Carbohydrate Research 405 (2015) 55–65

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Electrochemical synthesis of nanostructured gold film for the study of carbohydrate–lectin interactions using localized surface plasmon resonance spectroscopy

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ARTICLE INFO

Article history:
Received 30 April 2014
Received in revised form 15 July 2014
Accepted 30 August 2014
Available online 16 September 2014

Keywords:
Lectin
Mannose
Monolayer
Plasmon
Gold

ABSTRACT

Localized surface plasmon resonance (LSPR) spectroscopy is a label-free chemical and biological molecular sensing technique whose sensitivity depends upon development of nanostructured transducers. Herein, we report an electrodeposition method for fabricating nanostructured gold films (NGFs) that can be used as transducers in LSPR spectroscopy. The NGF was prepared by electrodepositing gold from potassium dicyanoaurate solution onto a flat gold surface using two sequential controlled potential steps. Imaging by scanning electron microscopy reveals a morphology consisting of randomly configured block-like nanostructures. The bulk refractive index sensitivity of the prepared NGF is 100 ± 2 nm RIU^{-1} and the initial peak in the reflectance spectrum is at 518 ± 1 nm under N_2(g). The figure of merit is 1.7. In addition, we have studied the interaction between carbohydrate (mannose) and lectin (Concanavalin A) on the NGF surface using LSPR spectroscopy by measuring the interaction of 8-mercaptooctyl-α-D-mannopyranoside (αMan-C_8-SH) with Concanavalin A by first immobilizing αMan-C_8-SH in mixed SAMs with 3,6-dioxa-8-mercaptooctanol (TEG-SH) on the NGF surface. The interaction of Con A with the mixed SAMs is confirmed using electrochemical impedance spectroscopy. Finally, the NGF surface was regenerated to its original sensitivity by removing the SAM and the bound biomolecules. The results from these experiments contribute toward the development of inexpensive LSPR based sensors that could be useful for studying glycan–protein interactions and other bioanalytical purposes.

1. Introduction

Localized surface plasmon resonance (LSPR) spectroscopy based on the development of noble metal nanostructures with tunable and responsive plasmonic behavior has become of broad interest.[1–5] LSPR spectroscopy can provide a label-free and sensitive technique for biosensing or assays that has great potential to be miniaturized or developed into array formats. The sensitivity of LSPR spectroscopy depends on the properties of the nanostructure used as a transducer. Nanostructures of the coinage metals such as copper,[6] silver,[7] and gold[8] are being actively studied as LSPR-based transducers. LSPR can be observed for nanostructures having features much smaller than the wavelength of the incident light. The LSPR response to change in refractive index in the medium surrounding the nanostructure depends on the composition, shape, size, and local dielectric properties. Although silver shows a stronger LSPR response compared to gold or copper, gold is preferred due to its chemical stability. A recent effort has been reported to electrodeposit gold around gold-silver core-shell nanoparticles on indium tin oxide coated glass to preserve the stronger response of silver.[9] Nanostructures having different shapes such as triangles, spheres, cubes, and rods produce different peak wavelengths, full widths at half maxima and hence different LSPR bulk sensitivity.[10] In general, nanostructures having sharper features yield higher refractive index sensitivity.[11] It has also been found that increasing the size of nanoparticles red shifts the resonance peak position and increases the bulk refractive index sensitivity; however, the peak becomes broader decreasing the figure of merit (FOM) due to radiation damping.[12,13]

Common techniques for fabricating nanostructured transducers include immobilization of nanoparticles on chemically modified substrates,[14,15] nanolithography (including nanosphere lithography,[16,17] and electron-beam lithography[18,19]), and evaporation of a thin layer of metal on a glass surface followed by thermal annealing.[20] Although immobilized nanoparticles (e.g., nanorods, nanostars, nanoprisms, nanorice) show good LSPR responses, there

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http://dx.doi.org/10.1016/j.carres.2014.08.019
0008-6215/

can be some disadvantages with regard to stability and reproducibility.[21] In addition, aggregation of free nanoparticles in solution is a potential challenge and nanoparticles may not be completely free from stabilizers used to avoid aggregation, which will affect the sensitivity measurements and binding experiments.[22] To avoid these limitations, nanolithography techniques have been developed using templates to fabricate different nanostructures. One of the popular nanolithography techniques is nanosphere lithography.[23] In this method, polystyrene nanospheres of various diameters are used as deposition masks on glass substrates. These nanospheres self-assemble in hexagonally close-packed pattern on substrate, such that metals can be deposited in gaps between the nanospheres. The nanospheres can then be removed by sonication of the substrate in organic solvents leaving behind the triangular or spherical nanostructures in a periodic array.[24,25] This method is popular because it is cheaper, simpler, and does not require sophisticated instrumentation.[26] However, there are possibilities for the formation of various types of defects in this method as a result of nanosphere polydispersity, site randomness, point defects, line defects, and polycrystalline domains.[24] The concentration of nanospheres directly plays a role in the arrangement of nanospheres on the substrate,[24] which means a variety of structures may be formed on the same substrate. An alternate strategy involves depositing gold caps on SiO_2 nanospheres randomly arranged on a gold surface, for which a good LSPR response was found.[27] Electron beam lithography can make nanostructures precisely without any defects;[28] however, this technique is expensive and requires more time and expertise.[24] Evaporating a thin layer of metal on glass surface followed by annealing is also a cheaper and simpler technique;[29] however, the nanostructures produced are polydisperse. Annealing of evaporated thin Au films at high temperatures can help to control the morphology and improve the LSPR response.[30] All of these examples show that more research remains to be done in this field for producing sensitive nanostructures so that LSPR spectroscopy can become a method of choice for biochemical sensing. Besides LSPR spectroscopy, these nanostructured transducers are also used in surface enhanced Raman spectroscopy (SERS),[24] a very sensitive analytical technique whose detection limit approaches the single molecular level,[31,32] which once again emphasizes the importance of research in nanostructure fabrication.

LSPR has been compared to traditional SPR[33] and is found to be quite competitive on the basis of a number of features, especially cost. SPR experiments are based upon propagating surface plasmons, often at the surface of a flat gold film, whose thickness should be near 50 nm, and supported on a prism or waveguide. Many of the SPR experiments reported use commercial Biacore instruments along with supplied sensor chips. SPR can be done in a variety of modes, the most popular being measurement of the shift of the resonance angle with analyte binding to the gold surface modified with some sort of recognition layer. Both SPR and LSPR can be conducted in imaging mode; for SPR the element size must be approximately 10 microns, while for LSPR single supported nanoparticles and changes occurring on them can be imaged.[34] For a basic LSPR measurement on an ensemble of nanostructures, either by transmission or reflection, the cost of instrumentation is a small fraction (as little as 1/60th) of the cost of a commercial Biacore instrument, thus far adopted as a standard by much of the life science community. Real-time detection is possible with LSPR as it is with SPR. For LSPR done in transmission mode, extinction at a specific wavelength or resonant wavelength versus time can be followed, while in reflection mode reflectivity at a chosen wavelength or resonant wavelength versus time can be followed. As noted by Van Duyne, the refractive index sensitivity of LSPR is much lower than that of SPR; however, the plasmon decay length is much shorter for LSPR (typically 5–15 nm) than for SPR (200–300 nm), and hence a high level of sensitivity to molecular binding at the surface can still be achieved. The lower bulk refractive index sensitivity of LSPR does provide an advantage of simplicity in that close temperature control is less essential. Recent reviews have covered the variety of nanostructures developed for use with LSPR.[35,36]

SPR has played a major role in probing many types of biomolecular interactions,[37] including protein–carbohydrate and lectin–glycoprotein binding. The applications of SPR to study carbohydrate binding interactions have been reviewed,[38] and compared with other analytical methods. The use of imaging SPR to study binding to carbohydrate arrays is especially promising for screening carbohydrate–protein interactions.[39,40] Approaches based on coupling derivatized carbohydrates to activated SAMs, often in the presence of a diluting species terminated in oligoethylene glycol units known to minimize non-specific protein adsorption, have been pursued using Diels–Alder reactions,[41] disulfide-thiol exchange,[42] and click chemistry.[43] Use of Biacore sensor chips pre-modified with a carboxymethylated dextran gel to which amine derivative glycans can be bound after NHS activation has been reported.[44] This widely used type of sensor chip has the potential complication that the lectin Con A, for example, has an affinity for the dextran component.[45] Mixed SAMs of a carbohydrate component and diluting species have also been prepared directly and studied using SPR.[46] Recently, a method for directly attaching underivatized glycans by photochemically activated C–H bond insertion onto SAMs terminated in a perfluorophenylazide group was reported.[39] Efforts have been reported to precisely control the spacing between sugars using cyclic peptides presenting a specified number of mannose units and to examine the influence of this on the multivalency and clustering effects that can occur during lectin binding.[47,48]

The studies reported in which LSPR has been applied to studying protein binding to a carbohydrate modified nanostructure have primarily been carried out in transmission mode. In an early study, the results for studying a protein–carbohydrate interaction using LSPR and SPR were directly compared.[49] Mixed SAMs of a triethylene glycol terminated disulfide and a maleimide terminated analog were formed on silver triangular nanoprisms formed by nanosphere lithography on glass slides. Reaction of maleimide with a mannose thiol derivative gave about 5% mannose coverage available for interaction with Con A. Experiments were conducted in transmission mode, and both the peak wavelength and the magnitude of its shift due to Con A binding were found to depend on the aspect ratio of the nanoprisms. The modified Ag triangular nanoprisms were resistant to non-specific protein binding and were suitable for following Con A binding in real-time by monitoring the peak wavelength as a function of time, with comparable results for SPR found by monitoring the resonance angle versus time using a Biacore instrument. The response during the dissociation phase was markedly different for LSPR than for SPR, and also dependent on the aspect ratio of the triangular nanoprisms which was found to influence the plasmon decay length. Au nanoparticles supported on glass have been modified by polymer brushes with many pendant glucose residues and LSPR was used to determine a binding constant from real-time analysis of $5.0 \pm 0.2 \times 10^5\ M^{-1}$ noted as larger than that for Con A binding to methyl α-D-glucopyranoside of $2.4 \pm 0.1 \times 10^3\ M^{-1}$ in solution and attributed to multipoint binding effects.[50] The use of supported gold nanoparticles modified with a polymer brush having pendant mannose units was applied to follow Con A binding,[51] resulting in an apparent association constant determined from analysis of real-time association kinetics data of $7.4 \pm 0.1 \times 10^6\ M^{-1}$, noted as much greater than that for Con A to methyl α-D-mannopyranoside in solution of $7.6 \pm 0.2 \times 10^3\ M^{-1}$, with the difference attributed to multipoint binding effects. Au nanoparticles bound to glass modified by 3-aminopropyltrimethoxysilane were modified by dodecanethiol SAMs into which a *N*-acetylglucosamine