Self-assembly of gold nanoparticles on e-beam nano-patterns towards protein nanoarray

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ABSTRACT

As our understanding of human diseases grows, so does our need to understand functions and pathways on a proteomic level. Protein arrays are a way of analyzing proteins and pathways within single molecule detection. A new concept of fabricating protein arrays based on self assembly of protein/gold nanoparticle conjugates onto nanometer patterns formed by e-beam lithography. Hydrophilic gold nanoparticles (AuNPs) are formed using the Brust-Schiffrin method without stabilizer, and are exposed to a model hydrophobic surface with hydrophilic patterns etched within. The AuNPs bind to the exposed hydrophilic surfaces, while the hydrophobic surfaces repel the gold. This method can be used to bind different proteins to specific size AuNPs, which in turn, when serially added to nanometer patterns, will self assemble onto their respective sized patterns.

INTRODUCTION

DNA microarray technologies have accelerated the abilities to sequence human genome in the past years. Recently, however, attention has been turned to the field of proteomics, and subsequently to protein arrays. There are good reasons for this change of interests; (1) many untreatable diseases are believed to originate from proteome level, not genome level, and (2) a single genome may express hundreds of different proteomes through post-translational modifications depending on environmental or developmental factors[1]. Protein nanoarrays are a way of analyzing proteins in small amounts and detecting signal pathways through single molecule detection.

A new concept towards fabricating a protein nanoarray is proposed here, based on the self-assembly of gold nanoparticles (AuNPs) onto the nanometer patterns generated by e-beam nanolithography. AuNPs, varying in size and surface hydrophobicity, will be added serially to the nanometer patterns consisting of hydrophobic polymers [e.g., poly(methyl methacrylate) (PMMA)] and hydrophilic metals [e.g., silicon (Si) wafer], and will self-assemble based on their size, hydrophobicity or the potential applied to the system. Several different types of AuNPs will be conjugated to several different antibodies, leading to the fabrication of multi-component protein nanoarrays. Easier and faster multi-component patterning is expected with the proposed method than with the scanning probe lithography (SPL) techniques such as dip-pen nanolithography (DPN), since alignment and calibration are not required between each serial patterning.[1,2] Single-molecule patterning, e.g., single antibody molecule per single array spot, is also possible through 1:1 conjugation of a AuNP and an antibody (Figure 1), enabling single molecule detection (SMD) thus eliminating complications of ensemble-averaged signals. Selective assembly of AuNPs through hydrogen bonding/electrostatic (polar) or hydrophobic (non-polar) interactions onto the nano-patterns will be assessed from scanning electron microscope (SEM) images.

MATERIALS AND METHODS

Substrate Formation: The model substrate used for these experiments was PMMA (495,000 MW, 3% in anisole) (Microchem, Newton, MA) spun on 4" doped silicon (Si) wafers, which contain a thin oxide layer. Wafers were placed on spin coater, then spun at 1000 rpm for 5 seconds while PMMA was added to center of wafers. After 5 seconds, the speed increased to 4000 rpm for 15 seconds to evenly disperse the PMMA on the wafers. The PMMA thickness was measured to be approximately 1200 Å. The wafers were then cut into 1 cm^2 chips. In order to show the proof of concept[7,8], preliminary experiments were performed on chipped polystyrene (PS) Petri dishes and Si chips. Next, Si chips and Si chips covered in PMMA were compared. The next set of experiments were performed using micro-sized trenches, formed by stamped lettering in the Si wafer blocking the spread of PMMA during the spin coating.

AuNP Self Assembly: For synthesizing AuNPs, the Brust-Schiffrin method[3,4], without stabilizer, was used. The lack of stabilizer causes the AuNPs formed to be hydrophilic[5]. All flasks, cylinders, and stir bars are pre-washed in 5 M H_2SO_4 and rinsed rigorously in deionized (DI) water (18.2 MΩ·cm^{-1}) water. 20 mL and 10 mL of DI water are placed into 25 mL cylinders, and N_2 is bubbled through the water, to eliminate the O_2 and prevent oxidation, for 15 min. Approximately 12.5 mg of $KAuCl_4$ is added to the 10 mL of DI water and 4 mg of $NaBH_4$ to the 20 mL of DI water. Solutions are bubbled for 15 minutes with N_2. For each set, 6 mL of the $NaBH_4$ solution was placed in 3 different flasks (AuNP were kept at uniform size) with a magnetic stir bar, then placed in an ice bath and stirred. 3 mL of $KAuCl_4$ was dropwise placed in each flask. The flasks were stirred for 3 hours in an ice bath, resulting in a dark pink/light purple coloration, signaling the synthesis of the AuNPs. The substrate chips were then stirred for 15 minutes within the AuNP suspension, then rinsed with DI water and allowed to air dry overnight.

Sample Preparation: Due to the fact that PMMA is made mainly for use in e-beam lithography, the PMMA coated Si chips needed to be sputter coated with gold. The gold allowed the sample to be more conductive, as well as provided a protective layer that allowed longer viewing time of the samples before the PMMA was deteriorated.

SEM Imaging: The scanning electron microscope (SEM) used was the Hitachi S-4500 field emission SEM[6], containing a cold field emission electron gun, with a maximum magnification capability of 500,000X. Samples were viewed using a secondary electron detector at 5 kV accelerating voltage, to allow both higher resolution and maximum viewing time of image, before deterioration of PMMA affected the image.

RESULTS AND DISCUSSION

Si and Polystyrene: Initial experiments using PS as the model hydrophobic substrate showed that the proof of concept, of compatibility of hydrophilic AuNPs bonding to Si and incompatibility to hydrophobic polymer surfaces, was successful. Many individual AuNPs bonded to the Si surface, with little to no aggregation. However, it was observed that a few of the AuNPs that remained on the hydrophobic surface tended to form larger aggregates. The aggregates seemed to be attracted to surface defects or scratches on the PS surface. This may be due to slight polarization of the defected or scratched areas.

Si and PMMA: The PMMA responded very similar to PS, by repelling the AuNPs or forming aggregates on the surface. Figure 1 shows that one of the PMMA surfaces scanned was completely clear of AuNPs. The Si responded similarly as well, by attracting the AuNPs. There were a few aggregates on the Si surface, but most of the observed AuNPs were single particles. The aggregation may be due to the lack of stabilizer used during AuNP formation. The numbers within the photos (Figure 1) represent places where X-ray analysis was used to determine if the particles were gold under this magnification. The PMMA shows a lot of noise within the picture, due to the lack of gold coating during the time of analysis, and the electron charging of the PMMA surface. The degradation of the PMMA also added to the noise within the picture. The Si surface has a few other particles that, when analyzed by the X-rays, showed that they were not AuNPs, but some form of contamination. Contamination was later found to be dust particles from the laboratory environment.

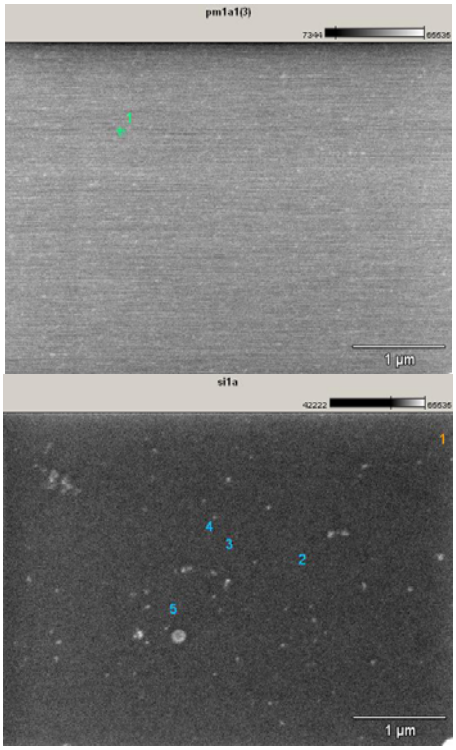

Figure 1. The AuNPs were repelled from the PMMA surface (left) and attracted to the Si surface (right). The PMMA surface is brighter and shows noise, due to both charging of surface and surface degradation.

Micro-channel on PMMA: Specific Si chips containing flaws in the PMMA spreading were used to simulate micro-trench of exposed Si within the PMMA surface. The micro-trench attracted a majority of the AuNPs (Figure 2), while very few AuNPs remained on the PMMA surface, and again the remaining AuNPs aggregated (Figure 3). There is a clear difference between the amount of AuNPs in the micro-trench versus the amount on the PMMA surface. Size distribution was able to be determined at higher magnification. The Brust-Schiffrin method without the stabilizer seems to yield single particles between 30-90 nm. The aggregates formed usually measured between 100-250 nm, depending on the number of particles aggregated.

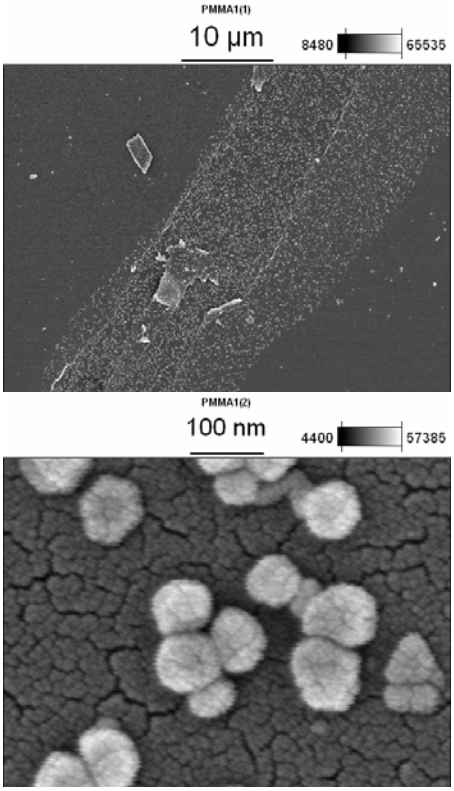

Figure 2. Micro-trench clearly shows a large amount of the AuNPs were attracted to the surface (top). Magnification of the particles in the trench shows both the size distribution and the slight aggregation of the particles (bottom).

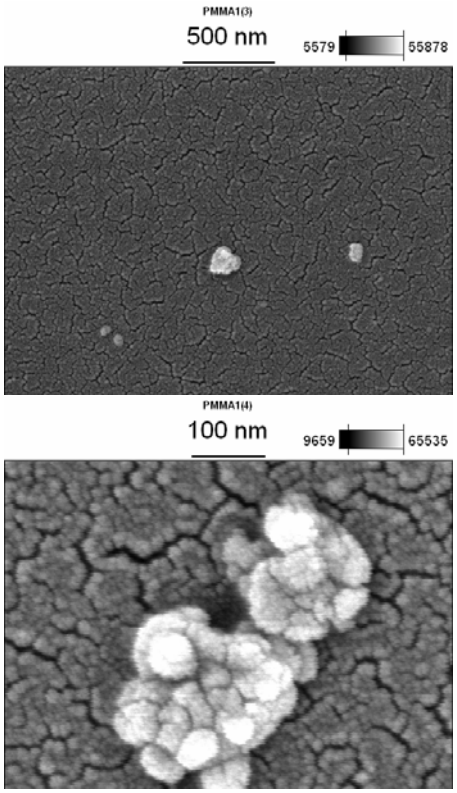

Figure 3. Average area of PMMA, containing very few AuNPS (top). The AuNPS on the PMMA surface tend to aggregate into large clumps (bottom).

CONCLUSIONS AND FURTHER RESEARCH

Hydrophilic AuNPs were formed using the Brust-Schiffrin method and exposed to both hydrophilic and hydrophobic surfaces. The AuNPs were then simultaneously exposed to model surfaces consisting of both hydrophilic and hydrophobic domains. SEM scans of the AuNPs showed compatibility with the hydrophilic Si surface and incompatibility with the hydrophobic PS and PMMA surfaces.

Further research will include the effects on variation in AuNP size and the formation of various nano-sized trenches. 'Trench' patterns shown in the left will be formed, followed by 'square' patterns shown in the right (Figure 4). The largest AuNPs will be added first onto these nano-patterns, followed by rinsing the excess AuNPs. The second largest AuNPs will be followed and the process will continue until the smallest AuNPs are self-assembled on the smallest nano-patterns. Hence the whole patterning process is serial in nature. The whole nano-patterning procedure will be monitored

again through the SEM images. Once successful in the above approach, several different types of AuNPs will be conjugated to several different antibodies, and the above process will be repeated with the antibody-AuNP conjugates, leading to the fabrication of multi-component protein nanoarrays (Figure 5).

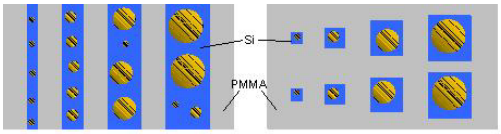

Figure 4. AuNPs self-assembles into 'trench' (left) and 'square' (right) nano-patterns.

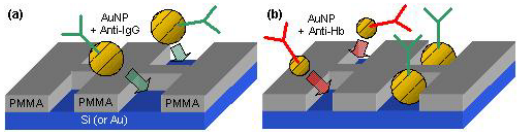

Figure 5. Serial patterning of anti-IgG and anti-Hb through self-assembly of differently-sized AuNPs on nanometer patterns.

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