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Improved surface plasmon resonance biosensing using silanized optical fibers

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ABSTRACT

Coupling surface plasmon resonance (SPR) to optical fiber (FO) technology has brought tremendous advancements in the field by offering attractive advantages over the traditional prism-based SPR platforms, such as simplicity, cost-effectiveness and miniaturization. However, the performance of the existing FO-SPR sensors widely depends on the adhesion of the gold (Au) layer to the FO silica core, thereby often representing a major limiting factor in achieving the properties of the benchmark SPR systems. In this paper, we used (3-marcaptopropyl)trimethoxysilane (MPTMS) as an adhesion promoter for developing robust Au surfaces on the three-dimensional (3D) FO-SPR sensing probe. Carefully prepared FO substrates were first silanized using a wet chemistry approach, with MPTMS concentrations ranging from 2.5 to 24 mM, and subsequently exposed to a drying treatment at room temperature (RT) or at 100 °C, before coating them with a ~50 nm Au plasmonic film. Differently prepared silanized FOs were next used for evaluating their sensitivities, by performing refractive index (RI) measurements in sucrose dilutions. Advanced statistical analysis of the obtained data indicated that using 8 mM MPTMS solution coupled with a RT post-drying treatment is an efficient way of producing FOs with dramatically improved Au adhesion properties. The role of the MPTMS underlayer was further investigated by exposing the reference and silanized FOs to stress conditions, such as strong mechanical (adhesion tape tests), chemical (piranha solution treatments) and thermal variations. Although additional studies using scanning electron microscopy (SEM) revealed changes in the Au film morphology after these endurance tests, the silanized FOs exhibited an enhanced robustness while retaining the overall sensor's capabilities. In contrast, the reference FOs consistently failed the mechanical and chemical tests, while only resisting under thermal variations. Moreover, the improved resistance of the silanized FO-SPR probes allowed them to be reused up to three times with no significant loss in the sensor performance, while implementing bioassays based on two types of bioreceptors (a DNA aptamer against thrombin protein and a polyclonal antibody against human immunoglobulin E – hIgE). All these results might represent a step forward in the fabrication of more robust and reusable FO-SPR biosensors, featuring great potential for developing highly-sensitive biochemical assays.

1. Introduction

The surface plasmon resonance (SPR) technology is a well-established detection and analysis tool with many proven applications in health care diagnostics [1], biotechnology and life-science research [2], the agro-food sector, security and environmental monitoring [3]. SPR sensors exploit the mono- or polychromatic polarized light, which excites the metal (typically gold – Au or silver – Ag)/dielectric (e.g. target sample – usually a liquid) interface to generate propagating plasmonic waves, highly sensitive to the refractive index (RI) changes in the sample [4,5]. These changes can be used to measure real-time interactions between proteins, lipids, nucleic acids or even low molecular

Abbreviations: FO-SPR, fiber optic - surface plasmon resonance; RI, refractive index; NPs, nanoparticles; APTMS, [(3-aminopropyl)trimethoxysilane]; MPTMS, [(3-mercaptopropyl) trimethoxysilane]; hIgE, human immunoglobulin E; MES, 2-(N-morpholino)ethanesulfonic acid; NHS, N-hydroxysulfosuccinimide; EDC, 1-ethyl-3-[dimethylaminopropyl]carbodiimide; SAM, self-assembling monolayer; DIW, deionized water; PBS, phosphate buffer saline; TGK, (3-hydroxyamino)methane-glycine-potassium; ANOVA, analysis of variance; RT, room temperature.

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weight molecules such as drugs [6], providing thus valuable information on target quantification and biomolecular kinetics. Despite all this potential, SPR devices that are already available on the market (e.g. Biacore [7,8]) are still rarely used outside the research centers because most of them are expensive bulky systems due to the associated complex optical equipment, precision mechanical components and sophisticated pump-driven microfluidic schemes [9,10]. In addition, although the SPR platform has been promoted as a promising tool capable of handling label-free bioassays [11], it still poses a number of challenges in developing sensitive and specific bioassays [12].

Mostly for portability purpose, Jorgenson and Yee [13] proposed in 1993 a novel SPR system based on a Au-coated fiber optic (FO) design. Numerous similar FO-SPR devices have been introduced since then [14–16] in an attempt to enhance the performance of the entire sensing platform and make it comparable with the traditional SPRs [17]. In this context, it has been shown that by accurately controlling the chemical interactions at the sensor surface [18], by implementing labeling approaches using Au or magnetic nanoparticles (NPs) [19], by patterning the active sensing area using well-controlled nanostructuring techniques [20], and/or just by simply improving the adhesion of the Au thin layer deposited on the glass substrate [21], the specificity, sensitivity and other features of the sensor can be further improved.

In our previous research it has been demonstrated that the in-house developed FO-SPR platform [22,23] has great potential for applications outside the specialized research environment due to the low-cost components, its capacity for multichannel measurements in screening applications and the possibility for improved sensitivity through the incorporation of NPs into bioassays, a feature not feasible with the classical microfluidics-based SPR systems [24]. Moreover, this sensing platform was successfully used both, for protein- [25] and DNA- [26] based bioassays, making it useful for different applications. However, the performance of the existing FO-SPR sensors mostly depends on the adhesion of the Au layer sputtered on the FO silica core within the sensitive zone, thereby often representing the major limiting factor in achieving the expected properties. Most commonly, a good adhesion can be achieved by introducing an intermediate metallic ultra-thin (several nm) layer of Cr or Ti [27]. However, these compounds may absorb part of the propagated light during the SPR excitation, altering further the sensor's signal response and thus lowering its sensitivity [28,29]. Moreover, the deposition of such extremely thin metallic underlayers with good evenness onto non-planar FO substrates requires specialized, complex and expensive equipment.

Recently, several groups reported the use of organosilanes as efficient attachment layers for either Au NPs [30] or films [31] on flat glass substrates. These organic compounds can be homogeneously deposited resulting in thin films with no additional light absorption, thus with minimal plasmon damping effects [32]. Two silane types are widely used for such purpose: (i) an amino functional silane [(3-aminopropyl)trimethoxysilane – APTMS] [33] and (ii) a thiol functional compound [(3-mercaptopropyl)trimethoxysilane – MPTMS] [34]. Although both compounds combine good chemical and physical properties with excellent transparency, the APTMS is a less preferred candidate for this purpose due to the weak electrostatic NH_2–Au interactions [35] compared to the strong covalent S–Au bonds formed by the MPTMS [36,37]. Such an approach might be an effective way to attract various Au architectures (e.g. NPs [37] or nanorods – NRs [38]) directly on the FO silica core.

In this paper, the MPTMS was used as an adhesion promoter for preparing robust and consistent Au thin films onto the three-dimensional (3D) surface of the in-house developed FO-SPR sensor [22,23]. We demonstrated that the presence of the MPTMS layer enhances the stability and robustness of the sensor under mechanical, chemical and thermal stress conditions. The robustness was further evaluated by implementing two different bioassays on the FO substrate: (i) an immunoassay for human immunoglobulin E (hIgE) [39] detection and (ii) an aptamer based bioassay for α-thrombin [40] detection. The silanized FOs featured a surface capable of resisting complete regeneration with piranha solution (a mixture of sulfuric acid and hydrogen peroxide) up to three times while retaining the overall bioassay performance and sensitivity. This research represents a step forward in the fabrication of robust and reusable FO-SPR biosensing platforms suitable for implementing highly-sensitive bioassays.

2. Materials and methods

2.1. Reagents

The chemicals in this work were of high quality analytical grade (99.99% purity, unless otherwise specified). Acetone, sodium hydroxide (NaOH), sulfuric acid (97% H_2SO_4) and acetic acid were purchased from Chem-Lab, Belgium. Hydrogen peroxide (30% H_2O_2), anhydrous toluene, ethanol, methanol, sodium acetate, ethanolamine, MPTMS, 2-(N-morpholino)ethanesulfonic acid [41], bovine serum albumin (98% BSA), trizma base, streptavidin and anti-hIgE (ε-chain specific) polyclonal antibody were supplied by Sigma-Aldrich, Belgium. Glycine, D(+)-sucrose, sodium chloride (NaCl), 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide (EDC) and N-hydroxysulfosuccinimide (Sulfo-NHS) were acquired from Fischer Scientific, Belgium. Biotinylated self-assembling monolayer (SAM) and carboxylic acid-SAM were obtained from GERBU Biotechnik GmbH, Germany. Tween 20 BioChemica and potassium phosphate dibasic (K_2HPO_4) were purchased from Applichem, Germany. Human α-thrombin was supplied by Bio-Connect, the Netherlands. The biotinylated anti-thrombin DNA aptamer [42] was purchased from Integrated DNA Technologies, Belgium. Myeloma hIgE kappa was obtained from Athens Research and Technology, USA. Au NPs (20 nm diameter, concentration of 7×10^{11} NPs/mL) were purchased from BBI international (Cardiff, United Kingdom). All dilutions were prepared with deionized water (DIW) purified by a Milli-Q 50 system (Millipore, USA). A nitrogen (N_2) gas stream with an estimated volumetric flow rate of 35 m^3/h was used to dry the samples during the different processing steps. A phosphate buffer saline (PBS) pH 7.4 was prepared by dissolving a 10 g PBS packet (Neogen Corporation, UK) in 1 L of DIW. The TGK buffer (pH 8.3) was obtained by preparing an aqueous solution containing 25 mM trizma base, 192 mM glycine and 5 mM K_2HPO_4.

2.2. Fabrication and silanization of the FO-SPR sensors

Optical probes were manufactured by cutting a TEQS multimode FO (Thorlabs, Germany) with a diameter of 400 μm in pieces with a consistent length of 3.6 cm. A SPR–sensitive zone of 0.6 cm was constructed at one side by mechanically removing the FO jacket and dissolving the polymer cladding in acetone, followed by drying the FO with dust free tissues. The hydroxyl groups (OH^-) on the FO surface were activated by immersing the samples in piranha solution (3:7% v:v H_2O_2:H_2SO_4) for 15 min. The FOs were subsequently rinsed with DIW and methanol, and afterwards dried under N_2 gas flow. The prepared probes were then subjected to a silanization process, using a wet chemistry approach. Briefly, the samples were ultrasonicated for 2 h in anhydrous toluene, containing 0.3 M acetic acid and MPTMS. The silane concentrations tested for optimizing the protocol were: 0, 2.5, 8, 16 and 24 mM. The acetic acid was used to favor the hydrolysis reaction while minimizing the condensation inside the solution. Next, the silanized probes were cleaned in methanol and dried using a N_2 gas stream. Additionally, half of the samples were in a subsequent step exposed to 5 min drying