular vesicles, their origin, composition, purpose, and methods for exosome isolation and analysis. *Cells* 8:727
120. Bebelman MP, Smit MJ, Pegtel DM, Baglio SR. 2018. Biogenesis and function of extracellular vesicles in cancer. *Pharmacol. Ther.* 188:1–11
121. Contreras-Naranjo JC, Wu HJ, Ugaz VM. 2017. Microfluidics for exosome isolation and analysis: enabling liquid biopsy for personalized medicine. *Lab Chip* 17:3558–77
122. Jiang Y, Shi M, Liu Y, Wan S, Cui C, et al. 2017. Aptamer/AuNP biosensor for colorimetric profiling of exosomal proteins. *Angew. Chem. Int. Ed. Engl.* 56:11916–20
123. Zhang K, Deng R, Teng X, Li Y, Sun Y, et al. 2018. Direct visualization of single-nucleotide variation in mtDNA using a CRISPR/Cas9-mediated proximity ligation assay. *J. Am. Chem. Soc.* 140:11293–301
124. Huang L, Wang DB, Singh N, Yang F, Gu N, Zhang XE. 2018. A dual-signal amplification platform for sensitive fluorescence biosensing of leukemia-derived exosomes. *Nanoscale* 10:20289–95
125. Liu C, Zhao J, Tian F, Chang J, Zhang W, Sun J. 2019. λ-DNA- and aptamer-mediated sorting and analysis of extracellular vesicles. *J. Am. Chem. Soc.* 141:3817–21
126. Dong H, Chen H, Jiang J, Zhang H, Cai C, Shen Q. 2018. Highly sensitive electrochemical detection of tumor exosomes based on aptamer recognition-induced multi-DNA release and cyclic enzymatic amplification. *Anal. Chem.* 90:4507–13
127. Xu H, Liao C, Zuo P, Liu Z, Ye BC. 2018. Magnetic-based microfluidic device for on-chip isolation and detection of tumor-derived exosomes. *Anal. Chem.* 90:13451–58
128. Zhang K, Yue Y, Wu S, Liu W, Shi J, Zhang Z. 2019. Rapid capture and nondestructive release of extracellular vesicles using aptamer-based magnetic isolation. *ACS Sens.* 4:1245–51

129. Huang R, He L, Li S, Liu H, Jin L, et al. 2020. A simple fluorescence aptasensor for gastric cancer exosome detection based on branched rolling circle amplification. *Nanoscale* 12:2445–51
130. Li P, Yu X, Han W, Kong Y, Bao W, et al. 2019. Ultrasensitive and reversible nanoplatform of urinary exosomes for prostate cancer diagnosis. *ACS Sens.* 4:1433–41
131. Zhao X, Zhang W, Qiu X, Mei Q, Luo Y, Fu W. 2020. Rapid and sensitive exosome detection with CRISPR/Cas12a. *Anal. Bioanal. Chem.* 412:601–9
132. He D, Ho SL, Chan HN, Wang H, Hai L, et al. 2019. Molecular-recognition-based DNA nanodevices for enhancing the direct visualization and quantification of single vesicles of tumor exosomes in plasma microsamples. *Anal. Chem.* 91:2768–75
133. He F, Wang J, BC Yin, Ye BC. 2018. Quantification of exosome based on a copper-mediated signal amplification strategy. *Anal. Chem.* 90:8072–79
134. Wang L, Pan Y, Liu Y, Sun Z, Huang Y, et al. 2020. Fabrication of an aptamer-coated liposome complex for the detection and profiling of exosomes based on terminal deoxynucleotidyl transferase-mediated signal amplification. *ACS Appl. Mater. Interfaces* 12:322–29
135. Zhang J, Shi J, Liu W, Zhang K, Zhao H, et al. 2018. A simple, specific and "on-off" type MUC1 fluorescence aptasensor based on exosomes for detection of breast cancer. *Sens. Actuators B Chem.* 276:552–59
136. Zhang Q, Wang F, Zhang H, Zhang Y, Liu M, Liu Y. 2018. Universal Ti_3C_2 MXenes based self-standard ratiometric fluorescence resonance energy transfer platform for highly sensitive detection of exosomes. *Anal. Chem.* 90:12737–44
137. Zhang Z, Tang C, Zhao L, Xu L, Zhou W, et al. 2019. Aptamer-based fluorescence polarization assay for separation-free exosome quantification. *Nanoscale* 11:10106–13
138. Sun Y, Jin H, Jiang X, Gui R. 2020. Assembly of black phosphorus nanosheets and MOF to form functional hybrid thin-film for precise protein capture, dual-signal and intrinsic self-calibration sensing of specific cancer-derived exosomes. *Anal. Chem.* 92:2866–75
139. Wang S, Zhang L, Wan S, Cansiz S, Cui C, et al. 2017. Aptasensor with expanded nucleotide using DNA nanotetrahedra for electrochemical detection of cancerous exosomes. *ACS Nano* 11:3943–49
140. Huang R, He L, Xia Y, Xu H, Liu C, et al. 2019. A sensitive aptasensor based on a hemin/G-quadruplex-assisted signal amplification strategy for electrochemical detection of gastric cancer exosomes. *Small* 15:1900735
141. Zhou YG, Mohamadi RM, Poudineh M, Kermanshah L, Ahmed S, et al. 2016. Interrogating circulating microsomes and exosomes using metal nanoparticles. *Small* 12:727–32
142. Wang Y-M, Liu J-W, Adkins GB, Shen W, Trinh MP, et al. 2017. Enhancement of the intrinsic peroxidase-like activity of graphitic carbon nitride nanosheets by ssDNAs and its application for detection of exosomes. *Anal. Chem.* 89:12327–33
143. Chen J, Xu Y, Lu Y, Xing W. 2018. Isolation and visible detection of tumor-derived exosomes from plasma. *Anal. Chem.* 90:14207–15
144. Zeng Z, Zhang P, Zhao N, Sheehan AM, Tung CH, et al. 2010. Using oligonucleotide aptamer probes for immunostaining of formalin-fixed and paraffin-embedded tissues. *Mod. Pathol.* 23:1553–58
145. Pu Y, Liu Z, Lu Y, Yuan P, Liu J, et al. 2015. Using DNA aptamer probe for immunostaining of cancer frozen tissues. *Anal. Chem.* 87:1919–24
146. Li WM, Bing T, Wei JY, Chen ZZ, Shangguan DH, Fang J. 2014. Cell-SELEX-based selection of aptamers that recognize distinct targets on metastatic colorectal cancer cells. *Biomaterials* 35:6998–7007
147. Yuan B, Jiang X, Chen Y, Guo Q, Wang K, et al. 2017. Metastatic cancer cell and tissue-specific fluorescence imaging using a new DNA aptamer developed by Cell-SELEX. *Talanta* 170:56–62
148. Li X, An Y, Jin J, Zhu Z, Hao L, et al. 2015. Evolution of DNA aptamers through in vitro metastatic-cell-based systematic evolution of ligands by exponential enrichment for metastatic cancer recognition and imaging. *Anal. Chem.* 87:4941–48
149. Li S, Xu H, Ding H, Huang Y, Cao X, et al. 2009. Identification of an aptamer targeting hnRNP A1 by tissue slide-based SELEX. *J. Pathol.* 218:327–36
150. Bukari BA, Citartan M, Ch'ng ES, Bilibana MP, Rozhdestvensky T, Tang TH. 2017. Aptahistochemistry in diagnostic pathology: technical scrutiny and feasibility. *Histochem. Cell Biol.* 147:545–53