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Characterization of Drop Cast as Strategy for the Biofunctionalization of Plasmonic Sensors Based on Highly Doped Ge-Based

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Summary:

Conventional metal-based plasmonic biosensors in the Vis/IR range rely on flat or nanostructured metallic surfaces. CMOS-compatible novel biosensors operating in the THz range would benefit from their integration with mm-wave electronics to greatly reduce size, price and/or increase functionality. In this work, we investigate the adhesion of albumin to the surface of Ge plasmonic antennas operating in the THz spectral region. The sample surfaces were pre-treated with oxygen plasma or isopropanol as cleaning agents, before investigating their properties using various methods. We observed an increase of surface hydrophilicity after surface interaction with oxygen plasma, leading to improved attachment of albumin to the Si_3N_4 surface.

Keywords: biofunctionalization, plasmonic antennas, THz resonance

Background, Motivation and Objective

Human serum albumin (HSA) is the most abundant protein in plasma. The binding sites of HSA allow it to bind to metal ions, hormones, nucleic acids, heme proteins, as well as to affect the pharmacokinetics of many drugs. HSA display also antioxidant capacities [1–4]. Moreover, HSA has a key role in human innate immunity, because it can render potential toxins harmless [5–7]. Considering the physiological and pharmacological functions of HSA, we exploit the possibility to immobilize albumin on a substrate to develop an albumin-based biosensor. Within the framework of this goal, we have recently demonstrated a low-cost biosensing platform for label-free detection of various concentrations of molecules such as BSA and alpha lipoic acid (ALA) [8–10]. The platform is based on 6 mm x 6 mm chiplets of CMOS-compatible highly-doped (~$1x10^{19}$ cm^{-3}) Ge plasmonic bow-tie antennas encapsulated in Si_3N_4 and realized on a Silicon-on-isolator (SOI) substrates, operating in the Terahertz (THz) range [8,9].

Towards a lab-on-a-chip strategy, miniaturized sensor chiplets fabricated by conventional lithography techniques can benefit from size and compatibility with complementary metal-oxide-semiconductor) CMOS technology ecosystem, such as generation/reception of the operating frequency, data storage and processing. In contrast with conventional plasmonic sensors using flat metallic surfaces, our CMOS THz sensor is based in micrometer-sized structures, which sensitivity is the highest at the gap between the two antenna's arms. A localized surface plasmon resonance (LSPR) is associated to an enhancement of the electromagnetic (EM) field intensity in the antenna hotspot, if the antenna is illuminated by a THz electromagnetic radiation at its resonant frequency (0.5-1.5 THz) possessing the proper polarization [11]. The resonance is seen in the THz transmission spectra as a ~200 GHz-wide-peak with reduced intensity.

One of the biggest challenges in the biofunctionalization of the antennas are chemical surface pre-modifications, which are responsible for the orientation of the immobilized biomolecules and the selectivity of surface coverage [12]. A self-assembled monolayer (SAM) is a common surface pre-modification method before immobilization of the target molecule [13–16], but in this work not applied to increase

speed and reduce complexity on the strategy. To this end, the drop casting of solutions onto the sensor chiplets would be the most straightforward approach for biofunctionalization and/or the delivery of analytes. Albumin is used for the specificity of analyte recognition, such as heme after various pre-treatments. Thus, in this work, several pre-treatment methods were used such as oxygen (O_2) plasma treatment [17] and isopropanol cleaning (IPA). Among these, we show that a 600 seconds-long O_2 plasma treatment of the Si_3N_4 antenna outer layer has the inherent advantages of compatibility with drop cast biofunctionalization method due to high surface activity and the ability to use a variety of biomolecules. Due to the structural and functional similarities between HSA and bovine serum albumin (BSA) (~ 70% homology) [18], the latter can be used as low-cost model protein to study the effect on plasmonic antennas resonance. Finally, we show that the functionalization of as little as 12.6 µg of BSA indicates a measurable antenna resonance shift of~ 11 GHz, i.e. a sensitivity of 9 GHz/(mg/mL).

Drop Cast Biofunctionalization Method

The plasmonic THz resonance is caused by Si_3N_4-coated ultra-high n-doped ~1x10^{19} cm^{-3} bow-tie Ge-microantennas on SOI substrates and amplified by LSPR. Fabrications details can be found here [8]. Droplet deposits were prepared by pipetting 10 µL of BSA solution onto antennas pre-treated with 600 s O_2 plasma. BSA solution was prepared by dissolving BSA (A4612; Merck KGaA) in ultrapure water (UPW) with a resistivity at 25°C >18 MΩ cm at three different concentrations (i.e. 1.26, 2.53 and 4.06 mg /ml). After the solutions dried for approximately 30-60 minutes, THz measurements and analysis were performed using THz-Time-Domain Spectroscopy (TDS) and Teralyzer software (Menlo GmbH, Munich, Germany), respectively. Subsequently, to detach BSA from the samples after drop cast functionalization, three samples with the same BSA concentration were rinsed in 1.0 mL UPW for 18 h at room temperature (RT). The wash solutions were analyzed by UV/Vis spectroscopy at 280 nm to determine the exact concentration of BSA detached from the antennas using the Beer-Lambert law. In addition, 20 µl of this solution were analyzed by Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) for confirmation of the UV/Vis analysis qualitatively (see Fig.1).

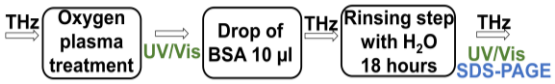

Fig. 1. Schematic representation of the procedures for the drop cast biofunctionalization method.

Subsequently, BSA marked with fluorescein isothiocyanate (FITC) (A9771, Merck KGaA) (here after named FITC-BSA) were used to visualize protein distribution on the antennas due to drop cast biofunctionalization by using optical light microscopy (Nikon Eclipse-LV100 ND, Intensilight C-HGFI, ultrahigh pressure 130 W mercury lamp and 100 % light intensity) (see Fig.1).

Results

Characterization of Surface After two Pre-treatment Strategies

Before the starting of BSA deposition, two pre-treatment strategies were tested: (i) antennas were treated by Reactive Ion Etching (RIE SI591 compact system, SENTECH, Germany) at 90 W, 10 sccm, O_2 gas and 5 Pa pressure for 0, 10, 60, and 600 seconds at RT, and (ii) antennas were sonicated in isopropanol for 5 minutes as described here [8]. Both O_2 plasma treatment and IPA cleaning are known to reduce the content of different impurities groups on the surface of Si_3N_4 layer [19]. The characterization of untreated (0 sec) and pre-treated samples (*i.e.,* 10, 60, 600 sec of plasma treatment or IPA cleaning) was performed by Atomic Force Microscopy (AFM) (neaSNOM system from NEASPEC in non-contact AFM mode) and Wetting Contact Angle technique (WCA) (Surftens Automatik) at RT.

The possible pre-treatment effect on surface roughness and morphology was analyzed by AFM for all pre-treatment methods (see Fig. 2a and 2c). It was observed that the surface morphology itself was not significant affected by any pre-treatments. The average height and mean roughness extracted from the AFM vary not significantly between the studied treatments. The average roughness is equal to ~12-13 nm for un- and 10 sec-treated surfaces and ~20 nm for the other pre-treatments, suggesting some increasing trend for increase plasma time. In case of mean roughness, the variation is slightly increased for O_2 plasma 60 and 600 sec pre-treatments ~6-7 nm, where for the other pre-treatments ~3-4 nm (see Fig. 2a). However, from Scanning Electron Microscope (SEM) analysis (not shown), it is concluded that Ge growth itself has a larger effect in its surface than the effect of the pre-treatment. Also, the manufacturing affects or/and accumulation of foreign particles, such as these white dots on all AFM images, are responsible for distorting the AFM measurements (see Fig.2c).

By WCA measurement is investigated the spreading and distribution of 1 µl water drop direct after drop fall. Thus wettability/hydrophilicity of the pre-treated surface can

precise investigated. As an average, the 1-point WCA measurement was carried out on three samples with the highest antenna density and is approximately 50° for an untreated surface (see Fig. 2b). The observed WCA value for Si_3N_4 relates with data reported in the literature, where the WCA is ca. 38° on as-grown Si_3N_4 surface without structures [19].

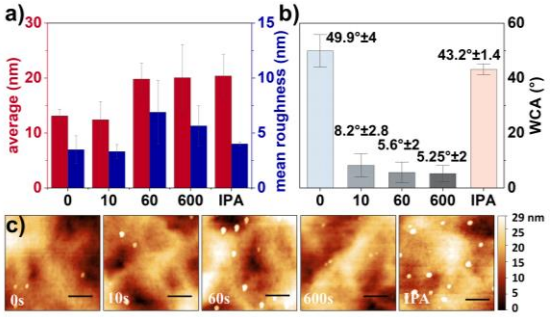

Fig. 2. (a) AFM surface roughness and (b) WCA measurements of the corresponding O_2 plasma treated or IPA cleaned substrates; (c) AFM surface morphology (scale bar 500 nm) for all pre-treatments (after 0, 10, 60, 600 sec O_2 plasma treatment and after IPA cleaning) of Si_3N_4 surface.

In conclusion, 600 sec O_2 plasma treatment gives more hydrophilic surfaces with a WCA of 5.25° ± 2. In the literature, a WCA value of approximately 2.7° is reported on Si_3N_4 surface without structures after plasma treatment [19].

Drop Cast Biofunctionalization

BSA drop cast functionalization immediately after plasma provides THz-TDS results directly proportional to the amount of loaded protein (see Fig. 3a). The rate of change of the resonant frequency defined as the resonance shift as a function of BSA concentration |Δf|/(mg/mL) was calculated and is 9 GHz/(mg/mL). In comparison with the previous results using IPA cleaning and 5 h incubation the sensitivity of same antenna design was ~6 GHz/(mg/mL) [8]. This represents an increase in sensitivity of semiconductor-based plasmonic platform due to O_2 plasma pre-treatment.

In detail, when loading 10 µl of BSA concentrated 4.06 mg/mL (a total amount of 40.6 µg) on sample, we measured an amount of protein detached of ~ 53.7 µg. Similarly, when loading 10 µl of BSA at a concentration of 2.53 mg/mL (total amount of 25.3 µg) and 1.26 mg/mL (total amount of 12.6 µg), we measured an amount of BSA detached of ~29.7 µg and ~17.7 µg, respectively. The slight increase in the amount of BSA measured in the washing solution may indicate the sample contamination as experiments were performed under non-sterile conditions. Remarkably, the analysis of the amount of albumin washed from antennas by SDS-PAGE revealed a trend from a darker to a lighter band with decreasing BSA concentration. This was further confirmed by the UV/Vis data (see Fig. 3b).

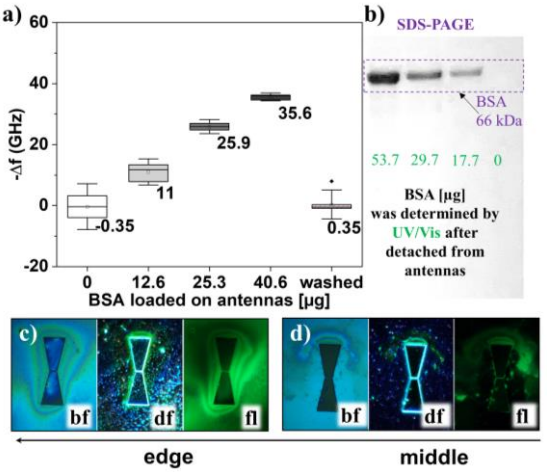

*Fig. 3. **Drop cast biofunctionalization strategy.** (a) THz measurement of antennas loaded with different amounts of BSA (i.e., 12.6, 25.3 and 40.6 µg). (b) After washing three samples with 1 mL UPW, the solution was first analyzed by UV/Vis spectroscopy to quantify the concentration of BSA detached. Then twenty microlites of the washing solution were analyzed by SDS-PAGE. (c-d) Representation of FITC-BSA distribution (c) in the edge, and (d) in the middle of the antenna (bf, bright field; df, dark field; fl, fluorescence, 100x).*

To further study the drop cast biofunctionalization method, fluorescence measurements were performed to visualize the distribution of the 10 µl FITC-BSA drop (concentration: 5 mg/mL; total amount: 50 µg) after 600 sec of O_2 plasma treatment. FITC-BSA has peak excitation and emission spectrum wavelengths of about 495 nm and 519 nm, respectively, thus resulting in a green signal (see Fig. 3c and 3d). The amount of FITC-BSA decreased from the edge to the middle of the antennas, forming an annular ring where the FITC-BSA amount was higher at the edge (see Fig. 3c) and lower in the middle (see Fig. 3d).

Conclusions

A drop cast biofunctionalization method has been developed to enhance the sensitivity and accuracy in determining the quantity of immobilized albumin. We demonstrated the use of plasma pre-treatment for the activation and improvement of the hydrophily properties of the Si_3N_4 surface, which gives the possibility to simply control the BSA deposition. The sensitivity of the Ge-based antennas to BSA after O_2 plasma treatment was up to ~9 GHz/(mg/mL), which is 1.5 times more than previously reported [8]. This biosensing platform can be further enhanced to improve the specificity and sensitivity of serum biomarkers detection for personalized point-of-care medical applications.

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