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Review Article

Aptamer-based electrochemical approaches to meet some of the challenges in the fight against cancer

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Abstract

The development of simple, rapid, and cost-effective electrochemical strategies for the detection of cancer biomarkers would improve the ways we have to find cancer at the earliest stages or to monitor its treatment and recurrences, thus contributing to control the global cancer burden. Ongoing advances in the selection of aptamers recognizing different cancer biomarkers have greatly enhanced our ability to design these electroanalytical platforms. Herein, we give an overview of the developments in aptamer-based electrochemical assays through the past two years, mainly focusing in simple and highly sensitive approaches such as nanopore-sensing, label-free and signal amplification systems. All of them could contribute to overcome two important challenges in the fight against cancer: understand cancer risk and prognosis by fostering new ways to detect it, and the availability of analytical platforms for integrating these biomolecular data with health systems.

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Introduction

Cancer is a global health problem that knows no borders. According to the International Agency for Research on Cancer, the number of new cancer cases could rise to 30.2 million by 2040, while cancer deaths are expected to rise approximately 60% over the next two decades [1], mainly due to population aging. In this scenario, new progress in prevention, early detection, and treatment should reach health systems worldwide, helping to largely avoid many premature deaths, unnecessary suffering, and unacceptable inequalities.

To accelerate the fight to stop cancer, the European Commission and European cancer organizations have identified key challenges and different areas of action to address them and thus turn the tide against cancer [2]. Among the four key action areas that would allow further progress: i) prevention, ii) early detection, iii) diagnosis and treatment and iv) quality of life of cancer patients and survivors, it is in the field of early detection and diagnosis and treatment where analytical chemistry, and more specifically electrochemistry, could have a significant impact. New cost-effective methods of analysis that enable cancer detection at the earliest stages, the assessment of the stage of the tumor, the chemotherapy monitoring, or the detection of cancer recurrence, are necessary tools to control the global cancer burden.

Tissue biopsy continues to be one of the dominant technologies in the diagnosis and management of cancer. However, liquid biopsy, as a non-invasive alternative, is gaining momentum due to its ability to obtain the molecular signature of tumors, contributing to the individualization of treatments. This approach relies on the detection of different biomarkers in biological fluids that are uniquely associated with tumors. Some of these biomarkers include circulating tumor DNA, cell-free RNAs, circulating tumor cells (CTCs), exosomes, or proteins. A significant hurdle in the development of liquid biopsy technologies is the low abundance of most biomarkers in highly complex matrices (e.g., serum or blood). The need for molecular recognition elements with high affinity and selectivity is crucial, with antibodies serving as the gold standard. However, the use of antibodies is not always feasible due to their sensitivity to temperature, irreversible denaturation, short shelf life, or the extensive and costly *in vivo* procedures required for their development. In contrast, aptamers offer an accessible and growing alternative for a wide range of biomarkers. Aptamers are single-stranded oligonucleotides that are selected through an *in vitro* enrichment

process called SELEX from highly complex libraries of nucleic acids obtained by combinatorial synthesis.

Several features render these "chemical antibodies" well suited for the recognition of circulating cancer biomarkers. They are small size molecules chemically synthesized at relatively large scale, with a versatile structural design and remarkable chemical stability, and able to recognize the corresponding target with high affinity and selectivity. The coupling of aptamers to electrochemical transducers enables the development of rapid, accurate, and reliable tests, with sufficient sensitivity and specificity to be used as liquid biopsy to address cancer diagnosis and management. While several works have reviewed the different aptasensor designs for cancer diagnosis [3—6], in this work we highlight the recent efforts to develop aptamer-based electroanalytical platforms to overcome current challenges in the fight against cancer. Inspired by the roadmap for the early detection and diagnosis of cancer reported by Cancer Research UK [7], we focus the discussion on two of the four suggested themes for research in this field, in which we consider that electrochemical aptasensors and aptamer-based assays could help. The first challenge is to understand cancer risk and prognosis, where aptamer-based analytical tools may foster new ways to detect cancer at early stages. The second is to overcome the barriers posed by the lack of platforms for the integration of biomolecular data with health systems. In each case, we discuss the possibilities that aptamer-based electrochemical detection offer and where research in this field should be directed to address these challenges.

New ways to detect and diagnose cancers

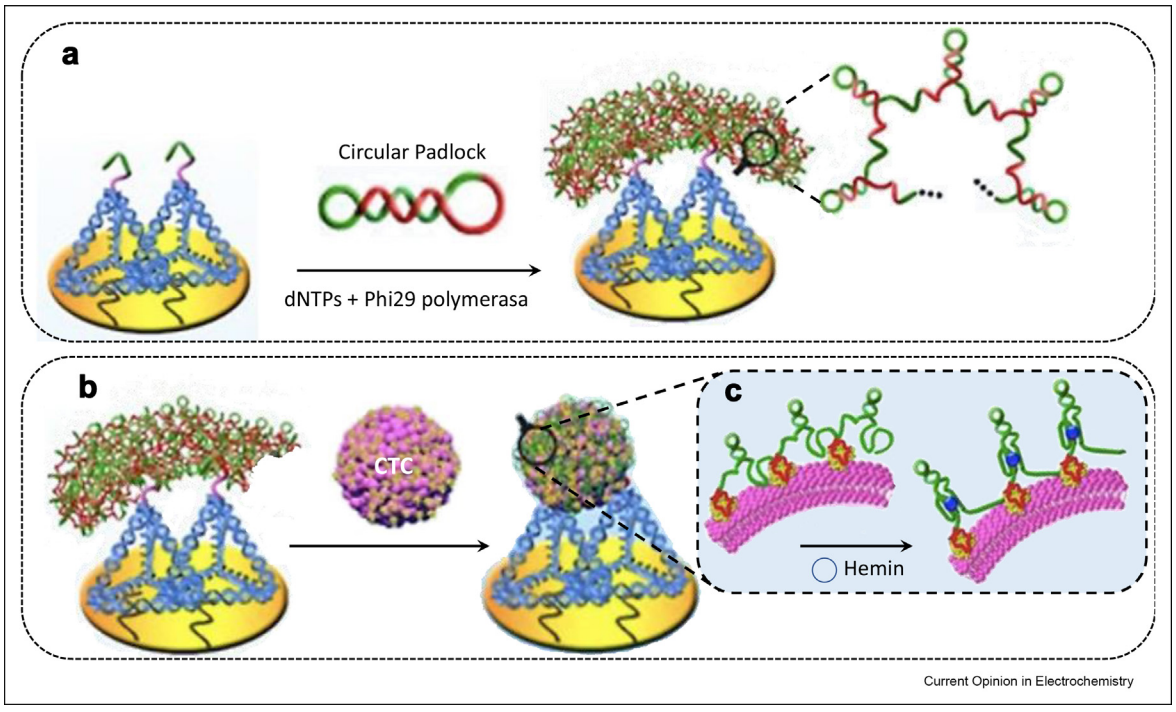
Given the attributes of aptamers, it is not surprising that a large number of electrochemical, aptamer-based assays have been reported for the detection of cancer biomarkers, as they provide minimally invasive and cost-effective solutions to detect and triage cancer at different stages. To realize their full potential as novel analytical tools in the fight against cancer, we have identified three areas of action: i) selection of aptamers against new biomarkers and development of methods by which aptamers can be modified to optimize their signaling; ii) design of simpler approaches to transduce the aptamer—target interaction into an electrochemical readout; and iii) amplification strategies easily integrated with these devices to improve their sensitivity. Primarily focusing the attention on works published between 2020 and 2022, we present a concise view of the newest and most promising developments in these aspects.

Selecting and optimizing the aptamers used as receptors

Our analysis of more than 475 works published between 2020 and 2022 containing the keywords "*aptamer*," "*electrochemistry*," and "*cancer*," reveals that the targets most used for the development of new diagnosis tests are circulating tumor cells (CTCs) and tumor-derived exosomes, specifically their tumor-associated cell surface proteins. Epithelial cell adhesion molecule (EpCAM) and human mucin-1 (MUC1) are overexpressed in most adenocarcinomas, and consequently well-characterized aptamers against these targets [3] are the most repeated for the selective entrapment and detection of CTCs and exosomes. However, capturing the large size cancer cells using monovalent aptamers is not trivial. To this end, a network of multivalent aptamers that can be generated directly on the electrode surface by extending a primer-immobilized sequence using rolling circle amplification (RCA) reaction was proposed [8,9*]. The cooperative binding of cells to the multiple repetitions of the EpCAM aptamer sequence leads to a fast and efficient capture process, enabling their isolation from whole blood in 20 min [8]. The inclusion of the primer in the vertex of a tetrahedral DNA structure allows to control the spatial orientation of the generated aptamer network on the electrode surface, reducing steric hindrances and further improving capturing efficiency [9*]. The combination of this approach with a smart design of the circular padlock that is used as template for RCA allows the capturing cell to be linked to a readily detectable electrochemical output. With that aim, the padlock design includes two divided G-quadruplex fragments, which after CTCs binding are switched into DNAzyme structures that, in the presence of hemin, catalyze the oxidation of tetramethybenzidine (TMB), leading to an amperometric output (Figure 1). Following this approach, as low as 23 cell/mL can be detected even in whole blood [9*]. A drawback of this strategy is that it is based on the identification of a single biomarker, thus compromising selectivity as the same biomarker is also present on the surface of normal cells with differences in the expression level. An alternative to this is the simultaneous recognition of two biomarkers using EpCAM and MUC1 aptamers [10]. This way, only cells containing both biomarkers trigger the final response, which in this case is based on an RCA amplification that leads to a DNAzyme chronoamperometrically detected. However, the chosen biomarkers are general of cells and exosomes associated with cancer and it would be desirable to combine them with more specific ones.

Similarly, exosomes can be captured using aptamers against surface proteins, being the aptamer against CD63 one of the most widely used for this purpose [11,12]. However, to predict the origin of the tumor-derived exosomes, a profile of their surface proteins should be obtained, which is achieved by combining different signaling aptamers in a sandwich format. This strategy has been used to analyze the differences in protein expression in exosomes derived from different subtypes of breast cancer cells [12]. The entrapment of

Figure 1



Electrochemical sensor using a multivalent aptamer network for enhancing the selective entrapment of CTCs. (**a**) A tetrahedral DNA nanostructure incorporates a primer in the vertex, which is elongated with multiple repeats of aptamer (red sequence) and a DNAzyme (green sequence). (**b**) The binding of the CTCs through multiple surface proteins reveals in the network multiple G-quadruplex as magnified in (**c**). In the presence of hemin, this leads to a peroxidase-mimicking structure, responsible for the final readout (Reprinted with permission from ref. [9*]. Copyright 2022 American Chemical Society).

the exosomes on magnetic beads modified with anti-CD63 aptamers is combined with silica particles modified with aptamers against EpCAM, MUC1, human epidermal growth factor receptor (HER2) and carcinoembryonic antigen (CEA) as signaling receptors. The silica particles contain ferrocene moieties, which are chemically released after the recognition step and detected electrochemically. One caveat to this approach is that it uses the same redox-active label for all proteins; therefore, even if the measurement is performed simultaneously on multiple electrodes, the assay cannot truly be simultaneous, requiring independent calibration for each protein.

Regarding the detection of proteins that tumor cells shed into the bloodstream, five targets are commonly selected for the fabrication of electroanalytical, aptamer-based platforms: CEA, HER2, prostate-specific antigen (PSA), cancer antigen 125 (CA125) and alpha-fetoprotein (AFP). Although the detection of single-protein biomarkers is often not sufficient to distinguish tumor from non-tumor tissues, multiplex assays for the simultaneous detection of these proteins are still scarce [13]. It is also important to note that these targets, along with many other biomarkers, are glycoproteins, and differential glycosylation of the core proteins confers tumor cells many of their distinctive properties. However, our ability to detect aberrant glycosylated forms of proteins associated to cancer development is hampered by the lack of glycan-specific affinity reagents. Carbohydrates are characterized by a poor immunogenicity, making it difficult to obtain antibodies directed towards the sweet spot of the glycoproteins. Aptamers may be an option but the number of aptamers specific for a particular protein glycoform is still scarce. Soh group recently developed a method to obtain base-modified aptamers specifically recognizing glycoforms of proteins [14*]. Though the selected aptamers rival the binding affinity of lectins, they are not able to simultaneously recognize the carrier protein. The possibility of directing the selection of aptamers against the glycosylation site of a protein has already been demonstrated [15]. The rational design of counter-selection steps, combining the use of human-PSA and a recombinant non-glycosylated form of the protein, together with a selective elution of the bound sequences led to an aptamer able to recognize the

innermost sugar residues and the peptide region surrounding the glycosylation site of PSA. In our group, we used this aptamer to develop a label-free impedimetric platform for the dual recognition of PSA, which supports the measurement of different fractions of PSA in serum after a simple dilution of the sample, greatly improving the clinical selectivity of PSA tests for prostate cancer diagnosis [16**]. Similar approaches could be explored for the detection of other post-translational protein modifications associated to tumorigenesis.

One of the main challenges in the field is finding the best circulating cancer biomarkers to be tested in liquid biopsy. Besides the tumor cells themselves, the tumor microenvironment is a source of circulating proteins and peptides that may act as cancer biomarkers [17]. An example of this is the 16-mer peptide from α1 chain of human collagen XI, a minor collagen dysregulated in several carcinomas. However, obtaining antibodies recognizing this peptide is especially challenging. As an alternative, in our group, we have selected aptamers against this fragment that are able to capture collagen XI from cell lysates [18], enabling its quantification even in diluted serum samples [19].

An important aspect in the selection of aptamers is their characterization in terms of affinity and selectivity towards their target. In addition, aptamers are subjected to post-SELEX modifications to improve these aspects and their performance on the electrochemical assay/sensor. This typically involves, for example, the truncation of the sequence to reduce their size to the minimal structure needed for recognition. Docking and molecular dynamics calculations are extremely useful tools to guide the truncation [15]. Another critical aspect to keep in mind is the aptamer stability in biological environments. In this sense, electrochemical, aptamer-based sensors have demonstrated their suitability to assess the nuclease activity of a potential cancer biomarker, human cyclophilin B (CypB), highlighting the importance of developing strategies that make aptamers nuclease-resistant in certain applications [20*]. Machine learning methods also provide new opportunities to refine the selection of aptamers, accelerating the obtention of high affinity aptamers with a minimal size [21**].

Innovative transduction technologies

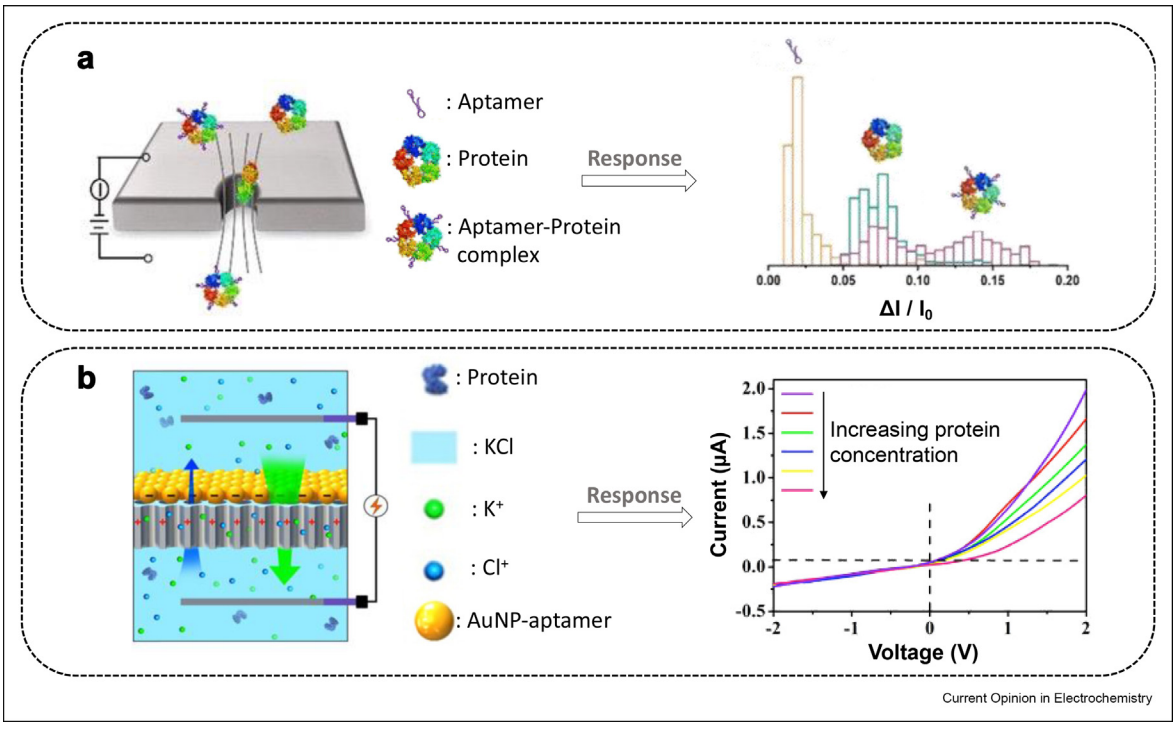
The development of aptamer-based electrochemical platforms for the detection of cancer biomarkers requires that target binding induces a readily detectable electrical output. A simple approach to achieving this is to combine the recognition event with nanopore-sensing [22,23**]. The molecular transport through nanoporous membranes is altered by the recognition step, observing two possible detection schemes: (i) resistive sensing, and (ii) current–voltage (I–V) measurements (Figure 2).

In resistive sensing, a nanoporous membrane is placed between two reservoirs with their respective electrodes. By applying a potential difference between them, a steady-state ion current is generated. The translocation of a molecule through the nanopores modulates the properties of this current (e.g., amplitude, duration, or frequency), being these changes related to the concentration of target. An example of this approach consisted on the use of commercially available SiNx membranes, which under dielectric breakdown led to nanopores with a size (14 nm diameter) matching that of human C-reactive protein (CRP). This nanoporous membrane showed high enough resolution to distinguish the translocation of CRP from that of its complex with an anti-CRP-aptamer [22]. It is also possible to use larger nanopores, such as those in glass nanopipettes (100 nm), but combined with nanocarrier structures to reduce the translocation speed of smaller molecules and thus increase the sensitivity. This has been achieved by designing a DNA origami with a central cavity where an aptamer against CRP is fixed by hybridization. The binding of the target to this nanostructure leads to a change in the translocation current through glass nanopores, which allows to easily distinguish occupied and unoccupied nanocarriers (Figure 2**a**). Counting the events corresponding to the occupied carriers' translocation, it is possible to detect up to 9 nM CRP in 5 μL of diluted human plasma in 2 min [23**].

In the I–V measurement approach, aptamers are immobilized onto the nanopores, and the recognition of the target modulates the charge in their walls, leading to rectification changes in resistance than can be matched to a target concentration (Figure 2**b**). For example, Pan and colleagues demonstrate this by immobilizing MUC1 aptamers onto gold nanoparticles that are self-assembled on anodized aluminum oxide, leading to a high density of nanochannels. In the presence of MUC1 in serum samples, a current change is observed, leading to limits of detection in the nM range [24].

In addition to nanopore-sensing, other strategies have been described for the label-free transduction of aptamer recognition, either capacitive [25] or faradaic impedance [16**,26] measurements. Driven by advances in nanotechnology, the advantages offered by these readouts include high sensitivity, versatility, and ease of operation. As an example, the integration of carbon-based microelectrochemical devices with bipolar exfoliated reduced graphene oxide on which an aptamer against platelet-derived growth factor-BB (PDGF-BB) is covalently immobilized, leads to electrodes with sufficiently high capacitances for the label-free detection of PDGF-BB [25]. The capacitance of the interphase selectively decreases with the increase in PDGF-BB concentration, distinguishing it from the homodimeric form PDGF-AA, or even the heterodimeric one, PDGF-AB. Similarly, the use of new nanomaterials to obtain

Figure 2



Detection schemes in nanoporous sensing. (**a**) Resistive measurements with aptamers in solution (reprinted with permission from ref. [22], Copyright 2022 American Chemical Society), and (**b**) Current–voltage measurements, where aptamers are immobilized on the nanopores. (Reprinted with permission from ref. [24] Copyright 2021 American Chemical Society).

nanostructured surfaces improves the analytical performance of aptamer-based impedimetric sensors, in this case with an electroactive redox probe in solution. As an example, using a composite of bimetallic CuCo Prussian blue analogue and carbon dots for the immobilization of an aptamer recognizing the epidermal growth factor receptor (EGFR) allows the direct detection of as low as 80 cell/mL of MCF-7 cancer cells overexpressing EGFR [26]. However, non-specific adsorption may also increase, and special attention must be paid to proper blocking of these nanostructured surfaces. Several strategies have been reported by which antifouling and stable electrochemical aptamer-based sensors can be designed to prevent undesirable adsorption of biomolecules from complex biological fluids. Among them, the use of hydrophilic synthetic peptides, with zero net charge that include a terminal cysteine for self-assembling onto gold nanostructures, where thiol-aptamers are also chemisorbed, supporting direct measurement of CA125 in 10% serum [27]. The anti-fouling biotinylated peptides have also been conjugated to the aptamer by click-chemistry and arranged in a branched nanostructure on the electrode surface through the streptavidin-biotin interaction. This improves the anti-fouling capabilities, enabling the detection of CA125 in undiluted serum samples [28].

Another attractive approach that is reagentless, but not label-free, is the design of electrochemical, aptamer-based (E-AB) sensors. First described by the Plaxco group [29], they rely on the immobilization of aptamers modified with a redox reporter, generally methylene blue (MB) or ferrocene (Fc), onto a gold electrode. Upon binding, aptamers undergo a conformational change that alters the electron transfer rate of the reporter with the electrode. This approach, widely used for *in vivo* molecular monitoring [30], has also been applied for the detection of different molecules, including cancer biomarkers [31]. The effectiveness of this approach is particularly noteworthy in detecting tumor cells when the electrode surface is nanostructured. For example, gold-electrode surfaces have been coated with nanopillars from the dielectric hydrogen silsesquioxane, HSQ (200 nm diameter and 20 nm height with pitch of 500 nm), making possible the chemisorption of SYL3C aptamers modified with a thiol at one end and a Fc moiety at the other, with a good control on their surface distribution because the thiols are not chemisorbed at HSQ. At the same time, the achieved nanoconfinement effect maximized the signal due to the redox reporter, while the nanopillars keep the cells at few nanometers above the electrode surface, minimizing non-specific adsorptions by suppressing the cell downward forces.

Binding of cells selectively recognized by the aptamer blocks the electron transfer of the redox reporter, decreasing the electrochemical signal. Thus, a LOD of 13 cells has been demonstrated for EpCAM expressing cancer cells, with excellent selectivity [32*].

Despite the success of E-AB sensors, quantification of the target using the electrochemical signal from a single redox reporter is affected by changes in the microenvironment of the aptamer and its local distribution, in detrimental of reproducibility and reliability of measurements. To overcome this, a dual-signal response scheme has been proposed, using a second redox reporter that is not affected by the aptamer—target interaction as an internal reference. To explore this approach for the detection of cancer derived exosomes, indium tin oxide (ITO) surfaces were modified with a nanocomposite of black phosphorous nanosheets and Fc-doped metal—organic frameworks. The immobilization of an anti-CD63 with a terminal MB enables the quantification of exosomes in 10-fold diluted plasma samples, using as readout the ratio between the signal from Fc (unchanged) and that from MB (decreasing as the concentration of exosomes increases), with an LOD of 100 particles/mL and low relative standard deviations [33]. The ratiometric approach has also been tested with other detection schemes, such as displacement assays with the signaling aptamer labelled with AuPt nanoparticles [34]. However, this strategy does not preclude the need for an external calibration.

Advances in nanotechnology have also boosted the use of spectroelectrochemical transduction approaches such as electrochemiluminescence (ECL) and photoelectrochemical (PEC) aptasensing strategies, which have had a remarkable effect on the analytical performance of such sensing platforms, achieving extraordinary sensitivities and very low limits of detection.

One of the most widely used ECL systems is luminol with hydrogen peroxide as co-reactant. Following this strategy, luminol has been incorporated into metallic nanoparticles to catalyze the decomposition of H_2O_2, thus enhancing its ECL emission and improving sensitivity. This readout mechanism has been employed in aptasensing to detect lymphoma cells, using an anti-CD20 aptamer chemisorbed on gold surfaces, in combination with chitosan-Pt-luminol nanoparticles. The positively-charged nanoparticles are electrostatically bound to the aptamer-modified sensing phase, and the specific interaction with B cells leads to their release, with a subsequent decrease in the ECL signal. This simple approach showed good sensitivity with a LOD of 31 cells/mL in blood samples [35]. However, the reliability of the platform is compromised due to the signal-off response, which can be masked by the gradual loss of the reporter nanoparticles just electrostatically bound.

A different approach consists of using light as excitation followed by electrochemical detection. This is the working principle of PEC, which is also characterized by its high sensitivity exploited for the design of aptasensors. ITO surfaces coated with a photoactive material, $La_2Ti_2O_7$ perovskite nanosheets sensitized with $Cd:Sb_2S_3$, are linked to a DNA oligonucleotide complementary to an anti-PSA aptamer. The aptamer modified with V_2O_5 nanospheres is immobilized on the surface by hybridization and both steric and catalytic effects lead to the suppression of the PEC signal. In the presence of PSA, the aptamer is released from the surface, leading to a signal-on response with excellent sensitivity (LOD 4.3 fg/mL) [36]. Although these platforms have been tested in biological samples, their validity has been established by recovery assays. In order to apply them in practice, it would be necessary to validate them by comparison with other well-established methods.

Signal amplification strategies

Despite the new electrochemical transduction technologies, the detection of cancer-specific biomarkers in biological fluids remains a complex and time-consuming challenge due to their low abundance. Successful detection relies, many times, on a suitable protocol for the isolation of biomarkers, for which the use of aptamer-modified magnetic particles has demonstrated to be a good choice [37,38]. Moreover, several strategies have been used to amplify the final readout. These strategies include: i) the use of natural or artificial enzymes to obtain multiple copies of the redox reporter responsible for the electrochemical signal; and ii) the nucleic acid nature of aptamers to trigger the amplification process.

Aptamers can be easily modified directly or indirectly with enzymes, being peroxidase, and its electroactive substrates TMB and hydroquinone, the most widely used strategy [11,19,37]. However, the cost and limited stability of these natural catalysts has prompted the search for enzyme-mimicking nanomaterials which are progressively adapted as labels in aptamer-based assays [39—41]. For example, nanomaterials with peroxidase [39,40] and catalase [41] activity have been used for detecting different biomarkers like PSA [39] or MUC1 [40,41]. Nonetheless, much work remains to be done, not only to obtain materials that match the catalytic activity of natural enzymes, but also to improve their performance in biological fluids.

Taking advantage of the nucleic acid nature of the aptamers, their sequence can be easily modified to include a DNAzyme. Guanine-rich DNA sequences that form G-quadruplex and exhibit peroxidase activity after binding to hemin are the most common approach. The hemin covalently attached to these sequences has also shown oxidase activity, which was recently exploited in a

sandwich assay onto magnetic particles to detect HER2/neu with the electrocatalytic reduction of oxygen as signal [42]. Advantages offered by this readout include a simpler protocol that does not require any additional reagents and leads to shorter analysis times. Using this approach, authors demonstrated the detection of HER-2/neu in 20% human serum with a LOD of 10 fM in 1 h.

The sensitivity of these assays can also be improved by incorporating multiple tandem repeats of the DNAzyme. This is typically achieved through enzymatic DNA isothermal amplification, being RCA the most widely used [9*,10,43,44]. The simplest approach consists of linking the aptamer to a primer that triggers RCA [9*,43]. A suitable design of the circular padlock leads to the elongation of the aptamer with numerous repeats of a peroxidase-mimicking DNAzyme [9*] or metal ion binding sequences [43]. Using the latter, the presence of EpCAM- and HER2-positive exosomes was converted into a voltammetric signal of Pb and Cd ions, with a LOD of 1 particle/mL in 50% diluted serum samples. The RCA primer can be also incorporated into auxiliary DNA sequences, with a little gain in sensitivity but at the expense of greatly complicating the design, making it less robust and more expensive [10,44]. Terminal deoxynucleotidyl transferase (TdT) is another enzymatic amplification approach that allows the extension of the 3′-hydroxyl group of the terminal nucleotide in the aptamer in a template-independent manner. Combined with a redox-active molecule that electrostatically interacts with DNA, it makes possible to obtain an amplified electrochemical signal associated to the aptamer. The binding of a large target such as CTCs to the aptamer inhibits this extension and leads to a signal-off method for CTCs quantification, although with a limited dynamic range in 10% diluted serum samples [45].

Another strategy to enhance detection sensitivity is to link the aptamer to the self-assembly of auxiliary DNA structures by hybridization. The binding of multifunctional dendrimer-like DNA nanostructures containing DNAzymes [46] or the hybridization chain reaction (HCR) [47–49] may be triggered only in the presence of the target molecule for the generation of a detectable signal. The use of endonucleases to cleave specific parts of the DNA nanostructures also allows a further improvement in sensitivity. This approach can be implemented in DNA logic devices to evaluate the presence of multiple biomarkers [50]. Although effective, these are in general tedious, complex designs, with insufficient robustness for clinical diagnosis.

Platforms for integration biomolecular data with health systems

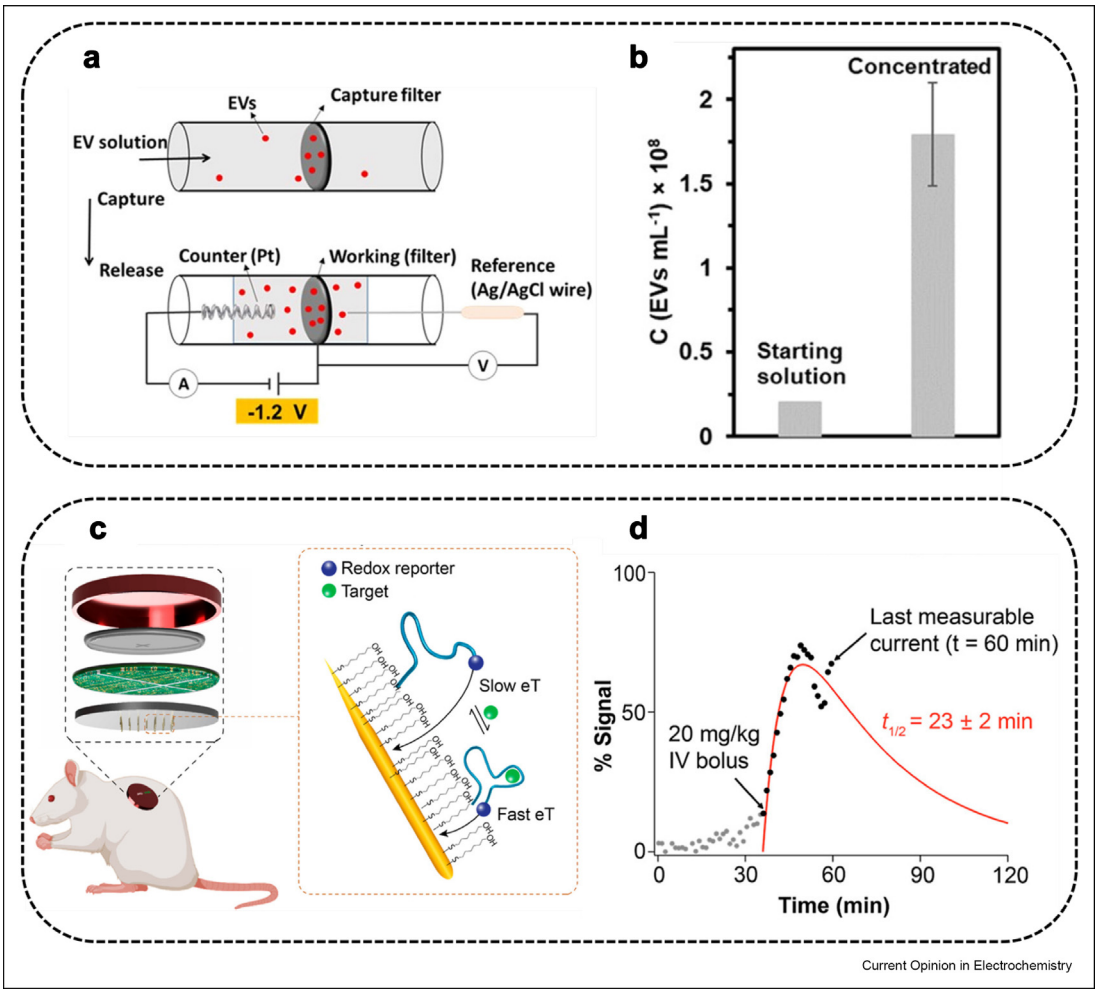
Another important aspect in the fight against cancer is the development of portable technologies, in different formats (e.g., wearable, implantable, smartphone-based, etc.), which enable early detection and diagnosis of cancer, risk stratification as well as its monitoring. Engaging citizens to regularly monitor their health condition has always been a challenge; however, the existence of convenient health monitoring devices such as cell phones or smartwatches, combined with the recent COVID-19 pandemic, are progressively changing the perception of the public, being now more open to monitoring their health.

The ideal portable, electrochemical platform for health monitoring uses an untreated biological fluid to provide a rapid and simple readout on the concentration of a given biomarker. The complexity of such fluids and the limited amount of target in the sample often makes it mandatory the use of integrated steps before obtaining a readout. Such an integration is mostly envisioned in microfluidic devices. While the detection step has traditionally caught the attention, the integration of pre-analytical steps is less frequently addressed, likely due to the variety of operations involved that precludes the design of a universal device for all body fluids and targets. A general platform for extracellular vesicle isolation potentially adaptable to different devices has been recently demonstrated [51]. This strategy relies on the selective capture of an aptamer immobilized on an electroactive cloth and subsequent electrochemical pulses to release the target (Figure 3).

Although different approaches have been reported in the literature to enable the use of these portable platforms for analysis in biological fluids, biofouling is still one of the main limitations. This is especially critical in situations where there is an extended contact with the sample in order to obtain sufficient target enrichment, thus overcoming its low concentration. Successful recent examples include the dielectrophoretic separation of red cells from whole blood [52] or an integrated microvortex that improves the accumulation efficiency of the target, reducing the contact time and so the fouling [53]. The latter device is designed for the exosome detection through EpCAM aptamers labeled with AgNPs for electrochemical readout. The device enables downstream analysis of exosome cargo (mRNA) just by device disassembling and exosome lysis. Though it is competitive with ELISA in time (50 min vs 5 h) and sample volume (10 μL vs 100 μL), it still needs whole blood pretreatment and exosome extraction outside the device.

Another approach to developing these portable platforms relies on the use of microfluidic paper. This strategy can be especially well-suited because it enables the passive transportation of sample through the system to the detection chamber without external forces, the separation of the different matrix components, and the immobilization of (bio)chemical reagents. Though

Figure 3



Potential approaches to the monitoring of cancer biomarkers (**a**) Microporous cloths coupled to electrochemical switches have been successfully used to capture and release extracellular vesicles (EVs) on-demand, (**b**) increasing their concentration in the surrounding of the working electrode. This approach could potentially be expanded to other biomarkers present at very low levels [51]. (**c**) E-AB sensors support the monitoring of molecular targets directly in ISF via minimally invasive, microneedle-based devices, (**d**) leading to continuous and real-time measurements. Although this recent approach has not been tested yet for cancer biomarkers, it highlights the potential of aptamer-based sensors to enable cancer biomarkers monitoring remotely [59]. (Reprinted with permission from refs. [51,59] Copyright 2020 and 2022 American Chemical Society).

fifteen years have passed since its conceptualization [54], aptamer-based detection of cancer biomarkers using paper is still scarce (exosomes [55], cells [56], or proteins [57]). A recent example is a folded continuous paper layer (origami) in which the inlet channel is connected to a graphene working electrode, keeping other electrodes separated to avoid contamination during the recognition event. Using this origami approach, sample volumes are greatly reduced to less than 5 μL [57]. The simplicity and cost-effectiveness of these paper-based architectures would ideally match with label-free, enzyme-free, and ultimately reagent-less formats. Replacement of enzymes is possible with DNAzyme-bearing DNA probes though the label-free feature is arguable. With this aim, a paper placed on a commercial SPE cell was functionalized with a Zr-based metal–organic framework (MOF) that captures the exosomes. The addition of the specific aptamer modified to trigger a hybridization cascade after the surface protein recognition in the presence of two DNA hairpin probes allows the signal amplification [55].

Wearable sensing devices also provide a very convenient way to monitoring health. Such devices can be developed to measure in less complex, relevant biofluids such as sweat or interstitial fluid (ISF), offering a non-invasive, or minimally invasive, approach to monitoring relevant biomarkers. However, only very preliminary works have been reported using aptamers as biorecognition elements for continuous monitoring of cancer biomarkers.

For example, a work recently published describes the use of a flexible, aptamer-based graphene sensor for the continuous monitoring the cytokine IFN-γ in sweat, with a reported LOD of 740 fM, which could potentially open the door for the monitoring of cancer biomarkers using skin patches [58]. However, a thorough study of biomarker concentration in sweat is necessary before assessing its full potential.

A very promising approach for the minimally invasive, continuous monitoring of molecular targets is ISF-based monitoring. Recently, aptamers have demonstrated their potential to enable ISF monitoring directly in the body of rodents using microneedle-based electrochemical devices [59,60], similarly to continuous glucose monitors (Figure 3). The clearer correlation between a biomarker blood levels and ISF levels makes this approach a strong candidate for health monitoring. Although no works have been published following this strategy to monitor cancer biomarkers, we acknowledge a highly promising work that demonstrates how E-AB sensors can monitor the biomarker receptor tyrosine kinase (KIT) [61], associated with different forms of cancer. Although still at a very incipient stage, we believe that such approaches can change the landscape of cancer monitoring, thus facilitating its early detection and treatment.

Conclusions and perspectives

Aptamer-based electrochemical platforms offer a wide range of approaches to improve our ability to sensitively find cancer biomarkers in biological fluids. Our overview of the recent articles published in the field reveals that aptasensors are well suited for the detection of promising circulating biomarkers, such as CTCs and exosomes. However, most detection strategies for these targets rely on the use of aptamers recognizing general epithelial markers like EpCAM and MUC1, upregulated in a variety of human cancers. To obtain additional information about the organ of origin of malignancy, it would be necessary to combine these targets with more specific ones. Aptamers have shown to be useful to detect subtle changes that occur as cells begins deregulated and then cancerous, as it is the case of the glycosylation machinery. As an example, aptamers targeting glycan structures simultaneously to peptide regions of aberrant forms of glycoprotein have shown the potential for designing diagnosis tests with improved clinical specificity. Advances in cancer detection require further exploring new selection approaches to obtain aptamers for recognizing specific changes associated to tumorigenesis like post-translational modifications, thus overcoming the technological limits for sufficient specificity of diagnostic tests for early detection of cancer.

The number of candidate biomarkers is exponentially increasing thanks to the use of omics technologies, but their predictive, diagnostic, or prognostic value still need to be verified by using suitable analytical platforms. The integration of aptamers with electrochemical transducers leads to generalizable platforms that host a wealth of benefits for this goal, such as high sensitivity and selectivity, ease of use, low cost, ease of miniaturization and applicability to a variety of formats that are compatible with simple detection devices such as glucometer-type devices. However, for this technology to mature and yield effective tools for research and clinical diagnosis there are still several challenges to address including: i) the design of simpler sensing approaches that are reagentless and non-invasive or minimally invasive, which fulfill the sensitivity and selectivity requirements; ii) to improve the robustness of the sensing platform to be used in complex biological fluids (e.g., sweat, ISF, urine, blood …) without the need for tedious sample treatment steps; and iii) an exhaustive validation of the proposed technologies in clinical specimens, to assess their ability to consistently produce rapid, analytical results useful in diagnosis and cancer management.

In recent years, efforts by the scientific community have focused on addressing some of these challenges like the fabrication of minimally invasive and reagentless sensing platforms for continuous ISF monitoring [59] or the development of electrode antifouling coatings to prevent non-specific interactions after prolonged exposure to biological fluids [62]. It is expected that these fundamental advances will be rapidly translated to the cancer management with aptasensors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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