Materials Science and Engineering C 33 (2013) 3609–3614

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Microcontact imprinted surface plasmon resonance sensor for myoglobin detection

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ARTICLE INFO

Article history:
Received 20 June 2012
Received in revised form 12 April 2013
Accepted 20 April 2013
Available online 26 April 2013

Keywords:
Molecularly imprinted polymer
Microcontact imprinting
SPR sensor
Myoglobin
Nanofilm

ABSTRACT

In this study, we prepared surface plasmon resonance (SPR) sensor using the molecular imprinting technique for myoglobin detection in human serum. For this purpose, we synthesized myoglobin imprinted poly(hydroxyethyl methacrylate-N-methacryloyl-L-tryptophan methyl ester) [poly(HEMA-MATrp)] nanofilm on the surface of SPR sensor. We also synthesized non-imprinted poly(HEMA-MATrp) nanofilm without myoglobin for the control experiments. The SPR sensor was characterized with contact angle measurements, atomic force microscopy, X-ray photoelectron spectroscopy, and ellipsometry. We investigated the effectiveness of the sensor using the SPR system. We evaluated the ability of SPR sensor to sense myoglobin with myoglobin solutions (pH 7.4, phosphate buffer) in different concentration range and in the serum taken from a patient with acute myocardial infarction. We found that the Langmuir adsorption model was the most suitable for the sensor system. The detection limit was 87.6 ng/mL. In order to show the selectivity of the SPR sensor, we investigated the competitive detection of myoglobin, lysozyme, cytochrome c and bovine serum albumin. The results showed that the SPR sensor has high selectivity and sensitivity for myoglobin.

1. Introduction

Molecularly imprinted polymers (MIPs) are artificial receptors with the ability to recognize and to bind specifically with the target molecules [1–4]. Due to their stability, ease of preparation and low cost, the researchers have assessed using these MIPs as substitutes for antibodies or enzymes in chemical sensors, catalysis, and separations [5–9]. Although creating MIP for all molecules is straightforward now, the imprinting of large structures, such as proteins, is still a challenge [10]. The major problem associated with the imprinting of large structures is their restricted mobility within highly cross-linked polymer networks and their poor efficiency in rebinding. Up to now, imprinting on the surface seemed to be the most promising way to overcome these difficulties [11,12]. Recently, Chou et al. [13] reported a microcontact imprinting (μCIP) method to prepare nanofilm with C-reactive protein on a glass surface. Using the μCIP approach, the researchers imprinted polymeric films with ribonuclease A [14], lysozyme [15], and myoglobin [16] on a glass surface and obtained promising results for potential sensor applications. Several studies have shown the potential of MIPs in combination with surface plasmon resonance (SPR) sensors that enable the analysis and detection of chemical and biological compounds without the need for biomolecule labeling [17–21]. But, MIP-based SPR sensors prepared for proteins are more rare than those prepared for small molecules, probably because of the challenge of protein imprinting. Matsunaga et al. [21] successfully prepared MIP-based SPR sensor for lysozyme detection. Uzun et al. synthesized hepatitis B surface antibody (HBsAb) imprinted nanofilm on a SPR sensor gold surface for the diagnosis of HBsAb in human serum [22].

Acute myocardial infarction (MI) remains the leading cause of mortality in the world and represents an enormous cost to the health care system. Cardiac markers have long been the cornerstone of diagnosis and continue to play an important role, especially in the group of patients with low to medium risk. A single marker that meets all of the criteria has yet to be found, therefore, a multi-analyte diagnostic approach would be the best. Biochemical markers used to diagnose acute MI are myoglobin, creatine kinase-myocardial band (CK-MB), and cardiac troponins. Myoglobin is a 17.6 kDa heme protein in the cytosol of skeletal and cardiac muscle but not smooth muscle. Because of its small size, myoglobin is rapidly released from the areas of ischemic injury. It is significantly elevated as early as 1 h after the onset of MI with peak levels occurring at 3–15 h [23]. At presentation and within the first few hours after chest pain, the sensitivity of myoglobin is greater than that of CK-MB and troponins (T and I) [24]. However, myoglobin lacks specificity. Patients with renal failure, skeletal muscle injury, or trauma may have abnormal concentrations of myoglobin in the absence of acute MI. This limitation may be resolved by the combined measurement of myoglobin and a skeletal specific marker (carbonic anhydrase III) or a cardiac specific marker (troponin I) [25].

In this study, a SPR sensor for myoglobin detection was prepared using the microcontact imprinting technique. For this purpose, myoglobin-imprinted poly(hydroxyethyl methacrylate-N-methacryloyl-L-tryptophan methyl ester) [poly(HEMA-MATrp)] nanofilm was synthesized on a gold surface of a SPR sensor. First, we modified the glass surface

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0928-4931/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.msec.2013.04.041

of lamella and the gold surface of SPR sensor and carried out the polymer film formation. The changes on the surfaces were determined using atomic force microscopy (AFM), contact angle (CA) and X-ray photoelectron spectroscopy (XPS) and ellipsometry. We studied the myoglobin detection from aqueous myoglobin solutions. In order to show selectivity of microcontact-imprinted poly(HEMA-MATrp) myoglobin (μCIP) SPR sensor, we used bovine serum albumin (BSA), lysozyme, and cytochrome c as competitor proteins. We calculated the kinetic and isotherm parameters and investigated the myoglobin detection capability of the prepared SPR sensor in human serum.

2. Experimental

2.1. Materials

Myoglobin was supplied by Sigma (Steinheim, Germany). We obtained L-tryptophan methyl ester, methacryloyl chloride, and allyl mercaptan from Sigma Chemical Co. (St. Louis, USA). We purchased hydroxyethyl methacrylate (HEMA), ethylene glycol dimethacrylate (EGDMA) and N,N′-azobisisobutyronitrile (AIBN) from Fluka A.G. (Buchs, Switzerland). We distilled HEMA and EGDMA under reduced pressure in the presence of hydroquinone inhibitor and stored at 4 °C until use. The gold surfaces (gold coated SF10 glass) for SPRimager II instrument were purchased from GWC Technologies (Madison, ABD). All other chemicals were of reagent grade and purchased from Merck A.G. (Darmstadt, Germany).

2.2. Synthesis of N-methacryloyl-L-tryptophan methyl ester (MATrp) monomer

The synthesis and characterization of functional monomer N-methacryloyl-L-tryptophan methyl ester (MATrp) were reported previously [26,27]. In the synthesis reaction, L-tryptophan methyl ester (5.0 g) and hydroquinone (0.2 g) were dissolved in 100 mL of dichloromethane. This solution was cooled to 0 °C. Triethylamine (12.7 g) was added into the solution. Methacryloyl chloride (5.0 mL) was poured slowly into this solution and then stirred magnetically at room temperature for 2 h. At the end of the chemical reaction, hydroquinone and unreacted methacryloyl chloride were extracted with a 10% NaOH solution. The aqueous phase was evaporated in a rotary evaporator. The residue (i.e., MATrp) was recrystallized in ethanol.

2.3. Preparation of the μCIP SPR sensor

The following procedures were used to prepare the poly(HEMA-MATrp) nanofilm on the gold surface of SPR sensor: (1) cleaning of lamellas used to make the protein stamp, (2) modifying glass surface with aminopropyl triethoxysilane (APTES) and glutaraldehyde (GA), followed by covalent immobilization of the myoglobin and pretreatment of monomer mixture, (3) allylmercaptan modification of the gold surface of the SPR sensor, (4) bringing the protein stamp into contact with the modified gold surface of the SPR sensor, allowing microcontact imprinting of the protein into the monomer solution [16], (5) UV irradiation to initiate polymerization and fix the imprinted orientation of monomers, and (6) removing the protein template.

2.3.1. Preparation of protein stamps

To prepare the protein stamps, we modified cover glasses in batches (each consisting of approximately 10 pieces) and cleaned them sequentially (30 min each step) in 100 mL of neutral cleaner (2 vol%; SoDosil® 02), deionized water, isopropanol, ethanol, and, finally, with deionized water in an ultrasonic bath at 55 °C. Each cover glass was then dried with nitrogen gas [15].

Prior to use, the cleaned glass slides were immersed in a 70/30 (v/v) mixture of H_2SO_4 and 30% H_2O_2 and dried in a vacuum oven for 2 h at 80 °C followed by washing with deionized water. The slides were immersed in 5% (v/v) APTES in acetone for 30 min at room temperature to introduce amino groups onto the surfaces of slides, then rinsed thoroughly with acetone and deionized water to remove any non-bound silane compounds. The prepared slides were dried in a vacuum oven at 80 °C for 2 h. The amino groups on the APTES silanized surfaces of the slides were activated via GA to covalently immobilize myoglobin. Specifically, the silanized slides were immersed in 2.5% (v/v) GA solution in a 0.1 M phosphate buffer (pH 7.4) and kept quiescent for 2 h at room temperature. Then, the slides were rinsed thoroughly with a phosphate buffer (pH 7.4) and dried with nitrogen gas. After that, the cover glasses were then incubated with 0.01 mg/mL myoglobin solution (pH 7.4, phosphate buffer) at 4 °C for 24 h and then dried with nitrogen gas. The cover glasses used for preparing non-imprinted nanofilm were incubated with phosphate buffer (pH 7.4) and dried with nitrogen gas.

2.3.2. Allyl mercaptan modification of the SPR sensor

The surface of the gold SPR sensor was modified with allyl mercaptan (CH_2CHCH_2SH). Before its modification, the SPR sensor was cleaned with alkaline piranha solution (3:1, NH_4OH:H_2O_2, v/v). The SPR sensor was immersed in 20 mL of alkaline piranha solution for 5 min. Then, it was washed with pure ethyl alcohol and dried in a vacuum oven (200 mm Hg, 40 °C) for 3 h. After that, the SPR sensor was immersed in an ethanol/water (4:1, v/v) solution containing 3.0 mM allyl mercaptan for 12 h. Finally; it was thoroughly rinsed with ethanol and dried with nitrogen gas under vacuum (200 mm Hg, 40 °C).

2.3.3. Preparation of myoglobin-imprinted nanofilm on SPR sensor

Myoglobin imprinted nanofilm on allyl mercaptan modified SPR sensor was prepared using the following steps: First, HEMA, MATrp, and EGDMA (in mol ratio of 1:1:5) were mixed at room temperature for 2 h. Then, AIBN (5 mg) was dissolved in a monomer mixture to prepare the stock monomer solution. After that, 5 μL aliquot was taken from the stock monomer solution and dropped onto the surface of the protein stamp, which was stored at 4 °C for 30 min to pre-organize the template (myoglobin) with functional monomer (MATrp). Then, the gold face of the SPR sensor was placed into this solution. Polymerization was initiated using UV light at room temperature (100 W, 365 nm) and was continued for 4 min at room temperature under nitrogen atmosphere. After the polymerization process, the protein stamp was stripped from the SPR sensor surface. The nanofilm coated SPR sensor was then washed, first with 0.1 M HCl/methanol (1:1, v/v) and then with methanol. Finally, it was dried in a vacuum oven.

2.4. Surface characterization studies

2.4.1. Contact angle measurements

KSV Attention Tetha instrument (Hamburg, Germany) was used to determine the contact angles (CA) of the SPR sensor and glass surfaces. The contact angles of the surfaces were measured via using taking 40 separate photos from the different parts of surfaces. In order to determine the surface free energy (SFE) of the SPR sensors, water and ethylene glycol were used by applying the Owens–Wendt method.

2.4.2. Atomic force microscopy studies

In order to characterize the SPR sensor and glass surfaces, the non-contact mode atomic force microscope (AFM) (Nanomagnetics Instruments, Oxford, England) was used. Visualization studies were carried out in non-contact mode. Sample area of $1 \times 1\ \mu m^2$ was displayed with a 2 μm/s scanning rate and a resolution of 256×256 pixels.

2.4.3. X-ray photoelectron spectroscopy studies

We analyzed the chemical composition of the bare glass and protein stamp using an XPS apparatus (PHI-5000, USA). The energy of excitation source, monochromatic Al K_α radiation, was 1486.6 eV,