Directed Self-Assembly of Virus-Based Hybrid Nanostructures

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ABSTRACT

Viruses of various geometrical shapes have been exploited as higher hierarchical biomolecules in self-assembled nanoelectronic structures. We have demonstrated several organic virus particle and inorganic nanoparticle peptide-directed conjugations, including cylindrical tobacco mosaic virus (TMV) with quantum dots (QD) and single-walled carbon nanotubes (SWCNT) and icosahedral poliovirus (PV) with SWCNTs using ethylene carbodiimide coupling (EDC) procedure. In order to exploit these nanostructures as interconnects in nanoelectronics, metallization was also performed by reducing platinum particles onto these conjugations to make them conductive. Characterizations such as scanning and tunneling electron microscopy, fluorescent imaging and Fourier transform infrared (FTIR) spectroscopy were shown to prove the organic-inorganic connected heterostructures

Keywords: biological self assembly, functional carbon nanotubes, tobacco mosaic virus, poliovirus

1 INTRODUCTION

Virus-based self-assembly has aroused numerous interests in the field of nanoelectronics in recent years. Viruses are composed of a nucleic acid core surrounded by capsid which is made up of encoded proteins. Different viruses have different ways to encode the protein structure of the capsid in that they have various shapes. Viruses of different geometrical shapes have been exploited as higher hierarchical biomolecules in self-assembled nanostructures due to their manifold configurations, well characterized surface protein properties, and nanoscale dimension. Along with the special antibody-antigen recognition ability and site-directed conjugation, viruses exhibit the uniqueness as self-assembled materials. Many self-assembled or directed self-assembled viral nanostructures have been reported, such as viral self-assembled semiconductive and magnetic nanowires [1-3] and oriented quantum dot nanowires [4, 5]. Viral nanosensors based on the principles of viral induced self-assembly were also demonstrated [6]. These results have proven that virus-based biotemplate is a promising technique in directed nanostructure synthesis.

In this paper, we present several peptide-directed organic-inorganic heterto-nano-structures, including cylindrical tobacco mosaic virus (TMV) with quantum dots (QD) and single-walled carbon nanotubes (SWCNT) and icosahedral poliovirus (PV) with SWCNTs using ethylene carbodiimide coupling (EDC) chemistry [7]. Meanwhile, electrodeless chemical deposition [8] of platinum metallized poliovirus and tobacco mosaic virus particles is also reported.

2 MATERIALS AND METHODS

2.1 Coupling of viruses and nanoparticles

For exploring possible combinations of virus particles with inorganic nanoparticles, we chose wild type icosahedral poliovirus [9] and wild type rod-shaped tobacco mosaic virus [10] due to their dissimilarity in physical shape. Among three poliovirus serotypes, type one was selected owing to it generality. Type one poliovirus was replicated in monkey kidney cells and modified for vaccine use so that they are less toxic than normal wild type polioviruses and not harmful for human beings with vaccine shot. The original poliovirus samples that we acquired had the concentration of 10^5 particles in one μL. Tobacco mosaic viruses purchased from American Type Culture Collection (ATCC) were common strain with concentration of 26 μg/μL.

Single-walled carbon nanotubes that we purchased were refluxed in 2N HNO_3 for 5 hours followed by 30 minutes sonication so that we had proper oxidizes sites on SWCNTs. After sonication, SWCNTs were filtered using a vacuum filter unit with one micron Millipore filter. During the filtration, excess amount of deionized water was added at a slow rate to ensure completely removal of the acid from nanotubes. Filtered SWCNT cake was then vacuum dried overnight and added to deionized water for making nanotube suspension.

In order to functionalize quantum dots with carboxyl terminal groups on the surface, we mixed 50 μL ZnS capped CdSe quantum dots (Core Shell Topo Evidots, Evident Technologies) with 5 mg/mL concentration in toluene solvent with 11 μL mercaptoacetic acid (MAA) to activate the surface of QD for four hours. Then, the supernatant was removed from the sample after it was centrifuged for three minutes at 13,000 rpm. The pellet left after centrifugation was re-suspended with 50 μL MeOH and repeated the centrifugation-resuspension process

several time in order to completely remove the residual MAA. After MeOH wash, QD pellets were re-suspended in deionized water and transferred to Millipore tube (Millipore UFV5 BQK 25) for centrifugal filtration. The QD solution fluorescence was examined by ultraviolet excitation under optical microscope.

After the carboxyl functionalization of SWCNT and QD, they were added to deionized water followed by EDC (0.5M) addition to activate the carboxyl groups. Sulfo-NHS was added later on to initiate the covalent conjugation between carboxyl and amino groups. Virus was added to the coupling solution and incubated for 4 hours.

The mixture was desalted by centrifugal filtration for scanning electron microscopy (Zeiss Supra 55) and tunneling electron microscopy (Philips CM300) sample. Solution was dropped on a Si chip and dried in a desiccator for SEM analysis. Figure 1 summarized the process for EDC coupling between virus particles and inorganic nanoparticles. Fourier transform infrared spectra using AgCl window were acquired by dropping each sample onto the window analyzed by Bruker Equinox 55 FTIR.

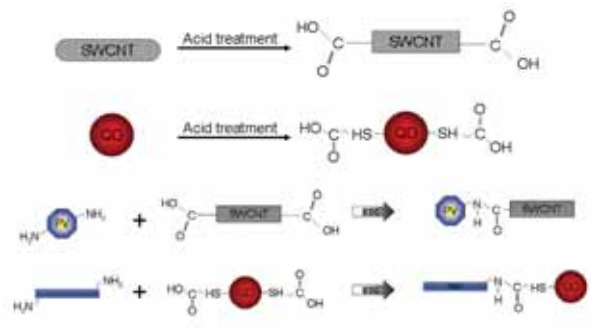

Fig. 1 (a) single-walled carbon nanotube oxidized by HNO_3 in order to open the cap at both end and introduce carboxyl functional group on nanotube (b) quantum dot functionalized with amino groups so that it can be used in combination formed by EDC chemistry (c) EDC coupling of poliovirus with carboxylated SWCNT (d) EDC coupling of tobacco mosaic virus with carboxylated QD

2.2 Platinum metallization of viruses

Virus metallization was originally accomplished by adding 65 μL of 1 mM Potassium Tetrachloroplatinate (K_2PtCl_4) to 1μL of virus solution. The mixture was kept at room temperature for aprroximately 4 hours to let virus capsid be activated by Pt ion. After that, 1 μL 10 mM Dimethylaminobenzaldehyde (DMAB) was added to the sample to provide a reduction bath for Pt ions to form metal clusters on the surface of virus particles. The sample was kept 27 degrees centigrade for 2 to 3 hours. The schematic of the Pt metallization is shown in Figure 2. We also manipulated several parameters such as temperature of the reduction bath, concentration of every chemical and virus sample, and the time of incubation of sample mixtures. The samples were characterized by scanning and tunneling electron microscopies.

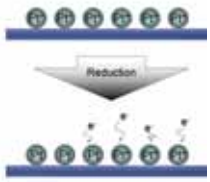

Fig. 2 Platinum ions first activated the surface proteins of viruses before reduction bath applied. After activation, DMAB was added to reduce Pt ions which attached on the capsids of viruses to Pt atom.

3 RESULTS AND DISCUSSION

For the purpose of self-assembled conjugation, we have applied several chemical reagents for different forms of conjugations, including EDC coupling with the aid of sulfo-NHS to stabilize the O-acylisourea generated by the reaction of EDC and carboxylate group and the reduction of platinum ion solution to deposit platinum clusters on the protein surface of virus. The results have been characterized by optical microscopy, scanning and tunneling electron microscopies, and Fourier transform infrared spectroscopy.

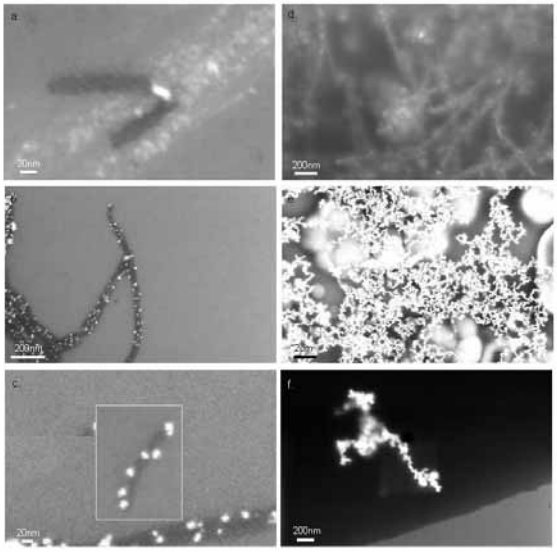

Fig. 3 SEM images of (a) TMV-QD-TMV structure (b) TMV with platinum clusters on capsid. (c) Close up image of a single TMV particle with six tiny platinum clusters on the surface. For self-assembled interconnect purposes, we can obtain conductive rod-shaped virus particle without sacrificing the targeting ability of virus by minimum metallization. (d) PV particles attached at the end and sidewall of carbon nanotubes. (e) Lower magnification of metallized PV particles is illustrated in this image. (f) A standout metallized PV aggregation. The size of the metal clusters depends upon how long the metal ion solution activates the virus sample. The longer the time, the larger the metal particle will be after reduction bath is introduced.

The scanning electron microscopy study of several conjugation formats of viruses with inorganic nanoparticles is illustrated in figure 3. In figure 3(a), a TMV-QD-TMV formation was formed by a QD cluster linking two small bundles of two to three TMV particles. Poliovirus particles attached on the end and the sidewall of SWCNT by EDC coupling is shown in figure 3(d). As we can tell, most of the PV particles aggregated at the end of carbon nanotubes. But there are also some virus particles affix to the sidewall of nanotubes due to the sidewall oxidization. We can control the oxidized sites by altering the oxidizing time and the acid normality. Therefore, depending upon the application, sidewall attachment on carbon nanotube is an alternative.

Figure 3(b) and 3(c) demonstrate the Pt metallization on TMV particles with Pt particle size of approximately 20 nm in diameter. The size of metal cluster is determined by the time that metal ions activate the protein surface and also the time of reduction bath. The SEM images shown in the paper imply the possibility of minimum metallization of virus particles so that the virus is conductive for the use of nanoelectronic interconnects while the recognition capability is maintained for selectively binding with its antibody counterpart to build a more complicated two or three dimension circuit network. Poliovirus particles metallized by Pt is shown in figure 3(e) and 3(f) with different magnification. Since PV particle has diameter of 30 nm, metal particles with similar size covered all around the virus capsid. With the freedom to control the degree of metallization and to choose the versatile geometry of viruses, such as rod-shape TMV and icosahedral PV, the configuration of bio-nanoelectronics is beyond one's imagination.

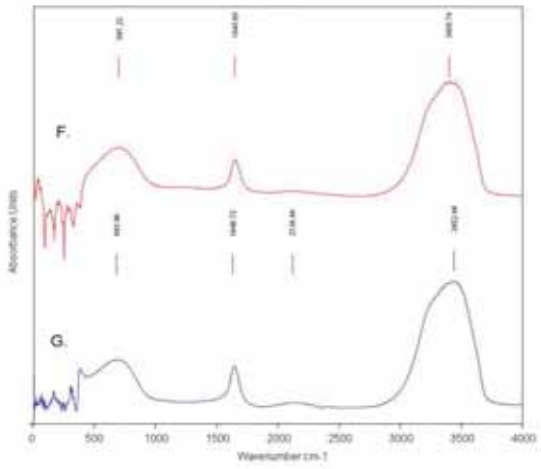

Fig. 4 Fourier transform infrared spectra of TMV-QD using AgCl window for background. (a) after EDC coupling reaction applied. (b) before applied EDC coupling. The alkyne bond in TMV was suppressed after conjugation.

FTIR spectra of TMV-QD covalent coupling using AgCl window shown in figure 4 indicates the amide (1645 cm-1) of TMV and R-CONH-R'(3405 cm-1) bonding, compared to FTIR spectrum of TMV in figure 4(b). Alkynes bond in TMV is suppressed after conjugation. The hybridization of Pt and QD cluster with TMV is also examined under tunneling electron microscopy as shown in figure 5. Figure 5(a) indicates QD cluster surrounding TMV particle bundles forming a large QD-TMV network. The metallization of TMV by Pt ion is shown in figure 5(b). The dark spots in the TEM image are the platinum metal clusters lying quite uniformly on the scaffold constructed by TMV particles. Furthermore, to confirm the existence of QD, we identified the QD in our covalent coupling virus samples by fluorescence imaging shown in figure 6. Although the visualization of virus particles is not feasible under optical microscope, we still can see, in figure 6(a), the fluorescence of QD forming a line in QD-TMV sample comparing to QD-PV sample which just randomly dispersive on the substrate.

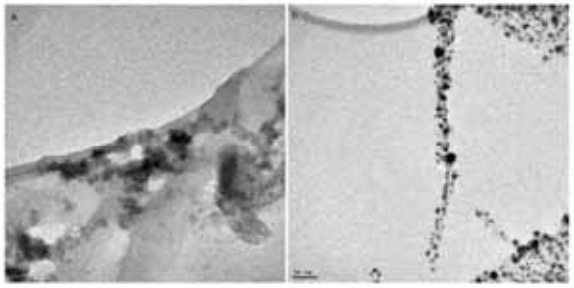

Fig. 5 Tunneling electron microscopy images of (a) TMV-QD (b) TMV-Pt under 100kV. Because of the EDC coupling chemistry, QD and TMV tends to bundle together forming a network. Instead in Pt reduction on TMV particle, Pt clusters distribute more uniformly along the axis of TMV particle.

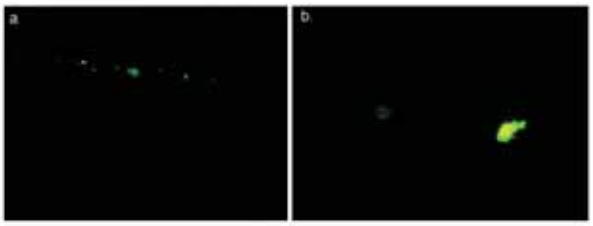

Fig. 6 Optical microscopy images of (a) TMV-QD (b) PV-QD. In (a), there are several small bright green spot forming a belt which could be the TMV underneath. We did not observe this belt-like bright cluster arrangement in PV-QD sample.

We have reported not only the covalent conjugation of different viruses with SWCNTs and QDs using EDC with sulfo-NHS but also the metallization of viruses by Pt salt reduction. Besides various approaches of hybridization, we have also provided several degrees of freedom to manipulate the structure of complexes, such as the choice of sidewall attachment of carbon nanotubes and

the targeting ability of minimum metallized virus particles. Future work to utilize antibody for the selective metallization and bioconjugation is underway.

4 ACKNOWLEDGEMENT

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