Toward Controllable Self-assembly of Microstructures: Selective Functionalization and Fabrication of Patterned Spheres

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The ability to fabricate self-assembled three-dimensional (3D) structures is potentially useful for the development of diffractive optical devices, micromechanical systems, and sensory elements. Recently, techniques such as two-photon absorption and multilayer photolithography have been successfully employed to produce 3D structures.[1−3] In addition, colloidal particles have also been used in conjunction with sol−gel techniques to self-assemble nano- or microspheres through gravity or pressure to form closely packed periodic structures.[4−7] However, these processes are generally time-consuming, do not allow selective introduction of defects, and are not applicable to a wide range of materials, and more importantly, there is little control over the lattice structure of the self-assembly (e.g., simple cubic or diamond).

Structures of millimeter-scale self-assembled objects have recently been reported using elements individually functionalized in selective areas.[8−15] It is expected that selective functionalized elements are needed to realize controlled assembly of 3D structures. Herein, novel approaches toward selective functionalization of defined areas on spheres are described. In particular, a general protocol is described here to selectively functionalize micro- or nanospheres with molecules that can potentially direct the self-assembly of these spheres through H-bonding, electrostatic or hydrophilic−hydrophobic interaction, or chemical reaction. These self-assembling sites are introduced via chemical reaction on exposed areas of the spheres; alternatively, a metal layer, such as gold, can be first deposited on the sphere for attaching the desired functional moieties.

In this paper, spheres with gold caps on selective areas have been produced, and complementary strands of DNA were used as potential self-assembly linkers.[16−20] Two methods are reported here to fabricate patterned spheres. In the first approach, a polymer layer was used to control the size of the exposed area on a sphere; whereas in the second approach, controlled metal etching defines the size of a metal cap on a sphere. Both approaches require a monolayer of noncontacting spheres to be spin-cast onto a polished silicon wafer. This was achieved with a specially formulated colloidal silica solution.[21] Figure 1a depicts our approach involving a photoresist-protecting layer. A readily available photoresist (Novolac AZ5214 from Clariant Co., Somerville, NJ) was used because it is formulated to have good planarization properties, and the reactive ion etching (RIE) rate is easily controlled by time and plasma power. Figure 2 shows an atomic force microscope (AFM) image of partially buried spheres after such oxygen-reactive ion etching. As an example, very thin noncontinuous Ti (10 Å) and Au (25 Å) layers were subsequently deposited and the photoresist was dissolved to give spheres functionalized with a gold cap on one side. With gentle sonication, the spheres can be released from the substrate. Depending on the self-assembly strategy, other metals (Ag or Cu) or metal oxides (Al_2O_3 or TiO_2) may also be deposited, as can different types of sphere materials, such as metals and other inorganic oxides. Furthermore, a reactive mono-

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(21) It was found that contacting spheres were difficult to separate after all the processing procedures. Therefore, a specially formulated solution was used and it was prepared as follows: a mixture of 1 g of 1-mm SiO_2 solution (2 wt % in water as received from Duke Scientific), 9 g of methanol, 0.3 g of surfactant FC-430 (3M Co.), 15 drops of *N*,*N*-dimethyl formamide (DMF), and 25 drops of aqueous ammonium hydroxide (concentration 37 wt %). It was then sonicated for 5 min before spin coating.

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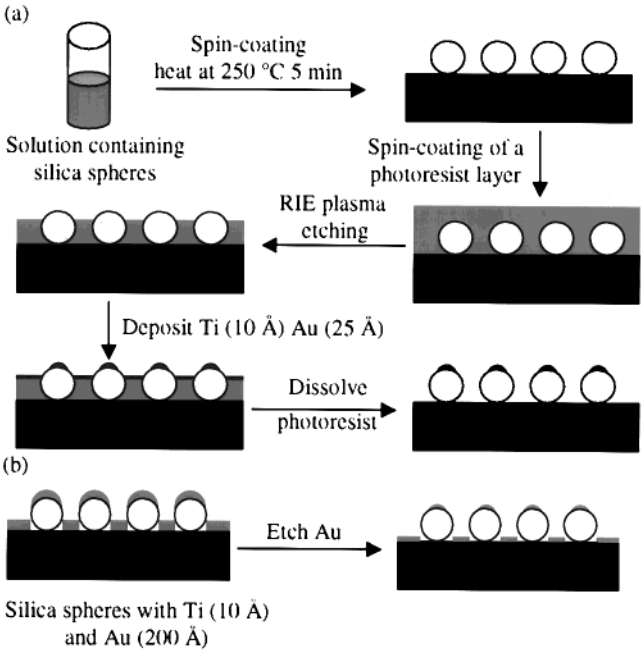

Figure 1. (a) Schematic procedures for selective patterning of spheres using a photoresist layer. A Si wafer covered with a monolayer of SiO_2 spheres was prepared from a colloidal solution.[21] A spin rate of 1000 rpm was used for 90 s. The wafer was heated and held at 200 °C for 15 min to ensure good adhesion between SiO_2 spheres and Si wafer. The photoresist (Novolac AZ5214 from Clariant Co., Somerville, NJ) was then spin-coated using the following spinning profile: spreading at 75 rpm for 5 s; spinning at 3000 rpm for 40 s followed by baking at 120 °C for 90 s to remove the solvent. This spin-coating procedure gives rise to a smooth thick photoresist film (1.6 μm). O_2 plasma was then used to etch the photoresist for 5 min until the thickness was reduced to 950 nm. Ti (10 Å) and Au (25 Å) were subsequently evaporated using e-beam sputtering. The photoresist layer was removed by soaking in an acetone solution for 10 min followed by rinsing with acetone. (b) Schematic procedures for producing spheres with various sizes of gold caps using the gold etching method. The etching solution for Au was prepared by dissolving 16.8 g of KOH, 1 g of $K_3Fe^{III}(CN)_6$, 0.13 g of $K_4Fe^{II}(CN)_6$, and 7.4 g of $Na_2S_2O_3$ in water (300 mL). This solution is stable up to 2 h.

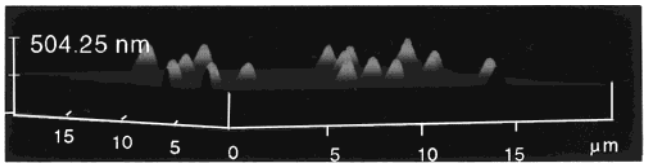

Figure 2. AFM image of spheres with partially exposed upper surfaces from the photoresist layer after O_2 plasma etching for 5 min. About 250-nm height of the 1-μm-diameter sphere is exposed.

layer, such as alkyl trimethoxysilanes for oxides and alkanethiols for gold spheres, can be directly attached to the exposed regions. This photoresist-based approach is versatile for spheres of various sizes of which the smallest ones we have successfully patterned are ≈100 nm in diameter.

In the second approach, we have employed Ti (10 Å) and Au (200 Å for 1 μm spheres) layers deposited *directly* onto the monolayer of spheres. The substrate was placed above the gold evaporation source so that the gold vapor rises vertically, leaving spheres with half of their surfaces being covered (Figure 2). As a result, the gold layer is thickest at the top of the spheres and

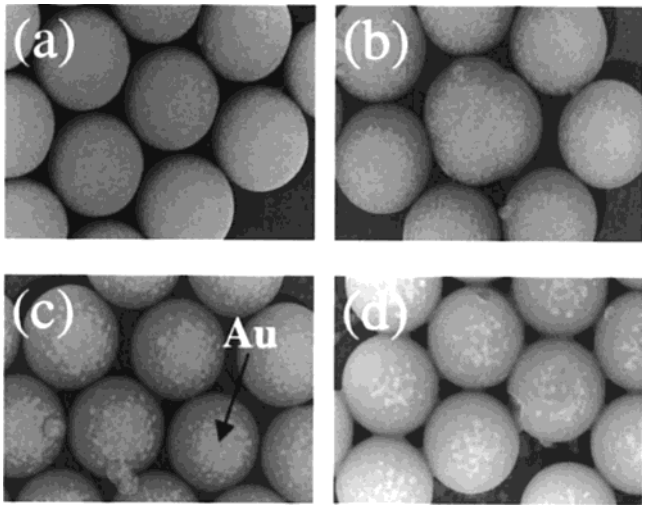

Figure 3. SEM images of 1-μm SiO_2 spheres with gold caps underwent different etching times: (a) 30 s, (b) 2 min, (c) 3 min, and (d) 4 min.

thinnest at the sides. When the etching time is controlled, spheres with various sizes of gold caps can be obtained for further functionalization. Figure 3 shows a series of scanning electron microscope (SEM) images of SiO_2 spheres with Au caps that have undergone different etching times. After an etching period of 30 s, an Au cap with a radius of 480 nm was observed (Figure 3a). Longer etching time leads to a smaller Au cap. The radius of the Au cap was 470 nm after 1 min, 410 nm after 2 min (Figure 3b), 320 nm after 3 min (Figure 3c), and 200 nm after 4 min (Figure 3d). Finally, the Au film was completely removed after a 5-min exposure. Bradley et al. had shown that metal wires can be grown selectively on gold particles using electrochemical reactions.[22] Our methods apply to metal particles and a variety of other particles, such as TiO_2 and Al_2O_3. Compared with the photoresist approach, this approach is simpler if the particles used are table to Au etchant.

With the functionalized side of the sphere attached to the substrate through either H-bonding, electrostatic interaction, chemical bonding, or hydrophilic−hydrophobic interaction, the corresponding upper side of the sphere can also be patterned via methods similar to those described above. Single-stranded (ss) DNA with thiol-derivatized end groups (−SH) was chosen to be attached to a patterned gold substrate, allowing subsequent binding to their complementary strands with its thiol ends attached to the gold caps on the SiO_2 spheres.[17,23] Figure 4 illustrates the selective absorption of DNA-derivatized spheres on a patterned gold substrate that has been derivatized with complementary ssDNA. Control experiments with unmodified spheres did not show any preferred absorption in the DNA-derivatized Au region, which indicates that potential nonspecific binding between DNA and gold is not strong enough to hold gold particles in place. Nonspecific binding between DNAs could also be responsible for selective absorption. However, repeated rinsing of the

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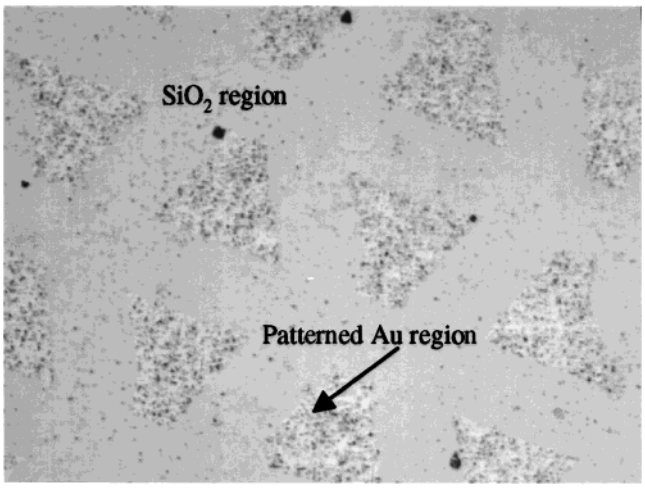

Figure 4. Spheres with single-stranded DNA (ssDNA) modified gold caps showed preferential attachment to areas with their complementary ssDNA modified gold regions observed using an optical microscope (500×). The thiolated ssDNA (from Research Genetics) is a 25-base oligonucleotide with the following sequence: 5′-HS−$(CH_2)_6$−CAC GAC GTT GTA AAA CGA CGG CCA G-3′. The complementary thiolated ssDNA has the following sequence: 5′-HS−$(CH_2)_6$−CTG GCC GTC GTT TTA CAA CGT CGT G-3′. The procedures for the preparation of DNA-modified patterned gold substrates and spheres have been reported in the literature.[27] The DNA-derivatized spheres were collected from the wafers in Figure 1 by sonication for about 5 min in a buffer solution (pH 7.0, TE−1 M NaCl buffer). The hybridization was carried out by immersing the DNA-derivatized patterned gold substrate in DNA-derivatized spheres in TE-1 buffer solution for 120 min at 50 °C.

substrate with buffer solutions did not remove particles from the DNA-derivatized Au region. At this point the gold caps are facing the substrate, and the upper surfaces of the spheres can subsequently be functionalized using similar methods as described above. It was observed that some of the spheres were adsorbed in the Si region due to nonspecific binding. To minimize undesired nonspecific binding, gentle sonication and repeated rinsing with buffer solutions were found to enhance the overall selectivity by more than 50%. Alternatively, a patterned photoresist layer was also applied to cover up large portions of the Au regions, and the photoresist layer was dissolved away together with nonspecifically bounded spheres. Other surface modification methods have been reported previously and can potentially be exploited to further reduce nonspecific binding.[23−25] In this instance, the advantage of using ssDNA is that it offers a reversible self-assembly tool for the preparation of functionalized objects because DNA dehybridization can readily occur above 90 °C. The DNA employed here contained only 25 base pairs, and it is expected that longer DNA strands may be necessary for spheres of larger sizes because of their heavier mass. In addition, other bioconjugate interactions such as biotin and streptavidin or antibody and enzyme can also be used as potential tools for such self-assembly studies.[26]

For the formation of a second gold cap on the same sphere, we utilized the same technology in which gold etching was again applied in conjunction with the photoresist approach outlined earlier. After the photoresist layer was first etched to approximately half the height of the sphere, it was followed with deposition of Ti and Au and subsequently etching of the gold. Gold caps similar to those seen in Figure 4 can be obtained. The advantage of this approach is that it prevents degradation of both the DNA and the first gold cap (now buried in the photoresist) from the etching solution.

In summary, novel approaches for preparing site-specific functionalized spheres have been demonstrated. This effort is applied in combination with DNA-directed self-assembly, which could potentially open new avenues for generating self-assembled structures with controlled parameters, such as periodicity and composition. More importantly, these methods could potentially be applied toward the production of useful quantities of simple micrometer- or nanoscale building blocks for the self-assembly of 3D structures. The selectively patterned spheres can also be used to make core−shell structures with defined pores in the shell controlled by the areas of the functionalized region (in this case, Au caps). The method described here could potentially be extended to fabricate spheres with up to six functionalized sites. Future research efforts are directed toward the exploration of various surface chemistries that allow specific and reversible binding.

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