A modified nanosphere lithography for the fabrication of aminosilane/polystyrene nanoring arrays and the subsequent attachment of gold or DNA-capped gold nanoparticles

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A modified nanosphere lithographic method for producing arrays of silanized structures at silicon surfaces is described. Polystyrene (PS) particles (600 nm or 1000 nm in diameter) were self-assembled onto a silicon substrate to form a hexagonal close-packed pattern. The resultant patterned surface was then exposed to a solution of 3-aminopropyltriethoxysilane (APTS), which deposited gradually in the interstitial voids of the PS particle array. When such a surface was sonicated in toluene to dislodge the PS particles, a mesoporous network containing truncated PS nanorings/shells was produced. Gold nanoparticles or DNA-capped gold nanoparticles, which are both negatively charged, can be electrostatically attached onto the PS/APTS nanoring array. Atomic force microscopy (AFM) was used to image the surface pattern and structure after each step of the procedure, while X-ray photoelectron spectra (XPS) and UV-visible spectrometry were used to determine the composition of the surface patterns. The mechanisms for forming the PS/APTS nanostructures are discussed. These structures could potentially be used as biosensors, heterogeneous catalysts, and functionalized nano-devices.

1. Introduction

The fabrication of functionalized nanostructures and nanodevices demands development of building blocks with structural and compositional complexity and the invention of simple and reliable methodology.1-3 Template-directed methods for producing well-ordered surface patterns and structures with variable sizes and tunable properties continue to evolve due to their important applications in areas such as catalysis, sensors, and nanoelectronics.4-11 The assembly of 2-dimensional and 3-dimensional arrays of nanospheres (e.g., latex particles and silica spheres) and the subsequent use of the arrays as templates for designing novel devices and for producing meso- and microporous thin films have attracted a great deal of attention.12-14 Materials such as ceramics,15 carbon,16 metals,17,18 and polymers19,20 or semiconducting nanoparticles21 can be filled into the interstitial voids of the arrays. Nanosphere lithography (NSL)4 has proven to be a versatile way for fabricating arrays of nanoparticles on diverse substrates, including, but not limited to, gold, aluminium, indium–tin oxide, glass, and silicon.22,23 When the templates are removed, various types of patterns, such as honeycomb,24 hexagonally packed nanoparticles and nano-triangles, and evenly distributed nano-rings and nanochains6,25 can be produced. However, unless a core–shell template is used26 the formation of arrays constituting interconnected ring-like structures has not been achieved by using NSL. Such ring-like structures may serve as porous electrodes, separation media, heterogeneous catalysts with increased surface areas, and sensors with enhanced sensitivities.6,24

Among the substrates, silicon is particularly attractive because of its low cost, flat and clean surface properties, and versatility in terms of chemical modification.27 For example, organosilanes bearing different functional groups can be attached to the silicon surface, rendering the surface desirable physical and chemical properties.28-32 Recently, our group has assembled polyelectrolyte (PE)-coated nanospheres onto various substrates and utilized the highly ordered core–shell particles to create different surface patterns.24,26-33 This provides additional versatility to NSL in that the dimensions of the core–shell structures can be varied by altering both the thickness of the PE shells and the diameters of the nanosphere cores. Other patterns that are difficult to fabricate by conventional NSL can thus be created. For example, an array of evenly truncated egg-shell structures composed of PE/polyaniline was produced.26 Despite the versatility and the possibility of generating PE/metal13 and PE/conducting polymer composite materials,24,26 some special patterns (e.g., the arrays composed of interconnected rings) cannot be produced without the PE shells. In addition, we have not explored the possibility of attaching functionalized materials onto the prefabricated arrays or networks for potential applications in biological and chemical sensing.

Herein we describe a simple and effective approach for producing nanoring arrays using a modified NSL procedure. The modification was made by treating the silicon surface covered with the hexagonal close-packed PS nanoparticles with a silane reagent prior to the nanoparticle removal. Owing to the formation of a siloxane film within the interstitial voids and possibly around the PS particles, the nanoparticle array becomes more robust. Upon sonication in toluene, a nanoring array is produced. We further implanted gold and DNA-capped gold nanoparticles onto the arrays. The impetus behind this implantation stems from the unique properties associated with gold nanoparticles34 and the diversity of derivatizing or functionalizing gold nanoparticles.35-49 As a demonstration, gold nanoparticles capped with DNA molecules were grafted onto the arrays of PS nanorings.

2. Experimental

2.1 Materials

Ammonia, H2O2, ethanol, toluene, NaCl, NaOH, and propanol were all purchased from Fisher Scientific (Fair
Lawn, NJ). EDTA and Tris-HCl were acquired from Sigma (St. Louis, MO). 3-aminopropyltriethoxysilane (APTS) was obtained from Aldrich Chemical (Milwaukee, WI) and vacuum-distilled before use. A silicon (111) wafer polished on one side was acquired from Silicon Valley Microelectronics Inc. (San Jose, CA). Polystyrene (PS) particles with diameters of 600 nm and 1000 nm (4.2%/3.9 wt.) suspension in water, respectively, were received from Interfaclonal Dynamics Corp. (Portland, OR). The ratio between carbon and sulfur was specified by the vendor to be 71.5/1. All of the solutions were prepared with water purified by a Millipore Milli-Q Plus 185 purification system. The thiolated 30-mer probe with a sequence of 5'-AGA GGA TCC CCG GGT ACC GAG CTC GAA TTC-3' (CH$_2$)$_2$SH was obtained from Integrated DNA Technologies, Inc. (Coralville, IA).

2.2 Solution preparation

The DNA solution preparation followed our published procedure.$^{38,49}$ The synthesis of gold nanoparticles was carried out according to the literature procedure$^{50}$ and the nanoparticle size was determined by UV-visible spectrometry to be 13 nm in diameter.$^{49}$ The DNA-capped gold nanoparticles were prepared by mixing 200 µL of a 9.2 nM gold nanoparticle solution with 80 µL 2.5 mM 30mer DNA. 1% APTS/99% H$_2$O/90% ethanol (v:v:v) was prepared daily and used to submerge the PS particle-covered silicon surface.

2.3 Instruments

All AFM images were obtained using a PicoScan Model (Molecular Imaging, Tempe, AZ) operated in the contact mode. Particle diameter and height were measured using the Picoscan software program (Version 5.3; Molecular Imaging Corporation). The UV-visible spectrometry was obtained with a Cary 100 UV-visible spectrometer (Varian Instruments). X-ray photoelectron spectroscopy (XPS) were collected with a M-probe surface spectrometer (VG Scientific, UK). Electrons were excited with monochromatized Al Kα X-rays incident at 35° from the horizontal sample surface. The pressure in the chamber was 5 × 10$^{-9}$ Torr. The incident X-ray and the analyzer axis were in vertical planes at right angles to each other. All samples were sufficiently conductive and consequently reported binding energies could be referenced to the Fermi level of the spectrometer. Data collection and analysis were performed using the M-probe package software Version 3.4. Survey scans were collected in the scanned mode with an 800 µm × 1500 µm elliptic spot. High-resolution scans were performed in the unscanned mode with the same spot size. The instrument had a resolution (FWHM for the Au 4f$_{7/2}$ peak) of 1.50 ± 0.01 eV and 1.00 ± 0.01 eV, for the survey and high-resolution scans, respectively.

2.4 Procedures

Modification of silicon substrate. The silicon wafers were cut into 1.0 cm × 1.0 cm pieces. They were cleaned with the RCA protocol$^{51}$ (1%HF/5%NH$_4$OH/H$_2$O$_2$/H$_2$O at 80 °C for ca. 15 min), followed by extensive rinsing with a copious amount of deionized water. The modified substrates were either used immediately or stored in water for less than one week.

Fabrications of arrays of PS particles and PS shells and subsequent attachment of gold nanoparticles or DNA-capped gold nanoparticles. Prior to assembling the PS particles onto silicon substrates, the suspensions containing the 600 and 1000 nm diameter PS particle solutions were diluted 100 and 90 fold, respectively. These dilution factors are greater than the literature values because the simple casting procedure for assembling the PS particle template requires a more diluted PS particle solution than the high-speed spin-coating protocol, which tends to diverge the particles on the surface due to the centrifugal force.$^5$ Therefore, in using the simple casting procedure, 50 µL of the diluted PS particle solution was prepared and spread$^{33}$ onto the substrate where the nanospheres were allowed to self-assemble into a hexagonally close-packed monolayer for 2–3 days. To prevent rapid water evaporation, substrates covered with the PS particle solution were housed in a humidified container at 20 °C with about 75% humidity. We and others have shown that this approach generally produces PS particle arrays of higher quality.$^{24,26}$ Subsequently, the resultant substrates were soaked with 1% APTS overnight. Nonspecifically adsorbed APTS was rinsed off with ethanol and water. Finally, the APTS-treated substrates were sonicated in toluene for 15 s.

For the attachment of gold nanoparticles or DNA-capped gold nanoparticles, a substrate that had been subjected to sonication in toluene was exposed to 50 µL of the respective nanoparticle solution for 30 min, followed by rinsing with water and drying with N$_2$.

3. Results and discussion

Fig. 1 illustrates the procedure for creating a well-ordered array of PS rings onto which gold nanoparticles or DNA-capped gold nanoparticles can be subsequently attached. First, a hexagonally close-packed PS pattern was formed by immersing the silicon substrate in an aqueous dispersion (~0.05% V/V) of PS particles (600 nm or 1000 nm in diameter) (Fig. 1a). When such an array is exposed to an APTS solution, APTS is covalently attached to the silicon substrate within the interstitial voids by silanization (Fig. 1b).$^{52–55}$ In addition, the positively charged APTS are electrostatically attracted by the negative charges on the PS particles.$^{56}$ Interestingly, unlike the conventional NSL procedure in which the PS particles are readily removed by exposure to toluene, this procedure produces a more robust PS particle array. We found that a relatively short period of sonication in toluene only dissolves the centers/cores of PS particles, and the hexagonal pattern of the original template is retained (Fig. 1c). Such a structure can be further decorated with other species. For example, as depicted in the final step of Fig. 1, gold nanoparticles or DNA-capped gold nanoparticles can be attached to the PS shells through electrostatic force and/or hydrogen bonding (vide infra). Similar to other studies we have previously published,$^{24,26,33}$ the organization of the final array is highly dependent on the template (i.e., the diameter and the regularity of the packed particles as well as the core extraction time).

3.1 Fabrication of well-ordered PS arrays and deposition of APTS into the interstitial voids

Fig. 2a is an AFM image of a well-ordered hexagonal close-packed pattern of PS particles formed on top of a silicon surface. The treatment of the silicon surface using the RCA protocol prior to the PS particle assembly is carried out for two reasons: (1) negative charges such as OH$^-\$ will be introduced onto the silicon substrate, and generate coulombic repulsion with the small number of negative charges on the PS particles (sulfonate groups), preventing aggregation or stacking of the PS particles and favoring the formation of a uniform monolayer of PS particles,$^{31}$ and (2) the creation of additional silanol moieties at the silicon surface should provide more anchoring sites for the APTS attachment.$^{57}$

The as-prepared template surface was then submerged in an APTS solution. In our work, we judiciously chose the experimental conditions that are propitious to the formation of uniform APTS films within the interstitial voids. Vallant et al.$^{58}$ found that a prolonged aging of the APTS solution results in the formation of preorganized aggregates of silanol molecules as the primary hydrolysis products in solution.
Fig. 1 Schematic representation of the procedures employed for the fabrication of well-ordered arrays of PS nanorings and the subsequent attachment of gold or DNA-capped gold nanoparticles. The sticks at the surface after the first step represent the APTS molecules that withhold the PS shells via covalent and hydrogen bonds and/or electrostatic interactions. For clarity, the PS particles and the APTS molecules are not drawn to scale.

Fig. 2 AFM images of silicon substrates covered with (a) a well-ordered hexagonal close-packed 600 nm diameter PS particles, (b) an array composed of well-packed 600 nm diameter PS nanorings, (c) an array of PS shells that also contains large undissolved 600 nm diameter PS particles formed with a short period of sonication (<15 s), and (d) isolated 1000 nm diameter PS rings produced after a relatively long period of sonication (>15 s). The cross-sectional contour of (d) corresponds to the height difference between the regions inside and outside of the ring. The scale bar in all images is 800 nm. The grayscale bars for heights are provided for all the images.
Therefore, in our work, fresh APTS solutions were prepared daily and used within 1 h after preparation. Since the deposition time is also an important factor affecting the thickness and porosity of the APTS film, different times were also investigated. A 12 h soaking was deemed to be optimal for the creation of a highly regular APTS array. A shorter time causes the formation of monolayers with more defects58 (data not shown). We also found that a relatively dilute APTS concentration (1%) tends to reduce the possibility of forming “mounds”.59 As will be delineated below, the adsorption of APTS in the interstitial voids increases the mechanical stability of the PS particles.

3.2 Formation of PS nanoring arrays

Fig. 2b depicts an AFM image of a PS particle array that had been sonicated in a toluene solution for 15 s. As can be seen from this image and the dimensions of the rings (Table 1), unlike what is typically encountered in NSL4 which completely dislodges the particles off the surface, only the apices of the PS particles are truncated. If the sonication is shorter than 5 s, some intact PS particles remain on the surface in Fig. 2c. Note that the diameter of the PS particles shown in this AFM image appears to be greater than the actual size. We believe that the unremoved PS particles, upon exposure to toluene, may have swollen more dramatically than the nanoring structure, owing to the absence of materials surrounding these particles. On the other hand, longer sonication times (e.g., 1 or 5 min) would rid most of the PS particles of the substrate, leaving some isolated circular features behind (Fig. 2d). The average thickness of the APTS film within the void space can be estimated by measuring the difference between the surface between a nanoring (not covered by APTS) and that between two adjacent rings (modified with APTS). A representative cross-sectional contour revealing such a difference is provided as an inset of Fig. 2d. Since the height displayed in this AFM image is between 0.2–1.2 nm, compared with the thickness of an APTS monolayer (0.2 nm60,61), it is apparent that both monolayered and multilayered APTS films have been formed.

As shown in Table 1, the average outer diameters of the rings originated from the 600 nm diameter particles (578 ± 112 nm) and that from the 1000 nm diameter particles (1002 ± 169 nm) suggest that the hexagonal pattern of the original template is preserved. The thicknesses of the rings (171 ± 32 nm and 292 ± 37 nm from the 600 and 1000 nm diameter particle templates, respectively) indicate that the cores of the PS particles are extracted in the middle of the particles. Thus, it appears that the PS particle walls are somewhat more resistant to the toluene dissolution or the mechanical perturbation by the sonication. While the patterns are similar to those generated from our published procedure using polyelectrolyte-coated nanosphere lithography (PE-NSL),33 the mechanism for the nanoring array formation is different. In PE-NSL, the template is based on a core–shell structure in which the PE shell is left behind on the surface upon the core extraction. In the present case, the formation of the APTS film around parts of the PS particles retards the dissolution of the outer portions of the PS particles that are in contact with the APTS film. As pictorially displayed in Fig. 1b, APTS may have served as a “glue” to adhere the PS particles to the silicon surface by linking the silanol groups on the silicon surface to the functional groups present at the PS particles. As specified by the vendor, the coverage of sulfonate groups on the PS particles is 8% and hydroxyl and carboxyl groups are also present in small amounts. In other words, not only could APTS fill the interstitial voids, it also would link the silanol groups on the silicon wafer to the hydroxyl groups on the PS particle. Moreover, the amino groups on APTS could form hydrogen bonds53,62 with the hydroxyl and sulfonate groups at the PS particles. These two possible interactions are illustrated in Fig. 3a and Fig. 3b, respectively. In the first (Fig. 3a), if the APTS film does not cover the entire particle surface, the uncovered top portion of the PS particle will be truncated first, exposing the middle of the PS particle. The particle will further dissolve in the center and leave a ring-like...

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Table 1  Dimension of the array structures formed with 600 nm and 1000 nm diameter PS particles

<table>
<thead>
<tr>
<th>Shell array formed from 600 nm diameter PS particles/nm</th>
<th>Shell array formed from 1000 nm diameter PS particles/nm</th>
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</thead>
<tbody>
<tr>
<td>Pore inner diameter</td>
<td>Ring thickness</td>
</tr>
<tr>
<td>---------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>PS/APTS</td>
<td>236 ± 48</td>
</tr>
<tr>
<td>PS/APTS/Au</td>
<td>203 ± 43</td>
</tr>
<tr>
<td>PS/APTS/Au + DNA</td>
<td>259 ± 51</td>
</tr>
</tbody>
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* More than 100 nanorings were measured in various AFM images for the reported values.

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Fig. 3  Schematic representation of the two possible types of interactions that could strengthen the attachment of the PS particles and prop up the PS nanorings or thin shells after silanization of the silanol groups on silicon and the hydroxyl groups at the PS particle surface: (a) hydrogen bonding between the amino groups on the APTS and the hydroxyl or sulfonate groups at the PS particles and (b) the formation of APTS polymer films around the PS particles.
structure on the surface. In the second scenario (Fig. 3b), owing to the relatively scarce density of the hydroxyl groups, a patchy APTS film is produced over most of the PS particle surface. However, the toluene solution can still penetrate through the porous APTS film and dissolve the PS materials away. The presence of these bonds has been confirmed by a variety of surface techniques, such as NMR, FTIR, and XPS. Both types of bonds could be present and concertedly consolidate the surfaces of the PS particles during the template extraction step.

It is well known that trifunctional silanes can oligomerize and even polymerize at a surface or in solution, depending on the experimental conditions. The polymerization of APTS, being a bimolecular reaction, proceeds much faster at high concentrations than at low concentrations. Thus, the negatively charged nanospheres, upon preconcentrating the positively charged APTS monomers, could promote the formation of APTS films around the particles. The presence of the APTS polymer films retards the toluene dissolution, creating the ring-like structures.

To show that the array of the nanorings is composed of polystyrene and that small amounts of APTS are also present on the surface, we collected XPS spectra from an array-covered silicon surface (Fig. 4). In the broad survey scan, the peaks of C(1s) and O(1s) were discernable at 286 and 533 eV, respectively. The presence of Si(2p) and Si(2s) peaks suggests that there also exist some bare silicon areas. This is expected, since the center of the PS nanorings is the area of silicon that has never been exposed to APTS (vide supra). The Si(2p) peak, given rise by the sulfonate groups on the PS particles (169 eV) provides evidence about the residual PS materials. The small intensity of the Si(2p) peak can be interpreted based on the low abundance of sulfonate groups of the PS particles (wt% ≈ 8%). Furthermore, a weak N(1s) peak is visible at 402 eV in the high-resolution XPS scan (inset of Fig. 4). This peak indicates it is an ammonium-like chemical form. The low intensity can be ascribed to the fact that the area containing APTS mainly exists within the interstitial voids of the original template whose accumulative area is only 25% of the entire substrate.

The presence of residual PS materials was further supported by UV-visible spectrometric measurements of the materials stripped off the silicon surface. In conducting such an experiment, a surface similar to that shown in Fig. 2b was sonicated in ethanol for 5 min to completely desorb the residual PS shells. The peak at 260 nm in the UV-Vis spectrum acquired from the ethanol solution (not shown) confirms the existence of the aromatic rings associated with the styrene units.

To further demonstrate that the adsorption of APTS molecules plays an important role in affixing the PS shell, we carried out the same procedure as that in Fig. 1 at a thin gold film. AFM did not reveal any partially broken PS particles or PS residues at the final surface, indicating that the PS particle array present at the Au substrate could not sustain the mechanical perturbation. In a different experiment, the step involving immersion of the PS particle array situated on top of a silicon substrate into an APTS solution was skipped. Consequently, the subsequent sonication step completely removed the PS particles. These observations are not entirely surprising, as no covalent or hydrogen bonds are present to attach the outermost PS shell onto the gold or silicon surfaces. The only forces that facilitate the attachment and packing of the PS particles onto surfaces are the capillary force and the weak van der Waals’ interactions between adjacent PS particles. The capillary force will become absent once the water layer over the PS particles is evaporated, and the van der Waals’ interaction is not nearly as strong as the covalent or hydrogen bonds depicted in Fig. 3.

3.3 Attachment of gold nanoparticles or DNA-capped gold nanoparticles

Finally, as a preliminary study, we investigated the possible use of the PS nanoring array as a new template for the fabrication of potential sensor surfaces. Figs. 5a and b display the surface patterns upon grafting gold nanoparticles and DNA-capped gold nanoparticles onto the arrays of PS nanorings produced with the 600 nm diameter PS particles, respectively. The negatively charged gold nanoparticles can be attached onto the sites where the positively charged APTS predominates (i.e., interstitial voids and the exteriors of the PS rings). Possibly blurred by the attached Au nanoparticles, the interstitial voids in Fig. 5a are not as well-resolved as those in Fig. 2b. The AFM image of the PS nanoring array covered with the DNA-capped gold nanoparticles (Fig. 5b) is even less clear than that of the array surface modified with gold nanoparticles. These observations suggest that the Au nanoparticles, being somewhat mobile, may have interacted with the AFM tip to broaden the AFM images. We envision that tapping mode AFM should yield clearer AFM images. AFM measurements of the array shown in Fig. 5b show an increment of approximately 4 nm in
the thickness of the PS nanorings. Such an increase is smaller than the diameter of the gold nanoparticles (13 nm), suggesting that the gold nanoparticles may have been partially imbedded into the PS shells. The thickness of the rings in Fig. 5b was difficult to estimate, due in part to the attachment of a greater number of DNA-capped gold nanoparticles. This is understandable, because the more abundant negative charges on the DNA-capped Au nanoparticles (phosphate groups on the oligonucleotides) can enhance its electrostatic attraction with the APTS moieties.

Fig. 5c is an XPS broad survey scan of the nanoring array partially covered with DNA-capped Au nanoparticles. The characteristic Au peaks were clearly observable. However, the phosphorus peak was not found, perhaps owing to the lower abundance of phosphate groups in DNA and the small surface coverage of oligonucleotides capping the Au nanoparticles. Thus, both AFM images and XPS data suggest that adsorption of the negatively charged Au nanoparticles or DNA-capped gold nanoparticles onto the PS nanoring array can provide a convenient and viable means for the fabrication of 3D constructs that possess a greater surface area and smaller steric hindrance than planar surfaces modified with these nanoparticles. Both features are known to be beneficial for the enhancement of signals arising from specific molecular recognition events.

4. Conclusion

In this work we demonstrate that nanosphere lithography can be modified to fabricate well-ordered arrays of PS nanorings/shells at silicon substrates. By depositing APTS onto the silicon surface within the interstitial voids of the close-packed PS particles and around the nanospheres the affixation of the PS particles is strengthened due to the formation of siloxane structures at the silicon surface and parts of the PS particles. The APTS film helps link the silanol groups on the silicon surface and the hydroxyl and sulfonate groups on the PS particles via covalent bonding, electrostatic attractions, and possibly hydrogen bonding. As a consequence, when the silicon substrate covered by the PS particle array is exposed to a toluene solution with a short period of sonication, well-ordered arrays of PS nanorings/shells can be produced. The dimensions of the ring-like structure were characterized by AFM and the presence of PS materials was verified by XPS and UV-visible spectrometry. The preparative parameters were investigated by changing the size of the PS particles, the APTS concentration,
and the sonication time. We show that the nanoring-covered structure can be further derivatized with gold nanoparticles and DNA-capped gold nanoparticles via electrostatic interaction between the positively charged amino groups at the APTS film and the negatively charged nanoparticles. Such nanoparticle-covered arrays may find potential applications in sensor development and fabrication of functionalized substrates and heterogeneous catalysts. The procedure is simple and tunable, and does not require sophisticated equipment.

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