Expert Opinion

Evolution of phage display: from bioactive peptides to bioselective nanomaterials

VA Petrenko
Auburn University, Department of Pathobiology, AL 36849, USA

Background: New phage-derived biorecognition nanomaterials have emerged recently as a result of the in-depth study of the genetics and structure of filamentous phage and the evolution of phage display technology. *Objective*: This review focuses on the progress made in the development of these new nanomaterials and discusses the prospects of using phage as a bioselectable molecular recognition interface in medical and technical devices. *Methods*: The author used data obtained both in his research group and sourced using Science Citation Index (Web of Science) search resources. *Results/conclusion*: The merging of phage display technologies with nanotechnology over the past few years has proved promising and has already shown its vitality and productivity by contributing vigorously to different areas of medicine and technology, such as medical diagnostics and monitoring, molecular imaging, targeted drug and gene delivery, vaccine development, as well as bone and tissue repair.

Keywords: biosensors, drug delivery, gene delivery, molecular imaging, nanobiotechnology, phage display

Expert Opin. Drug Deliv. (2008) 5(8):825-836

1. Introduction

George Smith, the recognized founder of phage display, first used fusion phage as an alternative vector to screen expression libraries for a recombinant clone of interest using antibodies as probes [1]. The concept was to link the expressed proteins to the genetic material in a much more direct way than in existing vector systems, such as λgt11, where the gene and protein have to be isolated and analyzed separately as parts of the same infected bacterial clone. In filamentous phage, expressed proteins could be tightly linked to the encoding gene as parts of the same infectious particle. George Smith first proved this idea and isolated target fusion phage from a mixture of non-related clones using the antibody as the 'bait'. In this affinity selection procedure, later named 'biopanning', extremely rare binding clones were captured out of a huge library of recombinant fusion phage [2].

As an established concept, the phage display emerged later, adapting the principles of combinatorial chemistry developed first by Mario Geysen and Richard Houghten [3,4]. The combinatorial concept replaced the traditional collections of natural or individually synthesized compounds for libraries of peptides obtained in parallel synthesis as grouped mixtures [5]. In the phage display approach suggested by George Smith, random peptide libraries were made on infectious filamentous phage, making this kind of random peptide technology much more effective. In the past two decades, phage display libraries have become a standard tool to identify peptides and proteins that bind antibodies, cellular receptors and other biological molecules [6]. In particular, this paradigm has

informa
healthcare

Expert Opin. Drug Deliv. Downloaded from informahealthcare.com by Hacettepe Univ. on 12/02/12
For personal use only.

Copyright © 2008 Informa UK Ltd
Not for Sale or Commercial Distribution
Unauthorised use prohibited. Authorised users can download, copy, view and print a single copy for personal use

10.1517/17425240802280275 © 2008 Informa UK Ltd ISSN 1742-5247
All rights reserved: reproduction in whole or in part not permitted

RIGHTSLINK

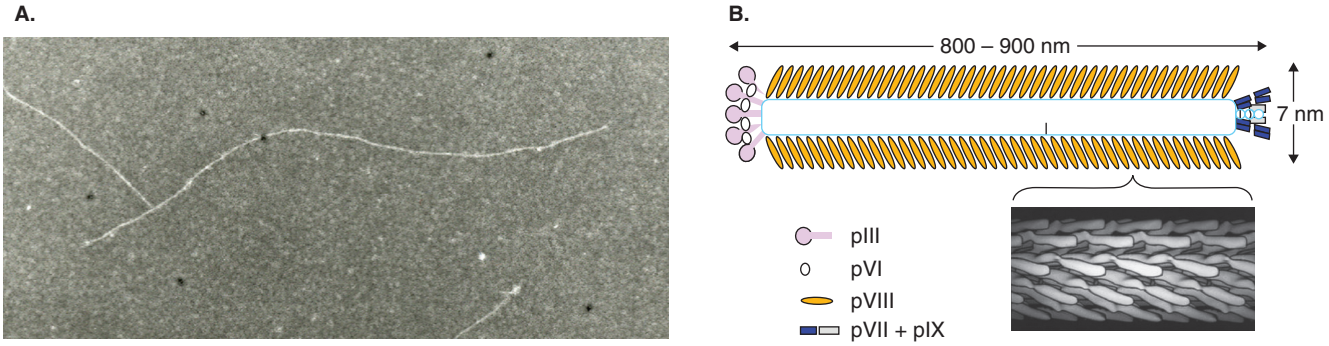

Figure 1. Structure of filamentous phage. A. Transmission electron micrograph of phage (original magnification ×57,000). **B.** Composition of phage and arrangement of major coat protein surface domains (shown as electron densities of the proteins; the image is provided by Gregory Kishchenko).

Expert Opin. Drug Deliv. Downloaded from informahealthcare.com by Hacettepe Univ. on 12/02/12
For personal use only.

been successfully employed for the identification of potent agonists and antagonists of pharmacologically significant receptors [7,8].

In the traditional phage display approach, binding molecular entities are identified by screening libraries of different kinds of binding structures: short peptides, or randomized regions within a fixed protein scaffold, such as antibodies or their shortened single-chain versions [9]. In this approach, phage serve only as a growth-supporting and peptide-exposing vehicle, which can be replaced by other replicating packages if necessary. In a distinct *phage nanobiotechnology* paradigm, fusion phage serve not only as a genetic carrier for foreign peptides, as in the traditional phage display approach, but operate as a nanoparticle (nanotube) decorated by thousands of foreign peptides, whose composition determines the integrated physicochemical characteristic of the whole nanoparticle. In this concept, virus capsids serve as versatile building blocks or scaffolds that can be used for producing nanomaterials [10-12]. Naturally spread viral particles demonstrate a remarkable diversity of shapes with well-defined geometry and uniformity in the nanometer range ideal for nano-fabrication. The atomic structures of many viruses have been resolved, allowing precise engineering of their modified forms with predetermined shapes and functions and a precise spatial distribution of virus-fused functional ligands at a nanoscale level [13]. In this review, we will outline progress in the development of the phage-derived nanomaterials and their use as bioselectable interfaces in medical and technical devices. We will focus on the use of filamentous phage as a vector for nano-manipulation, although different viruses have been used successfully by other laboratories [14].

2. Phage-derived bioselective nanomaterials

Bacteriophage – the viruses that infect bacteria – are ideal materials for nano-manipulations because their structure and life cycle are encrypted in their small genomes, which can be readily engineered using advanced chemical and biochemical methods, such as DNA synthesis, site-directed mutagenesis and molecular cloning. In particular, the filamentous phage of class Ff (M13, fd, f1), the most popular display system, have their genomes encoded as DNA in a single-stranded form which is ideally suited for genetic engineering. The phage DNA is enclosed in a tubular capsid composed predominantly of the pVIII major coat protein with a few copies of minor coat proteins at the ends of the virion (**Figure 1**). In-frame splicing of foreign oligonucleotides into one of the coat protein genes results in the expression of the foreign amino acids as part of the corresponding coat protein, creating a hybrid fusion protein displayed on the surface of the phage particles. Thus, the foreign peptide is firmly linked to the phage genotype and this forms the cornerstone for the creation of phage display libraries. Phage display libraries are obtained by splicing numerous randomized oligonucleotides into individual phages so that each phage displays a unique peptide displayed on the phage surface in multiple copies.

2.1 The major coat protein pVIII – a key building block in phage engineering

The major coat protein pVIII dominates the phage capsid, comprising 87% of its total mass and 98% of its protein content. About 2,700 copies cover the viral DNA, forming a symmetrical repeating pattern. Our research group at first challenged the structural integrity of the major coat protein, which was previously believed to be an extremely conservative domain that does not allow even tiny modifications of its structure [15]. We found, 'to the surprise of most phage biologists' [16], that small peptides added to all pVIII protein domains of the M13 phage did not compromise phage viability. Similar observations were subsequently reported by other laboratories [17-19]. Using pVIII-fusions we obtained the first phage-derived nanomaterial – a polyvalent immunogen containing epitopes of p17 Gag protein of the HIV1 virus [20]. Antibodies obtained from a rabbit immunized

RIGHTSLINK

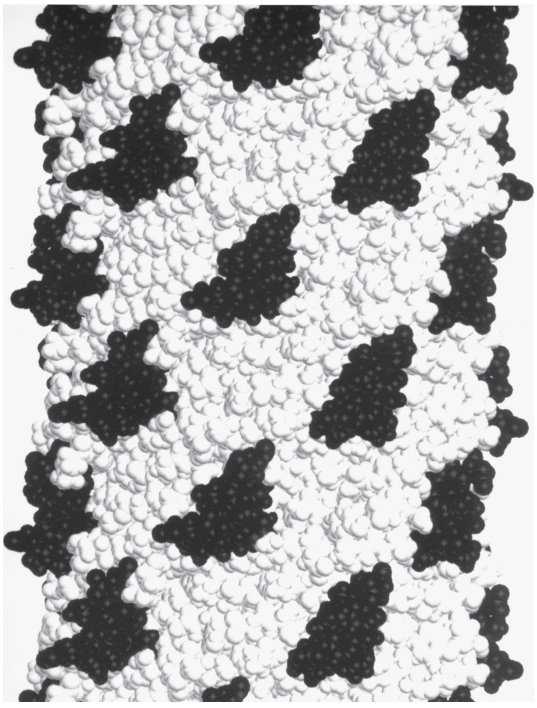

Figure 2. Model of landscape phage. About 1% of the phage length is shown. Foreign peptide inserts are pictured with dark atoms, their specific structural and positional details are entirely speculative but their overall arrangement is presumably accurate. The distance between neighboring peptides is ~ 2.7 nm.
Adapted from Petrenko *et al.* [28].

Expert Opin. Drug Deliv. Downloaded from informahealthcare.com by Hacettepe Univ. on 12/02/12
For personal use only.

with the pVIII-fusion phage reacted on western blots with the immobilized 17-kDa core protein of the HIV1 virus and with its precursor, protein p55. These results allowed us to propose using pVIII-fusion phage in vaccine development, a concept that was later confirmed by other researchers [21-26,27].

Phage with pVIII-fusions were eventually given the name 'landscape phage' to emphasize the dramatic change in surface architecture caused by arraying thousands of copies of the guest peptide in a dense, repeating pattern around the tubular capsid (**Figure 2**). The foreign peptides decorating the phage body create defined organic surface structures (landscapes) that vary from one phage clone to the next. A landscape library is a huge population of such phage, encompassing billions of clones with different surface structures and biophysical properties [28]. The binding peptide, comprising up to 20% of the phage mass and up to 50% of the phage surface, may be easily prepared by precipitation from a culture of infected bacteria and purified, if necessary, by hydroxyapatite chromatography [29].

2.2 Landscape phage as substitute antibodies

Affinity selection is a characteristic aspect of phage display technology that allows obtaining ligands against any receptor, including biopolymers, organic compounds or inorganic materials. Therefore, the landscape phage is a unique microfibrous material that can be selected with desired properties by an affinity-binding protocol [30]. To obtain a specific phage ligand, an immobilized target molecule or a cellular particle, called the 'selector', is exposed to a phage display library (**Figure 3**). Phage particles whose displayed peptides bind the selector are captured, while all other phages are washed away. The captured phage, generally a $1/10^8$ – $1/10^7$ fraction of the initial library population, can then be eluted from the support with mild acid, alkaline or detergent solutions without affecting phage infectivity and propagated by infecting bacterial host cells. A single round of affinity selection is able to enrich for selector-binding clones by many orders of magnitude; a few rounds suffice to survey a library with billions of initial clones for exceedingly rare guest peptides with particularly high affinities for the selector. After several rounds of affinity selection, individual phage clones are propagated and their ability to bind the selector is confirmed. Phages can be selected from landscape phage display libraries with affinities for a wide range of simple targets such as dioxin, cibacron blue, β-galactosidase, streptavidin, neutravidin and fibrinogen [31], as well as for more complex targets such as cancer cells [32], serum antibodies [33], or inorganic materials [34].

3. Phage-derived recognition interfaces

3.1 Phage-driven targeted drug delivery systems

It is commonly accepted that selectivity of drug and gene delivery systems can be increased by their coupling with ligands targeted to differentially expressed cellular receptors. In particular, it has also been demonstrated in experimental and clinical settings that tumor-specific delivery of therapeutics allows optimization of dosages while moderating unwanted side effects [35]. A plethora of ligands including antibodies or their fragments, peptides, growth factors, glycoproteins, carbohydrates or receptor ligands have been proposed for drug targeting [36]. Although antibodies dominate in most drug targeting studies, their clinical use may be limited because of their large size (~ 160 kDa), which precludes efficient penetration, a problem aggravated by elevated tumor interstitial pressure [37]. Furthermore, optimal tumor penetration may be hindered by a high affinity interaction between the antibody and the first few antigen molecules it comes in contact with, thereby preventing uniform diffusion, a phenomenon first described as 'binding site barrier' [38] and later demonstrated conclusively [39]. In addition, immunogenicity and non-specific uptake by the reticulo-endothelial system contribute to less than favorable clinical outcomes. Advances in antibody engineering have partly alleviated these problems by creating more compact functional fragments of target-specific antibodies, such as single chain Fv fragments (~ 25 kDa) [40], VHH domains or nanobodies (~ 15 kDa) [41], which tend to increase tumor penetration and decrease immunogenicity and non-specific

RIGHTSLINK

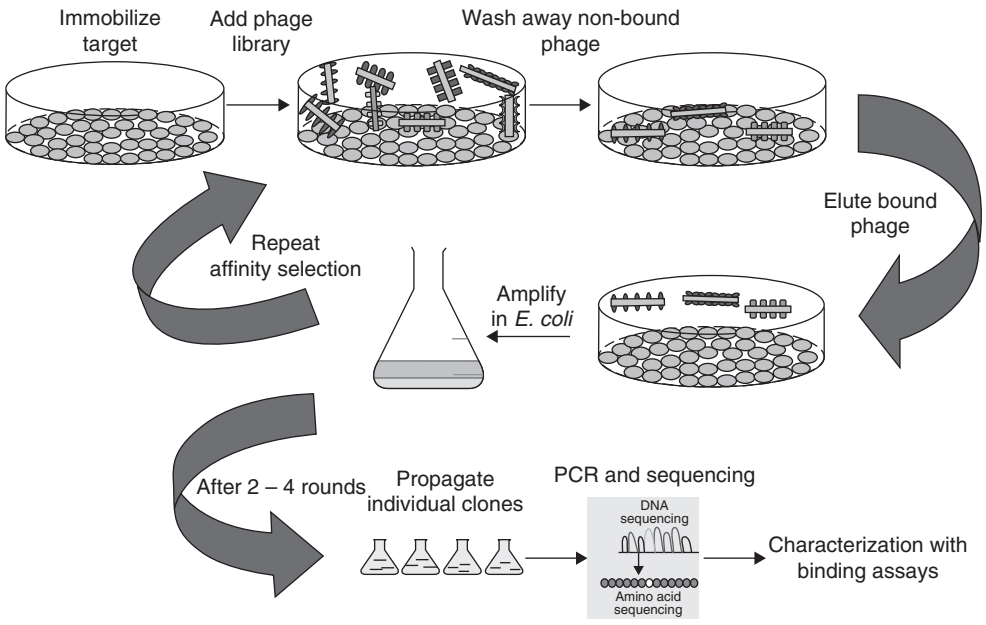

Figure 3. General scheme of affinity selection of target-specific peptides. Described in the text.

Expert Opin. Drug Deliv. Downloaded from informahealthcare.com by Hacettepe Univ. on 12/02/12
For personal use only.

removal. The improved pharmacokinetic properties of smaller molecules justify their preference in tumor-directed therapies [42]. Peptides in the range of 1 – 2 kDa demonstrating acceptable affinity and specificity to their targets represent an attractive alternative to antibodies. Many of the problems related to the use of antibodies appear to be resolved with the use of smaller peptides. They possess impressive tumor penetrating capacity, are generally not recognized by the mononuclear phagocytic system (MPS) and are less likely to instigate immune responses. In addition, they exhibit a higher activity per mass and greater stability [8].

Potential therapeutics can be extracted by affinity selection from combinatorial peptide libraries – an inexhaustible supply of binding peptides [2]. The use of phage displayed peptide libraries for obtaining tumor-specific peptides has been illustrated with several examples in recent reviews [43-45]. Tumor targeting by drugs conjugated to phage-derived peptides has demonstrated the power and promise this approach holds. For example, tumor blood vessel-specific peptides conjugated with doxorubicin showed targeting to the tumor vasculature *in vivo*. This regimen, in addition to enhanced drug efficacy, also lowered drug toxicity [46]. Furthermore, the coupling of a pro-apoptotic domain to the peptides that home to human breast cancer cells or human prostate cancer cells could specifically destroy the respective human tumor xenografts in mice [47].

A critical factor within this scenario is that peptides identified by the phage display technique, and conjugated with chemotherapeutics, can be hindered in their anticancer propensity. Furthermore, the stoichiometric ratio of a drug and a targeting ligand may not be optimal for creating a critical cytotoxic concentration of the drug within the target cells. This problem can be solved by the conjugation of the targeting peptide with a drug-encapsulating nanoparticle. Numerous covalent coupling techniques, including the formation of a disulfide bond, crosslinking between primary amines, reactions between a carboxylic acid and primary amine, between maleimide and thiol, between hydrazide and aldehyde, or between a primary amine and free aldehyde can be used for peptide conjugation [48]. These procedures are quite efficient for the preparation of various targeted liposomes on a small scale – for their preliminary laboratory and clinical studies. However, the cost and reproducibility of these derivatives in quality and a quantity sufficient for pharmaceutical applications are challenging problems. Therefore, the use of peptide and protein conjugates may be less efficient as reactions are scaled up, where more standardized and pharmaceutically acceptable preparations are required [49,50]. In addition, preparative conditions for the addressed vesicles of different formulations differ markedly from one targeted particle to another.

These considerations led us to evaluate the potential of intact phage fusion coat proteins, isolated in biopanning experiments, as easily available targeting components of drug preparations [51,52]. In this approach, the phage specific for the target organ, tissue or cell is selected from the multibillion-clone landscape phage libraries *in vitro* or *in vivo*, according to established procedures [30,53-55], and is converted to liposomes or micelles exploiting the intrinsic 'membranophilic' properties of phage proteins. As a result, the targeting probe – the tumor-specific peptide fused to the major coat protein – is displayed on the shell surface of the drug-loaded vesicle as illustrated in **Figure 4**. Using the assembly of the phage proteins with lipid membranes, we

RIGHTSLINK

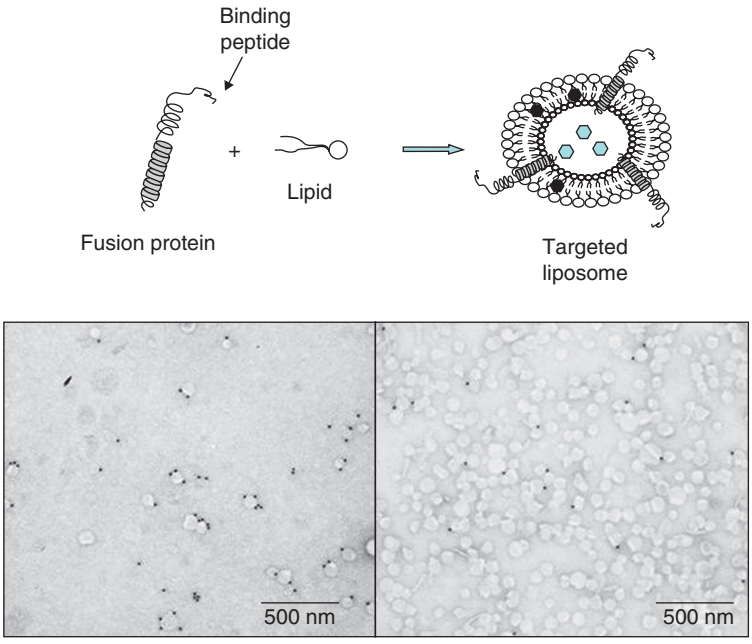

Figure 4. Liposomes targeted by the pVIII protein. Top: The scheme of liposome targeting with phage proteins. The hydrophobic helix of the pVIII spans the lipid layer and binding peptide is displayed on the surface of the carrier particles. Drug molecules are shown as hexagons. Bottom: The specificity and functionality of liposome-incorporated phage coat proteins. Targeted and non-targeted liposomes loaded onto E/M grids were incubated with streptavidin-conjugated gold particles and analyzed by transmission electron microscopy. The left and right panels show correspondingly targeted and non-targeted liposomes treated with gold particles. The ratio of gold beads bound to targeted liposomes is 1.8 versus 0.09 in control.
Adapted from Jayanna *et al.* [52].

Expert Opin. Drug Deliv. Downloaded from informahealthcare.com by Hacettepe Univ. on 12/02/12
For personal use only.

incorporated a model streptavidin-targeted protein into ~ 80 nm liposomes. The streptavidin-binding phage was affinity selected from the landscape phage library [28,31], stripped and incubated with liposomes. As a result of fusion with the targeted phage proteins, the liposome acquired a new emergent property – the ability to bind streptavidin and streptavidin-conjugated fluorescent molecules, as was evidenced by protein microarray, fluorescent microscopy and fluorescence-activated cell sorting (FACS). The complex of the targeted liposomes with streptavidin-coated gold beads was visualized by transmission electron microscopy (**Figure 4**).

In contrast to sophisticated and poorly controllable conjugation procedures used for the coupling of peptides and antibodies to targeted vesicles, the phage-based approach relies on very powerful and extremely precise mechanisms of selection, biosynthesis and self-assembly. When landscape phage serve as a reservoir of the targeted membrane proteins, one of the most troublesome steps of the conjugation technology is bypassed. Furthermore, it does not require idiosyncratic reactions specific for each new shell-decorating polymer or targeting ligand and may be easily adapted to a new nanoparticulate composition and a new addressed target. No reengineering of the selected phage is required at all: the phage themselves serve as the source of the final product–coat protein genetically fused to the targeting peptide. The major coat protein constitutes 98% of the total protein mass of the virion – a level of purity hardly attainable in normal synthetic or bioengineering procedures. A culture of phage-secreting cells is an efficient, convenient and discontinuous protein production system. The yield of phage particles reaches 300 mg/l (20 mg/l for the engineered landscape phage described here) and they are secreted from the cell nearly free of intracellular components. Purification of the phage is easily accomplished by simple, routine steps that do not differ from one clone to another. As normal intestinal parasites, phage and their components are not toxic and have already been tested for safety in preclinical and clinical trials [54,56]. In contrast to immunization procedures, phage selection protocols frequently require tiny amounts of target material for obtaining the tumor-specific phage ligands (e.g., thousands of tumor cells available from biopsy procedures [55]). The affinity and selectivity of phage-derived probes can be improved, if necessary, by exploring well-developed depletion and affinity maturation procedures.

3.2 Phage-targeted gene delivery

The demonstrated ability of targeted fusion filamentous phage to penetrate into mammalian cells, survive inside cellular compartments and express transgenes in target cells has aroused considerable interest in them as gene delivery systems [57-62]. Indeed, filamentous phage could be exemplary vectors for delivery of foreign genes in mammalian cells.

RIGHTSLINK

Expert Opin. Drug Deliv. Downloaded from informahealthcare.com by Hacettepe Univ. on 12/02/12
For personal use only.

Phage nanoparticles can be genetically modified through rational or combinatorial design to target them to distinct cells in the body and to tune up their behavior in biological fluids and inside target cells by harnessing the power of biological selection. Furthermore, unlike animal viruses that have broad natural tropisms, phage have no known natural receptors on mammalian cells [63]. Receptor-mediated internalization of phage vectors by mammalian cells can occur if they display foreign cell-specific ligands, such as fibroblast growth factor (FGF2), anti-ErbB2 scFv F5 antibody or integrin-binding peptides [58,60,61,64,65]. Phage-derived delivery vehicles are not toxic to mammals and can be manufactured using very simple and safe technologies. Bacteriophage have a long history of practical use as antibacterial therapeutics in humans [63] and some have been approved recently by the FDA as antibacterial food additives [66,67]. Furthermore, clinical trials have shown that serial library administration in cancer patients during the screening of phage libraries for cancer-specific ligands can be accomplished without major untoward clinical side effects [54].

Tumor-specific pVIII- and pIII-fusion phage can be easily transformed into a gene delivery system–phagemid infective particles (PIPs) encapsulated within bacteria by phage-encoded peptides [68-71]. These particles are considerably smaller than normal phage (~ 700 nm vs ~ 1300 nm but have all the elements necessary for delivery and expression of genes in mammalian cells. This approach, shown first for glioma cells [68], differs from others in that a phagemid expressing a model marker or particular therapeutic gene can be easily exchanged for a phagemid expressing a different therapeutic or reporter gene. In addition, a different helper phage selected from a phage display library can target any cell type and direct the encapsulation of any suitable phagemid. Because of its versatility, the PIP system may be readily used for optimization of the gene delivery strategies applied to specific cell and tissue targets. An as yet unsolved problem in using phage-derived gene delivery vectors is their low transformation efficiency. A new approach, demonstrated by Hajitou *et al.* [72], allows dramatic improvements in post-targeting expression of filamentous phage-borne transgenes by genetic hybridization of the phage vector with compatible *cis*-elements (such as inverted terminal repeats (ITRs)) from adeno-associated virus (AAV) – a mammalian single-stranded DNA virus. The hybrid vector, termed AAVP, targeted to αv integrins by pIII-fused RGD-4C peptide, exhibited much higher mammalian transduction efficiency over corresponding phage-based vectors, probably because of better persistence of episomal DNA in the mammalian cell and the multimerization of the entire transgene cassette during hybrid DNA maintenance [72]. In particular, when mammalian cell cultures were incubated with targeted AAVP for 4 h in serum-free medium at a ratio of at least 10^6 TU per cell, transgene expression was observed after 48 – 72 h and reached a maximum level approaching 10 – 20% of transduced cells by one week. The AAVP particles have been shown to serve as molecular imaging probes *in vivo*, allowing monitoring and prediction of drug response in a nude rat model of human sarcoma [73]. These recent breakthrough advances in the development of targeted phage-derived delivery systems demonstrate their enormous potential for systemic targeted delivery of therapeutic genes and molecular imaging reporter transgenes in solid tumors of cancer patients.

3.3 Phage probes for molecular imaging

Modern clinical cancer treatments require not only precise positional information on tumor location, size and possible spread to lymph nodes and organs, which can be obtained using traditional anatomical imaging methods such as computed X-ray tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET) and ultrasound (US), but also need a more detailed molecular characterization of tumors to help optimize the treatment regimen for individual patients [74]. Molecular imaging would allow clinicians to visualize the expression and activity of specific molecules (e.g., proteases and protein kinases) and biological processes (e.g., apoptosis, angiogenesis and metastasis) that influence tumor behavior and/or response to therapy [75]. This information is expected to have a major impact on cancer detection and individualized treatment [76]. A general approach to improving the accumulation of the imaging agents at the target site is to conjugate the imaging label to a ligand that binds to a specific molecular target (active probes). The probes bind the targets and are retained at the target site, while unbound probes are cleared from circulation. This approach is most useful for tumor imaging, as cancer cells often overexpress certain surface receptors.

Some of the newest challenging goals of molecular imaging lie in the competency of phage display methods, which can promote discovery, validation and visualization of molecular markers in a disease-specific way. It was demonstrated recently that *in vivo* phage selection allows the obtaining of disease-specific and organ-specific phage clones [54,77-80], which can serve successfully as molecular recognition interface for non-invasive *in vivo* imaging agents [81-85]. Phage and other viruses can function as imaging agents in four different formats: i) as a scaffold for chemical attachment of targeting peptides and imaging labels [13]; ii) as a preselected *in vitro* or *in vivo* phage probe genetically linked with multiple copies of cancer-specific peptides and conjugated with imaging labels [83]; iii) as a preselected biotinylated anticancer probe, which can be used for pretargeting and subsequent imaging of the phage-bound cells with streptavidin conjugated to a radioactive label [85]; and iv) as a gene-delivery vector with a reporter transgene [72,73].

The performance of phage-derived imaging probes was demonstrated using different model systems. For example, Newton *et al.* selected *in vivo* phage that bind human PC-3 prostate carcinoma xenografts in mice and showed that fluorescently labeled phage itself can serve as a replenishable

RIGHTSLINK

Expert Opin. Drug Deliv. Downloaded from informahealthcare.com by Hacettepe Univ. on 12/02/12
For personal use only.

molecular imaging agent [84]. The identified phage, labeled with the near-infrared AlexFluor 680, retained target specificity on labeling and demonstrated *in vivo* a high tumor-to-muscle fluorescence ratio. Using other models – nude mice with Lewis lung carcinoma cell xenografts expressing Secreted Protein Acidic and Rich in Cysteine (SPARC) – Kelly *et al.* showed that distribution of the fluorescently labeled SPARC-specific phage can be quantified *in vivo* starting from thresholds of ~ 300 phage/mm^3 of tissue throughout the entire depth of the tumor using fluorescent tomographic imaging [83]. Thus, fluorescently labeled phage particles can be remarkably efficient as *in vivo* imaging agents, akin to peptide-decorated nanoparticles. Another phage-derived contrast reagent, called 'magnetophage', has been obtained by Segers *et al.* by covalently coupling ultra-small particles of iron oxide (USPIO) and their PEGylated forms to phage capsids [86]. Magnetophages specific for phosphatidylserine were tested as a potential magnetic resonance imaging agent (MRI) in a model system of apoptotic and control cells in which magnetophages allowed the discrimination of phosphatidylserine from phosphatidylcholine and of apoptotic cells from normal control cells. Although non-protected magnetophages were rapidly cleared from the bloodstream *in vivo* and internalized by the phagocytic cells of the liver, the stealthy PEG-magnetophages, which are largely invisible to phagocytic cells, were successfully targeted to apoptotic cells of the liver.

Another approach to molecular imaging, through the convergence of ligand-directed targeting and a reporter transgene delivery, was suggested recently by Hajitou *et al.* [73]. A hybrid vector that incorporates genomic *cis*-elements of adeno-associated virus (AAV) and filamentous phage (AAVP) (described in the previous subsection), and targeted to integrins through an incorporated RGD peptide, was studied as an imaging tool to monitor and predict drug responses in a rat model bearing human soft-tissue sarcoma xenografts. It was shown that AAVP allows ligand-directed targeting of human sarcoma xenografts in a preclinical setting and provides molecular imaging of reporter genes. The technique was suitable for serial non-invasive monitoring of soft-tissue sarcoma and enabled response prediction to a model cytotoxic agent. The authors believed that this potential strategy would be successfully translated to human sarcoma patients. All of these results provide proof-of-concept of phage nanoparticles as bioselective molecular imaging agents and hold great promise for their future use in clinical applications.

3.4 Phage-driven biosensors

Prompt monitoring of physiological liquids is crucial for early diagnosis, prevention and treatment of most human and animal diseases and is of critical significance for the development of personalized medicine. Miniature wireless biosensors interfaced with bioselective bacteriophage hold promise as an innovative approach to this challenging problem. Sensors are analytical electronic devices that transduce a molecular recognition event into a measurable signal. Their principal components are a sensing interface that interacts with an analyte and a signal processor that transduces the binding impulse into an observable signal. When a sensor interface is composed of biological entities, or when a sensor is designed to detect a biological agent, they are generically called 'biomolecular sensors' or 'biosensors'. The desired characteristics of biosensors – sensitivity, selectivity, robustness and prompt performance – are determined mostly by the nature of the biorecognition interface. The most commonly used recognition elements in biosensors are antibodies, although a variety of other bio-organic molecules have also been effectively used as interfaces in biosensors, for example peptides, enzymes, lectins, carbohydrates, nucleic acids, aptamers, recombinant proteins or molecularly imprinted polymers. In a more recent detection format, whole cells were explored as binding entities. None of these types of recognition interfaces, however, could meet the sensor performance requirements completely.

Recombinant phage with multiple fusion bioselective peptides provide another source of high quality detection reagents in the arsenal of biomedical analytical methods [30]. It was demonstrated that phage display libraries contain many potential probes for various types of biomolecules, including surface markers of cells and blood components [30]. Experiments with different antigens have shown that phage may be used as a new type of substitute antibodies – filaments that can bind protein and glycoprotein antigens with nanomolar affinities and high specificity [87]. The performance of the probes in the detection of biological agents was demonstrated using a quartz crystal microbalance (QCM) [88,89], electrochemical sensors [90], wireless magnetoelastic biosensors and microcantilevers [91-93], and PSR sensors [94] in which phage immobilized on a gold electrode reacted with their analytes in solution-phase [95]. Phage allow different means of immobilization onto the sensor surfaces: by attachment of a biotinylated or stripped phage to the Langmuir-Blodgett (LB) phospholipid [88,96], by chemical immobilization [89] or by direct physical adsorption of phage to the sensor surface [91,94,97-100]. The phage-loaded sensors demonstrated specific dose-dependent binding of β-galactosidase from *E. coli* [88,94,98], antibodies [89], spores and bacteria [91,93,97,99,100]. For example, a miniature (500 × 100 × 5 μm) wireless magnetoelastic biosensor interfaced with phage specific for *B. anthracis* spores, or *S. typhimurium* bacteria, demonstrated a linear dose-response with the detection limit on the order of 10^3 cell/ml [91,93,99]. Binding of the analytes to the sensors was confirmed by scanning electron microscopy (SEM) (**Figure 5**).

Medical, biological and environmental monitoring require advanced biosensors with robust biorecognition probes that can operate in non-controlled conditions and can be readily reused after cleaning [101]. These requirements can be

RIGHTSLINK

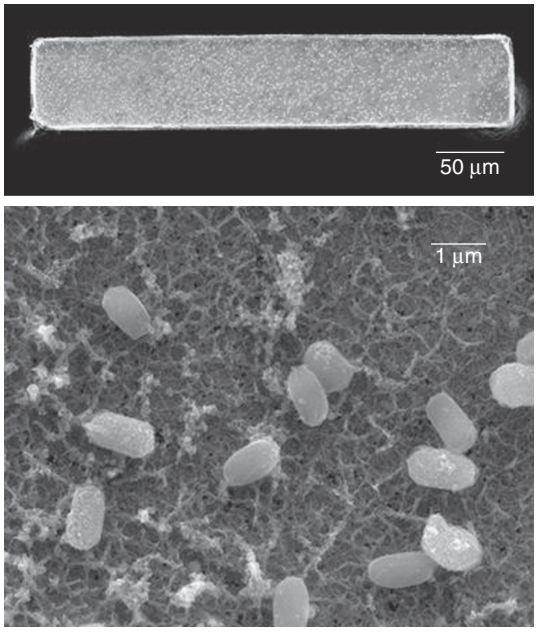

Figure 5. Scanning electron microscopy micrographs of a gold-coated, 500 × 100 × 5 μm, biosensor after loading with phage and spores. The network of the gold-immobilized phage interacting with spores can be seen in the bottom image.
Adapted from Johnson ML, Wan J, Huang S, et al. A wireless biosensor using microfabricated phage-interfaced magnetoelastic particles. Sensors Actuators A Phys 2008 ;144 (1): 38-47 [93]. Copyright (2008), with permission from Elsevier.

satisfied by using phage interfaces, which are much more robust than traditional antibodies. For example, when thermostability of a landscape phage and a monoclonal antibody (mAb), specific for *E. coli* β-galactosidase were examined in parallel, they were both stable for 6 months at room temperature, but at higher temperatures the mAb degraded more rapidly than the phage probe [102]. At 37°C, phage degraded only slightly (half-life 950 days), while mAb lost virtually all activity over 30 weeks (half-life 107 days). At 50°C mAb activity was undetectable after 5 weeks while phage retained more than 50% of its activity. At 63°C mAb completely inactivated after 24 h while phage maintained detectable activity for 6 weeks. Phage retained binding activity even after short incubations at 76°C (half-life 2.4 days).

Similarly, phage-coated magnetoelastic biosensors preserved about 49, 40, and 25% of their binding activity after three months of storage at 25, 45 and 65°C, respectively [100]. In comparison, the antibody-coated sensors showed no binding affinity after only five days at 65 and 45°C. In another experiment, the biosensor with immobilized phage was shown to retain complete activity after multiple acid treatments [89]. These results clearly demonstrate that the filamentous fusion phage can serve as a source of highly selective and robust molecular recognition interfaces in biosensors and can operate in non-controlled conditions even after exposure to chemical stresses or high temperatures.

4. Conclusion

Genetically driven phage nanobiotechnology has allowed the development of libraries of diverse nanostructures expressed on the phage surface providing a rich resource of diagnostic, detection and pharmaceutical probes. Phage engineering, which is based on natural mechanisms of selection, amplification and self-assembly, allows directed nano-fabrication of bioselective materials with possible applications in gene/drug-delivery, biosensors, nanoelectronics, biosorbents and other areas of medicine, technology and environmental monitoring. In particular, landscape phage expressing tumor-specific peptides fused to all copies of the major coat protein pVIII can be converted easily into gene-encapsulating particles or drug-loaded vesicles that acquire the ability to recognize the same receptors, cells, tissues and organs that have been used for selection of the precisely targeted phage. A culture of phage-secreting cells is an efficient, convenient and discontinuous production system. Phage particles are secreted from the cell nearly free of intracellular components, and their further purification can be easily accomplished by simple steps that do not differ from one clone to another. The major fusion coat protein constitutes 98% of the total protein mass of the virion – a purity hardly attainable in normal synthetic and bioengineering procedures. As a normal intestinal parasite, phage and its components are not toxic and have already been tested for safety in preclinical and clinical trials. In contrast to immunization procedures, phage selection does not suffer from the problem of tolerance, which can hamper obtaining antibodies to 'self' antigens, and frequently require tiny amounts of target material. All these unique characteristics of phage commend it very well as a very promising nanomaterial for a variety of medical and technical applications.

5. Expert opinion

The more than 20-year evolution of phage display has dramatically affected the potential of this technique, amid other bioengineering methods. First, due to the commercial success of the phage display libraries, phage has been brought to the bench of many researchers working in very different and diverse disciplines. Second, the marriage of combinatorial chemistry and biological selection has been very powerful in changing the methodology of biochemical research by allowing selection simultaneously among billions of genetic species in one test tube. Third, and most important for our analysis, phage display evolved into the discipline of material science, presenting phage not only as an instrument for peptide and antibody discovery, but also as a prospective nanomaterial that can be easily tailored using routine genetic engineering manipulations. This merge of phage display technologies with nanotechnology over recent years is very promising and has already shown its vitality and productivity in contributing vigorously to different areas of medicine and

Expert Opin. Drug Deliv. Downloaded from informahealthcare.com by Hacettepe Univ. on 12/02/12
For personal use only.

RIGHTSLINK

technology, such as medical diagnostics and monitoring, molecular imaging, targeted drug and gene delivery, vaccine development, as well as bone and tissue repair. Further development of applications of phage nanotechnology in these areas will require the collective efforts of specialists in many fields – medical doctors, microbiologists, structural biologists, chemists, pharmacists and many others. Although very encouraging preliminary results have been obtained that demonstrated efficient targeting and safety of phage-derived preparations for patients, more efforts are required to monitor their behavior in the organism. Thus, phage nanomedicine is likely to grow in the near future into a discipline with its own methods and strategies. One of the first challenging goals of phage nanomedicine may be the development of non-immunogenic and stealthy phage with an increased lifetime in the patient's body. Technical applications of phage will require expanding the available assortment of phage 'on display' that are presently limited to a few representatives with quite monotonous structures and shapes, and will lead to discovery of new phage variants with unexpected chemical and physical properties. Harnessing the power of natural phage diversity in combination with phage tailoring will be a challenging goal of phage nanobiotechnology in the near future.

Declaration of interest

The author states no conflict of interest and has received no payment in the preparation of this manuscript.

Expert Opin. Drug Deliv. Downloaded from informahealthcare.com by Hacettepe Univ. on 12/02/12
For personal use only.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Smith GP. 1988 – A year of discovery. In: Kay BK, Winter J, McCafferty, editors, Phage display of peptides and proteins. San Diego, London, Boston, New York, Sydney, Tokyo, Toronto: Academic Press; 1996. p. xvii-xix
2. Smith GP, Petrenko VA. Phage display. Chem Rev 1997;97(2):391-410

•• **A comprehensive review of phage display technique.**

3. Geysen HM, Meloen RH, Barteling SJ. Use of peptide synthesis to probe viral antigens for epitopes to a resolution of a single amino acid. Proc Natl Acad Sci USA 1984;81(13):3998-4002

• **This is the first study of multiple peptides in one combinatorial set-up.**

4. Houghten RA. General method for the rapid solid-phase synthesis of large numbers of peptides: specificity of antigen–antibody interaction at the level of individual amino acids. Proc Natl Acad Sci USA 1985;82(15):5131-5

• **This was the first high throughput synthetic method that allowed the study of groups of peptides in one experiment.**

5. Houghten RA. Combinatorial libraries. Finding the needle in the haystack. Curr Biol 1994;4(6):564-7
6. Petrenko VA, Smith GP. Vectors and modes of display. In: Sidhu SS, editor, Phage display in biotechnology and drug discovery. Bo Raton, FL, USA: CRC Press, Taylor & Francis Group; 2005. p. 714
7. Lowman HB. Bacteriophage display and discovery of peptide leads for drug development. Ann Rev Biophys Biomol Struct 1997;26:401-24
8. Ladner RC, Sato AK, Gorzelany J, de Souza M. Phage display-derived peptides as therapeutic alternatives to antibodies. Drug Discov Today 2004;9(12):525-9
9. Bradbury ARM, Marks JD. Phage antibody libraries. In: Clackson T, Lowman HB, editors, Phage display a practical approach. Oxford University Press; 2004
10. Suci PA, Berglund DL, Liepold L, et al. High-density targeting of a viral multifunctional nanoplatform to a pathogenic, biofilm-forming bacterium. Chem Biol 2007;14(4):387-98
11. Brumfield S, Willits D, Tang L, et al. Heterologous expression of the modified coat protein of Cowpea chlorotic mottle bromovirus results in the assembly of protein cages with altered architectures and function. J Gen Virol 2004;85(Pt 4):1049-53
12. Klem MT, Willits D, Young M, Douglas T. 2-D array formation of genetically engineered viral cages on au surfaces and imaging by atomic force microscopy. J Am Chem Soc 2003;125(36):10806-7
13. Manchester M, Singh P. Virus-based nanoparticles (VNPs): platform technologies for diagnostic imaging. Adv Drug Deliv Rev 2006;58(14):1505-22
14. Singh P, MJ G, Manchester M. Viruses and their uses in nanotechnology. Drug Dev Res 2006;67:23-41
15. Ilyichev AA, Minenkova OO, Tatkov SI, et al. Construction of M13 viable bacteriophage with the insert of foreign peptides into the major coat protein. Doklady Biochemistry (ProcAcad Sci USSR)-EnglTr 1989;307:196-8

• **This is the first study demonstrating multivalent phage display of foreign peptides fused to the major coat protein pVIII.**

16. Russel M, Model P. Filamentous phage. In: Calendar R, editor, The bacteriophages. 2nd edition. Oxford University Press; 2006. p. 146-60
17. Greenwood J, Willis AE, Perham RN. Multiple display of foreign peptides on a filamentous bacteriophage. Peptides from Plasmodium falciparum circumsporozoite protein as antigens. J Mol Biol 1991;220(4):821-7
18. Felici F, Castagnoli L, Musacchio A, et al. Selection of antibody ligands from a large library of oligopeptides expressed on a multivalent exposition vector. J Mol Biol 1991;222(2):301-10
19. Markland W, Roberts BL, Saxena MJ, et al. Design, construction and function of a multicopy display vector using fusions to the major coat protein of bacteriophage M13. Gene 1991;109(1):13-9
20. Minenkova OO, Ilyichev AA, Kishchenko GP, Petrenko VA. Design of specific immunogens using filamentous phage as the carrier. Gene 1993;128(1):85-8

• **This was the first study of phage as an artificial specific immunogen and a potential vaccine.**

21. Fang J, Wang G, Yang Q, et al. The potential of phage display virions expressing malignant tumor specific antigen

RIGHTSLINK

Expert Opin. Drug Deliv. Downloaded from informahealthcare.com by Hacettepe Univ. on 12/02/12
For personal use only.

MAGE-A1 epitope in murine model. Vaccine 2005;23(40):4860-6

22. Wang G, Sun M, Fang J, et al. Protective immune responses against systemic candidiasis mediated by phage-displayed specific epitope of Candida albicans heat shock protein 90 in C57BL/6J mice. Vaccine 2006;24(35-36):6065-73
23. Yang Q, Su QP, Wang GY, et al. Production of hybrid phage displaying secreted aspartyl proteinase epitope of Candida albicans and its application for the diagnosis of disseminated candidiasis. Mycoses 2007;50(3):165-71
24. Wu Y, Wan Y, Bian J, et al. Phage display particles expressing tumor-specific antigens induce preventive and therapeutic anti-tumor immunity in murine p815 model. Int J Cancer 2002;98(5):748-53
25. Wan Y, Wu Y, Bian J, et al. Induction of hepatitis B virus-specific cytotoxic T lymphocytes response in vivo by filamentous phage display vaccine. Vaccine 2001;19(20-22):2918-23
26. Guardiola J, De Berardinis P, Sartorius R, et al. Phage display of epitopes from HIV-1 elicits strong cytolytic responses in vitro and in vivo. Adv Exp Med Biol 2001;495:291-8
27. Manoutcharian K, Diaz-Orea A, Gevorkian G, et al. Recombinant bacteriophage-based multiepitope vaccine against Taenia solium pig cysticercosis. Vet Immunol Immunopathol 2004;99(1-2):11-24
28. Petrenko VA, Smith GP, Gong X, Quinn T. A library of organic landscapes on filamentous phage. Protein Eng 1996;9(9):797-801

• **This paper describes the first phage display library with multivalently displayed random peptides fused to all copies of the major coat protein pVIII.**

29. Smith GP, Gingrich TR. Hydroxyapatite chromatography of phage-display virions. Biotechniques 2005;39(6):879-84
30. Brigati JR, Samoylova TI, Jayanna PK, Petrenko VA. Phage display technique for generating peptide reagents. In: Dunn BM, et al, editors. Current Protocols in Protein Science. John Wiley & Sons; 2008. Chapter 18:Unit 18.9
31. Petrenko VA, Smith GP. Phages from landscape libraries as substitute antibodies. Protein Eng 2000;13(8):589-92
32. Samoylova TI, Petrenko VA, Morrison NE, et al. Phage probes for malignant glial cells. Mol Cancer Ther 2003;2(11):1129-37
33. Kouzmitcheva GA, Petrenko VA, Smith GP. Identifying diagnostic peptides for lyme disease through epitope discovery. Clin Diagn Lab Immunol 2001;8(1):150-60
34. Lee SK, Yun DS, Belcher AM. Cobalt ion mediated self-assembly of genetically engineered bacteriophage for biomimetic Co-Pt hybrid material. Biomacromolecules 2006;7(1):14-7
35. Torchilin VP. Targeted pharmaceutical nanocarriers for cancer therapy and imaging. AAPS J 2007;9(2):E128-47
36. Immordino ML, Dosio F, Cattel L. Stealth liposomes: review of the basic science, rationale, and clinical applications, existing and potential. Int J Nanomed 2006;1(3):297-315
37. Jain RK. Physiological barriers to delivery of monoclonal antibodies and other macromolecules in tumors. Cancer Res 1990;50(3 Suppl):814-9
38. Fujimori K, Covell DG, Fletcher JE, Weinstein JN. Modeling analysis of the global and microscopic distribution of immunoglobulin G, F(ab′)2, and Fab in tumors. Cancer Res 1989;49(20):5656-63
39. Adams GP, Schier R, McCall AM, et al. High affinity restricts the localization and tumor penetration of single-chain Fv antibody molecules. Cancer Res 2001;61(12):4750-5
40. Adams GP, Schier R. Generating improved single-chain Fv molecules for tumor targeting. J Immunol Methods 1999;231(1-2):249-60
41. Cortez-Retamozo V, Backmann N, Senter PD, et al. Efficient cancer therapy with a nanobody-based conjugate. Cancer Res 2004;64(8):2853-7
42. Reilly RM, Sandhu J, Alvarez-Diez TM, et al. Problems of delivery of monoclonal antibodies. Pharmaceutical and pharmacokinetic solutions. Clin Pharmacokinet 1995;28(2):126-42
43. Craig R, Li S. Function and molecular mechanism of tumor-targeted peptides for delivering therapeutic genes and chemical drugs. Mini Rev Med Chem 2006;6:757-64
44. Krumpe L, Mori T. The use of phage-displayed peptide libraries to develop tumor-targeting drugs. Int J Peptide Res Ther 2006;12:79-91
45. Rezler EM, Khan DR, Tu R, et al. Peptide-mediated targeting of liposomes to tumor cells. Methods Mol Biol 2007;386:269-98
46. Arap W, Pasqualini R, Ruoslahti E. Cancer treatment by targeted drug delivery to tumor vasculature in a mouse model. Science 1998;279(5349):377-80

• **This was the first use of phage display technique for selective targeting of tumor cells *in vivo*.**

47. Arap W, Haedicke W, Bernasconi M, et al. Targeting the prostate for destruction through a vascular address. Proc Natl Acad Sci USA 2002;99(3):1527-31
48. Nobs L, Buchegger F, Gurny R, Allemann E. Coupling methods to obtain ligand-targeted liposomes and nanoparticles. Drugs Pharm Sci 2006;158:123-48
49. Nellis DF, Ekstrom DL, Kirpotin DB, et al. Preclinical manufacture of an anti-HER2 scFv-PEG-DSPE, liposome-inserting conjugate. 1. Gram-scale production and purification. Biotechnol Prog 2005;21(1):205-20
50. Nellis DF, Kirpotin DB, Janini GM, et al. Preclinical manufacture of anti-her2 liposome-inserting, scFv-PEG-lipid conjugate. 2. Conjugate micelle identity, purity, stability, and potency analysis. Biotechnol Prog 2005;21(1):221-32
51. Petrenko VA. Phage-derived bioselective nanovehicles for drug and gene delivery. Nanotechnology Conference and Trade Show. Santa Clara; 2007. p. 703-6
52. Jayanna PK, Torchilin VP, Petrenko VA. Liposomes targeted by fusion phage proteins [submitted]. Nanomedicine
53. Shukla GS, Krag DN. Phage display selection for cell-specific ligands: Development of a screening procedure suitable for small tumor specimens. J Drug Target 2005;13(1):7-18
54. Krag DN, Shukla GS, Shen G-P, et al. Selection of tumor-binding ligands in cancer patients with phage display libraries. Cancer Res 2006;66(15):7724-33

• **This is the first study of safety and specific targeting activity of phage display libraries in human tumor patients.**

55. Shukla GS, Krag DN. Selection of tumor-targeting agents on freshly excised

RIGHTSLINK

Expert Opin. Drug Deliv. Downloaded from informahealthcare.com by Hacettepe Univ. on 12/02/12
For personal use only.

human breast tumors using a phage display library. Oncol Rep 2005;13(4):757-64

56. Krag DN, Fuller SP, Oligino L, et al. Phage-displayed random peptide libraries in mice: toxicity after serial panning. Cancer Chemother Pharmacol 2002;50(4):325-32

57. Larocca D, Witte A, Johnson W, et al. Targeting bacteriophage to mammalian cell surface receptors for gene delivery. Hum Gene Ther 1998;9(16):2393-9

• **This is the first proof-of-concept experiment demonstrating that filamentous bacteriophage can serve as a vehicle for targeted gene delivery to mammalian cells.**

58. Poul MA, Marks JD. Targeted gene delivery to mammalian cells by filamentous bacteriophage. J Mol Biol 1999;288(2):203-11

59. Kassner PD, Burg MA, Baird A, Larocca D. Genetic selection of phage engineered for receptor-mediated gene transfer to mammalian cells. Biochem Biophys Res Commun 1999;264(3):921-8

60. Ivanenkov VV, Felici F, Menon AG. Targeted delivery of multivalent phage display vectors into mammalian cells. Biochim Biophys Acta 1999;1448(3):463-72

61. Larocca D, Kassner PD, Witte A, et al. Gene transfer to mammalian cells using genetically targeted filamentous bacteriophage. FASEB J 1999;13(6):727-34

62. Burg M, Ravey EP, Gonzales M, et al. Selection of internalizing ligand-display phage using rolling circle amplification for phage recovery. DNA Cell Biol 2004;23(7):457-62

63. Barrow PA, Soothill JS. Bacteriophage therapy and prophylaxis: rediscovery and renewed assessment of potential. Trends Microbiol 1997;5(7):268-71

64. Monaci P, Urbanelli L, Fontana L. Phage as gene delivery vectors. Curr Opin Mol Ther 2001;3(2):159-69

65. Sergeeva A, Kolonin MG, Molldrem JJ, et al. Display technologies: application for the discovery of drug and gene delivery agents. Adv Drug Deliv Rev 2006;58(15):1622-54

66. Daniells S. FDA approves viruses as food additive for meats. FOOD USA. Navigatorcom, 23-Aug-2006. Available from: http://foodproductiondaily.com/news/ng.asp?id=70066.

67. Ricks D. Spray to quell E. coli. AM New York; 2007

68. Mount JD, Samoylova TI, Morrison NE, et al. Cell targeted phagemid rescued by preselected landscape phage. Gene 2004;341:59-65

69. Jiang H, Cai XM, Shi BZ, et al. Development of efficient RNA interference system using EGF-displaying phagemid particles. Acta Pharmacol Sin 2008;29(4):437-42

70. Li Z, Jiang H, Zhang J, Gu J. Cell-targeted phagemid particles preparation using Escherichia coli bearing ligand-pIII encoding helper phage genome. Biotechniques 2006;41(6):706-7

71. Li Z, Zhang J, Zhao R, et al. Preparation of peptide-targeted phagemid particles using a protein III-modified helper phage. Biotechniques 2005;39(4):493-97

72. Hajitou A, Trepel M, Lilley CE, et al. A hybrid vector for ligand-directed tumor targeting and molecular imaging. Cell 2006;125(2):385-98

73. Hajitou A, Lev DC, Hannay JA, et al. A preclinical model for predicting drug response in soft-tissue sarcoma with targeted AAVP molecular imaging. Proc Natl Acad Sci USA 2008;105(11):4471-6

74. Contag CH. In vivo pathology: seeing with molecular specificity and cellular resolution in the living body. Ann Rev Pathol 2007;2:277-305

75. Stephen RM, Gillies RJ. Promise and progress for functional and molecular imaging of response to targeted therapies. Pharm Res 2007;24(6):1172-85

76. Weissleder R. Molecular imaging in cancer. Science 2006;312(5777):1168-71

77. Ludtke JJ, Sololoff AV, Wong SC, et al. In vivo selection and validation of liver-specific ligands using a new T7 phage peptide display system. Drug Deliv 2007;14(6):357-69

78. Pasqualini R, Ruoslahti E. Organ targeting in vivo using phage display peptide libraries. Nature 1996;380(6572):364-6

• **The first use of phage display libraries for selection *in vivo*.**

79. Rajotte D, Arap W, Hagedorn M, et al. Molecular heterogeneity of the vascular endothelium revealed by in vivo phage display. J Clin Invest 1998;102(2):430-7

80. Valadon P, Garnett JD, Testa JE, et al. Screening phage display libraries for organ-specific vascular immunotargeting in vivo. Proc Natl Acad Sci USA 2006;103(2):407-12

81. Kelly K, Alencar H, Funovics M, et al. Detection of invasive colon cancer using a novel, targeted, library-derived fluorescent peptide. Cancer Res 2004;64(17):6247-51

82. Kelly KA, Clemons PA, Yu AM, Weissleder R. High-throughput identification of phage-derived imaging agents. Mol Imaging 2006;5(1):24-30

83. Kelly KA, Waterman P, Weissleder R. In vivo imaging of molecularly targeted phage. Neoplasia 2006;8(12):1011-8

• **One of the first uses of filamentous phage for imaging of tumors *in vivo*.**

84. Newton JR, Kelly KA, Mahmood U, et al. In vivo selection of phage for the optical imaging of PC-3 human prostate carcinoma in mice. Neoplasia 2006;8(9):772-80

• **One of the first uses of filamentous phage for imaging of tumors *in vivo*.**

85. Newton JR, Miao Y, Deutscher SL, Quinn TP. Melanoma imaging with pretargeted bivalent bacteriophage. J Nucl Med 2007;48(3):429-36

86. Segers J, Laumonier C, Burtea C, et al. From phage display to magnetophage, a new tool for magnetic resonance molecular imaging. Bioconjug Chem 2007;18(4):1251-8

87. Petrenko VA, Sorokulova IB. Detection of biological threats. A challenge for directed molecular evolution. J Microbiol Methods 2004;58(2):147-68

88. Petrenko VA, Vodyanoy VJ. Phage display for detection of biological threat agents. J Microbiol Methods 2003;53(2):253-62

• **The first use of phage as a biospecific interface in biosensors.**

89. Yang LM, Diaz JE, McIntire TM, et al. Covalent virus layer for mass-based biosensing. Anal Chem 2008;80(4):933-43

90. Yang LM, Tam PY, Murray BJ, et al. Virus electrodes for universal biodetection. Anal Chem 2006;78(10):3265-70

91. Wan J, Johnson M, Guntupalli R, et al. Detection of Bacillus anthracis spores in liquid using phage-based magnetoelastic micro-resonators. Sensors Actuators B 2007;127:559-66

92. Fu L, Li S, Zhang K, et al. Magnetostrictive microcantilever as an advanced transducer for biosensors. Sensors J 2007;7:2929-41

RIGHTSLINK

93. Johnson ML, Wan J, Huang S, et al. A wireless biosensor using microfabricated phage-interfaced magnetoelastic particles. Sensors Actuators A Phys 2008;144(1):38-47

94. Nanduri V, Balasubramanian S, Sista S, et al. Highly sensitive phage-based biosensor for the detection of β-galactosidase. Anal Chim Acta 2007;589:166-72

95. Petrenko VA. Landscape phage as a molecular recognition interface for detection devices. Microelectron J 2008;39(2):202-7

96. Olsen EV, Sykora JC, Sorokulova IB, et al. Phage fusion proteins as bioselective receptors for piezoelectric sensors. ECS Transactions 2007;2(19):9-25

97. Olsen EV, Sorokulova IB, Petrenko VA, et al. Affinity-selected filamentous bacteriophage as a probe for acoustic wave biodetectors of Salmonella typhimurium. Biosens Bioelectron 2006;21(8):1434-42

98. Nanduri V, Sorokulova IB, Samoylov AM, et al. Phage as a molecular recognition element in biosensors immobilized by physical adsorption. Biosens Bioelectron 2007;22(6):986-92

99. Lakshmanan RS, Guntupalli R, Hu J, et al. Phage immobilized magnetoelastic sensor for the detection of Salmonella typhimurium. J Microbiol Methods 2007;71(1):55-60

100. Wan J, Shu H, Huang S, et al. Phage-based magnetoelastic wireless biosensors for detecting bacillus anthracis spores. IEEE Sensors J 2007;7(3)

101. Draper WM. Biological monitoring: exquisite research probes, risk assessment, and routine exposure measurement. Anal Chem 2001;73(12):2745-60

102. Brigati JR, Petrenko VA. Thermostability of landscape phage probes. Anal Bioanal Chem 2005;382(6):1346-50

Affiliation

VA Petrenko
Professor,
Auburn University,
Department of Pathobiology,
AL 36849, USA
Tel: +1 334 844 2897; Fax: +1 334 844 2652;
E-mail: petreva@auburn.edu

Expert Opin. Drug Deliv. Downloaded from informahealthcare.com by Hacettepe Univ. on 12/02/12
For personal use only.

RIGHTSLINK