Gold Nanowire Fabrication with Surface-Attached Lipid Nanotube Templates

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A high-throughput approach to fabricate gold nanowires on surfaces with a lipid nanotube template is demonstrated. Streptavidin-coated gold nanoparticles are attached to the biotin-tagged lipid nanotubes. After the chemical fixation, the samples are dried and treated with oxygen plasma to remove the organic template and connect the particles. The created nanowires are characterized by cryo-transmission electron microscopy, atomic force microscopy, and electrical measurements.

1. Introduction

Nanoparticles are used in multifield applications such as cosmetics, food, agriculture, medical, and pharmaceutical industries. For their surface-based applications, a variety of general methods to align or pattern nanoparticles on substrates have been demonstrated, including self-assembly, dip-coating, electric field induction, magnetic induction, liquid crystal assisted orientation, 3D assembly using DNA origami, and colloidal lithography. Especially the colloidal lithography became a popular sample fabrication approach in surface plasmon resonance sensors and cell adhesion experiments due to its simplicity and high throughput. Atomic force microscopy (AFM)-based approach is a lower throughput but more controlled approach where the exact position of individual particles can be specified. Alternatively, nanofabrication using organic templates is an emerging field. Among the templates, lipids are a good candidate because they are safe, cheap, and their self-assembly offers material-efficient nanofabrication with a good throughput compared to the top-down lithography. Shimizu and co-workers encapsulated gold and silver nanoparticles inside hollow lipid nanotubes (LNT). Through thermal treatments the lipid template was removed and the particles were connected, forming metal wires. Schnur used LNTs assembled in bulk solution as a template to create metal nanotubes by electroless-metallization techniques. The metal nanotubes were successfully used for a cathode for vacuum field emission and as a capsule for controlled drug release. These ideas to use self-assembled lipid objects as templates for fabricating metal tubes or wires are innovative. However, in both cases the formed metal nanotubes and wires are either dispersed in solution or obtained as powder because the original lipid template assembled in solution. Lack of efficient method to pick and place these formed metal objects on substrates limited the further development of applications.

In this work, we fabricate surface-assembled gold nanowires with a LNT template that is preattached on substrates. Previously we have discovered that the main component of bacterial cell membranes, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), self-assembles into single-wall tubes with an outer diameter of 19.1 ± 4.5 nm. DOPE is a zwitterionic conical-shaped lipid. In aqueous solution, they form lipid blocks in inverted-hexagonal phase \( (H_{II}) \). The \( H_{II} \) lipid blocks adsorb on surfaces coated by cationic polyelectrolytes such as polyethyleneimine (PEI) and polylysine (PLL). Upon solution flow, the lipid blocks move while a part of the block is attached to the substrate, protruding LNTs. The method enables the LNTs to be patterned in a simple, straightforward, and high-throughput manner with solution flow. We use these surface-assembled LNTs to fabricate gold nanowires by attaching gold nanoparticles (GNP)
to them, followed by oxygen plasma cleaning to remove the lipid template. The fabricated nanowire has a diameter of around 20–50 nm, which is as small as the feature size of the conventional electron beam lithography with much higher throughput.

2. Results and Discussion

Figure 1 shows our strategy to attach gold nanoparticles to LNTs (see the Experimental Section for the detail). The basic protocol for the LNT surface assembly is described elsewhere.\[20\] We incorporated two main modifications to it. 1) Biotin-tagged PE lipids were added to the LNTs to attach streptavidin-coated GNPs. 2) The substrate was functionalized with PLL grafted polyethylene glycol (PLL-g-PEG)-biotin-streptavidin to attach LNTs but to passivate GNPs to avoid their background adsorption. In brief, first DOPE, DOPE-Biotin, RhodaminePE were mixed in chloroform, dried, and rehydrated with HEPES (4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid) buffer solution with streptavidin-coated GNPs. Upon sonication, lipids form H-II blocks where the streptavidin-coated GNPs are incorporated in the lipid matrix. The H-II lipid blocks were adsorbed on a glass coverslip functionalized with PLL-g-PEG-biotin-streptavidin. The exposed streptavidin on the surface is used to attach H-II lipid blocks via DOPE-Biotin, while the rest of PEG-coated area acts as a passivation surface to prevent nonspecific adsorption of GNPs. Solution flow was induced by a pipette, and the sheer force formed a LNT network. For achieving a high density of GNPs along LNTs, the formed LNTs were further incubated in streptavidin-coated GNPs solution to attach GNPs from outside of LNTs.

Figure 2 shows a fluorescent image of GNP-attached LNTs. LNTs self-assemble because of the conical shape of DOPE lipids that takes H-II phase. Therefore, mixing different types of lipids could disturb the LNT formation. We found that the addition of DOPE-Biotin at 1%–5% did not affect the LNT self-assembly, while LNT formation was disturbed when DOPE-Biotin was added at higher concentrations.

Figure 3 shows cryo-transmission electron microscopy (cryoTEM) images of GNP-encapsulated H-II lipid blocks (Figure 3A) and a LNT (Figure 3B). GNPs are incorporated inside (and are possibly attached outside) of the lipid blocks as we expected (Figure 3A). GNPs were also found on or inside LNTs (Figure 3B). The GNP density along the LNTs was somewhat lower than expected. The procedure to create LNTs on the TEM grid may have affected the density.

Next, LNTs were chemically fixed with osmium tetroxide and glutaraldehyde (see the Experimental Section), so that the LNTs withstand the following drying procedure. Chemical fixation is widely used in biology to prepare samples for electron microscopy.\[22\] Glutaraldehyde cross-links proteins and also reacts with free amino groups of phospholipids. Osmium tetroxide primarily reacts with double bonds in unsaturated fatty acid chains of phospholipids.\[23\] Therefore, the combined use of glutaraldehyde and osmium tetroxide cross-links both the head groups and the tails of the lipids in LNTs, making them possible to dry without destroying their alignment on surfaces. Without fixation, LNTs were completely destroyed upon drying. With such dried samples, we obtained absorption spectra of LNTs with and without GNPs (Figure 4). A clear peak from the surface plasmon resonance\[24\] was observed only after the attachment of GNPs, proving their presence.

Figure 5A shows an AFM image of GNP-attached LNTs after fixing and drying. Note that AFM tends to overestimate the size of structures in xy because it is a scanning microscopy technique, while it has a sub nm resolution in z direction. The height of the LNTs observed in AFM (several
nm) is smaller than what we have seen in cryoTEM images (20–25 nm in Figure 3B). It is because the fixation and drying procedure flattened LNTs. We observed some pits on the background (see the cross section in Figure 5A,Bi,C). They are the footprints of GNPs that nonspecifically attached to the PEG-coated substrates but detouched upon rinsing procedure. Interestingly, GNPs attached to LNTs were not visible in most images. It may be because the strong interaction between the streptavidin coated GNPs and the biotin-functionalized LNTs resulted in engulking many GNPs through an endocytosis-like uptake instead of the deposition on top of the LNT surfaces. After the oxygen plasma treatment for 10 min, these embedded GNPs became visible (Figure 5B).

The zoom-in image of the white dotted square in Figure 5B shows particles aligned along LNTs. Figure 5Bi shows a cross section of a pit (the footprint of particles that have been removed) and a GNP along the LNT. The depth of the pit is around 2 nm, which corresponds to the height of the PLL-g-PEG-biotin monolayer. The height of the particle is around 5 nm from the substrate (considering the 2 nm PLL-g-PEG-biotin monolayer), which matches to the diameter of the GNPs (5 nm). The height of the LNT template relative to the polymer-coated substrate (cross section in Figure 5Bii) was decreased into 1 nm compared to that before oxygen plasma treatment (4 nm from Figure 5A). It also supports the successful partial removal of the lipid template by plasma cleaning. Further oxygen plasma cleaning for 1 h removes the template almost completely (Figure 5C). Gold was left on the substrate as droplets. We attempted to connect these gold droplets into gold nanowires by annealing. However, we found out that it is impossible because at high temperature (350–500 °C) surface-attached gold prefers the formation of bigger droplets rather than connecting themselves into wires due to the high surface tension of liquid gold. In fact, this pearling effect of gold is well-known and used to pattern gold droplets on substrates.25,26 Note that at nanometer scale the melting point of gold is much lower than the bulk gold (1064 °C). In addition, the plasma treatment itself has been reported to induce morphological changes in gold.27 In our case, probably the pearling effect already took place during the oxygen plasma cleaning since the chamber temperature increases during the procedure. Therefore, next we rather shorten the duration of the oxygen plasma treatment to capture the moment before pearling starts and characterized their electrical conductivity.

To characterize the electrical properties of the samples, the same fabrication procedure was repeated on glass substrates with micropatterned gold electrodes (interdigitated electrodes with both 10 µm electrode width and the pitch). Figure 6 shows an AFM image of such LNTs fabricated on the substrate with electrodes after fixing. AFM characterization suggested that the LNTs covered both gold electrodes and glass substrate area.

The conductance of the samples was measured with the two-electrode setup (Figure 7). The control sample, where the GNP-attached LNTs were fixed and dried, presented no conductivity (black line in Figure 7). It suggests that the attachment of GNPs on LNTs only does not permit the electrical current to flow, because there is a gap between GNPs. We found that the sample conductivity increases after a short oxygen plasma treatment (2 min 30 s for both sample 1 and 2 in Figure 7). It implies that the removal of the lipid templates and the annealing effect due to the heating of the chamber connected the GNPs slightly. Even if we observed electrical connection, the sample resistivity \( \rho = 1.5 \times 10^4 \mu \Omega \text{ cm} \) supposing a single LNT with a dimension of 3 nm × 50 nm × 10 µm from the AFM images was four orders of magnitude higher than the reported values.
(2–6 $\mu\Omega\text{cm}$ for gold nanowires\cite{28,29} and 2.2 for bulk gold). It is probably because the organic template is still remained in between gold interfaces, disturbing the current. We attempted to remove those organic residuals by piranha cleaning, however, the procedure destroyed the patterned electrodes. Therefore, we could not perform the subsequent electrical characterization. In addition, $I-V$ curves presented a hysteresis (red and blue lines in Figure 7). It implies the presence of redox reactions during the voltage application, which also indicates the remained organic molecules on the gold surfaces. Further increase in the sample conductivity was difficult, because longer plasma treatment or annealing induced...
pearling rather than connecting the GNPs. Those samples with longer oxygen plasma treatment or annealing did not present electrical conductivity.

3. Conclusion

We demonstrated an approach to surface-assemble nanoparticles with an LNT template. The main advantage of the method is that the small size of the LNT (\(\phi = 20\) nm) enables the fabrication of solid nanostructures without using expensive electron beam lithography with a higher throughput. The presented work also clarified the advantages (high throughput, cheap) and the challenge (pearling effect of liquid gold) of the method. The approach can be used to align different particles, proteins, and peptides by replacing GNPs with other molecules. It can also be combined with the single LNT patterning with a micromanipulator\(^{[30]}\) to create defined objects instead of random networks.

4. Experimental Section

Buffer Solution (HEPES): All the experiments were performed in HEPES buffer solution at pH 7.4. The buffer solution was prepared with \(10 \times 10^{-3}\) M 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (Sigma-Aldrich, Switzerland) and 0.15 M sodium chloride (Sigma-Aldrich, Switzerland) in ultrapure water filtered through MilliQ Gradient A10 filters (Millipore AG, Switzerland). The pH was adjusted to 7.4 using 6 M NaOH (Sigma-Aldrich, Switzerland). It was sterile filtrated through 0.22 µm porous membranes before use.

Sample Chambers: The sample consists of a donut-shaped polydimethylsiloxane (PDMS) block and a glass coverslip cleaned by an oxygen-plasma cleaner (TePla IoN 3Mz oxygen plasma machine, PVA TePla, Germany) just before the experiments. PDMS blocks adhere to the glass surface acting as a wall to confine liquid inside.

Surface Functionalization with Polyelectrolytes: PLL-g-PEG-biotin (Polylysine (20 kDa) grafted with polyethyleneglycol (2 kDa), #PLL(20)-g[3.5]-PEG(2)) was purchased from SuSoS AG (Switzerland). PEI (\(M_w = 25.000\) g mol\(^{-1}\), branched, #408727) and polystyrene sulfonate (PSS) was purchased from Sigma-Aldrich, Switzerland. PEI and PSS were dissolved in HEPES buffer at concentrations of 1.0 mg mL\(^{-1}\). PLL-g-PEG-biotin was dissolved in MiliQ at 0.1 mg mL\(^{-1}\). For the single layer PLL-g-PEG-biotin coating, an oxygen-plasma-treated glass coverslip was incubated in PLL-g-PEG-biotin/PLL-g-PEG solution (1:1) for 30 min and rinsed with MiliQ. The mixture of biotin-tagged PLL-g-PEG and PLL-g-PEG permits to control the density of biotin on surfaces. Positively charged PLL attaches to the negatively charged glass surfaces, forming a monolayer of PLL-g-PEG-biotin. The same method was used for the coating of chips with interdigitated gold electrodes (#ED-IDE-Au, Micrux Technologies, Spain). To increase the adhesion of PLL-g-PEG-biotin to the substrates, an adhesion layer (PEI/PSS)\(^{[n]}\) between the chip and PLL-g-PEG-biotin was added. Nevertheless, no significant difference between two protocols was observed, and both protocols worked. No clear difference was observed between the number of layers \((n = 1, 5, 10)\). It is partly because the LNT density varies between different samples and different sample areas even if the fabrication condition is the same, thus the small difference due to \(n\) was difficult to detect. After the functionalization of the surfaces with PLL-g-PEG-biotin, the substrates were incubated in streptavidin (#S4762, Sigma-Aldrich, Switzerland) solution (50 µg mL\(^{-1}\)) for 45 min and rinsed with MilliQ.

Lipid Nanotube Assembly: 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (\#850725), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(cap biotiny) (BiotinPE, #870273), and
Electron micrographs were recorded at an accelerating voltage of 200 kV and a nominal magnification of 50 000×, using a low-dose system (20 e− Å−2) and keeping the sample at −175 °C. Defocus values were about −2.5 μm. Micrographs were recorded at 4K × 4K CMOS camera (TVIPS, Germany).

**UV–Vis Spectroscopy:** Absorption spectra were obtained with V-670 (Jasco, USA). The samples deposited on glass coverslips were fixed with a hand-made sample holder. The absorption spectra shown in Figure 4 were taken with LNTs after fixing and drying (black line), or with GNP-attached LNTs after fixing, drying, and plasma cleaning (red and blue lines).

**Electrical Measurements:** I–V curves were obtained with Autolab (Metrohm AG, Switzerland) with the two-electrode measurement setup. For the electrical contact between the chip and the potentiostat, we used a chip holder (#ED-DROP-CELL, Micrux Technologies, Spain).

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