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Nanomaterials

Viruses as Building Blocks for Materials and Devices

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From the viewpoint of a materials scientist, viruses can be regarded as organic nanoparticles. They are composed of a small number of different (bio)polymers: proteins and nucleic acids. Many viruses are enveloped in a lipid membrane and all viruses do not have a metabolism of their own, but rather use the metabolic machinery of a living cell for their replication. Their surface carries specific tools designed to cross the barriers of their host cells. The size and shape of viruses, and the number and nature of the functional groups on their surface, is precisely defined. As such, viruses are commonly used in materials science as scaffolds for covalently linked surface modifications. A particular quality of viruses is that they can be tailored by directed evolution by taking advantage of their inbuilt colocalization of geno- and phenotypes. The powerful techniques developed by life sciences are becoming the basis of engineering approaches towards nanomaterials, opening a wide range of applications far beyond biology and medicine.

1. Introduction

Viruses are infectious agents that replicate only within living cells. After entering their host cells they are able to control the transcription/translation machinery of the cell, which is then employed for the production of their constituents. The viral biopolymers self-assemble into functional virions—mature viral particles—ready to infect other cells. The genome of viruses can be made up of different types of nucleic acids. It contains the genetic sequences that encode the structural proteins of the virus and also sequences whose function is to control the cellular metabolism, redirecting it towards an efficient replication of the viral genome. This modular quality of viral genomes, combined with the ability of the synthesized elements to self-assemble, provides enormous possibilities for molecular-biology-based engineering. The properties of the virus can be readily modified by changing the underlying construction plan—the nucleic acid sequence of the viral genome. For example, foreign polypeptides can be displayed on their structural proteins. If the sequences of these structural proteins are inserted into plasmids and expressed in cells it is possible to obtain virus-like particles (VLPs) that either do not carry any genetic material or that incorporate only selected pieces of code. It is also possible to produce viral chimeras that carry proteins of different viral origins. Viruses can be used in methods of directed evolution for screening libraries of nucleic acid sequences. These so-called surface-display systems are based on the colocalization of the genotype and the phenotype in every single virus particle and allow the isolation of a functional peptide in physical conjunction with its encoding nucleic acid sequence. This concept is widely used in life sciences, for example, to explore the unknown encoded function of the multitude of sequences provided by genomics. In materials science, the same approach can be used, for example, to create novel peptides with the capacity to bind to selected technical materials. Although viruses can be multiplied in appropriate tissue and cell cultures, they do not have any metabolic activity of their own. This, in principle, allows the use of virions as durable building blocks for composite materials. In combination with powerful molecular-biology approaches, all these features provide novel and far-reaching possibilities for the production and engineering of hybrid composite materials from these nanoparticles.

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1.1. Concepts for Using Viruses in Nanotechnology

1.1.1. Viruses as Scaffolds for Chemical Synthesis

In materials science, viruses are currently predominantly used as scaffolds for chemical synthesis. Most studies involve viruses without enveloping membranes. Molecules of interest

are connected to the viral surface by means of bioconjugation chemistry approaches. In this way, nanoparticles of viral origin can be fabricated that show a defined number and arrangement of functional molecules on their surface. Viruses are further used as scaffolds for mineralization or metallization. Some viruses can be conveniently disassembled and reassembled by changing the environmental conditions, such as the pH value of the medium. This feature provides the unique possibility to use viruses for encapsulation, which is appropriate for the design of nanocontainers (see Section 2 and Figure 1).

1.1.2. Designing the Viral Scaffold

Viruses can be modified to a large extent by means of molecular-biology techniques. For example, foreign peptide sequences can be inserted into the surface proteins of the viruses (see Section 3 and Figure 1). This is accomplished on the level of the genetic code rather than through conjugation chemistry. Thus, particles can be produced that display peptides with specifically designed functions on their surface. Engineering approaches based on molecular biology range from inserting selected functional groups by site-directed mutagenesis to the display of entire proteins. Displayed polypeptides may be additionally modified after protein translation. Whether or to what degree post-translational modification, such as glycosylation, takes place is a host-cell-defined parameter (see Section 4.1.2). Once the proper modification of the nucleic acid sequence has been achieved, the tailored virus nanoparticles can be produced at any time in a cell culture.

The strategy of engineering the viral surface by modifying the underlying genetic code finds an analogy in modern machining in which a program controls the manufacturing process. This concept is known as the computerized numerical control (CNC) principle. The host cell employed for nanoparticle fabrication would be the analogue of the machine tool in industry, whereas the virus genome provides the controlling program.

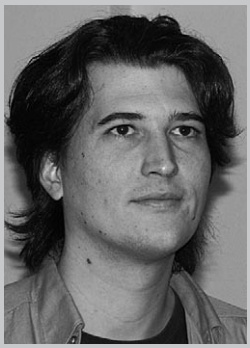
1.1.3. Evolving Surface Chemistry

Surface-engineered viruses can be regarded as nanoparticles in which the introduced peptides are physically connected to the encoding nucleic acid sequence that resides inside the virion. By introducing fragments of nucleic acid libraries into viral genomes, a huge variety of different surface-engineered viruses can be produced. Screening these particles for a displayed protein functionality allows selection of those virions that only carry the appropriate peptides. These can be enriched and subsequently multiplied in their host cells. After a number of screening/propagation cycles, one or a small pool of viral clones displaying the desired functionality is obtained. The surface-display technique allows the isolation of polypeptides together with their encoding nucleic acid sequence without any a prior knowledge of the sequence–function relationship. Even sequences encoding peptides with artificial functionalities, that is, not present or at least not yet known in the biological realm, can be selected. An example of this approach would be the production of peptides capable of specifically binding to noble metals or semiconductor materials (see Section 4, Figure 2).

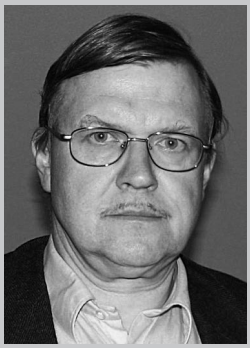
1.1.4. Integrating Viral Particles into Composite Materials

Instead of chemically engineering functions into a composite material, it may be more convenient to take advantage of nanoparticles as carriers of the desired properties. These nanoparticles can then be used as building blocks for the fabrication of a composite material with the required qualities. Engineered viruses may fulfill the role of the nanoparticles and once a convenient and general strategy to attach them to an interface is found, the setup can be standardized. The fabrication of a large variety of functionalized surfaces becomes possible by bringing together the potential of viruses for combinatorial surface display and a general strategy for surface attachment (see Section 5, Figure 3).

Although there are already a few examples in which viruses have been used as building blocks for composite materials, the comprehensive toolkit of the different viral systems developed in biotechnology, especially with respect to eukaryotic viruses, is far from being fully tested in materials science and chemistry. Although eukaryotic viruses are more difficult to handle than, for example, bacteriophages, they offer great possibilities for the design of virus particles with very sophisticated surface chemistries. The specific advantage



Martin Fischlechner was born in Innsbruck, Austria, and studied food and biotechnology at the University of Natural Resources and Applied Life Sciences, Vienna. His PhD work focused on engineering viral functions on colloidal particles. Current research deals with designing tools for displaying peptides and proteins on particles and interfaces employing viral building blocks.



Edwin Donath was born in Schorbus, Germany. He studied physics at Moscow State University and received his PhD in Biophysics from the Humboldt-University, Berlin, where he was also a lecturer in Biophysical Chemistry. Later he joined the Max-Planck-Institute of Colloid and Interface Science in Golm/Potsdam, Germany, where he headed with Prof. Helmuth Möhwald the research on LbL colloids and capsules. In 2001 he was appointed to a professorship at Leipzig University. His research interests include polyelectrolyte multilayers, biomimetic materials, and interfacial engineering.

is that post-translational modifications of functional polypeptides may fine-tune the interactions with relevant targets in eukaryotic organisms.

2. Virions as Nanoparticles/shells

Viruses can be regarded as monodisperse core/shell nanoparticles. The shell consists either of only proteins or proteins embedded in a lipid membrane. The functional groups provided by the amino acid residues on the surface of the virus particle observe a precise spatial arrangement. By using the various techniques available for covalent coupling of molecules onto proteins, viruses can be used as a platform for spatially defined chemical surface modifications. The advantage of using viruses instead of artificial nanoparticles is the defined number and orientation of the accessible functional groups on their surface. This provides the possibility to arrange different chemical functionalities with nanometric precision. The concept of utilizing viruses as a synthesis platform can thus be seen as a nanoparticle-based 3D equivalent of nanotechnology methods used for arranging molecules in two dimensions. The 2D approach has been demonstrated, for example, with crystallized bacterial surface layers (S-layer proteins).[1] With respect to covalent modifications of viral surfaces, lysine, cysteine, or tyrosine residues are commonly used as the chemically reactive sites for further coupling reactions.[2] Combining viral scaffolds with bioconjugation chemistry[3] thus represents a general means of displaying functional modules in defined spatial arrangements (Figure 1).[4]

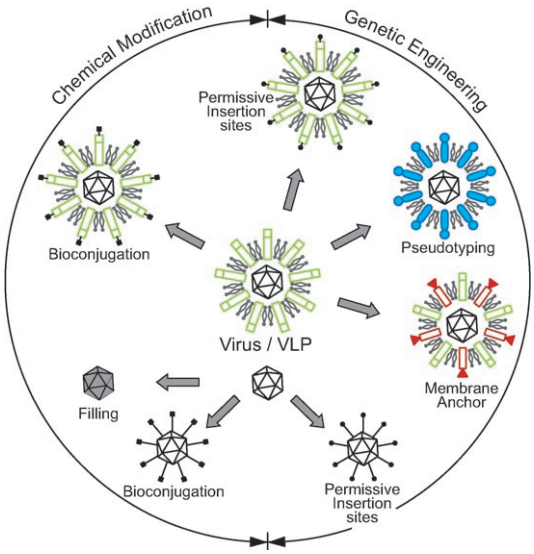


Figure 1. Chemical- and molecular-biology approaches to engineer viral surfaces. The described techniques can also be combined.

The use of viral scaffolds as nanoparticles and nanoshells has been recently reviewed with an emphasis on both the chemistry[5] and biomedical applications of the viruses used.[6] Most of the viruses so far employed, with the notable exception of bacteriophages, were nonenveloped plant viruses, such as tobacco mosaic virus (TMV), cowpea mosaic virus (CPMV), or cowpea chlorotic mottle virus (CCMV). The rationale behind their common use as scaffolds is their lack of pathogenicity towards humans as well as the possibility to isolate them in large quantities with low effort. Recent materials-science applications range from displaying redox-active, organometallic complexes that act as electron-transfer mediators,[7] metal nanoparticles,[8] or 3D conductive molecular networks[9] to the decoration with carbohydrates.[10] In the biomedical field, viral vectors for gene therapy can be retargeted, for example, by covalent binding of poly(ethylene glycol) receptor conjugates.[11] The tropism of virions can be broadened by attaching polylysines.[12] Viruses carrying gold nanoparticles have been targeted to cells for subsequent photothermal treatment in cancer therapy.[13]

Viruses have been employed as scaffolds for metallization or for the growth of minerals, resulting in metallized or mineralized building blocks.[14] Viruses can also be used as nanocages for the entrapment of substances.

Many VLPs can be assembled in vitro from their protein constituents. In this way, a molecule can be confined inside the viral shell.[15] The possibility of controlling the size of the pores in assembled capsid shells by shrinking and swelling as a function of the pH value or by using osmotic shock offers a practical means to use viral shells as nanocontainers. This makes the mineralization of the capsid interior[16,18] as well as the loading of VLPs with nucleic acids possible.[17] As nature has designed capsid shells as envelopes for the negatively charged nucleic acids, it is relatively straightforward to refill them with other negatively charged polyelectrolytes.[18] A similar concept has been applied to reconstitute a viral shell upon surface-functionalized gold cores.[19] Membrane proteins from lipid-enveloped viruses can be reconstituted into liposome membranes. These reconstituted systems, known as virosomes, have been applied for targeted delivery of entrapped substances[20] (taking advantage of the cellular specificity of membrane proteins of viral origin) as well as for vaccination.[21]

3. Displaying Proteins on Viral Scaffolds

The true power of using viral systems originates from their unique quality as nanocomposites that carry all the necessary information for the production of their components within their host cell. This allows the display of specific polypeptides on the viral surface by engineering the viral nucleic acid sequence inside. The most straightforward engineering concept aimed at modification of the viral surface is thus to modify the genetic code of the viral genome itself. If successful, the product would be a genetically modified virus that is still able to replicate. If the virus scaffold is, however, derived from a pathogen, the production of VLPs could be more appropriate. VLPs are essentially viruses that lack their genetic code and, naturally, their infectivity, thus being safe systems.[22] Only plasmids encoding the structural proteins with the desired sequence modifications are employed for the production of the particles.

Introducing engineered modifications into a viral protein generally involves finding a permissive insertion site for the desired peptide. This is a site where the insertion of extra code does not cause an interference in the natural function or replication of the virus. Once such a site has been found, virtually any sequence can be introduced through recombinant DNA techniques. A plethora of functional polypeptides can be displayed by taking this approach, although the use of permissive insertion sites often implies certain limitations concerning the size of the peptides.

The direct use of viruses as scaffolds for the display of functional proteins or peptides has certain advantages towards producing the proteins themselves and subsequently attaching them onto a surface. Viruses or VLPs are continuously released from the producing cells into the culture medium and can be easily harvested, for example, by means of ultracentrifugation on a sucrose cushion, whereas the isolation of proteins would be more demanding. Costly and time-consuming fractionation and purification steps are not necessary for the recovery of viruses or VLPs.

Devices for detecting biomarkers most often rely on the attachment of proteins as catcher molecules onto a surface.[23] If a device comprising many different functions is required, it is likely that the immobilization protocols will have to be optimized individually for the different proteins to ensure their functionality on the surface. By using virions as scaffolds for display, the introduced functionalities are presented in a spatially defined fashion. They can be designed as needed, purified by a single protocol despite their different engineered features, and used as a functionalized nanoparticle. Once a particular clone is engineered, it can be produced at will by infecting new cells, requiring very little effort compared with the synthesis of chemically modified particles.

3.1. Nonenveloped Viruses

If chemical modifications on virus surfaces are intended, nonenveloped viruses may represent the system of choice. Additional amino acid residues are frequently engineered on the viral surface through site-directed mutagenesis. This is a straightforward approach to expand the number and nature of spatially defined addressable functional groups for subsequent conjugation chemistry.[24] Genetic-engineering techniques for introducing functional polypeptides into the surface proteins of, for example, bacteriophages, are well developed. Despite the limits regarding the length of the peptides that can be introduced into the viral coat proteins[25] and although the display of more-complex protein features is difficult, these constructs represent the viral system of choice for many purposes.

Concerning mammalian nonenveloped viruses, recent research activities have focused on developing strategies for engineering the viral coat proteins in the context of their application as vectors for gene therapy.[26] Adenoviruses[27] or adeno-associated viruses[28] are widely used for this purpose. One of the main challenges is to alter their tropism. By displaying additional receptors or antibody fragments on their surface, adenoviruses and adeno-associated viruses can be targeted towards cells that lack the native receptors for virus binding.

3.2. Enveloped Viruses

Many mammalian viruses are surrounded by a lipid envelope that, in most cases, is derived from the cytoplasmic membrane of the host cell, which is acquired during the final stage of replication (budding).[29] The lipid envelope serves as a matrix for the viral transmembrane proteins and is supported by the viral capsid. The function of the transmembrane proteins is to bind to specific receptors present at the cell surface and to induce membrane fusion, an essential step during infection.[30] Altering the capsid proteins through genetic engineering may strongly interfere with the self-assembly of the virus, whereas transmembrane proteins are generally less sensitive to modifications.

3.2.1. Permissive Insertion Sites

Techniques for genetic engineering of the membrane proteins of lipid-enveloped viruses are well established with several viral systems. A number of viral membrane fusion proteins are known to have permissive insertion sites for the introduction of foreign peptide sequences. Examples of these are the membrane fusion proteins from baculovirus,[31] avian leukosis virus,[32] vesicular stomatitis virus,[33] murine leukemia virus,[34] influenza A,[35] and others. Although the modified viruses maintain their infectivity, it can be diminished if long polypeptides are inserted resulting in steric hindering of the dynamics of the fusion process. This often imposes a size limit for the introduced modifications.

3.2.2. Pseudotyping—Anchor Molecules

If two different enveloped viruses simultaneously infect a cell, viral chimeras can be produced. This phenomenon, known as pseudotyping, is associated with the mechanism of enrichment of similar viral membrane proteins in lipid rafts of the cytoplasmic membrane during budding.[36] With respect to genetic engineering of the viral surface, this property can be used to an advantage for the fabrication of chimeric viruses or VLPs. This is an important concept in current gene-therapy research. For example, vectors based on retroviral elements, which are fundamental for stable insertion of DNA sequences into host genomes, often do not address the designated cell types. The tropism of these vectors is then altered by means of inserting membrane proteins of foreign origin, such as native or genetically engineered fusion proteins of vesicular stomatitis virus, sindbis virus, and many others.[37]

The cytoplasmatic and transmembrane domains of truncated viral fusion proteins can serve as an anchor for the display of proteins or peptides. This approach does not necessarily have the inherent limitations of a permissive insertion site. The viral particle remains infective provided that a functional viral membrane fusion protein[38] is displayed together with the truncated form used for display.

4. Combinatorial Methods/Directed Evolution

The outlined genetic-engineering techniques for displaying functional peptides or proteins on the surface of virions are applicable with infective viruses as well as with VLPs. Directed evolution approaches, however, can only be applied to infective viruses as an amplification step follows the screening for a selected function (see Figure 2). Notably,

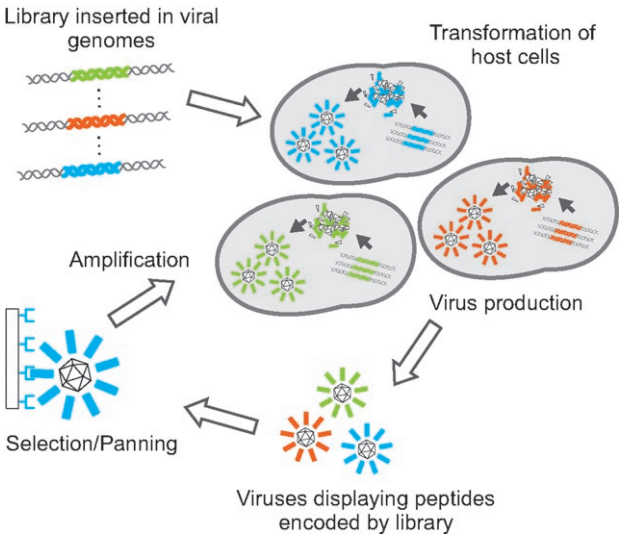


Figure 2. Virus surface display. Cells are transformed with virus genomes carrying fragments of a nucleic acid library. The viruses produced display the introduced genetic sequence as peptides on their surfaces. By screening (panning) for a desired protein functionality followed by amplification of the binders, one or a small pool of viral clones can be obtained after several rounds of selection that display the desired property.

surface-display techniques are by no means restricted to viruses as carriers of nucleic acid libraries. Display systems engineered on the basis of prokaryotic[39] as well as eukaryotic cells[40] have been described. In vitro systems based on messenger RNA (mRNA) have also been reported.[41] The need to develop a variety of display systems is caused by limitations of protein synthesis and subsequent modifications in different organisms. For example, complex eukaryotic proteins with the correct folding, disulfide bonds, and proper glycosylation can only be produced in cells from higher organisms. On the other hand, these systems are considerably less effective in terms of the volume of genetic sequences that can be conveniently screened.

4.1. Searching Nucleic Acid Libraries

4.1.1. Phage-Display Systems in Life and Materials Sciences

The most prominent surface-display systems developed to date have been based on bacteriophages.[42] M13, MS2, lambda phage, and some of the T-series phages were employed.[43] Antibody production, enzyme technology, protein–protein interactions, and vaccine development are some areas in which phage-display systems are widely used. There are a number of recent reviews on this issue.[44]

In the field of materials science, the trend towards building organic/inorganic hybrid composite materials created a demand for novel peptides. These peptides are intended to bind with inorganic materials like noble metals, semiconductors, polymers, and other technically relevant compounds. Through the use of display techniques, in particular phage display, a number of such peptides have been isolated.[45]

Surface-display techniques can also be used to obtain binding sequences based on D-amino acids. In a scheme known as mirror-image phage display, viruses are screened on a chemically synthesized target consisting of D-amino acids. The resulting binding sequence, synthesized with D-enantiomers, is then able to interact with the L-form of the target used for screening.[46] The use of such mirror-image techniques allows the production of functional peptides that are more stable towards degradation by enzymes and have different immunogenic qualities in relation to their natural counterparts.

Viruses use the transcription/translation machinery of their host cells for replication. Recently it has been shown that it is possible to introduce amino acids that are not used in the natural genetic code into the metabolism of cells.[47] The use of such cells as host cells for virus surface display extends the technique towards polypeptides containing artificial amino acids.[48]

4.1.2. Trends Towards Eukaryotic Systems

Although phage display offers exciting possibilities for working on sequence–function relationships, it also has some drawbacks. Because phages are produced in bacteria, the proteins or peptides displayed on the phage suffer from general limitations associated with the expression mechanisms in prokaryotic organisms. Owing to the reducing environment in the bacterial cytoplasm, it is often not feasible for proteins to form disulfide bonds. Eukaryotic post-translational glycosylation, which is rather important for many functions of the displayed polypeptides,[49] is not possible either.[50]

These limitations can be overcome if either eukaryotic-cell surface display, or viral surface-display strategies based on eukaryotic viruses are used. Although nonenveloped rhinovirus[51] and adeno-associated virus[52] have been established for surface display, the majority of eukaryotic virus-display systems relies on enveloped viruses. Baculovirus,[53] murine leukemia virus,[54] and avian leukosis virus[55] have been successfully employed in display systems so far. Further to screening on the level of the virus, it is also possible to screen on the level of the producing cells as they present the viral proteins on their surface during virus production. Although these systems do not allow the screening of sequence libraries in sizes comparable with phage display and are still in an early stage of development compared with phage display, they will certainly have a major impact on surface-display techniques because of their ability to display eukaryotic proteins in an authentic manner.

The problem of limited library size with display systems based on eukaryotic cells can be circumvented by improving

the quality of the library, which is the basis of screening.[56] DNA shuffling and similar methods based on recombination of related nucleic acid sequences in vitro make it possible to obtain libraries smaller in size but enriched in meaningful sequence variations.[57] This approach, predominantly used for the improvement of enzymes for industrial applications, has been applied to viral vector systems for gene therapy aiming to improve their tropism,[58] avoid neutralization by antibodies,[59] and enhance their stability.[60]

5. Fabricating Composites with Functionalized Viruses as Building Blocks

Viruses have been used as building blocks for the fabrication of composite materials. They have found their way into commercially available products, as it is the case for ELISA plates or latex beads in immunological assays, which rely on nonspecific adsorption of viruses to various substrates. If surfaces are, however, coated with compounds that provide sites for specific interaction with a viral component, selective immobilization becomes possible. This is the strategy followed in immunochromatography for purifying purposes or in biopanning procedures for surface-display techniques.[61] In combination with patterning techniques,[62] such as microcontact printing,[63] dip pen nanolithography,[64] Langmuir–Blodgett lithography,[65] molecular combing,[66] and other related methods, the fabrication of two-dimensional arrangements of viruses on a large scale becomes possible. Antibodies,[67] binding proteins like avidins,[68] immobilized DNA,[69] or chemically reactive groups[70] as connecting elements have been used for the selective binding of viruses.

Viruses can also be arranged in three-dimensional structures. Crystals formed by viruses were used as templates for the synthesis of materials.[71] Some viruses form regular assemblies upon centrifugation or sedimentation and have been used for building colloidal photonic crystals.[72] Lamellar structures have been fabricated from lipid/phage arrangements.[73] The aggregation of viruses can be controlled by the temperature dependence of the pairing of covalently attached oligonucleotides.[74] It is also possible to arrange viruses on the oil/water interface by using pickering emulsion techniques.[75] The viruses can subsequently be cross-linked at the interface, resulting in micron-sized droplets with virus-like surfaces.

Applying these techniques to viruses that display engineered functional polypeptides, or by directly using viruses that were evolved by surface-display techniques, as elements for such assemblies provides a very general means of creating surfaces with functional polypeptides.

Viral building blocks carrying special peptides have been selected by surface display on the filamentous phage M13. The displayed peptides have been screened for their interaction with various inorganic materials. The M13 phages themselves have been used as templates for nucleation and subsequent crystal growth.[76] Inorganic rod-shaped building blocks were obtained and were then assembled into fibers, films, and liquid-crystalline materials.[77] These nanowires can also be shaped into branchlike structures.[78] A promising application might be in the field of nanoelectronic devices.

Furthermore, viruses have been used as layer constituents in polyelectrolyte multilayers fabricated by layer-by-layer (LbL) technology on flat substrates.[79] Tuning the binding of charged filamentous phages in competition with weak polyelectrolytes as a top layer, it was possible to form a liquid-crystalline monolayer of viruses.[80] The density of this floating film of viruses depended on the charge of the viruses, which could be adjusted by altering the pH value. This concept has been applied to metallized virus particles to build thin and flexible electrode materials for lithium-ion batteries.[81]

Along with the use of virus surface-display methods for materials applied in technical devices, another important area of application is the design of interfaces with biological matter. Viruses carrying selected biological functions can serve this purpose. In phage-based microarray technology, solutions containing peptide-displaying phages as capturing agents instead of proteins are spotted on the array. If phages are used as the detection agent, like a secondary antibody within an ELISA setup, the inbuilt genome of the phages can be directly used for signal amplification through immuno-PCR.[82]Recently, phages screened on displaying peptides for binding of autoimmune antibodies from the sera of cancer patients have been used in phage microarrays for the diagnosis of breast and prostate cancer (see Figure 3).[83] As it has already been demonstrated that viral particles displaying peptides can substitute proteins in conventional microarray technology, a vigorous development toward their use as an interface between technical materials and biological systems can be foreseen.

A natural way of integrating viruses into interfaces is to take advantage of the fusion capacity of enveloped viruses with lipid membranes (see Figure 3). Lipid layers can be assembled on various substrates like silica, polymer cushions, and others forming so-called supported bilayers. They can also be formed on polyelectrolyte multilayers, providing an add-on for layer-by-layer technology while maintaining the possibilities for engineering the layers underneath. Lipid-enveloped viruses, which infect their host cells by the endosomal pathway, fuse with lipid membranes at low pH values. The fusion with a supported lipid layer can thus be triggered by lowering the pH value. If the bilayer constituted the top of a polyelectrolyte multilayer-covered colloid, composites in the colloidal dimension with a virus-like surface can be fabricated.[84] These colloids display the viral envelope proteins, which may be genetically engineered, in an authentic manner.[85] As an example, a bead array for the simultaneous detection of viral antibodies in sera has been recently fabricated, demonstrating the feasibility of combining LbL technology on colloids with virus functions.[86]

6. Outlook

Viruses represent composite nanoparticles that offer many degrees of freedom to design their surface properties. This can be achieved by either techniques based on chemical conjugation or by genetic-engineering methods. The surface-display approach is a powerful means of retrieving displayed

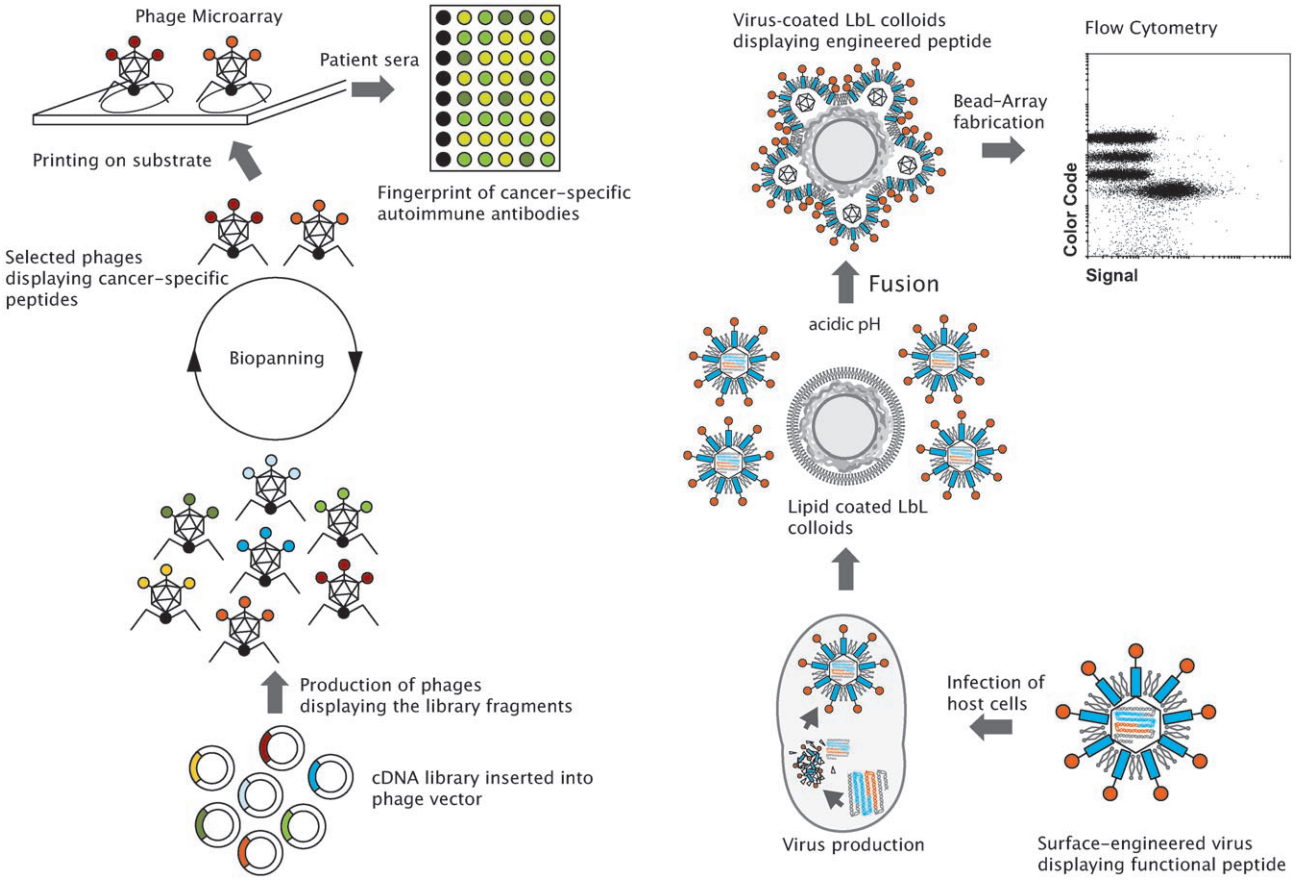


Figure 3. Left: Fabrication of a phage microarray for detecting prostate-cancer-specific autoimmune antibodies in sera. A complementary DNA (cDNA) library constructed from mRNA derived from prostate-cancer tissue is inserted into a phage vector and the surface-modified bacteriophages produced in *E. coli*. After production of phages displaying the library fragments, they are selected by several rounds of biopanning. The selection procedure involves panning against sera from healthy donors to remove nonspecific clones followed by the enrichment of binders against a pool of sera derived from prostate-cancer patients. Some of the selected phage clones are then spotted on a glass slide. Upon incubation of the biochip with patient sera followed by incubation with a fluorescent anti-human IgG antibody, an autoantibody signature of the patient's sera can be obtained, thereby facilitating prostate cancer diagnosis.[83b] Right: A similar approach to fabricate detection devices in colloidal dimensions. Lipid-enveloped viruses that fuse with lipid membranes at low pH values can be used as building blocks for the assembly. Native or surface-engineered viruses, which were obtained by rational engineering or surface-display technique, are fused with lipid-coated LbL colloids. The beads can be color coded by using polyelectrolytes labeled with fluorescent dyes as layer constituents. A bead array for the detection of virus specific antibodies in sera has been constructed following this protocol. [85,86]

functional polypeptides that can be evolved beyond the naturally occurring biological structures.

Virus technologies based on genetic-code design are likely to see a steep development in the near future. The enormous progress in gene-synthesis techniques has been recently demonstrated by the synthesis of complete viral genomes from nucleic acid monomers.[87] On the other hand, advances in the understanding of nucleic acid sequence code allowed the systematic redesign of viral genomes, as was recently shown with the creation of the phage T7.1.[88] Genetic engineering has a clear analogy to software production in that pieces of code can be multiplied at low cost, put together in an artificial genome, and processed afterwards in the cellular machinery. Repositories for standard (genetic) building blocks ready for customization for special needs are about to be established.[89]

Materials that rely on protein functions can be directly fabricated from viral building blocks instead of employing the proteins themselves. This is a tempting alternative, especially if the fabrication of devices with many different protein functions is desired. Recent developments in microfluidics[90] will certainly contribute to the parallel production of surface-engineered viruses at low cost and with a great diversity of functions. Although this field is still in its infancy, automatic parallel production of viral building blocks seems possible when recent progress ranging from chip-based DNA manipulation[91] to cell-culture techniques[92] is considered.

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