Nanoscale Assembly of Gold Nanowires Templated by Microtubules

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Abstract. There is increasing interest in using biostructures as templates to construct nanoscale-architectures. Many biosystems can self-assemble into regular structures of less than 100 nm and contain functional groups that bind to inorganic particles. In this study, fabrication of microtubule (MT)-based Au nanowires is investigated. Microtubules are fibrous proteins that are rigid hollow tubes about 25 nm in outer diameter and microns in length. We deposit nanoparticles of Au on microtubules by a new method involving photochemical reduction, in which MTs are mixed with Au³⁺ and the surfactant poly(oxyethylene) isoctylphenyl ether (Triton X-100 or TX-100). Exposure of the solution to UV irradiation reduces Au³⁺ to Au⁰. By varying the Au³⁺ and TX-100 concentration in the solution, Au particle size can be well controlled and uniform Au coverage on microtubules is observed. Multiple UV exposure steps induce Au particle growth and leads to dense coverage of Au on the MT.

1. Introduction

There is considerable interest in using biological structures as templates to pattern inorganic materials. Many biomolecules are attractive templates because they self-assemble into periodic structures of less than 100 nm and contain functional groups that bind to inorganic materials[1, 2]. Fibrous structures such as DNA[3, 4], M13 bacterial phage[5, 6], Tobacco Mosaic Virus[7, 8], and NM fiber of yeast[9] have been studied as templates for nanowires. Each of these templates showed the ability to bind Au. Electrical conductivities reported for the M13 virus templated Au nanowire[6] and NM fiber[9]-templated Au wires showed resistor-like behavior, but the resistivities were orders of magnitude higher than the bulk materials. Among the common problems experienced with biotemplated systems are that the bound particles are usually granular and discontinuous. The templates are deformed after deposition and extensive agglomeration usually occurred. It would be very difficult to build an ordered structure with such nanowires.
We are using microtubules (MTs) as templates to fabricate Au nanowires. MTs are protein filaments found in nearly all eukaryotic cells. As part of the cytoskeleton system, microtubules play important roles in supporting cell structures, positioning organelles, and providing intracellular movement. Microtubules are long hollow tubes about 25 nm in diameter and microns in length. They have the advantage of being straight and rigid. When they are injected into a flow field, they align nicely in the direction of the flow[10, 11]. This provides means to form nanosized patterns such as arrays. Structurally, microtubules are composed of dimerized protein subunits called α- and β-tubulin. The tubulin proteins self-assemble into microtubules in vitro at 37°C in presence of guanosine triphosphate (GTP) and Mg\(^{2+}\). The outer surface of a microtubule contains several amino acids, such as histidine, methionine, and tryptophan that can serve as potential binding sites for gold[12].

Au deposition on MT has been reported using Au colloids[13, 14] and chemical reduction methods [14, 15]. These studies demonstrated the ability to bind Au at the MT surface, but the coverage produced was far from continuous. In this paper, we establish a new method for depositing Au nanoparticles on MTs by using photochemical reduction[16]. The Au particles produced were monodispersed and Au coverage on MT was greatly improved. The fabrication of MT-templated Au nanowire was demonstrated.

## 2. Experimental

### 1. Tubulin polymerization

Twice cycled α-tubulins are isolated from porcine brain tissue using procedures modified from the protocol of Williams et al.[17]. Concentration of the tubulins varied from 1 mg/ml to 3 mg/ml depending upon the batch preparation. The tubulins were stored in a buffer (100 mM 1,4-piperazinediethanesulfonic acid (PIPES) pH 6.8, 1 mM ethylenediaminetetraacetic acid, 2 mM MgCl\(_2\)) at -80°C. To form the microtubules in vitro, the tubulin aliquots were thawed at room temperature and mixed with 1 mM GTP and incubated at 37°C for 30 min. At the end of the incubation, 20 μM taxol was added to stabilize the MTs and 0.02% NaN\(_3\) was added to prevent bacterial growth. The assembled tubulins were stored at room temperature.

### 2. Gold nanoparticle formation by photochemical reduction

A 200 μl solution containing 1 mM HAuCl\(_4\) and 5 mM TX-100 in double distilled water was mixed in UVvette (Fisher Scientific) and exposed to 254 nm of UV (Fisher Biotech) for 5 min. The solution was left at room temperature for 2 hours before UV/vis spectroscopy (Varian, Cary100 Bio) was performed. The same procedure was followed in evaluating the addition of 20 mM PIPES, to the HAuCl\(_4\) solution.

### 2.3. Au deposition on MTs

MTs were diluted to 5 μg/ml using a solution containing 20 mM PIPES and 20 μM taxol. 1 mM HAuCl\(_4\) and 5 mM TX-100 were added to the MT solution. The mixture was exposed to UV for 5 min. UV/vis spectroscopy was performed 2 hours after the UV irradiation.

To further increase the number and size of Au particles, a second photochemical reduction was performed. After the first UV irradiation, the solution was centrifuged at 600 x g for 1 min to pelletize the Au-bound MT. The pellet was resuspended in double distilled H\(_2\)O. 2 mM HAuCl\(_4\) and 2 mM TX-100 were added and the solution was exposed to UV again for 5 min.

We also prepared Au MT nanowires on silicon substrates. In this case, the Au decorated MTs formed after the second UV irradiation were deposited and air-dried on the substrate. The solution containing 0.5 mM HAuCl\(_4\) in 20 mM PIPES was added to cover the silicon substrate and exposed to UV for 5 min. The wafer was then rinsed with distilled water and air-dried. Transmission (TEM) and scanning (SEM) electron microscopies were used to image the various MT templates and Au MT nanowires. The TEM used was Jeol JEM-1200EX, while SEM characterization was carried out using Jeol JSM 6700F.
3. Results and Discussion
We used a new photochemical approach to generate gold particles in this study. The method was previously described in the literature[16] and was successful in producing Au particles in a well controlled manner. Nonionic surfactant TX-100 acted both as a reducing agent and Au stabilizer. When Au$^{3+}$ ions and TX-100 are mixed and exposed to UV, hydroxyethyl radicals generated by photolysis of TX-100 reduces Au$^{3+}$. The Au atoms formed in the solution coalesce into clusters. The photochemically generated clusters can be used as nucleation centers and catalyze the reduction of the remaining metal ions present in the adsorbed state or in the bulk. The result is the autocatalytic growth of the nucleation center. The Au clusters are monodispersed due to the stabilization of TX-100 micelles.

Au nanoparticles were formed in the solution containing 1 mM HAuCl$_4$ and 5 mM TX-100 after UV irradiation. The resulting pink solution showed an absorption maximum at around 530 nm (Figure 1a). TEM images showed Au nanoparticles with sizes between 8 nm and 18 nm (Figure 1b). Some of the Au nanoparticles were aggregated into clusters. In the presence of 20 mM PIPES, the solution containing the same amount of HAuCl$_4$ and TX-100 produced more monodispersed and less aggregated Au particles with sizes varying from 10 nm to 12.5 nm (Figure 1c). The corresponding absorption curve showed a sharper and more intense peak at 520 nm, indicating that smaller and more monodispersed Au nanoparticles were produced. These results suggested PIPES acted as an additional stabilizing agent and made the Au nanoparticles more monodispersed and less aggregated. PIPES contains negatively charged sulphonic groups that possibly stabilized the Au through electrostatic interaction.

Photochemical reduction of Au was also successfully performed when microtubules were present in the solution containing HAuCl$_4$ and TX-100. The UV spectrum (Figure 2a) showed a peak at 520 nm for a solution containing 5 µg/ml microtubule, 1 mM HAuCl$_4$, 5 mM TX-100 and 20 mM PIPES. The Au particles were less uniform in size as compared to the same reaction conditions without the MT. TEM showed that Au particles deposited on the MTs were uniformly distributed along the length of the MT (Figure 2b). The use of a second UV irradiation step leads to further growth of the Au nanoparticles and increases the Au coverage on the MTs (Figure 2c). By adjusting the concentration of the TX-100 and HAuCl$_4$, the amount of coverage can be well controlled (data not shown).

Electron diffraction (Figure 3c) taken from the Au MT after the second photochemical reaction showed that the Au MT nanowires are polycrystalline. Comparing the bright field and dark field TEM

![Figure 1](image1.png)

**Figure 1.** (a) UV/vis spectra of Au nanoparticles formed by photochemical reduction. Dashed line: Au nanoparticles formed without PIPES. Solid line-Au nanoparticles formed with 20 mM PIPES. (b) TEM image of the sample giving the UV spectra in dashed line. (c) TEM image of sample giving the UV spectra in solid line.
images (Figure 3a and 3b), it seems that larger Au particles are composed of agglomerations of smaller Au single crystals with different lattice orientations.

![Graph](image)

Figure 2. Au deposition on MT. a) UV/vis spectrum of Au particles formed in the presence of MT (dashed line) and without MT (solid line). (b) TEM image of Au nanoparticle decorated MT after first photochemical reaction. (c) Increased Au coverage on MT after second UV irradiation. (d) SEM image of Au covered MT after third UV irradiation.

We have successfully prepared Au-MT nanowires on silicon substrates. The use of a third photochemical reaction leads to a higher density of Au deposition. Au nanoparticles completely cover the MT, and Au nanowires are produced as shown in Figure 2d. The coverage of Au was still uniform and the diameter of the Au nanowires produced this way ranged between 50 and 80 nm. We are currently performing the electrical conductivity measurements of these Au MT nanowires.

4. Conclusion

We have produced Au nanoparticles in PIPES buffer using photochemical reduction. The presence of the PIPES improved the monodispersity of the Au particles. We then used this approach to obtain Au nanoparticle deposition on MTs. Uniform Au coverage was observed. Multiple steps of UV irradiation were used to increase the Au coverage on the MT and the fabrication of MT-templated Au nanowire was demonstrated.
Figure 3. (a) Bright field TEM image of Au MT after the second UV irradiation (b) The same area imaged with dark field TEM. (c) Electron diffraction pattern taken from the area shown in (a) and (b).

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References
[1] Dujardin E, Mann S 2002 Adv Mater 14 775-788
[2] Niemeyer C M 2001 Angew. Chem. Int. Ed. 40 4128-4158
[3] Becerril H A, Stoltenberg R M, Monson C F, Woolley A T 2004 J. Mater. Chem. 14 611-616
[4] Richter J, Mertig M, Pompe W 2001 Applied Physics Letters 78 536-538
[5] Mao C, Flynn C E, Hayhurst A, Sweeney R, Qi J, Georgiou G, Iverson B, Belcher A M 2003 PNAS 100 6946-6951
[6] Huang Y, Chiang C-Y, Lee S K, Gao Y, Hu E L, Yoreo J D, Belcher A M 2005 Nano Letters 5 1429-1434
[7] Dujardin E, Peet C, Stubbs G, Culver J N, Mann S 2003 Nano Letters 3 413-417
[8] Shenton W, Douglas T, Young M, Stubbs G, Mann S 1999 Adv. Mater. 11 253-256
[9] Scheibel T, Parthasarathy R, Sawicki G, Lin X-M, Jaeger H, Lindquist S L 2003 PNAS 100 4527-4532
[10] Stracke R, Bohm K J, Burgold J, Schacht H-J, Unger E 2000 Nanotechnology 11 52-56
[11] Zhou J C, Luo T-J M, Gao Y, Xue M, Lau J, Hamasaki T, Hu E, Wang K, Dunn B 2006 Mater. Res. Soc. Symp. Proc. 910E p 0901-Ra16-41-Rb16-41
[12] Nogales E, Whittaker M, Milligan R A, Downing K H 1999 Cell 96 79-88
[13] Bekeredjian R, Behrens S, Ruef J, Dinjus E, Unger E, Baum M, Kuecherer H F 2002 Ultrasound in Medicine and Biology 28 291-295
[14] Behrens S, Unger E, 2004 Nanostructured materials synthesized by deposition of metals on microtubule supports, Dekker Encyclopedia of Nanoscience and Nanotechnology (New York: Marcel Dekker, Inc.) p 2563-2569.
[15] Boal A K, Headley T J, Tissot R G, Bunker B C 2004 Mat. Res. Soc. Proc 823 W4.3.1-W4.3.6
[16] Pal A 1998 Talanta 46 583-587
[17] Williams R C, Lee J C 1982 Methods in Enzymology vol 85 (Academic Press), p 376-392.