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Phage as a Genetically Modifiable Supramacromolecule in Chemistry, Materials and Medicine

Binrui Cao[†], Mingying Yang^{*,§}, and Chuanbin Mao^{*,†,‡}

[†]Department of Chemistry & Biochemistry, Stephenson Life Science Research Center, University of Oklahoma, 101 Stephenson Parkway, Norman, Oklahoma 73019, United States

[§]Institute of Applied Bioresource Research, College of Animal Science, Zhejiang University, Yuhangtang Road 866, Hangzhou, Zhejiang 310058, China

[‡]School of Materials Science and Engineering, Zhejiang University, Hangzhou, Zhejiang 310027, China

CONSPECTUS

Filamentous bacteriophage (phage) is a genetically modifiable supramacromolecule. It can be pictured as a semiflexible nanofiber (~900 nm long and ~8 nm wide) made of a DNA core and a protein shell with the former genetically encoding the latter. Although phage bioengineering and phage display techniques were developed before the 1990s, these techniques have not been widely used for chemistry, materials, and biomedical research from the perspective of supramolecular chemistry until recently. Powered by our expertise in displaying a foreign peptide on its surface through engineering phage DNA, we have employed phage to identify target-specific peptides, construct novel organic–inorganic nanohybrids, develop biomaterials for disease treatment, and generate bioanalytical methods for disease diagnosis. Compared with conventional biomimetic chemistry, phage-based supramolecular chemistry represents a new frontier in chemistry, materials science, and medicine.

In this Account, we introduce our recent successful efforts in phage-based supramolecular chemistry, by integrating the unique nanofiber-like phage structure and powerful peptide display techniques into the fields of chemistry, materials science, and medicine: (1) successfully synthesized and assembled silica, hydroxyapatite, and gold nanoparticles using phage templates to form novel functional materials; (2) chemically introduced azo units onto the phage to form photoresponsive functional azo-phage nanofibers via a diazotization reaction between aromatic amino groups and the tyrosine residues genetically displayed on phage surfaces; (3) assembled phage into 2D films for studying the effects of both biochemical (the peptide sequences displayed on the phages) and biophysical (the topographies of the phage films) cues on the proliferation and differentiation of mesenchymal stem cells (MSCs) and induced pluripotent stem cells (iPSCs) and identified peptides and topographies that can induce their osteogenic differentiation; (4) discovered that phage could induce angiogenesis and osteogenesis for MSC-based vascularized bone regeneration; (5) identified novel breast cancer cell-targeting and MSC-targeting peptides

*Corresponding Authors: cbmao@ou.edu., yangm@zju.edu.cn.

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and used them to significantly improve the efficiency of targeted cancer therapy and MSC-based gene delivery, respectively; (6) employed engineered phage as a probe to achieve ultrasensitive detection of biomarkers from serum of human patients for disease diagnosis; and (7) constructed centimeter-scale 3D multilayered phage assemblies with the potential application as scaffolds for bone regeneration and functional device fabrication.

Our findings demonstrated that phage is indeed a very powerful supramacromolecule suitable for not only developing novel nanostructures and biomaterials but also advancing important fields in biomedicine, including molecular targeting, cancer diagnosis and treatment, drug and gene delivery, stem cell fate direction, and tissue regeneration. Our successes in exploiting phage in chemistry, materials, and medicine suggest that phage itself is nontoxic at the cell level and can be safely used for detecting biomarkers *in vitro*. Moreover, although we have demonstrated successful *in vivo* tissue regeneration induced by phage, we believe future studies are needed to evaluate the *in vivo* biodistribution and potential risks of the phage-based biomaterials.

INTRODUCTION

Filamentous phage, a virus that specifically infects and replicates within bacteria, is a nanofiber-like biosupramacromolecule composed of a single stranded (ss) DNA wrapped in a viral protein coat.¹ The viral protein coat can be genetically engineered to display peptides or proteins by inserting DNA encoding peptides/proteins into the genes of the coat proteins.^{1,2} In addition, the surface display of foreign peptides on the phage enables the construction of a phage-based random peptide library made of billions of phage clones, each displaying a unique peptide sequence.¹ Phage libraries have been widely used for the affinity selection of peptides that can specifically bind to a target (e.g., inorganic materials,³ small proteins,⁴ or mammalian cells^{5,6}). Although the natural functions of phage are to store ssDNA and transfer the DNA to host bacteria, we have recently intensively applied the filamentous phage (M13 and its close relative fd) to a variety of fields in chemistry, materials, and medicine, including protein–protein interaction,⁴ synthesis of novel nanomaterials,^{3,7–17} construction of matrix for stem cell studies,^{18–20} fabrication of scaffolds for tissue regeneration,²¹ discovery of cell-targeting peptides,^{5,6,22–24} drug and gene delivery,^{23–25} and development of novel biosensors.^{26,27} In this Account, we summarize our efforts in these aspects.

CHEMICAL VIEW OF PHAGE

Filamentous phage is a virus composed of several thousand highly ordered copies of major coat protein (pVIII) and five copies each of the four minor coat proteins (pIII, pVI, pVII, and pIX) surrounding a circular ssDNA, which encodes all the coat proteins (Figure 1). The pVIII constitutes the side wall of the phage body. Five copies each of pVII and pIX are at one tip, and five copies each of pIII and pVI are at the other distal tip. Inserting DNA encoding foreign peptides into the genes of specific coat proteins results in the display of the peptides on the outer surface of the phage at the tip by genetic fusion to the N-terminus of minor coat proteins (e.g., pIII display) or along the length by genetic fusion to the N-terminus of pVIII (i.e., pVIII display) (Figure 1).¹ Since the surface (side walls or tips) of phage can be genetically modified to display designed peptides, the surface chemistry of the phage can be precisely controlled, which is a unique property that differentiates phage from other macromolecules. Moreover, through peptide display on the phage, a phage library can be constructed for the identification of target-binding peptides by a process called phage display or biopanning (Figure 2).^{4,28}

MATERIALS VIEW OF PHAGE

From a materials science perspective, filamentous phage is a bionanofiber (~900 nm long and ~8 nm wide) with its side wall made of self-assembled highly ordered pVIII. It is an excellent building block for materials synthesis and assembly for several reasons. First, phage display can identify material-binding peptides or phages, which can be used to synthesize or assemble nano- and biomaterials.¹ Second, the phage itself is chemically stable in acidic, basic, and even many organic solvents and remains intact at high temperatures up to 80 °C.²⁹ Third, the biological nature of phage replication guarantees the error-free production of monodisperse phage.³⁰ Fourth, different peptides can be codisplayed on the surface of a single phage, enabling the phage to become a multifunctional nanofiber.³¹ Fifth, phage tends to self-assemble into differently ordered structures by simply controlling factors such as concentrations.¹⁶

PHAGE AS A TEMPLATE FOR NANO- AND BIOMATERIALS SYNTHESIS

Earlier it was demonstrated that semiconductor-binding peptide displayed on the phage could result in the production of single-crystalline semiconductor nanowires.³ The general strategy includes three steps. First, a target-binding peptide is identified by biopanning. Second, the peptide is genetically displayed on the side wall of phage. Third, the resultant phage is used as a biotemplate to synthesize or assemble nanoparticles. Inspired from this strategy, we have recently prepared unique nanostructures of silica,^{8,11} hydroxyapatite,^{9,10,17} and gold¹³ using phage as a template.

Silica

We first developed a general method of using surface proteins of biotemplates to catalyze the hydrolysis of organosilicate precursors and subsequent polycondensation to produce silica.¹¹ Then we applied this method to prepare well-ordered mesoporous silica fibers with hexagonally arranged pores using fd phage as a biotemplate (Figure 3).⁸ First, the surface

proteins of anionic phage initiate the hydrolysis of 3-aminopropyltriethoxysilane (APTES) to form oligomers, which link neighboring phage nanofibers in parallel manner and result in parallel-aligned ordered phage assemblies. Then the prehydrolyzed tetraethoxysilane (TEOS) was added to the APTES–phage mixture to initiate the polycondensation of TEOS into a layer of silica to form well-ordered phage–silica composites. Finally, the phage–silica composites were calcined to remove the biotemplates and generate well-ordered mesoporous silica fibers with hexagonally arranged pores (center-to-center pore spacing of 7.6 nm, Figure 3).⁸ In addition, we found that wild-type phage could lead to the formation of phage bundles within silica fibers whereas bioengineered cationic phage tended to form a silica fiber with only one phage nanofiber embedded.⁸ The phage-templated mesoporous silica has potential applications in drug release, photonic nanodevices, and bioreactors.³²

Hydroxyapatite

Extracellular matrix (ECM) of bone is mainly made of hydroxyapatite (HAP) crystals embedded in self-assembled highly ordered collagen fibrils. Inspired from this fact, we used phage, morphologically mimicking the collagen fibrils, to arrange HAP crystals into ECM-like matrix by two different strategies. In the first strategy, positively charged calcium ions were used to trigger the self-assembly of negatively charged phage (e.g., wild type phage¹⁰ or phage displaying a cationic peptide such as a contiguous sequence of 8 Glu¹⁷) into bundles, which in turn served as a calcium source to react with phosphate ions to form nanocrystalline HAP with preferred crystal orientation along the phage bundles. In the second strategy,⁹ we fabricated two types of engineered HAP-nucleating phage nanofibers with each displaying one of the two HAP-nucleating peptides (QESQSEQDS and ESQES) derived from a HAP-nucleating protein, Dentin Matrix Protein-1. The resultant two HAP-nucleating phage nanofibers self-assembled into bundles by forming beta-structure between the two peptides displayed on them, which promoted the site-specific nucleation of HAP with the preferred crystal orientation and the subsequent bone ECM-like organization of phage and HAP. Because phage nanofibers have a higher degree of monodispersity than the traditional polymer nanofibers, the resultant building blocks are expected to self-assemble into scaffolds with more well-defined architectures for bone regeneration than the traditional polymer-HAP composite nanofibers.

Gold

We also demonstrated that anionic phage could interact with cationic polyethylenimine (PEI)-coated gold nanoparticles (AuNPs) to form AuNP–phage composites, in which AuNPs and phage nanofibers are assembled into bundles.¹³ Anionic phage and cationic AuNPs can also be assembled into a composite film through a layer-by-layer assembly method.²⁶ Such AuNP–phage composites will have great potential in sensing through surface plasmon resonance spectroscopy.

PHAGE AS A SUBSTRATE FOR ORGANIC REACTION

The super stability of phage in acidic, basic, or even organic solutions enables the functionalization of phage into functional organic nanofibers through organic reactions. To demonstrate this idea, we developed a phage-based photoresponsive organic nanofiber by

first displaying a tyrosine-rich peptide (YYGYYGYYGY) on the phage surface, followed by a site-specific chemical reaction to form an azo structure (Figure 4).¹⁵ Specifically, aromatic amino groups from the aromatic amines interacted with the tyrosine residues on the phage surfaces via a diazotization reaction to introduce azo units to the phage surface, forming azo-phage nanofibers. The azo-phage showed a unique reversible photoresponsive behavior due to the UV-light-triggered switches between cis and trans isomerization of the azo chromophores (Figure 4). Moreover, the morphology of phage nanofibers before and after chemical reactions remained the same, confirming the chemical stability of phage. The azo-phage nanofibers with unique reversible photoresponsive properties will likely find applications in reversible holographic recording, nonlinear optics, and optical data storage.

PHAGE AS A MATRIX FOR STEM CELL GROWTH AND DIFFERENTIATION

Stem cell niche controls stem cell function and fate by stimulating the cells through chemical and physical cues. Biomaterials are considered as a type of stem cell niche. However, currently used peptide-coated biomaterial substrates do not allow us to conveniently vary the biochemical and biophysical cues to tune the stem cell fate.³³ Peptide is usually attached to the biomaterials surface by chemical conjugation, but the site of peptide conjugation cannot be precisely controlled. In contrast, a peptide can be introduced onto the surface of phage by genetic means in a site-specific manner. Thus, we assembled phage nanofibers displaying different peptides into films with different nanotopographies and then studied the effects of both biochemical (the peptide sequences displayed on phage) and biophysical (the topographies of phage films) cues on the proliferation and differentiation of both mesenchymal stem cells (MSCs)^{18,19} and induced pluripotent stem cells (iPSCs).²⁰

MSCs

To prepare phage films, we adopted a layer-by-layer self-assembly method in which poly(lysine) and phage nanofibers are alternatively coated on the glass cover-slide with phage nanofibers as a terminating layer (Figure 5A).^{18,19} The resultant films were made of parallel phage bundles as ridges separated by grooves. We found that the parallel-aligned phage bundles induced the MSCs to become elongated and aligned along their length direction, suggesting that the topography of phage films affected the morphology of the MSCs. We also found that cell proliferation was greatly regulated by both the topography of phage films and the peptides displayed on the phage nanofibers. Films with narrower phage bundles and displaying the cell-adhesive RGD peptide favored the proliferation of MSCs. Moreover, we discovered that films assembled from a mixture of phage nanofibers displaying two fibronectin-derived peptides (RGD and PHSRN) could induce the osteogenic differentiation of MSCs more efficiently than individual phage nanofibers alone.

iPSCs

To develop a biomaterial for inducing the osteogenic differentiation of iPSCs for bone regeneration, we investigated the use of phage films to regulate osteogenic differentiation of iPSCs.²⁰ First, we displayed one of the following peptides on the side walls of phage: RGD (termed FN1-phage), PHSRN (termed FN2-phage), KIPKASSVPTELSAISTLYL (derived

from bone morphogenetic protein 2 (BMP2), termed BMP2-phage), and ALKRQGRTLYGFGG (known as osteogenic growth peptide (OGP), termed OGP-phage). Then we assembled phage nanofibers into films by our layer-by-layer method.¹⁹ We found that phage films with random topography could not induce the osteogenic differentiation of iPSCs no matter what peptides were displayed on the phage nanofibers. We also found that phage films with parallel ridge/groove topography could induce the osteogenic differentiation of resident iPSCs with phage displaying BMP2 and OGP peptides showing the best inducing capability (Figure 5). This work verified that our phage film system is a powerful platform for studying stem cell niche.

PHAGE AS A SCAFFOLD FOR BONE REGENERATION

Natural bone is a tissue containing a high density of blood vessels for delivering blood and nutrients. However, current bone regeneration methods usually cannot generate new bones with enough blood vessels. To overcome the challenge, we introduced phage nanofibers displaying RGD peptide on the surface, which is capable of regulating activated endothelial cell migration and adhesion, into the pores of a 3D printed MSC-seeded bioceramic scaffold to form a virus-activated matrix (VAM) for the regeneration of vascularized bone in vivo (Figure 6).²¹ The results showed that scaffolds filled with RGD-phage (VAM group) induced bone formation more than those filled with wild-type (WT)-phage (negative control) or without phage (blank control) but less than the scaffolds seeded with vascular endothelial growth factor (VEGF)-expressing MSCs (positive control) (Figure 6). Moreover, VAM-induced bone tissues contained more blood vessels than negative and blank controls but fewer than the positive control. We believe phage displaying other functional peptides could also be used for the regeneration of other tissues, opening up a new avenue in phage-based regenerative medicine.

PHAGE AS A TOOL FOR DISCOVERING CELL-TARGETING SMALL MOLECULES

Cell-targeting small molecules should specifically target, bind to, and even enter target cells. When conjugated to imaging probes or drug or gene carriers, they can greatly enhance the specificity and efficiency of cell imaging and drug or gene delivery. Biopanning (Figure 2) is a powerful technique for discovering cell-targeting peptides from a phage library.²⁸ It can identify two types of cell-targeting peptides: one that selectively recognizes the surface of target cells and another that can be selectively internalized into the target cells. To enhance the nonviral MSC-based gene therapy and develop new breast cancer therapy, we applied the biopanning to discover MSC-binding⁵ and SKBR3 breast cancer cell-internalizing⁶ peptides, respectively.

MSCs

We selected rat MSC (rMSC)-homing peptides from a major coat-displayed phage library, where an 8-mer peptide was fully displayed on the major coat of each fd phage clone and billions of phage clones are present.⁶ A peptide (VTAMEPGQ) was found to have the highest frequency and its rMSC-binding capability was verified by capture ELISA assay and

rMSC labeling test (Figure 7A,B). Strong green fluorescence could only be found from rMSCs treated with FITC-VTAMEPGQ but not from those treated with control peptides or from control cells, suggesting the high affinity and specificity of VTAMEPGQ (Figure 7B). By integrating this rMSC-binding peptide into MSC-based gene therapy, we found that the transfection efficiency in rMSCs was significantly enhanced.^{5,24,34}

Breast Cancer Cells

We selected SKBR3 cell-internalizing peptides from the same phage library through several rounds of biopanning.⁶ A peptide (VSSTQDFP) showed the highest frequency among the randomly picked clones and was found to target cell surface and then become internalized. This peptide entered the SKBR3 cells by mechanisms of energy-dependent uptake and reorganization of actin cytoskeleton. The phage displaying the peptide has been successfully applied to develop targeted breast cancer therapy.²³

PHAGE AS A CARRIER FOR DRUG AND GENE DELIVERY

Targeted delivery of drugs or genes to target sites remains a major hurdle in precision medicine. We have used phage as a carrier for delivering drugs or genes to target cells.^{23–25,35} In addition, we conjugated the specific cell-targeting peptide (alone or fused to pVIII) onto gene carriers for enhanced gene delivery to stem cells.^{24,34}

Drug Delivery to Breast Cancer Cells

Photodynamic therapy (PDT), a promising breast cancer therapy, is limited by the selective enrichment of photosensitizer (PS) drug around cancer cells. To overcome this limitation, we chemically conjugated PS (pyropheophorbide-a, PPa) onto the side walls of a phage surface displaying our identified SKBR3-cell-targeting peptide (VSSTQDFP)⁶ to form a PPa–phage complex (Figure 8A).²³ We found that when treated with PPa–phage complex followed by washing and light irradiation to initiate PDT, ~85% of target SKBR3 cells were killed, much higher than the percentage of killed control MCF-7 cells (Figure 8B), indicating that our PPa–phage complex can selectively and efficiently destroy the target SKBR3 cells by PDT without harming nontarget cells.²³ This study demonstrated the possibility of using cancer-targeting phages to achieve targeted cancer therapy.

Gene Delivery to MSCs

Nonviral MSC-based gene therapy, in which MSCs are transfected and then implanted, holds promise for treating many diseases. However, MSCs are hard to transfect by nonviral vectors. We successfully presented phage-borne rMSC-targeting pVIII on the surface of gene-carrying nanoclusters and developed virus-mimetic magnetic/silica nanoclusters (VMSNCs),²⁴ which reached a significantly improved transfection efficiency of ~40% in rMSCs compared with commercial transfection reagents (Figure 9). We also integrated rMSC-targeting peptide into the lipid-based nanoparticles for the enhanced delivery of gene encoding therapeutic proteins.^{5,34} Surprisingly, we discovered that the peptide, either alone or in the 3-fold reiterated fashion, significantly improved the transfection efficiency (up to ~50%) and achieved long-lasting gene expression (for over 170 h). Our work suggests that

the stem cell-targeting peptides selected from biopanning can be conjugated to nanocarriers for enhancing stem cell-based gene therapy.

PHAGE AS A RECOGNITION ELEMENT IN BIOSENSING AND DISEASE DIAGNOSIS

Phage can display more than one peptide and become multifunctional, a feature we found very useful in developing phage into a biosensing probe in the ultrasensitive detection of disease (e.g., cancer or fungal infection) biomarkers in sera collected from human patients.^{36,37} For example, *Candida albicans* infection causes high mortality rates within cancer patients due to the low sensitivity of the current diagnosis systems. To improve the detection of such infection, we employed phage nanofibers genetically displaying two functional peptides, one for binding magnetic nanoparticles (MNPs) and another for recognizing and capturing the biomarker (anti-secreted aspartyl proteinase 2 IgG, anti-SAP2 IgG) in the serum of cancer patients.³⁶ The resultant phage nanofibers were decorated with MNPs, allowed to capture the biomarker from the serum, and magnetically enriched. Then the captured biomarker was analyzed by ELISA assay. Our method lowered the detection limit to 1.1 pg/mL, about 2 orders of magnitude lower than that of the conventional ELISA method (Figure 10), and thus successfully detected human anti-SAP2 IgG in sera from *Candida albicans*-infected human cancer patients with ultrahigh sensitivity (Figure 10). Our most recent work further demonstrates that biomarker-recognizing phage nanofibers are also a powerful probe in detecting cancer biomarkers (e.g., p53 antibody) from human cancer patients for early cancer diagnosis.³⁷

PHAGE AS A BUILDING BLOCK FOR DEVELOPING NEW MATERIALS

Phage is a bionanofiber with genetically tunable surface chemistry and also an excellent building block for constructing functional materials. Inspired by the natural magnetically guided orientation of organisms containing MNPs, we decorated phage nanofibers with MNPs and then used external magnetic fields to align them into single-orientation horizontally aligned arrays (Figure 11).⁷ On such arrays, we could apply more single-orientation layers, each with a twisted magnetic field, and thus build a multiorientation multilayered phage assembly with each layer made of single-oriented phage arrays (Figure 11). Such long-range-ordered phage nanostructures have a great potential to be used as scaffolds in directing stem cell fate, inducing tissue formation, and building functional devices.

CONCLUSIONS AND OUTLOOKS

Powered by our expertise in phage engineering, we have employed phage to identify cell-specific peptides, synthesize novel organic-inorganic nanohybrids, direct stem cell fate, induce tissue formation, develop nano- and biomaterials for treating diseases, and generate bioanalytical methods for disease diagnosis. Our findings indicate that phage is truly a supramacromolecule that can be applied not only to develop novel organic, inorganic, or hybrid materials but also to advance the important fields in molecular targeting as well as disease diagnosis and treatment. From this perspective, the phage-based supramolecular

chemistry is complementary to conventional biomimetic chemistry and represents a new and unique frontier in chemistry, materials science, and medicine and a new area that needs to be further exploited in the era of precision medicine. The integration of phage with conventional fields is still at an early stage. It is now clear that applying phage in vitro is safe because phage has been proven to be nontoxic to mammalian cells.³⁸ Although some breakthroughs have been made in the use of phage-based materials in tissue regeneration at animal levels, challenges still impede the development of this field, such as a good understanding of the immune response to phage-based biomaterials and potential risks in applying phage-based biomaterials in vivo. We believe efforts from a variety of researchers with multidisciplinary backgrounds are necessary to further advance this exciting field. Although this Account is focused on filamentous phage, our most recent work³⁹ shows that the other types of phage also hold promise for use in chemistry, materials, and medicine.

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Biographies

Binrui Cao received his Ph.D. in 2010 from University of Oklahoma and has stayed there as a postdoctoral research fellow until now. His research interest is to use bioengineered filamentous biostructures to synthesize novel bionanomaterials for medical applications.

Mingying Yang received her Ph.D. in 2005 from Tokyo University of Agriculture and Technology, followed by postdoctoral studies at the same university. Her research interests include protein engineering and protein-based biomaterials.

Chuanbin Mao received his Ph.D. in 1997 from Northeastern University in China. He carried out postdoctoral studies at Tsinghua University and the University of Texas at Austin before taking a faculty position at the University of Oklahoma. His research interests include phage display, nanobiotechnology, biomaterials, and nanomedicine.

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Figure 1.

Scheme of phage structure and phage display technique. Filamentous phage (~900 nm long and ~8 nm wide) is a nanofiber-like virus composed of ~3000 highly ordered copies of major coat protein (pVIII) and 5 copies of the minor coat proteins (pIII, pVI, pVII, and pIX) surrounding a circular ssDNA, which encodes all the coat proteins. By insertion of DNA encoding foreign peptides into the genes of specific coat proteins, the peptides can be displayed at the tip by genetic fusion to the N-terminus of pIII (pIII display) or along the length by genetic fusion to the N-terminus of the pVIII (pVIII display).

Figure 2.

Scheme of the selection of mesenchymal stem cell (MSC)-targeting peptides by screening a phage-displayed random peptide library against target cells. The process is called biopanning. Reproduced with permission from ref 6. Copyright 2010 American Chemical Society.

Figure 3.

(A) Scheme of the phage-directed formation of hexagonal mesoporous silica; (B) TEM images of phage-silica composites before (B-1) and after (B-2) calcination; (C) cross-sectional TEM images of phage-silica composites before (C-1) and after (C-2) calcination. Reproduced with permission from ref 8. Copyright 2012 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

Figure 4.
 Schematic representation of photoresponsive azo-phage and reversible photoisomerization under UV exposure and dark conditions. Reproduced with permission from ref 15.
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Figure 5.

(A) Fabrication of phage films by a layer-by-layer self-assembly method for inducing the osteoblastic differentiation of iPSCs. Real-time PCR (B) and immunofluorescence staining (C) assays for evaluating the expression levels of the osteogenic marker proteins, including osteocalcin (OCN) and osteopontin (OPN). Collagen (COL) was used as a reference marker, and plates without phage films were the control (CON). Reproduced with permission from ref 20. Copyright 2014 American Chemical Society.

Figure 6.

(A) Scheme of the integration of RGD-phage and rMSCs into a 3D printed bioceramic scaffold for bone regeneration. (B) Micro-CT images showing bone regeneration using the scaffold filled with wild-type phage (negative control, B-1), with RGD-phage (VAM, B-2), and with both RGD-phage and VEGF (positive control, B-3). (C) Quantification of bone volume density (bone volume/tissue volume) within the scaffolds. The bone volume density within the VAM scaffold is higher than that in the blank and negative control groups. (D) Number of newly formed blood vessels within the scaffolds. There are significantly more blood vessels formed in VAM scaffold than negative control. Reproduced with permission from ref 21. Copyright 2014 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

Figure 7.

(A) ELISA results for the evaluation of selected rMSC-binding peptide by biopanning, showing that the peptide VTAMEPGQ has the highest rMSC-binding affinity. (B) Affinity and specificity tests of FITC-labeled VTAMEPGQ with rMSCs. Fluorescence was detected from 1 μ M (B-1) and 10 μ M (B-2) FITC-VTAMEPGQ in rMSCs. But no specific fluorescence was observed from either 1 μ M (B-3) or 10 μ M (B-4) FITC-labeled control peptide in rMSCs. No specific fluorescence was observed from 10 μ M FITC-VTAMEPGQ in either rat fibroblastic cells (B-5) or rat smooth muscle cells (B-6). Scale bar = 100 μ m.

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Figure 8.

(A) Schematic of photosensitizer conjugation onto phage for developing targeted PDT. (B) In vitro evaluation of PDT on control MCF-7 cells (B-1, B-2, B-3) and target SKBR-3 cells (B-4, B5, B-6): (B-1, B-4) light microscopy images of MCF-7 and SKBR-3 cells; (B-2, B-5) fluorescent images of MCF-7 and SKBR-3 cells using live/dead cell viability kit upon incubation with photosensitizer and laser irradiation; (B-3, B-6) fluorescent images of MCF-7 and SKBR-3 cells using live/dead cell viability kit upon incubation with phage–photosensitizer complex and laser irradiation. All images were captured at 10× objectives.

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Figure 9.

(A) Scheme for using VMSNCs for gene delivery to MSCs; (B) Mechanism of the magnetic/silica nanocluster formation and cleavage with intracellular glutathione (GSH); (C) The EGFP-VEGF (vascular endothelial growth factor tagged with green fluorescence protein) gene transfection efficiency by VMSNCs and control vectors. MSNCs: magnetic silica nanoclusters, WT: wild-type, MPTS: (3-mercaptopropyl)-trimethoxysilane, CTAB: cetyltrimethylammonium bromide, PEI: polyethylenimine. Reproduced with permission from ref 24. Copyright 2013 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

Figure 10.

(A) Schematic of using phage displaying anti-Sap2-IgG targeting (ASIT) peptide and decorated with magnetic nanoparticles (MNP) (ASIT-MNP-phage) (A-1) for the ultrasensitive detection of anti-Sap2-IgG from human serum (A-2, A-3). (B) The detection of anti-Sap2 IgG in serum of *Candida albicans*-infected cancer patients: (B-1) percentage of anti-Sap2 IgG positive population among all cancer patients (1, *Candida albicans*-infected patients; 2, healthy control); (B-2) percentage of anti-Sap2 IgG positive population among patients of each specific cancer type (1, lung cancer; 2, breast cancer; 3, intestinal cancer; 4,

others). WT: wild-type, HRP: horseradish peroxidase, TMB: 3, 3', 5, 5'-tetramethylbenzidine, PK: MNP-binding peptide (PTYSLVPRLATQPFK). Reproduced with permission from ref 36. Copyright 2015 American Chemical Society.

Figure 11.

(A) Schematic for using a magnetic field to guide the alignment of MNP-decorated phage (MNP-phage); (B) TEM image of the MNP-phage; (C) SEM image of the single-orientation horizontally aligned MNP-phage; (D) centimeter-scale single-orientation horizontally aligned MNP-phage; (E, F) optical images of double-orientation multilayered MNP-phage arrays. Reproduced with permission from ref 7. Copyright 2013 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.