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Self-assembled gold nanoparticles for impedimetric and amperometric detection of a prostate cancer biomarker

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ABSTRACT

A label-free dual-mode impedimetric and amperometric aptasensor platform was developed using a simple surface chemistry step to attach gold nanoparticles (AuNPs) to a gold planar surface. As a case study, the strategy was employed to detect prostate specific antigen (PSA), a biomarker for prostate cancer. An anti-PSA DNA aptamer was co-immobilised with either 6-mercapto-1-hexanol (MCH) or 6-(ferrocenyl)hexanethiol (FcSH) for both impedimetric or amperometric detection, respectively. We show that the use of AuNPs enables a significant improvement in the limit of impedimetric detection as compared to a standard binary self-assembled monolayer aptasensor. A PSA detection of as low as 10 pg/mL was achieved with a dynamic range from 10 pg/mL to 10 ng/mL, well within the clinically relevant values, whilst retaining high specificity of analysis. The reported approach can be easily generalised to various other bioreceptors and redox markers in order to perform multiplexing.

1. Introduction

There is an increasing demand for simple, low-cost, reliable and rapid screening of biomarkers for the early detection of diseases such as cancer and this has led to a scurry of activity towards label-free biosensors. Removing the labelling step can provide significant savings in cost and time, making point-of-care sensing more viable than labelled alternatives. However, the removal of the label can lead to more difficult determination of the analyte due to non-specific interactions in complex media, resulting in decreased sensitivity and sometimes a system incapable of meeting the clinical requirements.

Given that a biosensor's signal is generally proportional to the surface coverage, most methods for increasing the sensitivity of label-free biosensors revolve around surface modifications to increase probe loading. Forming meso- and micro-porous surfaces with methods such as electrodeposition can provide increased surface area whilst still maintaining detection with low sample volumes. However, it is often easier and more controllable to increase surface area by anchoring nanoparticles to the surface. Nanoparticles may be formed from metals, oxides, semiconductors and conducting polymers, but it is the use of gold nanoparticles (AuNPs) which has attracted most attention for biosensing applications, in particular for biosensors based on optical and electrochemical transduction [1,2].

The wide adoption of AuNPs has in part been down to their excellent biocompatibility, conductivity, and catalytic properties. AuNPs offer an important structural surface, amplifying the resulting electrical response. They can act as electroactive intermediates between electrodes and solution and hence increase the sensitivity of biosensors. AuNPs offer also a suitable platform for multi-functionalization with a wide range of organic or biological ligands for the selective detection of small molecules and biological targets [3–5]. Whilst antibodies remain the molecular recognition workhorse of choice for many biosensing devices, their use can impose limitations on both technology adoption

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and resulting applications [6]. One long-championed alternative to antibodies has been DNA aptamers. DNA aptamers are short, stable oligonucleotide sequences possessing high affinity and specificity for particular targets. DNA aptamers have many advantageous properties compared to their biological antibody counterparts such as long-term stability, affordability and ease of development compared to antibodies [7,8]. They can also be regenerated without loss of integrity or selectivity [9] providing a platform to develop multi-use sensors. Aptamers are however prone to protein fouling in serum due to DNA binding proteins [10] which means that the surface chemistry should be considered to provide optimal performance.

The use of both AuNPs and aptamers for improved specificity and signal amplification have been previously demonstrated for electrochemical [11–13], optical [14,15] and mass-based [16] biosensors. In this work we show how a simple step to attach AuNPs to a planar gold surface results in a significant amplification of the biosensor response. The key focus of this work is to keep the number of fabrication steps to a minimum with low complexity. By doing this, we ensure a robust surface chemistry is achieved. Such an approach has been demonstrated by using a prostate cancer (PCa) biomarker as a case study. PCa is the most commonly diagnosed cancer amongst men worldwide. One of the key issues surrounding PCa diagnosis is that it develops very gradually over time and the absence of symptoms often results in late diagnosis of the tumour which puts pressing needs on the development of reliable and sensitive diagnostic platforms. Currently, changes in levels of prostate specific antigen (PSA), a biomarker for PCa, in the blood can be used for PCa screening; levels higher than the cut-off level of 4 ng/mL prompt biopsy procedures to be considered [17–20]. PSA is a 30–33 kDa serine protease secreted by the prostate gland. Despite well-documented controversies linked with PSA testing [21–23], PSA still remains the most commonly used biomarker for PCa screening, monitoring the effectiveness of treatment and post treatment [22,24].

Taking a previous system based on an impedimetric aptasensor which used a planar gold surface with co-immobilised DNA aptamer/capto-1-hexanol (MCH) probe layer [25], we show how sensitivity can be significantly improved by the addition of a single fabrication step to attach AuNPs to the planar gold electrode. As a result, we shift the limit of quantification from 60 ng/mL to 10 pg/mL, i.e. nearly 4 orders of magnitude improvement, so that it aligns with the clinically relevant range of 1–10 ng/mL. The fabricated aptasensor was successfully tested with spiked human serum samples and a detection of PSA as low as 10 pg/mL was achieved. Furthermore, simply by replacing MCH with 6-(ferrocenyl)hexanethiol (FcSH), a thiolated redox marker, during the co-immobilisation of the aptamer, the aptasensor could be similarly used for sensitive amperometric detection of PCa at clinically relevant concentrations. Such a dual-detection approach could potentially reduce false positives, providing additional validation of the signals.

2. Experimental

2.1. Reagents

Thiolated anti-PSA aptamer, 5′-HS-$(CH_2)_6$-TTT TTA ATT AAA GCT CGC CAT CAA ATA GCT TT-3′ and a random DNA sequence non-specific to PSA (5′- HS $-(CH_2)_6$-AAA AAT TAA TTT CGA GCG GTA GTT TAT CGA AA-3′) used as control DNA probe were obtained from Sigma Aldrich (UK). Prostate specific antigen (PSA) from human semen was obtained from Fitzgerald (MA, USA). Human serum albumin (HSA), human serum, 11-aminoundecanethiol hydrochloride, 6-mercapto-1-hexanol (MCH), 6-(ferrocenyl)hexanethiol (FcSH), potassium buffer saline tablets (pH 7.4), potassium hexacyanoferrate (III), potassium hexacyanoferrate(II), gold nanoparticles (20 nm, stabilized suspension in 0.1 mM PBS, reactant free) were all purchased from Sigma Aldrich (UK). All other reagents were of analytical grade. All aqueous solutions were prepared using 18.2 MΩ cm ultra-pure Milli-Q water with a Pyrogard® filter (Millipore, MA, USA). For binding studies, different concentrations of PSA were prepared in 10 mM PBS, pH 7.4. The specificity of the aptamer was evaluated by studying its interaction with 10 ng/mL HSA as a control protein dissolved in 10 mM PBS, pH 7.4. For experiments with serum, different concentrations of PSA were prepared in 1:10 diluted human serum (diluted in 10 mM PBS, pH 7.4). 10 times diluted human serum solution was further filtrated through a 0.22 μm pore filter.

2.2. Apparatus

The electrochemical measurements were performed using a μAUTOLAB III/FRA2 potentiostat (Metrohm Autolab, Netherlands) using a three-electrode cell setup with a Ag/AgCl reference electrode (BASi, USA) and a Pt counter electrode (ALS, Japan). The impedance spectrum was measured in 10 mM PBS (pH7.4) containing 4 mM ferro/ferricyanide $[Fe(CN)_6]^{3-/4-}$ in a frequency range from 100 kHz to 100 mHz, with a 10 mV AC voltage superimposed on a bias DC voltage of 0.2 V *vs.* Ag/AgCl. Cyclic voltammetry was performed in 10 mM PBS (pH 7.4) containing 10 mM ferro/ferricyanide $[Fe(CN)_6]^{3-/4-}$ and scanning the potential between −0.3 V to 0.5 V *vs.* Ag/AgCl. Square wave voltammetry was performed in 10 mM PBS (pH 7.4) in the potential range from −0.4 V to 0.65 V *vs.* Ag/AgCl with a conditioning time of 120 s, modulation amplitude of 20 mV and frequency of 50 Hz.

Surface characterisation of gold electrodes modified with gold nanoparticles was performed using a scanning electron microscopy (JSM-6480 Jeol, Japan) on gold evaporated chips at 100,000 × magnification with an acceleration voltage of 5 kV. Ambient contact mode (tapping mode) atomic force microscopy (AFM) imaging was carried out with a MultiMode NanoScope with IIIa controller in conjunction with version 6 control software (Bruker, Germany). Gold evaporated chips modified as described in the fabrication section for gold electrodes were imaged with a 10 nm diameter AFM ContAl-G tip (BudgetSensors®, Bulgaria), images were then processed by the NanoScope Analysis software, version 1.5.

2.3. Electrode preparation

Prior to functionalisation, gold disc working electrodes with a radius of 1.0 mm (ALS, Japan) were cleaned by mechanical polishing for 5 min with 50 nm alumina slurry (Buehler, UK) on a polishing pad (Buehler, UK) followed by 5 min sonication in ethanol and then in water. The electrodes were then subjected to chemical cleaning with piranha solution (3 parts of concentrated H_2SO_4 with 1 part of H_2O_2 for 5 min). The electrodes were then rinsed with Milli-Q water. Thereafter, electrodes were electrochemically cleaned in 0.5 M H_2SO_4 by scanning the potential between 0 V and +1.5 V *vs.* Ag/AgCl for 50 cycles until no further changes in the voltammogram were observed. After electrochemical cleaning, electrodes were extensively washed with MilliQ water to remove any acid residues. Finally, electrodes were cleaned with ethanol and were left to dry in an air-filtered environment for several minutes.

2.4. Sensor fabrication

An overview of the aptasensor fabrication for both impedimetric and amperometric determination is illustrated in Fig. 1. The protocol for the modification of planar gold electrodes with gold