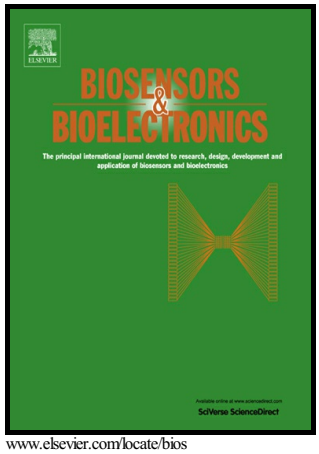

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Shifting paradigm of cancer diagnoses in clinically relevant samples based on miniaturized electrochemical nanobiosensors and microfluidic devices

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Abstract

Cancer is one of leading causes of death in the world and occurs in more than two hundred types according to the National Cancer Institute. Its early diagnosis has been remained a prime focus amongst scientists and clinicians since long, not only to understand the complications but also to mitigate its chance of further proliferation. Nowadays, tremendous advances in nanotechnology-empowered diagnostics are serving a substantial input to identify biomarkers associated with various cancers. These biomarkers are found in different forms including overexpressed proteins/surface antigens, metabolites, miRNA, and the entire cell as well. Several approaches have been adopted to detect such cancer biomarkers, where electrochemical sensors have widely been appreciated due to its high sensitivity, selectivity, robustness, and miniaturized point-of-care cancer diagnostics. Due to its immense importance, the present review has been formulated describing classicle concepts of cancer biomarker discovery followed by the recent status of electrochemical biosensors for cancer diagnoses. Particularly, we have summarized the state-of-the-art technologies based on potentiometric, impedimetric, amperometric, voltammetric biosensors for the detection of different biomarkers viz. protein, miRNA, and whole cell and biomarkers generated by metabolic shift in response to carcinoma population. Apart from these, we have also highlighted different deliverable microfluidics-based approaches and recent prototypes for cancer detection. To put various perceptive insights on the recent advancements in cancer diagnostics, an extended table is incorporated, which includes sensor fabrication strategies, type of biomarkers, detection strategies, and analytical performance of the cancer biosensor since last five years (2013-2017).

Keywords: Cancer; Nanobiosensor; Microfluidics. Electrochemistry; Biomarker; Clinical Diagnosis; Analytical Performance

Introduction

Among all the life-threatening diseases, cancer is one of the most common cause of death worldwide and can exist in about two hundred diverse forms including the cancers of bladder, bone, breast, colon, lung, neuron, ovary, etc (Chandra et al., 2017). Researches have proven that various factors including genetic (viz. hereditary mutations etc.) and environmental (viz. alcohol, radiation, smoke, carcinogenic chemical exposure, etc.) play a crucial role in its fatal progressions (Jemal et al., 2011). Down the line, microorganisms have also been reported as the causative agents in some forms including the gastric and cervical cancers (Cummins and Tangney, 2013). From the minute alteration in molecular level to the tissue level changes takes a prolong duration due to which the appearance of symptoms is much delayed from onset of the disease. In cancer, it is caused due to the lengthy process of malignant growth which comprises the series of cellular events such as; specified genomic alterations in the healthy cell (initiation), tumor formation, and metastasis (Levenson, 2007). Such genomic changes are caused by the mutations in regulatory genes eventually unbalances the rates of altered cell proliferations to apoptotic mass which is a normal process of maintaining the integrities of tissues and organs. Mostly, the mutation occurs in DNA repair system and tumor suppressor genes seed the cancer forms (Brücher and Jamall, 2014; Burnet, 1957). In addition, proto-oncogenes are other factors usually show normal behavior in healthy cellular conditions, which get mutated to form cancerous cells (Fearon and Vogelstein, 1990). For instance, on average fifteen driving mutations and sixty passenger mutations have been reported in the case of colon cancer (Wood et al., 2007)**.** There are various causes associated to such mutations are comprehensively explained elsewhere (Knudson and Strong, 1972; Watson et al., 2013). Initiation is then followed by clonal evolution, proliferation, and eventually metastasis. The stages involve in cancer progression are comprehensively described in **figure 1** (Burnet, 1957).

The early diagnosis of cancer is necessary not only for preventing the spread of malignancy but also to rescue the patients from its fatal severity (Chandra et al., 2017). Nowadays, such

preliminary diagnoses are being pursued by using various high-end sophisticated methods such as X-ray, nuclear magnetic resonance, and ultrasound based imaging; mammography, computed and positron-emission tomography based scanning methods (El-Salam et al., 2013; Taralli et al., 2013). These methods are majorly dependent on the size of the cancerous growth where the threshold size detectable by such techniques is limited to the mass of billion cells which is a quite high number indicating the advanced stages of cancer forms (Selvarajan et al., 2013). Apart from these, several advances in analytical molecular biological techniques such as; polymerase chain reactions, mass spectrometry, chromatography, electrophoresis, and surface plasmon based methods etc. have paved the innovative detections of cancer forms with approximately thousand times lesser cell numbers than the conventional primary diagnoses (Selvarajan et al., 2013). In spite of having major advantages of such improved detections, these demand time, dedicated laboratory space, and skilled personnel for authenticated results. Some other critical concerns appear, especially when it comes to the molecular biology techniques such as; sample processing, handling conditions, and storage time, where the complex nature of human sample may lead to the inadequate results (Diamandis, 2004).

The recent advancements in nanotechnology have empowered miniaturized, onsite, and early diagnoses of cancer in an efficient manner (Selvarajan et al., 2013)**.** Such advancements have paved the clinical aspects of cancer treatment to a new era where the precise and quick estimation of the patient's condition is of prime importance. Electrochemical (EC) techniques owing the better analytical performance and customizability have found inclusive usage in several biosensors and therefore, acclaimed for remarkable acceptance not only by clinicians but also for the daily health monitoring (Chandra, 2016; Zhu et al., 2012a). Inspired by these facts, EC based cancer biosensors have remained a prime focus among the researchers worldwide since last decade. Several literature surveys have also been reported summarizing various aspects of the cancer biosensing (Hasanzadeh et al., 2017; Topkaya et al., 2016; Wang et al., 2017a). However, emphasis on the suitability of various EC biosensors have not discussed in detail. Importantly, information related to cancer progression and opportunities for biomarkers are very critical which may provide valuable knowledge to researchers for designing the translational cancer biosensors. Unfortunately, there are no reports describing the importance / discovery of clinically important cancer biomarkers together with EC biosensors / microfluidics. Therefore, this review has been formulated to address these unaddressed issues, which may be helpful for

wider range of readers. The review comprehensively describes various cancer biomarkers; its genesis in the sub-cellular events, importance and need of these biomarkers in cancer diagnosis. Moreover, this review also discusses about the type of EC biosensors and state-of-the-art microfluidic devices for point-of-care (POC) cancer diagnoses.

1. Types of cancer biomarkers for EC detection

Biomarkers are either chemical or biological moieties, which are usually found in body tissues and fluids (e.g. blood, urine, saliva, etc.) in various clinical conditions exhibiting a genuine real-time indicator of a disease (Group, 2001). In cancer studies, the biomarkers usually come from cellular, sub-cellular or supramolecular origin which are either the products or by-products of various intracellular events i.e. mutation in genomic level, anomalies in transcriptional or post-transcriptional level, etc. **Figure 2** shows various opportunities of utilizing the cancer biomarkers produced in the process of carcinogenesis for therapeutics, diagnostics, and prognostics (Bhatt et al., 2010). In fact, such biomarkers play a significant role in judging the explicit instantaneous physiological state of a human body (Chandra, 2016; Ueda, 2013). In clinical diagnoses, these are generally obtained from embryonic tissues and tumors (Sedgwick and D'Souza-Schorey, 2016). Furthermore, circulating tumor cells (CTCs) have also been used as a biomarker, which usually migrate from primary malignant tissue to the various other body parts via circulation of body fluids leads the secondary malignant tumors (Alix-Panabieres and Pantel, 2014). These are exceedingly rare in blood, found one cell per 10^9 hematologic cell counts during early metastasis. Thus, the whole cell based detections are not only tedious but also full of irrationalities due to its tremendous technical challenges (Nagrath et al., 2007). Further, advances in molecular techniques found another set of biomarker known as micro ribonucleic acid (miRNA), which has also been studied for the functional significance not only in regular biological processes but also in various human diseases including cancer (Peng et al., 2016). The miRNAs are found in body fluids such as plasma, serum, urine, etc. and possesses various properties viz. temperature resistance, tolerance to extreme pH, and resistance to ribonuclease activities. So far, some tissue and organ-specific biomarkers are reported, few of them are shown in **figure 3**. In conventional clinical approaches, the diagnosis of cancer is based on biopsy and traditional genomic and proteomic methods where biomarkers play an important

role (Fredriksson et al., 2007; Zhang et al., 2016). In clinical contexts, the confirmatory diagnoses are reliant on the particular biomarker or the set of biomarkers depending on the origin of tumors, some of them are depicted in **figure 3 (blue-boxes).** In addition to this, various multitudinous complications in cancer sometimes introduce a gray area in their diagnoses, which is essentially due to the existence of different forms and stages. The overlap of symptoms, origin, and position of the tumor often misguide the effective diagnoses. To overcome such associated discrepancies, biomarker-based diagnoses have greatly been acclaimed in contemporary medicine and are usually categorized under proteinaceous, miRNA, and whole cell based indicators (Tiss et al., 2013). Apart from these, specific immune responses or metabolic changes due to the presence of altered cells have also been considered as biomarkers (Chandra et al., 2017; Wu and Qu, 2015). The fundamental doctrine of detecting the overexpressed proteins on the cell surface, whole cell, and miRNA is to facilitate the diagnosis of the different type of cancer, whereas targeting the small molecules of metabolic by-products, or immune responses give indirect way of analyzing the proliferation of cancer. This section describes various type of biomarkers usually used for precise cancer diagnosis.

1.1. Proteins / surface antigens

Proteins are one of the building blocks of structural and functional unit of life, maintaining various cellular and subcellular functions such as; vital syntheses, energy generation, cell signaling, replication, and cellular regulation (Perutz, 1962). In addition, the synchronous activities of cells and tissues are also controlled by the major mass of proteins. Such moieties also involve in various regulatory pathways employing the healthy proliferation of cells. In some cases, mutations in such genes turn on the unconstrained escalation of cells transforming a cancerous mass (Greenblatt et al., 1994). Apart from this, sometimes epigenetic alterations also introduce the mutations in proteins which may lead to cancer. At the cellular level, the altered proteins are also expressed on the cell surface which is also considered as a biomarker for cytosensing e.g. human epidermal growth factor receptor 2 (HER2), epithelial cell adhesion molecule (EpCAM) etc. Also, various correlational studies have shown that the occurrence of cancer forms are closely related to the unusual expression level of cellular proteins (Thompson et al., 1991; Zhu et al., 2013). Moreover, serum protein biomarkers have also found considerable attention due to the ease of testing and specificity. Availability of such biomarkers in body fluids preferably in blood has a specific range in the case of a healthy individual, where on the other

side, the patient has either elevated or depleted levels depending on the expression profile. For example, carcinoembryonic antigen (CEA) is an immunoglobulin, present in the serum of < 2.5 ng mL^{-1} in an adult non-smoker, whereas in the case of smoker adult it has been reported 5.0 ng mL^{-1}. In carcinoma cancer patients such as colorectal, pancreas, stomach, breast, lung, thyroid and ovarian cancers have much higher CEA level (over 20 ng mL^{-1}) (Websource, 2015).

1.2. The miRNA

The miRNA is a short RNA sequence (~ 22 nucleotides) transcribed from non-coding genes found in various forms in diseased or healthy conditions (Calin et al., 2002). Since the last couple of decades, enormous researches have been carried out to explore their physiological functions such as; regulation of target genes, cellular differentiation, proliferation, apoptosis, cell death as well as various translational and transcriptional regulations (Esquela-Kerscher and Slack, 2006). Apart from the physiological roles, miRNAs have also been recognized in human diseases including different cancer forms (He et al., 2005). For instance, miR-155 and miR-21 were reported to be upregulated while miR- 91 was found to be downregulated in the case of breast cancer. Moreover, the miR-145 is reported as a tumor suppressor, which was found in low level in the various type of cancer of diverse origin including colon, ovary, bladder, breast, and prostate (Larrea et al., 2016). Although, cancer detection using miRNA is in its nascent stage, but has a great potential in noninvasive diagnostics due to its resistance to ribonuclease activity and organ specificity (Ren et al., 2015).

1.3. CTCs

CTCs are cancerous cells found in body fluids (mostly reported in the blood) originates during the metastasis of the malignant cells from the primary tumor, thus are considered as the primary biomarker of malignancy. These are, however, exceedingly rare even in blood, having few cells in the count, a range of 1-10 cells mL-1of the blood (Contributors, 2017; Miller et al., 2010). The variation of range depends on the stages and forms of cancer, where the higher counts have been correlated with the aggression of the disease, increased metastasis, and decreased time to relapse (Chaffer and Weinberg, 2011). In cancer diagnosis, CTCs indeed play a vital role as the authentic confirmatory biomarker, but are not so easy to realize due to tedious isolation processes. These are due to the fact that different type of cancers have their own form of CTCs which possess a range of surface antigens (van de Stolpe and den Toonder, 2014). Thus, requires

a dedicated immunoassays for isolation and their detection. In clinical prospect, CTCs are not only limited by the specificity but also restricted by the available diagnostic tools. However, preliminary qualitative detection of metastasis can certainly be authenticated by positive test for CTCs (Alix-Panabières and Pantel, 2013).

1.4. Other biomarkers

Several other common indicators of cancer have also been reported including nuclease enzymes and volatile organic molecules. One of such indicator is telomerase which is a specialized ribonucleoprotein polymerase containing an integral RNA with a short template element that guides the synthesis of telomeric repeats at the end of chromosomes to protect the degradation, recombination, and fusion of chromosome (Wu and Qu, 2015). In cancer cells, the expression pattern of telomerases is found different than the healthy cells, which make telomerase a promising biomarker for early diagnosis and prognosis of cancer (Hirano et al., 1998; Yoshida et al., 1998). Furthermore, the enzyme activities in tissues, serum, blood, and isoenzyme changes with malignancy suggests that such altered activities can also be used as an effective indicator for a particular type of cancer diagnosis. So far, in clinical contexts, the large molecules such as protein, miRNA, and cell have been targeted, however, various other small molecules appear in cancerous environments due to the changes in pathophysiological states. In a study, it has been reported that the volatile organic content of breathing in lung cancer patient is different from a healthy individual due to the difference in metabolism (Haick et al., 2014). These accounts such nonconventional biomarkers carry enormous potentials for easy, noninvasive, and efficient diagnostic, but unfortunately are not well understood in terms of specificity of the cancer forms.

2. Cancer diagnostics: a shifting paradigm towards miniaturization

Most commonly used biomolecular analytical methods in cancer diagnosis involve the invasive excisional biopsy, which possesses various limitations in understanding the dynamics of cancer progression and metastasis, therefore, these are not always preferred (Marrinucci et al., 2009). Alternatively, the more informative technique liquid based biopsies suffer for less sensitivity. Furthermore, commonly used enzyme-linked immunosorbent assay (ELISA) are time-consuming, non-robust process shows incompatibility to POC applications. Hence, an efficient, robust, handy, and ultrasensitive detection technique for cancer diagnoses is of

clinically high demand and has tremendous commercial interest. Focus on cross-disciplinary researches has shifted the paradigm of such conventional processes into the new class of analyses using nano-biomolecular diagnostics (also called as biosensors or nanobiosensors). These devices have been found a considerable attention since recent past for the recognition of various clinically relevant biomolecules including metabolites, cancer biomarkers, CTCs, miRNA, etc. (Chandra et al., 2017; Smith et al., 2014; Tothill, 2009). The importance of such devices are not only limited to academic purpose, but it has also accounted incredible commercial attention. In a recent research survey done by Persistence Market Research, USA in July 2015 forecasts the global market of biosensors in Asia-Pacific to be US$ 22,551.2 million until 2020. Thus, it is evident that such diagnostic technologies have tremendous promises not only on the commercial side but also in terms of applicability. Among all forms of nano-biosensors, EC based biosensors are widely accepted due to its high sensitivity, robustness, specificity, portability, and wider economic feasibilities, which uses various nanomaterials / nanocomposites (nanoparticles, carbon nanotubes (CNT), graphene, quantum dots, conducting polymer matrices etc.) and biological receptors (enzyme, antibody, half antibody fragment, peptide, aptamer etc.) for the biomolecular analyses (Agrawal et al., 2013; Yadav et al., 2014; Zhu et al., 2013). The integration of microfluidic technologies has further miniaturized these biosensors allowing it for various onsite analyses. Nowadays, such integrations have tremendously been employed to the EC biosensors which not only provides remarkable analytical performances but also empowers the simplified user-friendly detection (Chandra, 2016).

3. EC biosensors

EC biosensors have been comprehensively studied more than fifty years back when Clark and Lyons introduced the pioneering work of enzyme electrode having immobilized glucose oxidase (Clark and Lyons, 1962). Springs Instruments (Ohio, USA) manufactured the first commercial biosensor in 1975 for glucose detection in diabetic patient's blood samples based on amperometric detection of hydrogen peroxide (Newman and Turner, 2005). At present, there are number of commercially available biosensing device for pathogens and toxins (Kumar and Rani, 2013; Pohanka et al., 2007). A classical EC biosensor comprised of three electrode system viz. working, auxiliary, and reference electrodes, where the bio-functionalized working electrode or probe is coupled with the physicochemical transducer that facilitates electron mobility to obtain

the quantifiable signals (Chandra et al., 2015; Chandra et al., 2013b; Noh et al., 2012b; Zhu et al., 2012b). There are several advantages of EC biosensors over the other forms comprising enormous capabilities of detections without being affected by the matrix interferences which usually appears in the presence of fluorophore, chromophore, or any other interfering entities that commonly affect optical detections (Chandra, 2016; Mahato et al., 2017). That adds the credibility to EC biosensors for sensing the analyte even in colored and turbid real samples viz. blood, urine, serum, sweat, tears, saliva etc. EC biosensors use different transducers including potentiometric (potential measurement), amperometric (current measurement), impedimetric (measurement of charge transfer resistance), and voltammetric to convert the generated biochemical information into a measurable digital signal (Ronkainen et al., 2010).

3.1. **Potentiometric biosensor based cancer diagnostics**

Potentiometric techniques are based on the measurement of accumulated charge potential on the working electrode when no current flows through the EC cell assembly. These biosensors are widely used to detect various analytes including proteins, metabolites, and a number of electroactive species. Recent advancements in potentiometric techniques have added the suitability of label-free detection of ultralow concentration of biochemical species (Liang et al., 2017). It offers low detection limit (DL) (between 10^{-8} and 10^{-11} M) which is an important for cancer detection as the concentration of biomarkers are very low in the early stages (Grieshaber et al., 2008). So far, various potentiometric techniques have been utilized to detect cancer biomarkers. For example, Jia and coworkers have reported a light-addressable potentiometric sensor (LAPS) for the detection of human phosphatase of regenerating liver-3 (hPRL-3), a prognostic biomarker of cancer. In this work, hPRL-3 and mammary adenocarcinoma cells (MDAMB231) have been detected with linear range (LR) of 0.04 - 400 nM and 0 - 10^5 cells mL^{-1}, respectively (Jia et al., 2007). In another work, CEA was detected from CEA-producing LoVo human colon cancer cells using surface molecular imprinted self-assembled monolayers (SAM) of hydroxyl-terminated alkanethiol and template biomolecules on gold-coated silicon chip as bio-recognition element for a potentiometric biosensor with reported LR of 2.5 - 250 ng mL^{-1} (Wang et al., 2010). In malignant pleural mesotheliomas (MPM) the overexpressed proteinaceous biomarker, hyaluronan-linked protein 1 (HAPLN1) has also been targeted for using label-free potentiometric detection and achieved the DL in pM range with a quick response

time of 2-5 minutes in real sample (Mathur et al., 2013). Over the time, potentiometric biosensors have also been designed for the detection of other cancer biomarkers including miRNA and whole tumor cells or cluster. Since miRNA possess several advantages over the proteinaceous biomarkers including thermal stability and availability in almost all types of body fluids, they have been exploited for non-invasive detections. Several studies are done on miRNA detection using potentiometric techniques. For instance, in a study carried by Goda and coworkers, a hybridization-based potentiometric microarray was developed to detect the exosomal miRNA (Goda et al., 2012). Furthermore, cancerous cells have also been targeted using potentiometric techniques to study the electrochemistry of proximal micro-environments, since the cancerous cells usually release lactate which introduces the fluctuation of pH of media. Based on this concept, Shaibani and coworkers targeted cancer cells (MDA MB231) with the DL of 10^3 cells mL^{-1}. Also, this work confirms changes in pH flux surrounding the neoplasm consisting cancer cells which infers the correlation to the metabolism of altered cells **(shown in figure 4(i))** (Shaibani et al., 2017). Similarly, anti-EpCAM functionalized graphene oxide potentiometric biosensor was developed for the selective detection of CTCs of prostate cancer using the LAPS technique (Gu et al., 2015). Thus, potentiometric detections can be adopted as the prospective diagnostic strategies for carcinomas which exploits the extracellular environment.Although the detection based on this technique are able to give accurate estimation of the presence of living carcinoma cell, but will certainly be limited in case of the other instances such as miRNA, protein, and dead carcinoma cell in real sample due to the prominent interferences introduced by coexisting compounds in test sample.

3.2. Impedimetric biosensors based cancer diagnostics

The existing cancer biosensors are generally proteins, miRNA, and cells that are usually non-conducting in nature. Therefore, when these moieties come into the electrical circuit, introduces resistance that has been utilized to design electrochemical impedance spectroscopy (EIS) based biosensor. So far, using EIS techniques, sensitive detection of several chemical and biochemical reactions have been achieved (Chang and Park, 2010). Similar to the other EC biosensors, it is also performed using three or two electrode systems where the working electrode is functionalized with various receptors for the selective detection. Due to its simplistic nature, these techniques are widely being expanded to monitor the enzyme catalyzed reactions as well as biomolecular recognition events for proteins, aptamers, lectins, receptors, nucleic acids, whole

cells, antibodies, and antibody binding fragments (Carvalho et al., 2014). Furthermore, EIS has been utilized for cancer detection by targeting either whole cancer cell or associated biomarkers (Cho et al., 2009; Zhang et al., 2017). Using impedimetric detection, so far various trials have been taken for the detection of cancer forms such as; breast, bladder, ovarian, bone, glial cancer etc. targeting different analytes mainly comprising serum protein, cell surface antigens, miRNAs, and the whole cells (Chandra, 2016). Protein biomarkers are easy to detect by the probe using affinity-binding properties with anti-analyte, which are immobilized over the electrode surface. In a study, vascular endothelial growth factor (VEGF) overexpression has been detected in wide range of carcinomas of breast, bladder, and ovarian epithelia by Sezginturk et al. In this study, EIS technique was used for the detection of VEGF and reported with a wide LR of 10-70 pg mL^{-1} in human serum samples (Sezginturk, 2011). Similarly, murine double minute 2 (MDM2), another most abundant biomarker found in gastric, colon, lymphocytic leukemia etc. has also been detected using EIS techniques (Elshafey et al., 2013b; Okamoto et al., 2015). For instance, Elshafey et al. have reported cysteamine SAM based technique to detect MDM2 with LR and DL of 1-10^6 pg mL^{-1} and 0.29 pg mL^{-1}, respectively in PBS, whereas it has shown a remarkably comparable DL of 1.3 pg mL^{-1} in homogenate sample of mouse brain tissues (Elshafey et al., 2013b). The antibodies used are susceptible to the temperature and constrained to various environmental conditions. Thus, the functionalities of these bio-recognition elements are not reliable for a wide range of temperature. To achieve thermostability, another type of bioreceptor known as aptamer is introduced which is comparable to the antibodies in terms of specificity. Using aptamers, several cancer biosensors have been developed in recent times. For example, Feng et al. have reported an impedimetric aptasensor using a clinically recognized aptamer AS1411, which shows specificity towards nucleolin, an overexpressed surface protein on cancer cells with the reported LR and DL of 10^3 - 10^6 cells mL^{-1} and 794 cells mL^{-1}, respectively (Feng et al., 2011). Furthermore, EIS techniques have also been used for the detection of cancer by targeting the whole cell. The most common approach for detecting the cancer cells is to target the surface antigens that are not innately found in high concentration over such cells. The principal advantage of targeting such moieties is that one can achieve higher selectivity. For example, a EIS based biosensor has been developed by Chandra et al. which was not only able to discriminate the cancerous cells from the healthy ones but also precisely detected various cancerous cells viz. Hela, MCF-7, HT-29, and SKBr-3 in real samples, providing a universal

method of cytosensing (Chandra et al., 2013a). In another example, a bi-functional impedimetric biosensor for detecting prostate-specific membrane antigen [PSMA (+) and PSMA (-)] have been reported by Min and coworkers using RNA/peptide dual-aptamer probe (Min et al., 2010). The miRNAs for various cancer forms have also been targeted to overcome biopsy like painful process, as these are easily available in various body fluids. So far, reported miRNA-based biosensing mechanisms are mostly relying on the hybridization assay based techniques (Zhou et al., 2016). For example, a miRNA-based impedimetric biosensing prototype has been reported where the capture probe was hybridized by–miRNA (miR-21) in a dual-mode detection mechanisms, where the recognition and amplification occurred concurrently. This technique has shown a wide LR of 1-1000 pM and remarkable DL of 0.3 pM. The detailed scheme of this prototype has been shown in **figure 4(ii)** (Azzouzi et al., 2017). Although miRNA-based biosensing techniques give ease in various aspects over the conventional biomarkers and cell-based approaches; several limitations are also associated, which include the usage of expensive and corrosive chemicals for electrode modification, requires more time, and several issues with the reproducibility. (Kilic et al., 2012). Although, the enormous developments of EIS based prototypes for cancer detection have been reported, but the realization for signal amplification and trace analysis are still challenging because the real samples can significantly introduce matrix interferences and contribute to non-specific impedances.

3.3. Amperometric biosensor based cancer diagnostics

Amperometric biosensors are another class of EC analyzers which are widely used for cancerous biomarker detection where the signals are greatly amplified and transduced by conjugated bio-recognition / reporter elements of either enzymatic or non-enzymatic origin (Chandra et al., 2015). For cancer detection, the amperometric techniques have widely been employed targeting various protein biomarkers. For instance, Annexin II and MUC5AC, the profound diagnostic biomarkers in lung cancer have been detected by Kim and coworkers using amperometric immunosensing methods with the DL of 280 ± 8.0 pg mL^{-1} (Kim et al., 2009). For this amperometric sensor, the probe was fabricated using antibody-functionalized gold nanoparticles (AuNPs) doped with dendrimers composite material. An overexpressed protein found in the head and neck squamous carcinoma cells, interleukin 8 (IL-8) has been detected using HRP-ELISA coupled amperometric technique, which has been reported with the 30000 fold improved DL (1 fg mL^{-1}) in a clinical serum sample. Although, a number of amperometric

prototypes have been reported showing several advantages such as; greater selectivity, sensitivity, etc. yet these are not remained untouched by the limitations, which includes the enzyme dependency that makes them vulnerable for the usage in the wide range of temperature and pH. Moreover, the presence of thermally unstable antibodies as a bio-recognition element, competitive molecules in the sample and intrinsic properties of enzymes also attributes vulnerabilities in some extent (Gessei et al., 2015; Weinryb, 1966). To overcome such limitations, various groups have been working for strengthening the capabilities of amperometric biosensors. One class of thoughts follow after the environmentally benign prototypes while another class is seeking for a novel exclusive and explicit biomarker for existing formats. Continuing with the former, the thermo-labile enzymatic bio-recognition elements are now constantly being replaced with nano-catalysts and aptamers enabling the biosensors operable in the wide range of temperature and pH (Chandra et al., 2010; Prasad et al., 2016). While later hunts for the biomarkers found in various bio-fluids such as miRNA, CTCs, and other small molecules. This class is not only seeking for the novel biomarker discovery but also finding the non-invasive way of detection which adds the various possibilities of POC products (Chandra, 2016; Mahato et al., 2016). For example, a selective and sensitive biosensing prototype based on graphene nanocomposite with functionalized AuNPs has been reported for the detection of human cervical cancer biomarker miR-21 (Zhang et al., 2014). Signals were obtained by the help of locked hairpin DNA and amplified by using HRP tags, eventually offering LR and DL of 1-5000 nanomol mL^{-1} and 0.4 nanomol mL^{-1}, respectively (Zhang et al., 2014). In another example, nanostructured probes composed of tetrahedral DNA based on hybridization chain reaction amplification has been developed to detect miR-122b with DL of 10 aM. Furthermore, the capture DNA is also employed for molecular recognition of miRNA to form molecular beacons (MB) (Cai et al., 2013). For instance, a miRNA-based novel biosensor has been developed using label-free functional allosteric MB where the HRP system was used for the signal amplification. In this model, the HRP-coupled 3,3',5,5'-Tetramethylbenzidine (TMB) played an important role in electron transfer to detect let-7a miRNA with the LR and DL of 100-100000 pM and 13.6 aM, respectively (Cai et al., 2013). The CTCs are also targeted using amperometric techniques; for example, Moscovici et al. have reported rapid CTC detection, where circulating prostate cancer cells were detected using gold array electrodes (Moscovici et al., 2013). In another work, Chandra et al. have developed an amperometric biosensor for the detection of cancer cells using

anti-permeability glycoprotein functionalized nanoprobe, which has shown LR and DL of 50 and 100,000 cells mL^{-1} and 23±2 cells mL^{-1}, respectively (**figure 4(iii)**) (Chandra et al., 2015). Apart from these, over the years various physiological and pathological markers related to cancer have also been detected using amperometric biosensors (Chandra et al., 2012; Koh et al., 2011; Sharafeldin et al., 2017; Tang and Ma, 2017).

3.4. **Voltammetric biosensor based cancer diagnostics**

Voltammetry is potentiodynamic techniques where current is monitored as a function of biomarker concentrations. It has also been widely utilized for cancer biomarkers detection in numerous matrices. For cancer detection, various voltammetric methods i.e. cyclic voltammetry (CV), linear sweep voltammetry (LSV), differential pulse voltammetry (DPV), square-wave voltammetry (SWV), stripping voltammetry (SV) have been adapted; however, SWV, SV, and DPV are often used due to their high sensitivity (Chandra, 2016). The voltammetric techniques have been used to detect numerous cancer biomarkers including, IL-10, HER2, Osteopontin (OPN), HEp-2, HT29, PSA, CA153, HCT, etc. (Hasanzadeh et al., 2017; Raji et al., 2015). For example, Ramgir et al. have reported lung cancer detection by using CV targeting the IL-10 with the DL of 1 fg mL^{-1} and 1 pg mL^{-1} in ideal and serum sample, respectively. (Ramgir et al., 2007). In another example, an oral cancer biomarker CYFRA-21-1 has been targeted using DPV technique with the LR of 0.01-29 ng mL^{-1} and remarkable DL of 0.01 ng mL^{-1}. This prototype has been fabricated using nanostructured zirconia nanoparticles, showing a quick response time of six minutes in clinical saliva sample (Kumar et al., 2016). In addition to this, various other protein-based biomarkers have been targeted for the detection of different cancer forms including oral, prostate, gastrointestinal, glial, skin, lungs, etc (Kokkinos and Economou, 2017; Parnsubsakul et al., 2017). Several groups have also attempted cancerous cell detection using voltammetric techniques. For instance, a real-time EC detection of liver cancer cell HepG2 has been reported using CV which has targeted the overexpressed surface protein CD-133 (Damiati et al., 2017). The alteration of glyco-signatures of the proteins on cell surfaces is very commonly found in the diseased cases, which were also targeted to identify the cancerous cells (Kim and Varki, 1997). For instance, Dai et al. have reported EC based biosensor for detecting the surface sugar using DPV technique with the LR and DL of 1 pg mL^{-1} - 100 ng mL^{-1} and 0.57 pg mL^{-1}, respectively (You et al., 2017). Another bifunctional cancer cytosensor has been developed by

Zhu et al. which not only target HER-2 protein in human serum samples but also detects HER-2 overexpressed breast bourn malignancies using SV technique. In this biosensor, the sensing probe was fabricated using anti-HER-2 functionalized self-assembled 2,5-bis(2-thienyl)-1H-pyrrole-1-(p-benzoic acid) on AuNPs, which has not only shown excellent analytical performances but also remarkably differentiates the cancerous cells from normal ones (Zhu et al., 2013). Furthermore, voltammetry is also applied to detect miRNAs related to various cancers. For instance, an ultrasensitive detection of miR- 21 has been reported by Zhang et al. where the characterization of the biosensing probe was done using SWV with LR and DL of 1 pM - 25 nM and 0.6 pM, respectively (Zhang et al., 2015b). In another work, the same biomarker miR-21 has been targeted using voltammetric techniques in spiked serum sample and reported LR and DL of 10×10^{-15} - 1×10^{-9} M and 4×10^{-15} M, respectively. Here the probe was fabricated using biotin-MB-AuNPs labeled neutravidin electrode (**figure 4(iv**)) (Fredj et al., 2017).

4. Cancer biomarker detection using EC microfluidic technology

In recent years, the EC biosensors are coupled with various microfluidic chip for several reasons, including the precise sample fluid handling which provides user friendly miniaturized POC diagnoses (Chandra, 2016; Chandra et al., 2017). In microfluidics, small-scale properties of tiny dimensional channels are exploited to achieve efficient and controlled fluid movements. It encompasses the domain knowledge of various fields including engineering, physics, chemistry, biochemistry, nano-biotechnology etc. in order to resolve the real-time practical problems (Morton and Bridle, 2016). Microfluidics provide multiplexing, high-throughput, and robotic integration to these POC devices, facilitating tremendous applications in various kind of sample analysis viz. surface water, blood, saliva, urine etc (Chandra, 2013; Chandra et al., 2011a; Noh et al., 2012a). In recent years, these microfluidic-based prototypes have been found a great attention for the detection of various cancer biomarkers as well as whole cell (Bunyakul and Baeumner, 2015) coupled with the several existing strategies such as; colorimetric (Yu et al., 2009), fluorescence (Hu et al., 2010), chemiluminescence (Wang et al., 2012), electrochemiluminescence (Sardesai et al., 2013), and EC assay (Su et al., 2014). Among all, EC based assays are considered to have the most sensitive and translational compatibility. So far, various microfluidic EC prototypes have been proposed for cancer detection targeting several biomarkers. An elastomer, polydimethylsiloxane (PDMS) based machine assembled micro-channels have been developed to detect PSA and IL-6 offering DL of 0.23 pg mL^{-1} and 0.30 pg

mL^{-} for PSA and IL-6, respectively (Chikkaveeraiah et al., 2011). Similarly, a PDMS based microchip has been developed by Tang and coworkers for the high-throughput multiplexed detection for biomarkers including PSA, PSMA, platelet factor-4 (PF-4), and IL-6 (detailed scheme has been shown in **figure 5 (i)**) (Tang et al., 2016). This biosensor is based on the HRP coupled immunoassay where the HRP-labeled superparamagnetic particle and antibodies were used as detector-antibody immobilized on eight-electrode system based quantifying chip. The analytical amperometric signals were obtained by the injection of HRP-substrate H_2O_2 offering the DL of 0.23 pg mL^{-1} and 0.30 pg mL^{-1} for PSA and IL-6, respectively. In addition, the detection time for this prototype was 1.15 hours, which is reasonable for the clinical POC devices. The validation of the method was also done using ELISA-based technique. Using the similar approach, a multiplexed amperometric microfluidic sensor has been designed by Malhotra et al. for the simultaneous detection of oral cancer biomarkers (viz. IL-6, IL-8, VEGF, and VEGF-C) which offers ultra-low DL between 5 and 50 fg mL^{-1} in serum samples within 50 minutes (Malhotra et al., 2012). Nowadays, inkjet fabrication process is adopted for the efficient use of raw materials facilitating low-cost fabrication technology. An example of such fabrication technique has been reported by Krause et al. where the nanoimmunosensor was developed to detect IL-6 and IL-8 with the detection time of 8 minutes (Krause et al., 2013). The clinical potential of this sensor was evaluated using head and neck squamous carcinoma cells. The fabrication of EC microfluidic system is not only limited to the detection of cancer biomarkers, but it has also been applied to detect various cancerous cells. Recently, an in situ microfluidic EC-ELISA for cancer cells detection has been reported where CTC was detected by using anti-EpCAM tagged magnetic nanoparticles immobilized in a microfluidic device (Safaei et al., 2015). In the next step, the captured CTCs were treated with biotinylated anti-CK18 and streptavidin-ALP conjugate which then provided the analytical signal converting p-aminophenyl phosphate to p-aminophenol, an electrochemically active molecule, where the signals were proportional to the captured CTC counts. The detailed scheme of this integrated sensor system has been shown in **figure 5(ii**) (Safaei et al., 2015).

Another class of emerged microfluidic system for cancer detection is microfluidic paper based analytical device (μPAD) which is considered to be simple, user-friendly, cost effective, disposable, and has ability to be applied for instrument free onsite total analysis (Mahato et al., 2017; Martinez et al., 2010). Wu and coworkers for the first time developed μPAD for the

multiplex detection of AFP, CEA, CA125, and CA153 by integrating a signal amplification strategy using graphene (Wu et al., 2013). The reporter antibody and signal producing HRP enzyme were co-immobilized onto monodispersed SiO_2 nanoparticles. A precise, quick, and cheap POC detection of four cancer biomarkers were demonstrated by a photoresist-patterned μPAD as shown in **figure 5(iii)**. The analytical signals were obtained using a standard HRP-O-phenylenediamine-H_2O_2 EC detection system and the DL of AFP, CEA, CA125, and CA153 were found to be 0.001, 0.005, 0.001, and 0.005 ng mL^{-1}, respectively. Furthermore, the real clinical application of developed μPAD was demonstrated by detecting these biomarkers in the serum samples of cancer patients. The interesting feature of this μPAD was its regeneration by incubating it with 0.1 M glycine-HCl (pH 2.2) which dissociates the antigen-antibody complex. Due to the regeneration step, the μPAD was reused for 24 times without any apparent loss in its analytical performance (Wu et al., 2013). The use of aptamers has also offered significantly stable, cost effective, and reusable sensor platform compared to other bioassays (Chandra et al., 2011b). In one such example, Tian et al. have developed a paper based lung cancer detection prototype which is capable of delivering excellent detection performances with the LR and DL of 0.5 -500.0 nM and 0.167 nM, respectively (Tian et al., 2017). This prototype qualifies for the low-cost POC cancer diagnostics as it delivers quick saliva analysis. Furthermore, Su and coworkers, for the first time developed a simple μPAD for the detection of the cancer cell with high sensitivity using aptamers (Su et al., 2014). In addition, the aptamer has been utilized in another work for the detection of human acute promyelocytic leukemia cells (HL-60) (Sefah et al., 2009). Thus, it has been used as a model for microfluidic paper-based EC cyto-device (μPECD). In this μPECD, a three-dimensional biocompatible macroporous Au-paper (Au-PE) was modified with aptamers to construct the working electrode for efficient capturing of cancer cells (HL-60) with the LR and DL of 5.0×10^2 to 7.5×10^7 cells mL^{-1} and 350 cells mL^{-1}, respectively. The abundance of foliate receptor on the cancer cell surface was exploited to design the sensing probe (Krystofiak et al., 2013; Su et al., 2014).

A microfluidic EC biosensor has also been applied for miRNA detection. For instance, a study performed by Bettazzi and coworkers, a biotinylated DNA detector probe which was attached on streptavidin layered paramagnetic beads has been developed to detect miRNA (Bettazzi et al., 2013). The miRNA obtained from cell samples were biotinylated followed by its hybridization with the detector probe. The complete hybrid was incubated with streptavidin-ALP

and treated using an adequate enzyme substrate to obtain the EC signal for miRNA detection. This proof-of-concept methodology was also examined in a microfluidic device for multiplex detection of miRNA from various cancer cell lines (Human non-small-cell lung cancer Calu-1 cell line, glioblastoma (U87MG and T98G), and human non-small-cell lung cancer (H460) cell lines) with DL of 7 pM. In another example, miR-210 was detected by combining microfluidics and MB-supported isothermal circular-strand-displacement polymerization (details has been shown in **figure 5 (iv))** (Giuffrida et al., 2015). The method was able to discriminate between target from the nonspecific and single mismatched sequences. The reported DL of this method was 3.3×10^{-18} mole miRNA in 20 nL droplets. The applicability of the developed method was validated by detecting miR-210 in K562 leukemia cells.

5. Conclusions and future prospects

In this review, we have been inclined to address the detailed picturesque of cancer diagnoses, from the genesis of biomarkers in various cellular or subcellular events to the designing of miniaturized EC biosensors. Herein, we have compiled the illustrative figures that summarize the origin of cancer biomarkers generated at the various levels including genomic, transcriptomic, and post-transcriptomic levels. To put more insights, we have added the tissue and organ specific biomarkers details. We have summarized the EC sensors with the compilation of various illustrative prototypes and proceedings. We have also assessed the performances of sensors with different parameters including, suitability, sensitivity, selectivity, LR, and DL for different biomarkers family viz. cancer protein biomarker/surface antigens, cells, miRNAs etc. (examples are shown in **Table 1(a-b**)). In **Table 1(c),** several recent microfluidic prototypes have been compiled to add insights of the emerging microfluidic cancer biosensors. Microfluidic platforms offer several advantages over conventional bioanalytical approaches as they require low sample volume, supports portability, and are easy to use. However, these prototypes are in their nascent stage; thus, extensive researches are required to shape up with commercial viabilities. Moreover, despite various available strategies for signal amplification, cancer detection and estimation are still a challenging domain and required to exercise more rigorously to find the improved and rational way for cancer diagnostics. Thus, more stimulating works are desired from the close collaboration and knowledge exchange between biotechnologist, material scientist, clinicians, engineers, nanotechnologist to translate the existing diagnostic devices for

making personalized POC based cancer diagnostic device. This review may induce great impact on strategies of development of cancer detection techniques, post-treatment monitoring, and personalized health care.

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6. References

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Figure and table captions

Fig 1: Schematic representation of various steps of the cancer proliferation

Fig.2. Line diagram showing of different steps of the central dogma in carcinogenesis and the opportunities of utilization of the different bio-markers for the cancer diagnoses [adopted from (Bhatt et al., 2010)]

Fig.3. Schematic representation of various cancer biomarkers available for the diagnoses

Fig.4. (i) schematic representation of cancer cell detection using light addressable potentiometric technique, where the detection has been employed by fluctuation in pH in presence of live cancer cells, reprinted with permission from Shaibani et al. copyright (2017) American Chemical Society; **(ii)** miRNA based cancer detection using dual functional recognition/amplification

based impidimetric technique, reprinted with the permission Azzouzi et al. copyright (2017) Elsevier Inc.; **(iii)** cancer cell detection using the amperometric technique where overexpressed surface antigen has been targeted, reprinted with the permission of Chandra et al. copyright (2015) Elsevier Inc.; **(iv)** miRNA based cancer detection using voltammetric technique (A) shows the design of biosensor and (B) shows working principle of the probe, permission of Fredj et al. copyright (2017) Elsevier Inc.

Fig.5. (i) PDMS based EC multiplexed microfluidic device of cancer cell detection (A-D) describes fabrication and components of the chip (E-F) shows the multiplexing strategy adopted for the simultaneous detection of multiple biomarkers (G) describes the detection processes, reprinted with the permission Tang et al. copyright (2016) American Chemical Society; **(ii)** EC detection of EpCAM based cancer cell detection, where (A) shows the chip design (B) simulated data for the design of microfluidic features (C) the micrograph of features (D) fabricated chip, and (E) working principle, reprinted with the permission Safai et al. copyright (2015), American chemical society; **(iii)** pictorial representation of EC μPAD for the CEA detection, reprinted with permission from Wu et al. Copyright (2013) American Chemical Society; **(iv)** miRNA based cancer detection using PDMS based microfluidic platform, reprinted with the permission from Giuffrida et al. copyright (2015) Springer.

Table 1. (a) Cancer biosensor for detection of protein and nucleotide, (b) cancer cells, and (c) microfluidic biosensing prototypes for cancer diagnosis. NR = not reported

Sensor Design Specifications	Target	Detection Technique	Analytical Performance		Real Sample	Reference
			LR	DL		
Sandwich mechanism is followed for the detection of target molecule "CEA" a carcinogenic antigen found in various carcinomas CEA is sandwiched by thiol-terminated aptamer 1 (attached to the electrode surface) and thiol terminated aptamer 2 and hexenthiol	CEA	DPV	1 to 200 ng ml^{-1}	0.5 ng ml^{-1}	Serum	(Shu et al., 2013)

(Fc) **Excellent sensitivity and good stability towards the target analytes**						
Reported the detection of hydrogen peroxide released from human breast cancer cells thereby estimation of the cancer cells **Sequence specific peptide was used for the detection** **Usage can be in physiological and pathological detections**	Extracellular H_2O_2	Chronoamperometry	1.0×10^{-7} M to 1.0×10^{-4} M	3.0×10^{-8} M	NR	(Zhao et al., 2013)
Reported the detection of protein biomarker p53 using functionalized nanofiber composite probe of mwCNTs-PA6-ppy **Reported high sensitivity, speed, and biocompatibility due to the large surface area** **Proof of concept strategy that can be adoptable for various type of analytes**	p53	DPV	NR	50 fm for wild type sequence	NR	(Wang et al., 2013a)
Electrochemical impedance based cancer detection where the probe was fabricated using Cysteamine/ PDITC/protein G Functionalized AuNP. **Specificity was attributed by antibody used and AuNP facilitated the improved loading capacity for antibody immobilization**	EGFR	EIS	1 pg ml^{-1} and 1 µg ml^{-1}	0.34 pg ml^{-1} (PBS) and 0.88 pg ml^{-1} (human plasma)	Plasma	(Elshafey et al., 2013a)
Electrochemical immunosensor for label free detection of CA 15-3 using the	CA15-3	EIS	0.1-20 U ml^{-1}	0.012 U ml^{-1}	Serum	(Li et al., 2013)

graphene modified electrode assisted by AuNP for immobilizing signaling antibodies						
Electrochemical Impidimetric biosensor for the detection of HER-3 based on the HER-3 and anti-HER-3 interaction using single frequency impedance technique	HER-3	EIS	0.2- 1.4 pg ml^{-1}	NA	Serum	(Canbaz et al., 2014)
Functionalized nanoparticles and Graphene matrix assisted electrochemical biosensor for the detection of breast cancer biomarker BRCA1	BRCA1	CV, Chronoamperometry	NA	1 fm (5.896 fg ml^{-1}).	NR	(Rasheed and Sandhyarani, 2014)
Electrochemical immunosensor fabricated using factionalized AuNP on SPCE for the detection of HER2 ECD Reported detection time was 2 hr / 50 min with the reported LOD below the established cut off	HER2 ECD	LSV	15-00 ng ml^{-1}	4.4 ng ml^{-1}	Serum	(Marques et al., 2014)
PAMAM dendrimer/cdse–cds-QD based electrochemical biosensor was reported for the detection of cancer biomarker and cells Aptamers for cell detection were immobilized using magnetic Fe_3O_4 coated AuNP	DNA, Cancer cells	ASV, DPV, and EIS	160 - 15360 cells ml^{-1}	89 cells ml^{-1}.	NR	(Jie et al., 2014)
Electrochemical aptasensor for cancer detection using methylene blue as electrochemical indicator on o-	MUC1 protein	CV, DPV ,and EIS	1-12 ppb	0.62 ppb	NR	(Taleat et al., 2014)

aminobenzoic acid films						
Electrochemical biosensors for DNA detection based on impidimetric and differential pulse voltammetric techniques. Reproducible prototype with better selectivity and high sensitivity Reported satisfactory results in the genome samples. Easy fabrication of the prototype	DNA	DPV EIS	1.0×10^{-19} - 1.0×10^{-7} M	4.6×10^{-20} M	NR	(Benvidi et al., 2015)
Label-free capacitance based electrochemical aptasensor for cancer biomarker detection.						
Signal generation was achieved by the charge distribution upon the interaction of aptamer–protein molecules by applying AC of frequency 50–350 Mhz	HER 2/ erbb2	EIS	0.2 - 2 ng ml^{-1}	NR	Serum	(Qureshi et al., 2015)
Electrochemical immunosensor using anti-HE4 IgG functionalized magnetic beads for detection of ovarian cancer biomarker. Sensitivity was obtained by enzymatic system (alkaline phosphatase) converting the substrate to various electroactive entities	HE4	SWV	NR	6.8 fm	NR	(Čadková et al., 2015)
Cell surface mannose mimicking Thiomannosyl dimer was used to fabricate the biosensing probe for cell surface	Cell surface glycan	DPV	1.0×10^{2} - 1.0×10^{6} cells ml^{-1}	40 cells ml^{-1}	NR	(Zhang et al., 2015)

glycan detection Sensitivity enhancement was introduced by MWNT/ AuNP, and signal amplification was obtained by the Con A–MWNT–HRP bio-conjugates.						
A sandwiched aptamer assisted electrochemical immuosensing technique was used for the detection of cancer biomarker (EGFR) Selectivity was introduced by EGFR aptamers, and quantificaition was done by DPV method	EGFR	DPV	1-40 ng ml^{-1}	50 pg ml^{-1},	Serum	(Ilkhani et al., 2015)
An amperometric magneto-immunosensor for the determination of breast cancer. DL reported with 1000-fold lower than the clinically approved	ErbB2	Amperometry	0.1- 32.0 ng mL^{-1}	26pg mL^{-1}	Serum and Cell lysate	(Eletxigerra et al., 2015b)
Electrochemical biosensor for folate receptor was reported using the immobilization-free strategies. The biosensor combines the terminal protection of small molecule liked DNA with the immobilization less strategies Biomarker and cell detection have been studied	DNA and cancer cells	DPV	10 fm - 10 nm 100 - 10000 cell ml^{-1}	3.8 fm 50 cell ml^{-1}	NR	(Ni et al., 2016)
Amperometric immunosensor was fabricated using electric field mediated method Dual responsive signals for the confirmatory detections. Reported with high	PSA, CEA, and CA125	Amperometry, and Colorimetry	NR	Amperometry / Colorimetric 0.7/0.8 pg ml^{-1} (PSA) 0.8/0.6 pg ml^{-1} (CEA) 0.005/0.006 U ml^{-1}	Patient plasma	(Hong et al., 2016a)

specificity and sensitivity for both the detection modes (electrochemical and colorimetric signals) Improved performences were reported				(CA125)		
An electrochemical immunosensor was reported for the detection of various cancer biomarkers using the direct deposition of PNIPAAm Reusable and multiplexed support provided with improved specificity and sensitivity Regeneration of the probe was facilitated by the Altering currents	CA125, CEA, and PSA	CV, EIS.	0.0064 - 256 U ml^{-1} (CA125) 1 pg ml^{-1} - 100 ng ml^{-1}(CEA) 10 pg ml^{-1} - 10 ng ml^{-1} (PSA)	0.007 U ml^{-1} (CA125), 0.7 pg ml^{-1} (CEA), and 0.9 pg L^{-1}(PSA)	Serum	(Hong et al., 2016b)
Impidimetric immunosensor for the detection of various pancreatic biomarkers using Cu2+, Cd2+, Pb2+ or Zn2+ doped chitosan nano-spheres	CEA, CA199, CA125 and CA242	DPV, EIS	0.1-100 ng ml^{-1} (CEA), 1-150 U ml^{-1} (CA199, CA125 and CA242)	0.02 ng ml^{-1} (CEA), 0.4 U ml^{-1} (CA199), 0.3 U ml^{-1} (CA125), and 0.4 U ml^{-1} (CA242).	Patient serum	(Rong et al., 2016)
Anti-CD 59 functionalized self-assembled Lcysteine deposited on probe for the oral cancer detection for oral cancer biomarker CD59	CD 59	CV, EIS	1 - 1000 fg ml^{-1}	0.38±0.03 $fgml^{-1}$	Saliva	(Choudhary et al., 2016)
Electrochemical Impidimetric biosensors for the breast cancer biomarker BRCA 1 using the self-assembled antifouling peptides deposition on electrode surface. Supports the quantification of the target molecule in real human serum	BRCA 1	EIS	1.0 fm - 10.0 pm	0.3 fm	Serum	(Cui et al., 2017)

sample						
Simultaneous detection prostate cancer biomarkers (VEGF and PSA) using the ss-DNA functionalized graphene oxide incorporated with dual-antibody modified PLLA NPs. High sensitivity and detection and multiplexed detection of PSA and VEGF	PSA and VEGF		0.05-100 ng ml^{-1} (VEGF) And 1–100 ng ml^{-1} (PSA)	NA	Patient serum	(Pan et al., 2017)
Electrochemically synthesized hierarchical gold nanostructure based biosensing for the detection of cancer biomarker (CA125) pH based growth mediated deposition have been used. Improved performances have been reported due to the large effective area of the probe	CA125	CV and EIS	10 - 100 U ml^{-1}	5.5 U ml^{-1}	NR	(Torati et al., 2017)
magnetic-controllable electrochemical miRNA biosensor	Oral cancer-related microRNA (miRNA)	Chronoamperometry	1aM - 10fM	0.22 aM (2.2×10−19 M)	Saliva	(Wang et al., 2013b)
A DNA concatamer-based electrochemical biosensor for cancer-associated circulating miRNAs detection. The biosensor reported with high sensitivity and selectivity for target miRNA	miRNA	EIS,	100 aM - 100 pM	100 aM	Serum	(Hong et al., 2013)
Label free direct electrochemical nanobiosensor based on CNT-conductive polymer based nanocomposites for prostate cancer detection	miRNA-141	Voltammetry	10^{-15} - 10^{-8} M	8 fM	NR	(Tran et al., 2013)

A label free electrochemical biosensor for miRNA-24 detection The probe was fabricated using functionalized MWCNTs shows excellent guanine oxidation signal thus , promoting greater sensitivity (4.963 μA cm^{-2} $decade^{-1}$)	miRNA-24	CV DPV EIS,	NR	1 pM	Artificial complex miRNA	(Li et al., 2014)
An electrochemical ELISA-like immunodetection of miRNA based on antibody functionalized RGO-CNT nanocomposites targeting DNA-RNA hybrids	DNA-RNA hybrids	SWV	10 fM - 1 nM	10 fM	NR	(Tran et al., 2014)
An electrochemical detection of microRNA based on dual signal amplification using hairpin shaped capture probe based on functionalized AuNPs – Graphene	miRNA	CV DPV	10 fM - 1 nM	3.3 fM	Biological	(Wu et al., 2014)
A label free electrochemical biosensor based on triple signal amplification using Aminophenylboronic acid/biotin – functionalized AuNP for cancer miRNAs detection Redox cycling has been incorporated for the triple detection	miRNA	CV, Amperometry	10 fM - 5 pM	3 fM	NR	(Liu et al., 2014b)
A label free, low-cost electrochemical strategy was adopted using the MB binding on ssDNA and miRNA/DNA duplex for the selective	miRNA-21	CV DPV EIS	0.1 - 500.0 pM	84.3 fM	NR	(Rafiee-Pour et al., 2016)

detection of miRNA						
An electrochemical biosensor for the detection of breast cancer targeting miRNA on screen printed electrodes with high sensitivity	miRNA-155	CV SWV EIS	0 aM - 1.0 nM	5.7 aM	Serum	(Cardoso et al., 2016)
An electrochemical nanobiosensor for gastric cancer detection, reported with high sensitivity using two-stage cyclic enzymatic amplification method Specificity and sensitivity was introduced by using T7 Exonuclease and T4 RNA ligase 2	miRNA21	DPV	1–100000 fM	0.36 fM	Real tumor, serum miRNA	(Li et al., 2016a)
An electrochemical nanobiosensor was developed based on graphene oxide and gold nanorods as signal amplifiers using anthraquinone, Oracet Blue for electroactive and intercalative label for the detection of breast cancer in early stage	miRNA-155	DPV	2.0 fM - 8.0 pM	0.6 fM	Plasma	(Azimzadeh et al., 2016)
A sandwich enzyme amplification based electrochemical nanobiosensor using low-cost pencil graphite modified electrode AuNP composites was reported for miRNAs detection	miRNA-21	DPV EIS,	200 pM - 388 nM	100 pM	NR	(Mandli et al., 2017)
A dual-mode electrochemical biosensor for miRNA-21 detection based on AuNPs@MoS_2 probe with high sensitivity and selectivity	miRNA-21	EIS, DPV	10 fM - 1 nM	0.45 fM	Serum	(Su et al., 2017)

Table 1(a):

Sensor Design Specifications	Target (Cell lines)	Detection Technique	Analytical Performance LR	DL	Real Sample	Reference
Label free impidimetric detection concanavalin A (Con A), a mannose specific lectin targeted for the cancer cell detections	BEL-7404	EIS	NR	234 cells mL^{-1}	NR	(Hu et al., 2013)
Self-assembled monolayer of succinimidyl 6-(3-[2-pyridyldithio]-propionamido) hexanoate (LC-SPDP) was used for detection probe fabrication Cell identification was done by using magnetic beads and molecular profiling	MCF-7	Voltammetry	1×10^5-1×10^8 cells ml^{-1}	1×10^5 cells ml^{-1}	NR	(Arya et al., 2013)
Cytosensor based on the folic acid detection was reported Ferrocene was used for electrochemical indicator Cell viability was enhanced using the fast DPV techniques	HeLa cells	DPV	10 -10^6 cells ml^{-1}	10 cells ml^{-1}	NR	(Liu et al., 2013)
ITO based portable photo-electrochemical cytosensing device was fabricated using ZnO/graphene and S6 aptamers Specificity for cancer cells is attributed by S6 aptamer	SK-BR-3	CV, EIS	of 1×10^2-1×10^6 cells mL^{-1}	58 cells mL^{-1}	NR	(Liu et al., 2014a)
Electrophoretically deposited spongy graphene (Mg2+-charged) electrodes were used for electrochemical detection of leukemia single cell	Human myelogenous leukemia K562	DPV	1.0×10^5 - 0.1 cells mL^{-1}	~0.02 cells mL^{-1}	Serum	(Akhavan et al., 2014)
Disposable electrochemical sensor using ITO glass for studying the behavior of drug interacting cancer cells for cost-effective, simple, rapid and proof of concept model	leukemia K562	CV DPV	$2.0\times l0^3$ - $1.0\times l0^7$ cells mL^{-1}	$1.2\times l0^3$ cells mL^{-1}	NR	(Yu et al., 2014)
Ferrocenyl carboranes (FcCB) based droplet system was reported for electrochemical cytosensing of cancer based on pH fluctuation	Leucocytes	CV, DPV EIS,	1.0×10^3 - 3.0×10^4 cells mL^{-1}	800 cells mL^{-1}	NR	(Wu et al., 2015)
Antibody functionalized conducting polymer –AuNP nanocomposites based electrochemical biosensors for detecting drug resistant cancer cells Sensitivity comparable to conventional ELISA based highly selective cytosensors	SKBr-3 and HeLa	CV EIS,	50 - 100,000 cells mL^{-1}	23±2 cells mL^{-1}	Biological	(Chandra et al., 2015)

An amperometric cancer cytosensor using antibody functionalized conducting polymer-AuNP bio-conjugates	Ep-MCCs	CV, EIS, amperometry	45 - 100,000 cells mL^{-1}	28±3 cells mL^{-1}	Biological	(Pallela et al., 2016)
Label free detection of cancer cells was reported following electrochemical mode of detection using aptamer immobilized in the probe surface using polyadenine **High sensitivity and selectivity was reported**	MCF-7	DPV	10 - 10^5 cells mL^{-1}	8 cells mL^{-1}	Serum	(Wang et al., 2017)
Acousto-electrochemical immunosensors have for hepato-carcinoma detection. Probe fabrication have been done with the deposition of antibodies on S-layer fusion protein (rSbpA/ZZ) that allows efficient spatial immobilization	HepG2	CV	1×10^5 - 3×10^6 cells mL^{-1}	NR	NR	(Damiati et al., 2017)
Electrochemical cancer cytosensor using Boronic acid functionalized polythiophene coated probe was reported **The cytosensor shows high sensitivity and selectivity**	AGS cells	EIS	1×10^1 to 1×10^6 cells mL^{-1}	10 cells mL^{-1}	NR	(Dervisevic et al., 2017)
A sandwich-type electrochemical immunocytosensor is reported using rGO-TPA/FeHCF nano labeled Anti-HCT signal tag **Quantification was done by differential pulse voltammetric techniques**	SKBR-3 cancer cells	CV, EIS	500 - 30,000 cells mL^{-1}	21 cells mL^{-1}	NR	(Amouzadeh Tabrizi et al., 2017)
A impedimetric sandwich type aptasensor using AuNPs/SBA-15-pr-NH2 nanocarrier was developed for CT26 cancer cells **Fabrication of such probe using Aptamer/AuNPs/SBA-15-pr-NH_2 was reported for the first time to detect Cells**	CT26	CV, EIS	CV 10 - 1.0×10^5 cells mL^{-1} and EIS 1.0×10^5 - 6.0×10^6 cells mL^{-1}	2 cells mL^{-1}	NR	(Hashkavayi et al., 2017)

Table 1(b):

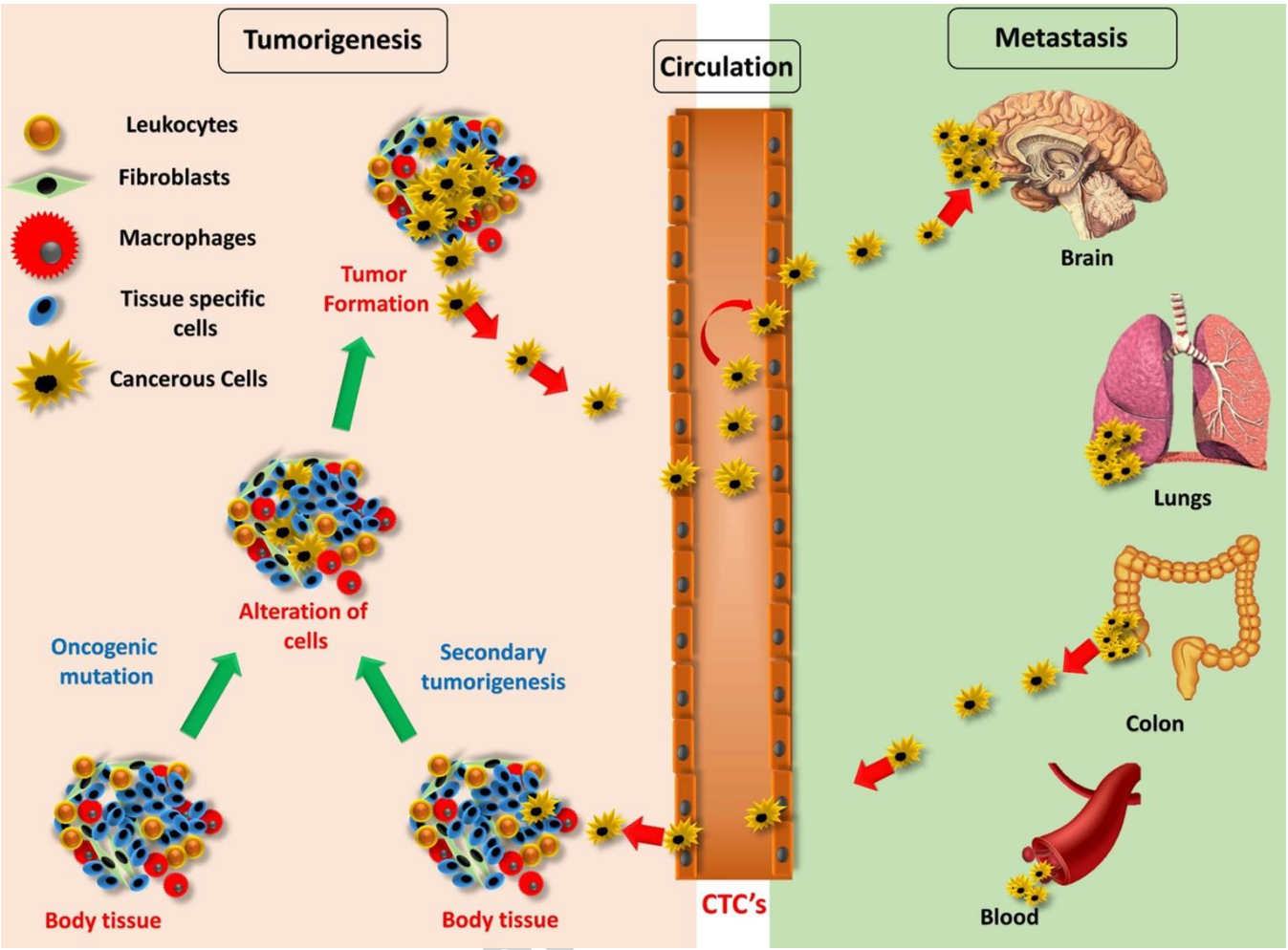
Sensor Design Specifications	Target	Detection Technique	Analytical performance LR	DL	Real Sample	Reference
A PDMS based flexible microfluidic platform was reported for the label free detection HeLa cells Dynamic analysis on proliferation was also reported for these cell lines	HeLa cells	Voltammetry	NR	NR	Cell	(Cao et al., 2014)
A paper based electrochemical biosensor was reported using 3D Au NPs and graphene composite	K-562	CV, EIS	1.0×10^{3} – 5.0×10^{6} cells mL^{-1}	200 cells mL^{-1}	NR	(Ge et al., 2015a)
Micro-PAD based electrochemical biosensor was repored with high sensitivity using 3D macroporous Au-PCE.	K562	DPV, EIS	550 - 2.0×10^{7} cells mL^{-1}	NR	NR	(Su et al., 2015)
Multiplexed disposable immunosensing chip fabricated using the chromium and gold film deposition over glass wafer	CEA, CA19-9, Helicobacter pylori CagA protein (H.P.), P53, PG I, PG II	DPV	0.37–90 ng mL^{-1} (CEA), 10.75–172UmL $^{-1}$ (CA19-9), 10–160 UL^{-1} (H.P.), 35–560 ng mL^{-1} (P53), 37.5–600ng mL^{-1} (PG I) 2.5–80 ng mL^{-1}(PG II)	0.37 ng mL^{-1} (CEA), 10.75 UmL^{-1} (CA19-9), 5 UL^{-1} (H.P.), 35 ng mL^{-1} (P53), 37.5 ng mL^{-1} (PG I) 2.5 ng mL^{-1}(PG II)	Patient Serum	(Xie et al., 2015)
A disposable microfluidics based immunosensing device for the detection of tumor necrosis factor based on the electrochemical techniques Biomarker:	TNF- α	DPV,	NR	4.1 ng mL^{-1}	Serum	(Eletxigerra et al., 2015a)
A lab on a paper electrochemical device is reported using the trimetallic dendrites (Au@PtPd) for the detection of cancer cell	K562	CV, DPV, EIS	1.0×10^{2} - 2.0×10^{7} cells mL^{-1}	31 cells mL^{-1}	NR	(Ge et al., 2015b)
An electrochemical multiplexed biosensor on printed circuit board with MLPA-barcode approach detection of breast cancer biomarker was reported	mRNA markers	CV, EIS	NR	25 pM	NR	(Sánchez et al., 2016)
Paper based microfluidic electrochemical inmmunosensor for CEA detection	CEA	CV, DPV	50 pg mL^{-1} - 500 ng mL^{-1}	10 pg mL^{-1}	Patient serum	(Wang et al., 2016)

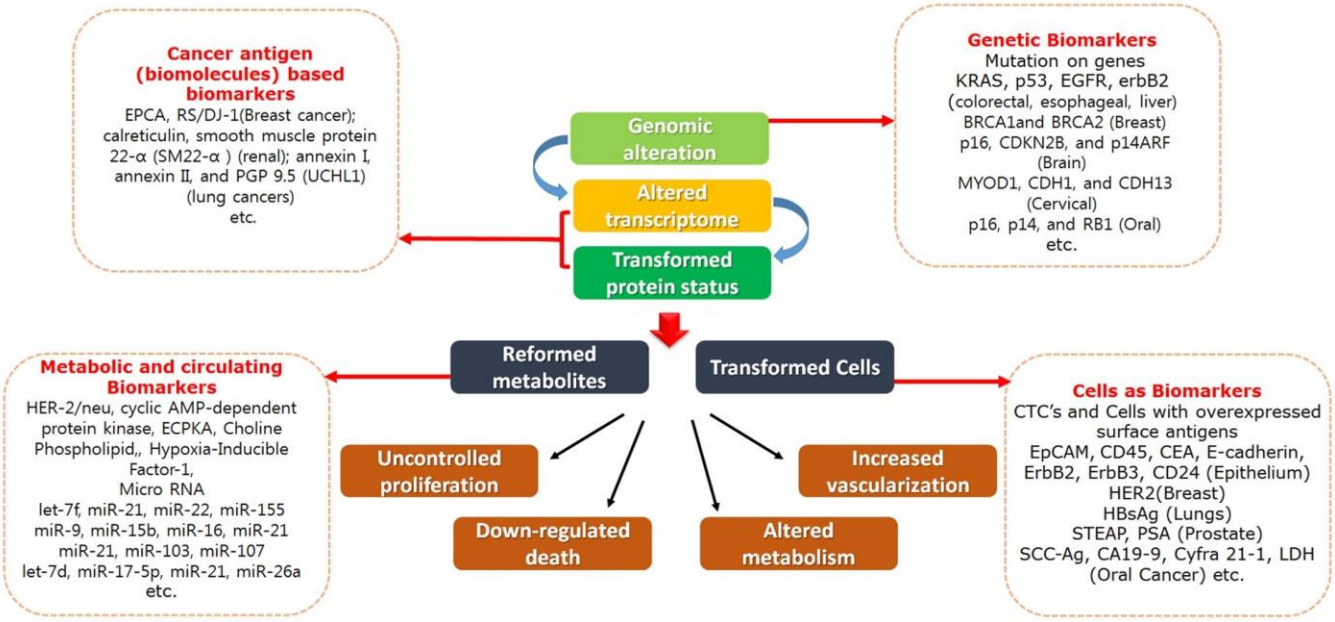
Microfluidic device fabrication by screen printing on paper substrate using wax barrier Anti-CEA used for selective detection making faster and cheaper detection						
A microfluidic immunosensor was reported for cancer surface biomarker EpCAM quantification using antibody functionalized PVA coated AgNPs	EpCAM	CV	2-2000 pg mL^{-1}	0.8 pg mL^{-1}	Biological	(Bravo et al., 2017)
A chip based electrochemical immunosensor for the detection epidermal growth factor receptor (EGFR) was reported using CMK-3/poly-acrylamide-co-methacrylate of dihydrolipoic acid nanocomposite over porous silica substrate	EGFR	CV	0.01 - 50 ng mL^{-1}	3.03 pg mL^{-1}	NR	(Regiart et al., 2017)
A multiplexed on chip detection of cancer protein biomarkers was reported using Fe_3O_4@graphene oxide nanocomposite **This prototype have been reported with the tailoring of the LOD and dynamic range as per the sample requirements**	PSA, PSMA	Amperometry	1.25–1000 pg mL^{-1} (PSA) 9.7– 5000 pg mL^{-1} (PSMA)	1.25 pg mL^{-1} (PSA) 9.7 pg mL^{-1} (PSMA)	Serum	(Sharafeldin et al., 2017)

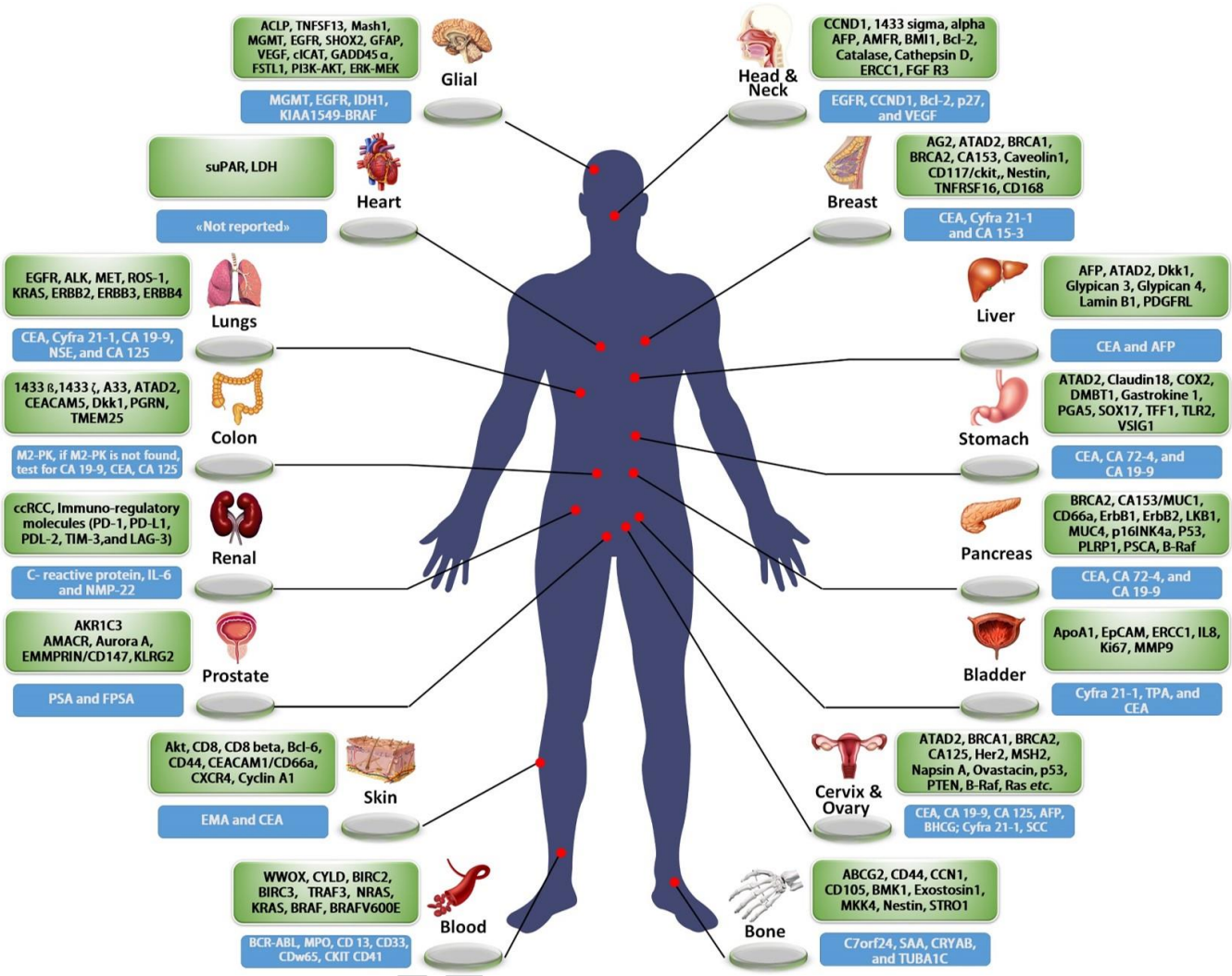
Table 1(c):

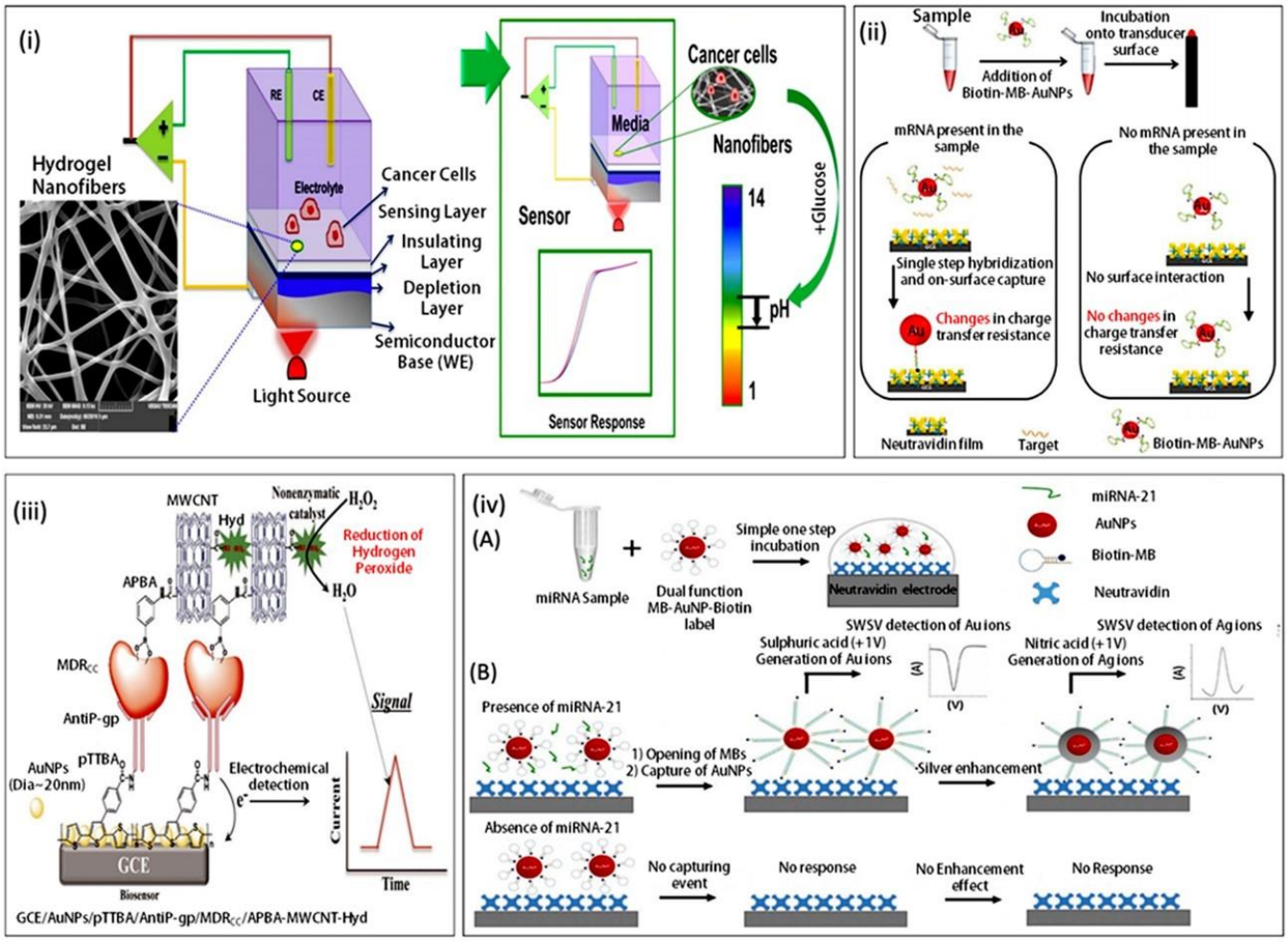
Highlights:

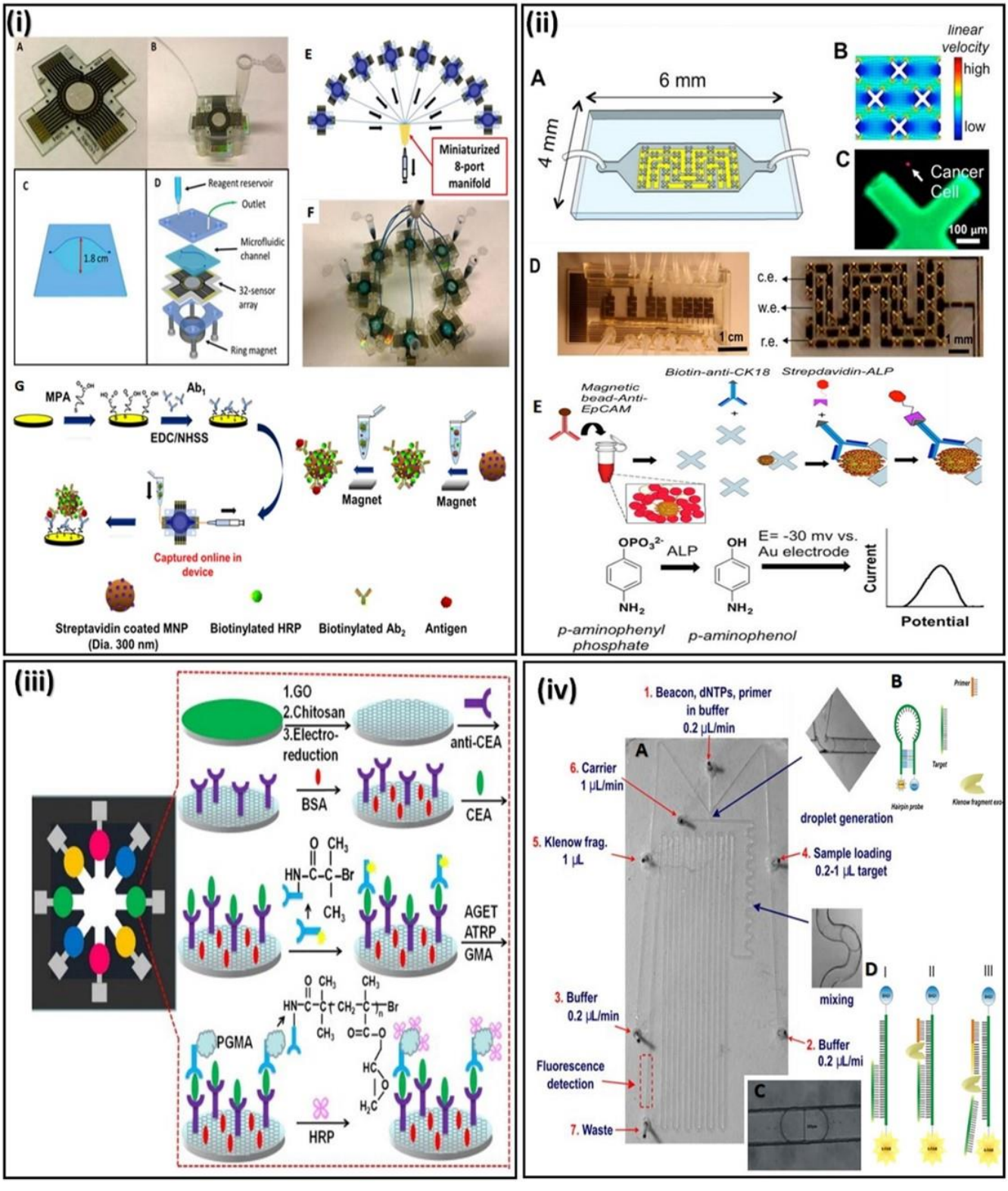
- Concept of cancer progression, central dogma of cancer biomarkers discovery in various organs / tissues have been discussed.
- Most recent miniaturized electrochemical biosensors and microfluidic devices for cancer diagnoses have been collated.
- Specification, target molecule, detection mode, analytical performance of biosensors have been documented.
- Implication of clinically relevant samples have been emphasised.


Tumorigenesis
Circulation
Metastasis
Leukocytes
Fibroblasts
Macrophages
Tissue specific cells
Cancerous Cells
Tumor Formation
Alteration of cells
Oncogenic mutation
Secondary tumorigenesis
Body tissue
Body tissue
CTC's
Brain
Lungs
Colon
Blood




ACLP, TNFSF13, Mash1, MGMT, EGFR, SHOX2, GFAP, VEGF, cICAT, GADD45α, FSTL1, PI3K-AKT, ERK-MEK
Glial
MGMT, EGFR, IDH1, KIAA1549-BRAF
CCND1, 1433 sigma, alpha AFP, AMFR, BMI1, Bcl-2, Catalase, Cathepsin D, ERCC1, FGF R3
Head & Neck
EGFR, CCND1, Bcl-2, p27, and VEGF
suPAR, LDH
Heart
«Not reported»
AG2, ATAD2, BRCA1, BRCA2, CA153, Caveolin1, CD117/ckit,, Nestin, TNFRSF16, CD168
Breast
CEA, Cyfra 21-1 and CA 15-3
EGFR, ALK, MET, ROS-1, KRAS, ERBB2, ERBB3, ERBB4
Lungs
CEA, Cyfra 21-1, CA 19-9, NSE, and CA 125
AFP, ATAD2, Dkk1, Glypican 3, Glypican 4, Lamin B1, PDGFRL
Liver
CEA and AFP
1433 ß,1433 ζ, A33, ATAD2, CEACAM5, Dkk1, PGRN, TMEM25
Colon
M2-PK, if M2-PK is not found, test for CA 19-9, CEA, CA 125
ATAD2, Claudin18, COX2, DMBT1, Gastrokine 1, PGA5, SOX17, TFF1, TLR2, VSIG1
Stomach
CEA, CA 72-4, and CA 19-9
ccRCC, Immuno-regulatory molecules (PD-1, PD-L1, PDL-2, TIM-3,and LAG-3)
Renal
C- reactive protein, IL-6 and NMP-22
BRCA2, CA153/MUC1, CD66a, ErbB1, ErbB2, LKB1, MUC4, p16INK4a, P53, PLRP1, PSCA, B-Raf
Pancreas
CEA, CA 72-4, and CA 19-9
AKR1C3 AMACR, Aurora A, EMMPRIN/CD147, KLRG2
Prostate
PSA and FPSA
ApoA1, EpCAM, ERCC1, IL8, Ki67, MMP9
Bladder
Cyfra 21-1, TPA, and CEA
Akt, CD8, CD8 beta, Bcl-6, CD44, CEACAM1/CD66a, CXCR4, Cyclin A1
Skin
EMA and CEA
ATAD2, BRCA1, BRCA2, CA125, Her2, MSH2, Napsin A, Ovastacin, p53, PTEN, B-Raf, Ras etc.
Cervix & Ovary
CEA, CA 19-9, CA 125, AFP, BHCG; Cyfra 21-1, SCC
WWOX, CYLD, BIRC2, BIRC3, TRAF3, NRAS, KRAS, BRAF, BRAFV600E
Blood
BCR-ABL, MPO, CD 13, CD33, CDw65, CKIT CD41
ABCG2, CD44, CCN1, CD105, BMK1, Exostosin1, MKK4, Nestin, STRO1
Bone
C7orf24, SAA, CRYAB, and TUBA1C


(i)
Hydrogel Nanofibers
RE
CE
Electrolyte
Cancer Cells
Sensing Layer
Insulating Layer
Depletion Layer
Semiconductor Base (WE)
Light Source
Media
Sensor
Sensor Response
Cancer cells
Nanofibers
+Glucose
14
pH
1
(ii)
Sample
Addition of Biotin-MB-AuNPs
Incubation onto transducer surface
mRNA present in the sample
No mRNA present in the sample
Single step hybridization and on-surface capture
Changes in charge transfer resistance
No surface interaction
No changes in charge transfer resistance
Neutravidin film
Target
Biotin-MB-AuNPs
(iii)
MWCNT
Hyd
Nonenzymatic catalyst
H_2O_2
Reduction of Hydrogen Peroxide
H_2O
APBA
MDR_{CC}
AntiP-gp
Signal
AuNPs (Dia~20nm)
pTTBA
Electrochemical detection
e^-
GCE
Biosensor
Current
Time
GCE/AuNPs/pTTBA/AntiP-gp/MDR_{CC}/APBA-MWCNT-Hyd
(iv)
(A)
miRNA Sample
Dual function MB-AuNP-Biotin label
Simple one step incubation
Neutravidin electrode
miRNA-21
AuNPs
Biotin-MB
Neutravidin
(B)
Presence of miRNA-21
1) Opening of MBs
2) Capture of AuNPs
Sulphuric acid (+1V) Generation of Au ions
SWSV detection of Au ions
Nitric acid (+1V) Generation of Ag ions
SWSV detection of Ag ions
Silver enhancement
Absence of miRNA-21
No capturing event
No response
No Enhancement effect
No Response


(i)
A
B
C
D
E
F
G
Reagent reservoir
Outlet
Microfluidic channel
32-sensor array
Ring magnet
1.8 cm
Miniaturized 8-port manifold
MPA
Ab1
EDC/NHSS
Magnet
Magnet
Captured online in device
Streptavidin coated MNP (Dia. 300 nm)
Biotinylated HRP
Biotinylated Ab2
Antigen
(ii)
A
6 mm
4 mm
B
linear velocity
high
low
C
Cancer Cell
100 μm
D
1 cm
c.e.
w.e.
r.e.
1 mm
Biotin-anti-CK18
Strepdavidin-ALP
E
Magnetic bead-Anti-EpCAM
OPO3 2-
ALP
OH
NH2
NH2
E= -30 mv vs. Au electrode
Current
Potential
p-aminophenyl phosphate
p-aminophenol
(iii)
1.GO
2.Chitosan
3.Electro-reduction
anti-CEA
BSA
CEA
AGET
ATRP
GMA
PGMA
HRP
(iv)
1. Beacon, dNTPs, primer in buffer 0.2 μL/min
A
B
Primer
Target
Hairpin probe
Klenow fragment exo-
6. Carrier 1 μL/min
droplet generation
5. Klenow frag. 1 μL
4. Sample loading 0.2-1 μL target
D
I
II
III
mixing
3. Buffer 0.2 μL/min
2. Buffer 0.2 μL/mi
Fluorescence detection
C
7. Waste