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Electroanalytical and surface plasmon resonance sensors for detection of breast cancer and Alzheimer's disease biomarkers in cells and body fluids

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Cancer and neurological disorders are two leading causes of human death. Their early diagnoses will either greatly improve the survival rate or facilitate effective treatments or modalities. Detection of biomarkers in body fluids and some tissues (*e.g.*, blood, urine and cerebrospinal fluids) is relatively non-invasive and provides useful chemical and biological information that is complementary to tomographic imaging (*e.g.*, magnetic resonance imaging, positron emission tomography and X-ray computed tomography). Recent years have witnessed the contributions from and potential applications of bioanalytical methods for early detection of major diseases. In this review, we survey some recent developments of electroanalytical (as a representative label-based technique) and surface plasmon resonance (SPR) (as a representative label-free technique) biosensors for detection of biomarkers relevant to etiologies of breast cancer and Alzheimer's disease (AD). While breast cancer is representative of cancers of complexity (multiple biomarkers, false positives from tomographic scans, and a need for more effective early diagnostic methods), AD is the most prevalent neurological disorder that is also linked to multiple biomarkers. Both electroanalytical and SPR-based sensors have attractive features of sensitivity, portability, obviation of large sample volumes, and capability of multiplexed detection. Various sensing protocols developed in the past five years are reviewed, demonstrating the feasibility of both techniques for diagnostic purposes. Problems inherent in these two techniques that must be overcome before being clinically viable are also discussed.

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1. Introduction

Sensitive and selective detection of disease biomarkers is of great importance for early diagnosis, personalized treatment, and molecularly targeted therapy of major diseases.[1–3] Three of the leading debilitating types of diseases are cardiovascular

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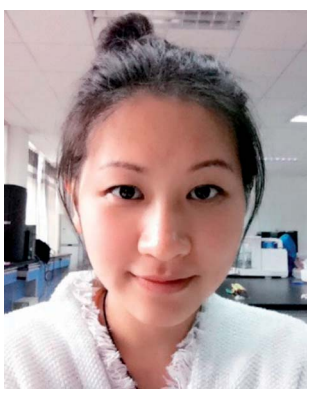

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diseases, cancers, and neurological disorders, the latter two of which are difficult to diagnose in the early stages. In normal persons or patients whose cancers are in the early stage or those who suffer from mild cognition impairment, the expression level of many biomarkers in human fluids (*e.g.*, urine, blood, saliva, and cerebrospinal fluids) and tissues (*e.g.*, tumor cells) is usually at trace levels (*e.g.*, pg mL^{-1} range). Consequently, early diagnoses of these diseases require techniques and methods with high sensitivity and selectivity. Several reviews have already summarized the detection of various cancer biomarkers.[4–8] Some reviews have also been published on the possible use of various biomarkers for assessment of the severity of neurological disorders.[9,10] However, these reviews are rather broad and tend not to focus on biomarkers related to a specific type of cancer or neurological disorder.

In this review, we chose to survey bioanalytical methods that could serve as alternative means for early diagnosis of breast cancer and neurological disorders. We chose these two major types of diseases based on the facts that both are closely related to aging as well as genetics, and environmental factors are also implicated in their etiologies.[11,12]

Bioanalytical techniques that have been applied to assays of the above-mentioned biomarkers can be classified into label-based and label-free ones. Table 1 lists select techniques in each of the two categories. We should mention that, for biomarkers that contain inherent signaling moieties (*e.g.*, fluorophores or redox moieties), some of the techniques (*e.g.*, fluorescence or electrochemistry) can also be used in the label-free fashion. However, generally speaking, most biomarkers need to be tagged in order for these techniques to operate. Given the scope of a short critical review, we therefore further limit our surveys on the recent developments of electroanalytical and surface plasmon resonance (SPR) biosensors within the past five years. Although electroanalytical and SPR-based sensors rely on a binding event between the biomarker and a surface-tethered capture molecule, the detection (or signal transduction) of these events differs considerably (*cf.* Table 1). Electrochemistry is a technique that studies the charge transfer process between the redox moiety (or tag) on the biomarker and the electrode, while SPR measures the refractive index change (adsorption of a biomarker) on the sensor chip. The former[33,34] is a good representative of the label-based techniques, while the latter[13,14] has features that are common among label-free techniques. Both electroanalytical[15] and SPR biosensors[13] possess promising characteristics for early clinical diagnosis in their sensitivity and speed. Their low cost and portability are also attractive for point-of-care assays.[16–18] Another consideration in choosing to review these two types of sensors is that their detections occur at the solid–solution interface (*i.e.*, heterogeneous biosensors). Specifically, in both types of sensors, target biomarkers are captured by molecules (also referred to as probe molecules in the bioanalytical community or bait molecules in the medical field) as varied as antibodies,[19,20] aptamers,[21] peptides[22] and DNA.[23] SPR and electrochemistry are also complementary. For example, SPR provides information about binding kinetics and affinity between a capture probe and a specific disease biomarker. Such information is important for the development of reliable and effective sensors including electrochemical biosensors.[24,25] In electrochemical detection, a redox label or moiety has to be in close proximity to the electrode to generate detectable signals. On the other hand, a cleverly designed electrochemical sensor can yield higher sensitivity than many

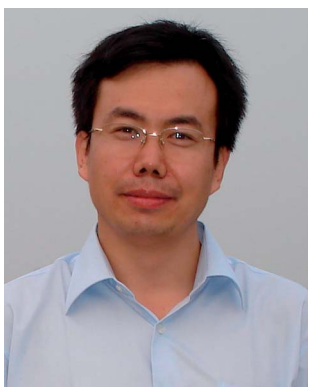

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Table 1 Select label-based and label-free methods for cancer sample analyses

	Method	Detection principle	Sensitivity	Limitation
Label-based	Fluorescence[98]	Fluorescent light emission	High	Static and dynamic quenching
	Electrochemistry	Charge transfer	High	Nonspecific adsorption, sluggish charge transfer
	Electrogenerated chemiluminescence[99]	Charge-transfer-stimulated light emission	High	Complicated procedures
	Chemiluminescence[100]	Light emitted from chemical reactions	Moderate	Limited dynamic range
Label-free	Surface plasmon resonance	Refractive index change/ mass loading	High	Bulk refractive index change, nonspecific adsorption
	Surface acoustic wave/quartz crystal microbalance[101]	Mass change	Moderate–high	Viscoelastic/temperature effects, non-uniform sensing area
	Impedance spectroscopy[102]	Conductivity	Moderate	Complicated modeling and data interpretation
	Cantilever sensors[103]	Atomic force	High	Limited dynamic range and semi-quantitative

SPR-based sensors. We should also mention that electrochemistry and SPR can be combined into a hybrid technique to study protein conformational and structural changes accompanying with charge transfer[26,27] or to enhance the binding events with an applied potential.[28] To help readers to assess the potential of electroanalytical and SPR sensors for breast cancer and AD biomarker detection, we have summarized some key performance criteria (*e.g.*, dynamic ranges and detection limits) at the end of Sections 2 and 3. Improvements to be made and challenges to be overcome for implementing these sensors as viable diagnostic tools are critically reviewed in Section 4, Conclusions and outlook.

2. Breast cancer biomarkers

Cancer is the second leading cause of mortality worldwide following cardiovascular disease. More than 100 types of cancers have been classified according to the type of cells initially affected. Breast cancer is the most common invasive cancer in females, accounting for 16% of all female cancers and 22.9% of invasive cancers in women in 2008.[29] About 18.2% of all cancer deaths in both males and females worldwide are from breast cancers.[30] It is a disease with several distinct subtypes.[31] Among the various types of cancers, breast cancer is representative because, while its clinical diagnosis is easier than those of many other cancers, there still remain many challenges for early detection to guide effective therapies. The following issues are not exhaustive but certainly problematic: (1) there exist multiple proteins as possible biomarkers and relying on the analysis of a single biomarker for diagnosis is insufficient and unreliable,[32] (2) current medical imaging methods are still prone to producing false-positive results and could be relatively insensitive to tiny tumors[33] and (3) some of the existing diagnostic tools are still invasive.[34] The common definition of breast cancer is whether or not it is human epidermal growth factor receptor 2- (HER2-) positive, estrogen receptor- (ER-) positive and/or progesterone receptor- (PR-) positive. Triple positive groups mean that the patients have a close correlation with the three biomarkers, while triple negative ones suggest that doctors cannot diagnose patients based on these biomarkers. Another biomarker for women with a family history of breast cancer is *BRCA1*, which has been proven to be extremely useful for preventive measures.[35] The five-year survival rate of breast cancers is substantially higher if detected early. During the past few decades, the treatment of breast cancer has been greatly advanced due to the discovery of these biomarkers that could direct more individualized therapies to different molecular subgroups. The breast cancer biomarkers discussed in this review include HER2, carbohydrate antigen 15-3 (CA 15-3), ER/PR, *BRCA1*/*BRCA2* and p53.[36,37] HER2 and CA 15-3 are membrane proteins and the rest of the above biomarkers are intracellular proteins.

2.1 HER2

The expression level of HER2 increases in approximately 20–30% of breast cancer tumors.[38,39] Breast cancer patients who are positive to HER2 have increased HER2 concentrations in blood (15–75 ng mL^{-1}) compared to normal individuals (2–15 ng mL^{-1}).[40] The HER2-positive breast cancer tumors tend to be more aggressive and fast-growing than the HER2-negative ones.[29]

Shim and co-workers developed a sandwich-type electrochemical immunosensor for the detection of HER2 and HER2-overexpressing breast cancer cells.[41] The immunosensor label was prepared *via* functionalization of gold nanoparticles (AuNPs) with hydrazine and an aptamer that is specific to HER2. After HER2 and the immunosensor label (AuNPs coated with the reductant hydrazine) were sequentially captured with the anti-HER2 antibody-modified electrode, the electrode was exposed to silver ions, which are reduced by hydrazine. The deposited silver could be clearly seen under a microscope and analyzed by square wave stripping voltammetry (SWSV), and the amount of HER2 or HER2-overexpressing cells was determined (Fig. 1). This method provides a simple alternative to

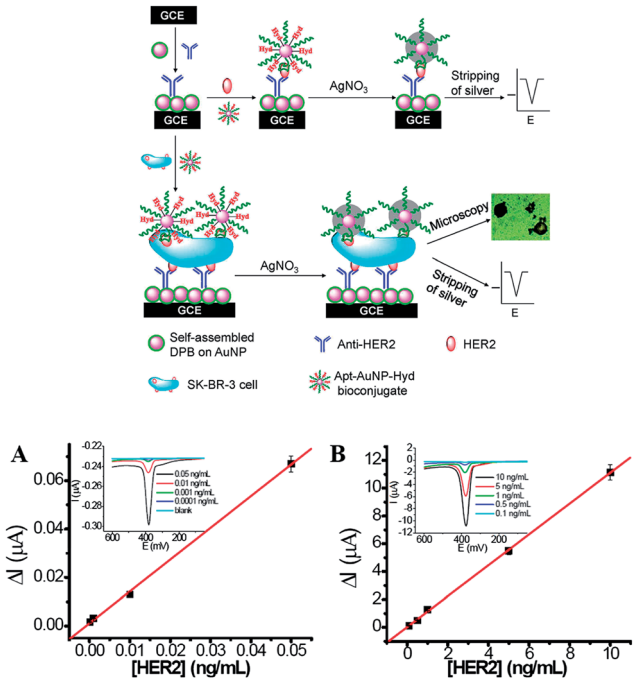

Fig. 1 Top: schematic representation of the immunosensor for detection of HER2 protein and HER2-overexpresing breast cancer cells. GCE = glassy carbon electrode; DPB = 2,5-bis(2-thienyl)-1*H*-pyrrole-1-(*p*-benzoic acid). Bottom: calibration curves of the immunosensor in the range of (A) 0.0001–0.05 ng mL^{-1} and (B) 0.1–10 ng mL^{-1}. The insets show the corresponding square wave stripping voltammograms for HER2 with varied concentrations. (Adapted from ref. 41 with permission. Copyright© 2013 American Chemical Society.)

selectively stain breast cancer cells, thus making cancer cells easily detectable.

Marrazza *et al.* constructed an electrochemical immunosensor for the detection of HER2.[42] Protein A-modified magnetic beads were utilized for the immobilization of capture anti-HER2 antibodies and alkaline phosphatase (AP) was chosen as the label on the detection anti-HER2 antibody. The electrochemical signal was generated *via* oxidation of 1-naphthol, which was produced by AP-catalyzed hydrolysis of 1-naphthyl phosphate.

Ugo's group developed an electrochemical immunosensor for HER2 based on nanoelectrode ensembles (NEEs) prepared in track-etch polycarbonate membranes.[43] After construction of the sandwich immuno-structure on the NEEs, horseradish peroxidase (HRP)-catalyzed electrochemical current in response to H_2O_2 was recorded. The advantage of the NEEs is the highly improved signal-to-background current ratio. Consequently, the detection limit was two to three orders of magnitude lower than that obtained with conventional electrodes. However, the electrode preparation is complicated.

Lee and co-workers fabricated a label-free electrochemical impedance aptasensor for HER2 by immobilizing the HER2-specific single-stranded DNA aptamer onto the electrode.[44] After capture of different concentrations of HER2, the impedance of the electrode was increased, and the increased resistance is proportional to the concentration of HER2. In comparison with the sandwich-type immunosensors, the label-free sensors are simpler and cost-effective. However, the sensitivity is lower than that of the sandwich-structured sensors.

2.2 ER/PR

ER and PR are both endocrine receptors.[45,46] Estrogen and progesterone are essential for the growth and development of mammary glands and have been associated with the promotion and growth of breast cancers.[47] Both estrogen and progesterone exert many effects *via* their receptors, ER and PR. There are two kinds of ERs, ERα and ERβ.[48] Given the ill-defined role of ERβ in human breast cancer development, ERα is more commonly used as the target receptor for clinical decisions. PR levels are primarily regulated in response to ER.[49]

Li and collaborators constructed an electrochemical biosensor for the detection of ER relying on the ability of exonuclease III (Exo III) to digest DNA duplexes.[50] Two complementary DNA probes were designed, one for selective binding of ER and the other for the subsequent hybridization and intercalation of methylene blue (MB) as the redox reporter. After formation of a stable duplex onto the electrode, a high electrochemical current of MB was observed. When Exo III was introduced to digest the duplex, the electrochemical response was decreased because of the removal of MB from the electrode surface. However, in the presence of ER, the digestion of Exo III was blocked due to the formation of the ER–DNA complex, leading to an increased electrochemical signal. Such a method possesses high sensitivity and the detection limit was estimated to be 0.38 nM.

Miyashita *et al.* reported an SPR-based competitive immunosensor for the detection of estrogen and measurement of ER-binding activity.[51] The pre-immobilized estrogen–BSA conjugates were captured by anti-estrogen antibody, yielding SPR signals. When different concentrations of estrogen are present with the antibody solution, the binding of the antibody to the chip is inhibited, leading to the change in the response signal. The method is rapid and simple, with a detection limit of 200 pg mL^{-1}.

2.3 CA 15-3

CA 15-3 belongs to the large family of glycoproteins encoded by the MUC1 gene. CA 15-3 has been clinically analyzed for breast cancer diagnosis of at-risk women, whose CA 15-3 level is typically greater than 30 U mL^{-1}, the threshold value.[52,53] Typically, high concentrations of CA 15-3 were observed in up to 80% of metastatic breast cancers.[54] The biomarkers detected in the CA 15-3 assay are the soluble forms of MUC-1 protein, which is a transmembrane protein consisting of two subunits that form a stable dimer. Several papers have reviewed the structure and functions of MUC-1.[55,56]

Lin's group reported an SPR-based immunosensor for the detection of CA 15-3 with a detection limit of 0.025 U mL^{-1}.[57] The SPR chip was modified with a gold/zinc oxide (Au/ZnO) nanocomposite to enhance the chip performance. The sensitivity of the method was increased by at least 2 fold over that of the traditional gold/chromium (Au/Cr) film.

Li *et al.* developed a label-free electrochemical immunosensor for the detection of CA 15-3.[58] Capture anti-CA 15-3 antibody was immobilized onto the carboxylic acid group-functionalized graphene surface. The electrode conductivity was decreased after capture of CA 15-3, as the antigen is non-conductive. This label-free immunosensor has a low detection limit of 0.012 U mL^{-1}, which is significantly lower than the aforementioned threshold value.

By examining the direct electrochemistry of glucose oxidase (GOD), Yuan and co-workers designed a label-free immunosensor for CA 15-3.[59] Electrodes modified with carbon nanotubes and core–shell organosilica@chitosan nanospheres were further coated with GOD to achieve direct electron transfer between GOD and the electrode. The formation of the antibody–antigen immuno-complex onto the electrode decreased the electrochemical current of GOD due to the increased spatial blocking and dielectric constant of the microenvironment around the GOD molecules. The current is inversely proportional to the concentration of CA 15-3 and a detection limit of 0.04 U mL^{-1} was estimated. This method has a relatively high variability because of the difficulty in achieving direct electrochemistry of GOD. In addition, the immunosensor is rather complex to prepare.

Breast cancer cells can also be detected by monitoring the overexpression of MUC-1 proteins on the cancer cell surface. For example, Liu and co-workers developed an immunoassay for the breast cancer cells (MCF-7) utilizing MUC-1 aptamer-modified magnetic beads to capture MCF-7.[60] Quantum dot-modified silica nanoparticles were used as a detection label. Quantitative detection of MCF-7 cells was achieved by both photoluminescence and square wave voltammetric measurements. In another study, they proposed a strategy for the detection of MUC-1 protein and MCF-7 cells based on the electrochemiluminescence resonance energy transfer (ERET) between the MUC-1 aptamer-functionalized ruthenium complex and graphene oxide (GO).[61] Furthermore, Li *et al.* reported on a sandwich-type immunosensor for the detection of MCF-7 cells based on the MUC-1 aptamer and HRP label.[62]

2.4 BRCA1/BRCA2

BRCA1 and *BRCA2* are human genes, capable of producing tumor suppressor proteins. Genetically inherited mutations of the *BRCA1* or *BRCA2* gene put women at high risk of breast and ovarian cancers.[63] *BRCA1* and *BRCA2* mutations account for about 20 to 25% of hereditary breast cancers and about 5 to 10% of all breast cancers.[64,65] Wei and colleagues developed a sandwich-type electrochemical immunosensor for the *BRCA1* assay. They immobilized capture anti-*BRCA1* antibody onto a graphene-modified electrode and used HRP-functionalized silica nanomaterials as a detection label. Under the optimized conditions, the electrochemical immunosensor exhibited a wide linear range from 0.01 to 15 ng mL^{-1} and a detection limit of 4.86 pg mL^{-1} (Fig. 2).[66] In another study, a label-free electrochemical immunosensor for the *BRCA1* assay has been developed using a toluidine blue (TB)–mesoporous carbon nanosphere nanocomposite-modified electrode.[67] The specific antibody–antigen immunoreaction on the electrode surface resulted in a decrease in the redox signal of TB. However, the possibility of distinguishing between wild-type and mutated *BRCA1* has not been attempted. By using interdigitated gold nanoelectrodes, Valle *et al.* described the electrochemical impedance assay of *BRCA1*, and demonstrated the differentiation between the wild-type and mutated *BRCA1* genes.[68]

2.5 p53 protein

p53 is a tumor suppressor protein and a transcription factor. The function of p53 involves elimination and inhibition of the proliferation of abnormal cells. In more than 50% of cancer cases, the p53 gene has been found mutated.[69] In breast cancers, p53 mutation is associated with more aggressive cancer development and worse overall survival. Gasco *et al.* have reviewed the p53 pathway in breast cancers.[70] Recently, our group designed an electrochemical biosensor for quantification of the wild-type p53 protein.[71] Consensus DNA duplexes were modified onto the electrode to capture wild-type p53 protein and the electrochemical signal was then amplified using ferrocene-capped gold nanoparticle–streptavidin conjugates. This method affords the sensitivity and selectivity necessary for detecting wild-type p53 protein in normal and cancer cell lysates. However, such a protocol did not allow an accurate determination of the extent of p53 mutation. In our other study, simultaneous and label-free detection of wild-type and mutant p53 present in cancer cell lysates was carried out using a dual channel SPR instrument.[72] One channel of the SPR chip was modified with a consensus double-stranded (ds-) DNA while the other with a monoclonal antibody (Fig. 3). The high affinity of the antibody to total p53 protein (wild-type and mutant combined) and the consensus ds-DNA to wild-type p53 protein results in remarkably low detection levels (10.6 and 1.06 pM for the wild-type and total p53, respectively). We found that for liver and colon cancers, the extent of p53 mutation (>60%) is substantially greater than control cell lines. This method is label-free and does not involve additional amplification steps to enhance the sensitivity. Such SPR-based assays can potentially serve as a viable alternative for facile and sensitive clinical analyses. It has been reported that mutation of p53 also occurs in breast cancer patients.[70] Thus we envision that our methods should be extendable to breast cancer studies and diagnosis.

A summary of the dynamic ranges and detection limits of electroanalytical and SPR-based sensors for detection of breast cancer biomarkers is shown in Table 2.

3. Biomarkers related to neurological disorders

Neurodegenerative disorders are characterized by neuronal impairment that eventually leads to neuronal death.[73] They are commonly linked to the pathological aggregation of misfolded proteins and accumulation of intracellular and

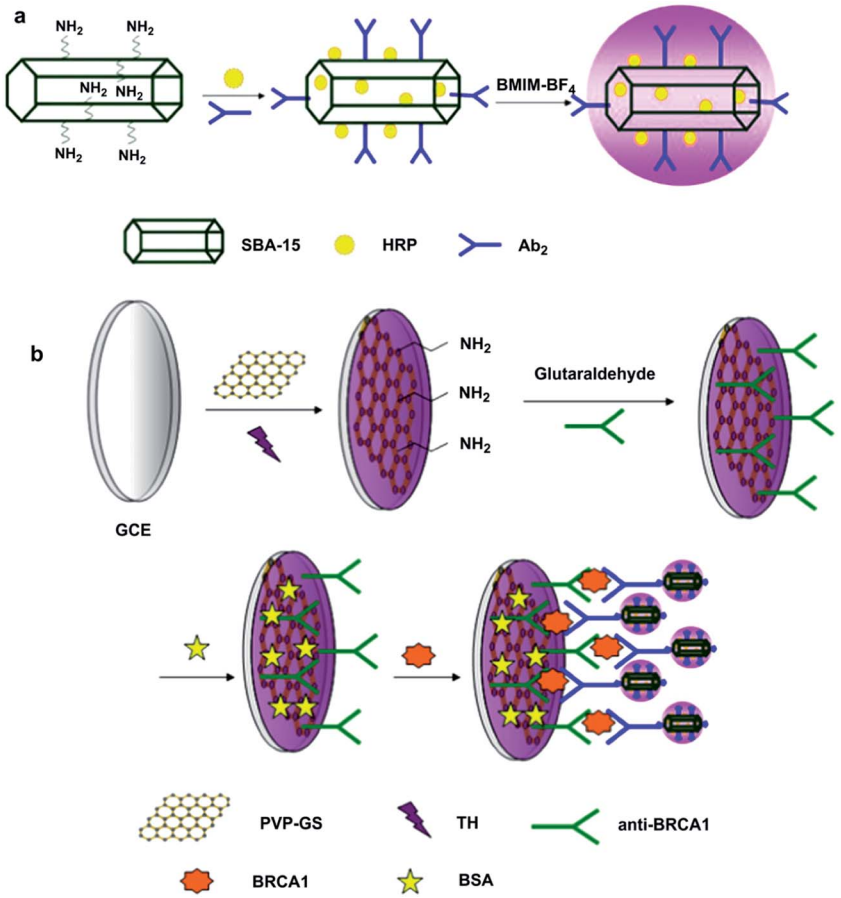

Fig. 2 Schematic representation of the fabrication of the immunosensor. (Adapted from ref. 66 with permission. Copyright© 2011 Elsevier.)

extracellular amyloid inclusions or fibrils in the central nervous system.[74] For neurological disorders, body fluids are perhaps the only samples that are available clinically, as tissue samples are only accessible postmortem. Assays of body fluids also offer biological and chemical information, which complements information obtained with imaging techniques (*e.g.*, magnetic resonance imaging, positron-emission tomography and X-ray computed tomography) about morphological changes in specific regions of an organ. Similarly, proteins and peptides are either overproduced or down-regulated or cannot be effectively cleared in neurological disorders. The major neurological disorders include Alzheimer's disease (AD), Parkinson's disease, Huntington's disease, and Creutzfeldt–Jacob disease.[75–77] We decided to focus on the assays of clinical samples for biomarkers related to AD, given that AD is the most prevalent neurological disorder and the fact that the two major hypotheses (amyloid beta cascade and tau atrophy) have biomarkers (amyloid beta peptide for the former[75,78] and the tau protein for the latter[79]) that interact with many proteins in an extra- and/or intracellular milieu. In addition to proteins and peptides, other biomarkers, such as phospholipids, nucleic acids, small molecules (including neurotransmitters), and metal ions, are also indicative of a neurodegenerative disorder. A more in-depth survey of non-peptide or -protein biomarkers related to neurological disorders is provided in another review paper by our group.[80]

3.1 Aβ peptides

Aβ peptides of 39–43 amino acid residues, proteolytically cleaved from the amyloid precursor protein (APP), are major constituents of neuritic plaques. Both postmortem analyses of the senile plaques from AD patient brain extracts and *in vitro* Aβ peptide aggregation studies have been extensively carried out.[75] Vestergaard *et al.* reported on the first electrochemical assay of Aβ aggregation with the detection limit estimated to be approximately 0.7 μg mL^{-1} for both Aβ(1–40) and Aβ(1–42).[81] However, the detection limit is not low enough for the Aβ assay in human fluids (Aβ(1–42) is present at 500 pg mL^{-1} in cerebrospinal fluid (CSF)).[82] Oh *et al.* performed a multiplexed electrochemiluminescence assay of Aβ(1–40) and Aβ(1–42) levels in plasma.[83] The method possesses a large dynamic range, consumes a small amount of plasma (0.5 mL) and can be completed within a short period of time (shorter than that needed for an ELISA measurement). Along this line, Islam *et al.* developed a simple microfluidic device coupled with cyclic voltammetric detection of very small quantities of Aβ(1–42) peptide.[84]

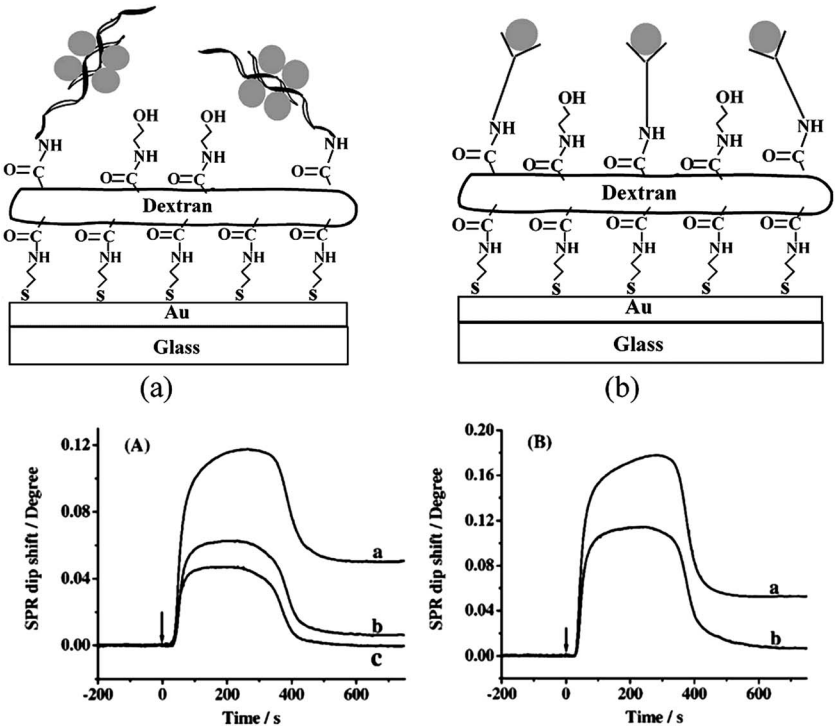

Fig. 3 Top: schematic representation of the simultaneous SPR detection of wild-type and total p53 proteins by consensus DNA duplexes (a) and monoclonal antibody (b) in two separate fluidic channels covering a dextran-modified Au sensor chip. Bottom: SPR sensorgrams correspond to (A) injections of 50 μL of 0.266 nM wild-type p53 solution into fluidic channels covered with consensus double-stranded (ds-) DNA (curve a), single-stranded (ss-) DNA (curve b), and nonconsensus ds-DNA (curve c) and (B) injections of 50 μL of 0.106 nM wild-type p53 solution (curve a) and 0.106 nM IgG solution (curve b) into fluidic channels covered with the monoclonal antibody. The arrows indicate the time when the injections were made. (Adapted from ref. 72 with permission. Copyright© 2009 American Chemical Society.)

Lee *et al.* utilized a gold nanoparticle–antibody complex to amplify SPR detection of synthetic Aβ(1–40) peptide in buffer solution.[85] A detection limit of 1.0 fg mL^{-1} was achieved. However, the extent of nonspecific adsorption and selectivity of the method were not examined. Recently, we have accomplished sensitive and simultaneous SPR quantification of Aβ(1–40) and Aβ(1–42) peptides in human CSF.[86] The signal amplification was achieved by using a conjugate formed between streptavidin and a biotinylated antibody that is selective to the common N-terminus of the Aβ peptides (Fig. 4). The

Table 2 Dynamic ranges and detection limits of electroanalytical and SPR sensors for detection of breast cancer biomarkers

Biomarker	Technique	Dynamic range	Detection limit	Reference
HER2	Electroanalytical chemistry	0.1 pg mL^{-1} to 10 ng mL^{-1}	0.037 pg mL^{-1}	41
		0–15 ng mL^{-1}	6 ng mL^{-1}	42
		Not specified	Not specified	43
		0.01 pg mL^{-1} to 100 ng mL^{-1}	0.01 pg mL^{-1}	44
ER/PR	Electroanalytical chemistry	0.5–100 nM	0.38 nM	50
	SPR	Not specified	Not specified	51
CA 15-3	SPR	1–40 U mL^{-1}	0.025 U mL^{-1}	57
	Electroanalytical chemistry	0.1–20 U mL^{-1}	0.012 U mL^{-1}	58
		0.1–160 U mL^{-1}	0.04 U mL^{-1}	59
MCF-7	Electroanalytical chemistry	250–10 000 cells per mL	85 cells per mL	60
		100–2500 cells per mL	30 cells per mL	61
		100–10 000 000 cells	100 cells	62
BRCA1	Electroanalytical chemistry	0.01–15 ng mL^{-1}	4.86 pg mL^{-1}	66
		0.01–15 ng mL^{-1}	3.97 pg mL^{-1}	67
		Not specified	Not specified	68
p53	Electroanalytical chemistry	2.2 pM to 5.6 nM	2.2 pM	71
	SPR	1.06 pM to 53.2 nM	1.06 pM	72

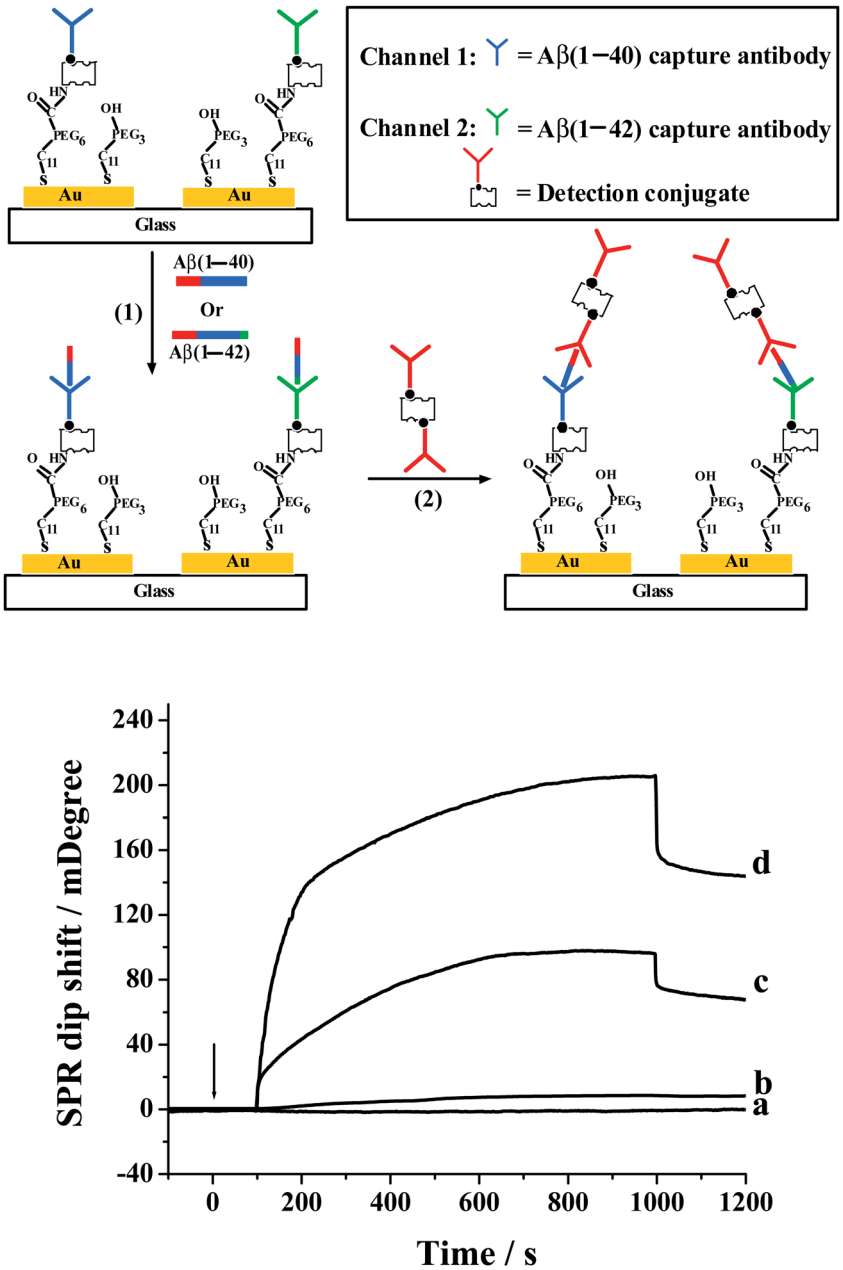

Fig. 4 Top: schematic diagram showing the simultaneous SPR detection of Aβ(1–40) and Aβ(1–42). Fluidic channels are covered with the capture antibody for Aβ(1–40) and Aβ(1–42) respectively. Injection of Aβ samples results in the attachment of Aβ to the respective channel (Step 1), and injection of the detection conjugate that can recognize the common hydrophilic domain of Aβ(1–40) and Aβ(1–42) leads to signal amplification (Step 2). Bottom: SPR sensorgrams after injections of (a) 1.00 and (b) 50.00 nM of Aβ(1–40) into a precoated channel with an Aβ(1–40) capture antibody, (c) curve (a) + 30.00 nM of Aβ(1–16) detection antibody, and (d) curve (a) + 30.00 nM of the detection conjugate. The arrow indicates the time when the injections were made and the flow rate was 10 μL min^{-1}. (Adapted from ref. 86 with permission. Copyright© 2010 American Chemical Society.)

concentration ratio between Aβ(1–40) and Aβ(1–42) in CSF samples from AD patients has been found to be almost twice as high as that from healthy donors. Using an array of Ag nanotriangles, Van Duyne's group developed a localized SPR for sensitive detection of amyloid-β derived diffusible ligands (ADDLs) in both CSF and brain extracts.[87] The SPR biosensors can potentially serve as a viable alternative to gain a better understanding of AD pathology and for AD diagnosis.

3.2 Tau proteins

Tau proteins promote assembly and maintain structural integrity of microtubules, support axon outgrowth, and regulate transport of vesicles and organelles.[88] Levels of tau have been found to be increased in CSF of AD individuals compared to those in age-matched controls, probably due to neuronal and axonal degeneration or accumulation of neurofibrillary tangles

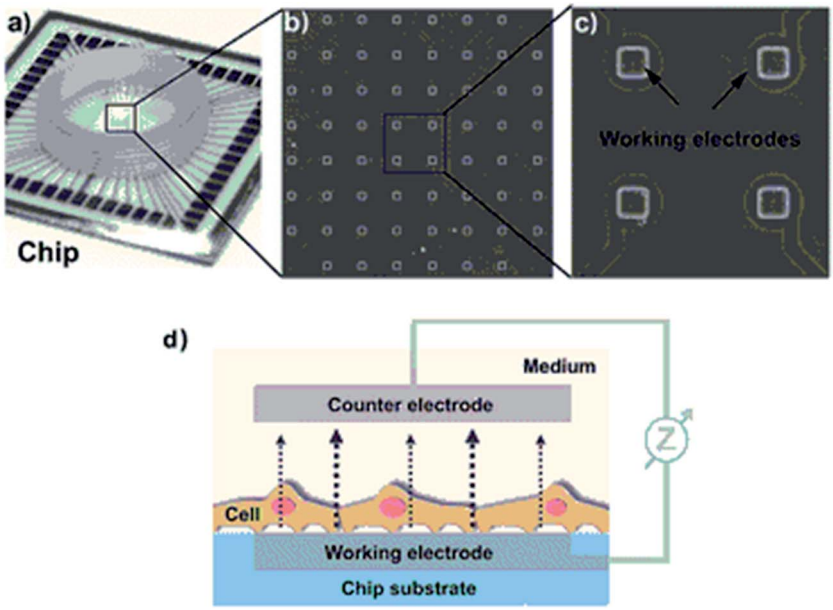

Fig. 5 Setup and measuring principle of cells by microelectrode-based impedance spectroscopy. (a) Microelectrode sensor chips comprise of a ring-like culture reservoir for maintaining cells onto microelectrodes. (b) Signals from 60 transparent indium tin oxide (ITO) can be recorded at once. (c) Each of the squared electrodes have sizes of 40 μm × 40 μm. (d) For recording impedance signals, a small alternating voltage (10 mV) is applied. The resulting alternating current is frequency-dependent and flows from a working electrode through or between the cells to an opposite counter electrode. (Adapted from ref. 90 with permission. Copyright© 2009 RSC Publishing.)

(NFTs).[89] Jahnke *et al.* reported a microelectrode-based impedimetric array sensor for label-free detection of hyperphosphorylated tau in the human neuroblastoma cell line, SH-SY5Y (Fig. 5).[90] Such a method could offer fast and sensitive testing of the efficiency of tau kinase inhibitors *in vitro*. Vesterdgaard *et al.* reported on multi-spot localized SPR detection of tau protein with a detection limit of 10 pg mL^{-1}, which is lower than the typical concentration of tau (around 195 pg mL^{-1} in CSF).[91]

3.3 Aβ-binding proteins

It has been suggested that Aβ aggregation and polymerization are reduced by a broad range of Aβ-binding proteins, including β-trace/prostaglandin D2 synthase (β-trace),[92] transthyretin (TTR),[93] cystatin C (CysC)[94] and apoE.[95] Aβ-binding proteins may play an important role in preventing amyloid formation or clearing preformed oligomers, which are believed to be more toxic than Aβ fibrils. Therefore, the concentrations of Aβ-binding proteins in body fluids are also indicative of the etiology of AD. As a powerful technique, SPR has also been utilized to detect the interaction between Aβ and Aβ-binding proteins to gain insight into the role of these proteins in the pathology of AD.[92,96,97]

Table 3 lists the dynamic ranges and detection limits of electroanalytical and SPR-based sensors for detection of the above-mentioned AD biomarkers.

4. Conclusions and outlook

A number of electroanalytical and SPR biosensors have been successfully developed for the detection of breast cancer and neurological biomarkers. Owing to the exceedingly low levels of many biomarkers, a variety of signal amplification schemes

Table 3 Dynamic ranges and detection limits of electroanalytical and SPR sensors for detection of select AD biomarkers

Biomarker	Technique	Dynamic range	Detection limit	Reference
Aβ(1–42)	Electroanalytical chemistry	Not specified	0.7 μg mL^{-1}	81
		10–3000 pg mL^{-1}	10 pg mL^{-1}	83
		100–300 μM	10 μM	84
	SPR	0.02–150.00 nM	3.5 pM	86
Aβ(1–40)	Electroanalytical chemistry	Not specified	0.7 μg mL^{-1}	81
		74.9–5344.4 pg mL^{-1}	74.9 pg mL^{-1}	83
	SPR	1.0–10^9 pg mL^{-1}	1.0 fg mL^{-1}	85
		0.02–150.00 nM	3.3 pM	86
Tau	Electroanalytical chemistry	Not specified	Not specified	90
	SPR	Not specified	10 pg mL^{-1}	91

have been used. For such a purpose, functionalized nanomaterials are particularly powerful. However, most of the reported methods can only detect one analyte at a time. As mentioned in the Introduction, relying on the detection of a single biomarker is insufficient to reach a definite diagnostic conclusion of many cancers. Clinically, more reliable information can be gleaned from high-throughput analyses of multiple disease biomarkers. In this regard, multiplexed detection of different analytes can be conveniently performed with multichannel electrochemical cells with micro-fabricated and nanofabricated working electrodes. Such electrodes are becoming quite reliable, disposable, and convenient to use. Similarly, imaging SPR using multiple microfluidics-based channels or preprinted microarray chips has matured and can be readily implemented for multiplexed sensing. We envision that more applications of these devices for simultaneous detection of multiple biomarkers will appear in the coming years.

As stated earlier, both electroanalytical and SPR-based sensors operate with recognition or detection events occurring at the surface–sample solution interface (*i.e.*, heterogeneous sensors). As a consequence, overcoming non-specific adsorption of interferents in complicated sample matrices (*e.g.*, blood samples) is paramount, as uncontrolled adsorption can produce false-positive results and in severe cases can even completely "foul" the SPR chips or electrode surfaces. Fortunately, a number of antifouling molecules or films (*e.g.*, dextran, chitosan, polyethylene glycol, *etc.*) have proven to be effective in eliminating or significantly reducing adsorption of interfering species. In this aspect, implantation of these molecules to SPR chips is generally more straightforward than to electrode surfaces, as the attachment of antifouling molecules to electrochemical sensors could potentially block or impede electron transfer between the redox label and the underlying electrode. Another problem inherent in heterogeneous biosensors is the variability from sensor to sensor. It is not difficult to imagine that reproducibility in fabricating sensors with uniform coverage of probe molecules and well controlled surface orientation is key to the quality of the ultimate clinical results. Validation of diagnoses of cancers and neurological disorders by different laboratories or hospitals requires that identical responses be obtained for the same samples. These two major challenges are perhaps the most serious roadblocks for electrochemical and SPR-based sensing to be accepted as standard clinical methods. We should mention that many analytical figures of merit can be improved by combining SPR with electrochemistry or coupling either with other important analytical techniques (*e.g.*, HPLC and mass spectrometry). We envisage that more biomarker detections will be achieved with such hybrid techniques involving either SPR or electrochemistry.

Finally, for most, if not all, of the existing bioanalytical methods to be commercially viable, many clinical trials and tests must be conducted to establish their robustness and reliability. Thus far, most of the published or even patented methods are evaluated only with limited sample pools for proof-of-concept purposes. The ultimate goal is to apply these methods to clinical assays and point-of-care testing. With the ongoing intensive effort as well as the advancement of nanotechnology, fabrication methods (including reliable mass production of chips and disposable electrodes), and electronics (for reducing noise and enhancing robustness), we believe that simple, reliable, and cost-effective analytical devices will eventually be available for clinical diagnosis of various diseases.

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