FOCUS REVIEW

Filamentous virus-based soft materials based on controlled assembly through liquid crystalline formation

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This focus review describes the utilization of M13 phage, a filamentous virus, for the development of a novel class of materials. Historically, M13 phage has been widely utilized as a scaffold to display desired peptides or proteins on the surface through genetic engineering. This biotechnology is well known as 'phage display' and has generally been used for constructing peptide libraries on M13 phage surfaces to screen peptides with a specific affinity for desired molecules or materials. Recently, the preparation of ordered structures composed of M13 phages based on liquid crystal formation has generated great interest as a means of utilizing the outstanding properties of phages for the development of novel soft materials. The combination of phage display technologies and liquid crystal formation can be effectively used to develop structurally regular hybrid materials composed of M13 phages and inorganic or organic materials. M13 phages can be used, for example, as polymeric materials to construct structurally regular hybrid hydrogels, hydrogel matrices for controlled release systems, selective adsorbents for rare earth elements and protein-detecting microarray systems. The excellent properties of M13 phages should further contribute to novel attractive opportunities of next-generation soft materials for science and technology.

INTRODUCTION

The controlled assembly of desired molecules into ordered structures for functional expression is an important challenge in various fields, including chemistry, physics, biology and materials science. For the development of functional molecular systems, bottom-up self-assembly approaches, which involve the autonomous organization of individual components, have been used to integrate various molecules, including low molecular compounds, synthetic polymers, biopolymers and inorganic nanoparticles.[1,2] For the past several decades, self-assembly has proved to be a powerful technique for material design and synthesis in nanoscience and nanotechnology.[3–5] In nature, a number of biomolecules, such as nucleic acids, peptides (or proteins) and saccharides, exhibit the functions required for nanoscale self-assembly, resulting in supramolecular architectures assembled through numerous weak intermolecular interactions. Inspired by natural systems, the self-assembly of various biomolecules is now emerging as a novel method to produce new materials, whereas controlling or programming the self-assembly is still difficult. Various disciplines, including interdisciplinary fields, have approached the design of biopolymers, such as DNA, peptides and proteins, to construct architectures with desired functions.[1,6,7] Designing DNA molecules with a specific sequence (such as DNA origami and DNA tiles)[8,9] and peptides with designed β-sheet structures[10,11] are typical strategies for constructing novel, smart, bio-based nanomaterials. In the case of DNA, two- or three-dimensional nanostructures have been designed and constructed,[12,13] and in the case of peptides, various smart materials, such as fibers, tubes, capsules and hydrogels, have been achieved.[14–17] However, it is still a great challenge to precisely design and control the assembly of biomolecules in order to achieve multi-level hierarchical structures for desired functions.

Viruses, which are biomacromolecular assemblies composed of proteins and nucleotides (DNA or RNA) with different shapes and sizes ranging from tens to thousands of nanometers, are emerging as powerful components for controlled self-assemblies. Viruses are infectious agents that replicate only within living cells. Biologically, they can be classified as enveloped and nonenveloped viruses. Enveloped viruses have a phospholipid membrane surrounding their capsid proteins, whereas nonenveloped viruses do not. Although several studies using enveloped viruses for material applications have been reported,[18,19] almost all viral studies toward the construction of novel materials have been based on simple nonenveloped viruses. These nonenveloped viruses share two basic structures: either spherical (icosahedral) or filamentous helical structures.[20] Among them, typical filamentous viruses such as the tobacco mosaic virus and M13 bacteriophages (phages) are regarded as useful biological building blocks for novel self-assembled materials due to their liquid crystalline properties.[21,22] Filamentous viruses have precisely assembled structures of up to 1 µm in size due to their molecular and assembled structures

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defined by their genomic DNA; such viruses are superior from the viewpoint of controlling the assembly at the nano to micrometer scale toward materials construction. To widen the applicability of virus-based materials, controlling viral assemblies and introducing functionality are essential requirements.

Over the past few decades, M13 phage, which is a filamentous virus that replicates itself and propagates its genetic information by infecting bacteria host cells, has gained considerable attention as a typical scaffold material for constructing a biological peptide library. In 1985, it was reported that peptides with desired amino acid sequences could be genetically displayed on the coat proteins of the phage surfaces by inserting the corresponding DNA fragment into the phage genome.[23] Importantly, the infectiousness of the resultant genetically engineered phages against host cells was maintained; therefore, the genetically engineered phages could be easily amplified. When peptides with different sequences are displayed on the surfaces of each phage, a molecular library of peptides can be constructed. Today, such 'phage display methods' are a versatile and important tool for screening and identifying the peptide ligands of biomolecules, such as peptides and proteins.[24,25] This approach to screen specific peptide ligands has also been utilized to obtain artificial material-binding peptides.[26,27] The identification and application of phage-displayed material-binding peptides has attracted great interest, especially due to their wide utilization in material engineering and nanotechnology.[17,28–31] Recently, M13 phage was reported to act as a component for constructing virus-based materials due to its specific characteristics. M13 phages have proved to be excellent components for constructing various materials in various fields[30,32–35] due to their defined sizes and dimensions, ease of surface modification through chemical methods[36,37] genetic engineering potential[24,25] and ability to self-assemble into regularly ordered liquid crystalline structures.[38,39] In this focus review, the recent advances of M13 phages are summarized.

CHARACTERISTICS OF M13 PHAGE
Structural features of M13 phage
M13 phage is 4.5 nm wide and 900 nm long, with a molecular weight of 16.3 MDa. Its genome consists of 6.4 kb single-stranded circular DNA containing 11 genes of well-known sequences and structures.[39] The outer proteins of M13 phage consist of ~ 2700 major coat proteins (pVIII), which are helically wrapped around the DNA, and minor coat proteins (pIII, pVI, pVII and pIX) at each end (Figure 1, top side). Due to their highly anisotropic shape and monodispersity, M13 phage has
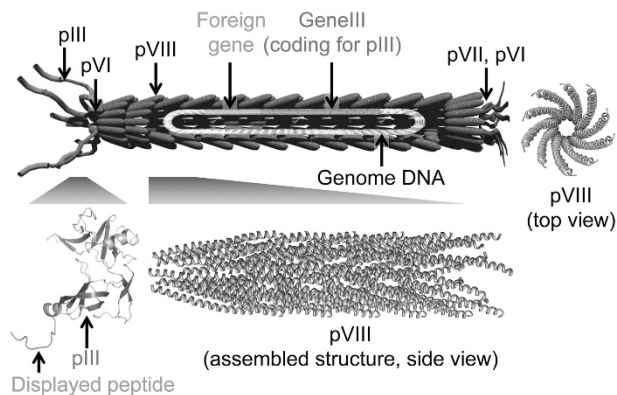
Figure 1 Schematic illustration of filamentous M13 phage structures. A foreign gene was genetically inserted into the 5′ end of gene III, which was coded for coat protein III (pIII) to display the peptide on pIII. A full color version of this figure is available at *Polymer Journal* online.

the capability to exhibit liquid crystalline properties in highly concentrated solutions (and suspensions in some cases).[38] Therefore, M13 phage has attracted great interest as an experimental model system for studying liquid crystalline formation and structures because this phage is sufficiently large to be observed using various microscopic technologies. As determined from experimental studies, the liquid crystal behavior of M13 phage is highly dependent on various external factors as well as the concentration of the phage.[38–40] It is well known that the most essential factor is the concentration, and M13 phage behaves as a lyotropic liquid crystal. The liquid crystal phases formed by M13 phage change from isotropic to nematic, cholesteric and smectic phases with the increasing concentration (Figure 2).[39,41,42] Recently, these liquid crystalline properties have been effectively utilized to construct functional materials with hierarchically ordered structures.[32]

Surface modification of M13 phage
As mentioned in the introduction, the surfaces of M13 phage can be modified with desired peptides or proteins using genetic engineering. In other words, any functions achieved by natural systems through peptides or proteins can be genetically introduced onto the phage surface. Furthermore, by screening using phage display methods, phages displaying desired material-binding peptides can be realized (Figure 1, down side). Indeed, various materials such as metals, metal ions, semiconductors, inorganic oxides, nanocarbons, organic compounds, natural and synthetic polymers, and molecular assemblies have been applied as targets for peptides, and the acquisition of certain peptides with affinities for each target has been reported.[27,29,31] The identified material-binding peptides not only act as material binders but also can act, for example, as functional nanomaterials for surface modifiers, adsorbents for patterning, catalysts for preparing inorganic nanoparticles, additives for controlling polymeric fluorescence and nuclei for preparing polymeric nanoparticles,[17,26,28,43] indicating that phages with various functions have been obtained.

However, in order to introduce functions that are impossible for peptides or proteins, organic chemistry-based surface modifications have also been developed.[37,44,45] In this strategy, active functional groups such as amino (lysine), carboxy (aspartic or glutamic acids), thiol (cysteine) and phenol (tyrosine) groups on the phage surfaces are generally used for further chemical conjugation using relatively mild reactions and/or conditions such as amine coupling, maleimide-thiol coupling and diazonium reactions. These nongenetic phage manipulations have extended viruses into various applications ranging from components for energy materials to biomedical applications.[35] In various chemical methods, unnatural amino acids bearing azide or alkyne groups are effectively being used to expand the applicability of phages as material components.[37,44,46]

DRY OR SOLID MATERIALS COMPOSED OF M13 PHAGES
Liquid crystal formation-based self-assembly of M13 phages
M13 phages possess structural advantages as building blocks for one-dimensional fibers due to their anisotropic features. Lee and Belcher[47] prepared fibers with submicron- and micrometer scale diameters using crosslinking reagents. In this study, M13 phage suspensions with a liquid crystalline orientation were injected into a solution of glutaraldehyde through capillary tubes, resulting in M13 phage-only fibers. The same group further developed M13 phage-based fibers to introduce functions through genetically engineered gold-binding peptides (sequence: Val-Ser-Gly-Ser-Ser-Pro-Asp-Ser).[48] Interestingly, the mechanical properties of the fibers varied depending on the assembled structures and concentrations of the crosslinking reagents; therefore, conjugation with functional compounds offers various
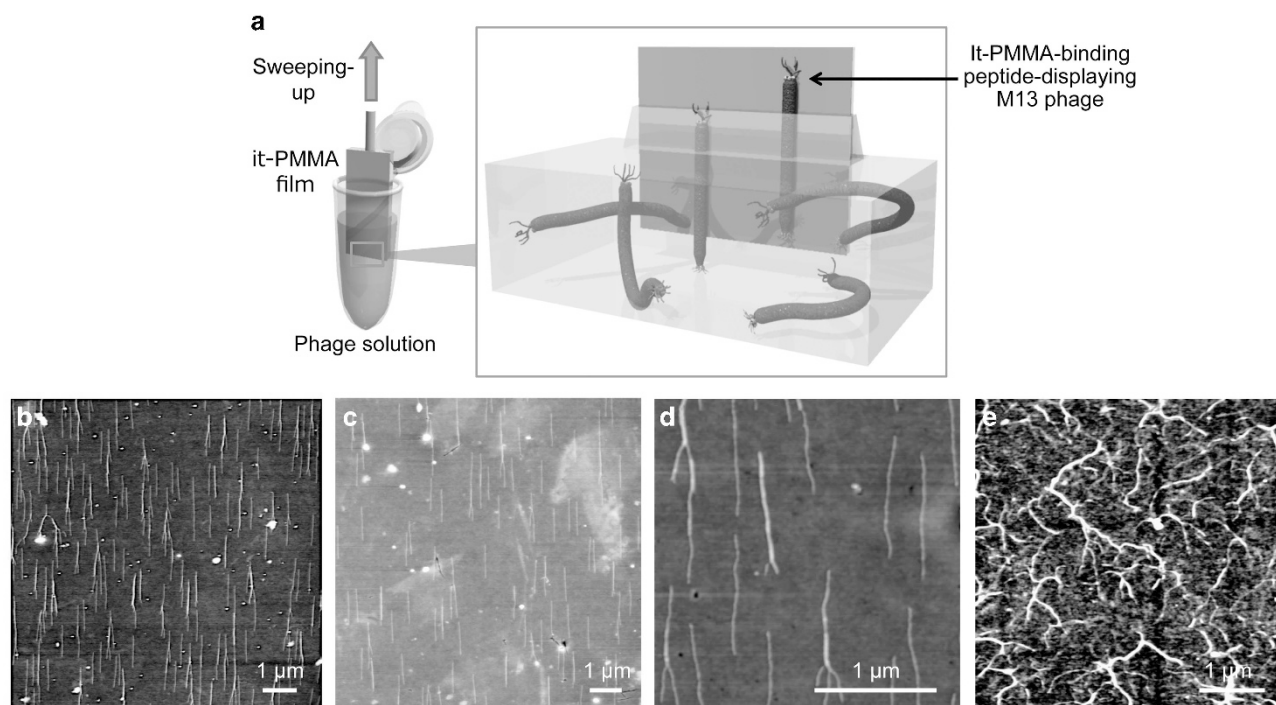
applications. Because filamentous biopolymeric structures are one of the most common shapes in natural extracellular matrix components, such as collagen and elastin, there is great interest in using fibrous M13 phage to construct biomimetic artificial extracellular matrix. A flow-induced injection method using liquid crystalline M13 phage solutions in agarose hydrogels was successfully utilized to prepare liquid crystalline-anisotropic fibrous materials.[49] When neural progenitor cells were incubated within materials containing phages that displayed bioactive (promoting cell adhesion, proliferation or differentiation) peptides (sequences: Arg-Gly-Asp or Ile-Lys-Val-Ala-Val) constructed through genetic engineering, the direction of the neuronal growth could be controlled along the direction of the M13 phage length within the extracellular matrix.
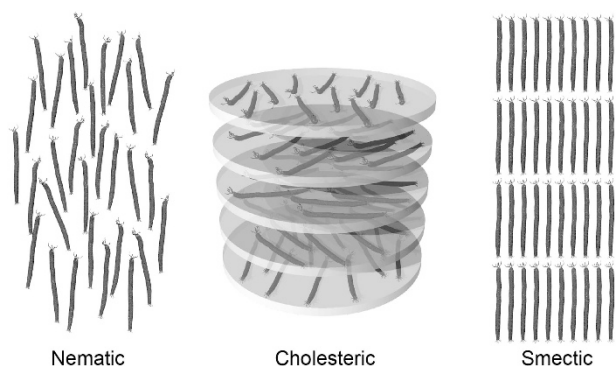
In addition, films and assemblies with a liquid crystalline orientation that are only composed of M13 using meniscus phenomena have been reported. Due to the specific micrometer length of M13 phages, long-range-ordered films were achieved using a flow-induced method,[50] and the liquid crystalline orientation of the films could be controlled by varying the concentration of M13 phage. Importantly, the M13 phage-based films were effectively utilized to hybridize inorganic nanomaterials such as zinc sulfide (ZnS) nanocrystals[51] and gold nanoparticles (GNPs),[52] driven by liquid crystalline ordering in the genetically engineered M13 phages (see below). More recently, Lee and co-workers[53] described a versatile method for the biomimetic self-template-based assembly of M13 phages into hierarchically organized structures for functional materials. A single-step process was used to successfully prepare M13 phage-based films on substrates with various structural colors based on the liquid crystalline-oriented structures, such as nematic orthogonal twists, cholesteric helical ribbons and smectic helicoidal nanofibers. In addition, genetically engineered M13 phages have been utilized with bioactive peptides (sequence: Arg-Gly-Asp) to control directional cell growth on substrates. The substrate-supported M13 phage-based films were further applied as colorimetric sensors for various volatile organic chemicals and explosive trinitrotoluene based on the swelling and shrinking of the nanobundled structures on the substrates.[54] Furthermore M13 phages were adsorbed and self-assembled using shear forces to directionally organize structures on substrates composed of amorphous carbon or silicon dioxide.[55] These results suggested M13 phage assemblies provide a powerful toolkit for designing specific functional solid fibers or film materials from standard techniques to achieve functional materials.

As mentioned above, the ordered assemblies of M13 phages on substrates can act as excellent biologically tunable scaffolds for material

Figure 2 Schematic illustration of the liquid crystalline phases of M13 phages. A full color version of this figure is available at *Polymer Journal* online.

Figure 3 Immobilization of the isotactic poly(methyl methacrylate) (it-PMMA) binding phages in an oriented manner. (**a**) Schematic illustration of the dipping/sweeping method to immobilize the phages onto a polymeric substrate. (**b**, **c**) Atomic force microscopy (AFM) images of the it-PMMA-binding phages (c02 phages) on an it-PMMA film using phage concentrations of (**b**) 100 and (**c**) 10 pM. (**d**, **e**) Images of (**d**) wild-type (WT) phages on an it-PMMA film using a phage concentration of 1 nM and (**e**) c02 phages on an it-PMMA film using a solution casting method with a phage concentration of 100 pM. Reprinted with permission from Sawada *et al.*[56] Copyright 2013 RSC. A full color version of this figure is available at *Polymer Journal* online.

integration. To effectively express the potential of phage assemblies, an essential requirement is to construct a versatile method to immobilize flexible M13 phages into desired configurations. Sawada and Serizawa[56] reported an extremely simple dipping/sweeping process using substrates to immobilize M13 phages in a highly oriented manner through specific interactions between the peptides displayed on the phage termini and the substrates. In the study, we used a previously identified isotactic poly(methyl methacrylate) (it-PMMA)-binding peptide (sequence: Glu-Leu-Trp-Arg-Pro-Thr-Arg) that was displayed on the pIII minor coat proteins of the M13 phages (termed c02 phage).[57,58] The it-PMMA films were prepared on silicon wafer substrates using a spin cast method and were dipped into the c02 phage solutions. After incubation, the films were swept at a precisely controlled speed (10–100 mm min^{-1}) (Figure 3a). Using atomic force microscopy, aligned filamentous objects (that is, the c02 phages) were clearly observed (Figure 3b). The direction of the aligned phages was nearly parallel to the sweeping direction. Lower concentrations of the c02 phages yielded a decrease in the immobilized amount and fused and/or winding configurations (Figure 3c). An increase in the sweeping speed resulted in a clear increase in the ratio of highly oriented phages. Importantly, when wild-type phage (without additional peptides) solutions with higher concentrations were utilized instead of the c02 phage solution, winding configurations were observed (Figure 3d). Furthermore, when using a conventional solution cast method with the c02 phage, the orientation completely disappeared (Figure 3e). Hence, we demonstrated the effectiveness of achieving oriented immobilization using the appropriate method. The orientation immobilization was considered to originate from the pIII side, possibly due to the preferential interactions between the c02 phage-displaying peptides and the polymeric substrate. Because various material-binding peptides have been identified, this immobilization method, which uses specific interactions, can be applied using desired materials to immobilize phages with regular configurations.

Self-assembled phage materials hybridized with inorganic or organic compounds

It is well known that structural ordering generally enhances the performance of individual compounds or materials; hence, we strongly expected to construct hybrid materials with excellent properties beyond those offered by each component individually by combining the M13 phage assemblies with other functional inorganic or organic materials. Belcher and co-workers[51] reported the fabrication of highly ordered composite films composed of genetically engineered M13 phages displaying ZnS-binding peptide (sequence: Cys-Asn-Asn-Pro-Met-His-Gln-Asn-Cys) and ZnS nanocrystals. Solutions of M13 phages displaying peptides with an affinity for ZnS on the pIII proteins were mixed with ZnS precursor to form liquid crystalline phases. After a drying process, three-dimensionally ordered and self-supporting hybrid films composed of the M13 phage and ZnS nanocrystals were prepared. The hybrid films exhibited micrometer scale ordered patterns of fluorescent lines, possibly due to the ordered ZnS nanocrystals located at the phage termini by peptide interactions. This alignment strategy can be expanded to other nanomaterials using streptavidin-binding peptide (sequence: Trp-Asp-Pro-Tyr-Ser-His-Leu-Leu-Gln-His-Pro-Gln)-displaying phages and streptavidin-modified GNPs through the liquid crystal formation of M13 phages.[52]

The same group further developed M13 phage-based hybrid materials that can be utilized as an electrode for flexible lithium-ion batteries.[59] The genetically engineered M13 phages displayed tetra-glutamate or gold-binding peptides (sequence: Leu-Lys-Ala-His-Leu-Pro-Pro-Ser-Arg-Leu-Pro-Ser) on the pVIII major coat proteins and were utilized to prepare tricobalt tetroxide or gold-tricobalt tetroxide NP-based hybrid nanowires templated using engineered M13 phages. By applying the phage-templated inorganic nanowires to an anode electrode of a lithium-ion battery, the capacity was improved by over 30%. They suggested that this improvement could be explained by the participation of the GNPs in the redox reaction to enhance the electronic conductivity of the tricobalt tetroxide NPs. Furthermore, the potential capabilities of using M13 phage-templated inorganic hybrids as surface-enhanced Raman scattering nanoprobes were reported.[60] In this study, a dual-functionalized M13 phage, which displayed antibodies as ligands on the pIII and cetyltrimethylammonium bromide-coated GNP-binding peptides (sequence: Pro-Asp) on the pVIII proteins, was genetically constructed and utilized as an surface-enhanced Raman scattering probe. The M13 phages preferentially interacted with gold nanocubes to form ordered assemblies with one-dimensionally assembled fibers. The structures resulted in strong surface-enhanced Raman scattering signals, possibly due to the closely assembled gold nanocubes on the phages. These results clearly demonstrated that hybrid materials composed of M13 phages and inorganic materials will offer powerful tools for various fields, such as devices and sensors.

To develop separate fields of conducting polymer-based electrochemical sensors, nanowires composed of M13 phages hybridized with organic conductive materials such as poly(3,4-ethylenedioxythiphene) (PEDOT) were prepared.[61] To prepare the phage-PEDOT nanowires, anion-charged M13 phages competed with conventional perchloric acid to electrostatically interact with the nanowires during the electropolymerization of EDOT, resulting in lithographically patterned nanowire electrodeposition. The nanowire-patterned devices realized real-time, reagent-free and selective biosensing of positively charged antibodies under physiological conditions. Because molecular recognition capabilities for various molecules can be genetically incorporated into M13 phages, this strategy shows a high applicability for sensing a wide range of analytes, such as important disease biomarkers.

WET MATERIALS COMPOSED OF M13 PHAGES

Despite the developments and recent progress in controlling the self-assembly of M13 phages, it is still challenging to construct M13 phage-based three-dimensional structures at a large scale due to the electrostatic repulsion caused by the acidic amino acids at their surfaces. Hydrogels are one of the most common three-dimensional network structures. They are composed of crosslinked polymers or supramolecular assemblies of low-molecular weight compounds. The filamentous structure of M13 phage inspired us to generate hydrogels with suitable strategies. Pasqualini and co-workers reported a strategy based on the effective combination of viruses and other materials. The approach was to utilize the nonspecific electrostatic interactions between the pVIII proteins on M13 phages and negatively charged GNPs in acidic conditions.[62] When genetically engineered M13 phage-displaying peptides containing seven basic amino acids (sequence: Pro-Arg-Gln-Ile-Lys-Ile-Trp-Phe-Gln-Asn-Arg-Arg-Met-Lys-Trp-Lys-Lys-Pro) on the pVIII proteins were utilized, M13 phages and GNPs were directly assembled to easily form a hydrogel that was stable against pH and salt concentrations.[63] Mixtures of GNPs and magnetic iron oxide have been used for hydrogel formation, and the hydrogels showed biocompatibility with cell cultures and magnetic field responsiveness.[64] The magnetized hydrogels containing M13 phages achieved levitated three-dimensional cell cultures, resulting in more consistent and repeatable *in vivo* protein expression and long-term multicellular studies.
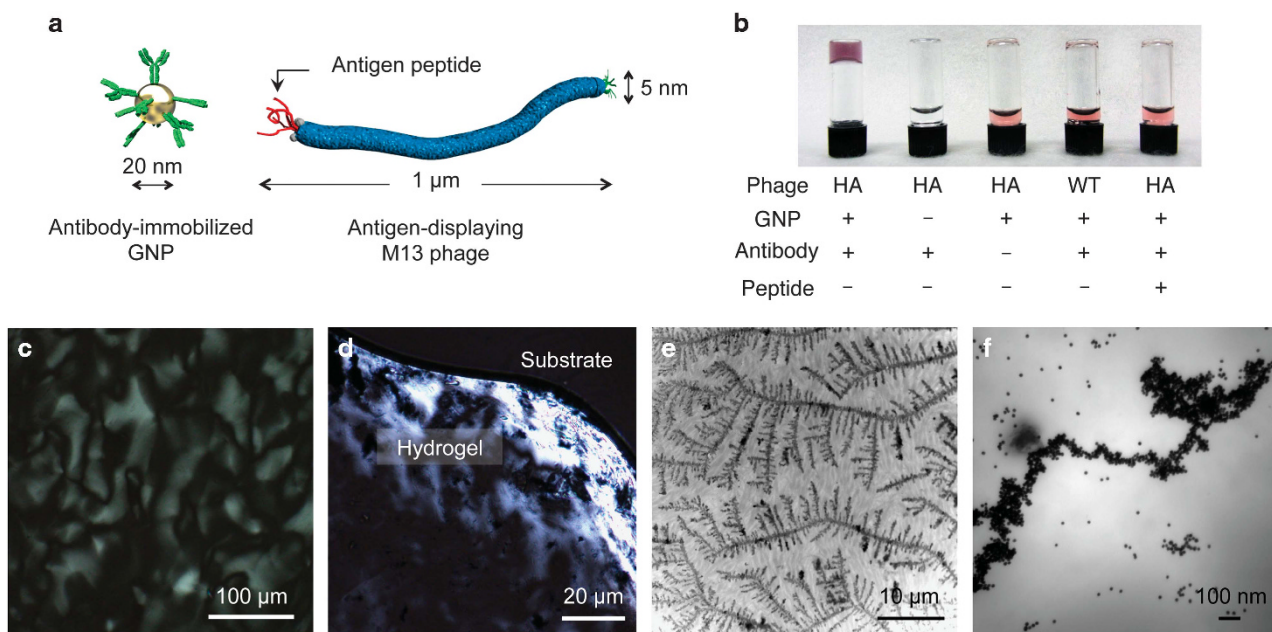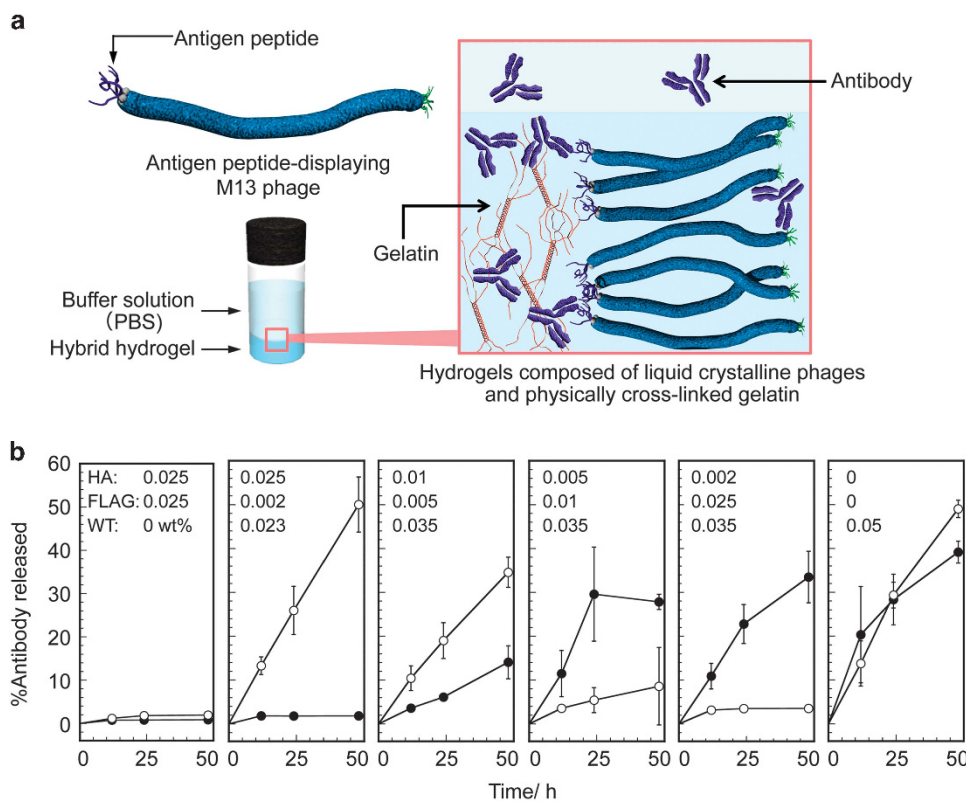
Figure 4 Construction of hybrid hydrogels composed of antigen peptide-displaying phages and antibody-immobilized gold nanoparticles (GNPs). (**a**) Schematic illustration of the GNPs (left) and M13 phages (right). (**b**) Optical photograph of the mixed solution. The different types of phages and the presence/absence of the GNPs, antibodies and chemically synthesized hemmagglutinin (HA) peptides are shown in the figure. (**c, d**) Polarized optical microscopy (POM) images of the hydrogels composed of the GNPs and M13 phages at different magnifications. (**e, f**) Transmission electron microscopy image of the well-ordered networks composed of the GNPs in the hydrogel at different magnifications. Reprinted with permission from Sawada et al.[65] Copyright 2014 ACS.

To further widen the applicability of M13 phage-based materials, the ability to precisely control the phage assemblies at the nano to macro scale is an essential requirement. Sawada et al.[65] reported novel hybrid hydrogels composed of genetically engineered M13 phages and surface-modified GNPs through 'specific' interactions between both the components. A hemmagglutinin (HA)-tag peptide (sequence: Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala), well known as a tag peptide, was genetically fused to the N-termini of the pIII proteins on the M13 phages, and anti-HA peptide antibodies (HA antibodies) were immobilized on the GNPs with a diameter of 20 nm (Figure 4a). Mixed solutions containing the HA peptide displaying M13 phages (HA phages) and the HA antibody-immobilized GNPs (anti-HA GNPs) self-assembled into transparent and uniform self-supporting hydrogels (Figure 4b, far left image). Importantly, mixed solutions during control experiments in the absence of GNPs or HA antibodies, using wild-type phages instead of HA phages, or in the presence of chemically synthesized HA peptides did not self-assemble into hydrogels (Figure 4b), demonstrating that the specific interactions between the HA peptides at the phage termini and the HA antibodies on the GNPs were essential for forming hydrogels. The hydrogel structures were characterized using polarized optical microscopy and transmission electron microscopy observations, which revealed that the HA phages and GNPs in the hydrogel formed liquid crystalline and well-ordered structures, respectively (Figure 4c–f). The concentration of the M13 phages in the hydrogels was three times lower than that typically required for the liquid crystal formation of M13 phage.[32,38] Therefore, the crosslinking interactions between the HA phage termini and the anti-HA GNPs were considered to induce the liquid crystal formation of the HA phages. By contrast, the main chains of the well-ordered network structures of the GNPs nearly reached the sub-centimeter scale, and the branched side chains tended to extend perpendicular to the main chains in a self-similar manner, demonstrating highly regular mesoscale structures.

To demonstrate the generality of the antigen–antibody interaction systems for M13 phage-based hydrogel formation, we further utilized M13 phages displaying other tag peptides (FLAG and Myc peptides; sequences: Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys and Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu, respectively) and antibody-immobilized GNPs instead of the HA peptide system.[65] As a result, hydrogelation was also observed under the same conditions, indicating that the specific interactions between the antigen peptides at the termini and the antibodies on the GNPs generally induced hydrogelation, possibly due to the formation of a network. The mechanical strength measurements of the hybrid hydrogels prepared under various conditions revealed that well-assembled M13 phages with higher concentrations were effectively entangled and promoted the hydrogel strength. Interestingly, comparing the strengths between the peptide species under the same conditions suggested that the strength was simply dependent on the affinity constant values between the phage-displayed peptides and their antibodies. Therefore, molecular-level interactions between the peptides and antibodies affected the macroscopic mechanical strength of the hydrogels. Hence, regular structures composed of M13 phages and GNPs were successfully constructed using specific interactions between the M13 phages displaying gold-binding peptides and bare GNPs and subsequent chemical crosslinking.[66] In summary, crosslinking interactions between the M13 phage termini and spherical nanoparticles cooperatively and effectively regulate the assembled structures of the two heterogeneous components to produce hybrid hydrogels.

Affinity-based controlled release systems using desired molecules within hydrogels have attracted attention for drug delivery applications owing to their ability to prevent bursting or fast release and because they provide tunable release properties. To widen the applicability of

Figure 5 Construction of hybrid hydrogels for controlled release composed of antigen peptide-displaying phages and physically crosslinked gelatin. (**a**) Schematic illustration of the controlled release of antibody molecules from the hybrid hydrogels. (**b**) Release behaviors of the hemmagglutinin (HA) (closed) and FLAG (opened) antibody molecules from the hybrid hydrogels with different mix ratios of the HA, FLAG and wild-type (WT) phages. The ratios are shown in the figure. Reprinted with permission from Sawada et al.[65] Copyright 2017 RSC.

these release system, universal hydrogel matrices with the capability to control the release of drug molecules are required. Sawada et al.[67,68] constructed liquid crystalline hydrogels with controlled release capabilities for high–molecular weight antibodies using tag peptide displaying M13 phages. Hybrid hydrogels composed of HA phages and physically crosslinked gelatin containing HA antibodies were prepared to construct a new controlled release system (Figure 5a). The HA antibodies released from the hydrogels into buffer solutions were quantified. The results indicated that the specific interactions between the HA peptides on the M13 phages and the HA antibodies were essential for suppressing the HA antibody release. The importance of the liquid crystalline-ordered lamellar structures of the HA phages was clearly evident after dialyzing the HA antibodies from the mixed solutions of the HA phages and subsequent polarized optical microscopy observations. The release rates and amounts of the HA antibodies could be controlled by varying the concentration of the HA phages. However, the released amount of the M13 phages was lower than the detection limit using a titer counting method, possibly due to the high molecular weight of M13 phage. This antigen–antibody system could be expanded to other combinations of antigen–antibody complexes, and the orthogonally controlled release of multiple antibodies from a single hybrid hydrogel composed of HA phages, FLAG peptide-displaying M13 phages and antibodies was achieved (Figure 5b). Because various drug molecule-binding peptides have been identified, the utilization of genetically engineered M13 phages as hydrogel matrices should lead to opportunities for constructing universal hydrogels with the capability of controlling the release of various drug molecules.

Furthermore, hydrogel structures have been useful for fabricating three-dimensional porous nanostructures as templates. Belcher, Hammond and co-workers[69] reported a genetically engineered single-walled carbon nanotube (SWNT)-binding peptide (sequence: Asp-Ser-Pr-His-Thr-Glu-Leu-Pro) displayed on the pVIII proteins of a M13 phage-templated nanocomposite of single-walled carbon nanotube and polyaniline (PANI) with enhanced electrical conductivity and electrochemical performance. The affinity of the peptide-modified pVIII for the single-walled carbon nanotubes enabled the formation of complexes of single-walled carbon nanotube and M13 phages to further improve the electrical conductivity of prepared thin films toward various applications. This biotemplated system was developed using an aqueous-based preparation of M13 phages and is expected to adapt to large-scale production with a low cost.

OTHER UTILIZATIONS OF M13 PHAGES TOWARD MATERIAL APPLICATIONS

The selective recovery of rare earth elements from industrial waste is essential for green economic and recycling processes. Conventional methods to recover rare earth ions have considerable disadvantages, such as low environmental friendliness and a lack of specificity.[70] Sawada et al.[71] demonstrated a M13 phage-based selective recovery system for neodymium ions (Nd^{3+}), which are generally used in common rare earth magnets composed of neodymium-iron-boron alloys. The Nd^{3+}-binding M13 peptide (sequence: Gly-Leu-His-Thr-Ser-Ala-Thr-Asn-Leu-Tyr-Leu-His) was identified by an affinity-based screening procedure using phage display methods (Figure 6a), and the peptide showed a specific affinity for Nd^{3+} rather than iron ions
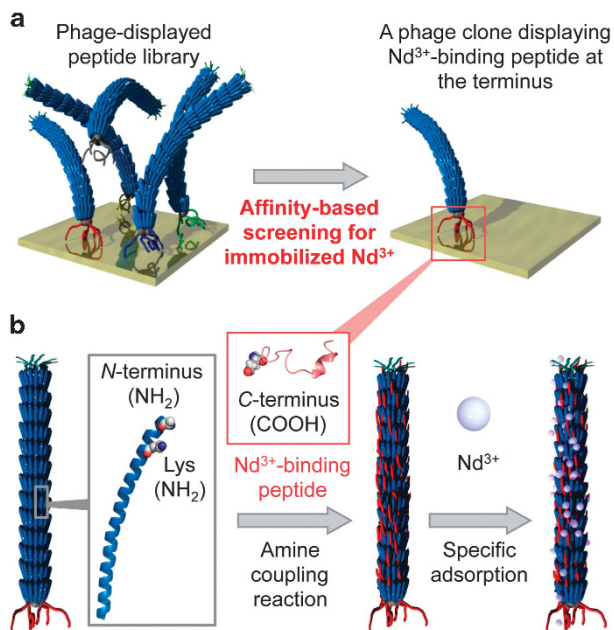
Figure 6 Schematic illustration of (a) peptide screening against Nd^{3+} and (b) the subsequent surface modification of M13 phages by the screened Nd^{3+}-binding peptide to construct an Nd^{3+}-specific adsorption system based on M13 phages. Reprinted with permission from Sawada et al.[71] Copyright 2016 Wiley.
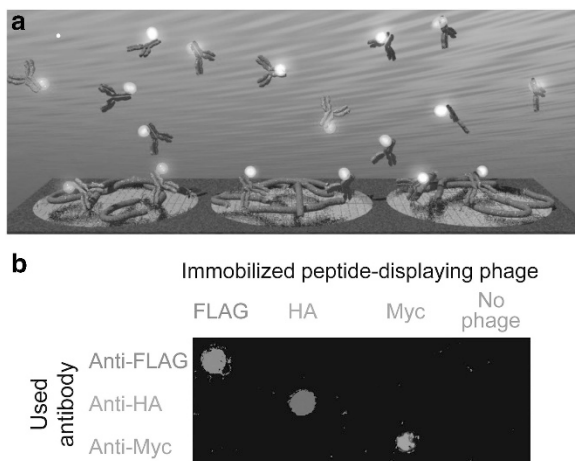
Figure 7 Construction of a protein-detecting microarray based on peptide-displaying M13 phages. (a) Schematic illustration of the selective detection of various antibodies using antigen peptide-displaying M13 phages in glass wells. (b) A fluorescence image of the various phage-immobilized surfaces detected after mixing with three kinds of antibody molecules. Partially reprinted with permission from Sawada et al.[72] Copyright 2011 CSJ. A full color version of this figure is available at Polymer Journal online.

(Fe^{3+}). To clarify the potential applicability of M13 phages as adsorbent scaffolds as well as the specific affinity of the peptide for Nd^{3+} recovery, the peptides were chemically introduced to the surfaces of the M13 phages because greater numbers of peptides could be introduced to the surfaces of M13 phages compared to using genetic engineering methods (Figure 6b). The amount of Nd^{3+} adsorbed on the peptide-modified M13 phages was 15 times greater than that of Fe^{3+} from each single ion solution, demonstrating the high specificity for the target Nd^{3+}. Importantly, over six times more Nd^{3+} adsorbed onto

the peptide-modified M13 phages than Fe^{3+} from a solution containing excess Fe^{3+}, which mimicked a dissolved solution of neodymium-iron-boron ($Nd_2Fe_{14}B$), demonstrating the high potential capability of using M13 phages as selective recovery scaffolds. Because biological screening using phage display methods can produce various rare earth, metal or toxic ion-specified peptides, various ions can interact with the M13 phages for selective ion recovery or removal systems.

Protein or peptide microarray technology is expected to be a powerful high-throughput tool for the direct analysis of protein structures, functions and interactions with target molecules. Sawada et al.[72] constructed a novel peptide array system for protein detection systems based on genetically engineered M13 phages (Figure 7a). Three kinds of tag peptide-displaying M13 phages were immobilized onto each well of a glass slide. When mixed solutions of the three kinds of anti-tag peptide antibodies were applied to each the M13 phage-immobilized wells, the M13 phage-displayed peptides were selectively detected by their antibodies with a minimum signal/noise ratio of 5 (Figure 7b). Therefore, M13 phage-displayed peptide microarray systems will be useful and effective tools for constructing protein characterization systems due to the ease of genetically engineering the proteins or peptides displayed.

CONCLUSIONS

Over the past two decades, filamentous M13 phages have gained increasing attention due to their ability to display foreign peptides or proteins through genetic engineering, and they have been utilized to construct a peptide molecular library for screening peptides with a specific affinity for desired molecules or materials. In particular, various material-binding peptides screened from a phage-displayed peptide library have greatly contributed to materials science and technology as novel bionanomaterials. Recently, the fabrication of ordered structures of M13 phages has been of great interest as a means of utilizing the excellent properties of phages to develop new materials. Indeed, self-assembled M13 phages with fiber or film structures have been utilized as functional materials. Importantly, the combination of genetic engineering and liquid crystalline formation expands the applicability of M13 phages hybridized with inorganic or organic materials to achieve novel classes of soft materials. Because various material-binding peptides have been obtained using biotechnology, a number of metal nanoparticles have been applied to construct regularly assembled M13 phage-based materials. Furthermore, desired functional molecules can be conjugated to the surfaces of M13 phages using genetic engineering and chemically synthetic methods; therefore, various functionalized assemblies have been constructed. Although M13 phage is only one species of biologically constructed virus, M13 phage is resistant to high temperatures (~80 °C),[73] a wide range of pH values (3 ~ 11),[62] organic solvents,[74,75] chaotropic reagents[76] and shear forces.[77] Accordingly, M13 phage-based assembled materials will contribute to novel imaginative opportunities for the exploitation of structurally regular assemblies in materials science and technology.

CONFLICT OF INTEREST
The author declares no conflict of interest.

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