

# Controlling clinically relevant biofilms using bacteriophages

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Bacteriophages are viruses that infect bacteria. Each phage particle (virion) contains a nucleic acid genome that is enclosed in a protein or lipoprotein coat known as the capsid. Phages are obligate parasites. The phage nucleic acid encodes all of the information necessary to direct its reproduction within the host bacterium, but phages are unable to generate energy and have no ribosomes to make proteins. Virulent phages multiply by means of a lytic cycle, in which the virion adsorbs to the host cell surface, injects its genomic material, and hijacks the host metabolic machinery resulting in intracellular phage multiplication. Cell lysis and liberation of progeny phages completes the lytic cycle.

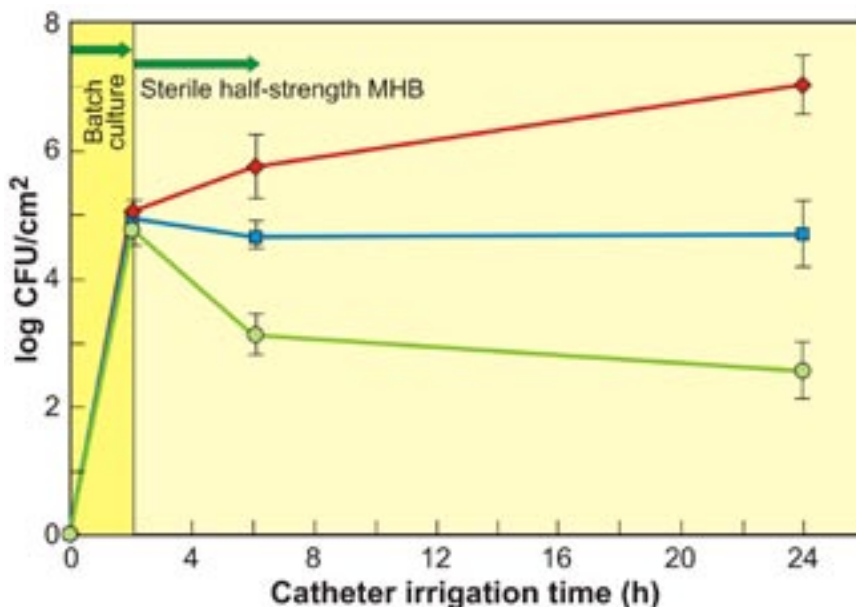
Early on, phages were recognized for their potential in the treatment of infectious diseases through the lysis of infecting bacteria.

An advantage of phage treatment over antimicrobial agents is that phages can replicate at the site of infection and thus become available in abundance where they are most required.

A number of published studies have demonstrated the potential of phage for the treatment of infectious disease in plants (Fox, 2000), animals (Barrow et al., 1998), and humans (Slopek et al., 1987), including infections caused by multi-drug resistant bacteria (Weber-Dabrowski et al., 2000; O'Flaherty et al., 2005).

Biofilms that develop on the surface of indwelling medical devices may result in infections (Donlan and Costerton, 2002). Since it is known that organisms growing in biofilms exhibit extreme tolerance to antimicrobial agents, the use of phages as a control strategy appears promising for several reasons. Phages produce enzymes that can degrade the biofilm extracellular polysaccharide (EPS) matrix. Hughes et al. (1998) isolated a phage specific for *Enterobacter agglomerans* and demonstrated that its ability to control biofilms of this organism was in part due to the production of a polysaccharide depolymerase. Hanlon et al. (2001) demonstrated that a phage targeting *Pseudomonas aeruginosa* produced a depolymerase enzyme that reduced the viscosity of alginates and the EPS of this organism. In the phage lytic cycle, infection of a bacterial cell with a single phage virion will result in production of multiple progeny phages, depending upon the burst size of the particular phage strain. The burst size, or number of progeny phage produced per infected cell is of the order of magnitude of tens to hundreds. Using fluorescent dyes to label phage particles, Doolittle et al. (1996) showed that progeny phage propagate radially through a biofilm. A single dose of phage should be able to treat an infection, since each progeny phage should, in theory, be able to infect adjacent cells in the biofilm.

How might phage treatment be used to prevent or control biofilms on the surfaces of indwelling medical devices? One strategy



**Figure 1.** Effect of phage pretreatment of catheter surface on biofilm formation. Red diamonds represent mean log CFU/cm<sup>2</sup> of viable *S. epidermidis* 414 recovered from hydrogel-coated catheters; blue squares, mean log CFU/cm<sup>2</sup> of viable *S. epidermidis* recovered from hydrogel-coated catheters pretreated with phage 456; green circles, mean log CFU/cm<sup>2</sup> of viable *S. epidermidis* recovered from hydrogel-coated catheters pretreated with phage 456 and in media supplemented with divalent cations). Error bars represent  $\pm$  standard deviation (n = 3).

## BIOFILM PERSPECTIVES AUTHOR



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is Team Leader of the Biofilm Laboratory in the Division of Healthcare Quality Promotion at the Centers for Disease Control and Prevention (www.cdc.gov) in Atlanta, Georgia, a position he has held since 1998. Current research in the Biofilm Lab is focused on the evaluation of strategies for the prevention or control of catheter-associated biofilms, and on the role of biofilms in the transfer of antimicrobial resistance in the healthcare setting.

Prior to coming to CDC, Dr. Donlan worked in private industry, where he studied biofilms in drinking water and industrial water systems. He has had a long association with the Center for Biofilm Engineering and with other national and international biofilm research groups, and has been fortunate to mentor a number of talented guest researchers and fellows. He received his Ph.D. in 1987 from Drexel University, and his M.S. and B.S. degrees from Virginia Polytechnic Institute and State University.

would be to coat or impregnate the surface of a device with phages to provide a barrier against potential colonizing bacteria. This same approach, incorporating more traditional antimicrobial agents and/or silver salts, has been reported (Donlan and Costerton, 2002) and such devices are commercially available. Recent laboratory studies have shown that incorporation of a certain phage into the hydrogel coating of a catheter can significantly reduce attachment and biofilm formation by *Staphylococcus epidermidis*, the host organism for this phage, as shown in Figures 1 and 2 (Curtin and Donlan, 2006). Another approach would be to instill the catheter with a suspension of phage, much like an antimicrobial lock treatment occasionally used by healthcare practitioners to salvage colonized catheters (Berrington and Gould,

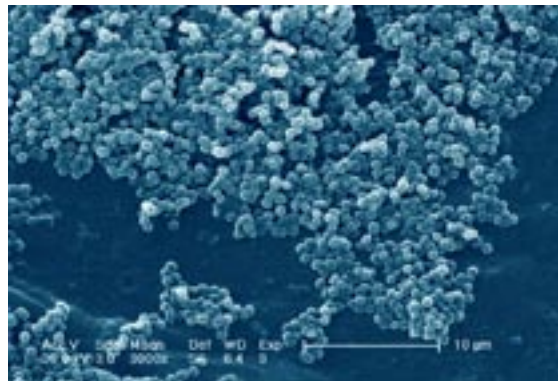
2001). Organisms isolated from the colonized device could be screened against a bank of phages to determine the specific phage strain with greatest lytic ability. This strain could in turn be grown to a high titer, purified, and added as a "phage lock" treatment. Several published studies have simulated this "phage lock" approach using in vitro model systems and demonstrated efficacy against biofilms of different organisms (Doolittle et al., 1995; Hanlon et al., 2001; Hughes et al., 1998; Sillankova et al., 2004). Phage therapy might also be suitable for the topical treatment of biofilm infection, such as those in chronic wounds.

What are the potential concerns with using phage for the control of biofilms? As with other biofilm control strategies, the treatment must be compatible with the patient. One question that often arises is how the immune system will respond to the introduced phages. Phages are antigenic and will elicit a serum antibody response and stimulate the innate immune system.

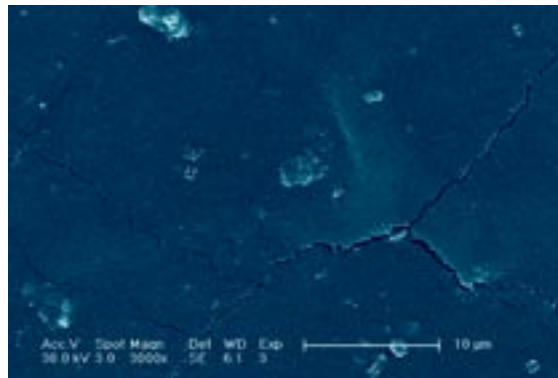
Phages are inactivated following contact with serum, although the rate of clearance is dependent on the degree of prior exposure of the serum to the phage. Inchley (1969) found that >99% of T4 Phages were phagocytosed within the first 30 minutes, but  $10^2$  plaque-forming units remained even after 48 hours. Geier et al. (1973) showed that Phage  $\lambda$  was cleared from the blood of infected mice within 48 hours; Uhr and Weissmann (1965) showed the rapid removal of Phages  $\Phi$ X 174 and T2 from the bloodstream of guinea pigs and rabbits primarily by the liver and spleen.

However, studies have also shown that phages will quickly associate with biofilms when introduced into flowing or static systems (Hughes et al., 1998; Doolittle et al., 1995; Doolittle et al., 1996). It is possible that the biofilm EPS could protect the introduced phages from being cleared and inactivated by the immune system. For example, Landy et al. (1958) demonstrated that various polysaccharides, including polysaccharides produced by bacteria, would block the phage-neutralizing activity of human serum.

Medical devices such as catheters will rapidly become coated with serum proteins when they are inserted (Donlan, 2002). How might these materials affect the ability of phages to either prevent (in the case of a phage coating) or control (in the case of a "phage lock") biofilm formation? We have shown, for *S. epi-*



**Figure 2a. Scanning electron micrograph of the surface of a hydrogel-coated catheter after biofilm formation by *S. epidermidis* 414 for 24 h (X3000 magnification).**



**Figure 2b. Scanning electron micrograph of the surface of a hydrogel-coated catheter pretreated with phage 456 after biofilm formation by *S. epidermidis* 414 for 24 h (X3000 magnification).**

*dermidis*, that the activity of phages embedded in a hydrogel coating was unaffected by exposure to whole serum in a catheter model system (Curtin and Donlan, 2006).

Phage lysis of gram-negative bacterial cells may result in the production of endotoxins. Endotoxin contamination of phage preparations could be overcome by using better purification methods such as cesium chloride density-gradient centrifugation (Merril et al., 1996). Endotoxin release from biofilm-associated cells following phage treatment could be minimized by using engineered phage strains that would kill their bacterial hosts without lysing the cells (Hagens et al., 2004).

Important issues that need to be addressed before we can hope to use phage treatment to prevent or treat device-associated infections are:

- the effect of the biofilm in protecting introduced phages from the immune system;
- the stability of phage coatings and the long term protective effect of phage-treated catheters or other devices;
- the efficacy of “phage cocktails” containing multiple phage strains for treating polymicrobial biofilms;
- whether the expected tolerance of biofilms resulting from reduced growth rates of biofilm-associated cells will affect the phage treatment;
- the potential for development of host resistance to phages when sequential therapy is used and;
- whether phages can act synergistically with antimicrobial agents for the control of clinically relevant biofilms.

The resolution of these issues should help propel phage technology from the laboratory to the bedside.

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