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REVIEW

DNA aptamer-based detection of prostate cancer

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The use of aptamers in biosensing has attracted considerable attention as an alternative to antibodies because of their unique properties such as long-term stability, cost-effectiveness and adjustability to various applications. Among cancers, the early diagnosis of prostate cancer (PCa) is one of the greatest concerns for ageing men worldwide. One of the most commonly used biomarkers for PCa is prostate-specific antigen (PSA), which can be found in elevated levels in patients with cancer. This review presents the gradual transition of research from antibody-based to aptamer-based biosensors, specifically for PSA. A brief description on aptamer-based biosensing for other PCa biomarkers is also presented. Special attention is given to electrochemical methods as analytical techniques for the development of simple, sensitive and cost-effective biosensors. The review also focuses on the different surface chemistries exploited for fabrication and their applications in clinical samples. The use of aptamers represents a promising tool for the development of point-of-care biosensors for the early detection of prostate cancer. In view of the unmatched upper hand of aptamers, future prospects are also discussed, not only in the point-of-care format but also in other novel applications.

Keywords: DNA aptamer, biosensor, electrochemical detection, prostate-specific antigen, prostate cancer, surface chemistry

Introduction

Prostate cancer (PCa) is a type of cancer that develops in the prostate gland, which is a part of the male reproductive system. PCa is the most commonly diagnosed cancer amongst men in Europe and the United States and is the second highest cause of cancer-related morbidity worldwide. It has been reported that PCa is predominant in older men above the age of 50 (Kirk, 1997; Hoffman, 2011) and among black men (Stanford et al., 1999; Greenlee et al., 2000). It has been also projected that PCa will be the commonest cancer in the UK by 2030 (Greenlee et al., 2000; Jeong et al., 2010).

Most PCas are generated in the epithelium cells (Bostwick, 1989). As androgens regulate the cell division of the gland epithelium (Ross et al., 1998), these hormones are believed to be the main cause of PCa. However, no study demonstrating a consistent correlation between androgens and prostatic carcinogenesis has been reported to date and the precise causes that lead to PCa remain inadequately understood (Kufe et al., 2003).

PCa often develops very slowly and the absence of symptoms during the early stages of the disease leads to late diagnosis of the tumour. Moreover, if diagnosed at a late stage, no effective treatments are currently available. In many cases, PCa does not show any clinical manifestation during the lifetime of a patient, who might die for non-related PCa causes. However, for those patients that develop a more aggressive cancer form, PCa cells can break away from a prostate tumour and metastasise. Since the prostate is well-connected to numerous lymph nodes, the spread is easy and some of the most common sites of the PCa metastatic process are bones (Chou & Simons, 1997).

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Current detection methods

There is no single test for the diagnosis of PCa. Moreover, all the tests currently used to diagnose have pros and cons which are usually discussed by the doctors with their patients. The methods most commonly used for PCa detection are: digital rectal examination (DRE), transrectal ultrasound (TRUS), biopsy and PSA blood test.

In DRE, a doctor inserts a gloved finger into the rectum and examines for bumps or swelling of the prostate gland. It is an inexpensive method and can also detect PCa irrespective of changes in the level of prostate specific antigen (PSA) in blood. Accuracy of the diagnosis can be increased when DRE is combined with PSA tests and biopsy results (Uzzo et al., 1995; Basler & Thompson, 1998; Jeong et al., 2010). In comparison with DRE, in the TRUS method an ultrasound probe is inserted into the rectum, emitting energy sound waves to image the prostate gland. It is a very useful tool for understanding the pathology of tumours and in guiding needle biopsies for sampling of tissue (Aus et al., 1996; Irani et al., 1997). For a biopsy, a small section of the tissue is removed through the rectum using a needle and is examined by pathologists microscopically. It requires a high number of samples from the prostate making it a painful protocol. Not only are the results from biopsies controversial, there is also a high risk of severe infections with subsequent biopsies (Jeong et al., 2010; Loeb et al., 2013).

The test most frequently used for PCa screening is quantification of the levels of PSA in blood. If PSA levels are higher than the cut-off levels of 4 ng mL^{-1}, biopsy procedures are considered (Catalona et al., 1991, Jeong et al., 2010; Savory et al., 2010). However, the levels of PSA in blood in ageing men can also be raised due to other factors such as benign prostatic hyperplasia (BPH) and prostatitis, which could lead to an over-diagnosis in men (Carter et al., 1992). Consequently, due to faulty diagnosis, patients undergo biopsy surgery, making PSA testing a controversial diagnostic tool. Due to these controversies with PSA testing, in May 2012 the US Preventative Services Task Force recommended against PSA screening in all men. This emphasised the need for more reliable biomarkers for diagnosis of the disease (Moyer, 2012).

Prostate specific antigen (PSA): PCa biomarker

PSA belongs to the family of kallikrein proteins defined as serine proteases. Approximately 15 kallikrein family members have been identified in humans. PSA is the only kallikrein specific to prostate (hK3), although pancreatic renal kallikrein (hK1) and human glandular kallikrein (hK2), which are androgen regulated, are also expressed in the prostate (Balk et al., 2003).

PSA is synthesised in its inactive form: a 244-amino acid long protein denoted as pro-PSA. Pro-PSA is cleaved from the N terminus in the prostate by the hK2 enzyme leading to active PSA which is a 237-amino acid long protein (Takayama et al., 1997). The active PSA is a 30 kDa protein which can be found in both semen and serum of men. PSA is present in semen in the range of 0.5–2 mg mL^{-1} and its physiological role is to de-coagulate semen by breaking down the proteins semenogelin I and II (Lilja et al., 1987; Lövgren et al., 1999). In prostate cancer, there is release of both active PSA and pro-PSA due to rupture of the basal membrane. Moreover, internally cleaved forms of PSA (with no enzymatic activity) also enter the bloodstream but remain un-complexed and are taken into the free PSA (fPSA) count. However, when active PSA enters the bloodstream, it immediately forms complexes with protein inhibitors. Most of the assays employing antibodies measure the total amount of PSA (tPSA) (Takayama et al., 1997).

Many studies have reported that PSA levels are directly proportional to the stage of the cancer and to the volume of the tumour (Stamey et al., 1987; Grossklaus et al., 2002; Pinsky et al., 2007; Lilja et al., 2008). Nowadays PSA detection results are highly sensitive (Madu & Lu, 2010) and reasonably inexpensive. Moreover, PSA testing is a procedure more readily accepted by patients than DRE and this has augmented the early detection of PCa (Balducci et al., 1997). However, even though PSA testing induced a decrease in PCa mortality of 20 %, its screening led to over-diagnosis and over-treatment (Andriole et al., 2009) of patients who would have not been clinically affected by the tumour during their lifetime. Over-diagnosis can, in fact, lead to unnecessary treatments and increase the state of anxiety in patients. Conversely, clinicians are not currently able to discriminate between a harmless or a lethal form of prostate cancer and so to decide whether the patient needs treatment. Once a prostate cancer has been definitively treated, PSA screening is the most reliable and rapid means enabling detection of a contingent recurrence of the tumour (Lilja et al., 2008).

Given the shortcomings of the current tests for PCa, including PSA testing, there is a concerted effort to look for alternatives. However, it would be a challenge to replace PSA entirely due to its minimally invasive nature and low cost. Instead, there is a pressing need to look for other biomarkers to complement PSA that can increase the specificity and sensitivity of PCa screening and inform the prognosis and treatment courses.

One path currently being considered when a high level of PSA is detected in patients with cancer is to differentiate PSA into different forms, namely free PSA (fPSA) and total PSA (tPSA) and to quantify them individually. One approach is to measure the ratio of free PSA to total PSA in the blood. It has been

proven that the levels of fPSA are lower in patients with PCa than in patients with BPH (Christensson et al., 1993); hence, this can be an indication of the aggressiveness of the cancer. However, the method can cause false negative results as the amount of fPSA can be higher in patients with a larger prostate volume (Stephan et al., 1997; Catalona et al., 1998). Nevertheless, the ratio of free to total PSA when combined with the total PSA levels increases the confidence of the diagnosis (Velonas et al., 2013).

Pro-PSA

Several studies are also focused on the detection of a distinct form of free PSA, denoted as proenzyme PSA (pro-PSA). Pro-PSA is an enzymatically inactive precursor of PSA obtained by the co-translational removal of an amino-terminal leader. The N-terminal of pro-PSA can be cleaved at various positions resulting in different forms of pro-PSA. Pro-PSA truncated between the third and second amino acid is denoted as [–2]pro-PSA and is believed to provide a better discrimination between cancerous and benign forms of prostate disorders (Mikolajczyk et al., 2001, 2004). Increased values of other forms of pro-PSA ([–5] and [–7]) have also been associated with PCa. A truncated precursor form of a prostate-specific antigen is, therefore, a more specific serum marker of prostate cancer.

PSA density

A better discrimination of BPH from PCa might be achieved by measuring the ratio of PSA to prostate volume. However, this parameter denoted as PSA density provided contradictory evidence on the tumour aggressiveness and malignity (Stamey et al., 1987; Ohori et al., 1995). Furthermore, in order to obtain prostate volume values, TRUS is required in addition to the standard PSA test with a consequent discomfort for patients as well as an increase in the cost and time required to perform the test. For these reasons, PSA density has not been extensively employed as a routine test for PCa.

PSA velocity and PSA doubling time

PSA velocity refers to the rate of serum PSA increase over time while PSA doubling time refers to the time required for a given PSA level to be doubled. As with the previous PSA derivatives, PSA velocity also can be used to distinguish a prostate cancer from a BPH (Carter et al., 1992). Both PSA velocity and PSA doubling time are used to monitor the recurrence of the tumour after treatment (D'Amico et al., 2004, 2005). Again, some studies have compared the responses from PSA velocity and PSA doubling time with biopsy results demonstrating how these two PSA derivatives can fail the diagnosis (Melichar, 2012).

Age-specific PSA reference ranges

Since the level of PSA increases with the age of men, scientists have studied this correlation in order to obtain a median value of PSA for given age-ranges. By comparing the PSA level with the median PSA for that patient's age (age-specific PSA), a better choice might been made prior to ordering biopsies (Loeb & Catalona, 2007).

Oligonucleotide aptamers

In recent years, a range of assays for PSA detection such as electrochemical assays (Okuno et al., 2007; Panini et al., 2008), enzyme-linked immunosorbent assays (Acevedo et al., 2002), cantilever assays (Wee et al., 2005), and chemiluminescent immunoassays (Albrecht et al., 1994; Seto et al., 2001) have been developed. These assays are mostly based on the use of antibodies as recognition elements. One of the alternatives to antibodies is aptamers which can offer several advantages over the former. However, an extensive research is being carried out to prove whether antibodies can be replaced by aptamers to develop a real biosensor for clinical applications. The scope of this review is to highlight the major developments on PSA aptasensors and their potential for use with real clinical blood samples.

Oligonucleotide aptamers are single-stranded DNA or RNA sequences that can bind to a target molecule with high specificity and affinity. Aptamers have already been widely used in drug delivery applications and are now being extensively studied as new emerging bioreceptors for biosensors (termed aptasensors) (Hianik & Wang, 2009; Iliuk et al., 2011). Aptamers have shown comparable or even stronger binding than antibodies towards a broad range of targets (e.g. proteins, peptides, amino acids, drugs, whole cells, etc.), especially with the development of novel selection technologies (Xiao et al., 2005). The high affinity of the aptamers towards the target molecule is defined by their capability to undergo conformational changes upon the binding event (Hermann & Patel, 2000; Song et al., 2008; Hianik & Wang, 2009). Although the use of aptamers has many advantages over antibodies, careful consideration is still needed when fabricating a biosensor. For instance, the binding of an aptamer to a protein might be affected by changing buffer conditions. Also, as aptamers are oligonucleotide sequences, special care is required as they are sensitive to DNase and RNase activity. Furthermore, the k_d value of aptamers is often not as good as that for antibodies.

Aptamers are developed using an in vitro selection process based on Systematic Evolution of Ligands by EXponential enrichment (SELEX) (Fig. 1). Briefly, this consists of three steps that are repeated systematically in order to identify the oligonucleotide sequence that binds to the target better. The first step

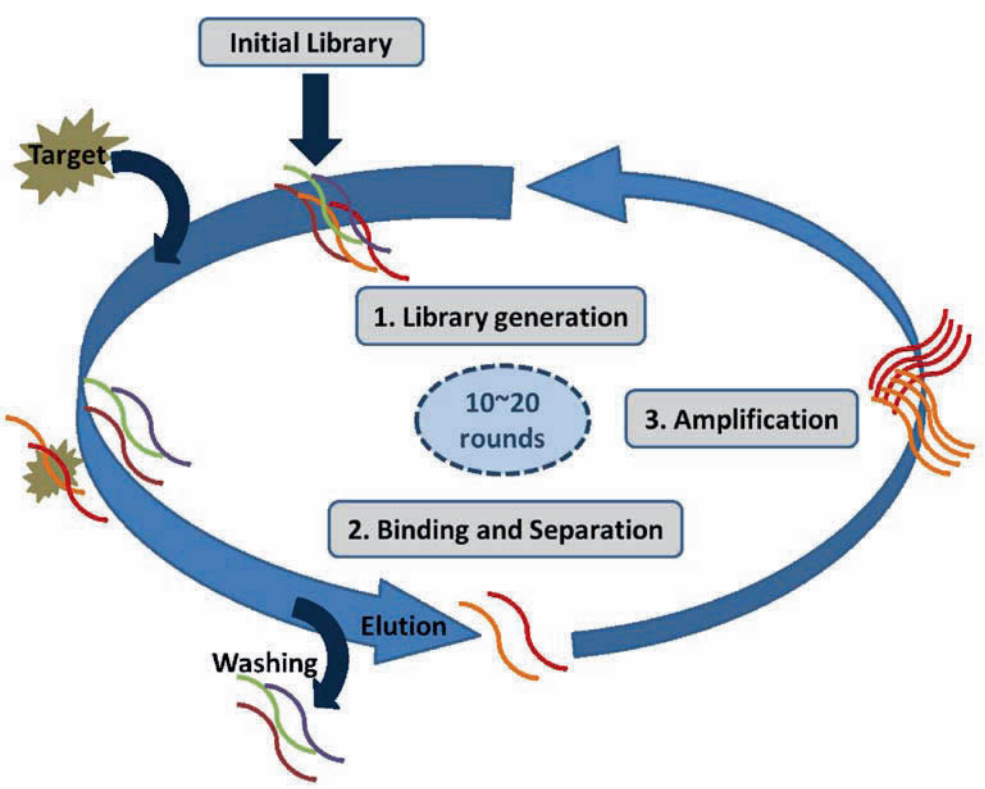

Fig. 1. General SELEX protocol. Starting with random library followed by incubation with the target. Later, the bound sequences are separated and further amplified for the next round of selection.

is known as library generation, where a library consisting of random DNA or RNA sequences (usually 30–40 base-pairs long) flanked by the primer binding site are used. The library is then incubated with the target molecule. Thereafter, the target-bound library is separated from the unbound library. Finally, the target-bound library is amplified using polymerase chain reaction (PCR) to create a new library for use in the next round. The aptamers' binding and conformation characteristics are identified using various biological assays (Syed & Pervaiz, 2010; Liu et al., 2012).

There has been intense interest in gaining in-depth understanding of ligand-binding and the conformational properties of aptamers. Aptamers possess many advantages over antibodies, making them very important molecular tools for both diagnostics and therapeutics. For instance, the selection of aptamers is an in vitro process and they can be raised to a wide variety of targets ranging from small molecules and toxins to large proteins and even whole cells. Secondly, aptamers, once selected, can be synthesised with high purity and reproducibility. Also, in comparison with antibodies, aptamers are usually highly chemically stable. Furthermore, they can undergo significant conformational changes in their structure upon binding with the target – a feature which can be exploited for biosensing applications. This offers great flexibility in designing novel biosensors (Clark & Remcho, 2002; Tombelli et al., 2005; Willner & Zayats, 2007; Mairal et al., 2008; Song et al., 2008; Liu et al., 2012).

PSA detection

Currently, PSA is detected in dedicated laboratory settings using automated analysers running antibody-based assays which are generally expensive and time-consuming (Lin & Ju, 2005; Healy et al., 2007). Cost-effective, easy to use and possibly portable devices are required to afford more powerful tools for the early detection of prostate cancer. To date, researchers have exploited several techniques for PSA detection such as optical (Besselink et al., 2004; Huang et al., 2005; Cao & Sim, 2007), piezoelectric (Weeks et al., 2003; Wee et al., 2005) and electrochemical (Sarkar et al., 2002; Fernández-Sánchez et al., 2004; Liu et al., 2013).

Although label-free-based biosensors can provide many advantages, label-based approaches are still intensively studied and can offer interesting features such as low limit of detection due to amplification strategies. An interesting magnetic bead-based detection system for PSA detection was developed by Zani et al. (2011): paramagnetic microparticles were adsorbed on an array of screen-printed electrodes and the PSA was sandwiched between two antibodies on the beads; the alkaline-phosphatase-labelled secondary antibody could be detected with differential pulse voltammetry (DPV) to achieve a limit of detection of 1.4 ng mL^{-1}. A limit of detection as low

Table 1. Performance comparison of different aptasensors for PCa detection

Method	Material	Biomarker	Limit of detection	Reference
QCM-D/EIS	Gold	PSA	–	Formisano et al. (2014)
EIS	Gold	PSA	1 ng mL^{-1}	Jolly et al. (2014)
Optical	AuNPs	PSA	32 pg mL^{-1}	Chen et al. (2012)
DPV/CV	AuNPs@GMCs	PSA	0.25 ng mL^{-1}	Liu et al. (2012)
EIS	Gold	PSMA cells	–	Min et al. (2010)

as 0.5 pg mL^{-1} in undiluted serum samples was obtained by Mani et al. (2009) by combining a multi-enzyme-labelled immunoassay with a sensing surface using gold nanoparticles: in this case the secondary antibody was bound to micromagnetic HRP-labelled beads, which massively amplified the current signals for a very low PSA limit of detection. A similar detection technique was improved and integrated in a microfluidic system by Chikkaveeraiah et al. (2011) achieving an even lower limit of detection. A remarkable electrochemiluminescence-based immunoassay was developed by Sardesai et al. (2011) for both PSA and interlukin 6 (IL-6) by using single-wall carbon nanotubes (SWCNT) fabricated on microwells and a sandwich assay where the secondary PSA antibody was functionalised with RuBYP-Silica particles: the limit of detection achieved was 1 pg mL^{-1} for PSA.

Label-free electrochemical sensors for PSA detection

Electrochemical techniques are widely employed in biosensing devices as they can be highly sensitive, simple to use and cost effective. An electrochemical biosensor involves an electrode surface that is functionalised with a molecular recognition element for sensing biomolecules. The binding of an analyte to this element results in an electrical change in current transfer (amperometric), voltage (potentiometric and field effect transistors), impedance (impedimetric), conductivity (conductometric) or ion charge across the electrode, which can be quantified and correlated to the amount of analyte captured. As stated previously, most of the biosensors for PSA detection currently available are antibody-based. Amongst the antibody-based electrochemical sensors, the particularly important results are those obtained by using label-free systems. Arya & Bhansali (2012) developed a gold biosensor modified with a cysteamine self-assembled monolayer (SAM) for PSA detection. Li et al. (2005), on the other hand, employed In_2O_3 nanowires and carbon nanotubes. Electrochemical impedance spectroscopy (EIS)-based sensors have been reported by Chiriacň et al. (2013) and Chornokur et al. (2011). The former exploits a combined use of two different antibodies for both free and total PSA, while the latter reported on a miniaturised sensor obtained with photolithographic techniques using a single monoclonal antibody. Another label-free antibody-based sensor which uses a polycrystalline silicon field-effect transistor was reported by Huang et al. (2013).

Aptasensor for PSA detection

An aptasensor biosensor comprises an aptamer as a biorecognition element (Lim et al., 2009). Aptasensors can be integrated with different sensing techniques, such as electrochemical, optical and mass-sensitive techniques. Among these, electrochemical aptasensors have been fabricated using several detection techniques, namely EIS, potentiometry and differential pulse voltammetry (DPV) (Clark & Remcho, 2002; Ikebukuro et al., 2005; Xu et al., 2005; Wang et al., 2007; Feng et al., 2008; Numnuam et al., 2008; Cho et al., 2009; Liu et al., 2012). For the detection of PSA, both RNA and DNA aptamers have been developed, although there are only a few reports on PSA biosensors using aptamers. A summary of aptamer-based biosensors for PCa detection is presented in Table 1.

The first aptamer developed was a RNA aptamer (Jeong et al., 2010) that was used to demonstrate the recognition of active PSA. Following that, a DNA aptamer was developed using a genetic algorithm with post-SELEX screening against PSA (Savory et al., 2010). To date, the literature contains no reports on the application of RNA aptamers for PSA biosensing; this could be due to the long length of the sequence rendering it difficult to be synthesised commercially.

The DNA-based PSA aptamer has been combined with different sensing techniques with sensitivities ranging from pg mL^{-1} to ng mL^{-1}. Chen et al. (2012) were the first to report the use of a PSA aptamer to develop an optical-based aptasensor. The conjugation of gold nanoparticles (AuNPs) with DNA aptamers was used to develop an aptasensor based on a resonance light-scattering (RLS) spectral assay. The novel technique relied on changes in resonance light-scattering on binding of the PSA to the aptamer, with a limit of detection of 32 pg mL^{-1}. Thiolated DNA aptamers were immobilised on AuNPs and then a blocking step with BSA was performed prior to use of the complex AuNPs–aptamers with PSA samples. In this configuration, the gold surface of the nanoparticles was covered with the flexible aptamer structure, hence no aggregation of particles occurred in the absence of PSA.

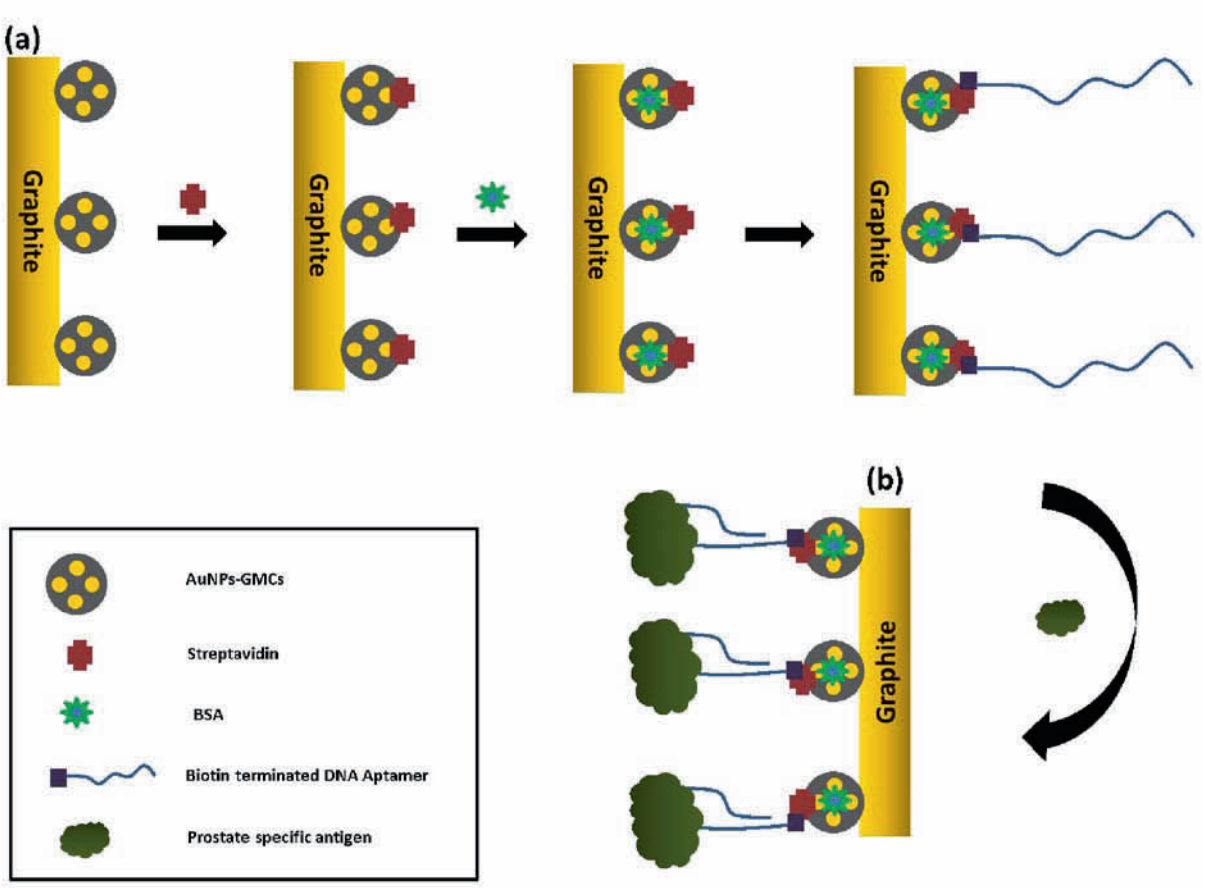

Fig. 2. Schematic illustration of fabrication process of aptasensor based on gold nanoparticles encapsulated by graphitised mesoporous carbon (a); PSA detection (b).

In the presence of PSA, aptamer–PSA complexes were formed and the aptamers underwent a conformational change in their structure from flexible to rigid. The changes in the aptamer conformation exposed some parts of the AuNPs that were thus available to form AuNPs aggregates upon the addition of potassium chloride. This resulted in an increase in the RLS signal. The assay exhibited good sensitivity and selectivity towards PSA and tests on human blood samples showed results comparable with those obtained with ELISA (relative deviation < 7 %).

With regard to the electrochemical aptasensor, modification of the electrode surface is one of the largest fields of investigation. Typically, research is focused on finding the most suitable recognition platform to give a stable organisation to the sensor interface, leading to optimised binding efficiency and signal outcome (Lee et al., 2005; Putzbach & Ronkainen, 2013). Liu et al. (2012) applied aptasensors based on amplification via AuNPs and graphitised mesoporous carbon (GMCs) combined with the streptavidin-biotin system for electrochemical detection of PSA (see Fig. 2). GMCs-encapsulated AuNPs formed the first layer on the cleaned pyrolytic graphite electrode followed by coating with streptavidin. All the non-specific sites were blocked with bovine serum albumin (BSA). Finally, biotinylated DNA aptamers were allowed to react with streptavidin immobilised on the electrode surface. The fabricated aptasensor was then used to capture the PSA which was measured via differential pulse voltammetry (DPV). The limit of detection of the aptasensor was 0.25 ng mL^{-1} with high specificity to the PSA. Despite the high sensitivity and specificity, the fabrication procedure entails a layer-by-layer development of the sensor surface is quite complex, which may be a drawback in fabricating a cost-effective sensor. The group also used Electrochemical Impedance Spectroscopy (EIS) to characterise the layer-by-layer fabrication of the aptasensor.

Electrochemical Impedance Spectroscopy is one of the most promising electrochemical techniques for DNA-based approaches but requires a careful design in order to optimise its signal. The formation of a well-organised self-assembled monolayer (SAM), which allows an optimal charge transfer to occur, is particularly important for EIS biosensors. For successful EIS measurements, it is necessary to have a good and reliable SAM layer on the gold electrode surface. One approach to this goal in general use is via alkanethiol chemistry. Alkanethiols can be eas-

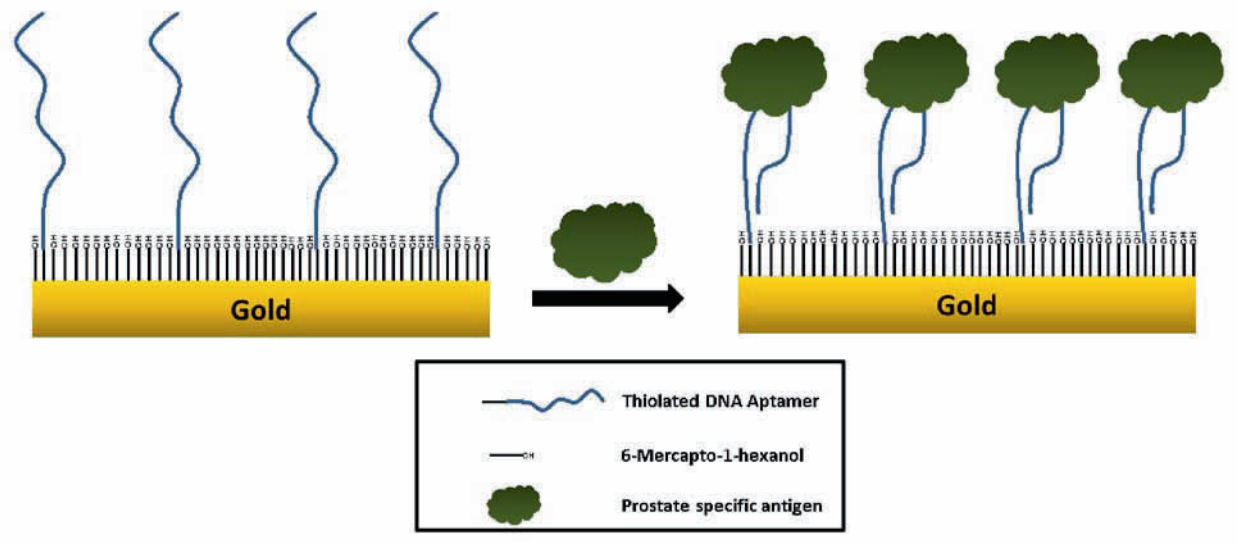

Fig. 3. Schematic illustration of fabrication process of aptasensor with 6-mercaptohexanol and thiolated DNA aptamer.

ily adsorbed and form SAMs (Love et al., 2005) on a clean gold surface through thiol bonds (see Fig. 3). It has been reported that longer alkane-chains give a more compact structure with minimal defects (Campuzano et al., 2006). Among different configuration of SAM, a mixed SAM of 11-mercaptoundecanoid acid (MUA), $HS(CH_2)_{10}COOH$, and 6-mercapto-1-hexanol (MCH), $HS(CH_2)_6OH$ exhibited reasonable starting impedance values and improved reliability (Herne & Tarlov, 1997). In order to gather or enhance the extent of a measurable signal of the recognition event occurring on the working electrode, marker molecules such as redox couples are exploited. The recognition events that occur on the SAM not only modify the charge transfer processes between redox couples present in the measurement solution and the sensor surface but also affect the double layer at the sensor interface. Both these events cause a change in the system charge transfer resistance (R_{ct}) which can then be quantified by using an appropriate equivalent circuit.

In EIS measurements using PSA aptamers, Jolly et al. (2014) and Formisano et al. (2014) reported a reduction in charge transfer resistance (R_{ct}) upon binding of the PSA to the immobilised DNA aptamers. This decrease conflicts with what has been reported in the literature for PSA, where an increase in R_{ct} was observed (Liu et al., 2012), even though these studies used EIS mainly to characterise the bio-recognition layer and not for dose response determination. A reduction in R_{ct} upon the aptamer–analyte interaction has also been reported for a different aptasensor using a lysozyme aptamer, where the reduction in charge transfer resistance upon binding of the lysozyme to its specific DNA aptamer was attributed as mainly due to the screening of charges on DNA (Rodriguez et al., 2005). The reduction in R_{ct} could arise for two reasons: firstly, upon binding, the PSA might screen the charges of the DNA aptamer; secondly, as PSA is also a charged protein, it could be that more positive charges are exposed because of the protein architecture itself. Consequently, as there is screening of the charges of DNA, there is a reduction in the electrostatic barrier to the ferro/ferricyanide anions towards the electrode surface, leading to lowering of the R_{ct} value of the system.

Earlier reports on DNA detection using DNA (Keighley et al., 2008a) and PNA probes (Keighley et al., 2008b) demonstrated the importance of optimisation of the oligonucleotide probe surface coverage in order to achieve efficient binding. On the same grounds, Formisano et al. (2014) first investigated the importance of optimisation of surface coverage by DNA aptamer for efficient binding using Quartz Crystal Microbalance with Dissipation mode (QCM-D). This study sought to optimise the conditions of an EIS aptamer-based sensor for PSA detection. In fact, EIS optimisation for DNA aptamers is somewhat complex due to the different characteristics that induce a signal change: namely DNA density, change in charge density close to the electrode upon DNA conformational changes, size and charge of the analyte, screening of DNA charges upon analyte-binding. The use of QCM-D provided valuable information on the conditions for maximal analyte-binding as well as the hydration, folding and behaviour of the aptamer distribution on the electrode. The system comprised a gold surface functionalised with a mixed SAM composed of a DNA aptamer and MCH which was used as a spacer molecule. The best conditions in terms of buffer solution and aptamer mole fraction (concentration of aptamers/concentration of total thiols) for binding the PSA to the aptamers were obtained by comparing the data from two techniques under similar conditions. With regard to the buffer conditions, the study demonstrated how the DNA aptamers' behaviour exhibited a strong depen-

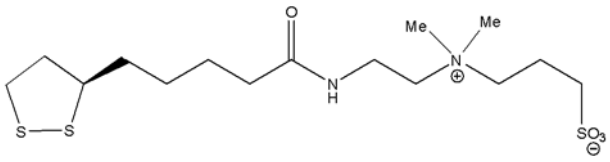

Fig. 4. Structure of thiol-terminated sulpho-betaine.

dence on the environment where it interacted with PSA.

In order to investigate an optimal surface chemistry that not only has a good anti-fouling effect but is also simple and cost-effective, a new molecule was investigated by Jolly et al. (2014) as a spacer molecule replacing MCH: a thiol-terminated sulpho-betaine (Fig. 4). This was the first report on a thiol-terminated sulpho-betaine application for an aptamer-based sensor. Thiol-terminated sulpho-betaine, which has a molecular mass of 398.6 g mole^{-1}, is a zwitterion due to the presence of both positive and negative charges with a flexible chain which makes it a good anti-fouling molecule (see Fig. 5). It been reported that sulpho-betaine not only reduces non-specific binding but also increases the sensitivity of the sensor (Bertok et al., 2013).

A comparison study between MCH and thiol-terminated sulpho-betaine thiol chemistry was carried out by monitoring non-specific binding using human serum albumin (HSA) as a control protein. A schematic representation of the fabrication protocol for surface chemistry with thiol-terminated sulpho-betaine is presented in Fig. 5. The co-immobilisation of 11-mercaptoundecanoic acid (MUA) and thiol-terminated sulpho-betaine formed the first SAM layer on clean gold electrodes. The carboxyl group of MUA was then activated with the conventional EDC/NHS-coupling reaction. The activated carboxyl groups were then used to immobilise amine-terminated DNA aptamers for PSA and finally the electrodes were

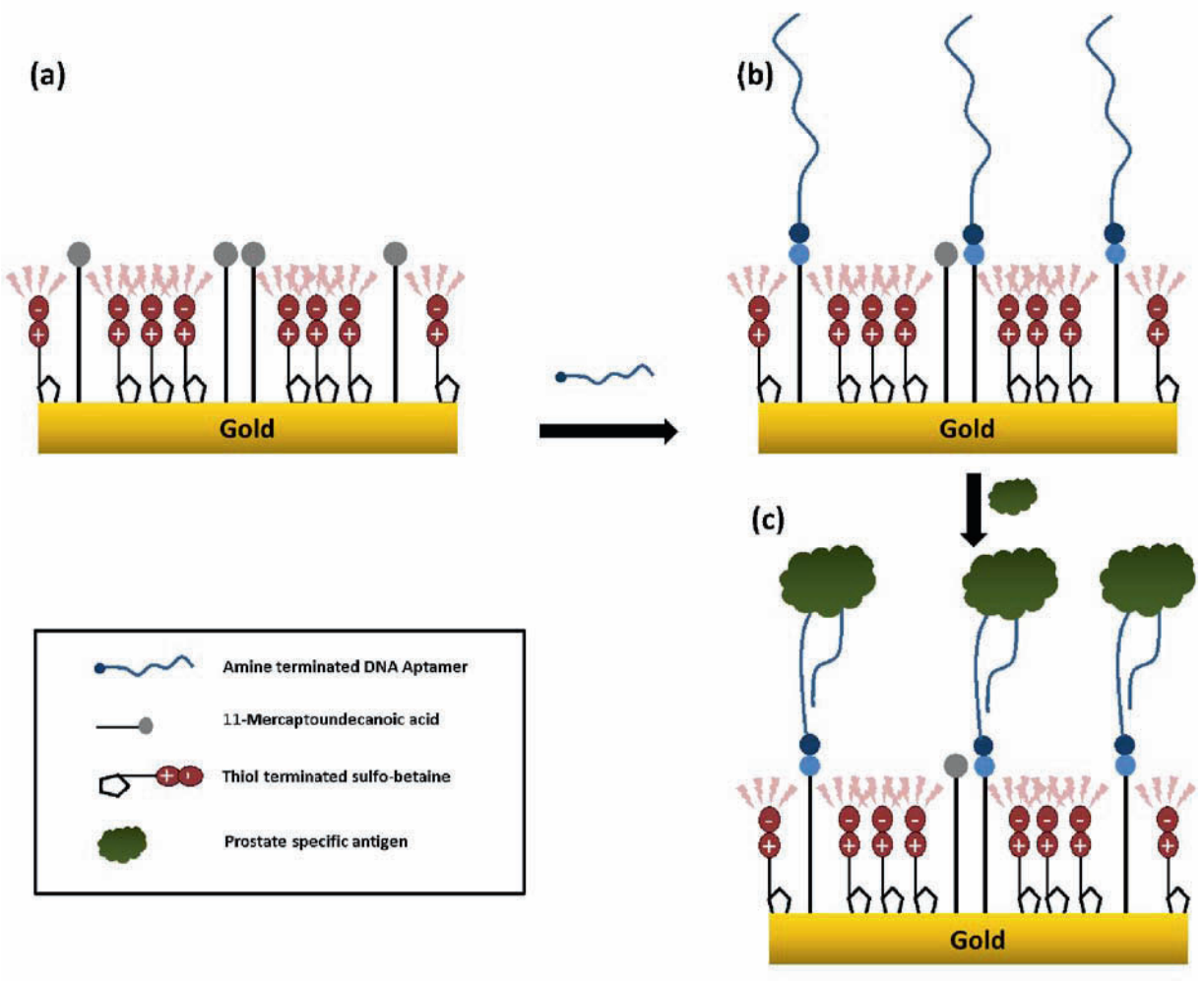

Fig. 5. Schematic representation of fabrication of thiol-terminated sulpho-betaine-based PSA aptasensor, consisting of co-immobilisation of 11-mercatoundecanoic acid and thiol-terminated sulpho-betaine (a), linking of amine terminated aptamer to SAM (b) and interaction of aptamer with PSA (c).

treated with ethanolamine to deactivate all the unreacted groups. The fabricated aptasensor with thiol-terminated sulpho-betaine surface chemistry can detected PSA levels down to 1 ng mL^{-1}, which falls within the lower clinical cut-off range of PSA in blood. The fabricated aptasensor with thiol-terminated sulpho-betaine also exhibited a significant reduction in the non-specific binding with HSA in comparison with the sensor where MCH was used instead as a spacer molecule. However, obstacles to the optimisation of the amount of DNA aptamers immobilised on the surface via EDC/NHS-coupling have also been reported. It was assumed that the charged thiol-terminated sulpho-betaine has an influence on the attachment of the DNA aptamer to the activated MUA via EDC/NHS-coupling, leading to a difference in the amounts of DNA aptamers in different electrodes fabricated under similar conditions.

Aptasensors for other PCa biomarkers

Besides PSA, other biomarkers for PCa are currently under investigation and can potentially be used for DNA/RNA-based detection systems. One is the prostate-specific membrane antigen (PSMA), which is a type II integral membrane glycoprotein found in human serum. It is overexpressed on prostate tumour cells and may play an important role in the progression of PCa. It can also differentiate between BPH and PCa (Feneley et al., 2000; Ghosh & Heston, 2004; Madu & Lu, 2010; Pircher et al., 2011). Furthermore, by analysing the expression of PSMA, two cell lines can be distinguished among PCa cells: PSMA (–) and PSMA (+) cells (Ghosh & Heston, 2004). Min et al. (2010) reported on an RNA/peptide dual-aptamer-based biosensor able to detect both PSMA (–) and PSMA (+) cells by using EIS. The biosensor consists of an anti-PSMA RNA aptamer (Lupold et al., 2002) which can target PSMA (+) cells and a DUP-1 peptide aptamer (Zitzmann et al., 2005) specific for PSMA (–) cells.

Another emerging biomarker is α-methylacyl-CoA racemase (AMACR), which is a racemic-type of protein found in urine and blood (Rubin et al., 2002; Jiang et al., 2003; Wu et al., 2004). Its function is to metabolise fatty acids in the human body. It is also overexpressed in PCa and can be detected with a high sensitivity and specificity with a cut-off value of 10.6 ng mL^{-1}. It also has the potential to differentiate between BPH and PCa. Currently, AMACR aptamers have been independently developed by Base Pair Biotechnologies, Inc. (aptamer AM310_2) and by Yang et al. (2013). However, no reports on their application to biosensing have been published to date.

Future prospects and conclusions

Recent work on the development of PSA aptasensors has made possible the transition from using antibodies to aptamers as a recognition layer. Surface modification plays an important role in the development of promising biosensors which would be aided by the ongoing revolution in fabrication techniques. Easier fabrication would enable these biosensors to be mass-produced and commercially viable. The trend towards the development of aptasensors for PSA requires further investigation into their use as an alternative to antibodies. Also, the sensitivity of an aptasensor is most likely to be influenced not only by the surface chemistry but also by the analytical method used for the detection of the target molecule; to date no PCa aptasensors have been used in complex samples such as blood. Overall, the development of aptamer-based biosensors is expected to attract increasing interest because of its ease of synthesis and the possibilities of multiple modifications. For early diagnosis of PCa, detection of different biomarkers would be preferred; consequently, more work is expected on the development of aptamers for different isoforms of PSA and other biomarkers of PCa. An ideal biosensor for PCa detection would be based on a parallel sensing of different biomarkers using an array of sensors for more accurate diagnosis. In addition to the need for a simple surface chemistry, the scope for biosensors in future point-of-care devices will largely depend on integration of the format into a device that will enable easy and simple sample-handling and an efficient read-out system with rapid and accurate sample analysis of minimal blood-sample volumes.

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