Detection of DNA Immobilization and Hybridization on Gold/Silver Nanostructures Using Localized Surface Plasmon Resonance

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In this study, we used localized surface plasmon resonances (LSPR) to observe a phenomenon of binding of DNA on Au and Au/Ag nanostructure arrays. Au and Au/Ag nanostructures of various geometric sizes and metal compositions were fabricated by colloidal lithography technique. The immobilization of capture DNA and subsequent hybridization with target DNA on the nanostructures caused the shift of maximum peak in LSPR spectra of the nanostructures. Using the peak shift, the immobilization of capture DNA was clearly verified in a nondestructive manner and hybridization with complementary target DNA was reliably differentiated from the non-specific binding with noncomplementary DNA. This work firmly implies that the LSPR spectra of the nanostructures can be efficiently utilized to achieve a novel strategy for the detection of DNA on the nanostructures.

Keywords: Localized Surface Plasmon Resonance, DNA Hybridization, Colloidal Lithography, Nanostructure.

1. INTRODUCTION

In past few years, there have been great efforts to develop efficient detecting strategies for biomolecules such as fluorescent, electrochemical and optical methods. Of these, fluorescence detection method has been most widely used due to its high sensitivity, however, the alternative methodologies for biosensor have been introduced as solutions to overcome several disadvantages of the fluorescence-based method, including the requirement of complicated labeling procedures. Labeling process is usually time-consuming and quite expensive mainly due to the high cost for the fluorescent reagent. Furthermore, labeled fluorescent materials may cause a high background signal. Therefore, development of a label-free detecting strategy will give a great benefit for biosensors.

Localized surface plasmon resonance (LSPR) can be a promising candidate allowing sensitive detection of biomolecules without labeling. LSPR is an electron charge density wave phenomenon on the surface of metallic nanoparticles and nanostructures. The signal transduction of the LSPR is related with the geometric scales and shapes of gold and silver substrates. With the rapid growth of electronics industry, various technologies to make uniform and small nanostructures have been developed such as E-beam lithography, nanoinprinting and colloidal lithography. Nanostructures fabricated by these technologies can be applied as substrates for immobilization of capture DNA and subsequent detection of target DNA without labeling because the LSPR signals of the nanostructures are intimately affected by the interaction between biomolecules and the substrate.

Another advantage of using LSPR is that no additional step for verification of capture probe immobilization is necessary. Currently available methods to verify DNA immobilization on arrays are based on destructive sampling of arrays which results in the increase of the cost of array production. Furthermore, these methods do not guarantee the immobilization status of the arrays that are actually employed in the analysis because the immobilization is just assumed by the sampled ones. By using LSPR, however, a change of LSPR signal caused by adhesion of capture probe on the surface of the nanostructure can be simply used to verify the successful immobilization of the capture probe. All the chips can be inspected after the production because the detection method using LSPR does not destroy the capture probe on the chips.

In this study, we prepared nanostructures which will serve as substrates for DNA immobilization and subsequent hybridization. Gold (Au) and gold/silver (Au/Ag)
nanostructures were fabricated by colloidal lithography. We prepared Au nanostructures of approximately 100 or 150 nm in diameter and 40 nm in height. Additionally, Au/Ag composite nanostructures of same diameters were manufactured with 20/20 or 10/30 nm in height. A 25-mer single strand DNA (ssDNA) including a mutation site in breast cancer susceptibility gene BRCA1 was immobilized as a capture DNA. A complementary ssDNA was allowed to hybridize with the capture DNA immobilized on the nanostructure. The capture immobilization and target hybridization were successfully detected by using the shifted LSPR signals compared to those of bare nanostructures.

2. EXPERIMENTAL DETAILS

Water was deionized and filtered (MilliQ unit, Millipore, USA). Capture strand, 5′–thiol (C6)–AAAAAAAAAA GGTTGGTTATCACC–3′, complementary target strand, 5′–GGTGATAAGCAACC–3′ and noncomplementary target strand, 5′–TAACAGAGCAGACTTGTAC TT–3′, were synthesized by Bioneer (Daejeon, Korea). All nanostructures were prepared by colloidal lithography. Au layer was deposited on a slide glass using an e-beam evaporator. To make Au/Au nanostructures, Ag thin layer was first formed on a slide glass followed by the deposition of Au layer on the Ag layer. A thin chromium under-layer was deposited before the deposition of metal layers to enhance attachment between the metal layer and the substrate. Polystyrene homopolymer solution (PS) (0.5 wt%, Mw 45,730) was coated on the Au layer using spin-coating. The PS spheres which were spin-casted into a monolayer on the Au or Au/Ag thin films were tuned by the reactive ion etching (RIE) conditions. RIE was performed directly onto the well-ordered colloidal particle array with a mixture of O2 (60 sccm) and CF4 (40 sccm) at a pressure of 60 mTorr. A power density of RIE was 80 W, and the etching time was 60 or 70 seconds. Then, the RIE modified particle array was used as a mask for the Ar ion milling. The DC bias for the ion milling was 400 V, and the Ar pressure was kept below 5 × 10−3 Torr. Finally, by removing the colloidal mask using oxygen plasma and ultrasonic sonication, patterned magnetic Au and Au/Ag nanostructures (100 nm and 150 nm in diameter, respectively) were fabricated.

2.5 μl of 1.0 M dithiothreitol (DTT) solution was added to 2.5 μl of capture DNA solution, and incubated for 15 min at room temperature. And then, ethylene acetate (EA) (Sigma-Aldrich, USA) was used to remove DTT in the capture solution. 5 μl capture DNA solution including DTT and 25 μl EA were mixed and vortexed vigorously for 1 min. Remained EA was removed by a pipette and a vacuum pump. After reducing the thiol-group at the end of capture DNA, Au and Au/Ag nanostructures were exposed to 5 μl of 100 μM or 1 μM capture DNA solution, and then incubated at 37 °C for 24 hours in a humid chamber. After the immobilization, the nanostructures were washed by washing buffer containing 0.2% SDS and distilled water. 5 μl of 100 nM target DNA solution was allowed to hybridize at 37 °C for 4 hours. After hybridization, the nanostructures were rinsed with washing buffer containing 0.005% Triton X-100 and 6X SSPE (0.45 M NaCl, pH 7.4) for 5 min followed by rinsing with triple distilled water at room temperature.

Scanning electron microscope (SEM) (JSM-5600, JEOL Inc. USA) was used to obtain optical images of the nanostructure arrays. LSPR spectra were measured on UV-visible-near IR spectroscopy (Jasco V-570, JASCO Inc. USA) of double-beam system with a monochromator. The scanning wavelength ranged from 500 nm to 1300 nm. The light source was an unpolarized halogen lamp (200~2500 nm). LSPR spectra data were obtained after the fabrication of nanostructures and biomaterial interactions on the nanostructures.

3. RESULTS AND DISCUSSION

To select a proper nanostructure to verify immobilization of capture DNA and to detect hybridization of target DNA, Au and Au/Ag nanostructures of 6 different geometries were manufactured. All the hexagonal nanostructures were fabricated on 2 cm × 2 cm glass slides by colloidal lithography. Au nanostructures have an average height of 40 nm and diameter of either 100 or 150 nm by controlling RIE time. Two Au/Ag nanostructures with the same diameters (100 nm or 150 nm) were also manufactured. The relative height of each component in the Au/Ag nanostructures was designed to be 20/20 nm or 10/30 nm (Au/Ag) by controlling the deposition amount of gold and silver. The typical SEM images of Au/Ag nanostructures of an average 20/20 nm in height and 100 or 150 nm in diameter (Fig. 1) show that the nanostructures were quite uniformly fabricated. Other nanostructures were also successfully manufactured.

Through preliminary optical characterization of the nanostructures, the wavelength showing maximum LSPR signals with bare nanostructures were determined. Figure 2 shows the distributions of maximum peaks (λmax) of the experimental extinction spectra. Bare nanostructures exhibited λmax of LSPR spectra in the 650~850 nm range. The LSPR peaks of Au/Ag nanostructures were detected at above 750 nm, while those of Au nanostructures were detected at near 700 nm. The λmax of nanostructures of larger diameter were always larger than those of corresponding smaller nanostructures. The Au/Ag nanostructure
having larger portion of Ag showed a little higher $\lambda_{\text{max}}$ but the difference was not significant. A problem observed during the preparation of the nanostructures was that $\lambda_{\text{max}}$ of nanostructures of same geometry showed some variation up to 100 nm in the case of B2 at Figure 2. However, the $\lambda_{\text{max}}$ of a bare nanostructure was only used as a baseline and the detection of DNA was determined by the difference between the $\lambda_{\text{max}}$ values of the nanostructure before and after the reactions on the surface. Therefore, we consider that this variation of $\lambda_{\text{max}}$ is not a critical obstacle for the detection of DNA on the nanostructures. Nevertheless, additional consideration or strategy to diminish this variation of $\lambda_{\text{max}}$ should be addressed for more reliable analysis based on the nanostructures.14–16

After measurements of LSPR spectra of bare nanostructures, capture DNA was immobilized on each nanostructure. Table I compares the $\lambda_{\text{max}}$ of each nanostructure before and after capture DNA immobilization using 100 μM DNA solution. The immobilization of capture DNA on nanostructures induced plasmon resonance peaks to be red-shifted relative to those of spectra of bare nanostructures. In particular, capture DNA immobilization on Au nanostructures resulted in less than 10 nm red-shift, while red-shifts by 50–150 nm were observed after the immobilization step.
employed in further experiments to check the utility of the strategy for the detection of complementary target DNA and to investigate the effect of the capture probe concentration for immobilization.

Figure 3(A) shows the spectra of the Au/Ag nanostructure before and after capture DNA immobilization using 100 μM solution, and after hybridization of complementary or noncomplementary target DNAs. For the evaluation of the specificity, \( \lambda_{\text{max}} \) change obtained from hybridization of perfectly matched DNAs was compared to that from mismatched DNAs. In both cases, the red-shift of \( \lambda_{\text{max}} \) was caused by DNA immobilization confirming that the capture DNA was successfully immobilized on the nanostructures. The following hybridization with complementary DNA caused the further red-shift by 71 nm, while there was small \( \lambda_{\text{max}} \) shift (blue-shift by 17 nm) observed upon the application of noncomplementary target DNA, which is supposed not to be hybridized with the immobilized DNA. We assume that the \( \lambda_{\text{max}} \) shift from complementary DNA was mainly resulted from the specific hybridization interaction between the target DNA and the immobilized capture DNA, but one can raise a question about the possibility that the target DNA may be nonspecifically and directly attached on the surface of the nanostructure, consequently resulting in the misleading \( \lambda_{\text{max}} \) shift. However, considering that 71 nm \( \lambda_{\text{max}} \) change by immobilization of capture DNA was quite larger than 71 nm \( \lambda_{\text{max}} \) change obtained from complementary target application and the application of noncomplementary DNA did not cause any significant red-shift of \( \lambda_{\text{max}} \), we can conclude that the \( \lambda_{\text{max}} \) shift was almost exclusively caused by the specific interaction between the DNAs. These results clearly indicate that Au/Ag nanostructure can be efficiently used for discrimination between perfectly matched and mismatched samples.

One disadvantage of our strategy is the requirement of relatively large amount of capture DNA solution compared with other microspotting-based methods because the surface of nanostructure should be fully covered with capture DNA solution for capture immobilization. This requirement may seriously increases the cost of array production. To overcome this problem, we prepared the DNA array using 1 μM capture DNA solution instead of normally employed concentration level of 100 μM and checked its capability for the detection of the complementary target DNA again.20 As shown in Figure 3(B), the array showed 51 nm red-shift of \( \lambda_{\text{max}} \) caused by the hybridization of the complementary target DNA, which was smaller than that from the array prepared using 100 μM capture solution. However, the peak shift still seems to be quite enough for the correct determination of the complementary target DNA if we consider that the binding of noncomplementary target DNA generated small peak shift less than 20 nm. This result indicates that very low concentration of capture DNA could be employed to our strategy without significant loss of final detecting capability.

4. CONCLUSIONS

In summary, this work demonstrates that Au/Ag nanostructures can be successfully used for the detection of DNA immobilization and subsequent hybridization based on a LSPR-based spectroscopic analysis. By incorporating silver to gold component, the \( \lambda_{\text{max}} \) shift of LSPR spectra was greatly increased and a clear discrimination between specific hybridization with complementary target and non-specific binding was successfully demonstrated. Since this strategy enables us to verify capture immobilization in a nondestructive manner and also conveniently detect a target DNA binding without the requirement of time-consuming labeling procedure, it would greatly benefit the array-based technology for the detection of biomolecules including DNA and protein.

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References and Notes


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