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Label-free femtomolar cancer biomarker detection in human serum using graphene-coated surface plasmon resonance chips

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ABSTRACT

Sensitive and selective detection of cancer biomarkers is vital for the successful diagnosis of early stage cancer and follow-up treatment. Surface Plasmon Resonance (SPR) in combination with different amplification strategies is one of the analytical approaches allowing the screening of protein biomarkers in serum. Here we describe the development of a point-of-care sensor for the detection of folic acid protein (FAP) using graphene-based SPR chips. The exceptional properties of CVD graphene were exploited to construct a highly sensitive and selective SPR chip for folate biomarker sensing in serum. The specific recognition of FAP is based on the interaction between folic acid receptors integrated through π-stacking on the graphene coated SPR chip and the FAP analyte in serum. A simple post-adsorption of human serum:bovine serum albumin (HS:BSA) mixtures onto the folic acid modified sensor resulted in a highly anti-fouling interface, while keeping the sensing capabilities for folate biomarkers. This sensor allowed femtomolar (fM) detection of FAP, a detection limit well adapted and promising for quantitative clinical analysis.

1. Introduction

Analytical methods that measure cancer biomarkers (e.g. proteins over-expressed in blood and serum) with high sensitivity and selectivity have become widely accepted as diagnostic tools for the early diagnosis of cancer (Perfézou, et al., 2012; Wu and Qu, 2015; Yang, et al., 2014). Surface plasmon resonance (SPR) based sensing, relying on the binding between the biomarker in solution and a surface-linked receptor, has shown to be highly promising in early clinical diagnosis of cancer. This is due to its high-throughput screening ability of protein biomarkers (Chang et al., 2010; Krishnan et al., 2011; Piliarik, et al. 2010; Sim et al., 2010; Uludag and Tothill, 2012; Vance and Sandros, 2014; Xia et al., 2010; Yang et al., 2014). The use of aptamer and antibody receptors immobilized onto the SPR chip allowed for selective detection of different protein biomarkers in serum samples (Piliarik et al., 2010; Uludag and Tothill, 2012). As most biomarkers are present at very low concentrations in the early stage of cancer, SPR sensors need to show in addition to selectivity extremely high sensitivity towards the biomarkers. SPR sensing sensitivities ranging from picomolar down to zeptomolar were achieved using nanoparticle-based signal amplification strategies (Krishnan et al., 2011; Sim et al., 2010; Uludag and Tothill, 2012; Vance and Sandros, 2014). Uludag and Tothill reported 8.5 pM detection limit for prostate specific antigen (PSA) biomarkers by performing a sandwich assay using antibody-modified gold nanoparticles (Uludag and Tothill, 2012). Attomolar (300 aM) detection of PSA biomarkers in serum was reported by Rusling and co-workers using antibody-modified super paramagnetic particles for signal amplification (Krishnan et al., 2011). A similar strategy was used to achieve attomolar detection of immunoglobulin E (IgE) on aptamer-functionalized SPR interfaces using anti-IgE modified gold nanorods for signal amplification (Sim et al., 2010). More recently, the use of aptamer-modified quantum dots as SPR signal enhancer allowed the sensing of C-reactive proteins in serum with a zeptomolar detection limit (Vance and Sandros, 2014).

A different approach for achieving sensitivities required for the detection of protein biomarkers in serum could be based on the use of graphene-based SPR interfaces, shown to exhibit high sensitivity towards proteins and DNA (Singh et al., 2015; Subramanian et al., 2013; Wang et al., 2011; Zagorodko et al., 2014).

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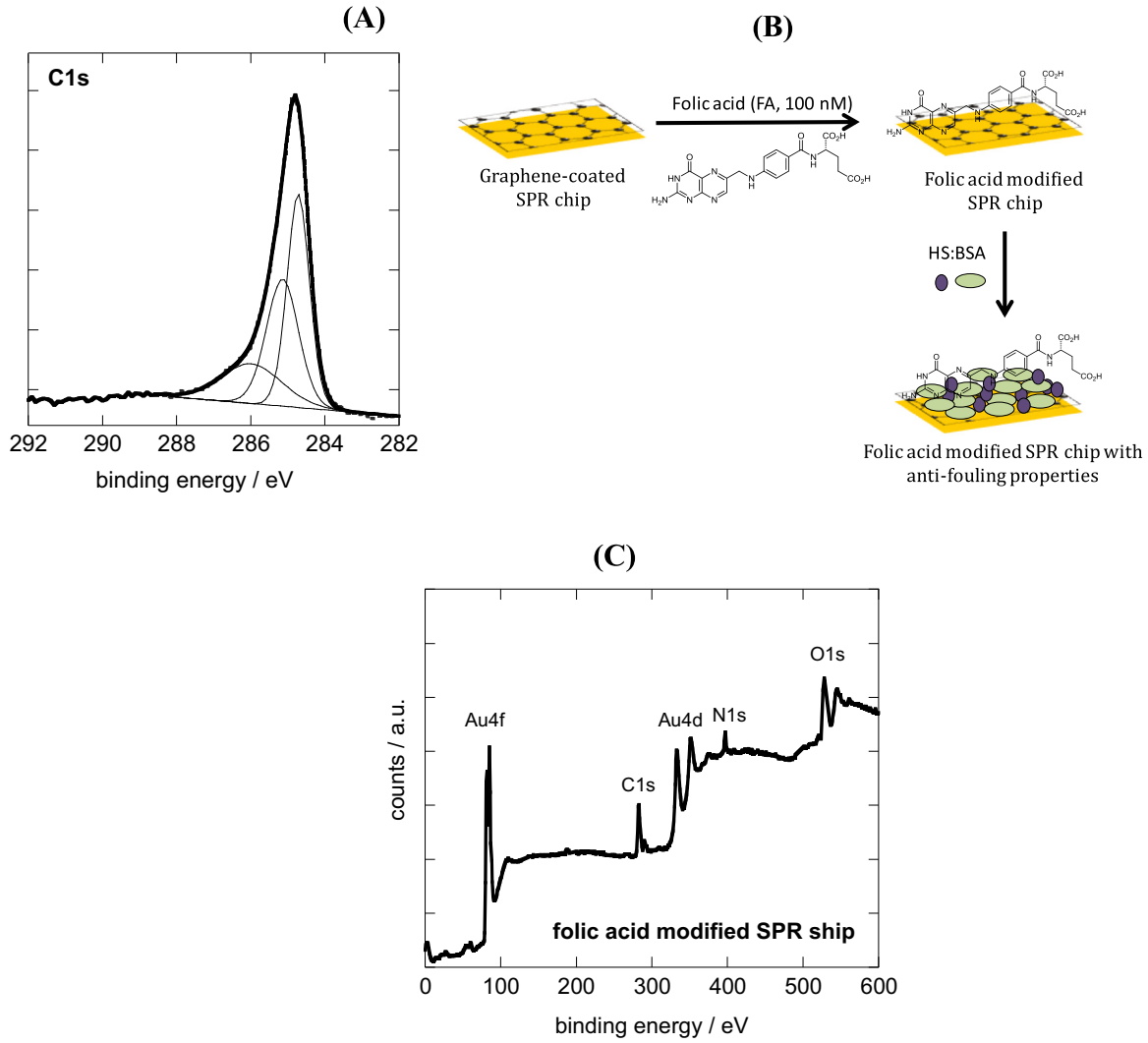

Fig. 1. (A) XPS C_{1s} high resolution core level spectrum of graphene-coated interface; (B) Schematic presentation of the formation of an anti-fouling folic acid modified SPR sensor; (C) XPS survey spectrum of folic acid modified graphene-coated SPR chip.

The excellent sensing properties of graphene-coated SPR chips are related to the spontaneous adsorption of hydrophobic domains or π-systems on graphene, enabling easy immobilization of the target bioreceptor units (Singh et al., 2015; Szunerits et al., 2013; Zagorodko et al., 2015). Monolayers of graphene on SPR interfaces have been used by us (Szunerits et al., 2013; Zagorodko et al., 2015, 2014) and more recently by Cosnier and co-workers (Singh et al., 2015) for the construction of sensitive DNA, protein and immuno sensors. However, the advantage of such interfaces for the sensitive detection of biomarkers in serum has not been addressed yet.

This study reports on the beneficial properties of graphene-based SPR for folic acid protein (FAP) sensing in human serum. Folic binding proteins, also known as folate receptors, are associated with numerous malignancies and are over-expressed in many human epithelial-derived tumors where levels as high as 22 pM can be reached (Eichner et al., 1978). Given that human serum is free of FAP, detection of FAP in serum has been used as a strategic target for the early state detection of cancer. A variety of different methods have been investigated for FAP detection such as fluorescence imaging and radio-labeled assays, which are rather costly and time consuming (Eichner et al., 1978; Jiang et al., 2015; Moon et al., 2003; Song et al., 2012). Quartz crystal microbalance (QCM) and electroanalytical approaches have been proposed as alternatives (Castillo et al., 2013; He et al., 2016; Henne et al., 2006; Jiang and Wang, 2014; Li et al., 2014; Wang et al., 2014; Wu et al., 2009) with detection limits in the picomolar range. SPR has been used for the determination of FAP in milk using a folic acid modified SPR interface (Nygren et al., 2003) with a detection limit of 2.63 nM and a linear range between 3 and 26 nM.

We show here that a femtomolar detection limit of FAP in human serum can be reached using anti-fouling folic acid modified graphene SPR interfaces. A number of strategies have been developed to reduce nonspecific binding of clinical serum samples such as the incorporation of ethylene glycol units onto the sensing surface (Ayela et al., 2007; Krishnan et al., 2011). In this study, we demonstrate that human serum itself in combination with bovine serum albumin (BSA) can be used for blocking non-specific interactions. The graphene-based SPR sensor is thus, well adapted to be used for the analysis of biomarkers in clinical samples.

2. Experimental part

2.1. Materials

Bovine serum albumin (BSA), polyethylene glycol (Mw = 1.5 kDa), fibrinogen from human plasma, folic acid (FA), folic acid protein (FAP), lysozyme and sodium dodecyl sulfate