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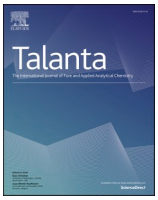

Review

Point-of-care cancer diagnostic devices: From academic research to clinical translation

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ABSTRACT

Early and timely diagnosis of cancer plays a decisive role in appropriate treatment and improves clinical outcomes, improving public health. Significant advances in biosensor technologies are leading to the development of point-of-care (POC) diagnostics, making the testing process faster, easier, cost-effective, and suitable for on-site measurements. Moreover, the incorporation of various nanomaterials into the sensing platforms has yielded POC testing (POCT) platforms with enhanced sensitivity, cost-effectiveness and simplified detection schemes. POC cancer diagnostic devices provide promising platforms for cancer biomarker detection as compared to conventional in vitro diagnostics, which are time-consuming and require sophisticated instrumentation, centralized laboratories, and experienced operators. Current innovative approaches in POC technologies, including biosensors, smartphone interfaces, and lab-on-a-chip (LOC) devices are expected to quickly transform the healthcare landscape. However, only a few cancer POC devices (e.g. lateral flow platforms) have been translated from research laboratories to clinical care, likely due to challenges include sampling procedures, low levels of sensitivity and specificity in clinical samples, system integration and signal readout requirements. In this review, we emphasize recent advances in POC diagnostic devices for cancer biomarker detection and discuss the critical challenges which must be surmounted to facilitate their translation into clinical settings.

1. Introduction

Cancer is now the second leading cause of death globally, with an expected 7.6 million people dying per year, which constitutes 13% of all deaths worldwide. The prevalence of cancer is predicted to grow in the future, with the cancer-related mortality rates expected to grow to more than 16.1 million by 2030 [1].

Cancer is a complex and highly heterogeneous disease with unique genomic and proteomic properties that differ among individual patients, and even between individual tumor regions and cancer types [2,3]. Unlike reactive medicine which aims to cure people after they become ill, proactive medicine focuses on prediction, prevention and early detection of the disease. Accordingly, offering the most effective diagnostic and therapeutic approaches is a task of paramount importance in proactive personalized medicine for cancer [3]. This procedure enables classifying cancer subtypes and, finally, evaluating the best treatment approach according to patient characteristics, medical history and observed response to therapy. Early diagnosis and personalized medicine (precision medicine) are now reasonable approaches to affect the expectancy and quality of life in patients with various types of cancer [4,5].

In recent years, much effort has been devoted to the development of various approaches toward creating successful POCT devices for early cancer screening and diagnosis [6,7]. The move to POC systems is in fact a paradigm shift from existing conventional diagnostic methods which employ medical laboratories to at or near the patient settings, providing clinicians with timely diagnostic information which enables them to make better informed decisions about diagnosis and treatment [8–11]. This could also pave the way for house-hold smart healthcare monitoring system with enhanced selectivity and sensitivity, and add powerful tools for personalized medicine, including pandemic surveillance. An ultimate goal for POC devices is in fact to establish multiplexed and simultaneous detection of several biomarkers on a single platform, providing a rapid, low cost and reliable quantitative analysis of

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biomarkers [12–14]. In this regard, nanomaterial based diagnostic assays are expected to play a pivotal role in overcoming the various drawbacks of conventional approaches by offering more accuracy and precision [15–17]. This review provides an overview of the different types of cancer biomarkers, including nucleic acids, proteins, circulating tumor cells (CTCs), and exosomes. We next highlight recent advances in nanomaterial-based POC cancer diagnostic assays implemented in paper based microfluidic, screen printing, and smartphone devices over the past five years. Finally, the critical challenges which provide barriers to adoption of POC cancer diagnostic devices as real world products as well as future device development directions will be presented.

2. Biomarkers for cancer detection

2.1. Nucleic acid biomarkers

Less-invasive detection of circulating free nucleic acids (cfNAs) biomarkers including cell-free DNAs (cfDNA) and RNA in the plasma and serum is of great importance for early diagnosis of cancers, staging, evaluation of therapeutic response and detection of recurrence [17–20]. It is estimated that over 80% of cancer patients can be diagnosed by using a panel of DNA and RNA biomarkers [21]. Due to the low levels of NAs typically found in clinical samples, a very high level of specificity is usually required to differentiate specific sequences from related molecules.

Therefore, development of new approaches that can integrate sample preparation with nucleic acid amplification and detection in a faster, cheaper, and user-friendly format still remain desirable and challenging [22]. At this time, effective miniaturized platform or POC systems have attracted great attention for early analysis of NAs due to the convenient automation, rapid response, low cost and multiplexed analytical capability. The biggest challenges in this area relate to achieving high levels of sensitivity, specificity, and the validation of performance levels in clinical sample analysis [18,23,24].

2.2. Protein biomarkers

In addition to nucleic acid biomarkers, detection of protein biomarkers, including circulating antigens and autoantibodies, also provide valuable information for evaluating cancer progression and drug treatment. The predominant analytical technique for detecting target protein is immunoassay, which relies on antigen–antibody reactions coupled with signal transduction to provide an analysis of the biomarker of interest concentration with high specificity and sensitivity [2,25]. Enzymes, fluorescent probes, electrochemiluminescent tags, and nanoparticles are frequently used as labels in immunoassay techniques. Protein assays based on immunoreaction are relatively easier and faster to perform, compared with complicated nucleic acid analysis which usually requires time consuming multiple steps, including cell lysis, nucleic acid purification and amplification [26,27]. Despite those advantageous features, the accurate measurement of cancer-related proteins with low concentration faces several challenges. First, the stability of fragile proteins may be affected by operational conditions which are difficult to control (e.g. temperature, humidity percentages, and pH). If this leads to changes in the epitope, it can introduce difficulties into measuring the target accurately. A second issue originates from the unamplified nature of proteins which inherently limits the detection sensitivity. Finally, the complex biological sample matrices, with their much higher nontarget protein concentrations can lead to non-specific binding (i.e., generating the high background signal) and subsequent false-negative signals, limiting the direct detection of trace amounts of target protein. Thus, technological advances are still required to meet the appropriate sensitivity and specificity requirements for the detection of very low concentrations of protein targets in real matrices such as blood, urine, and saliva. Notably, the development of new and highly selective antibodies with reduced off-target binding motifs are essential for achieving the desired analytical performance [28].

While a large number of publications emphasize immunoassay-based protein detection, this review focuses on their application for POCT diagnostics.

2.3. Circulating tumor cells (CTC)

CTCs are cancer cells quite often originating from primary cells of solid tumors. CTCs are found in the peripheral blood and are considered to be one of the roots of metastasis. CTCs can be employed as prognostic and predictive marker of treatment response in patients with localized, metastatic, or recurrent disease. Studies have linked CTCs to tumor progression in a number of solid cancers, and the enumeration and profiling of CTCs from the blood of patients with cancers, provides insightful information in assessing the mechanistic investigation of cancer metastasis and the determination of molecular evolution of cancer during treatment and disease progression [2,29]. However, CTCs exist in peripheral blood in extremely low numbers (approximately 1 in 10^7-10^9 cells/mL), particularly, at the early stage of cancer, making CTC isolation and characterization a technical challenge [29]. Therefore, specialized isolation and enrichment procedures (filtration, centrifugation, or immunomagnetic separation) are needed for the detection of these cells in a POC setting. Recently, microfluidic platforms have been compared favorably against mainstream technologies for CTC isolation and detection due to advantages implicit to miniaturization, including portability, cost-effectiveness and the ability to integrate with other technologies to improve the device efficiency for online isolation/detection. Moreover, microchip-based technologies offer excellent platforms to achieve the purification, enrichment, and analysis of CTCs in one chip as compared to conventional methods, which require multiple steps to sort, enumerate, and analyze rare cells, with each step potentially leading to CTC loss [11,30].

2.4. Exosomes

The term extracellular vesicles (EVs) covers a wide range of vesicles with various dimensions including exosomes (~100 nm in diameter), microvesicles (100–1000 nm), and apoptotic bodies (1–5 μm), which are secreted by a variety of cell types into the extracellular environment. These vesicles typically possess DNA, RNA, lipids, and a diverse collection of proteomic cargo that reflect the status and environmental condition of their parent cells at the time of their creation [31,32]. EVs can be isolated from several biological fluids, such as blood, urine, saliva, tears, sweat, and cerebrospinal fluid. Cancer derived EVs carry various cancer associated molecules, such as mutated or overexpressed oncoproteins and oncopeptides, glycoproteins, RNA species (for example, microRNAs, mRNAs, and various non-coding RNAs), and DNA fragments.

Many studies have demonstrated that cancer derived EVs have great potential to be used as effective alternative biomarkers for cancer subtyping, staging and severity evaluation, as well as therapy monitoring [32–35].

To advance the potential translation of EV liquid biopsy analysis approaches for early cancer diagnosis into clinical settings, POC methods should have some common characteristics. Mainly, these devices should be capable of handling and processing relatively minimal sample volumes; as well as they should be rapid, sensitive, specific, high-throughput, inexpensive and amenable to automation. Ideally, POC devices for EV analysis should utilize direct samples, without pre-treatment process, or employ simple and reliable protocols for EV purification. In the past few years, some procedures have been established to purify, enrich, and analyze EVs by incorporating various technologies into integrated platforms [36–39]. These POC methodologies present a favorable path for bringing EV based biomarker testing into clinical settings. Table 1 presents a list of common cancer biomarkers which have been applied for cancer diagnosis and prognosis.

Table 1
Common tumor biomarkers applied for cancer diagnosis.

Types of tumor markers	Target biomarker	Cancer	Sample type	Thresholds/normal values	Detection method	Clinical application	Ref
Nucleic acid biomarkers	TWIST2+NID2	Bladder	Urine	8 copies for the TWIST gene & 30 copies for the NID2 gene per test	Methylation-specific polymerase chain reaction (MS-PCR)	Predictive	[127]
	MGMT promotor methylation	Brain	Tumor	–	MS-PCR	Predictive	[128]
	HPV DNA	Cervical	Cervical samples	1 pg mL^{-1} HPV DNA	Digene Hybrid Capture 2 High-Risk HPV DNA test	Screening	[129]
	Methylated SEPT9 DNA	Colorectal	Blood	Cycle threshold (Ct) of 39	Real-time PCR	Early detection	[130]
	VIM	Colorectal	Stool	–	MS-PCR	Early detection	[131]
	SHOX2	Lung	Bronchial fluid	Delta-delta Ct (ΔΔCt) of −4.56 or 0.04% methylation	Real-time PCR	Early detection	[132]
	GSTP1	Prostate	Urine	–	MS-PCR	Early detection	[133]
	Prostate cancer antigen 3 (PCA3) mRNA	Prostate	Urine	PCA3 score of 35[a]	Transcription-mediated amplification (TMA™)	Diagnosis & prognostic	[134, 135]
	HPV E6/E7 mRNA	Cervical	Cervical liquid-based cytology	≥567 copies/ml	Real-time PCR & Sandwich hybridization assay	Screening	[136, 137]
Protein biomarkers	Nuclear matrix protein 22 (NMP-22)	Bladder	Urine	10 kU L^{-1}	Immunoassay	Screening and monitoring	[138]
	Cancer antigen 15–3 (CA15-3)	Breast	Serum	25 kU L^{-1}	Immunoassay	Monitoring	[139]
	Human epidermal growth factor receptor 2 (HER2)		Tissue	2–15 μg L^{-1}	Immunohistochemistry	Monitoring	[140]
	Estrogen, progesterone receptors (ER and PgR)		Tissue	0.6 μg/g cytosol	Immunohistochemistry	Prognosis and prediction	[141]
	CEA	Colorectal	Serum, plasma	2.5 μg L^{-1}	Immunoassay	Monitoring	[142]
	AFP	Hepatocellular carcinoma (HCC)	Serum	10 μg L^{-1}	Immunoassay, microfluidic capillary electrophoresis	Diagnosis	[143]
	Carbohydrate antigen 125 (CA125)	Ovarian	Serum, plasma	35 kU L^{-1}	Immunoassay	Monitoring	[144]
	Fibrin/fibrinogen degradation products (FDP)		Serum	1.5–4.5 g L^{-1}	Immunoassay	Monitoring	[145, 146]
	Carbohydrate antigen 19–9 (CA19-9)	Pancreatic	Serum, plasma	37 kU L^{-1}	Immunoassay	Monitoring	[147]
	PSA	Prostate	Serum	4 μg L^{-1}	Immunoassay	Screening and monitoring	[10]
	Neuronspecific enolase (NSE)	Small cell lung cancer (SCLC)	Serum	12.3 μg L^{-1}	Immunoassay	Diagnosis	[148]
	Human chorionic gonadotropin-β (HCG-β)	Testicular	Serum	2.2 IU L^{-1}	Immunoassay	Diagnosis	[149]
	Thyroglobulin (TG)	Thyroid	Serum	3–40 μg L^{-1}	Immunoassay	Monitoring	[150]
Circulating tumor cells (CTCs)	Epithelial cell adhesion molecules (EpCAM)	Breast	Blood	5 CTCs/7.5 mL	CellSearch	Monitoring	[151]
	Glycan	Breast	Blood	5 CTCs/7.5 mL	Flow cytometry	Monitoring	[152]
	EpCAM & podoplanin (PDPN)	Colorectal	Blood	23 CTCs/mL for EpCAM & 7 CTCs/mL for PDPN	Immunofluorescence staining	Screening and diagnosis	[153]
	Vimentin	Gastrointestinal	Blood	–	Immunofluorescence staining	Predictive	[154]
	Major vault protein	HCC	Blood	–	Flow cytometry	Diagnosis	[155]
	EpCAM & FRα	Non-small cell lung cancer	Blood	0 CTC/2 mL	CellSearch	Diagnosis	[156]
	Vimentin + PD-L1	Prostate	Blood	5 CTCs/7.5 mL	CellSearch	Prognosis	[157]
	Epithelial-mesenchymal transition (EMT) CTC	Prostate	Blood	5 CTCs/7.5 mL	CellSearch	Predictive	[158]
Exosomal nucleic acids markers	miRNA 105	Breast	Blood	–	Real-time PCR	Predictive	[159]
	miR-1246 & miR-21	Breast	Blood	–	Real-time PCR	Diagnosis	[160]
	BRAF & KRAS (mRNA)	Colorectal	Blood	–	PCR	Diagnosis	[161]
	miR-18a, miR-221, miR-222, miR-224	HCC	Blood	–	Real-time PCR	Diagnosis	[162]
	KRAS (DNA)	Pancreatic	Blood	5×10^9 exosomes/mL	Digital PCR	Early detection	[163]
	ERG, PCA3, and SPDEF (RNA)	Prostate	Urine	ExoDx Prostate Intelliscore (EPI) score of 15.6[b]	Reverse-transcriptase PCR	Predictive	[164, 165]
	CD47	Breast	Blood	–	ELISA assay	Diagnosis	[166]
	HER2	Breast	Blood	–	Immunofluorescence staining	Diagnosis	[167]

(*continued on next page*)

Table 1 (*continued*)

Types of tumor markers	Target biomarker	Cancer	Sample type	Thresholds/normal values	Detection method	Clinical application	Ref
Exosomal protein markers	CD147	Colorectal	Blood	–	Flow cytometry	Diagnosis and monitoring	[168]
	CA-125, EpCAM, & CD24	Ovarian	Blood	–	Microfluidic ExoSearch chip	Diagnosis	[169]
	Glypican-1	Pancreatic	Blood	–	Flow cytometry	Early detection	[170]
	Prostate-specific membrane antigen (PSMA)	Prostate	Blood	–	Flow cytometry	Prognosis	[171]

[a] PCA3 score is calculated using the ratio of urinary PCA3 mRNA copy number/mL to PSA mRNA copy number/mL × 1000.
[b] The EPI score ranges from 0 to 100, with the cut-off of 15.6.

3. Nanobiosensors for detection of cancer biomarkers: key advantages over conventional methods

Existing methods for biomolecular detection involve the use of mass spectrometry, immunoassay, enzyme assay, real-time polymerase chain reaction (PCR) and microarrays. However, these procedures are time-consuming, expensive and complex, so not suitable to meet contemporary needs in the POC cancer screening and diagnosis [9,40].

Nano-biotechnology plays an important role in overcoming the limitations of traditional techniques for cancer diagnosis and treatment by offering further accuracy and precision. This technology has gained substantial popularity recently for in vitro as well as in vivo detection of various biomarkers and it is expected that nano-biotechnology could truly revolutionize POC and personalized healthcare monitoring. In this area, extensive varieties of nanomaterials have been widely employed and provided some of the most promising results in this field [15,16]. According to literature search in Web of Science, from 2016 to July 2020, gold nanoparticle-based biosensors are most investigated (49%) for detection of cancer biomarkers followed by quantum dots (QDs), and graphene (Fig. 1).

Use of nano-scaled materials in cancer diagnostics has opened the path to detect many cancers at an early stage in clinical settings, enabled by a new class of biosensors known as nanobiosensors. Nanobiosensors are nanoscale analytical devices that commonly consist of a bio recognition element or bioreceptor such as an antibody, nucleic acid, enzyme, and even larger biological units, such as whole cells interfaced with a nanomaterials-based sensor-element. In these systems different types of transducers, including optical, electrochemical, and piezoelectric, can be employed to convey the relevant binding information in the form of signals [15,23,41–43].

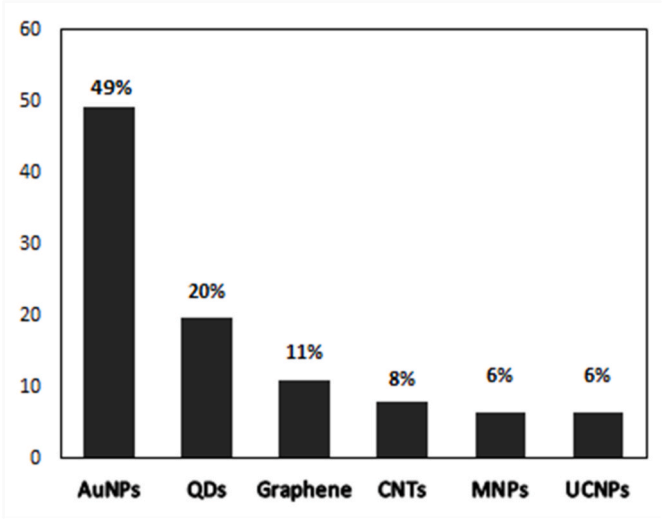

Fig. 1. Distribution of different types of nanomaterials applied in biosensors for cancer detection based on Web of Science database from 2016 to July 2020.

The use of nanomaterials in this field enables the further miniaturization of complex apparatus through integration with other methods, such as multiarray diagnostic platforms and to offer entirely new candidates for POC testing. Nano component materials incorporated into existing analytical devices are able to improve the detection sensitivity, specificity, reproducibility and reliability of assays due to their various specific characteristics such as high surface-to-volume ratio, good electrocatalytic properties, and chemical stability. These unique features, along with the capability of nanomaterials for reducing the cost of such systems, have made them one of the most interesting materials employed in POCT [16,26,44]. In the next section, we are going to review the use of nanomaterial combinations in POCT for early detection of cancer.

4. POCT approaches for cancer diagnostics

POC systems are integrated sensing platforms that enable one to run the multistep analytical procedures (such as sample preparation, signal amplification, and target detection) in one automated single unit. This analytical approach, which goes beyond traditional diagnostic tests supplies the clinician with a valuable source of molecular information to detect cancer via novel and efficient sensing platforms. Using POC devices, bio-diagnostic data can be obtained in a rapid, economical, and practical manner without requiring skilled technicians [11,45].

Recent advances in nano- and microfabrication techniques have driven more research into the development of low-cost and reliable POC cancer diagnostic tools. The resulting POC systems demonstrate many advantages, including those of reduced consumption of costly reagents, reduced analysis time, lowered power consumption, and ultrasensitive multiplexed analysis in an economical and fully portable sensing platform which can be used in both cancer diagnosis and prognosis development [12,46].

The global POC cancer diagnostics market was approximately USD 101 billion in 2013 and is expected to reach USD 168.6 billion by 2020 at an estimated compound annual growth rate (CAGR) of 7.60% from 2014 to 2020 [9].

There is demand and pressure for developing a POC platform in cancer diagnostics meeting the ASSURED diagnostics criteria, i.e. affordable, sensitive with very low false negative rates, specific with very low false positive rates, user-friendly tests that can be perform by anyone without needing any expertise, rapid and robust in situations outside of the lab, equipment free, i.e. can be performed without need of expensive instrument, and can be easily delivered to end user and remotely accessible in resource-limited settings [8,9].

In recent years, by integrating different types of biosensors with LOC, microfluidics and other techniques including arrays, POCT platforms have been rapidly developed to detect various classes of cancer biomarkers from proteins, nucleic acids, and cells [30,47,48].

A variety of detection strategies including electrochemical (i.e. amperometric and impedimetric), optical (i.e. surface enhanced Raman

scattering (SERS), fluorescent, and luminescent), colorimetric, magnetic, and other detection modalities have been integrated into POC systems for the detection of biomarkers. The signals developed in POC assays can be visualized in a variety of ways, including with the naked eye, glucometers, handheld light sources, camera phones, and electrophoresis [12,48]. In the following sections, several representative POCT devices developed for detecting cancer biomarkers are described and discussed.

4.1. Paper-based POC platforms

Paper-based platforms mainly include dipstick assays, lateral flow assays (LFAs), and microfluidic paper-based analytical devices (μPADs). These systems have been eagerly adopted in the POCT fields due to their innate advantages such as simplicity, low cost, ease of fabrication, independence from complex/external equipment, and capability of integration with other analytical devices [8,49–52]. The world market for paper diagnostics was nearly USD 5.69 billion in 2017 and will swell to over USD 9 billion by 2025 [53]. LFA-based POC devices are a major class of paper diagnostics with more than 100 companies manufacturing a wide range of LF devices [54]. Some of key players in the global LFA market are Alere, Inc., Hoffmann-La Roche AG, Abbott Laboratories, Danaher Corporation, Bio-Rad Laboratories, Dickinson and Company, bioMérieux SA, Siemens AG, Becton, Merck KGaA, Thermo Fisher Scientific, Inc, PerkinElmer, Inc, QIAGEN N·V., and Quidel Corporation. LFAs represent over one-third of the POC test market.

Among the main forms of paper-based platforms, LFAs and μPADS represent a unique opportunity for sensitive detection of cancer biomarkers as discussed in detail below. The lateral flow test strip is typically composed of four main pieces of paper, including a sample pad, conjugate pad, nitrocellulose membrane, and an absorbent pad which are assembled on a plastic backing card (Fig. 2a) [55]. The detection principle of an LFA relies on the flow of the liquid sample along a porous medium driven through capillary forces. The sample is first applied on the sample pad and then will flow through the conjugate pad where, in many cases, gold nanoparticles (Au NPs) conjugated with antibody/nucleic acid bind to the analyte in the sample to produce analyte-AuNP complexes. The resulting AuNPs complexes are then captured by a line of immobilized bioreceptors (i.e. antibodies or nucleic acid fragments) organized on the nitrocellulose membrane to generate easily recognizable red marks at positive test and control line locations (Fig. 2b). The qualitative results can be visualized by the naked eye without the use of any sophisticated instrument [8,54–59].

Most conventional LFAs use gold nanoparticles as a tag/label for colorimetric assay. In order to address the requirement of highly sensitive and specific quantification of targeted analytes, other types of nanomaterials such as QDs, magnetic nanoparticles (MNPs), upconversion nanoparticles (UCNPs), cellulose nanobeads, and silica have also been employed as colorimetric labels on LFAs [8,60,61].

Determination of nucleic acid biomarkers. LFA based POC devices have been successfully employed for detecting nucleic acid and protein targets due to their unique features including short detection time, low-cost, onsite operation, and user friendly formats [57,60,62,63].

Zheng et al. developed a nucleic acid lateral flow device for

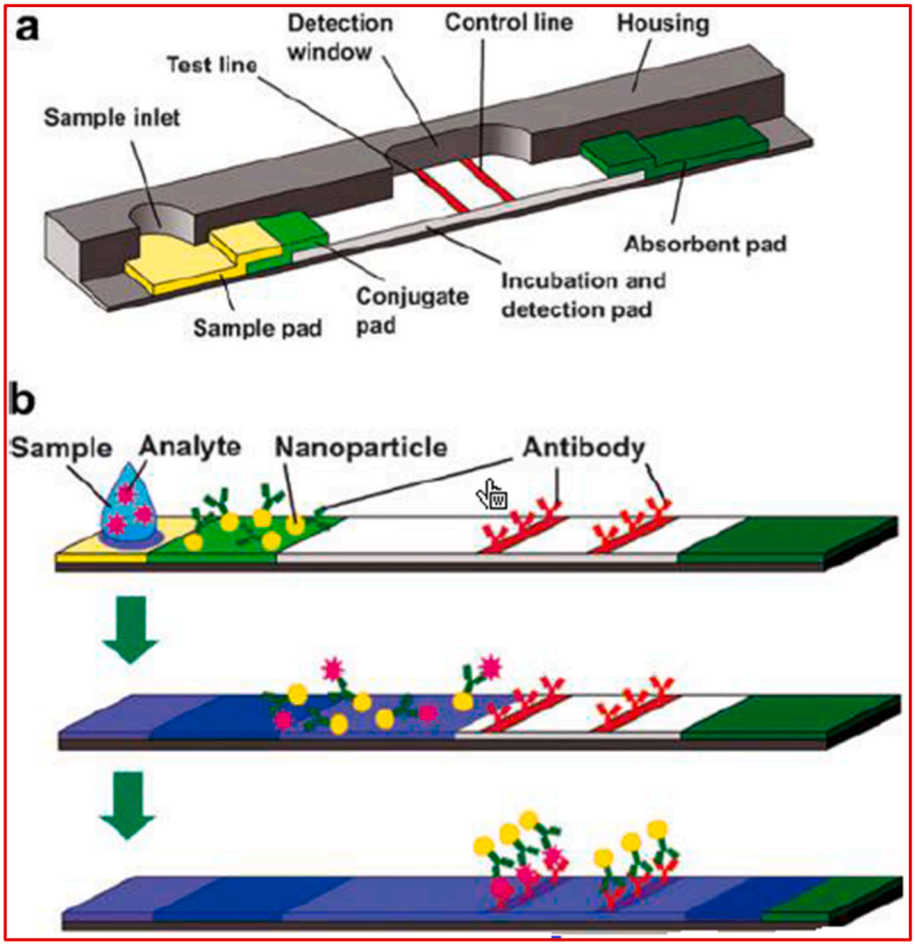

Fig. 2. (a) Schematic illustration of a lateral flow test. (b) Start of assay by adding liquid sample, in which analytes bind the antibody conjugated AuNPs. With the flow of sample, the analyte-bound particles are captured by antibodies in test line (positive result) and control line (proof of validity), respectively. "Reproduced from Ref. 55 with permission from the Royal Society of Chemistry."

simultaneous detection of three types of microRNAs (miRNA-21, miRNA-155 and miRNA-210) (Fig. 3a, b and c) in standard solution and in spiked serum samples (Fig. 3d-e). First, four capturing streptavidin-biotin DNA probes were immobilized on the nitrocellulose membrane to generate three test zones and one control zone. Thiolated DNA probes against three miRNA targets were covalently coated on the AuNPs, which subsequently bind to the miRNA targets in the sample to produce the sandwich complexes (DNA-miRNA-thiolated DNA/AuNPs). With the flow of sample, these complexes move along the test strip and were captured at the corresponding test lines. Red bands were observed with

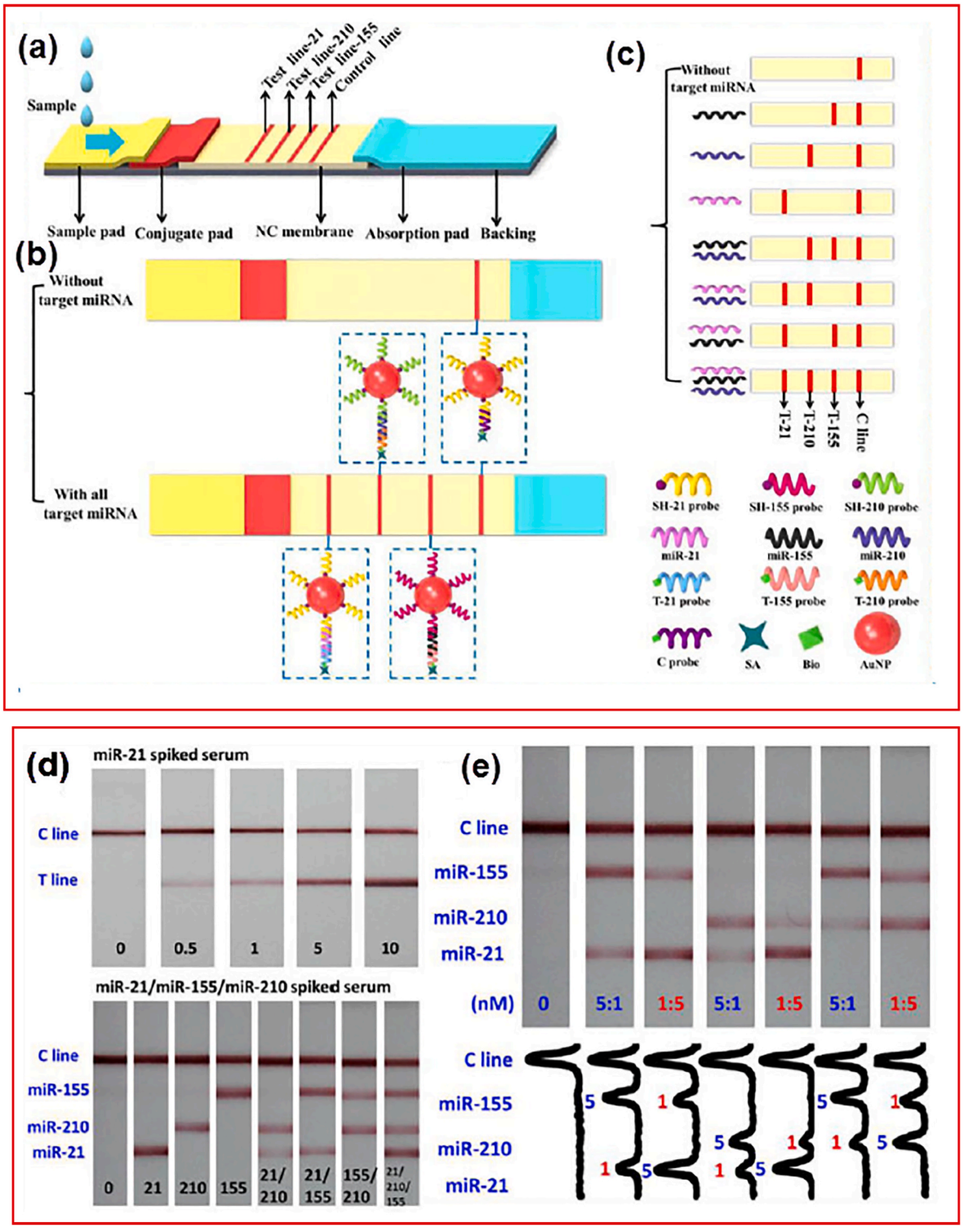

Fig. 3. (a) Schematic diagram of lateral flow nucleic acid biosensor (LFNAB) for multiplex microRNA assay, (b) in the absence and presence of three microRNA, (c) principle of detecting multiple microRNAs on LFNAB, (d) typical images of the LFNABs at different concentrations of miR-21 in spiked serum samples, and (e) typical images of the LFNABs in the absence and presence of individual microRNA and multiple microRNAs in spiked serum samples. "Reprinted from Sensors and Actuators B: Chemical, 264, W. Zheng, L. Yao, J. Teng, C. Yan, P. Qin, G. Liu, W. Chen, Lateral flow test for visual detection of multiple MicroRNAs, 320–326., Copyright (2018), with permission from Elsevier."

intensities proportional to the concentration of target RNAs (Fig. 3d-e) [64]. Recently, a LF device coupled with rolling circle amplification (RCA) was developed for simultaneous detection of miRNA 21 and let-7a. In this approach, AuNPs were primarily conjugated with thiolated DNA probes (prob 1) to capture the RCA products. Then, these sandwich type complexes were hybridized with captured probe sequences (prob 2) which had been immobilized on the NC membrane, resulting in the development of red bands. By adding the sample containing both miRNA targets, three red marks (two T lines and one C line) were observed on the LFA strip. A detection limit of 20 pM (for let-7b) and 40 pM (for miRNA 21) was achieved using this platform [65].

Another highly sensitive molecular diagnostic platforms relies on the new and upcoming technology which is known as the clustered regularly interspaced short palindromic repeats (CRISPR)-associated nuclease (Cas) system [66–69]. The CRISPR-Cas are the adaptive immune system in prokaryotes that uses RNA-guided nucleases to detect and destroy foreign/invader nucleic acids. In recent studies, CRISPR/Cas technology has been explored as a versatile tool for detection of nucleic acid targets due to the collateral cleavage activity of Cas effector proteins (Cas12, Cas 13, etc), allowing selective recognition and cleavage of target molecules with high efficiency. This platform can be integrated with many different read out systems to develop labor and cost-effective POC diagnostics devices [70–74].

The combination of CRISPR-Cas system with both electrochemical [72,75] and optical based biosensors [73,74,76] has been successfully applied for sensitive and specific detection of nucleic acids in pathogenic and nonpathogenic diseases, including cancer. Applications of CRISPR-Cas based biosensors have been comprehensively described in a number of recent reviews [67,69,77,78], so only a few examples are presented here.

For instance, by integrating cleavage ability of CRISPR-Cas12a, loop-mediated isothermal amplification (LAMP) [79,80] and AuNP-based LFA, Mukama et al. developed a sensitive paper-based platform for rapid detection of human papilloma virus (HPV) DNA in cervical exfoliated cell samples. In this work, genomic DNA was first extracted from heated clinical cervical samples followed by LAMP-amplification. LAMP-amplified products along with biotinylated ssDNA reporters were then used as the substrate for collateral cleavage activity of the Cas enzymes. In the presence of target, a ternary complex of Cas12a, CRISPR RNA/single guide RNA (crRNA or sgRNA), and target DNA was formed, leading to specific cleavage of its target DNA and collateral degradation of biotinylated ssDNA reporters in a non-specific manner. Subsequently, the cleavage activity was monitored by LFA readout. In LF strip, ssDNA capture probes complementary to the ssDNA reporter were immobilized on the test line while biotinylated rabbit polyclonal *anti*-IgG antibody were fixed on the control line. In the presence of the target, the degraded ssDNA reporter cannot hybridize with their complementary test-line probes (no test line) and are captured at the control line (rabbit

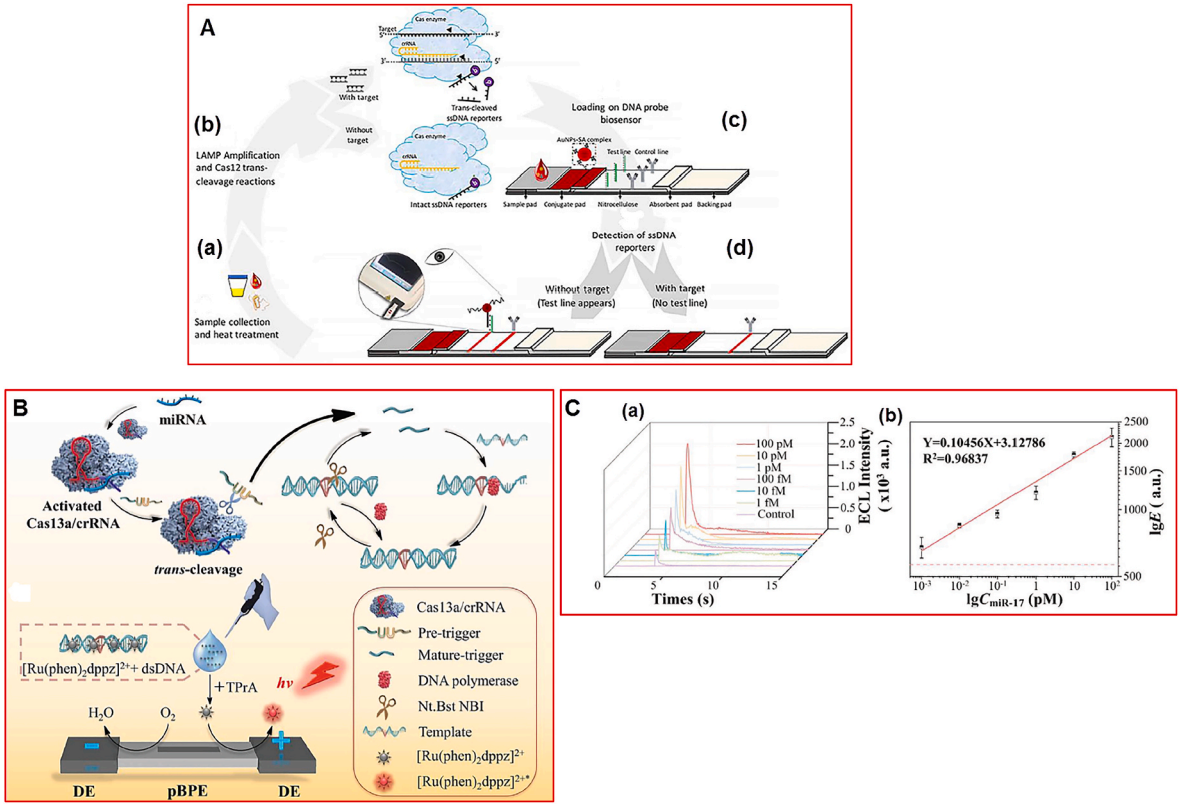

Fig. 4. (A) The working principle of CRISPR/Cas-isothermal amplification based lateral flow biosensor (CIALFB) approach. (a) Clinical samples can be heat-treated at 98 °C for 5 min to generate a crude genomic DNA template. (b) The template is LAMP-amplified and then used as a substrate for Cas12a (drawn in off-blue color) cleavage reaction. In the presence of the target, Cas12a collaterally cleaves the LAMP product as well as a biotinylated ssDNA reporter. (c) streptavidin-coated AuNP complex and ssDNA probe complementary to the complete ssDNA reporter are immobilized on the conjugate pad and test line, respectively. The biotinylated goat anti-mouse polyclonal IgG is fixed on the control line to capture excessive streptavidin-coated AuNP from the conjugate pad (d) In the presence of the target, the cleaved reporter cannot bind to its corresponding ssDNA probe (no test line), and vice versa (test line appears). "Reprinted from Sensors and Actuators B: Chemical, 316, O. Mukama, T. Yuan, Z. He, Z. Li, J. de Dieu Habimana, M. Hussain, W. Li, Z. Yi, Q. Liang, L. Zeng, A high fidelity CRISPR/Cas12a based lateral flow biosensor for the detection of HPV16 and HPV18, 128119., Copyright (2020), with permission from Elsevier." (B) Principle scheme of CRISPR/Cas13a powered portable ECL (PECL-CRISPR) chip for ultrasensitive and specific miRNA detection. (C) Sensitivity analysis of PECL-CRISPR system. (a) The analysis results of PECL-CRSIPR for various concentrations of target miR-17, (b) Linear analysis of the miR-17 detection results by the PECL-CRISPR system. The red dashed line represents the detection limit. Error bars are the standard deviation of triplicate measurements [72]. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

anti-IgG antibody), whereas in the absence of target, the intact/uncleaved reporter bound AuNPs were captured by the immobilized probes at the test line and induced visual red color (Fig. 4A). This Cas12a-based strip was capable of detecting a DNA target with a LOD of 3.1 amol (i.e., 1.8 copies) [73].

In another work, a powered portable paper based electrochemiluminescence (ECL) test was fabricated based on the combination of CRISPR/Cas13a and an exponential amplification reaction (EXPAR) for ultrasensitive detection of miR-17 from different human tumor cells. The EXPAR template was designed with two repeat regions that are complementary to target miRNA and separated by a short nicking endonuclease (NEase) recognition sequence. As shown in Fig. 4B, in the presence of RNA targets, collateral cleavage activity of Cas13a was activated to cleave the EXPAR specific pre-trigger primer (PT) into mature-trigger (MT). These cleavage products serve as primers to proceed the downstream exponential amplification, resulting in the formation of large amounts of double-stranded DNA (dsDNA) that could be bound with the ECL luminophore, [Ru (phen)2dppz]2+, for luminescence signal generation. The proposed device exhibited a linear dynamic range from 1 fM to 100 pM with a good detection limit (1 fM) and reproducibility (Fig. 4C) without the need for a tedious washing step, making it favorable for POC applications [72].

Tian et al. have developed a paper-based electrochemical platform via hierarchical assembly of nanomaterials onto a paper electrode for the simultaneous determination of two types of miRNAs, including miR-141 and miR-21. Silver nanowires (AgNWs) were first deposited onto paper surfaces to provide electrical conductivity pathways on the electrodes. High surface area molybdenum disulphide (MoS_2) nanosheets were decorated with AuNPs (MoS_2/AuNPs) layer). The gold particles enabled covalent attachment, through thiol groups, of strong binding DNA hairpin capture probes. Part of the target miRNAs hybridize with these electrode bound complementary DNA sequences, and the other half hybridizes with complementary DNA sequences bound to PtCu metal organic frameworks (MOFs). Two different characteristic electrochemical signals were measured, one for each type of target miRNA because the MOF bound DNA sequences were conjugated with different

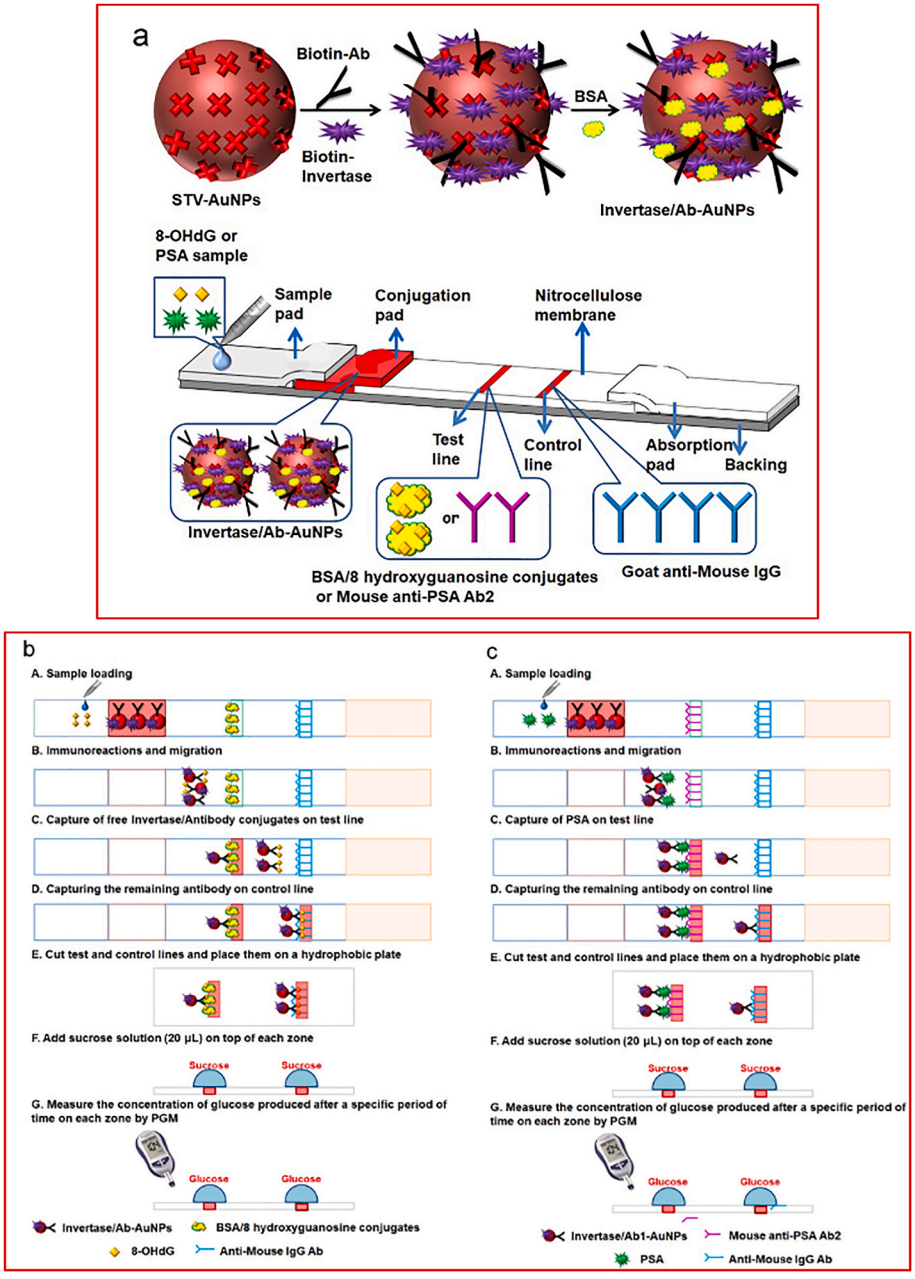

Fig. 5. (a) Design of test strips for quantitative detection of 8-OHdG or PSA, (b) Mechanism for 8-OHdG detection by a personal glucose meter (PGM), (c) Mechanism for PSA detection by a PGM. "Reprinted from Biosensors and Bioelectronics, 126, X. Zhu, M. Sarwar, J.-J. Zhu, C. Zhang, A. Kaushik, C.-Z. Li, Using a glucose meter to quantitatively detect disease biomarkers through a universal nanozyme integrated lateral fluidic sensing platform, 690–696., Copyright (2019), with permission from Elsevier."

electrochemically active reporters, either methylene blue (MB) or ferrocene (Fc). A femtomolar (0.1 fM) limit of detection (LOD) was obtained for both miRNAs with a minimal response to common interferents including uric acid, dopamine, and thrombin [81].

Determination of protein biomarkers. The combination of an LFA platform with a personal glucose meter (PGM) was employed for sensitive and rapid detection of both a small molecule marker, 8-Hydroxyguanosine (8-OHdG) and a large protein marker (PSA) in separate devices (Fig. 5a). In this work, 8-OHdG and PSA markers were detected using competitive and sandwich immunoassays formats respectively. In both cases, streptavidin-coated AuNPs were conjugated with both biotinylated invertase enzyme (to convert sucrose to glucose) and an antibody specific to the target analyte of interest in the sample. Next, bovine serum albumin (BSA)/8-Hydroxyguanosine conjugates and anti PSA antibodies were immobilized onto the nitrocellulose membranes at the test line positions. With the flow of the liquid sample, in the 8-OHdG competitive test, the analyte-bound AuNP complexes bypass the test line and are captured at the control line (Goat anti-Mouse IgG), whereas in the PSA sandwich assay, the analyte bound AuNPs were captured by the immobilized antibodies at the test line. The gold nanoparticle binding provides a qualitative response, with the intensity of the test line being inversely proportional to analyte concentration in the 8-OHdG case (Fig. 5b) and directly proportional to the concentration of PSA in their respective platforms (Fig. 5c). The noteworthy and important contribution is the use of a single enzyme type to provide a "universal" or common signal independent of the nature of the target molecule, i.e. small or large molecules can be determined as long as an antibody or antibody pair are known. Because the target-induced immunoreactions (sandwich/competitive) lead to the capture of multiple invertase enzymes addition of a sucrose solution leads to the production of detectable glucose concentrations which can be quantitatively measured by commonly available glucose meters. The linear dynamic range was 0.1–100 ng/mL for 8-OHdG and 1–100 ng/mL for PSA with a detection limit of 0.23 and 1.26 ng/mL for 8-OHdG and PSA respectively [82].

The incorporation of Förster resonance energy transfer (FRET) assay into the lateral flow strip was reported by Wang et al. for sensitive detection of carcinoembryonic antigen (CEA) cancer biomarker. The AuNPs were modified with *anti*-CEA monoclonal antibody, while fluorescein isothiocyanate (FITC)-labeled antibody was coated on the lateral flow strip. Upon addition of CEA antigens, the sandwich immunocomplexes were formed on the test line, leading to the fluorescence quenching of FITC and a visual red color on the strip surface [83].

Notably, the sensitivity of the LFA can also be improved by inducing fluidic delays in the LF strip, which in turn lead to optimal target capture efficiency. To regulate flow speed in the LF strip several strategies have been incorporated into the LFA, including polydimethylsiloxane paper hybrid, agarose, physical geometry of the paper, and magnetic probes. For example, a magnetic lateral flow device has been developed using Fe_3O_4/Au core-shell nanoparticles for ultrasensitive detection of valosin-containing protein (VCP) as a cervical cancer biomarker (Fig. 6a). The use of magnetic nanoprobes greatly increased interaction time between antigen−antibody, yielding optimal capture efficiency for sensitive detection of the target analyte. The detection limit of the reported device (25 fg/mL) is approximately 10^6 times more sensitive than conventional LF tests (Fig. 6b) [84].

Given the complexity of cancer, an ideal biomarker-based sensing platform should be capable of simultaneous quantification of multiple cancer biomarkers in a clinical sample. To address this issue, several researchers have attempted to develop suitable paper based devices on a single chip for multiplexed detection of cancer biomarkers.

Recently, Guo et al. have developed a multichannel paper-based microfluidic device to detect CEA, alpha-fetoprotein (AFP), and PSA simultaneously by utilizing their antibodies, immobilized on paper. The hydrophilic microfluidic channels, three channels with one detection zone per channel, were fabricated on paper by patterning paper sheets with a hydrophobic reagent (recycled polystyrene in chloroform) using an adhesive tape mask (Fig. 7A). CEA, AFP and PSA antibodies were then immobilized at their respective detection zones for capturing the target antigens, which subsequently bind with analyte specific HRP-labeled antibodies to form sandwich structures (Fig. 7B). This sandwich-type immunosensor, using HRP catalyzed luminol-H_2O_2 piodophenol (PIP) chemiluminescence (CL), was capable of detecting CEA, AFP and PSA over the range from 0.05 to 80 ng/mL, 5–80 ng/mL, and 1–50 ng/mL respectively [85].

A hybrid plastic-paper microfluidic chip was also adopted for impedimetric detection of AFP marker. A paper microchip consists of an oxidized cellulose-paper layered over a flexible plastic substrate, bonded together with double sided adhesive tape. The assembled layers were then cut into the electrode area using a laser cutter. Next, the finger type interdigitated electrodes were printed on top of the paper with an ink composed of a mixture of silver/graphene nanocomposite, followed by functionalization of the detection zone of the device with a diphenylalanine peptide nanotube containing amine groups. These peptides enable immobilization of AFP antibodies through the formation of covalent bonds. Finally, the specific interaction of the AFP antigens and antibodies generated changes in the magnitude of the impedance signal proportional to the concentration of the target biomarker (1 ng/mL to 10 μg/mL). A detection limit of 1 ng/mL (in PBS buffer) and 10 ng/mL (in plasma) was achieved using this platform [86].

Determination of circulating tumor cells. A paper based ECL

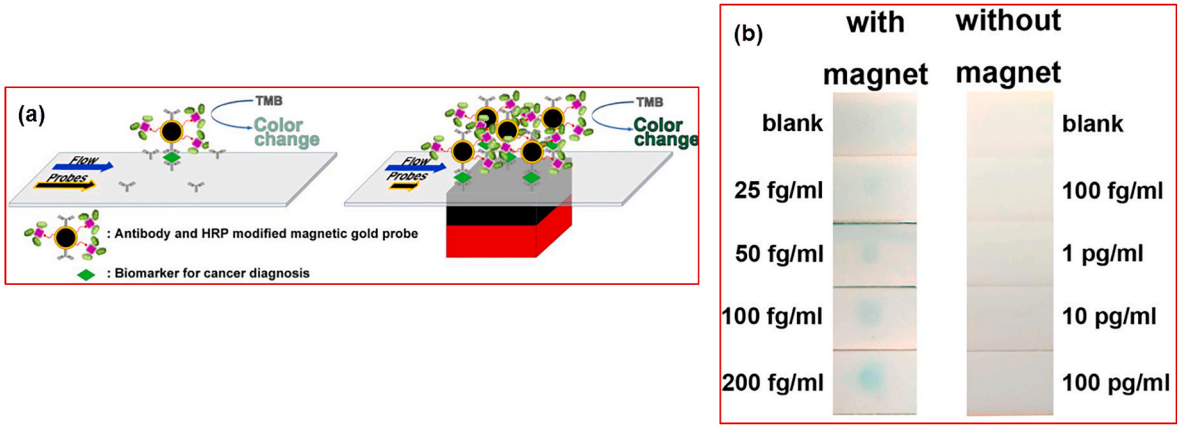

Fig. 6. (a) Schematic depicting the effect of magnets in magnetic focus lateral flow sensor (mLFS). Without magnet, the magnetic probe-labeled targets move along with the sample flow on the LF strip, resulting in a low capture efficiency (left). With magnet, the probe-labeled targets are focused at the signal generation zone due to magnetic focus thus increasing capture efficiency of labeled targets (right), and (b) comparison of detection results with and without magnet. Reprinted with permission from Ref 84. Copyright 2019 American Chemical Society.

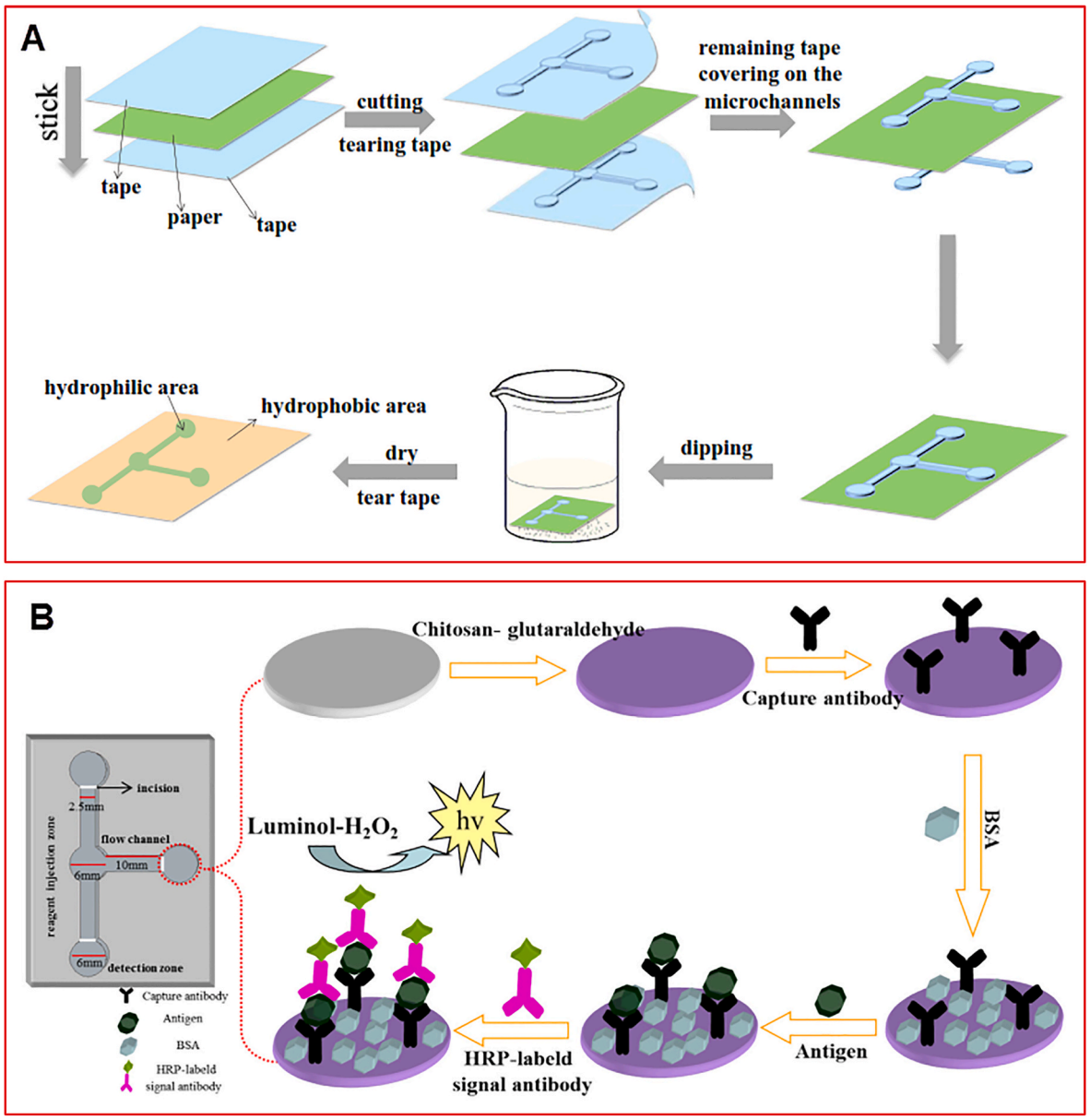

Fig. 7. (A) Schematic diagram of the dipping fabrication procedure, (B) schematic diagram of the size of the μPAD and the immunoassay procedure. "Reprinted from Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 223, X. Guo, Y. Guo, W. Liu, Y. Chen, W. Chu, Fabrication of paper-based microfluidic device by recycling foamed plastic and the application for multiplexed measurement of biomarkers, 117341., Copyright (2019), with permission from Elsevier."

cytosensor was fabricated using gold-palladium nanoparticles (AuPd NPs) modified bipolar electrode (BPE) for detection of MCF-7 breast cancer cells employing cellular H_2O_2. In this work, wax printing was used to pattern hydrophilic channels on a paper substrate and the carbon ink-based bipolar electrode and driving electrodes were screen-printed into the channels (Fig. 8A). Then, the BPE was modified with AuPd NPs which served as both a nanoscaffold for immobilization of aptamers and as a catalyst for the ECL reaction. When the target MCF-7 cells were captured on the aptamer-modified BPE, additional aptamer probes capable of nucleating hybridization chain reaction (HCR) were also captured, forming a sandwich type (capture probe-target cell-signaling probe) structure. Then, as a mechanism to amplify the ECL signal by locally immobilizing concentrated luminol, two different hairpin structured DNA sequences labeled with luminol/AuNPs were attached to the MCF-7 cells via a hybridation chain reaction (Fig. 8B). The cytosensor was able to detect MCF-7 in the range of 10^2-10^7 cells mL^{-1} with a LOD of 40 cells mL^{-1} (Fig. 8C) [87].

In another report, a nonenzymatic electrochemical platform was employed to monitor the H_2O_2 released from living breast and breast cancer cells. Gold nanoflower electrocatalytic nanostructures were synthesized through a dopamine-assisted one-pot hydrothermal synthesis method, where the reduction of the graphene oxide (GO) substrate and *in situ* growth of gold nanoflowers occurred concomitantly. This modified electrode showed excellent electrocatalytic activity for the nonenzymatic determination of H_2O_2. The real-time monitoring of H_2O_2 released from ~10^6 breast cells anchored to the modified electrode allowed the determination of peroxide released from the normal breast cell line (HBL-100) and from cultured breast cancer cells including MDA-MB-231 and MCF-7 cell lines [88].

Determination of exosome cancer biomarkers. Recently, an enhanced LFA have been developed using two different sized AuNP conjugates for sensitive detection of exosomes derived from MCF-7 cells. The first type of AuNP (15 nm) was modified with antibodies against exosomal proteins (CD9) which are selective to the CD9 marker on

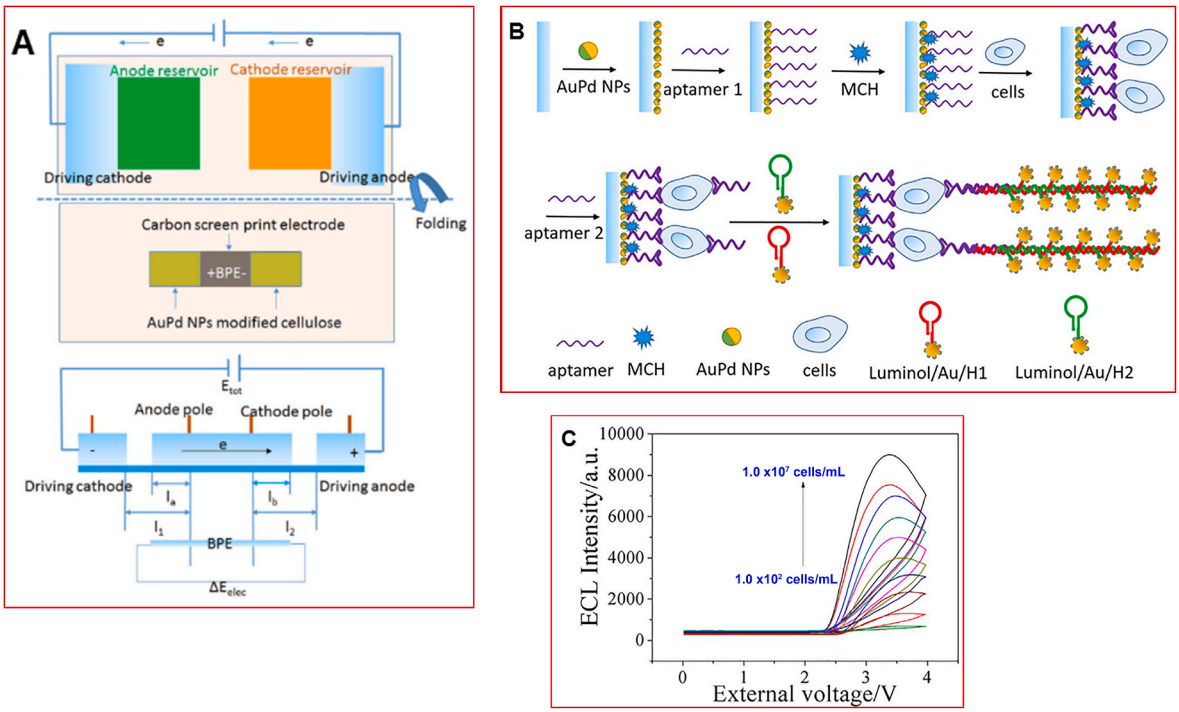

Fig. 8. Paper-based BPE device for ECL detection of MCF-7 cells: (A) Fabrication procedure for the paper-based BPE and potential difference across the BPE, (B) schematic illustration of the assembly process of the cytosensor, and (C) ECL intensities of the device at different concentrations of MCF-7 cells (1.0×10^2 cells/mL to 1.0×10^7 cells/mL). "Reprinted from Biosensors and Bioelectronics, 102, S. Ge, J. Zhao, S. Wang, F. Lan, M. Yan, J. Yu, Ultrasensitive electrochemiluminescence assay of tumor cells and evaluation of H2O2 on a paper-based closed-bipolar electrode by in-situ hybridization chain reaction amplification, 411–417., Copyright (2018), with permission from Elsevier."

exosomal membranes, while the second type AuNP (40 nm) was labeled with *anti*-BSA antibodies and subsequently bound to the first AuNPs, which had been covered with BSA antigen acting as a blocking agent. The detection limit of the dual-labeling scheme was 1.3×10^3 particle/μl, increasing detection sensitivity by 13-fold compared to the conventional LFA [89].

Chen et al. developed a fluorescent paper-based aptasensor using the luminescence resonance energy transfer (LRET) pair formed by UCNPs as fluorophores and gold nanorods (AuNRs) as quencher for detection of exosomes from hepatocellular carcinoma (HepG2) cells. The CD63 aptamer sequence was composed of two different parts called capture probe (CP) and detection probe (DP) to bind to the CD63 marker on the surface of the exosomes thus capturing them. The NH_2-CP and PEI-modified UCNPs were first covalently attached to the surface of the sodium periodate oxidized filter paper via the amine-aldehyde reaction. The AuNPs labeled with thiolated DP were subsequently mixed with exosomes and loaded onto the modified filter paper. In the presence of exosomes, two parts of the aptamer (CP & DP) formed a sandwich structure around the CD63 protein on the exosomes, shortening the distance between AuNRs and UCNPs, which leads to quenching of the green luminescence. Finally, the luminescence intensity of the analyte spot on the paper device, which is inversely proportional to the exosome concentration, was recorded by a CCD camera to quantify the exosome concentration. The high sensitivity (LOD of 1.1×10^3 particles/μL) and short response time (30 min) of this device presents a new strategy for clinical exosome detection [90].

Despite significant academic progresses in the active field of paper based POCT devices, they still cannot fulfill the end-user requirements and therefore are not in large-scale production. The major issues associated with paper based platforms include sample processing, lack of reproducibility, long-term instability, limited clinical accuracy, and the hurdle of compliance with regulatory guidelines, all of which need to be addressed for adoption as a clinical diagnostic devices.

Analysis of real clinical samples with different compositions present challenges for paper based sensing platforms. For example, unprocessed whole blood contain colored (red) cells, proteins, and other blood component and therefore require pretreatment using chemical reagent/external filtration to make the sample suitable for accurate downstream detection. The analytical sensitivity, specificity especially for low concentrations of analytes is another factor that require more development efforts to enable accurate detection of target analytes. Notably, the use of highly selective bioreceptors, inducing fluidic delays, and amplifying signals by incorporating nanomaterials are relatively newly available parameters which can be applied toward achieving the desired analytical performance. Moreover, paper devices are prone to batch-to-batch variability owing to the effect of different ambient conditions such as temperature and humidity. There are also several other factors including uncontrolled physical properties of paper (e.g., porosity, permeability, and capillary flow rate), requiring larger sample volume owing to liquid entrapment during the sample migration, and requiring chemical pretreatment of the paper surface for bioreceptor immobilization which can affect the analytical performance of these paper devices.

4.2. *Printed electrode based POC platforms*

Screen printing (thick film) technology is one of the methods most favored for large-scale production of electrochemical POC devices, due to its affordability, speed, and compatibility with different types of materials [6,91–94]. According to IDTechEx research, the total market for printed organic and flexible electronics will rise from $41.2 billion in 2020 to $74 billion in 2030 [95]. The most common products include organic light-emitting diodes (OLEDs), printed biosensors, and printed conductive inks. Advances in conductive ink composition, combined with the design flexibility and wide range of substrate choices (plastic, paper, glass, metal) has made screen printed electronics (SPEs), a very powerful option for construction of a range of useful electrochemical devices in both academic and industrial research laboratories [6,92,93,96,97].

Typically, the fabrication of SPEs includes the following steps: (i) selection of screen-mesh template to generate the desired pattern, (ii) selection of appropriate substrate material, (iii) selection and preparation of the printing media (i.e. inks/paste), (iii) printing on the solid substrate, (iv) drying and curing of the printing media [92,98]. In order to fulfill the specific analytical requirements, the surface of SPEs is modified with a range of compounds such as antibodies, enzymes, DNA strands, polymers, metals or electrochemical mediators [8,92,98].

Determination of nucleic acid biomarkers. Povedano et al. reported a simple and PCR free electrochemical method for the detection of methylated O-6-methylguanine-DNA methyltransferase (MGMT), the tumor suppressor gene, based on magnetic bead-modified screen-printed carbon electrodes (SPCEs). First, carboxylic acid-functionalized magnetic beads (HOOC-MBs) were covalently conjugated with anti-5-mC antibody, through 1-ethyl-3-(3 dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysuccinimide (NHS) chemistry, to capture 5-mC-methylated DNA sequences. The captured target sequence was then hybridized with a biotinylated probe, followed by further labeling with strepavidin-HRP. The subsequent enzymatic reduction of H_2O_2 produced current proportional to the concentration of methylated DNA. This sensitive biosensor was capable of detecting 1.2 pM methylated DNA with a relatively fast analysis time (45 min) without the need of laborious sample pretreatment and PCR amplification [99].

The combination of bisulfite treatment, asymmetric PCR amplification, and base-dependent adsorption of DNA at AuNP-modified SPCEs was recently reported for analyzing gene-specific methylation in hepatocellular carcinoma (HCC) cell lines and patient fresh tissue samples.

AuNPs were first deposited on the surface of screen printed working electrodes using a cyclic voltammetry method. Next, genomic DNA was extracted and purified from HCC cells and tissues followed by the bisulfite conversion and asymmetric PCR. PCR products were dropped directly onto the AuNPs-SPCE surface and allowed to adsorb. Due to higher adsorption affinity of the adenine-enriched unmethylated DNA than guanine-enriched methylated DNA to AuNPs, unmethylated DNA were strongly adsorbed on the SPCE surface and generated a lower differential pulse voltammetry (DPV) signal in the presence of $[Fe(CN)6]_3$. This biosensor was highly sensitive, enabling the discrimination of unmethylated from methylated DNA sequences with single methylated CpG sites (Fig. 9A) [100].

In addition to LFA readout, electrochemical reporters has been also integrated with Cas12a platform for portable and cost-effective detection of HPV DNA target and transforming growth factor β1 (TGF-β1) protein as HCC tumor marker. In this platform, ssDNA reporters were first modified with a thiol group at the 5′ end for covalent attachment onto gold screen printed electrodes (Au-SPE) and a MB at the 3′ end as electrochimacal signaling tag. In presence of target, the DNA reporters was degraded by Cas effectors, which caused the release of MB from Au-SPE surface and decreases the electrochemical signal. The amplification-free biosensor exhibited a linear range from pM to μM with a LOD of 50 pM for detecting HPV DNA target. This platform was then extended for detection of TGF-β1 protein biomarker in clinical sample with the aid of aptamer-based electrochemical CRISPR cascade. After incubation of the protein target with a fixed concentration of aptamer (50 nM for 30 min), the unbound aptamer was evaluated by electrochemical CRISPR, reflecting the amount of target antigen. A wide linear dynamic range, of 3 orders of magnitude, with an LOD of 0.2 nM was achieved [71].

On the basis of the double-specific probe strategy, Daneshpour et al. developed a simple and rapid electrochemical genosensor for simultaneous detection of miR-106a and let-7a gastric cancer biomarkers on a single electrode. This SPCEs-based electrochemical biosensor consists of two parts: the solid substrate with the captured probes (probe 1) and the electrochemical nanoprobe with detection probes (probe 2), which can form DNA/RNA heteroduplex structures with miRNA targets. The SPCEs were modified with polythiophene/rGO to improve the electrical conductivity of the electrodes and provide enough surface area for the immobilization of capture probes. The biotinylated DNA capture probes

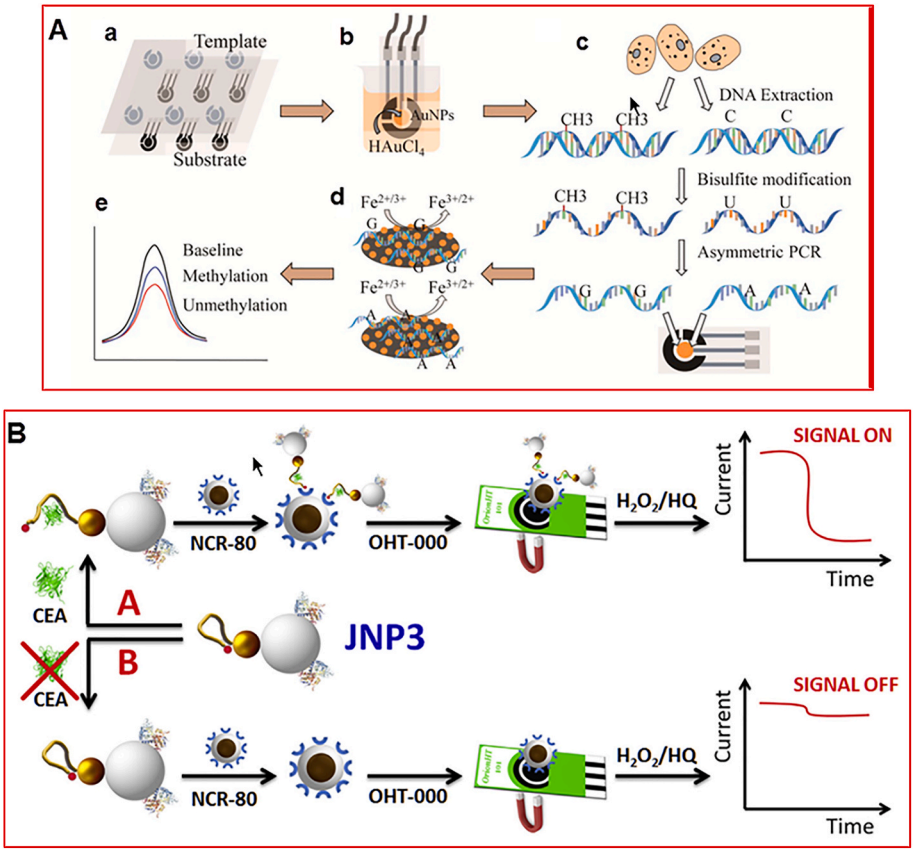

Fig. 9. (A) Scheme of AuNPs-SPCE fabrication and detection principle: (a) SPCE fabrication with various templates, (b) SPCE undergoes AuNPs modification, (c) genomic DNA is extracted and purified from cultured cells or clinical sample. Subsequently DNA undergoes bisulfite modification and asymmetric PCR to generate adenine-enriched and guanine-enriched sequences. Sample directly drops onto the AuNPs-SPCE for adsorption, (d) negatively-charged DNA on the electrode electrostatic repulses ferricyanide $[Fe(CN)6]^{3-}$ ions, and (e) methylated DNA generates higher DPV signal than unmethylated DNA. "Reproduced from Ref. 100 with permission from the Royal Society of Chemistry." (B) Schematic display of the Janus nanoparticle-based biosensing strategy for CEA detection. "Reprinted from Analytica Chimica Acta, 1061, G. Paniagua, A. Villalonga, M. Eguílaz, B. Vegas, C. Parrado, G. Rivas, P. Díez, R. Villalonga, Amperometric aptasensor for carcinoembryonic antigen based on the use of bifunctionalized Janus nanoparticles as biorecognition-signaling element, 84–91., Copyright (2019), with permission from Elsevier."

(probe 1) complementary to each target miRNAs (miR-106a & let-7a) were subsequently immobilized on the streptavidin-coated SPCEs and allowed to hybridize with target miRNAs. After that, Au/TMC/Fe3O4 and CdSe@CdS/TMC/Fe3O4 nanocomposites coupled with DNA detection probes (probe 2) were introduced in the system for detecting miR-106a and let-7a, respectively. Once the sandwich-type heterogeneous structure (signal probe/target miRNA/capture probe) was formed, the electrochemical signal generated by the nanocomposites/tracing tags was recorded. This multiplexed, PCR-free, miRNA biosensor exhibited a wide linear range and a low LOD of 0.02 fM and 0.06 fM for let-7a and miR-106a, respectively [101].

Determination of protein biomarkers. Kalyoncu et al. developed an electrochemical sandwich immunosensor for simultaneous detection of multiple cancer biomarkers, CEA, vascular endothelial growth factor (VEGF), and AFP. The capture antibodies were immobilized on the SPCE surface using glutaraldehyde cross-linking agent. The immunosensor was subsequently incubated with CEA, VEGF, and AFP target antigens and multiplexed detection of biomarkers was obtained by labeling their secondary antibodies with hybrid nanolabels, i.e. AbCEA-PbAu@ɣ-Fe2O3, AbVEGF-CuAu@ɣFe2O3 and AbAFP ZnAu@ɣFe2O3. Amperometric measurement relied on the different oxidation peaks of the labeling metals ((Pb, Cu, Zn) [102].

The stem-loop/hairpin DNA motif is a very versatile form of oligonucleotide probe which can play an important role in biomarker screening and has been widely used in electrochemical sensing platforms. For example, Paniagua et al. by employing a biotin thiol-modified hairpin aptamer as a recognition element and Au–SiO2 Janus nanoparticles as electrochemical labels, developed sensitive electrochemical aptasensing for CEA detection. In this work, the silica surface of Janus nanoparticles were functionalized with HRP (as signaling tags) while the Au surface was coated with a dual-labeled hairpin *anti*-CEA aptamer. The binding events between the CEA target analyte and its aptamer opens the stem-loop of the probe and leads to unmasking the biotin residues. Finally, capture of the CEA–Janus nanoparticle complex by avidin-modified Fe3O4@SiO2 magnetic nanoparticles (NanoCaptors) on the working electrode of SPCEs resulted in quantification of target analyte using the hydrogen peroxide/hydroquinone (H_2O_2/HQ) system (Fig. 9B) A linear range from 5.5 pM to 28 nM, with a LOD of 1.2 pM was achieved and this aptasensor was also capable of detecting the target biomarker in spiked human serum [103].

Freitas et al. developed a sandwich-type electrochemical immunosensor for detection of Human Epidermal growth factor Receptor 2 (HER2-ECD) using CdSe@ZnS core/shell QDs as an electroactive label. In this assay, antibody-modified SPCEs were used as substrate to capture HER2-ECD antigens followed by incubation with core/shell QDs-labeled secondary antibodies.

The immunological interaction between the cancer antigen and the antibody decorated on the QD nanoparticles was evaluated by the anodic stripping voltammetry technique, yielding a linear range of 10–150 ng/mL with a detection limit of 2.1 ng/mL [104].

Determination of circulating tumor cells. SPCEs were integrated with microfluidic channels for the electrochemical detection of circulating melanoma cells. In this system, the SPCEs were first modified with polyaniline nanofiber (PANI) followed by the covalent attachment of the cell surface melanocortin 1 receptor (MC1R) antibodies which specifically bind with the MC1R antigen. Subsequently, cell suspension was delivered onto antibody-modified SPCEs through the microfluidic channel. When cell suspension was delivered onto antibody-modified SPCEs, the antigen-antibody interaction caused changes in the DPV currents that were proportional to the cell concentration (range of 10–9000 melanoma cells/10 mL). To confirm cell binding affinity to the modified SPCEs, the green fluorescent protein (GFP)-tagged cells was also imaged with confocal microscopy. The proposed microfluidic device showed a low detection limit of 1 melanoma cell/mL both in buffered solutions and in cell-spiked samples [105].

In another study, Dou et al. reported the development of the multiplexed and ultrasensitive electrochemical detection of CTCs in human whole blood using aptamer-functionalized and gold nanoparticle array-decorated magnetic graphene nanosheet (AuNPs-Fe3O4-GS) capture probes and aptamer modified signal amplification probes which are AuNPs loaded with either ferrocene or thionine as electroactive species to provide unique signals for each cell type. The incubation of the capture and signal probes with the blood sample containing two target CTCs lead to the successful isolation of the rare cells and achieved two distinct voltammetric peaks on the SPCE. The combination of the two effects result in the efficient monitoring of rare CTCs in samples with detection limits down to 4 and 3 cells mL^{-1} [13].

Determination of exosome biomarkers. Recently, an amplification-free electrochemical platform has been developed using Au-SPE and magnetic beads to detect exosomal mRNA, miRNA-21, extracted from human serum/cultured cells. In this format, probe-modified magnetic beads were employed to capture target miRNA-21 from the samples. The captured hybrid was released from the magnetic beads and the purified target miRNA was subsequently adsorbed onto Au-SPE to allow electrochemical determination of the target miRNA in the presence of $[Fe(CN)_6]_3$. The developed method showed high reproducibility (RSD lower than 6%) and excellent sensitivity of 1 pM which is well within the range of levels of miRNA species observed in clinical samples (0.2 fM to 20 pM) [106].

Later, the same group reported use of a newly engineered gold-loaded ferric oxide nanocube (AuNPFe2O3NC) as a capture agent for electrochemical detection of the exosomal protein CD63. The synthesized AuNPFe2O3NCs were first modified with CD63 antibodies to enrich and separate exosomes from cell culture media. The exosomes-bound Au–NPFe2O3NC was subsequently captured by antibody-modified SPCEs. Due to the high density of targeted exosomes on the superparamagnetic nanoparticles coupled with the peroxidase-like activity of the AuNPFe2O3NCs, a low detection limit (10^3 exosomes/mL) and high sensitivity were obtained for these sandwich immunoassays for exosomes in cell culture media (Fig. 10) [107].

The representative examples described here highlight recent progress of SPEs to fabricate a range of suitable electrochemical sensing platforms and the promising future of such approaches for the implementation of proven laboratory research in real world settings. Although screen printed electrodes are the favorite choice for creating POC devices, some challenges need to be overcome, include optimization of substrates, printing methods, screen printing pastes, type of recognition elements and their immobilization on the electrode surface. (i) Significant damage of thin silver layers of commercially available screen-printed electrodes occurs after exposing the sensor to even a few cycles of CV and impedance measurements (ii) In order to enhance the chemical stability, analytical performance or enable the immobilization of analyte specific species on the surface, the electrodes need to be covered by thin gold films/gold nanoparticles, leading to an expensive sensing platform requiring time-consuming surface modification processes (iii) the non-specific binding with other physiological substances, making it difficult to provide convincing standard performance for quantitative measurement of targets (iv) Achieving fully reliable SPE based biosensors with prolonged operation and storage stability depends on several factors include the quality of receptor immobilization, surface chemistry, and storage conditions as such devices present poor stability even under refrigerated conditions.

4.3. Smartphone-based platforms

Smartphones represent a remarkable new platform for the development of next-generation POC diagnostics devices, providing portable and user-friendly mobile healthcare delivery and personalized IVD systems [108,109]. With over 6.8 billion cell phone subscribers, cell-phone technology has also opened up opportunities in the area of business and research [110].

Incorporation of mobile attachments or dedicated readers for LFAs

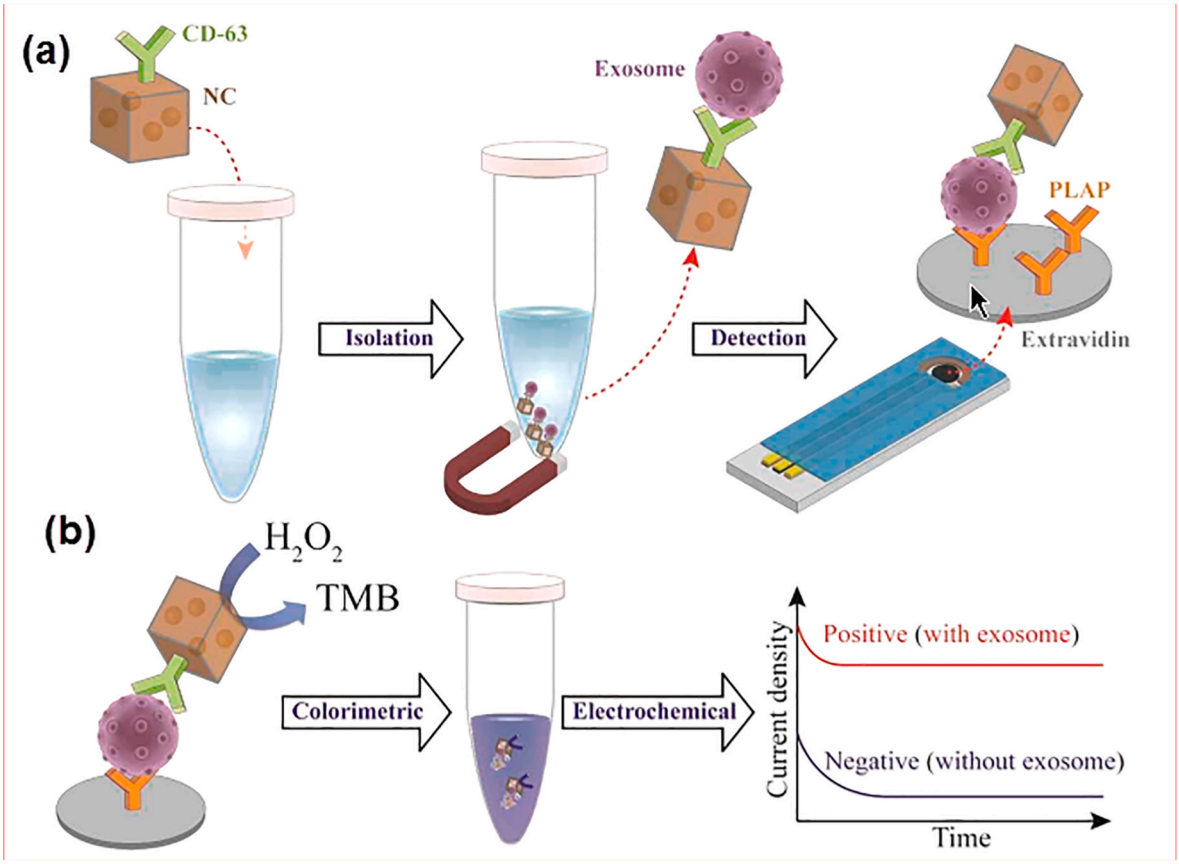

Fig. 10. Schematic representation of the assay for direct exosome isolation and detection from cell culture media: (a) the AuNPFe_2O_3NC were initially functionalized with CD63 antibodies and dispersed in sample fluids to capture bulk exosomes. After magnetic capture and purification, exosome-bound Au–NPFe_2O_3NCs are transferred to PLAP antibody-modified SPEs, (b) the peroxidase-like activity of Au–NPFe_2O_3NC was then used to achieve naked-eye detection along with UV–visible and electrochemical quantification of PLAP-specific exosomes present in the cell culture media. "Reprinted with permission from Ref 107. Copyright 2019 American Chemical Society. "

and paper-based devices leads to greatly increased qualitative and quantitative digital signals, providing an alternative for early disease monitoring at home, on-site and in remote locations without the need for advanced instrumentation [111].

Overall smartphone-based POCT has proven to be effective for improving patient safety and efficacy of care through providing real-time evaluation and enabling better diagnosis decisions.

So far, smartphone-based POCT have been developed for LFA, immunoassays, colorimetric and electrochemical sensing, microscopy, flow cytometry, and SPR-based biosensors. There are many companies offering smartphone-based devices for healthcare and diagnostics which are being used by millions of customers worldwide [45,109]. The main manufacturers for these devices are currently Mobile Assay, CellScope, iHealth, Nonin, GENTAG, Runtastic, among others. The present trend shows smartphone-based POCT has a key role to play in personalized healthcare monitoring and leads to the design of cost-effective IVD systems [109]. The results obtained with devices external to the phone are subsequently input through the smartphone cameras, smartphone-connected electrochemical analyzers, then processed in smartphone apps, and transferred to central systems [45,110,112].

Moreover, smartphones are promising tools for enabling portable, rapid, label-free, easy-to-use and on-site assessment of diseases in remote locations and resource-limited settings [113,114]. The following are some examples of smartphone devices developed for the four types of cancer biomarker testing as described in this review.

Determination of nucleic acid biomarkers. Recently, Tian et al. designed a fluorometric aptasensor for simultaneous detection of miRNA 21 and let-7a, based on intelligent DNA hydrogel coupled with a smartphone platform. First, UCNPs-embedded hydrogel microparticles with differently coding modes (shapes and color) were prepared using stop-flow lithography in polydimethylsiloxane (PDMS) microfluidic channels and subsequently modified with two specific aptamers to capture the target miRNAs. The miRNA aptamer sequence was composed of two different parts: sequences that are specific to the target miRNAs for capturing them (capture probe) and those that are specific to the universal biotinylated probe (adapter probe). After capturing target analyte in a sample, the universal biotinylated probe was introduced into the mixture and subsequently linked with the adapter probe sequences, followed by further labeling with streptavidin conjugated R-phycoerythrin (SA-PE). Finally, the fluorescent intensity of particles was recorded by the inverted fluorescence microscope and then converted to the analyte concentration via a mobile smart app. This platform was capable of detecting femtomole level of the target with a fast analysis time (10 s) [115].

Due to the limited brightness of fluorescent dyes, researchers have recently developed highly efficient multi-chromophore FRET systems that use a light-harvesting concept inspired by natural photosynthetic systems, where a large number of a donors can efficiently harvest and transfer the light energy to a few or even single acceptors through a FRET strategy. The high chromophore density, high extinction coefficient, and many-fold fluorescence brightness of artificial light-harvesting nanoparticles when compared to single organic dye donor make them a powerful tool for ultrasensitive bioanalytical assays and FRET imaging which could be detectable even using a smartphone camera. For example, the integration of light-harvesting FRET probe with the smartphone was employed for sensitive detection of target DNA

sequences of survivin as an anti-apoptotic cancer marker. In this approach, light-harvesting nanoantenna functionalized with DNA probe was based on 40-nm polymeric NPs loaded with ~6000 green-emitting octadecyl rhodamines and highly fluorinated bulky counterions, which was as bright as 135 QD 605. These DNA functionalized-nanoantenna particles serve as both a scaffold to capture the target DNA sequences and a giant FRET donor that efficiently deliver their excitation energy from a large number of a donor dye to a few FRET acceptors (red dye-tagged oligonucleotides) at the nanoparticle surface. Upon addition of target DNA, the fluorophore-tagged oligonucleotide acceptors were competitively displaced from the NP surface due to high affinity between DNA probe and their complementary target DNAs. The displacement of dye-labeled acceptor caused the decrease of FRET signal and simultaneous emission of green color which was subsequently recorded with a smartphone camera. The relative intensity of the acceptor decrease with increase in the DNA target concentration, which enables ratiometric imaging of the acceptor-to-donor (red/green, R/G) fluorescent intensities by smartphone RGB camera. This unique amplified energy transfer strategy has shown >20% FRET efficiency and a 25 antenna effect, allowing quantitative ratiometric detection of target analyte with a LOD of 3 pM [116].

In addition to fluorometric assays, smartphone based electrochemical platforms have also been extended to monitor nucleic acid cancer biomarkers as explained below.

A modified SPCEs combined with a circuit board of the smartphone was recently reported for sensitive detection of circulating miRNA-21 in saliva samples. A reduced graphene oxide/gold (rGO/Au) composite was first deposited on the surface of screen printed working electrodes

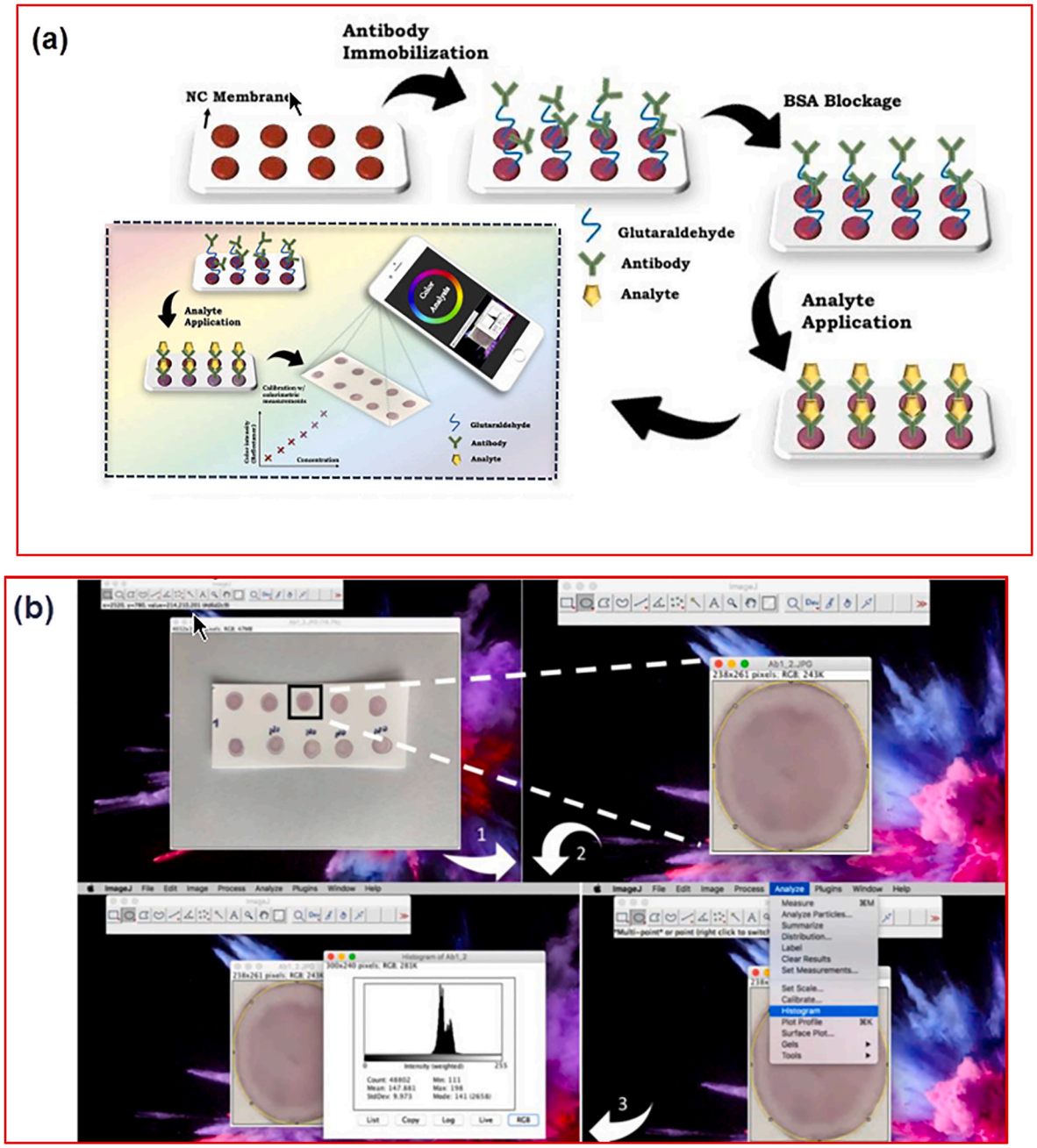

Fig. 11. (a) Schematic illustration of the proposed spot-like POC testing design, (b) analysis steps for the colorimetric measurements of the smartphone images via Image J software. "Reprinted from Talanta 208, E. Aydindogan, A.E. Ceylan, S. Timur, Paper-based colorimetric spot test utilizing smartphone sensing for detection of biomarkers, 120446., Copyright (2020), with permission from Elsevier."

using a cyclic voltammetry method. Next, thiolated DNA probes against miRNA targets were covalently coated on the AuNPs, which subsequently bind to the miRNA targets in the sample. Due to the negatively charged phosphate backbone of the DNA probe, the hybridization events between the target analyte and the DNA probe resulted in decreases of the electrochemical signal that were proportional to the concentration of miRNA. A linear response range (10–4 to 10–14 M) along with a low detection limit (1 pM) was achieved. The result showed that SPCEs in combination with smartphones can provide affordable analytical devices for the ultrasensitive detection of various biomarkers which could lead to the development of new devices adaptable to POC systems [117].

Determination of protein biomarkers. A dot blot assay on paper has been employed to detect the cancer protein biomarkers, AFP and mucin-16 (MUC16). In this assay, cysteamine modified AuNPs (cyst-AuNPs) were immobilized on nitrocellulose membranes followed by incubation with relevant antibodies (*anti*-AFP or *anti*-MUC16). The target antigens were then dropped on the spots and selectively bound to the antibody-modified AuNPs, inducing a red-to-purple color change (Fig. 11a). The intensity of color was recorded by the smartphone camera and then processed via Image J software (Fig. 11b) [118].

In another report, a near-infrared-based photothermal immunosensor was prepared by coupling Cu_xS nanocrystals with a smartphone-based thermal reader for detecting the PSA cancer biomarker. In this work, the Cu_xS nanocrystals (NCs) were synthesized by the one-pot method, followed by labeling with DNA aptamer units which were made by RCA. In the presence of PSA target antigen, the DNA-Cu_xSNCs assemblies were captured by the anti PSA antibodies on the plate surface and a sandwich-type immunoreaction product was formed. Under 808 nm near-infrared (NIR) irradiation, the Cu_xSNCs absorbed the light and converted it into thermal energy which was detected by the smartphone-based infrared thermal imager (Fig. 12A–a). Due to the combination of repeated aptamer sequences provided by the RCA products and the photothermal properties of Cu_xS NCs, the signal was amplified and a detection limit of 0.2 ng/mL was obtained (Fig. 12A–b) [119].

Determination of circulating tumor cells. Using MNP@QD multifunctional supraparticle assemblies coupled with smartphone-based imaging, Tran et al. developed a rapid immunomagnetic fluorescence assay for concurrent capturing and counting SK-BR3 breast cancer cells. In order to synthesize the three-layer nanocomposite (QD@dextran@Fe_3O_4), dextran-coated MNPs were first functionalized with imidazole groups and then densely coated with QDs through an affinity reaction, followed by further dextran coating to increase stability. To enable cell-specific capture, the assemblies (MNP@QD) were next conjugated with tetrameric antibody complexes (TACs), containing an antidextran antibody and anti-human HER2 antibody in order to crosslink to the dextran shell and to the HER2-positive SK-BR3 cells. After the immunomagnetic separation of specific cells, images of the immunofluorescent-labeled SK-BR3 cells were obtained using smartphone camera and analyzed. This developed platform showed a low limit of 10^2-10^3 cells/mL. As compared to existing FDA-approved CellSearch system for CTCs isolation and count, this QD-based immunofluorescent assay offers desirable features in terms of cost (decrease 2 orders of magnitude), size, and versatility, making it a promising future tool for the enumeration of CTCs [120].

Recently, another smartphone fluorescent imaging device integrated with a dual-layer paper-based microfluidic platform was fabricated to detect ROR1+ (receptor tyrosine-like orphan receptor one) cancer cells in the buffy coat blood samples. The paper microfluidic chip consists of two layers: the top porous fiberglass layer with the dye-labeled antibodies for selective capture of the target cancer cells from samples and a

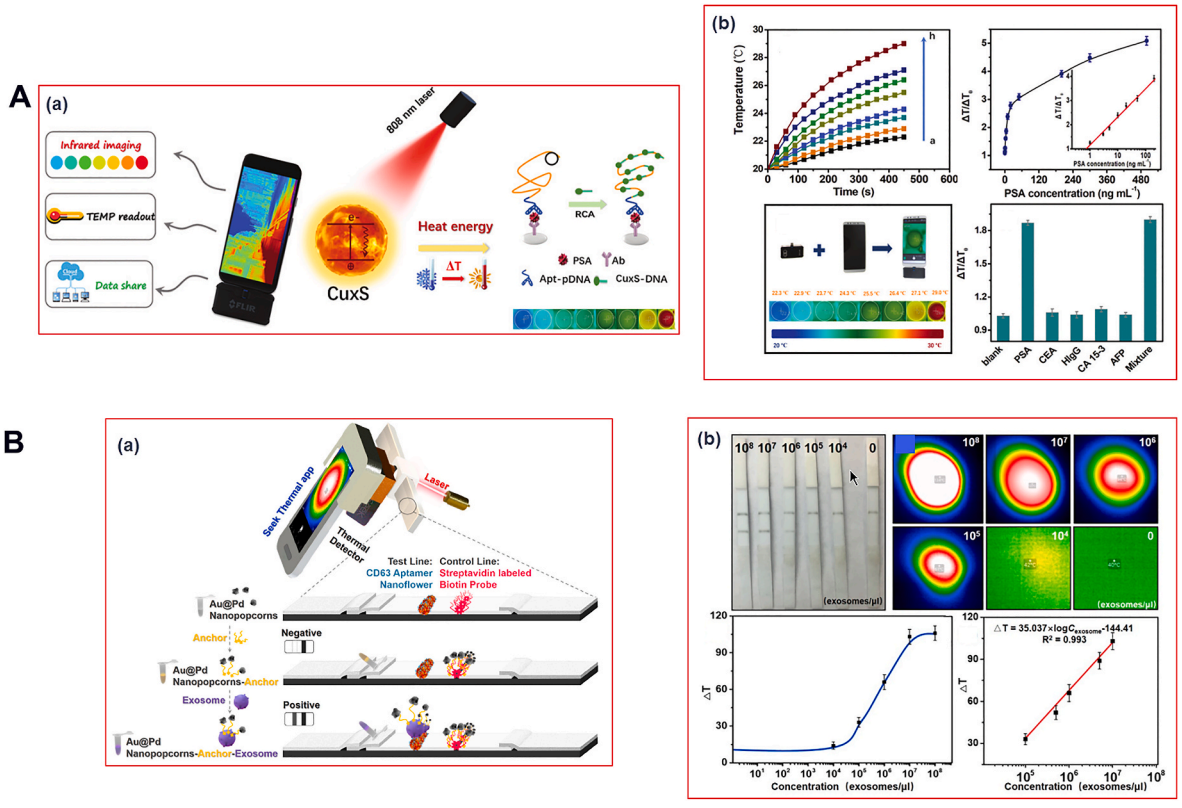

Fig. 12. (A-a) Schematic illustration of the CuxS nanocrystal-based photo-thermal immune-imaging assay of cancer biomarkers on a smartphone, (A-b) Changes in temperature versus time at different PSA concentrations under NIR laser (808 nm, 0.8 W) excitation for 450 s. Reproduced from Ref. 119 with permission from the Royal Society of Chemistry. (B-a) Schematic illustration of the strategy of integrating an Au@Pd nanopopcorn and aptamer nanoflower assisted lateral flow strip (ANAN-LFS) with a smartphone-based thermal reader, (B-b) Analytical performance of the ANAN-LFS at different concentrations of exosomes (0 to 10^8 exosomes/μL). Reprinted with permission from Ref 122. Copyright 2019 American Chemical Society.

bottom cellulose layer with four channel for detection of capillary flow velocity and wicking. The presence of target cells triggered an immunoagglutination process, resulting in a color change and flow velocity changes which were directly monitored using a custom-made smartphone fluorescence microscope and interpreted with appropriate apps. Overall, results showed that the flow rate analysis can provide higher detection sensitivity than imaging (LOD of 0.1 cells/μL vs. 1 cell/μL) [121].

Determination of exosome biomarkers. Cheng et al. developed a colorimetric LFA integrated with a smart phone for detecting exosomal CD63 using Au@Pd nanopopcorn and aptamer nanoflowers. The flower-shaped aptamers were produced from RCA products to capture the CD63 protein present on the exosomes at the test line. With the flow of sample, the exosomes-bound Au@Pd nanopopcorn from the conjugate pad and were then captured by the immobilized aptamer nanoflowers in the test zone. Next, a smartphone based thermal reader was used to quantify the thermal signal which was proportional to the concentration of the exosomes (Fig. 12B–a). The detection limit was 1.4×10^4 exosomes/μL, a 71-fold better sensitivity as compared with the conventional LF tests (Fig. 12B–b). The developed device also meets the clinically relevant level in human serum (10^6 exosomes/μL) [122].

The above mentioned studies have successfully demonstrated that smartphones can serve as detectors for electrochemical sensing, colorimetric, fluorescence, luminescence, and other optical signals. Next, the subsequent measurement signals/captured images can be transformed into quantitative data of targeted analytes. The mass market acceptance and the continuously increasing desired features of smartphones have attracted great attention from researchers and manufacturers toward building smartphone-based tools and smart technologies for clinical diagnostics and healthcare monitoring. Despite the great efforts made, there are some key challenges to be tackled prior to obtaining a worldwide penetration of clinical markets by smartphones serving as powerful medical diagnostic devices. First, in the case of optical detection, the quality of signals/captured images rely heavily on good performance of the smartphone camera and associated imaging-processing algorithm. Even though high-resolution (few to 41 megapixels) smartphone cameras are used for capturing the images, their resolution and sensitivity still do not meet specific requirements for acquiring the even higher-quality images required for desirable data acquisition. Moreover, ECL signals are susceptible to ambient light and must be performed in a light-tight environment/dark room, limiting its use for outdoor testing. Hence, a number of factors include exposure time, the pixel density, pixel size, image stabilization, designing new light-tight detection accessory, the speed of wireless connection (e.g., the use of higher-generation wireless system/5G), and processors must be optimized to maximize the performance of standard smartphone cameras, which are evolving rapidly. Second, electronic data and transmission via Bluetooth and wireless communication have raised the concerns related to the security and privacy of patients' data. Therefore, the development of advanced data storage strategies and encryption algorithms for securing this confidential data and personal health information is essential. In order to circumvent this issue, a number of ongoing initiatives target the creation of an international Cloud computing standard, such as Google Data Liberation Front and EuroCloud, to tackle technical challenges surrounding data ownership. Third, to achieve practical applications and regulatory compliance, the contamination of smartphone-based sensing platforms and the hygiene aspects need to be addressed. Forth, another key element to translate a technology into a commercial device is economic burden. Presently, mobile POCT devices are powered by external batteries, leading to high-cost sensing platform due to maintenance requirements and changing the batteries after prolonged use. The cost of a sensing platform can be dramatically reduced by using a built-in rechargeable smartphone battery as low-cost sources of power for basic operation of the device, signal processing, and data analysis. However, such internal rechargeable lithium batteries contain potentially toxic chemicals including nickel, lead and copper which may contribute to ecotoxicity and human toxicity. So, the development of solar-powered batteries can serve as an alternative solution, since the Sun provides an effective and abundant source for producing clean and sustainable energy without adverse human health effects and environmental toxic pollution. Fifth, most of the mobile POCT diagnostic devices are still on proof-of-concept stage where experiments do not meet criteria for application in a clinical practice. Therefore, to pass the performance requirements, the efficacy and safety of such devices must be evaluated via rigorous and randomized studies to allow them to go through clinical trials successfully. Finally, easy-to-use format, affordability, and compatibility with various smartphone models from different manufacturers may play critical roles in moving the technology out of academic settings and make them clinically viable.

Undoubtedly, the continuously improving technical specifications of mobile systems (i.e., processor, camera, security, and other technical issues), smart technologies, and Cloud computing would further improve the key features and capabilities of mobile health devices for use in enabling low-cost POCT and personalized healthcare. Furthermore, the smartphone equipped with a complementary technologies include LOC platforms, prolonged reagent storage concepts, encompassing rapid detection formats, and novel biosensor strategies will revolutionize bioanalytical applications of mobile POCT devices in the coming years.

5. Current status of point-of-care cancer diagnostic devices

POC platforms are emerging as powerful mechanism to accelerate delivery of high-quality health care in a timely manner and with optimal clinical and public health output [9,11,108]. In this review, we have described very recent reports of several types of POC devices that have been successfully employed for the quantification of different cancer biomarkers including nucleic acids, exosomes, proteins and cells (Table 2). Each biosensing approach was described based on the configurations of main component parts such as sensing, recognition and transduction elements, while focusing on the particular design reasoning employed along with detailed clarifications of the potential clinical applications. Out of thousands of devices developed in academic settings for cancer POC testing, only a very few of them have been used to quantify cancer biomarkers in the various untreated clinical sample matrices (i.e., fingerpick blood, urine) that are suitable for clinical/home applications. Among the cancer POC diagnostic platforms developed so far, LFAs are a mature technology, widely used in the clinic due to their low cost, simple working procedures, and their suitability for large scale manufacturing.

To date, a number of LFAs have been marketed for the detection of specific cancer biomarkers.

One of the cancer-related LFAs' applications is the determination of the level of PSA greater than 4.0 ng/mL. It is noteworthy that the total PSA cut-off value is 4 ng/mL in the blood, so less than 4 is healthy and levels above it requires further testing. CTK Biotech's "Onsite" is an immunochromatographic assay to be used for semi-quantitative determination of PSA in blood.

The test also recommends a biopsy for values greater than 10 ng/mL PSA [123]. Another example of cancer-related applications of LFAs is OncoE6™ from Arbor Vita for cervical cancer detection. This device detects an E6 oncoprotein produced by high-risk types of HPV. In order to measure cancer biomarkers in blood, the labeled-capture antibodies are immobilized on a nitrocellulose membrane to generate test and control zones. The test line is used for detection of target biomarker attached to the antibodies and the color intensity is closely associated with the relative amount of the cancer biomarker. Then, the sample migrates over the control line which verifies the validity of the assay [124].

For novel immunoassay systems such as microfluidic chips and LFAs, the use of nanomaterials with excellent properties can help in achieving real-time monitoring, when integrated with or even into mobile phones

Table 2
Summary of POC cancer diagnostic devices for cancer biomarkers detection.

POCT platforms	Nanomaterials	Cancer biomarker	Recognition element	Detection method	Detection limit	Sample type	Ref
Paper-based POC platforms	AuNPs	DNA	CRISPR	Fluorometric	0.24 fM	Cultured cell supernatant, plasma	[76]
	Cerium dioxide - Au@glucose oxidase (CeO2–Au@GOx)	miRNA-21	Hairpin probe DNA	Electrochemical	0.434 fM	Diluted human serum	[172]
	AuNPs@Cu-MOFs	miRNA-155	Hairpin probe DNA	Electrochemical	0.35 fM	Serum	[173]
	Platinum nanoparticles (PtNPs)	PSA	Antibody	Distance-based readout or LFA ruler	0.54 ng/mL	Serum	[174]
	Fe3O4@QDs nanobeads	Free & complexed PSA (f-PSA & c-PSA)	Antibody	Fluorescent LFA	0.009 ng/mL (f-PSA) 0.087 ng/mL (c-PSA)	Serum	[175]
	AuNPs- graphene	NSE & CEA	Aptamer	Electrochemical	10 pg mL^{-1} (NSE) 2 pg mL^{-1} (CEA)	Serum	[176]
	–	CEA	Bio-molecularly imprinted polymers	Electrochemical	0.32 ng/mL	Serum	[177]
	CdTe quantum dots (CdTe QDs)	CEA & PSA	Antibody	Fluorometric	0.3 ng/mL (CEA) 0.4 ng/mL (PSA)	Serum	[178]
	Polyhedral-AuPd nanoparticles (PH–AuPd NPs) & reduced graphene oxide (rGO)	MCF-7 cancer cell	Aptamer	Electrochemical	20 cells mL^{-1}	Serum	[179]
	AuNPs	Exosomal CD9, CD81, and CD63	Antibody	Colorimetric	8.54×10^5 exosomes μL^{-1}	Melanoma cell supernatants, plasma, urine	[180]
	AuNPs	Exosomal MICA & CD9	Antibody	Colorimetric	5×10^7 exosomes μL-1	Melanoma (Ma-Mel-55 and Ma-Mel-86c) cells and human serum	[181]
Printed electrode based POC platforms	–	Methylated DNA	Antibody	Electrochemical	–	Ovarian cancer cell lines (SKOV 3, OVCAR 3) and normal cell line (MeT-5A)	[182]
	Fe_3O_4 NPs & rGO	Methylated DNA	Antibody	Electrochemical	9×10^{-5} ng mL^{-1}	Standard solution	[17]
	–	Methylated DNA	Antibody	Electrochemical	1.2 pM	Paraffin-embedded colorectal tissues, cancer cells, and serum	[183]
	Au–NPFe2O3NC nanocomposites	miRNA-107	Oligonucleotide probe	Electrochemical	100 aM	Cancer cell lines ((HKESC-1, HKESC-4) and patient tissue samples	[184]
	CdSe@ZnS QDs	ExtraCellular Domain of the Human Epidermal growth factor Receptor 2 (HER2-ECD)	Antibody	Electrochemical	2.1 ng/mL	Spiked human serum	[104]
	Graphene nanoplatelets & Fe3O4@Au NPs	CEA	Antibody	Electrochemical	0.10 pg mL^{-1}	Spiked human serum	[185]
	Carbon nano-onions & graphene oxide (GO)	CA19-9	Antibody	Electrochemical	0.12 U mL^{-1}	Standard solution	[186]
	Flower like gold nanostructures	CA125	Aptamer	Electrochemical	5 pg mL^{-1}	Spiked human serum, urine and saliva samples	[187]
	Multiwall carbon nanotubes (MWCNTs)	Hepatic oval cells (HOCs)	Antibody	Electrochemical	100 cells mL^{-1}	Human hepatocellular carcinoma cell line (HepG2)	[188]
	AuNPs	Exosomal miRNA-21	p19 with biomarkers of exosome	Electrochemical	1 aM	Breast cancer cell line	[189]
	Silica nanoparticles (SiO2 NPs) & GO	Exosomal proteins	Aptamer	Electrochemical	9.46×10^2 particles μL^{-1}	Breast cancer cells and serum	[190]
	–	Exosomal proteins	Antibody	Electrochemical	15 ng μL^{-1}	Lung cancer cell line	[191]
Smartphone-based platforms	Upconversion NPs	Ephrin type-A receptor 2 (EphA2) & PSA	Antibody	Colorimetric	400 pg mL^{-1} (EphA2)89 pg mL^{-1} (PSA)	Standard solutions	[192]
	CuS NPs	CEA	Antibody	Fluorescent	0.05 pg mL^{-1}	Standard solutions & serum	[193]
	AuNPs	CA125	Antibody	Colorimetric	30 U mL^{-1}	Spiked human serum	[194]
	–	PSA	Anibody	Colorimetric/ fluorescent	0.08 ng/mL	Blood	[195]

or computers. In order to commercialize these integrated systems, the size must be decreased, sample introduction must be quite simple, and highly automated performance is to be expected. Portable, reliable, and multiplexed detection systems will almost certainly require integration with microfluidics in order to accurately measure panels of cancer biomarkers present in various clinical samples. Significant challenges related to materials synthesis remain. For example bioconjugation processes need to be carefully monitored in order to achieve high levels of reproducibility in large scale production. Differences in the size, shape, or composition of nanomaterials and in the reliability of bioconjugation during label preparation, may result in significant variations in quantitative values. The immune recognition activity can be processed into a detectable digital signal by mobile, wearable, and implantable tools, such as smartphones and wrist watches, which may themselves be generated using 3D printing methods. Widespread use of real-time personal health monitoring systems, coupled with communication of this data to large scale data networks, will lead to these immunoassay devices enabling not only new generations of POC analytical tools of the sort essential for monitoring individuals, but also yielding whole country protection via monitoring infectious diseases among the population, and improving public health.

The main challenge of existing colorimetric test pads for one-step multi-analyte detection is moderate detection sensitivity, where detection of biomarkers only occurs at relatively high concentrations. In order to achieve the desired higher level of sensitivity, novel single-step detection assay and/or probes must be constructed. The new test pads would be coupled with multiple functionalized particles to provide high performance for multiple target detection per strip. Recently, a capillary electrophoresis-based assay for DNA, RNA, protein and cell samples has beenreleased in the form of acommercial multiplex system, Agilent's Bioanalyzer.

Several beneficial attributes of LFAs reveal potential pathways to the resolution of critical challenges to the development of qualitative POCT devices. These features include easy analyte loading via capillary action, direct visualization by the naked eye, without the need of additional instrumentation, simple-to-use by patients or untrained individuals, and of great importance for potential ubiquitous use, being very cost-effective. However, for very sensitive and quantitative target detection, at this time they require a readout device. Additional obstacles impeding the development of LFAs into the clinically portable paper diagnostic devices sought for widespread use include insufficient reproducibility in disposable test panels, which leads to the requirement of using a control line for each test, the need for relatively high-analyte volumes, and limited flexibility in assay configuration. Addressing these shortcommings will lead to enhanceddevice commercialization.

Table 3 presents the existing commercially diagnostic devices in industry and their performances for cancer detection. Other POC diagnostic devices are still under development, are in clinical trials or are overcoming technical limitations.

6. Challenges in translation of point-of-care technologies to healthcare applications

The demand for POC devices is expanding rapidly, with a projected market size of US$ 36.96 billion by 2021 [125]. There are many aspects of this market, one of which is tumor and cancer biomarkers. At first, a biomarker needs to be found and a fast and simple way of identifying established to develop a new clinical-use test. In clinical environments, POC instruments have the ability to accelerate the transformation of traditional medicine into personalized medicine. Nonetheless, there are still many challenges hindering their advancement into clinically relevant, portable POC tools. Medical laboratories typically use industrial in-vitro diagnostic instruments which must produce test results at competitive or even fixed prices in accordance with strict standards. To

Table 3
Commercially available LFA for cancer biomarkers detection. Reprinted with permission from Ref. 63.

Company	Product name	Cancer type	Analyte	Required sample	Detection time (min)	Sensitivity	Specificity
CTK Biotech	On-Site PSA Semi-quantitative Rapid Test	Prostate	PSA	60–90 μL Blood/ Serum/Plasma	10	100%	99%
Alere	Alere NMP22 BladderChek	Bladder	Nuclear matrix protein (NMP22)	4 drops of Urine	30	99% when combined with cystoscopy	99% NPV along with cystoscopy
	On-Site FOB Rapid Test	Colorectal	hHB	Human fecal specimens	10	NR	NR
	Clearview iFOBT	Colon	Faecal Occult Blood	Faeces	5	93.60%	99.10%
Arbor Vita Corp.	OncoE6 Cervical Test	Cervical cancer	E6 oncoproteins	Cervical swab	150	84.6%	98.5%
	Onco*E6*™ Oral Test	Oral	E6 oncoproteins	NR	150	–	–
Quicking Biotech Co., Ltd	CA125 Rapid Screen Test Kit	Ovarian	CA125 Ag	100 μL of Serum	5–10	40 U mL^{-1}	–
Innovation Biotech	AFP Test	Hepatocellular Cancer	AFP	Serum Plasma	10	25 ng/mL	99%
Diagnostic Automation/Cortez Diagnostics Inc	CEA Serum Rapid Test	Adenocarcinoma of the colon	CEA	Serum	10	5 ng/mL	–
LifeSign, LLC	Status BTA	Bladder	Bladder tumor associated antigen	3 drops of Urine	5	67%	70%
ALFA SCIENTIFIC DESIGNS	INSTANT-VIEW®	Prostate	PSA	Serum	4–7	4 ng/mL	–
AccuBioTech Co.,Ltd	AFP Whole Blood Test Cassette	Hepatocellular Carcinoma	AFP	Blood/Serum/ Plasma	<10	99.3%	99.0%
	PSA Whole Blood Test Cassette	Prostate	PSA	Blood	<10	98.9%	98.6%
	CEA Whole Blood Test Cassette	Hepatocellular Carcinoma	CEA	Blood	<10	98.7%	99.3%
	FOB Faeces Test Strip	Colon	Human Occult Blood in faeces	Faeces	<10	94.6%	99.3%
Ulti med	FOB Cassette	Colorectal	Human Occult Blood	Faeces	<10	–	–

ensure their success in the market, these instruments must address several requirements. The device's performance must be checked in clinical practice and licensed for use by the appropriate regulatory authority (e.g., the FDA) before they are launched. To be competitive against such high throughput systems, POC devices must be proven to be more cost-effective, which will require development of efficient and consistent production methods for POC sensors and packaging. Each testing tool must be reliable and precise, and must provide multiple, or comprehensive, readouts. Diagnostic tools provided for medical examination outside of medical labs (at home without skilled operators) will require extra authorization by local regulators. While each nation has different rules, regulatory bodies generally require home usage diagnostic tools to be precise, be user-friendly for untrained people, and have little risk of user confusion or harm if performed incorrectly. In order to be most useful for health applications, diagnostic systems should be able to automatically and safely relay the test results to cloud-based systems, with appropriate maintenance of privacy. These diagnostics tools should have a low to medium price for purchase and use; they should be produced using readily available resources and be simple to produce via traditional automated systems. In order to keep the price per each test minimal, diagnostics should be comprised of three components (i) a disposable single-use element (e.g. paper unit, chip): in which the chemical analysis is conducted (ii) a specialized reader or smartphones used for signal development/reading, and (iii) a smartphone utility/program for signal processing and transfer of test results to the cloud. Diagnostic devices should be designed to carry out testing experiments in whole, untreated biological samples and ideally the disposable element should be completely integrated (i.e. have all the requisite reagents inside them). If it is necessary, an additional step such as applying a drop of a solution (using a dropper) to the tool may be appropriate. However any extra fluid manipulation steps, such as blending and pipetting solutions, may substantially raise the system's difficulty level (based on regulatory authorities' strict criteria) and may, thus, make the tool unsuitable for home use. To obtain the optimal level of user-friendliness and at the same time diagnose a pathological problem, diagnostics manufacturers can carefully select (i) a biological sample that can be conveniently acquired in a minimally invasive manner at home which includes a biomarker of disease and (ii) diagnostic POC technologies which could enable low-cost, simple-to-perform testing of untreated biological fluids.

Developing a new diagnostic tool is a daunting task. It is also very costly; a study revealed that in 2010 the total cost of translation of diagnostic tools from a research idea to market in the US is more than $30 million [126], and the majority of the cost was involved with the device's regulatory approval. In order to improve the chances of having a technology that could transfer from an academic concept to a diagnostic system, scientists and designers may need to consider the following points: (i) a new diagnostic tool must overcome an "actual" problem/diagnostic request, and the current solution to it should be inadequate or expensive. Then they should clearly define the problem and explain why the new diagnostic tool solves the need and contributes to a better result for health (ii) a diagnostic need which affects a large number of patients should be given greater priority than others. (iii) Local regulatory approval is a requirement for the implementation of health devices; it would therefore be helpful if the devices were targeted early in the design process to meet the criteria set by regulatory authorities.

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.

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