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Characterization of localized surface plasmon resonance transducers produced from Au₂₅ nanoparticle multilayers

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Abstract

This article reports the preparation of gold plasmonic transducers using a nanoparticle self-assembly/heating method and the characterization of the films using scattering-type scanning near-field optical microscopy (s-SNOM). Nanoparticle-polymer multilayer films were prepared by the layer-by-layer assembly on glass slides by alternating exposures to monodisperse Au₂₅ nanoparticles and ionic polymer linkers. Thermal evaporation of organic matters from the nanoparticle-polymer multilayer films at 600 °C allowed the nanoparticles to coalesce and form nanostructured films. Characterization of the nanostructured films generated from Au₂₅ nanoparticles using atomic force microscopy (AFM) showed that the films have rounded, small, island-like morphologies (d: 30-50 nm) with a pit in the center of many islands. However, further characterizations with s-SNOM revealed that the produced nanoislands contain a single gold cluster in a pit surrounded by donut-shaped dielectric species. Formation of such a structure is thought to be resulted from the embedding of gold clusters under the reorganized polysiloxane binder coatings and glass surfaces during heat treatment of the Au₂₅ nanoparticle multilayer films. The nanostructured films displayed strong surface plasmon resonance bands in UV-vis spectra with a peak absorbance occurring at ~545-550 nm. The optical sensing capability of the films was examined using *D*-glucose-functionalized gold island films with the interaction of Concanavalin A (ConA). The result showed that the adsorption of ConA on island films causes a large change in the LSPR band intensity.

Keywords

Nanoislands; Nanoparticle; Self-Assembly; Plasmonics; Sensor; Gold

1. INTRODUCTION

Localized surface plasmon resonance (LSPR) is a contemporary technique based mostly on gold (or silver) nanostructures and is attracting an intense interest in molecular and chemical

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sensing [1-3]. It has been known that the surface plasmon (SP) extinction band in discontinuous metal films is highly sensitive to changes in the dielectric properties of the contacting substance, thus allowing different functional groups to be tested for reactivity and adsorption under different biochemical conditions [1-8].

Since the morphology and optical property of nanostructured films have direct impacts on the sensitivity of LSPR measurement, other research groups have tried to control the size and shape of films using various methods including nanosphere lithography [9-11], nanocrystal growth [12-16], template synthesis [17-19], etc. Recently, our group reported the preparation of gold nanoislands through a process of self-assembly/heating of nanoparticle multilayer films [20-22]. This easy-to-control method did not require the use of special equipment such as a vacuum evaporation chamber and is entirely based on wet chemistry utilizing only common laboratory supplies available in most laboratories. The results suggested that the coalescence process of nanoparticles in multilayer films could be altered by using different heating temperatures and nanoparticles with different sizes (2 nm - 10 nm) [20]. In general, a decrease in grain area and heights with increasing heating temperature was observed. The study also proved that the high temperature (> 575 °C) heating dramatically improves the overall stability of nanoisland films [20]. The enhanced stability of nanostructured metal films is critical for the successful application, because the instability of the metal nanostructured films on the glass substrates has been a major problem and has limited a broader use of such systems in plasmonic sensing applications [21,23,24]. Several studies have now shown that the higher temperature heating causes a partial embedding of nanoisland arrays on the glass surface [20,24], so the morphological changes of the films can be prevented when the films are in contact with other chemical reagents or solvents.

The scope of this study is the preparation of gold island films using smaller and monodisperse nanoparticles (Au₂₅; ~1 nm) [25,26], the characterization of the films using scattering-type scanning near-field optical microscopy (s-SNOM), and the examination of the interactions of Concanavalin A (ConA) with *D*-glucose-functionalized gold island films.

S-SNOM utilizes the local field enhancement at the end of an illuminated metallic probe with a resolution only limited by the radius of curvature of the probe and characterizes nanosystems according to their known local dielectric function. S-SNOM has been used to detect the optical material contrast of single nanoparticles, spectroscopic mapping of single nanobeads and viruses, polydisperse nanoparticles, quantum dots, diamond and polymerlike nanoparticles [27-30]. In spite of various optical material contrast studies of nanoparticles using s-SNOM, near-field single particle investigation of complex nanostructures still remains largely unexplored.

The use of glucose-functionalized gold island films for the detection of ConA will serve as a model system to establish the schemes for the preparation of recognition interfaces [31,32]. Originally extracted from *Canavalia ensiformis*, ConA is a lectin protein that binds to terminal α -mannosyl or α -glucosyl groups of sugars and glycoproteins [33]. ConA also plays a significant role as a T-cell mitogen, stimulating mitosis and T-lymphocyte production in biological organisms [34].

2. EXPERIMENTAL SECTION

2.1. Materials

The following materials were purchased from the indicated suppliers and used as received: Hydrogen tetrachloroaurate (HAuCl₄•3H₂O), tetraoctylammonium bromide, toluene, sodium borohydride (NaBH₄), ethanol, methanol, acetone, acetonitrile, tetrahydrofuran

(THF), glass microscope slides, sulfuric acid, 30% hydrogen peroxide, *N,N'*-dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), and 3-mercaptopropyl trimethoxysilane (MPTS) were purchased from Fisher Scientific. 2-Phenylethanethiol, 4-mercaptobenzoic acid, sodium hydroxide, and poly(allylamine hydrochloride) (PAH; MW *ca.* 70,000) were purchased from Aldrich. Water was purified by Barnstead NANOpure Diamond ion exchange resins purification unit.

2.2. Synthesis of COOH-Functionalized Au₂₅ Nanoparticles

Phenylethanethiolate-protected Au₂₅ nanoparticles were synthesized by using the published method [25]. First, a solution of 2.50 g (6.36 mmol) of HAuCl₄•3H₂O and 100 mL of nanopure water was placed in a flask. Using 8.69 g (15.9 mmol) of tetraoctylammonium bromide, AuCl₄[−] was phase-transferred into 100 mL of CH₂Cl₂ and the aqueous layer was discarded. A 2.81 g (20.35 mmol) of 2-phenylethanethiol was added to the reaction mixture. The mixture was stirred for 10 min at room temperature before 2.4 g (63.4 mmol) of NaBH₄ in 30 mL of nanopure water was added for a period of 5 seconds. After stirring for 2 h, the water phase was discarded and the reaction mixture was evaporated to dryness using the rotary evaporator. The crude product was redissolved in 30 mL of acetonitrile, centrifuged for 30 min, and left for one day to allow larger particles to settle. The supernatant was carefully collected and condensed to dryness. The particles were washed with methanol several times and redissolved in acetone. The supernatant was again carefully collected and condensed to dryness. The p-mercaptobenzoic acid ligands were introduced by ligand exchange reaction. A 103.8 mg (0.013 mmol) of Au₂₅ NPs and 36.0 mg (0.233 mmol) of 4-mercaptobenzoic acid was added to 30 mL of acetone in a round bottom flask. The mixture was left stirring at room temperature for 24 h and then evaporated to dryness. The particles were purified using a methanol-toluene flocculation process. The resulting particles (Au₂₅ MBA-NPs) were characterized using UV-vis spectroscopy (Figure S1 in Supplementary Data), FT-IR, ¹H-NMR, and TEM.

2.3. Functionalization of Glass Slides

Glass slides were cut and cleaned in a “piranha” solution (3/1 H₂SO₄/30% H₂O₂) for 1 hour. The slides were then sonicated in nanopure water and then washed repeatedly with nanopure water and methanol. The slides were placed in a 50 mL methanol solution containing 2 mL MPTS and 1 mL nanopure water overnight. (Caution: Piranha solution reacts violently with organic materials and should be handled with extreme care.) The glass slides were rinsed with methanol and blown dry with N₂. The prepared glass slides were stored in a dry cabinet for future use.

2.4. Preparation of Gold Nanostructured Films

For multilayers containing polymer linkers, 10 mg of PAH (M.W. 70,000) was dissolved in 10 mL of nanopure water yielding *ca.* 14 μM solution concentration. The *ca.* 125 μM Au₂₅ nanoparticle solution used in the buildup of multilayers was made by dissolving 10 mg of Au₂₅ MBA-NPs in 10 mL of THF. To build the gold nanoparticle multilayers onto glass slides, the MPTS-functionalized glass slides were placed in the THF solution containing Au₂₅ MBA-NPs for 24 hours [20]. The slides were then alternately placed in the aqueous solution containing PAH and in the nanoparticle solution for ten minutes each to build the second layer of the Au₂₅ nanoparticle films. This last procedure was repeated six more times to build eight layers of the Au₂₅ nanoparticle multilayer films. The nanoparticle multilayer films were heated in a Barnstead Thermolyne 1300 furnace under air for one hour and characterized by monitoring the changes in the absorbance of nanoparticle films by UV-vis Spectrophotometer. The controlled temperature was set at 600 °C ± 5 °C. The heated slides were left to cool in air to room temperature and stored immediately in a dry cabinet.

2.5. Preparation of D-Glucose-Functionalized Gold Island Films and ConA Binding Studies

The nanoisland slides were first placed overnight in 1.0 mM MUA solution in ethanol. The MUA-functionalized slides were placed briefly in a 10 mM DCC in ethanol before placing in a THF solution containing 0.1 M *D*-glucose and 10 mM DMAP for 24 hours. Each slide was placed tightly in a home-made cell with nanopure water solution before measuring the absorbance. The water solution was removed carefully with a syringe and the ConA solution in nanopure water of varying concentrations (10 $\mu\text{g/mL}$ – 1,000 $\mu\text{g/mL}$ in nanopure water) was added to the cell. The UV spectra of the slides in ConA solution were recorded over wavelengths ranging from 400 to 900 nm. The sensitivity of 2 - 3 slides for each concentration was studied.

2.6. Measurements

UV-vis spectra of nanoisland films were acquired on a Shimadzu UV-2450 UV-vis spectrophotometer with a film (slide) holder. A baseline correction procedure (the spectrum of a standard glass slide was taken as baseline) was executed prior to each measurement session. Infrared spectra were obtained, using a Perkin Elmer Spectrum 100 FT-IR UATR spectrometer, from films on glass microscope slides. The spectra were recorded from 4000 to 600 cm^{-1} . Atomic force microscopy (AFM) images were acquired in an ambient condition with a Nanoscope IIIa Multimode scanning probe microscope (Digital Instruments, Santa Barbara, CA) using tapping mode. A silicon probe with the EV scanning head was employed. The nanoisland films were imaged with no further sample preparations. Most of the images were obtained using ACLA tips from AppNano. Scanning parameters varied with individual tips and samples, but typical ranges were as follows: tapping frequency of 150 kHz and scan rate of 1 – 3 Hz. Near-field scattering measurements in the visible (He-Ne laser at 633 nm) frequency were performed using a commercial s-SNOM setup (NeasNOM, neaspec.com). Development and details of s-SNOM are well reviewed in various references [31,32]. Briefly, s-SNOM is based on a tapping mode atomic force microscope (AFM) operated in the intermittent contact mode. S-SNOM is capable of simultaneous topography, amplitude, and phase contrast imaging by recording scattered laser light from commercial PtIr-coated cantilevered Si tips with a vertical oscillation frequency of 240 kHz and amplitude of ~ 20 nm. Near-field scattering data were acquired using a combination of demodulation of the detector signal at higher harmonics of the resonance frequency, $n\Omega$ (demodulation order $n > 1$) and a pseudoheterodyne interferometric signal detection scheme for greater background suppression [33]. In s-SNOM the scattering intensity is caused by the dielectric constants of the materials under the probe and the amplitude contrast is able to map the spatially varying dielectric properties of surfaces for complex material identification and characterization.

3. RESULTS AND DISCUSSION

We have previously shown that the stable gold nanoisland films can be prepared from nanoparticle-polymer multilayer films after heat treatment at 600 $^{\circ}\text{C}$ [20]. Heating at a temperature higher than 600 $^{\circ}\text{C}$ caused the deterioration (notable shape change) of the glass slides preventing the use of any higher temperature. The LbL films (8 layers) of Au_{25} nanoparticles-PAH linkers ($\text{Au}_{25}\text{NP-PAH}$) were grown on the surface of reactive glass substrates and heated at 600 $^{\circ}\text{C}$ for 1 h as described in Experimental Section. Nanoisland films generated from slightly larger Au nanoparticles ($\text{Au}_{314}\text{NP-PAH}$; 5 layers) were also prepared as described in the previous report [20]. Building 8 layers of $\text{Au}_{25}\text{NP-PAH}$ rather than 5 layers was necessary for the formation of gold nanoisland films with the SP bands at $\sim 540\text{-}550$ nm comparable to the island films generated from $\text{Au}_{314}\text{NP-PAH}$ as shown in Figure 1. This result agreed with our previous work that the nanoisland film were produced through the evaporation of organic molecules from nanoparticle multilayer films, which

allowing the Au₃₁₄ or Au₂₅ nanoparticles to coalesce into larger clusters on the surface of the glass slides [20-22]. The nanoisland films produced from Au₂₅ or Au₃₁₄ nanoparticles were visibly different each other. The nanoisland films generated from Au₂₅NP-PAH exhibited a faint pink color which was clearly distinguishable from the strong purple color of the nanoisland films generated from Au₃₁₄NP-PAH. UV-vis spectra of island films generated from Au₂₅NP-PAH showed relatively weaker SP bands despite being generated from 8 layers. The less intense SP bands of gold and a faint color of slides are most likely corresponding to the lower density of islands for the films generated from Au₂₅NP-PAH. Interestingly, a slightly longer maximum wavelength (λ_{max}) was observed for the nanoisland films generated from Au₂₅NP-PAH indicating a somewhat different chemical or physical environment surrounding the gold islands compared to that of films generated from Au₃₁₄NP-PAH.

Atomic force microscopy (AFM) has been used for the shape and size analysis of nanostructured films such as nanoislands [20,34]. Although AFM slightly overestimates the size of nanoislands due to the AFM tip convolution effect, it has provided an opportunity to directly study the relative size and morphology of nanostructured films. After heat treatment of the nanoparticle multilayer films (Au₂₅NP-PAH) for one hour, the films showed rounded morphologies with separations among the small island domains (d: 30-50 nm) in the AFM images (Figure 2). Interestingly, the AFM images also showed a pit in the center of many islands. The morphology shown in Figure 2 was quite different with that of nanoisland films generated from Au₃₁₄NP-PAH (Figure S2 in Supplementary Data), which had a typical morphology of discontinuous metallic nanostructures with a high density of islands [20]. Considering the inability of AFM in distinguishing metals from dielectric materials, a further investigation utilizing state-of-the art s-SNOM technology was necessary for accurate structural analysis of the nanoisland films generated from Au₂₅NP-PAH.

In Figure 3, we present results on the near-field optical imaging consisting of heat treated nanoparticle multilayer films (Au₂₅NP-PAH) described above (Figure 2) for further characterization of the surface. As shown in the topography image (Figure 3(a)), the films look identical with the AFM images in Figure 2 showing donut-shaped islands with a pit in the center. However, the s-SNOM optical amplitude image (Figure 3(b)) clearly distinguishes the donut-shaped islands surrounding the pit as being darker than the pit itself. The darker optical amplitude signal on the donut-shaped islands is because of its small dielectric value, in accordance with previous s-SNOM results that were explained by the dipolar near-field interaction between the tip and sample [35,36]. By taking line profiles (Figure 3(e-f)) we measured the heights of the dielectric layers surrounding an island to be about 5 nm. Furthermore, we found that the optical amplitude signal is reduced to about 20 % of the value of the pit which is about the same contrast as the substrate (Figure 3(f)). These observations imply the formation of such a structure is due to the reorganization of the polysiloxane binder coatings and glass surfaces that occur during the heat treatment of the Au₂₅ nanoparticle multilayer films. The complete removal of other organic materials such as thiol ligands and PAH polymer linkers are confirmed by FT-IR (Figure S3 in Supplementary Data). The formation of dielectric donut-shaped islands also likely involves an embedding of gold clusters and wetting of the cluster sides by the glass during heat treatments. Higher temperature annealing is known to cause an embedding of nanoisland arrays on glass surfaces at high temperature due to a partial melting of glass substrates over 575 °C [20,34]. Since heat treatments of Au₃₁₄NP-PAH films do not produce similar donut-shaped structures as shown in our previous studies [20], it appears that the lower density of small clusters generated from Au₂₅NP-PAH is a critically important factor for the formation of such morphology. The optical image in Figure 3(b) also shows several localized bright spots. Strikingly, the optical line profile taken on one of the spots represented by Line 1 (Figure 3(d)) shows bright localized features on the film with no counterpart in the

topography image (Figure 3(c)). The observation of these localized bright domains is evidence of the surface enhancement of electromagnetic fields in plasmonic hot spots formed on rough films that could be generated while heating. The results clearly demonstrated that the near-field optical contrast studies of nanoisland films on glass substrates using s-SNOM is useful for the high resolution investigation of complex nanostructures and overcomes the limitations of AFM.

Localized surface plasmon resonance (LSPR) sensing property of the gold nanoisland films prepared from Au₂₅NP-PAH and Au₃₁₄NP-PAH was examined by studying the *D*-glucose-ConA interactions. The gold island films were first functionalized with 11-mercaptoundecanoic acid (MUA), subsequently treated with *N,N*-dicyclohexylcarbodiimide (DCC) and then with *D*-glucose treated with *N,N*-dimethylaminopyridine (DMAP). This process resulted in the attachment of *D*-glucose on the surface of the gold nanoisland films [20]. Control experiments were set up using the nanoisland slides generated from Au₂₅NP-PAH and Au₃₁₄NP-PAH without the *D*-glucose attachment (therefore, only MUA on the island surface). A 100 µg/mL solution of ConA was used in these non-specific binding (control) experiments, in which no change in absorbance before and after exposure to ConA was observed (Figure 4 (a) and (c)). This indicates that ConA attachment to the MUA surface of the nanoisland films does not take place without the presence of *D*-glucose on the island surface. However, the intensity of the SP bands of gold was increased for *D*-glucose-functionalized nanoisland films after the exposure to the ConA solution (100 µg/mL) in nanopure water. For nanoisland films generated from Au₃₁₄NP-PAH, the change was only about 2% in the overall LSPR intensity (Figure 4(d)). Compared to this result, the change in the LSPR intensity for the donut island films generated from Au₂₅NP-PAH was about 80% (Figure 4(b)). The concentration of ConA solution was then varied to test for the lowest possible ConA concentration that could be detected by nanoisland arrays generated from Au₂₅NP-PAH films. The slides were exposed to solutions of 1,000, 100, 50, 20, and 10 µg/mL of ConA solution. The LSPR sensing studies for the island films generated from Au₂₅NP-PAH showed that the relative absorbance changes decrease quite linearly with the decrease in concentration of ConA in the range of 20 µg/mL - 100 µg/mL. The results suggested that the gold island films generated from Au₂₅NP-PAH could follow the binding of ConA with *D*-glucose down to the concentration of 20 µg/mL (~180 nM) in nanopure water.

4. CONCLUSIONS

The preparation of gold nanostructured films from Au₂₅ nanoparticle multilayers was successfully achieved through the self-assembly/heating protocol. When heated, the Au nanoparticles condensed into small nanoislands on the glass slide surface. The donut-shaped morphology of films could be accurately identified using s-SNOM. The LSPR spectra of *D*-glucose-functionalized island films showed significant increases in maximum absorbance after the attachment of ConA, clearly showing selective attraction between *D*-glucose and ConA. This model study using island-based LSPR transducers suggests that this research may give some insights into future experiments concerning proteins and their interactions with carbohydrates. With these findings, new techniques in biological and chemical sensors may be developed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

s-SNOM	scattering-type scanning near-field optical microscopy
LSPR	localized surface plasmon resonance
SP	surface Plasmon
ConA	Concanavalin A
DCC	<i>N,N'</i> -dicyclohexylcarbodiimide
DMAP	4-dimethylaminopyridine
MPTS	3-mercaptopropyl trimethoxysilane
PAH	poly(allylamine hydrochloride)
NPs	nanoparticles

6. REFERENCES

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Highlights

- Au₂₅ nanoparticle multilayers are generated by the layer-by-layer assembly.
- Heat treatments of Au₂₅ nanoparticle multilayers produce gold nanoisland films.
- The donut-shaped morphology of films is accurately identified using s-SNOM.
- The LSPR study confirms an attraction between *D*-glucose and Concanavalin A.

Fig. 1. UV-vis spectra of gold nanoisland films prepared from Au₃₁₄NP-PAH and Au₂₅NP-PAH.

Fig. 2.
AFM images of gold nanoisland films with a dimension of $1 \times 1 \mu\text{m}$ generated from $\text{Au}_{25}\text{NP-PAH}$

Fig. 3.

Near-nanostructured films ($\text{Au}_{25}\text{NP-PAH}$) (a) topography, (b) optical amplitude, (c) and (d) line profiles extracted from images (a) and (b) at position Line 1, and (e) and (f) line profiles extracted from images (a) and (b) at position Line 2.

Fig. 4. UV-vis spectra of (a) and (c) MUA- and (b) and (d) *D*-glucose-functionalized gold nanoisland films prepared from $\text{Au}_{25}\text{NP-PAH}$ and $\text{Au}_{314}\text{NP-PAH}$, respectively, before and after exposure to ConA solution (100 $\mu\text{g/mL}$).

Fig. 5. Relative LSPR absorbance changes of island films generated from Au₂₅NP-PAH at different ConA concentrations. The inset plot is a zoomed image with lower concentrations of ConA.