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Preparation of silica stabilized Tobacco mosaic virus templates for the production of metal and layered nanoparticles

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ABSTRACT

The use of biological molecules as templates for the production of metal nanoparticles and wires is often limited by the stability of the bio-template and its affinity for nucleating metal deposition. In this study, Tobacco mosaic virus (TMV) was used as a model bio-template to investigate the use of silica coatings as a means to both enhance template stability and increase its affinity for metal ions. Results indicate that the unmodified TMV particle can function as a template for the growth of thin (<1 nm) silica layers. However, this thin silica shell did not enhance the stability of the template during metal deposition. To increase silica growth on the TMV template, a pretreatment with aniline was used to produce a uniform silica attractive surface. Aniline pretreated templates yielded significant silica layers of >20 nm in thickness. These silica shells conferred a high degree of stability to the TMV particle and promoted the deposition of various metal nanoparticles through conventional silica mineralization chemistries. This process provides a simple and robust method for the layering of inorganics onto a biological template.

1. Introduction

The exploitation of biologically derived material for the assembly of micro- and nanoscale devices is a rapidly expanding field. At present the adaptation of biological molecules into nanodevices has generally been used to impart novel functionalities, such as nucleic acid recognition or antigen–antibody binding, for use in pathogen detection and gene surveillance. However, there are an increasing number of reports that investigate the use of select biological substrates as "bio-templates" for the patterning of inorganic materials. In particular, the macromolecular structures of viruses have proven to be useful scaffolds for the self-assembly of two- and three-dimensional nano-scaled structures that can be spatially patterned using genetic and/or chemical methods [1–6]. Inorganic deposition onto these bio-templates has been accomplished using a variety of methodologies including chemical cross-linking, genetic engineering, and electroless plating, resulting in the deposition of numerous inorganic compounds including metal particles, silica, metal oxides, and metal sulfides [7–10]. Virus-assembled inorganic nanostructures have been fashioned into conductive nanowires, field effect transistors, memory device components, and battery electrodes [11–14]. From these studies it is clear that inherent biologically properties of viruses, including self-assembly, genetic programmability and spatial patterning provide a novel scaffold for the assembly of inorganic compounds.

In this study, we utilize Tobacco mosaic virus (TMV) as a model biological template to examine methods for the multi-layering of inorganic materials. TMV forms a rod-shaped particle 18 nm in diameter and 300 nm in length with a 4 nm diameter hollow inner channel. TMV particles comprise ~2130 identical protein subunits of molecular weight 17.5 kDa that self-assemble in a helix around a single strand of genomic virus RNA [15]. Furthermore, TMV particles are stable in a wide range of temperatures (up to 60 °C) and pH values (pH ~ 2–10) [16], making TMV a viable template for a wide range of plating techniques.

Coating of materials onto the TMV surface has relied on electrostatic interactions in aqueous solvents [8,12,17,18]. In these instances, the solution pH was adjusted so the charge of the coating particle and that of the biological template were mutually attractive. Recently, two approaches have arisen to modify biological surfaces to increase their reactivity: genetic modifications of the coat protein to generate novel reactive amino acid and peptides [1–4,11,12,19,20], and chemical modifications attaching reactive groups directly to the bio-template [10,21,22]. However, one downside to using a biologically derived template is the lack particle stability at high metal ion concentrations [23]. Template instabilities reduce coating efficiencies, resulting in partial or incomplete metal coatings. To overcome this limitation, we propose an intermediary layer of silica to confer colloidal stability to the

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bio-templates prior to the metal coating. Such an intermediary silica shell would permit the use of metal ion concentrations that would normally disrupt the protein–protein interactions maintaining the stability of the virus particle. In order to achieve this, we investigate two silica-coated TMV templates; the first consists of a previously reported thin silica shell directly formed on the virus surface [22], and the second investigates the use of an aniline-precoat method [24] as a novel means to enhance the silica shell formation on the TMV surface.

Silica-coated TMV has been previously reported [7,10,22]; however, this is the first time an approach to produce thick shell silica-modified TMV templates with enhanced stability has been explored. Also, we investigate the creation of a TMV core with alternating silica–metal–silica shells to produce multi-layer coatings that take advantage of traditional silica chemistry and stability. The hydrolysis and condensation of silica are widely studied phenomena; the condensation of tetraethylorthosilicate (TEOS) has been exploited to create nanotubes from surfactant-based assemblies [25,26] and polymer templates [27]. Chemical modifications, such as polymers or silica shells, provide functionality to biological molecules increasing their reactivity and stability [21,22,28]. In the case of silica, the hydroxyl groups on hydrophilic silica surface provide reactive sites that permit interaction with inorganic ions [29–32]. Alternatively, the use of crosslinking molecules has been extensively investigated to facilitate coatings of gold and silver onto silica particle surfaces [32–35]. In this paper we examine the application of these techniques on the different silica-coated TMV templates.

2. Experimental

2.1. Pt and Pd mineralization of thin-shell silica-coated TMV

Thin-shell silica-coated TMV were prepared as described elsewhere [22]. The thinly silica-coated TMV particles were centrifuged and re-suspended in methanol to remove excess silica. Tin chloride ($SnCl_2$, Sigma-Aldrich 98%) in methanol was added to the solution to a final concentration of 1 mM and aged for 1 h. The sample was again centrifuged to remove excess Sn^{2+}, followed by the addition of hydrogen hexachloroplatinate hydrate ($H_2PtCl_6{\cdot}H_2O$, Aldrich, 99.9+%) or sodium tetrachloropalladate (Na_2PdCl_4, Aldrich, 98%) in methanol to a final concentration of 0.2 mM and incubated for 1 h. An aqueous borane–dimethylamine complex (DMAB) ($(CH_3)_2NHBH_3$, Aldrich, 97%) reducing agent was added post incubation to a final concentration of 0.4 mM. TEM and EDS samples were collected 15 min post DMAB addition. EDS samples were prepared by placing a drop of solution onto a TEM grid substrate and drying, prior to mounting on an SEM aluminum sample stub.

2.2. Preparation of thick-shell silica-coated TMV

Solutions of TMV in water are prepared following standard purification techniques described elsewhere [20]. Aniline-coated TMV particles are prepared as described by Niu et al. [24], where 900 μL of water are mixed with 100 μL of 10 mg/mL TMV solution, 10 μL of aniline, and 10 mg of ammonium persulfate. Samples are allowed to react overnight followed by centrifugation and resuspension in 100 μL of water. Aniline-coated TMV samples used for ethanol stability testing were mixed 1:10 with ethanol and allowed to incubate overnight. TEM samples were prepared on carbon–formvar grids and stained with UA. Silica-coating of aniline-coated TMV particles was carried out using the Stöber et al. method to produce silica spheres ∼100–200 nm in diameter [36]. Tetraethylorthosilicate (Aldrich, 98%) and ammonium hydroxide (NH_4OH, Aldrich, 5N) were used as received. For coating the TMV template in silica, 18 μL of aniline-coated TMV was mixed in 437 μL ethanol, 25 μL of 5 M ammonia solution (30 wt%), and 19 μL TEOS on ice, with ammonia being added last. Particle solutions were centrifuged and resuspended in water with sonication after 2 h. Multilayer silica-shells were deposited by repeating this procedure with the metalized silica-coated TMV templates rather than aniline-TMV particles.

2.3. Ag, Au, Pd, and Pt mineralization of thick-shell silica-coated TMV

Silver perchlorate hydrate ($AgClO_4{\cdot}xH_2O$, Aldrich, 99%), hydrogen tetrachloroaurate trihydrate ($HAuCl_4{\cdot}3H_2O$, Sigma-Aldrich, 99.9+%), Na_2PdCl_4, potassium tetrachloroplatinate ($K_2PtCl_4{\cdot}H_2O$, Aldrich, 99.9+%), DMAB, and 3-mercaptopropyl trimethylsilane (MPS) ($C_6H_{16}O_3SSi$, Aldrich, 95%) were used as received. Thick-shell silica-coated TMV particles were incubated overnight in a 1:10 ratio mercaptopropyl trimethylsilane (MPS) to ensure excess MPS in solution [33,37]. Functionalized silica templates (10 μL) were centrifuged and resuspended in 300 μL 0.1 M MOPS ($C_7H_{15}NO_4S$, Acros Organics, 99.5%) buffer, sonicated, and then placed on ice. To achieve platinum metalization, 0.05 M K_2PtCl_4 was added to the resuspended silica-TMV templates and incubated for 30 min, followed by reduction with 0.5 M DMAB. After 1 h, samples were centrifuged and resuspended in water. For silver, gold, and palladium a step wise procedure was used where metal salt addition followed by DMAB addition are broken up over 10 steps separated by 10 min incubations (for the case of Ag, this is performed in a darkroom) to the final concentrations of 0.05 M metal salt and 0.5 M DMAB. Particles were sonicated to break up aggregates. EDS samples were prepared by placing a drop of solution onto an aluminum SEM sample stub substrate and drying.

2.4. Growing surface-bound thick-shell silica-coated TMV

A gold-coated silicon chip was incubated overnight in the presence of 0.1 mg/mL TMV1cys in 0.1 M pH 7 phosphate buffer. The chip was then exposed to a solution consisting of 900 μL of water, 100 μL of 10 mg/mL wild-type TMV solution, 10 μL of aniline, and 10 mg of ammonium persulfate. Following aniline polymerization, treated gold surfaces were coated with silica by placing the chip into a solution of 18 μL of water, 437 μL ethanol, 25 μL of 5 M ammonia solution (30 wt%), and 19 μL TEOS on ice, with ammonia added last. Samples reacted for 1 h prior to being rinsed in ethanol and dried prior to imaging in the SEM. Both the TMV1cys mutant and wild-type TMV were prepared as previously described [20].

2.5. Characterization

TEM images of coatings on TMV templates were obtained using Zeiss EM 10CA TEM operated at 80 kV. All TEM samples were prepared without staining by using carbon/formvar coated copper grids. Platinum coated thick silica-shell TMV template samples were embedded in Spurr's resin sectioned to 70 nm thickness with a diamond knife. Sections were mounted on a carbon coated formvar copper grid. Energy dispersive X-ray spectroscopy (EDS), used to verify Ag, Au, Pd, Pt, and Si elemental presence, was conducted using an AMRAY 1820D SEM with an EDAX Genesis EDS system. EELS was obtained using an FEI Titan equipped with a Gatan Imaging Filter (GIF). High resolution TEM (HRTEM) was conducted using a JOEL 2100FE TEM operated at 200 kV. Diffraction analysis was carried out using the public domain ImageJ image processing software [38].