Assembly and characterization of hybrid virus-inorganic nanotubes

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(Received 14 March 2005; accepted 16 May 2005; published online 16 June 2005)

Recently, rod-shaped viruses have attracted attention as biological templates for assembly of nanostructures. Tobamoviruses such as the type strain of *Tobacco mosaic virus* (TMV-U1, or -common) have a cylindrical shape and dimensions suitable for nanoelectronic applications: 300 nm long and 18 nm in diameter with a 4 nm axial channel. TMV particles can be coated with metals, silica, or semiconductor materials and may also form end-to-end assemblies to be used as interconnects or device channels. In this letter, we report the preparation of TMV-U1 templated organic-metal nanotubes, and their structural characterization using transmission electron microscopy and micro-Raman spectroscopy. Reproducible phonon signatures different from that of native TMV-U1 were observed from the metal-coated TMVs. Our results indicate that Raman spectroscopy can be used for monitoring of the bio-assisted nanostructure assembly and for analyzing the vibrational modes of the resulting bio-inorganic junctions. © *2005 American Institute of Physics*. [DOI: 10.1063/1.1952587]

Fundamental limitations for conventional semiconductor technology scaling beyond certain limits motivate the search for alternative nanofabrication technologies and self-assembly concepts.[1] One of the serious problems associated with many actively pursued alternative nanofabrication techniques is the size dispersion of the components. It is extremely difficult to manufacture identical structures at the nanoscale. The stress-driven self-assembly using molecular-beam epitaxy (MBE) suffers from this problem. The MBE-grown nanowires and quantum dots, proposed as elements for future nanoelectronic circuits, come with size variation despite serious efforts on size, shape, and position control in the last decade.[2,3] Another promising approach, the assembly of nanostructures using electrochemically produced nanoporous templates, is also prone to this problem, although probably to a lesser degree.[4,5]

A recently emerging field in nanotechnology is the application of biological objects, such as viruses, as nanotemplates to fabricate organic-inorganic nanoscale materials or devices.[6,7] *Tobacco mosaic virus* (TMV-U1) has a cylindrical shape, which is convenient for applications as nanotemplates to fabricate nanowires for interconnects or elements of the device structure. This rod-shaped virus has suitable nanometer scale dimensions: 300 nm long, 18 nm in diameter, with a 4 nm inner diameter axial channel. The availability of different types of viruses, characterized by their size, surface structure, genome sequence, and coat protein kinetics[8] expands the range of materials that can be combined in assembly. The advantages of using TMV as a template for nanofabrication include: (1) TMV particles of the same type are identical in structure, shape, and dimension; (2) TMV virions can be self-assembled and can form certain organized structures, such as end-to-end assemblies; (3) they are highly stable both physically and chemically; and (4) they can be coated with metals, silica, or semiconductor materials. These advantages make TMV a promising candidate for application in nanotechnology.

The development of virus-assisted assembly of nanodevices faces similar challenges that have been experienced in the past for conventional solid-state technology; for example, material growth optimization and characterization of their properties. While using TMV as a template for nanotube fabrication, one may encounter a typical problem: it is difficult to monitor the coating process and control the quality. The adsorptivity and activity of metallic precursors with the TMV particles, speed of production, the shape, and the location of reduced metals on the virus, are sensitive to the chemical environment, such as the buffer, *p*H and *p*K values, etc. Structural characterization of the assembled metal-TMV nanotubes is another challenge. In the field of biochemistry, transmission electron microscopy (TEM) and x-ray crystallography are the most commonly used techniques to determine the structure and orientation of an organic molecule. Raman spectroscopy, on the other hand, provides supplemental information about the vibrational properties of various subunits and components in protein, DNA or RNA. For the structure of metal atom binding with specific functional groups in the TMV particles, Raman inspection is expected to be helpful in identifying the vibrational mode variation and further exploring the properties of such a metal-organic junction regarding its composition, binding, structure, etc.

In this letter, we report on the assembly of nanotubes on TMV-U1 virions used as nanotemplates, and on the characterization of the hybrid virus-inorganic nanostructures with TEM and Raman spectroscopy. We have prepared TMV nanotemplates using the following procedure. TMV-U1 was mechanically inoculated onto 35 *Nicotiana tabacum* "Xanthi" plants using a concentration of 25 μg/mL in 0.02 M potassium phosphate buffer, *p*H 7.2 (20 μL/leaf, 2 leaves/plant). Plants were grown under standard greenhouse conditions for 7 weeks. 250 g of infected leaves were harvested and virus

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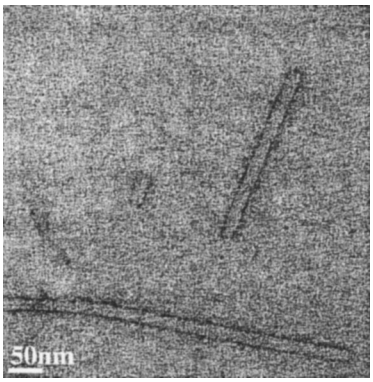

FIG. 1. Uranyl acetate-stained TMV-U1 particles. In the upper part of the photo is a single 300 nm TMV rod. Below is a native end-to-end, multiparticle TMV aggregate.

particles were extracted by mixing the plant tissue in a blender with 500 mL 0.2 M sodium phosphate buffer, *p*H 7.2 and 5 mL 2-mercaptoethanol. The mixture was strained through cheesecloth and centrifuged to remove large cellular debris (8000 g/15 min). The supernatant was adjusted to 8% butanol (v/v), stirred for 20 min at room temperature, and then centrifuged as before. The supernatant was removed and adjusted to 4% polyethylene glycol 8000 (PEG, w/v) and 0.1 M NaCl, incubated at 4 °C overnight, and then centrifuged to collect the precipitated virus. The pellets were resuspended overnight in a total volume of 200 mL sterile water, and then centrifuged for 10 min at 10 000 g to remove contaminants. The supernatant was again adjusted to 4% PEG/0.1 M NaCl, incubated overnight, centrifuged (8000 g/20 min), and the final pellets were resuspended and pooled in a total volume of 50 mL sterile water.

The nanofabrication—in this particular case, coating TMV particles with platinum—was done using our own modification of the method of Dujardin *et al.*[9]: 50 μL of a stock suspension of TMV-U1 in 0.01 M potassium phosphate buffer, *p*H 7.2 (30 mg/mL) was mixed with 1 mL of a 1 mM aqueous hydrogen hexachloroplatinate (IV) (H_2PtCl_6) solution, *p*H 3, and the final *p*H was adjusted to 5 using 0.2 M potassium phosphate buffer ($\sim$4 μL). The suspension was shaken at room temperature in the dark for 20 h before adding 500 μL of a 1 mM hydrazine hydrate solution and mixing well. A 10 μL aliquot was immediately removed ($T=0$), mixed with an equal volume of sterile water and a 10 μL aliquot was applied to a Formvar® and carbon-coated 200 mesh Cu TEM grid, allowed to stand for 2 min, and was then wicked off and air dried. Aliquots were similarly removed at 10, 20, 40, 60 min and 7 days after the addition of the hydrazine hydrate.

The formation and composition of virus-inorganic hybrid nanostructures with respect to the reaction time have been inspected using TEM equipped with an energy dispersion x-ray microanalysis system. Native purified TMV particles were examined by staining with uranyl acetate to enhance the electron transmission contrast since TMV is a weak electron conductor. Both single 300 nm long TMV rods (upper) and end-to-end assembled aggregates were seen (Fig. 1). Examination of the TEM grids containing platinum-coated TMV rods at different time intervals showed that the coating process was gradual and continual. At 10 min after the addition of the hydrazine hydrate, the Pt particles had begun to accumulate along the TMV nanorod [Fig. 2(a)]. At

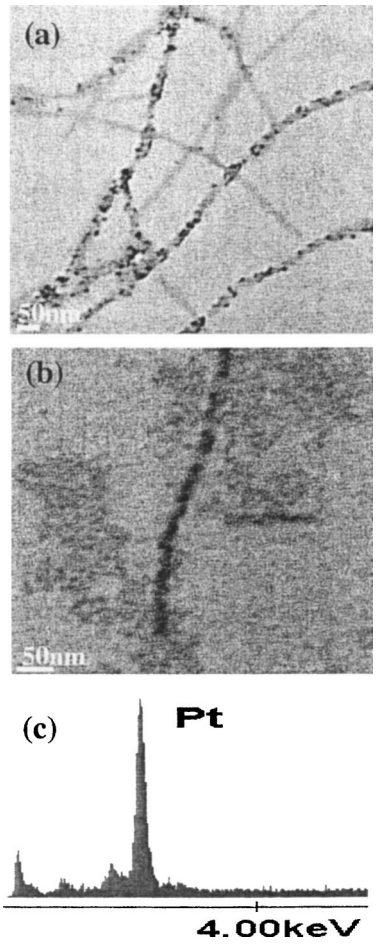

FIG. 2. TMV coated with Pt. (a) Ten minutes into the reaction. Pt nanoparticles appear along the TMV virion. (b) Twenty minutes into the reaction. The outer surface is almost completely covered by Pt. (c) Energy dispersive x-ray analysis shows a strong Pt peak 20 min into reaction.

20 min, the TMV was almost completely covered with Pt [Fig. 2(b)]. An increased Pt peak was observed at 20 min in the x-ray spectrum [Fig. 2(c)]. After seven days, all virions appeared to be completely coated and still intact showing that they are stable in this environment after an extended period of time (Fig. 3). A longer than monomer length, end-to-end Pt-coated TMV assembly is demonstrated in this figure. This self-assembling property and its stability are promising for forming interconnects or nanoelectronic circuit elements.

In another experiment, we used $HAuCl_4$ to fabricate Au-coated TMV nanowires following the method of Ref. 9. TEM inspection indicated that the TMV virions disas-

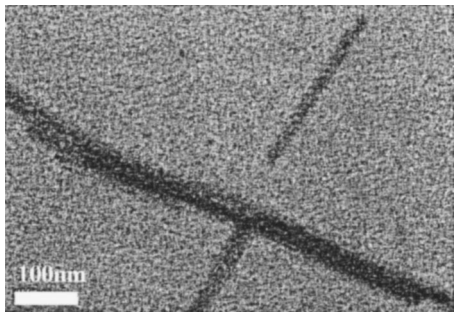

FIG. 3. Pt-coated TMV particles after seven days. End-to-end assembled, Pt-coated TMV virions are shown in the TEM image.

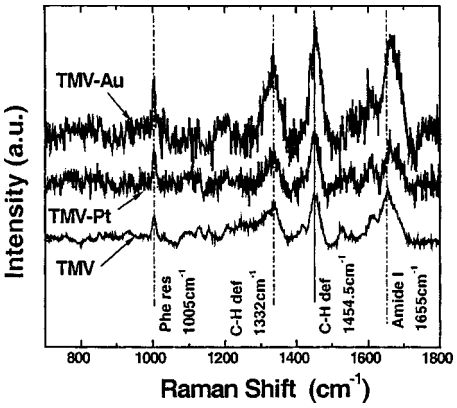

FIG. 4. Raman spectra of native TMV, Pt-coated TMV, and Au-coated TMV. Marked in the figure are the four most prominent peaks of TMV: the Amide I line at 1655 cm^{-1}, C–H deformation lines at 1454.5 and 1332 cm^{-1}, and the phenylalanine residue line at 1005 cm^{-1}. The Amide I lines of TMV-Pt and TMV Au are at 1664 and 1672 cm^{-1}, respectively.

sembled in the acidic environment that was used (measured $p\mathrm{H}=0$) since no intact virions were observed. However, there were observable Au nanoparticles reduced in the process and are believed to be combined with TMV protein subunits based on data obtained in the following section.

In order to optimize the viruses templated growth and fabrication, and to inspect the vibrational properties of the resulting bio-inorganic hybrid nanomaterials, one needs a characterization technique with potential for *in situ* monitoring. In this letter, we demonstrate that Raman spectroscopy might be a preferable technique for this purpose. Recent developments in instrumentation have strongly increased the usefulness of the micro-Raman spectroscopy for gaining insights into internal virus structure and viral assembly pathways.[10] Water, a medium of the assembly process, is also a notoriously strong IR-absorbing medium. As a result, biological samples generally can be investigated more favorably by Raman rather than by Fourier transform infrared methods. It has been shown theoretically that the low-frequency phonon modes of pure TMV, end-to-end TMV assemblies, and coated TMV particles are different.[11,12] The same is true for nanostructures coated with elastically dissimilar material.[13] Thus, signatures of these unique phonon modes observed in Raman spectra can be used to monitor the process of virus-assisted nanotube formation. With the binding of inorganic atoms into unit cells of different functional groups, the high-frequency spectrum is also expected to provide information that would allow one to assess the presence and quality of coating on the TMV nanotubes.

Micro-Raman spectroscopy has been carried out using a Renishaw micro-Raman spectrometer at room temperature. The spectra were excited with visible (488 nm) laser in the backscattering configuration. The micro-Raman spectra of the native TMV and TMV coated with Pt and Au are shown in Fig. 4. The metal coating, which can be viewed as an outside shell attached to the TMV sidewalls, may change the TMV characteristic phonon signatures. The four most prominent peaks of pure TMV; i.e., the Amide I line at 1655 cm^{-1}, C–H deformation lines at 1454.5 and 1332 cm^{-1}, and the phenylalanine residue line at 1005 cm^{-1} are marked in the figure. It can be seen that the most significant change in the Raman spectra of TMV coated with Pt and Au is the Amide I lines shift: 1664 cm^{-1} for TMV-Pt coating and 1672 cm^{-1} for TMV-Au coating. A previous study has demonstrated that the Amide I line is dominantly contributed from TMV coat protein capsid.[14] The apparent Amide I line shift observed in this study indicates the change of vibrational modes due to the binding of metal with that functional group in the shell protein. The shift for Amide I line is as large as 17 cm^{-1} for Au coated TMV. Other changes, including the less apparent line shifts for Phes, Trps, Tyrs, and the variations in the relative intensity of each individual line, are also observed in Raman spectroscopy.

In conclusion, we reported on the assembly of virus-inorganic nanotubes, and their characterization with TEM and micro-Raman spectroscopy. We show that phonon signatures for native TMV particles and metal-coated TMV in Raman spectra are different, consistent, and reproducible. The position of the Raman line shift identifies the vibrational mode change of the protein subunits on the sidewall of the coated TMV nanotubes as a result of their binding with metal. This indicates that Raman spectroscopy can be used as a monitoring tool for virus-assisted assembly of nanotubes.

The authors acknowledge the financial and program support of the DARPA-SRC Microelectronics Advanced Research Corporation (MARCO) and its Focus Center on Functional Engineered Nano Architectonics (FENA).

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