Published in final edited form as:

Angew Chem Int Ed Engl. 2009 ; 48(14): 2546–2548. doi:10.1002/anie.200805145.

Biom mineralization Nanolithography: Combination of Bottom-Up and Top-Down Fabrications to Grow Arrays of Monodisperse Au Nanoparticles along Peptide Lines on Substrates

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Abstract

Biom mineralization Nanolithography: Combination of Bottom-Up and Top-Down Fabrications to Grow Arrays of Monodisperse Au Nanoparticles along Peptide Lines on Substrates

Combination of the top-down (peptide nanolithography) and the bottom-up fabrications (biom mineralization) yielded arrays of monodisperse Au nanoparticles on the peptide lines on substrates. The number of particle lines was simply determined by the width of peptide pattern.

Keywords

Biom mineralization; Nanolithography; Bionanotechnology; Nanoparticle; Peptide

Superior and distinguished physical properties of nanomaterials have attracted investigators to construct complex architectures in device configurations in nanometer-scale. For these fabrications, two major approaches have been applied; top-down and bottom-up assemblies. The top-down fabrication has a longer history for microfabrication and still a major technique to produce electronics and optics, which requires creating nanoscale patterns at precise positions.[1,2] The dimension of further miniaturization with the top-down approach will be limited by the spacial resolution of lithography, and the use of the top-down approach for the nanofabrication is also not cost-effective. The bottom-up fabrication emerges to overcome these demands by achieving nanofabrication in high spacial resolution with the cost-effective and robust self-assembly,[3–9] however the difficulty for the precise immobilization of these nanoscale building blocks limits its practical application. Therefore, the combination of the top-down and the bottom-up fabrications could integrate advantages

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to create future nanoscale architectures with the precise and robust fabrication of nanoscale building blocks on substrates.[10–24]

Here we combine the top-down and the bottom-up fabrications to grow arrays of monodisperse Au nanoparticles (Au-NPs) on peptide lines patterned on substrates. The part for the top-down came from the patterning of peptides which can effectively mineralize Au-NPs. Then the bottom-up fabrications were applied to grow Au-NPs on underlying peptide lines to produce the array of Au-NP lines. This biomineralization nanolithography yielded monodisperse Au-NPs in the line-array due to the mineralization function of peptide. Previously, the array of NP-lines was grown on the peptides patterned by microfluidics or hologram,[10,11] however our method could reduce the particle size on the peptide array dramatically down to 5 nm in diameter. Another advantage for our integrated technique is the simplicity to design the number of Au-NP lines on substrates; The number of lines of Au-NPs was simply determined by the width of the mineralizing peptide lines patterned by nanolithography. We observed that the number of lines of close-packed Au-NPs was increased proportionally as the lines of the mineralizing peptides were patterned wider. This is one of the simplest methods to fabricate large-scaled arrays of monodisperse nanoparticles whose line width is less than 10 nm.

To develop Au NP-aligned wires in an array form, first the Au-mineralizing HRE peptide (AHHAHHAAD)[25,26] was patterned in a series of lines on Au substrate with nanolithography (Figure 1 (a) – (c)), and then Au nanoparticles were grown on this peptide array pattern via biomineralization (Figure 1 (d)). After the Au substrate was incubated overnight with MUEE ($\text{SHC}_{11}\text{H}_{22}(\text{CH}_2\text{CH}_2\text{O})_3\text{OH}$) to form the protective self-assembled monolayer (SAM) which resists the non-specific binding of peptides, the silicon cantilever of AFM was used as a pen to draw trenches by removing the MUEE SAMs on Au substrates (Figure 1 (b)). After the formation of trenches was confirmed by AFM imaging (Figures 2 (a)), we immobilized 16-mercaptohexadeconic acid (MHA) on Au surfaces in the trenches as a coupling agent to bind HRE peptide. In this process, MHA was immobilized in the trenches with the 12 hr-incubation, and then HRE peptide was covalently bound to MHA in the trenches via the EDC/NHS coupling reaction (Figure 1 (c)). After the resulting substrate was incubated in HAuCl_4 for 24 hrs, Au ions on the HRE peptide were reduced by NaBH_4 for 2 hrs to grow Au-NPs on the HRE peptide lines (Figure 1 (d)).

To study the correlation between the morphology of Au-NP arrays and the width of the mineralizing peptide lines, three different sizes of trenches were prepared such as 12 nm, 18 nm, and 32nm. The accurate measurements of the particle size and trench width are critical for this study, however these values are not reliably determined from AFM images because the radius of AFM tip is comparable with the size of Au-NPs. Since the particle radius was less than 4 nm from the height profile and the radius of the AFM tip was 15 nm, we applied Zenhausern model to calibrate these AFM images.[27] The Zenhausern model is one of the simple quantitative evaluation methods to determine the dimension of the nanoscale features on surfaces, and this model is especially suitable when the shape profile of nanoscale object is round and the radius of the nanoparticle is smaller than the radius of AFM tip.[28] For these reasons, the Zenhausern model was applied to calibrate the scales in x- and y-directions in all AFM images.

When the same concentrations of HAuCl_4 and NaBH_4 were applied to all trenches with different widths filled with the HRE peptides for the Au-NP growth, the diameter of the Au-NPs in the trenches was 5 nm throughout all sizes of substrates (Figure 2). However, the number of lines of Au-NPs in each trench varied and the number of the Au-NP lines was increased as the width of HRE peptide lines was widened. For example, when the peptide lines with the width of 12 nm were incubated with the Au precursor and the reducing agent,

two lines of Au-NPs in the diameter of 5 nm were grown on the peptide lines as shown in Figure 2 (b). Section analysis of this trench in Figure 2 (b) also showed two peaks, corresponding to the two Au-NPs on the peptide lines. Au-NPs on the peptide lines were closed-packed and ordered. The number of Au-NP lines was proportional to the trench size, and three lines of Au-NP wires were observed when the width of the peptide lines was increased to 18 nm (Figure 2 (c)). In the bottom graph of Figure 2 (c), the section analysis also showed three peaks for those particles. The number of Au-NPs apparently increased further when the width of the peptide line was expanded to 32 nm (Figure 2 (d)), and the section analysis in Figure 2 (d) indicates five Au-NP lines were formed in the 32 nm trenches.

We hypothesized that the formation of Au-NP arrays is controlled by the HRE peptide on the substrates. To confirm this hypothesis, we examined the control experiment to reduce Au ions with the same reducing agent on the patterned Au substrate without conjugating the HRE peptide on the MHA SAMs in the trenches. Without the HRE peptides, there were no Au-NPs grown in the trenches (Supporting Information). In this control experiment, fewer Au-NPs were observed on the background of substrate (i.e., non-trench regions), which indicates that Au-NPs on the backgrounds in Figures 1 (b) – (d) are due to the contamination of the HRE peptide attached on the MUEE SAM. This control experiment supports the hypothesis that the HRE peptides are necessary to grow Au-NPs in the trenches. Since it was interesting to observe that the Au-NP size did not change but just the number of the lines was increased as the peptide line width increased, we examined changing the concentrations of the peptide in trenches and Au precursors to correlate their influences on the particle size and/or the number of Au-NP lines. For example, MUEE or 16-mercaptohexadecane was introduced as a spacer in the trenches to dilute the concentration of HRE peptides on the surface. However, the growth of the Au-NP lines was only observed in the trenches at the concentration of 0.1 mM of HAuCl₄ when these spacers were not applied. The growth of metal nanoparticles on organic layers reflects complex factors such as the charge distribution on surfaces, the affinity of the peptide against metal ions, the precursor concentration, and the surface topology.[29, 30] The growth condition here seems to satisfy the ideal condition for the Au-NP line formation, but unfortunately the exact mechanism for the growth of the close-packed Au-NP lines from these complex conditions is still not well understood at this point. Since the HRE peptide contains many amino acids that are strong binders to Au ions, the fast nucleation of Au on the peptide lines could also contribute the uniformity of resulting Au NPs regardless of the line width. The topology of the trench is highly likely concave when the AFM tip scrapes the substrate for the nanolithography of the trench due to the tip shape, and the perfect alignment of Au-NPs along the trench direction could be assisted by the V-shape cross section of the trench running parallel to the long axis of the trench as a result of the nanolithography.

In conclusion, we successfully demonstrated the fabrication of Au-NP arrays in the line form by mineralizing Au-NPs on the HRE peptides patterned as arrays of lines by AFM nanolithography. The number of lines of Au-NPs in the peptide-filled trench was simply determined by the width of underlying Au-mineralizing HRE peptide patterns on substrates. Traditionally, to fabricate an array of monodisperse nanoparticles, separately-synthesized nanoparticles need to be assembled on substrates where binding motifs against capping ligands of nanoparticles are patterned.[31] Our biomineralization nanolithography approach reduces fabrication steps for the nanoparticles array formation by the direct growth of nanoparticles on substrates, and the dimension of line could be reduced to less than 10 nm due to the mineralization function of peptides. The writing of Au-NP lines via

Supporting information for this article is available on the WWW under <http://www.angewandte.org> or from the author.

nanolithography could be applied to wire electronic components in the complicated designs of logic gates, and the fabrication of line-arrays of the monodisperse nanoparticles could be useful in the fabrication of advanced photonic devices. The combination of biomineralization and nanolithography has a unique advantage to create the complex device geometries, and the types of nanoparticles can be expanded to semiconducting nanomaterials because recently various peptides were reported to behave as nanoreactors and they could catalyze semiconductor nanoparticle growths at room temperature.[32]

Experimental Section

Materials

1-Ethyl-3-[3-(dimethyl-amino)propyl]carbodiimide hydrochloride (EDAC), N-hydroxysuccinimide(NHS), 2-mercaptoethylamine, 16-mercaptohexa-decanoic acid (MHA) and octadecanethiol, HAuCl_4 from Aldrich. Annealed gold substrates from Molecular Imaging. Silicon substrate from Virginia Semiconductor. $\text{SHC}_{11}\text{H}_{22}(\text{CH}_2\text{CH}_2\text{O})_3\text{OH}$, 2-(2-(2-(11-mercaptopoundecyloxy)ethoxy)ethoxy)ethanol (MUEE) from SensoPath. HRE peptide (AAHAAHAAD) from Genescript. Si_3N_4 AFM tips were purchased from MikroMasch.

Nanolithography

To pattern Au-NPs as an array on Au substrates, an array of HRE peptide was patterned with the following sequence. First, $\text{SHC}_{11}\text{H}_{22}(\text{CH}_2\text{CH}_2\text{O})_3\text{OH}$ (MUEE), 1 mM, 2 ml, was self-assembled on Au substrates in 99% ethanol at room temperature for 12 h to prevent nonspecific binding of HRE peptide (Figure 1-a). When the substrate was taken out of the MUEE solution, it was diluted by ethanol in a 5–10 fold and removed quickly to avoid the multilayer formation. Then, a cantilever of atomic force microscope (AFM) was used as a pen to remove the SAM of MUEE to form trenches via AFM-nanolithography process (Figure 1-b). Before writing the trenches, the substrate was washed three times with ethanol. On the resulting substrate dried in a stream of nitrogen, an array of trenches was written by removing the thiol SAMs by the tip of AFM. This nanolithography was done in air with a tip force of 1–3 μN in the contact mode with the scanning speed of 1 $\mu\text{m/s}$. In the next step, this substrate was immersed in 16-mercaptohexadecanoic acid (MHA)/ethanol solution (1mM, 2ml) overnight to assemble MHA molecules on the shaved trenches via thiol-Au interaction (Figure 1-d). The substrate was taken out of the solution quickly after the dilution of ethanol, the same manner for the MUEE SAM formation, to reduce the multilayer deposition. After washed by ethanol and dried, the resulting substrate was incubated in 1 ml of the aqueous solution of NHS (50mM) and EDAC (200mM) for 15 min to covalently bind HRE peptide with carbonyl group of MHA. The substrate was rinsed with 10 mM ethanol amine and MES buffer thoroughly, and then immersed in 1 ml of 1 mg/ml of AAHAAHAAD (HRE) in a pH 7.4 PBS buffer solution for 12 h at 4 °C. After the substrate was passivated with 2 ml of 10 mM solution of ethanol amine, it was rinsed with PBS buffer and then incubated in 3 ml of 0.1 mM HAuCl_4 for one day. To grow Au-NPs on the HRE layer on the trenches, 30 μl of 1 % NaBH_4 was added to reduce Au ions. The substrate was removed from the growth solution after incubating for two hours and it was rinsed with water upon removal. Finally, the substrate was dried by flowing nitrogen gas. The topology of the resulting substrates was examined by AFM with the tapping mode.

Acknowledgments

This work was supported by the U.S. Department of Energy (DE-FG-02-01ER45935). Hunter College infrastructure is supported by the National Institutes of Health, the RCMI program (G12-RR-03037). N.N. thanks Drs. C.M. Drain and R. de la Rica for useful discussion.

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Figure 1. Procedure to grow the wires of Au nanoparticles on Au substrate by mineralizing Au nanoparticles on underlying HRE peptide array.

Figure 2.

(a) Top: AFM image of trenches patterned by AFM-based nanolithography. Bottom: Section analysis of the trench along the solid line. (b) Top: AFM image of Au nanoparticles grown in the 12 nm width-trenches filled with HRE peptide. Bottom: Section analysis of the trench along the solid line. (c) Top: AFM image of Au nanoparticles grown in the 18 nm width-trenches filled with HRE peptide. Bottom: Section analysis of the trench along the solid line. (d) Top: AFM image of Au nanoparticles grown in the 32 nm width-trenches filled with HRE peptide. Bottom: Section analysis of the trench along the solid line. Scale bar = 80 nm.