Localized Surface Plasmon Resonance Nanobiosensors for the Detection of a Prostate Cancer Biomarker

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Abstract

Silver nanoparticles exhibit distinctive optical properties. When exposed to electromagnetic radiation, a noble metal nanoparticle demonstrates the unique optical property of localized surface plasmon resonance (LSPR). LSPR is measured through an extinction spectrum, the sum of absorption and Rayleigh scattering, which results when the collective oscillation of electrons is resonant with the incident photon frequency. The specific binding of a prostate cancer biomarker, such as prostate specific antigen (PSA), to a PSA specific antibody can be monitored by using LSPR. This study contributes to the work toward detection and quantification of extremely low levels of PSA.

Introduction

According to the Prostate Cancer Foundation, prostate cancer is the most common non-skin cancer in America. The foundation estimates that more than 2 million American men are currently living with the disease and more than 186,000 will be diagnosed in 2008.[1] The Food and Drug Administration has approved the PSA test to detect cancer in men age 50 or older.[1] Such cancer is treated most commonly with surgery or radiation. A PSA test can monitor patients with a history of prostate cancer to see if the cancer has recurred by analyzing the PSA level in the blood.[1] An assay to quickly and accurately detect biomarkers such as PSA at low concentrations is essential for the well-being of a prostate cancer survivor. If cancer has returned, survival rate increases when detected at its earliest stage.

A promising approach is to use triangular silver nanoparticles as a platform for selective detection of biological proteins.[2–4] The LSPR nanobiosensor utilizes the optical properties of noble metal (Au, Ag, Cu) nanoparticles.[3] The LSPR nanobiosenor's main mechanism is its sensitivity to a change in the refractive index near the surface of the nanoparticle. Electromagnetic radiation upon the nanoparticle produces two unique phenomena. First, the electromagnetic field is enhanced near the surface of the nanoparticle[4] and the enhanced field is expected to have an exponential decay.[6] Second, the LSPR spectrum is highly sensitive to the refractive index of the local environment near the nanoparticle surface.[4]

The LSPR wavelength extinction maximum was observed using UV-vis spectroscopy.[2–4] The large absorption band is present only for the nanoparticle and not in the spectrum of the bulk metal.[3] The LSPR is the maximum extinction wavelength of the band (λ_{max}). The extinction peak shifts in wavelength corresponding to the change in refractive index within the enhanced electromagnetic fields of the nanoparticle.

This research demonstrates biomedical applications. The LSPR senses a single interaction of a molecule or protein with a single nanoparticle.[3] Detecting PSA levels through LSPR is an additional method of diagnosing prostate cancer. In this study, the nanobiosensor with PSA-specific antibodies was used to monitor the specific binding of PSA to its antibody as a function of antibody concentration.

Background

Van Duyne and coworkers studied three ligand/receptor systems to show the capability of the LSPR biosensor.[2–4] The systems are biotin/streptavidin, biotin/antibiotin, and amyloid-β derived diffusible ligands (ADDLs)/specific anti-ADDL antibodies.

The first, biotin/streptavidin, is a well-known system with a high surface-confined thermodynamic binding constant, $K_{a,surf} = 10^{11}\ M^{-1}$.[2] In this study, the Ag nanoparticles were functionalized with biotin, and thereafter streptavidin was added to measure a shift in the extinction peak. Using this system, the limit of detection for this specific LSPR biosensor was in the low picomolar to high femtomolar region.[2]

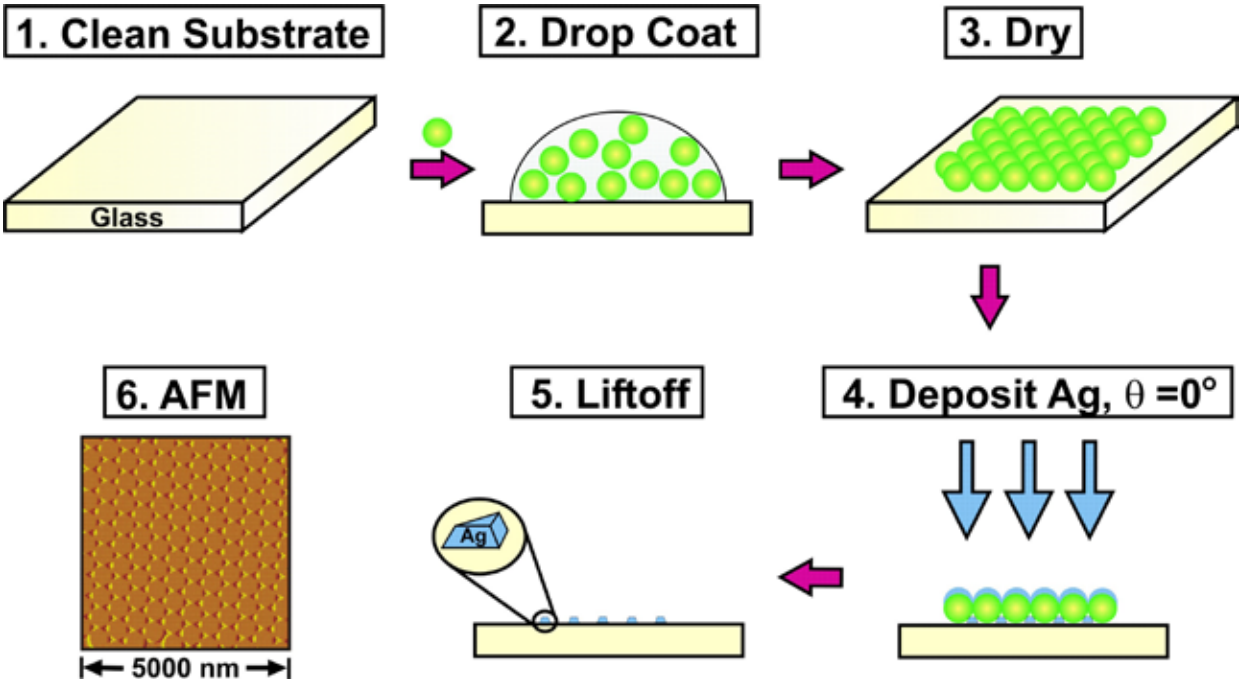

Figure 1. Fabrication of Ag nanoparticles via nanosphere lithography (NSL). (1) Glass substrate is cleaned. (2) Polystyrene nanosphere solution is drop-coated on to the glass. (3) Nanosphere solution is dried in ambient conditions. (4) Nanospheres form a hexagonally close-packed array. (5) Deposition of Ag onto nanospheres. (6) Removal of nanospheres by sonication. (7) AFM image of triangular Ag nanoparticles.

In the Van Duyne study of biotin and antibiotin, the interaction between an antigen and a solution-based antibody was investigated. They found that the surface-confined thermodynamic binding constant was significantly lower than that of biotin/streptavidin, $K_{a,surf} = 4.5 \times 10^7\ M^{-1}$.[3] Still, the LSPR biosensor was able to detect the binding between biotin and antibiotin. The limit of detection for antibiotin was in the high picomolar range.[3]

The Van Duyne study of ADDLs and ADDL antibodies brought attention to the clinical applications of LSPR nanobiosensors. In this study, the sandwich assay format maximized the amount of shift observed with the extinction peak. In the sandwich assay, an anti-ADDL antibody was covalently linked to the surface of a self-assembled monolayer (SAM)–functionalized Ag nanoparticle. Then the samples were incubated with ADDLs. To enhance the LSPR shift response, another layer of anti-ADDL antibodies was added. Also, this study used human extracts, such as cerebral spinal fluid, to demonstrate clinical applications in the diagnosis of Alzheimer's disease.[4]

The LSPR nanobiosensor must be explored with prostate cancer biomarkers, such as PSA, to provide an additional method of detecting PSA at low levels. The most common commercial method of analyzing PSA levels is the enzyme-linked immunosorbent assay (ELISA), which has a detection limit in the low picomolar range.[1] The long-term goal is to find a lower limit of detection for PSA than currently available methods. LSPR seems promising as part of this ongoing effort.

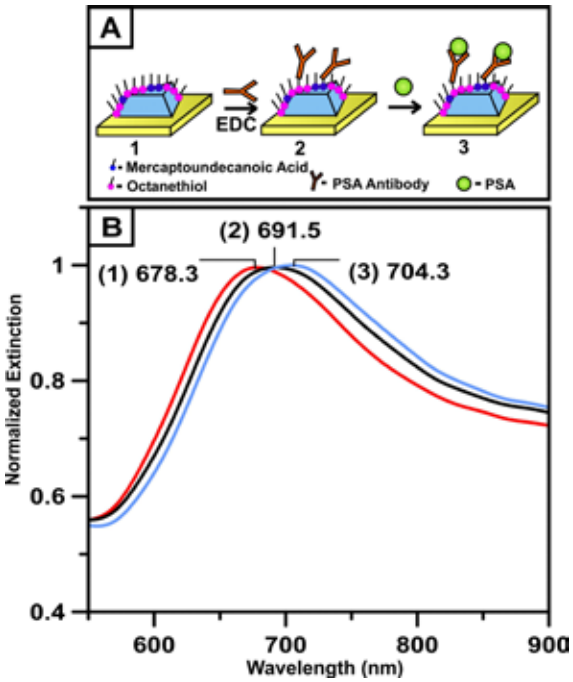

Figure 2: (A) A schematic representation of a PSA-antibody assay. (B) LSPR is the maximum extinction wavelength of the band, $\lambda_{max.}$, with extinction peak shifts in wavelength corresponding to the change in refractive index.

Approach

Nanosphere Lithography

Nanosphere lithography (NSL) is a low-cost and surface-independent technique that allows creation of a well-ordered array of triangular nanoscale silver particles.[3] Figure 1 shows each step of the process in fabricating the nanoparticle.[5] Glass substrates are treated in two preliminary steps: (1) piranha etch, 1:3 30% H_2O_2:H_2SO_4 for 0.5 hr to clean the substrate; (2) base treatment, 5:1:1 H_2O:NH_4OH:30% H_2O_2 with sonication for 1 hr to make the surface hydrophilic. Approximately 2.3 µl of 390-nm-diameter latex polystyrene nanospheres were coated onto the glass substrate to create a hexagonal close-packed array nanosphere mask. The nanosphere solution was allowed to dry in ambient conditions. Ag (20–25 nm) was deposited onto the nanosphere mask. Afterwards, the nanospheres were removed by sonicating for 3 min.

PSA Antibody Binding Study

Figure 2A shows a schematic representation of a PSA-antibody assay. The nanoparticles were first incubated in a solution of 1 mM 3:1 octanethiol: mercaptoundecanoic acid for at least 24 hr to form an SAM. PSA antibodies were

Table 1: PSA Assay		
	$\Delta\lambda_{max}$ (nm)	
Sample	Antibody	PSA
1 nM Antibody	-5.0	-
	6.3	-
10 nM Antibody	13.2	12.8
	12.6	5.0
100 nM Antibody	20.0	4.0
	30.0	4.0

Table 1. LSPR response to PSA binding to antibody-functionalized Ag nanoparticles. For each antibody concentration, a constant concentration of 100 nM of PSA was applied. Experiments were performed twice for each antibody concentration.

then covalently attached using 1-ethyl-3-[3-dimethylamino-propyl]carbodiimide hydrochloride (EDC). Different concentrations of antibodies — 1 nM, 10 nM, and 100 nM — were used to monitor the change in LSPR. For each sample, 100 nM of PSA was incubated for 1 hr on the antibody-covered surface. The optimal concentration of antibody had to be determined in order to maximize the shift of LSPR from PSA. All of the data in the results section were obtained in N_2 after rinsing.

Results and Discussion

Table 1 shows the PSA shift according to three different antibody concentrations. The experiment was performed twice for each concentration of antibody. When 1 nM of antibody was functionalized to the SAM surface, a small red shift of 6.3 nm occurred. More experiments using 1 nM of antibody must be repeated in the near future to obtain conclusive data. After exposure to 10 nM of PSA antibody, the LSPR shift was 12 and 17 nm. When PSA was applied, the LSPR shifts were 12.3 and 5 nm, respectively. The LSPR shifts were 20 and 30 nm after 100 nM of PSA antibody was functionalized. When PSA was applied, the red shifts were 4 nm both times.

The results demonstrate that the higher the concentration of antibody, the larger the red shift observed; the higher the concentration, the more antibody will covalently bond to the SAM surface. When greater amounts of adsorbates are near the surface of the nanoparticle, a greater refractive index change occurs.

Attaching the antibody to the SAM-functionalized nanoparticle surface ensures the specificity of the protein to bind. In this case, it was desirable to have

only the PSA bind to the antibody. Greater concentration of antibody does not necessarily correspond to increased binding of PSA. Instead, when large amounts of antibodies are present on the surface of a nanoparticle, steric hindrance can occur. This makes it difficult for the PSA to bind to the specific antibody, since the specific antibody is significantly greater in mass than the antigen PSA. The results indicated that when 10 nM of PSA antibody was present, a slightly higher red shift occurred, then when 100 nM of PSA antibody was used. For 10 nM of PSA antibody, a 12.8 nm red shift occurred; for 100 nM of antibody, a 4.0 nm red shift was observed. It is assumed a larger PSA shift occurs with 10 nM of antibody.

Maximizing the PSA shift will help showcase the sensitivity of the LSPR biosensor. Enabling this biosensor to detect PSA at the lowest level of concentration can lead to an earlier diagnosis of prostate cancer. Nonspecific binding experiments should also be initiated in the near future. Also, more experiments must be done in order to confirm the ideal range of the antibody concentration to maximize the PSA shift.

Conclusion

The purpose of this study of the LSPR bionanosensor was to analyze PSA and its interaction with its antibody. The PSA red-shift response is larger when 10 nM of antibody solution is used. This study will help screen potential cancer patients, and also help understand the interaction of key biomarkers of prostate cancer. This work shows one of many steps toward making such progress possible.

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