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Going viral: Designing bioactive surfaces with bacteriophage

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ABSTRACT

Bacteriophage-functionalized bioactive surfaces are functional materials that can be used as antimicrobial surfaces in medical applications (*e.g.*, indwelling medical devices or wound dressings) or as biosensors for bacterial capture and detection. Despite offering immense potential, designing efficient phage-functionalized bioactive surfaces is hampered by a number of challenges. This review offers an overview of the current state of knowledge in this field and presents a critical perspective of the technological promises and challenges.

1. Introduction

Bioactive surfaces are defined, for the purpose of this review, as substrates functionalized with specific recognition elements, used to detect, capture and/or deactivate microorganisms/analytes of choice. These substrates can find applications in biosensors (on platforms such as surface plasmon resonance sensors [1], quartz crystal microbalances [2], high throughput lab-on-chip systems [3], paper diagnostics and dip-stick assays [4,5], *etc.*) or as antimicrobial/antibiofilm surfaces in applications such as food packaging [6], indwelling medical devices (implants, stents, catheters) [7], wound dressings [8], antibacterial wipes, water treatment membranes [9] and coatings on surfaces in medical settings [10,11]. Although a rich and interesting field of science, the subject of biotemplate design by virus/phage immobilization/patterning on substrates is beyond the scope of this text. The interested reader can consult a number of comprehensive reviews on the subject [12–19].

Bacteriophages (phages) are viruses that infect bacteria [20,21]. Phages are the most abundant biological entity on earth and, being obligate parasites, propagate themselves by hijacking the host machinery [22]. They can have a broad host range, infecting several strains or species of bacteria [23], or infect a specific host, even down to a single bacterial strain [24]. A phage virion usually consists of a protein envelope (some also contain lipids in their envelope) encasing the genome, which can be a single or double stranded DNA or RNA molecule [25]. A phage virion may have a range of different shapes and sizes including tailed, filamentous, and icosahedral (Fig. 1) [26]. A phage infects its host bacterium by first attaching its "capture proteins" to specific receptors on the host cell surface (*e.g.*, lipopolysaccharide (LPS), pili, *etc.*) [27]. Phage capture proteins can be located symmetrically all around the virion [28] or located asymmetrically on one vertex [29], on a pole (filamentous phages) [30] or at the tip of tail fibers (tailed phages) [31]. Once attached to the host bacterium, phage injects its genome into the host and either takes over host machinery immediately and starts propagating (lytic phage), or the viral genome gets incorporated into the host DNA and stays dormant (lysogenic or temperate phage). The lytic life-cycle ultimately leads to host destruction, except for some filamentous lytic phage which only decrease host growth rate [32]. The genome of a temperate phage is passed on to daughter cells during bacterial replication, maintaining the lysogenic lifestyle. Certain environmental triggers (*e.g.*, heat, UV radiation, chemicals, *etc.*) can cause a switch from the lysogenic to the lytic lifestyle [33,34].

All phages have the potential to be used as specific recognition moieties in designing bioactive surfaces. Compared to other biological agents that provide similar specific interaction for pathogen capture/detection (*e.g.*, antibodies), phage offers the advantage of having a longer shelf life and being cheaper and more humane to produce. Most lytic phage also offer the dual functionality of capture and destruction of the host. Compared to other bactericidal agents (*e.g.*, antibiotics, antimicrobial peptides, silver nanoparticles, chemicals, *etc.*), phage offer the advantage of specificity. This characteristic could be very beneficial in applications such as food packaging. Food-borne infections continue to claim lives and cause significant economic loss in many developed countries [35]. Broad

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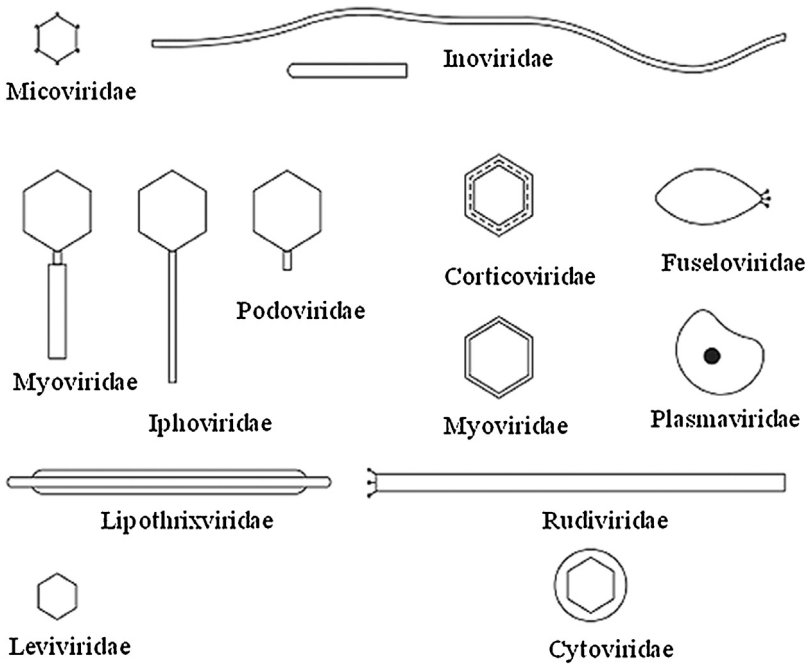

Fig. 1. Schematic representation of major phage groups. Reproduced from Ref. [25] with permission from Oxford University Press, USA.

action biocides must be used with caution in these settings because the taste, odor and texture of many food products depend on the presence of "good" bacteria. Thus, selective biocides are of particular interest. The same argument holds for many indwelling medical devices which must not disturb the body's microbiome, the collection of microorganisms living in our body that is responsible for maintaining physiological balance as well as the body's immunity towards infections [36]. Furthermore, phages are innocuous to humans, animals and plants, which is an added advantage over many biocides currently in use [37,38]. Bacteriophages exist all around us in the environment and even in our bodies. Because they have evolved over billions of years with their host bacteria, when the host develops resistance to the phage, the phage can mutate to develop the ability to infect its host, leading to a constant evolutionary arms race [39–41]. Thus, novel phages can constantly be isolated from the environment to tackle new infections.

To use bacteriophages for designing bioactive surfaces, there exist several challenges that must be addressed. Firstly, to design a bioactive surface with high efficiency for use as either a biosensor or an antimicrobial surface, the immobilized phage must retain its infectivity. Moreover, for biosensing applications, a high surface density of immobilized phage is desirable to increase sensitivity. Since more than 95% of the phages isolated to date are tailed [22], and thus asymmetric in terms of the placement of their capture proteins, it is crucial that the immobilized phage is oriented on the surface in a manner that leaves its capture proteins exposed to the host. In this paper, we review the current state of knowledge in designing bioactive surfaces with immobilized phage and offer a critical perspective of the challenges in this field.

2. Phage immobilization

2.1. Physisorption, electrostatic attachment and covalent bonding

Numerous approaches have been reported in the literature to effectively immobilize phage on a substrate. These immobilization methods are summarized with respect to bacterial biosensing, bacterial capture and antimicrobial activity in Tables 1–3, respectively. These tables present quantitative data on effectivity in terms of detection limits, bacterial capture per unit area and antimicrobial effect, respectively. Most of these approaches involve physisorption [7,42–67]. This is a very simple approach, but the physisorbed phage can detach from the substrate due to shear or changes in temperature, pH or ionic strength of the medium which is particularly troublesome for biosensing applications. Since most virions have an overall negative charge at neutral pH [68], several researchers have used electrostatic binding to immobilize phage [68,69]. This approach also suffers from instability and phage detachment in response to changes in physico-chemical properties of the medium. Covalent attachment of phage offers a much stronger bond and is not susceptible to easy phage detachment [70–82]. By targeting endogenous amino acids on the protein envelope of the virus, such as lysines, glutamic or aspartic acids, cysteines or (less common) phenol ring of tyrosines, many of the basic protein conjugation schemes can be used to attach phage to substrates of choice (Fig. 2) [83,84]. Appropriate chemistries can be tailored to the selected substrate and target application. Covalent binding results in a higher phage surface density, which is particularly desirable for biosensor applications [85–89]. Singh et al. report a 37-fold improvement of bacteriophage attachment with covalently bound phage when compared with physisorption (Table 2) [82]. Although covalent binding is able to provide a more stable immobilized phage layer, Klem et al. have shown that tightly bound viral cages did not allow for lateral mobility of the virus layer and ultimately hampered the formation of a tightly packed virus layer [90]. This could be of interest for biotemplate design applications. Furthermore, covalent binding is not by itself able to orient phage. The derivatized virions will bind to the substrate in an uncontrolled orientation resulting from their random diffusion to the surface. This would have direct implications for the bioactive performance of a phage modified surface. Phage orientation will be discussed in detail in Section 3.

It is important to bear in mind that some chemistries have the potential to damage phage integrity and/or decrease phage infectivity due to harsh reaction conditions (*e.g.*, pH, solvents, temperature) or over derivatization of phage capture proteins, as reported by Sun et al. [91]. For designing bioactive surfaces,