MMG 445
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Plant and bacteria virus use in nanotechnology

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Viruses have highly organized protein capsids, which are useful as scaffolds for building nanomaterials. Tobacco mosaic virus has applications as a building block for nanoelectronics, such as nanowires and conductive films, as well as in light-harvesting systems. Cowpea mosaic virus can be utilized in non-invasive imaging, biosensors, and in vaccines. Often, conjugation of moieties with the wild type is not possible, or can be enhanced, by chemical or genetic modification of the original viral structure. The future of nanobiotechnology lies in the potential for scaling-up of some current applications as well as with the discovery of viruses with novel properties.

Abbreviations: CPMV- Cowpea mosaic virus, FRET- fluorescence resonance energy transfer, ORF- open reading frame, QDs- quantum dots, TMV- Tobacco mosaic virus

Introduction

As major science sectors, such as biology, engineering, and chemistry, intersect with nanotechnology, a biologically focused subset of nanotechnology emerges. This emerging bionanotechnology field takes advantage of biomaterials to create materials and devices on the nanoscale level. Using bottom-up assembly techniques, biomaterials such as DNA, RNA, proteins, and viruses are used as building templates for nanomaterials [1•]. Of the many biomaterials used for building nanomaterials, viruses are of particular interest to the nanotechnology field because of their highly uniform structures, small size, and ability to self-assemble [2]. There are two useful properties for virus use as a nanoscale building tool; first, its genetic material directs self-assembly and can be modified to produce desirable proteins, secondly, the viral capsid, or protein coat, can be used as a scaffold for building nanomaterials. Science found in nature a convenient scaffold source for building on the nanoscale, for viruses can also be specialized, by genetic and chemical modification, to fabricate many different nanostructures [3]. Coupled with the ability to self-assemble, virus use as building blocks for nanostructures presents an opportunity for an emerging field to produce highly ordered and specific nanomaterials for use as building blocks for nanowires, nanoelectronics, biosensors, and biofilms [3,4].

Although many different plant and bacterial viruses have been used in a broad range of sciences, this review focuses solely on the building of nanomaterials using two particular viruses of different shapes: the Tobacco mosaic virus (TMV) and the Cowpea mosaic virus (CPMV). Several contributions to the nanobiotechnology field by each virus type are discussed, along with modifications made, genetically or chemically, to the viruses in the applications discussed.

Tobacco mosaic virus

Tobacco mosaic virus, with its rod-like shape, has found a niche as a biotemplate for metal deposition, mineralization, and the deposition of silica—particularly for the building of nanowires [1•,5]. Deposition of compounds for building of nanomaterials can occur on the outside of the virus or in the central cavity, allowing a great deal of flexibility in nanomaterial fabrication [2,6]. In its wild type form, Tobacco mosaic virus may require an activation agent, such as Pd (II), in order for metal particles to coat the viral particles successfully [7]. Therefore, genetic or chemical modification of TMV is useful in increasing its ability to bind metal particles [7,8••]. Several of such cases are discussed below, although there is one case discussed where the native TMV is used in its wild type form, without modification.

One genetic modification of the Tobacco Mosaic Virus is by PCR-based mutagenesis to incorporate cysteine residues in the viral coating protein, which improves the ability of the virus to bind metal more evenly. In this case, a TGT codon is inserted into the ORF of the coat protein at a specific site [9]. The thiol linkages from the synthetically added cysteine residues help stabilize the virion for mineralization and provide a binding site for the metal. Tobacco plants can then be inoculated with this mutant TMV. After an incubation period, the TMV mutant is purified, attached to a gold-patterned substrate, then conjugated with conductive compounds to form nanoelectrodes [9,10]. During attachment to the substrate in a particular experiment, researchers were able to expose the 5' end of the TMV RNA to bind the individual virions onto the substrate using nucleic acid hybridization with a virus-specific probe already linked to a chitosan-covered substrate, creating a spatially organized platform of immobilized Tobacco mosaic viral particles to which conductive particles can be conjugated [2,9]. The addition of a cysteine residue did not have an effect on the biological fitness of the mutant when compared to the wild type [9]. Conductive moieties often used in these fabrications include gold, nickel, palladium, silver, AlO_3, SiO_2, TiO_2, and platinum, and are critical for TMV use in nanowires and nanoelectrodes [2,8].

Tobacco mosaic virus can also be used in the fabrication of synthetic, self-assembling, light-harvesting systems [2]. Recombinant TMV with cysteine residues react with several types of thiol-reactive chromophores to form a complex capable of energy transport on the nanoscale via FRET events. Varying the chemical properties of pH and ionic strength of the TMV monomer solutions produces different rod and disc shaped nanostructures, which are FRET-capable. For example, data showed that increasing potassium phosphate concentration to 0.4M from 0.1M at neutral pH induced the formation of disc-shapes, which stacked together in groups of between 2-6 discs, while a buffer of 100mM sodium acetate at a slightly acidic pH induced rod-shape formation [11]. Natural light-harvesting systems use specific spacing of chromophores as well as different types of chromophores, which together act as energy donors via FRET events to one acceptor, to efficiently collect light from a wide spectrum; a TMV scaffold provides the rigid special structure and the ability to bind different chromophores necessary to achieve nature-like efficiency in its use as a synthetic light-harvesting system [11].

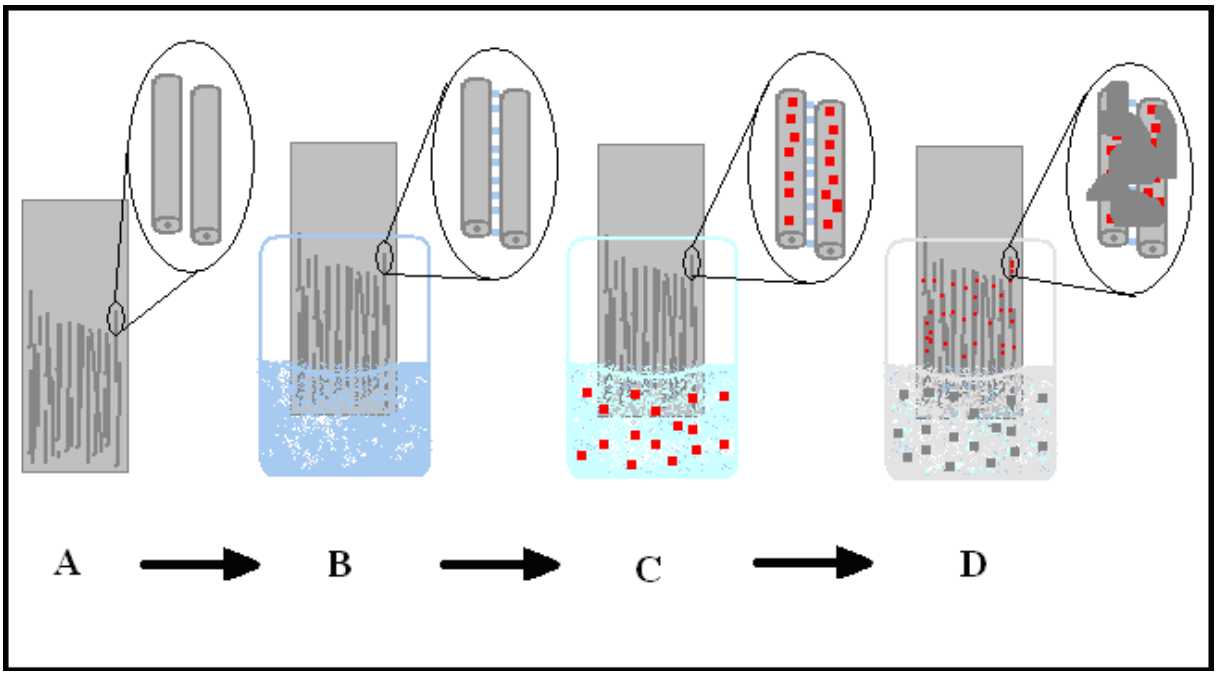

Figure 2. Stepwise preparation of nanowires from TMV-coated substrate. a) Model of a TMV-coated substrate plate, which is then placed in b) a glutaraldehyde solution to crosslink the TMV fibers. The resulting complex is then placed into a c) solution containing gold nanoparticles for conjugation onto the crosslinked TMV. Finally, the crosslinked, conjugated TMV in placed in a silver ion-hyrdoquinone solution for enhancement of the TMV nanowires. Adapted from [12].

On a larger scale, formation of highly ordered, electrowire films of Tobacco mosaic virus is also possible via meniscus-withdrawal deposition of virus bundles along a surface (Figure 1). This process is easy to scale up and does not require genetic alteration of the Tobacco mosaic virus particles. The hydrophobicity of the substrate onto which the TMV is depositing, shear forces, and dewetting are critical factors to achieving proper virion alignment. Once deposited, the TMV wires coating the substrate are cross-linked, conjugated with gold particles, then coated with silver particles to create conductive material with Tobacco mosaic virus nanowires up to several centimeters in length (Figure 2) [2,12].

More recently, the ability to sandwich the conductive layer of inorganic nanoparticles on Tobacco mosaic virus between two layers of silica has been demonstrated. The silica layers can then serve as insulation between differing conductive materials or between TMV and the environment, potentially increasing TMV applications as nanowires and in optical systems [13]. The wild type TMV can be used in this application without genetic modification, but must first be chemically treated. TMV treatment with aniline prior to silica coating prepares the protein surface for a more even coating of the first silica layer. The hydrophilic surface of silica and the hydroxyl groups on its surface provide ideal reactive sites for the inorganic conductive particles [13].

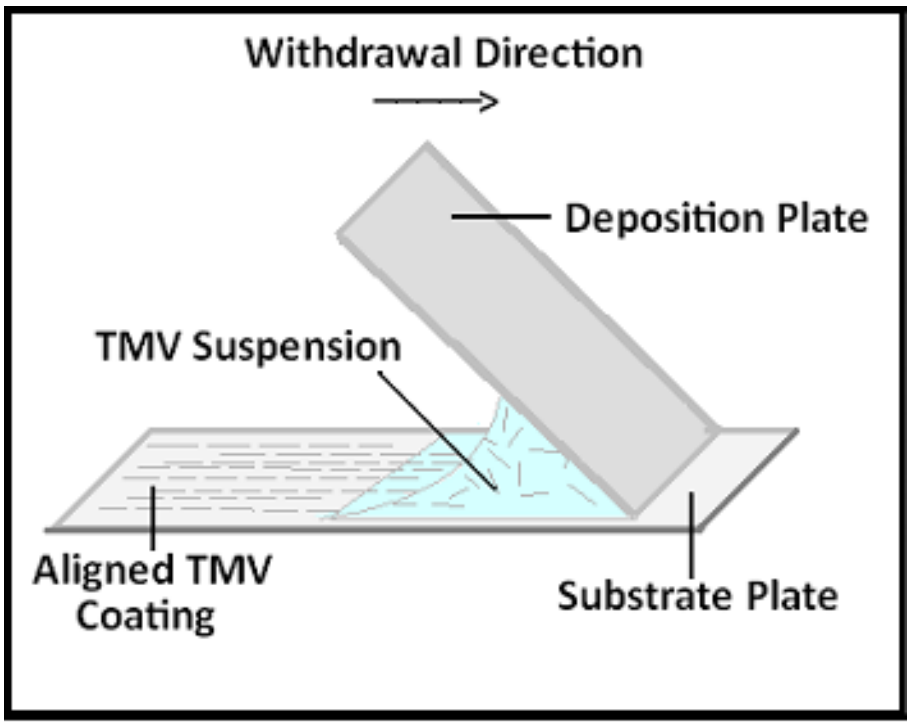

Figure 1. Deposition of TMV in an aligned coating. As the deposition plate withdraws to the right, the shear forces and receding meniscus order the TMV particles into a direction normal to the meniscus line. The speed by which the deposition plate withdraws is important in TMV alignment and clustering; a slower speed created less a less ordered coating. Adapted from [12].

Cowpea mosaic virus

With its icosahedral protein coat shape, which is capable of exterior display and encapsidation of molecules, Cowpea mosaic virus can serve as a powerful non-invasive imaging tool and as a delivery system for therapeutics [5]. Although CPMV lacks reactive cysteine residues on the outside of its capsid, it does contain as many as 300 usable lysine residues ready for conjugation and, as with TMV, CPMV can be modified, genetically or chemically, to increase reactivity with specific particles as needed for different applications [14]. As is the case with the genetically modified TMV, the cysteine residues added to CPMV are chosen for their reactive thiol groups.

The highly organized CPMV scaffold is ideal for carrying fluorescent dyes because the spacing of the dye particles is optimal so that no significant fluorescence quenching occurs [15••]. Prior to conjugation with the dye moieties, CPMV can be genetically modified by insertional or site-directed mutagenesis to express cysteine residues; however, this is only necessary if the dye moiety is thiol-reactive. Several mutants have been created (CPMV $_{CYS}$), which display genetically added cysteine residues, but the wild type CPMV has lysine residues with which to bind moieties that are not thiol-reactive [2]. Fluorescence by CPMV-attached QDs, in experiments with a surface-immobilized CPMV layer, showed increased fluorescence of up to 40% from fluorescence of the QDs alone [16]. A large part of this increase is attributable to the structure of CPMV, which creates more surface area than that of a flat surface for dye moieties to attach [17].

Another application of Cowpea mosaic virus-dye complexes is *in vivo* imaging. In one such study, CPMV was first chemically conjugated to fluorescent dyes by NHS ester chemistry. The resulting CPMV-A555 nanoparticle, named for the bound fluorescence agent, AlexaFluor 555, was injected into animal model vasculature, and was visualized in large and small vessels throughout the model. It was more successful than the dye alone at dispersing to all blood vessels, and did not aggregate. Over a period of 72 hours, the CPMV-A555 was excreted and no toxic effects were noted [18]. There exists a potential for use of this application in human systems as a non-invasive vascular imaging technique [5].

Since earlier research found that having many copies of epitopes on the surface of a carrier protein can improve immunogenicity, genetically modified Cowpea mosaic virus fused to epitopes developed as a possible new vaccination method [19•]. Inserting a specific foreign sequence into the genome on the site most surface-exposed, which is the βB-βC loop of the small (S) protein of CPMV, creates the EF-CPMV mutant, which displays 60 residues for epitope-binding, evenly distributed over the CPMV surface [2,19•]. There are other cysteine-enhanced CPMV mutants which can be utilized, such as DM-CPMV, which displays 120 residues, and BC-CPMV, which has the same number of cysteine residues as EF-CPMV, but limits the binding ability of moieties because of the smaller distance between binding sites [20]. One major vaccine application demonstrating use of epitopes with viral nanoparticles is that of CPMV/HRV-L1. This genetically-modified CPMV contains residues of Human Rhinovirus 14 (HRV14). Although CPMV/HRV-L1 generated an immune response in rabbits during which the antibodies bound successfully to HRV-14, the same insertion at other RNA points in CPMV were not as successful, resulting in incomplete or degraded display, which was only weakly recognized by antibodies. This indicates that epitopes may have ideal placement locations to solicit the desired immune response [21].

Cowpea mosaic virus-based nanomaterials also have uses as biosensors in antibody microarrays. CPMV labeled with binding molecules which are analyte-specific, such as DNA, antibodies, or peptides, and which are simultaneously labeled with fluorescent dyes have a niche in immunoassay use [22]. This function takes advantage of the multiplexing capability of CPMV, to conjugate CPMV with more than one moiety at one time. Antibody microarrays are one such application of multiplexing, where the normal tracer molecules are substituted for EF-CPMV scaffolds conjugated with fluorescent dyes and antibodies simultaneously. In this study, the viral based technology was much more sensitive than the other non-viral methods tested [23•].

Challenges for the Future of Viruses in Nanotechnology

As the field of bionanotechnology continues to grow, new research discovers, or designs, novel applications for virus platforms, as well as new hurdles to overcome. One such hurdle for virus-based nanotechnology is in the ability to withstand more extreme conditions, such as pH, temperature, and organic solvents. Therefore, the discovery of novel viruses from more extreme environments that may be naturally tolerant of extreme conditions is important to the future of the field [14]. Recently, a new group of viruses have been isolated from thermophilic bacteria of the genus *Sulpholobus*, that arenaturally heat-tolerant [14]. Genetic modification which creates more resilient viral particles is also a potential research topic for the future [14].

Novel methods for mass production of viral particles for self-assembly are also necessary for the future of the field. The ability to scale-up current and future applications, in economical ways, can play a large role in the growth of the field. Tobacco

mosaic virus mutants, for example, were recently successfully produced heterologously using yeast, although there were clear limitations [24].

Conclusion

The use of viruses in nanotechnology is creating new possibilities and novel approaches to many different fields, which run the gamut from electronics to biosensing. The possibilities for applications, thanks largely to the ability to modify the virus capsids, are limited primarily by the creativity by which researchers approach these natural building blocks. Applications in the biomedical field, such as non-invasive imaging in humans, and in the field of food safety, such as creation of bionanofilters for water filtration, are very likely potential future directions in bionanotechnology.

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