RESEARCH ARTICLE

The use of lytic bacteriophages in the prevention and eradication of biofilms of *Proteus mirabilis* and *Escherichia coli*

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Abstract

Antibiotics have been the cornerstone of the clinical management of bacterial infections since their discovery in the early part of the last century. Eight decades later, their widespread, often indiscriminate use, has resulted in an overall reduction in their effectiveness, with reports of multidrug-resistant bacteria now commonplace. Increasing reliance on indwelling medical devices, which are inherently susceptible to biofilm-mediated infections, has contributed to unacceptably high rates of nosocomial infections, placing a strain on healthcare budgets. This study investigates the use of lytic bacteriophages in the treatment and prevention of biofilms of bacterial species commonly associated with infections of indwelling urological devices and catheter-associated urinary tract infections. The use of lytic bacteriophages against established biofilms of *Proteus mirabilis* and *Escherichia coli* is described, whereby biofilm populations have been reduced successfully by three to four log cycles (99.9–99.99% removal). The prevention of biofilm formation on Foley catheter biomaterials following impregnation of hydrogel-coated catheter sections with a lytic bacteriophage has also been investigated. This has revealed an approximate 90% reduction in both *P. mirabilis* and *E. coli* biofilm formation on bacteriophage-treated catheters when compared with untreated controls.

Introduction

Scientific advancement has facilitated the elucidation of the pathophysiology and epidemiology of bacterial infections, with perhaps the most significant development in the fight against human infectious diseases being the discovery and development of antibiotics. These drugs quickly became the 'panacea' of medicine, used to treat even the most trivial of infections (Alanis, 2005). However, widespread, indiscriminate or suboptimal usage of antimicrobials has limited their effectiveness over the past decades. The emergence of multidrug-resistant pathogenic bacteria, combined with a concomitant increase in immunosuppressed patients, raises concerns that mankind may once again be entering a 'pre-antibiotic era' characterized by untreatable infectious diseases (Payne & Tomasz, 2004). Worldwide, modern healthcare systems are struggling with the burden of increasing hospital-acquired (nosocomial) infections. With pathogens such as methicillin-resistant *Staphylococcus aureus* and *Clostridium difficile* in the spotlight, attention has been distracted from other equally problematic hospital-acquired infections, such as those associated with medical devices. Medical devices (which include urinary or venous catheters, endotraceal tubes, synthetic heart valves, pacemakers and stents) are inherently susceptible to colonization and development of microbial biofilm infections, the nonshedding surface of these devices offering an excellent foundation for biofilm growth. Infection most likely occurs by inoculation with only a few cells from the patient's skin or mucous membranes during placement of the device; however, once attached to the polymer surface, proliferation and eventual formation of a mature biofilm community ensues (Von Eiff & Peters, 2003). Bacteria within biofilms are inherently tolerant to antimicrobial agents and therefore it is notoriously difficult to treat infections commonly associated with these indwelling polymeric devices. As modern medicine becomes ever more reliant on medical devices for both diagnostic and therapeutic procedures, further emphasis is placed on the necessity to safeguard effective antimicrobial therapies for future generations.

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The next stage in the battle against bacterial pathogens may involve the development of new classes of antimicrobial drugs, and of particular interest is nondestructive therapies that attenuate virulence rather than kill the pathogen, such that selection pressure is reduced and the threat of resistance is lessened (Potempa & Travis, 2000; Rasmussen & Givskov, 2006). Another attractive option is the use of bacteria's natural predators, the bacteriophages. Bacteriophages (or phages) are viruses that selectively infect bacteria, and in the case of lytic phages, disrupt normal bacterial metabolism in favour of viral replication and cause the bacterium to lyse rapidly. Bacteriophages are the most abundant biological entities on earth and it is estimated that phage predation reduces the global bacterial population by half every 48 h (Hendrix, 2002). Despite pre-dating the discovery of antibiotics by several decades, bacteriophage therapy was largely supplanted by antibiotics and vaccines in western medicine. Today, interest in phage therapy in western medicine is growing, as several advantages over conventional therapeutic agents have been recognized.

Bacteriophages are highly specific, thus unlikely to disturb normal flora in the same manner as current antibiotics, and are self-replicating at the site of infection, facilitating effective treatment by the delivery of a low phage dose. Although resistance has been reported, this can be avoided by using a 'phage cocktail' directed against numerous strains of the target species, whereas the easy manipulation of the phage genome provides the possibility of 'designer'-engineered phages to target problematic infections (Sulakvelidze *et al*., 2001). Furthermore, phages are capable of treating intracellular antibiotic-resistant pathogens such as *Mycobacterium avium* and *Mycobacterium tuberculosis* (Broxmeyer *et al*., 2002).

This study investigates the potential of bacteriophage therapy in the treatment and prevention of a common nosocomial infection, catheter-associated urinary tract infection (CAUTI). *Proteus mirabilis* and *Escherichia coli* are among the most common species isolated from biofilms on urinary catheters, with virtually every patient undergoing long-term urinary catheterization experiencing such an infection (Stickler, 1996). Approximately half of the cases are complicated by encrustation and blockage of the catheter, particularly when *P. mirabilis* is involved. At present, the only effective treatment for CAUTI is the removal of the catheter, which, in severe cases, must be removed surgically. However, with recatheterization, reinfection is likely to follow, and for many patients, there is no satisfactory resolution to the problem (Kohler-Ockmore & Feneley, 1996).

Two recent studies by Curtin and Dolan have shown the potential application of bacteriophages in the prevention of biofilm formation on medical devices, with a specific focus on *Stapylococcus epidermidis* and *Pseudomonas aeruginosa* (Curtin & Donlan, 2006; Fu *et al*., 2010). Through the selection of bacteriophages with specific activity against problematic urinary tract pathogens, it is envisaged that a similar approach may be of use on Foley catheters used for long-term urethral catheterization.

Materials and methods

Propagation of bacteriophages

Escherichia coli T4 phage ATCC 11303-B4 (LGC Standards, Middlesex, UK) and a coli-proteus bacteriophage, isolated from a commercially available bacteriophage preparation (Bacteriofag coli-proteic, Microgen Pharma, Russia), were used in these studies. The latter has the ability to cause lysis of both species of bacteria. Bacteriophage cultures were propagated using standard soft-agar overlay methods (Adams, 1959) on Müller–Hinton agar using *E. coli* ATCC 11303 (LGC Standards) or *P. mirabilis* 13 HER1094 (Felix d'Herelle Reference Centre for Bacterial Viruses, QC, Canada) as host strains. High-titre bacteriophage stocks were prepared by extracting bacteriophage from plate lysates after incubation with the appropriate host strain at 37 °C for 24 h. The soft agar layer was removed using a sterile scraper, and bacteriophages were extracted into 5 mL of Müller–Hinton broth (MHB) for at least 2 h at 4 °C with gentle agitation. The soft agar was removed by centrifugation (1700 ***g***, 20 min), and contaminating bacteria were removed by passing the supernatant through a 0.45-μm sterile filter. Phage stocks were protected from light and stored at 4 °C for use within 24 h. The titre of bacteriophage was confirmed before use using the standard agar-overlay technique, through serial dilution of stocks and calculation of plaque-forming units (pfu) mL^{-1}. Bacteriophage titres were adjusted as necessary by dilution with MHB before use.

Cultivation and bacteriophage treatment of biofilms

Biofilms of both bacterial species were cultivated using the Calgary Biofilm Device/MBEC Assay (Innovotech, Calgary) on a gyrotatory shaker (150 r.p.m.) at 37 °C. This device consists of a 96-well plate and lid-bearing polycarbonate pegs that protrude into each well containing bacterial culture, allowing for the growth of 96 identical biofilms per device (Ceri *et al*., 1999). Following the manufacturer's protocol (http://www.innovotech.ca/products_use.php), each well was inoculated with 1×10^7 CFU mL^{-1} (180 μL per well) of the appropriate bacteria in MHB and biofilms were allowed to develop over a 24-h period, after which both species produced an average biofilm viable count of 1×10^6 CFU per peg (data not shown). Biofilms of either *E. coli* ATCC 11303 or *P. mirabilis* 13 HER1094 were then exposed to 200 μL of bacteriophage at a high (1×10^6 pfu mL^{-1}), medium (1×10^3 pfu mL^{-1}) or a low

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(1×10^1 pfu mL^{-1}) bacteriophage titre (equating to a multiplicity of infection (MOI) of 0.2, 2×10^{-5} and 2×10^{-7}, respectively) by exchanging the base of the MBEC device for a 96-well plate containing bacteriophage at the appropriate titre in MHB, while for controls, the wells contained MHB alone. The biofilms were then incubated with bacteriophage for a further 24 h. Biofilm eradication was assessed via crystal violet (CV) staining of the MBEC device pegs. Staining of biofilms was performed using a method adapted from Stepanovic *et al.* (2000), with the exception that the MBEC pegs were exposed to the stain, rather than staining the wells of the device. Stain retained by the biofilm was dissolved into 200 μL of 33% glacial acetic acid, and $A_{570\,nm}$ was read. The mean value from at least six replicates was obtained and a statistical analysis was conducted using one-way ANOVA ($\alpha = 0.05$), followed by Dunnett's *post hoc* test for comparison between each bacteriophage titre with the control. Statistical computation was performed using GRAPHPAD PRISM version 5.01 (GraphPad Software Inc., San Diego). The reduction in viable biofilm cells was determined using a calibration plot of CV absorbance vs. viable count for the bacterial strain in question.

Calibration plots

In order to determine the correlation between CV absorbance and viable count, the MBEC device was inoculated as described previously, and identical biofilms were analysed using both techniques during their formation and growth. At regular time points over a 24-h period, 10 pegs were selected at random and broken from the lid of the device using sterile pliers. Of the biofilms obtained at each time point, five were subject to standard plate counting techniques on Müeller–Hinton agar (following 15 min of sonication from the pegs of the MBEC assay plate,) as described in the product literature, and five were analysed by direct staining with CV. A plot of CV absorbance vs. viable count was then constructed and coefficients of determination (R^2) were calculated.

Prevention of biofilm formation on Foley catheters

Sections of Foley catheter coated in a neutral hydrogel (Bard Lubri-Sil™) were treated with bacteriophage cultures, as described previously by Curtin & Donlan (2006), simply by incubating catheter segments in a bacteriophage culture. In this study, catheters were incubated for 1 h at 37 °C with a titre of 1×10^6 pfu mL^{-1} of either the T4 bacteriophage or the coli-proteus bacteriophage in MHB. Control catheter segments were incubated under identical conditions in MHB without bacteriophage. Loops of catheter were thoroughly rinsed in sterile 0.9% saline to remove excess bacteriophage, and then suspended with gentle agitation in MHB inoculated with *P. mirabilis* 13 HER1094 or *E. coli* ATCC 11303, as appropriate, for a 24-h period at 37 °C.

Microscopic examination of biofilms

Biofilms cultivated on the MBEC device

Following exposure of sessile bacteria to the phage for the desired contact time, three pegs, chosen at random, were broken from the lid of the device and rinsed in 0.9% saline for 1 min to remove loosely adherent bacteria. The pegs were then fixed in 3% glutaraldehyde in 0.1 M cacodylic acid (pH 7.2) overnight (18 h) at 4 °C. Following fixation, pegs were washed twice in 0.1 M sodium cacodylate buffer for several hours. After washing in cacodylate buffer, the samples were dehydrated in a graded series of ethanol solutions (50%, 70% and 90%) for 15 min each at room temperature and in 100% ethanol for 1 h, transferred to a microtitre plate containing 200 μL per well of hexamethyldisilazane and left to dry for 24 h in a fume cupboard. Fixed specimens were mounted on aluminium stubs, sputter coated in pure gold and examined using a scanning electron microscope (model JEOL JSM 840 SEM). Observations were performed at 15 kV and images were captured onto an Ilford black and white film. For each bacteriophage treatment or control, the entire surface of the three pegs was examined and images that represented the typical field of vision were selected.

Biofilms on phage-treated catheters

Following biofilm formation on phage-treated or control catheters, sections of catheter were immersed in Live/Dead Baclight™ (Molecular Probes) in the dark for 30 min at 30 °C, followed by immediate examination using a Leica SP2 AOBS confocal microscope. Examination of the catheter sections using a ×10 water immersion objective was conducted in order to determine the extent of biofilm development on the catheter surface.

Quantification of stained biofilm on Foley catheters

To quantify the extent of biofilm formation on phage-treated catheters and the corresponding untreated controls, IMAGEJ software-based analysis was used (National Institutes of Health, http://rsbweb.nih.gov/ij/). Five randomly selected fields of view (900 μm^2) were chosen per catheter section. Images were split into their red, green and blue fluorescent components using the 'RGB stack' function. With the green channel selected (attributed to a viable biofilm), thresholding (binary contrast enhancement) was applied with manual adjustment, and the area (μm^2) and percentage coverage of biofilm were assessed using the 'measure' function.

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Threshold limits were maintained at a constant value for all the images processed. Statistical analysis was carried out using a two-tailed Student's *t*-test with Welch's correction ($\alpha = 0.05$). Statistical computation was performed using GRAPHPAD PRISM version 5.01 (GraphPad Software Inc.).

Results

Bacteriophage treatment of mature biofilms cultivated on the MBEC device

A good linear correlation exists between viable biofilm population and CV, as illustrated by the calibration plots for both *P. mirabilis* 13 HER1094 and *E. coli* ATCC 11303 (Fig. 1), which produced R^2 values of 0.92 and 0.93, respectively. After staining with CV, it was observed that the two species of bacteria produced biofilms of differing morphologies when grown on the MBEC device. *Escherichia coli* biofilms developed in a discrete ring around the peg at the level of the air/liquid interface, while *P. mirabilis* biofilms extended over much of the peg surface, presumably due to the swarming ability of this species. For a given number of viable biofilm cells, it was found that the degree of CV staining was much greater for *P. mirabilis* than was obtained for *E. coli*. This observation highlights the necessity to perform a calibration for each strain or species of bacteria studied.

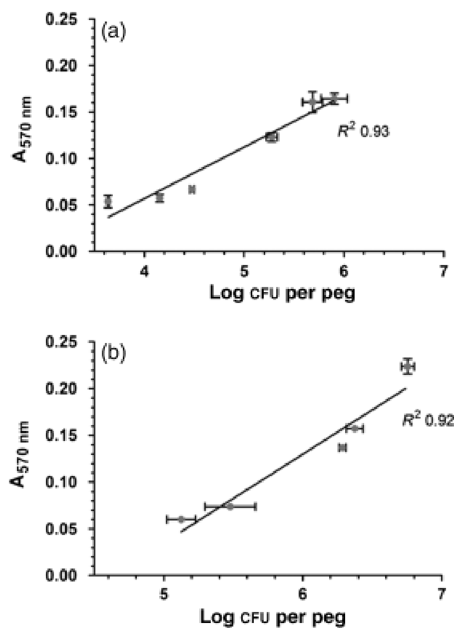

Fig. 1. Calibration plots, correlating the viable biofilm population (CFU per peg of MBEC device) with biofilm staining using CV ($A_{570\,nm}$). Data are means ± SDs ($n = 5$), (a) *Escherichia coli* ATCC 11303, (b) *Proteus mirabilis* 13 HER1094.

The populations of viable bacteria in biofilms of both species were successfully reduced through the use of lytic bacteriophages. Figure 2 demonstrates that the reduction in biofilm density, as determined by CV staining, is dependent on the titre of bacteriophage used. It is possible to gain an

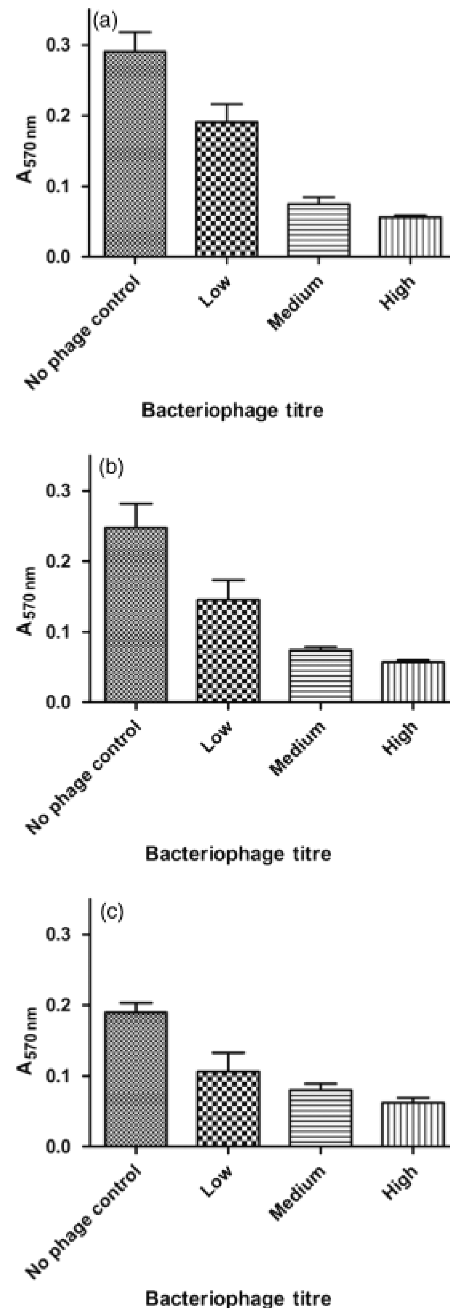

Fig. 2. Reduction of *Escherichia coli* and *Proteus mirabilis* biofilm using bacteriophage at low (1×10^1 pfu mL^{-1}), medium (1×10^3 pfu mL^{-1}) and high (1×10^6 pfu mL^{-1}) bacteriophage titres. Bars represent CV staining of the treated biofilm. Data are means + SDs ($n = 6$). (a) *Escherichia coli* treated with T4 bacteriophage, (b) *E. coli* treated with coli-proteus bacteriophage, (c) *P. mirabilis* treated with coli-proteus bacteriophage.

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insight into viable biofilm reduction with reference to the calibration plots, which display the relationship between staining intensity and viable biofilm. As shown in Fig. 3a, the highest titre used in this study (1×10^6 pfu mL^{-1}) was capable of reducing the viable *E. coli* biofilm population by approximately four log cycles (99.99% reduction). One-way ANOVA with Dunnett's *post hoc* test was used to analyse data, and in comparison with nonphage treated controls, the treatment of each biofilm with corresponding bacteriophage at high, medium and low titres significantly reduced the biofilm population ($P < 0.05$).

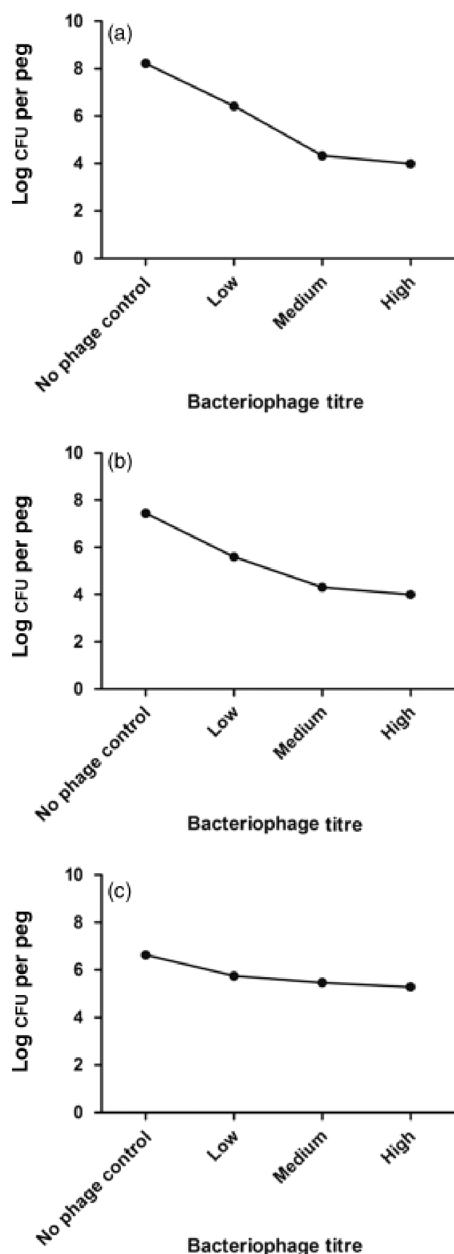

Fig. 3. Reduction of *Escherichia coli* and *Proteus mirabilis* biofilm using bacteriophage at low (1×10^1 pfu mL^{-1}), medium (1×10^3 pfu mL^{-1}) and high (1×10^6 pfu mL^{-1}) bacteriophage titres. Control values represent viable plate counts, while for bacteriophage-treated biofilms, values represent average CFU per peg, as translated from CV staining using calibration data. (a) *Escherichia coli* treated with T4 bacteriophage, (b) *E. coli* treated with coli-proteus bacteriophage, (c) *P. mirabilis* treated with coli-proteus bacteriophage.

Figure 3 shows that the treatment of *E. coli* ATCC 11303 biofilms with both coli-proteus and T4 bacteriophages led to a greater reduction in biofilm density than the treatment of *P. mirabilis* 13 HER1094 with the coli-proteus bacteriophage. Bacteriophages achieved a three- to four-log cycle reduction in *E. coli* biofilm at the high titre used, while the coli-proteus bacteriophage achieved only an approximate one-log cycle reduction of *P. mirabilis* biofilm. During the course of this study, we observed no development of resistance towards either bacteriophage used (data not shown). Therefore, the poor reduction in *P. mirabilis* biofilm may be a result of both biofilm- and bacteriophage-dependent factors. This may include polysaccharide depolymerase production and the ability to penetrate the biofilm extracellular polymeric substance (EPS), length of the lytic cycle and the burst size (i.e. the number of phage particles released per infected bacterium), and underscores the need for careful selection of the most efficacious phages for successful treatment.

SEM examination of biofilms after exposure to bacteriophage clearly illustrates cell lysis and destruction of the biofilm architecture compared with a nontreated control biofilm grown under identical conditions (Figs 4 and 5).

Prevention of biofilm formation on bacteriophage-treated Foley catheter sections *in vitro*

Visual examination by confocal imaging of catheter sections treated with bacteriophage showed a marked reduction in biofilm formation on the surface of Bard Lubri-Sil™ Foley catheters after 24 h as compared with untreated sections of the catheter. As shown in Fig. 6, biofilms have formed extensively on the surfaces of the untreated catheter segments, as indicated by the fluorescence emitted by the Live/Dead BacLight™ stain. Live cells are stained green (green-fluorescent Syto®9 stain), while dead cells are stained red (red-fluorescent propidium iodide only penetrates bacteria with damaged membranes). The pattern of staining observed in untreated controls in this instance displays the natural balance between live and dead bacteria within the matrix of a bacterial biofilm. In contrast, little evidence of bacterial staining/fluorescence can be observed on the surface of phage-treated catheters, indicating a significant reduction in biofilm formation on these catheter sections. Image analysis using IMAGEJ software provided a means to quantify the staining of biofilm on the surface of the catheter

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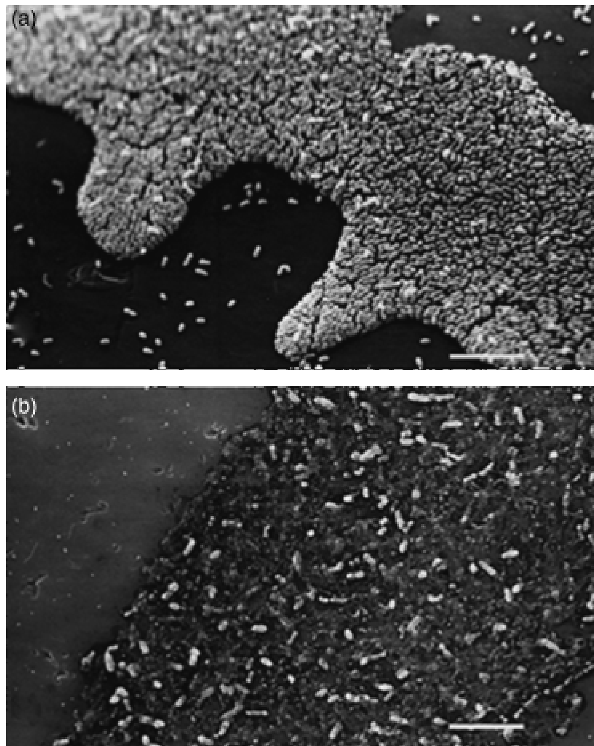

Fig. 4. Scanning electron micrographs of *Proteus mirabilis* 13 HER1094 biofilms grown on the MBEC device. (a) Nontreated control. (b) biofilm exposed to coli-proteus bacteriophage for 24 h. Scale bars = 10 μm.

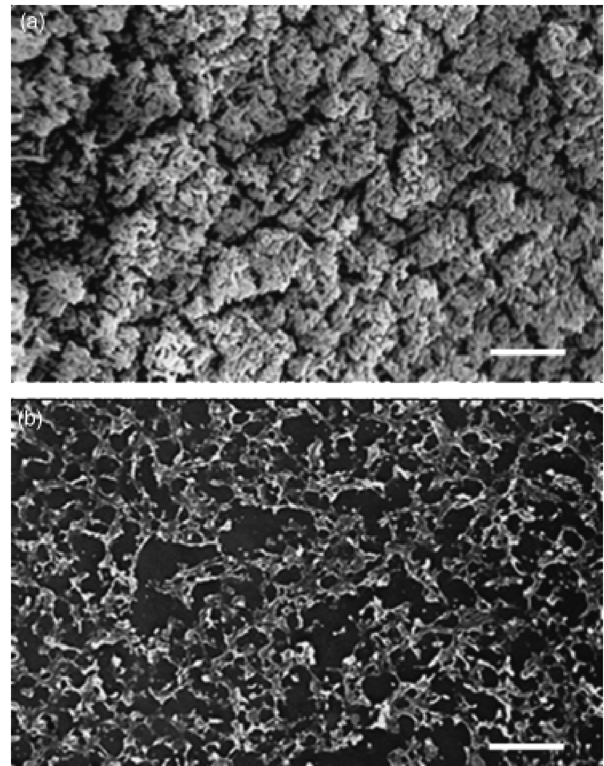

Fig. 5. Scanning electron micrographs of *Escherichia coli* ATCC 11303 biofilms grown on the MBEC device. (a) Nontreated control. (b) Biofilm exposed to T4 bacteriophage for 24 h. Scale bars = 10 μm.

segments. The data in Table 1 show a reduction in biofilm formation of approximately 90% on the surface of bacteriophage-treated catheters, in comparison with the control, for both bacterial species studied. Statistical analysis using Student's *t*-test with Welch's correction revealed a highly significant difference between the bacteriophage-treated catheter and the control in both cases ($P < 0.0001$). It is interesting that while the coli-proteus bacteriophage did not prove particularly effective in the eradication of established *P. mirabilis* biofilm, it nonetheless appears to be effective at preventing biofilm formation *in vitro*.

Discussion

Urinary tract infection is the most common hospital-acquired infection, with approximately 80% of these infections attributable to the use of an indwelling urethral catheter (Saint & Chenowith, 2003). While Gram-positive organisms (for example *S. epidermidis*, *Enterococcus faecalis*) are commonly isolated from infected catheters, Gram-negative bacteria are most commonly implicated in CAUTI, for example *E. coli*, *P. mirabilis*, *P. aeruginosa* and *Klebsiella pneumonia* (Gilmore *et al.*, 2009). A primary complication of CAUTI is the deposition of mineralized deposits (encrustation), stabilized by the biofilm matrix (Dumanski *et al.*, 1994), which can ultimately lead to blockage of the catheter lumen, urinary retention and severe discomfort for the patient. Encrustation associated with infection is composed of ammonium magnesium phosphate (struvite) and calcium phosphate (hydroxyapatite). The action of bacterial urease (which catalyses the hydrolysis of urea yielding ammonia and carbon dioxide) causes an elevation in urinary pH to pH 8–9. This alkalinization of both urine and the biofilm matrix initiates the precipitation of poorly soluble calcium and magnesium salts, resulting in surface deposition and crystallization (Gorman & Tunney, 1997). *Proteus mirabilis* is particularly problematic in terms of encrustation formation due to the production of a urease that is 6–10 times as potent as urease produced by other bacterial species (Jones & Mobley, 1987). In addition, *P. mirabilis* possesses the ability to differentiate into swarmer cells when flagellar rotation is inhibited, allowing for efficient migration across solid surfaces (Coker *et al.*, 2000). When this occurs on an abiotic surface (such as a urethral catheter), extensive biofilm formation can ensue and encrustation is promoted. Furthermore, swarmer cells are associated with a 30-fold increase in urease and increased polysaccharide production (Dumanski *et al.*, 1994; Rozalski

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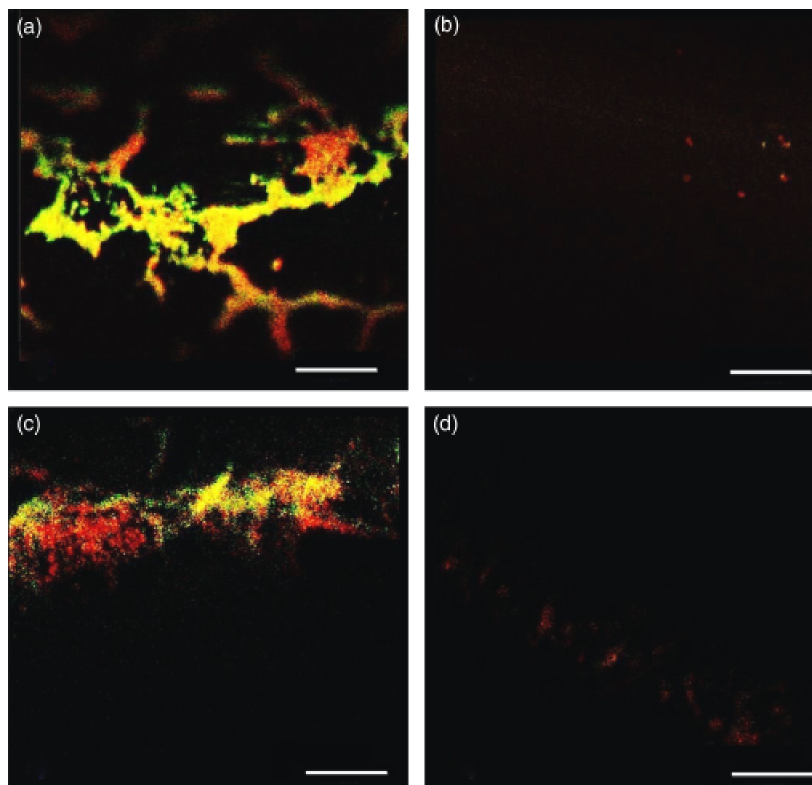

Fig. 6. Confocal scanning laser microscopy images of biofilm on the surfaces of Bard Lubri-Sil™ Foley catheters. Biofilms are stained with Live/Dead BacLight™. Left-hand images show biofilm growth of (a) *Escherichia coli* ATCC 11303 and (c) *Proteus mirabilis* 13 HER1094 on the surface of nontreated catheter segments. Right-hand images show a marked reduction in biofilm growth on the surfaces of bacteriophage-treated catheters. (b) *Escherichia coli* on a catheter treated with T4 bacteriophage and (d) *P. mirabilis* on a catheter treated with coli-proteus bacteriophage. Scale bars = 30 μm.

Table 1. Biofilm coverage on bacteriophage-treated Foley catheters, and on untreated controls

	Biofilm coverage on the catheter surface (900 μm^2 field of vision)	
	Mean area coverage (μm^2) ± SD	Mean % coverage ± SD
P. mirabilis/coli-proteus bacteriophage		
Untreated control	378.103 ± 36.034	42.011 ± 4.004
Bacteriophage-treated catheter	1.800 ± 1.663	0.2 ± 0.185
E.coli/T4 bacteriophage		
Untreated control	413.54 ± 23.861	45.949 ± 2.651
Bacteriophage-treated catheter	3.625 ± 2.659	0.403 ± 0.295

Data were generated using IMAGEJ software, with five fields of vision (900 μm^2 each) inspected.

et al., 1997). Successful treatment of the infection becomes practically impossible due to the inaccessibility and poor penetration of drug molecules into the inner structures of the biofilm (Stewart & Costerton, 2001). Such complications in the management of bacterial infections associated with urological devices are often only resolved by complete device removal.

The estimated cost of managing urinary encrustation and urinary tract infection associated with indwelling urinary devices amounts to somewhere in the region of £1 billion per annum in Western Europe, with similar, if not higher, estimations of cost associated with management of these conditions in the United States (Choong *et al.*, 2001), where catheter use is typically higher than in Europe (Weinstein *et al.*, 1999). With such a burden on healthcare systems, the importance of preventing these infections cannot be underestimated.

Many efforts have been made to overcome the infectious complications associated with indwelling urological devices, primarily by modification of the catheter biomaterial by, for example, impregnation or coating with conventional antimicrobials or silver (Johnson *et al.*, 1993; Ahearn *et al.*, 2000). However, in clinical practice, these approaches have not proved as successful as would have been desired (Lee *et al.*, 2004; Johnson *et al.*, 2006).

Management of human infections using bacteriophage therapeutics is proving increasingly attractive as the usefulness of many antibiotics declines, with many reports in the

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literature of the successful treatment of infections in both humans and animals with bacteriophage therapy (Sulakvelidze *et al.*, 2001). A number of reports also show the ability of bacteriophages to degrade biofilm matrices (Azeredo & Sutherland, 2008). Importantly, it has been demonstrated that pretreatment of hydrogel-coated catheters with bacteriophage has potential use in the prevention of *S. epidermidis* and *P. aeruginosa* biofilms, the former proving a particular problem on central venous catheters (Curtin & Donlan, 2006; Fu *et al.*, 2010). By careful selection of the appropriate bacteriophage and its successful incorporation into or onto the surface of the polymeric biomaterial, there is clear potential for this approach to be utilized for the prevention of biofilm-mediated infections on numerous types of implantable medical devices.

The data presented in this study suggest that lytic bacteriophages may be of use not only in the prevention of biofilm formation by bacteria commonly implicated in biofilm-mediated urological device infection and CAUTI by pretreatment of the catheter but also in the destruction/eradication of established biofilms (for example, in catheter lock solutions), where antibiotics are largely ineffective due to inherent tolerance and retarded penetration. It appears that the bacteriophages used in this study are capable of significantly reducing preformed *E. coli* biofilms, indicating their ability to access the biofilm and infect bacteria within its matrix, inducing cell death. This has been demonstrated by a 3–4-log cycle reduction of established biofilms of *E. coli* after exposure to a 1×10^6 pfu mL^{-1} titre of coli-proteus and T4 bacteriophages, respectively. This reduction equates to the killing of 99.9–99.99% of the biofilm. It is possible that access to the biofilm matrix in this instance has been assisted by bacteriophage depolymerases, which are thought to be responsible for the degradation of the EPS (Hughes *et al.*, 1998). In fact, Hanlon *et al.* (2001) have shown that the enzyme alone has the ability to disrupt the biofilm structure in the absence of the bacteriophage. Bacteriophages have also been engineered to express potent EPS-degrading enzymes (Lu & Collins, 2007), which could potentially allow for the development of tailored antibiofilm therapies.

The observed proportional reduction in biofilm by increasing bacteriophage titres detailed in this study correlates well with the observations of Corbin *et al.* (2001), who reported that increasing the MOI of the T4 phage by 100 resulted in greater disruption in *E. coli* biofilm integrity at 90 min than a 10-fold increase over the same time period.

The prevention of biofilm formation on the treated catheters is comparable with the work of Curtin and Donlan in that microscopic examination of bacteriophage-treated catheters demonstrates little evidence of biofilm formation (Curtin & Donlan, 2006; Fu *et al.*, 2010).

The potential of bacteriophage-releasing (or phage modified) medical device coatings (e.g. hydrogels, as used in this study) to prevent encrustation has not been studied as yet. However, successful prevention of biofilm formation would be expected to cause a reduction in, or the prevention of urinary encrustation, given the demonstrated role played by biofilm matrices in the stabilization of crystal growth and mineralization at biomaterial surfaces (Dumanski *et al.*, 1994; Rozalski *et al.*, 1997). The efficacy of phage treatment of established biofilms would be expected to be significantly diminished should the process of encrustation already be initiated with mineralized deposits present within the biofilm matrix, although further work is required in this area. In this case, bacteriophage treatment, in combination with bladder wash-out solutions, which can be used to dissolve encrusted deposits, may prove effective. This study demonstrates the potential utility of bacteriophage to reduce bacterial biofilms on medical device surfaces and to prevent their formation via direct incorporation and release of phage from device coatings. This approach, alone or in combination with standard therapeutic agents, may have the potential to reduce the significant, ubiquitous and burdensome spectre of device-associated infections.

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