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A novel double-layer molecularly imprinted polymer film based surface plasmon resonance for determination of testosterone in aqueous media

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ABSTRACT

This work aimed to prepare a novel double-layer structure molecularly imprinted polymer film (MIF) on the surface plasmon resonance (SPR) sensor chips for detection of testosterone in aqueous media. The film was synthesized by in-situ UV photo polymerization. Firstly, the modification of gold surface of SPR chip was performed by 1-dodecanethiol. Then double-layer MIF was generated on the 1-dodecanethiol modified gold surface. The non-modified and imprinted surfaces were characterized by atomic force microscopy (AFM), fourier transform infrared (FTIR) spectroscopy and contact angle measurements. Analysis of SPR spectroscopy showed that the imprinted sensing film displayed good selectivity for testosterone compared to other analogues and the non-imprinted polymer film (NIF). Within the concentrations range of 1×10^{-12}–1×10^{-8} mol/L, the coupling angle changes of SPR were linear with the negative logarithm of testosterone concentrations ($R^2 = 0.993$). Based on a signal/noise ratio of three, the detection limit was estimated to be 10^{-12} mol/L. Finally, the developed MIF was successfully applied to the seawater detection of testosterone. The results in the experiments suggested that a combination of SPR sensing with MIF was a promising alternative method for detection of testosterone in aqueous media.

1. Introduction

Surface plasmon resonance immunoassay is one of the main sensing methods of biological molecules. SPR sensors have attracted attention for last two decades [1–3]. SPR is an optical technique that measures changes in the refractive index occurring within approximately a few hundred nanometers from the sensor surface [4]. SPR was introduced in the early 1990s as the underlying technology in affinity biosensors for biomolecular interaction analysis [5]. Now it has become a widely used analytical technique to monitor not only for DNA [6], protein binding and environmental contaminants [7,8], but also for small molecule micro-arrays [9].

Although various methods are used to generate the sensitive SPR sensor, the most effective method is molecular imprinting technique. The method involves the synthesis of highly cross-linked polymers in the presence of template molecule. After the removal of template, complementary binding sites are obtained, which allow the binding of the template molecule with very high specificity [10]. Molecular imprinted polymers (MIPs) can be used in numerous applications, such as artificial antibodies [11], chromatographic separations [12], solid-phase extraction [13,14], sense technology [15], enzyme catalysis [16] and many other fields [17–19].

Testosterone is a kind of endogenous anabolic androgenic steroid which has also been found frequently in various water sources [20]. As it is an important indicator of a wide variety of pathological conditions such as male and female subfertility, prostate cancer and adrenal diseases, an accurate detection of the content of testosterone is of great importance for us. Conventional testosterone analysis performed in clinical laboratories is carried out mostly with immunoassays [21,22] and chromatography [23,24]. These methods have proven to be sensitive, but they have limitations concerning specificity. SPR sensors in combination with MIPs can address this problem for the detection of small molecules at high sensitivity and selectivity [25,26]. In addition, an SPR sensor chip with a three-dimensional binding matrix such as MIPs containing a porous structure could improve the sensitivity because it provides higher binding capacity [27] and allows the target molecules to absorb rapidly to the recognition sites.

In this study, we prepared an easily controlled layer structure water-compatible MIF on a gold chip of SPR chip. The previously developed MIF have demonstrated high selectivity for the

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recognition of target molecules mainly in organic solvent-based media [28,29]. However, many target molecules are predominately present in the aqueous environment, it is necessary to develop water-compatible MIF for binding the targets in the field of biotechnology [30]. To date, although some approaches are utilized to prepare the water-compatible MIF [31,32], few studies apply the layer structure polymer film modified on the sensor chips. The MIF was the double-layer structure film and the cross-linker concentration of the second layer pre-polymerization solution was higher than that of the 1st layer, so that the second layer could be solid enough to stabilize the binding sites and protect the hydrogen-bonding in the film while detecting the template in water solution. In addition, the novel SPR chips show high sensitivity and high selectivity for detection of testosterone in PBS buffer. In addition, the MIF exhibits good reproducibility in the later experiment. Furthermore, the proposed MIF was applied to the seawater detection of testosterone.

2. Materials and methods

2.1. Material

Testosterone and Benzophenone were purchased from Acros Organics, β-Estradiol, progesterone, Estriol, Estrone, Cinchonine and ethylene glycol dimethacrylate (EGDMA) were purchased from Aladdin Regent Company. Methacrylic acid (MAA) and 1-dodecanethiol were all purchased from J & K Scientific Ltd. (China). Ethanol and acetic acid was analytical grades and purchased from Beijing Tongguang Fine Chemicals Company. MAA and EGDMA were distilled under reduced pressure before used. All the other reagents except MAA and EGDMA were used as received. Phosphate buffered saline (PBS) used in the experiments was prepared from 140 mM NaCl, 10 mM phosphate and 3 mM KCl, and pH 7.4 was adjusted by HCl and NaOH.

2.2. SPR implementation

In the experiment, an optical setup based on SPR spectroscopy was employed. A light beam emitted from a stabilized HeNe laser (2 mW, $\lambda = 632.8$ nm) passed through a polarizer to become transverse magnetically polarized beam and was coupled to a LASFN9 glass prism (90°, $n_p = 1.845$) with optically matched sensor chip to its base by using immersion oil. A flow-cell with the volume of approximately 0.5 mL was pressed against the sensor surface to flow liquid sample over the substrate surface with a peristaltic pump.

2.3. Surface modification of the SPR chip

2.3.1. 1-Dodecanethiol modification of the SPR chip

To modify gold surface of the SPR chip with $C_{12}H_{25}SH$, the chip was washed with 30 mL of acidic piranha solution (3:1 H_2SO_4:H_2O_2, v/v). After the SPR chip was dipped in cleaning solution for 5 min, it was washed with ethanol and dried in vacuum oven for 2 h. To let the gold surface react with the benzophenone to form free radicals for anchoring polymers better, the chip was dipped in an ethanol solution (1 mmol/L) of 1-dodecanethiol for 24 h in order to form a self-assembled monolayer (SAM). Then it was cleaned with ethanol and dried with nitrogen gas. The alkanethiolate self-assembled monolayer on gold substrates will allow subsequent growth of surface-confined polymer.

2.3.2. In-situ preparation of MIF

Testosterone-imprinted film on $C_{12}H_{25}SH$ modified SPR chip was prepared as follows (Fig. 1). Firstly, testosterone (0.025 mol/L)

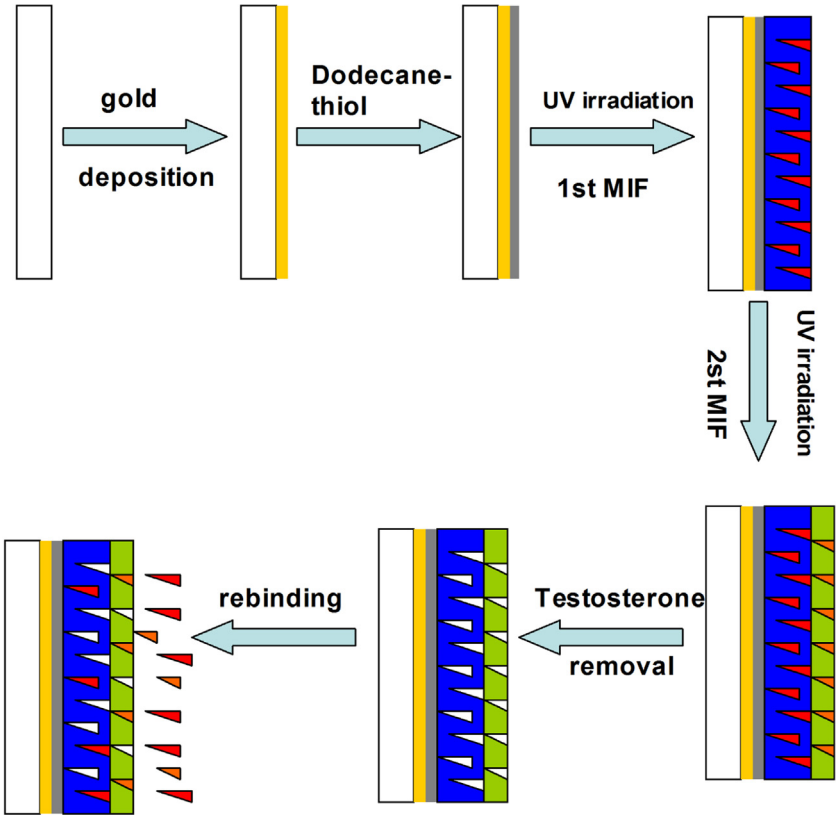

Fig. 1. The schematic illustration of the formation of MIF.