

REVIEW ARTICLE

Phage therapy against *Enterococcus faecalis* in dental root canals

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Antibiotic resistance is an ever-growing problem faced by all major sectors of health care, including dentistry. Recurrent infections related to multidrug-resistant bacteria such as methicillin-resistant *Staphylococcus aureus*, carbapenem-resistant *Enterobacteriaceae*, and vancomycin-resistant enterococci (VRE) in hospitals are untreatable and question the effectiveness of notable drugs. Two major reasons for these recurrent infections are acquired antibiotic resistance genes and biofilm formation. None of the traditionally known effective techniques have been able to efficiently resolve these issues. Hence, development of a highly effective antibacterial practice has become inevitable. One example of a hard-to-eradicate pathogen in dentistry is *Enterococcus faecalis*, which is one of the most common threats observed in recurrent root canal treatment failures, of which the most problematic to treat are its biofilm-forming VRE strains. An effective response against such infections could be the use of bacteriophages (phages). Phage therapy was found to be highly effective against biofilm and multidrug-resistant bacteria and has other advantages like ease of isolation and possibilities for genetic manipulations. The potential of phage therapy in dentistry, in particular against *E. faecalis* biofilms in root canals, is almost unexplored. Here we review the efforts to develop phage therapy against biofilms. We also focus on the phages isolated against *E. faecalis* and discuss the possibility of using phages against *E. faecalis* biofilm in root canals.

Keywords: *phage therapy; dental biofilm; E. faecalis*

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Antibiotics, ‘the magic bullets’, have proved to be one of the most revolutionary discoveries of the twentieth century (1, 2). However, their overuse and misuse in various cases, including viral and fungal infections, and patient failure to follow the prescribed course have led to a rise in antibiotic-resistant strains, the ‘post antibiotic era’ (3). Consequently, many resistant pathogens like MRSA (methicillin-resistant *Staphylococcus aureus*), CRE (carbapenem-resistant *Enterobacteriaceae*), VRE (vancomycin-resistant enterococci) (4, 5), multidrug-resistance *Pseudomonas* and *Acinetobacter* have developed into major threats. For instance, VRE exhibit resistance to vancomycin, which is considered ‘the last resort’ drug for Gram-positive bacteria, making their elimination almost impossible (6, 7). The rate of acquired antibiotic resistance is also alarming. For example, *Pseudomonas aeruginosa* was

shown to rapidly develop resistance against five relevant antibiotics upon exposure to stepwise increased concentrations (8). Apart from being life threatening, these antibiotic-resistant strains also lead to elevated health care costs (9). Moreover, failure in surgeries and other medical procedures related to untreatable infections is expected to increase. Having said that, should we be alarmed that we are about to face an era similar to the one prior to the discovery of antibiotics, in which mortality will be caused by common infections?

Today, it is accepted that yet another reason for the failure of antibiotics is the formation of bacterial biofilms (10). Biofilms are defined as dense aggregates of surface-adherent microorganisms that are embedded in a self-produced polymer matrix consisting of polysaccharide, protein, and extracellular DNA (11, 12). Biofilms are

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characterized by the following factors: the environmental conditions and surfaces that favor their formation, the gene products that are required for their formation, the genes that are activated and required to maintain the biofilm, the architecture of the biofilm, and the types of extracellular products that are concentrated in the biofilm matrix (13). According to the National Institutes of Health, biofilms account for more than 60% of the microbial infections in the body (14). These infections can be caused either by a single microbial species or by a mixture of species (multispecies) (15). Some of the common examples of biofilm infections are cystic fibrosis, native valve endocarditis, otitis media, periodontitis, and chronic prostatitis (16).

A major problem of biofilms is their resistance against phagocytosis and their inherent tolerance to the host defense system, to antibiotic therapy, and to disinfectants like chlorine and alcohol, as well as heat (17–19). Factors like poor antibiotic penetration, nutrient limitation and slow bacterial growth, adaptive stress responses in bacteria, and formation of persister cells constitute a multilayered biofilm defense, which cannot easily be overcome (20). The common techniques used for eradication of biofilms include mechanical disruption by physical means, such as tooth brushing and sonication (21), but these are not 100% effective. The difficulties in destructing biofilms necessitate development of alternative ways to prevent and control biofilm-associated clinical infections.

In dentistry, bacterial biofilms are involved in almost all major diseases. Plaques which are actually multispecies biofilms growing on the teeth contain primary colonizers like streptococci on the acquired pellicle and are later colonized by *Actinomyces*, which may lead to caries (21). Periodontitis is a classic example of a biofilm-mediated disease, which is refractory to antibiotic agents and the host defenses (22). Lastly, the most common biological reason for root canal disease is endodontic biofilm (23, 24), which is formed significantly by *E. faecalis* (25, 26) commonly found in previously treated root canals along with other microorganisms.

Currently, endodontic treatment against *E. faecalis* and other root canal infections involves removing bacteria by biomechanical cleaning, root canal shaping, and disinfection followed by sealing and crown restoration. The purpose of root canal sealing is to provide a tight fluid seal from the coronal and apical part of the tooth. Ideally, endodontic treatment should achieve a sterile root canal system, but given the available materials and techniques, this is undoubtedly impossible (26).

The case study of *E. faecalis*

E. faecalis is a commensal Gram-positive facultative anaerobic bacterium inhabiting the gastrointestinal tract of humans and various animals, but is also found in environments like soil and water (27–29). *E. faecalis* is

one of the most frequently isolated species from hospital-associated infections; it causes endocarditis, bacteremia, urinary tract infections, meningitis, and other fatal forms of systemic and local infection in humans (30).

The pathogenicity of *E. faecalis* can be attributed to the various virulence factors reported in clinical strains, including biofilm formation and the expression of surface adhesion components (31). Additional virulence factors identified are hemolysin/bacteriocin, aggregation substance, gelatinase, enterococcal surface protein (Esp), endocarditis-associated antigen, or capsular polysaccharides (32–36). The ability of *E. faecalis* to adhere to medical devices such as ureteral stents and catheters and to develop biofilms on these devices is likely associated with its pathogenicity (37).

Why is *E. faecalis* so hard to eradicate?

E. faecalis has become one of the most challenging bacteria to eradicate in the past few decades (30, 38). As mentioned above, in root canals it is hidden from the immune system and antibiotics. Various antiseptic and antibiotic materials are used for intracanal bacterial eradication, which include calcium hydroxide or antibiotic pastes to improve bacterial control before root canal sealing. Yet, viable *E. faecalis* cells were found even after many days in root canals following endodontic treatment, regardless of the use of calcium hydroxide (39–41).

In addition to root canals, *E. faecalis* is also hard to treat in the gut and other infection sites (30). First, it has many strains that are antibiotic resistant (42–44). Second, the increased use of antibiotics in hospitals worldwide causes dysbiosis, changes in the gut microbiota that are leading to subsequent alterations in the local immune system (45, 46). *E. faecalis* takes advantage of these alterations and takes over ‘the prized niche’ of the gastrointestinal tract, and this niche may be the primary source of organisms that cause enterococcal infections (46–48).

Persistence

Another reason for the difficulty to eradicate *E. faecalis* infections is its highly recalcitrant nature. This bacterium possesses exceptional surviving abilities and can persist in extreme conditions such as the gut (49) and root canal system (50) as a result of its ability to withstand an alkaline milieu and glucose starvation (51, 52).

Antibiotic resistance

In addition to all that, *E. faecalis* strains are sometimes genetically resistant to antibiotics. According to the Centers for Disease Control and Prevention, VRE strains of *E. faecalis* are some of the most difficult to treat bacteria (www.cdc.gov/drugresistance/biggest_threats.html). Various studies conducted worldwide have demonstrated increasing rates of VRE-acquired cases; for example,

more than 38% of such cases were revealed in Detroit Medical Center, Michigan, in 2009 and 11% of the cases were reported at the national level (53). Vancomycin resistance has five well-recognized phenotypes: VanA, VanB, VanC, VanD, and VanE (54–56). Interestingly, two of these, VanA and VanB, are mediated by newly acquired gene clusters that provide resistant phenotypes primarily in *E. faecalis* and *E. faecium* (57). Thus, when such resilient strains as *E. faecalis* also evolve to be antibiotic-resistant like VRE, it becomes almost impossible to control their infections. Nowadays, linezolid and daptomycin are the last resort drugs often used to treat infections caused by VRE *E. faecalis* (58, 59). However, strains resistant even to these antibiotics have emerged (60, 61). A recent study suggested that clinical samples not only had vancomycin-resistant *E. faecalis* but the isolates also showed resistance to tetracycline, linezolid, and ampicillin (62).

E. faecalis in root canals

In dentistry *E. faecalis* is one of the main bacteria associated with chronic apical periodontitis in failed root canal treatments. Despite the fact that endodontic infections have a polymicrobial nature, the root canal environment may favor and support the survival of one species, which is commonly *E. faecalis*. Although *E. faecalis* is actually seldom present in primary endodontic infections, in cases of postendodontic treatment with apical periodontitis, failed cases are approximately nine times more likely to harbor *E. faecalis* than cases of primary infections (63, 64). Furthermore, the prevalence of *E. faecalis* in periradicular disease including secondary endodontic infections was reported to be 33% (65) and 24 to 77% in persistent infections (50, 63, 66) resulting in the development of lymphadenitis abscesses and cellulitis (26, 46).

The way *E. faecalis* causes failure of root canal treatment is by entering via micro-leakage in faulty restorations, direct pulp exposure in cases of physical barrier breaks, and the gingival sulcus that reaches the pulp chamber through the periodontal membrane (67). After penetrating the dentinal tubules, the root canal serves as a reservoir for bacteria that remain in the root canals protected from the immune system. These bacteria cause constant intracanal infections, endodontic diseases, and refractory or persistent periapical diseases (52, 68–70). They can also adhere to dentin collagen (main organic component of dentine), invade the dentinal tubules, and therefore withstand root canal debridement (70).

In addition, *E. faecalis* contaminations were found to correlate with periodontitis where it was found to be prevalence in root canals of teeth with apical periodontitis requiring endodontic retreatment, or in saliva (71).

The current infection control techniques in root canals fall short of the desired effectiveness against persistent infections. As antibiotics are useless, the endodontic

treatment aims to eradicate bacteria from root canal and dentin tubules by mechanical removal of infected tissues and concomitant chemical treatment with antiseptic solution such as sodium hypochlorite and chlorhexidine (mechanochemical preparation). Despite these procedures, bacterial contamination, mainly *E. faecalis*, is histologically evident in dentine tubules (72, 73). Furthermore, one of the disadvantages of root canal debridement is that it cannot prevent root canal late reinfection that may originate from the previously infected dentinal tubules. *Ex vivo* and clinical studies have shown that in spite of a temporary absence of bacteria following chemo-mechanical preparation, bacteria reappear following successive endodontic appointments. Antiseptic rinsing or antibacterial dressing reduces the bacterial counts; however, it does not completely eliminate the infecting bacteria (74, 75). This suggests that intratubular bacteria may serve as a reservoir, out of reach of endodontic preparation.

Biofilms of *E. faecalis* in root canals

Biofilms, layers of bacteria growing together in a cooperative manner (76, 77), are mechanically and physiologically more protected from antibiotics than planktonic cells (78). This can be because most of the antimicrobial agents cannot penetrate into the deeply formed layers of bacteria in a biofilm. They can kill only the peripheral layers, and once the effect of the antibacterial agent has diminished, the surviving bacteria can form new layers of biofilm. The genetic basis of biofilm formation by *E. faecalis* is largely unknown. A recent study suggested that a specific enterococcus cell surface protein (Esp) is critical for biofilm formation by this organism (79). Complete sterilization of an infected root canal is an important challenge in endodontic treatment, as because of the complexity of the root canal system, the traditional methods often cannot achieve sterilization (80). Various protective measures of the *E. faecalis* biofilm increase its resistance to antibacterial treatment. This includes resistance to traditional antibacterial rinsing solutions such as chlorhexidine or sodium hypochlorite and the ability to adapt and grow in the presence of calcium hydroxide (51, 81). Biofilm islands were reported to exist between the root canal filling and dentin walls despite root canal treatment (82, 83). In general, it is found that resistance of biofilm to antibiotics may even increase up to 100–1,000-fold (84). The physical removal of biofilm by endodontic instruments is only partially effective as biofilm may hide in areas unreachable by these instruments (85). Moreover, any surviving biofilm may potentially recover, grow further, and spread apically, thus perpetuating the chronic apical periodontitis.

Phage therapy: is it the answer?

The increasing number of cases with infections related to antibiotic-resistant bacterial strains and biofilm

formation, coupled with the failure of conventional measures to deal with them, necessitate the development and implementation of alternative methods. The use of bacteriophages against pathogenic bacteria, termed ‘phage therapy’, is one of the most promising methods being explored by scientists around the globe. A bacteriophage or phage is a virus that specifically targets and destroys disease-causing bacteria by invading bacterial cells, disrupting their metabolism, and causing lysis. Their lifecycle could also be lysogenic; however, for phage therapy, only lytic phages are used.

Phages were first discovered by Fedrerick Twort in 1915. Felix d’Herelle developed the use of phages to treat various infectious diseases between 1917 and 1940. Along with George Eliava, he founded the George Eliava Institute in Tbilisi, Georgia, which uses phage therapy against bacterial infections even today. However, when Alexander Fleming discovered penicillin in 1928, its rapid success overtook the interest in phage therapy. The emergence of new resistant strains and the acceptance that biofilm formation is a major problem leading to treatment failure has recently rekindled the interest in phage therapy. Phage therapy offers various benefits over antibiotics (86):

1. **High specificity:** The phages target specific pathogenic bacteria and pose no harm to the commensal microbiome of the body (87).
2. **Ease of isolation:** Phages are bacteria-dependent and hence can be found wherever their target bacteria are present. It seems that each bacterium may have hundreds of phages as reflected, for example, in the collection of PhageDB, where 1,153 and 116 phages were isolated against *Mycobacterium* and *Arthrobacter*, respectively (PhageDB.org).
3. **Possibility for clinical improvement:** With the development of molecular biology and genetic engineer-

ing, the possibility of transforming phages increased in the past decades.

4. **‘Single shot’:** Phage multiplication occurs in correlation with the growth of the bacteria. Hence, theoretically only a single shot is required (88).
5. **No residual:** Once the pathogenic bacteria have been completely eradicated from the host cell due to phage-induced lysis, because the phages can no longer grow without the bacterial host, it is expected that they will be shed from the cell without any harm to the cell (89).
6. **Biofilms destruction:** Biofilm destruction by phages is much more efficient when compared with antibiotics (Fig. 1) (90). The phages can infect the bacteria present in the upper layer of the biofilm and replicate to form new phages, which can penetrate the inner layers and infect the remaining bacteria. The continuous replication of the phages and subsequent infection and killing of biofilm bacteria lead to their eradication. Moreover, when the target bacteria are killed, the phage particles are subsequently eliminated as they no longer have a host. As shown in Table 1, numerous experiments have been performed to date using single phages or phage cocktails or even a combination of phages and antibiotics against biofilms. To a large extent, all these trials successfully eliminated the targeted bacterial biofilms from the infection sites or reduced bacterial counts to noticeably lower numbers. Also, various reports of natural lytic phages with phage-borne polysaccharide depolymerases have shown that phage-induced lysis and extracellular polysaccharide degradation are used in combination in natural systems to reduce bacterial biofilms (91–95). These depolymerases appear to be carried on the surface of phages and degrade bacterial capsular polysaccharides to allow access

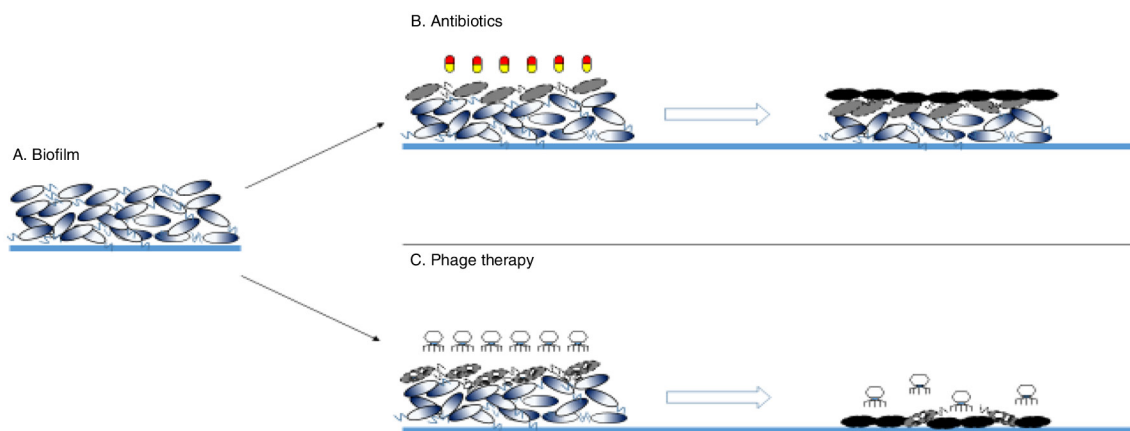


Fig. 1. Comparative analysis of the actions antibiotics and phages have on a mature biofilm. Antibiotics fail to penetrate the biofilm and only kill the bacteria superficially, and are thus unable to eradicate the biofilm. Phages, on the other hand, can infect bacterial cells on the outer layer of the biofilm, multiply, and in a chain reaction penetrate into the deeper layers, resulting in complete eradication of the biofilm in a single shot.

Table 1. Phage therapy trials on bacterial biofilms using different model systems

Bacteria	Model system	Phage treatment	Efficacy	References
<i>P. aeruginosa</i>	Catheters	Phage cocktail	99.9%	(97)
<i>P. aeruginosa</i>	Cystic fibrosis in lung airway cells	Single phage	75%	(98)
<i>P. aeruginosa</i>	Mouse wound model	Phage cocktail	Significant 2 log decrease	(99)
<i>Proteus mirabilis</i>	Catheters	3-phage cocktail	Complete prevention of blockage	(100)
<i>S. epidermidis</i>	Catheters	Single phage	–	(101)
<i>S. aureus</i>	Rabbit ear wound model	Single phage combined with debridement	Significant improvement in wound infection	(102)
<i>E. coli</i>	Urothelium	Single phage	45%	(103)
<i>P. aeruginosa</i>	<i>In vitro</i> biofilm from hospital isolates	Single phage	Highly efficient in prevention and dispersion of pre-formed biofilm	(104)
<i>S. aureus</i> and <i>S. epidermidis</i>	<i>In vitro</i> biofilm	Single phages and combined mixture of two phages	High efficiency in disrupting mono-species as well as dual-species biofilm.	(95)
<i>P. fluorescens</i>	<i>In vitro</i> biofilm grown on glass slides	Single phage	93% cell removal at early stage of biofilm formation and prevention of biofilm formation	(105)
MRSA and <i>P. aeruginosa</i>	Implant-related infection	Single phage combined with antibiotics	MRSA: biofilm absent <i>P. aeruginosa</i> : no significant difference	(106)
<i>S. aureus</i>	<i>In vitro</i> biofilm	Single phage with antibiotic	Highly efficient as combined effect	(107)
<i>P. aeruginosa</i>	<i>In vitro</i> biofilm and extracted tooth model for root canal treatment	Single phages and combined mixture of two phages	Highly effective against <i>in vitro</i> biofilm. No significant effect in extracted tooth model	(108)
<i>E. faecalis</i>	Human dental roots	Single phage	Substantial reduction in bacterial cell viability	(109)

The phage therapy here involves either use of single phages or phage cocktails or combination treatments where phages are used along with antibiotics or previous clinical treatments.

to bacterial cell surfaces (93). In addition, biofilm dispersion can be achieved by engineering a bacteriophage to express a biofilm-degrading enzyme during infection (96). Thus, using bacteriophages as single phage or a combination of phages in cocktails could be a good approach for the treatment of biofilms in infectious bacterial diseases.

Phage therapy and human safety

Given the numerous advantages and the potential benefits of bacteriophages over the failing antibiotics, it is no wonder that scientists worldwide are delving into phage therapy. To the uninitiated, phage therapy not only holds great promise, but sets off alarm bells as well: is it safe for humans? This concern, although understandable, is virtually unfounded, as this method has been in use treating humans for decades. Indeed, it was first used over a century ago in France by Felix d'Herelle in 1919 to treat children suffering from severe dysentery (110). Since then, many such trials have been conducted in France, Georgia, Poland, and many other places worldwide (111, 112).

A recent trial was successfully performed in a patient suffering from an eye infection by Fadlallah et al., in 2015

at the Phage Therapy Center in Tbilisi, Georgia (113). Many similar trials have achieved success without any harmful side effects (114–117). Phages were also found to have almost no harmful effects on the non-target microbiome (118). Interestingly, phages exist everywhere in nature, and although up to date they have never been found to cause any harm or diseases in humans, there are certain temperate phages that might contain exotoxins that have harmful effects (119–121). However, pharmacological studies coupled with genetic tools, which are well-established nowadays, can help choose and purify the right target phage and remove unwanted virulence genes. Thus, in principal, phage therapy can be considered as a relatively safe technique (122).

Phages against *E. faecalis*

For combatting VRE *E. faecalis* infections, phages have been isolated and tested for their efficacy by several researchers (Table 2). Most of these phages belong to the *Myoviridae* or the *Siphoviridae* families of tailed phages. In case of the phage IME-EF1, when administered intraperitoneally in a murine sepsis model, one dose of IME-EF1 or its endolysin was found to reduce the bacterial blood

Table 2. Phages isolated against *E. faecalis*, their accession numbers, and the family they belong to. This indicates the huge possibility of using these phages for phage therapy in the future.

Phages of <i>E. faecalis</i>	Lytic/lysogenic phage	Accession number	Family	References
phiEF24C	Lytic	AP009390.1	<i>Myoviridae</i>	(126)
ECP3	Lytic	KJ801817.1	<i>Myoviridae</i>	Unpublished
IME-EF1	Lytic	KF1920531	<i>Siphoviridae</i>	(123)
SAP6		JF731128.1	<i>Siphoviridae</i>	(76)
BC611		AB712291.1	<i>Siphoviridae</i>	(127)
EfaCPT1		JX193904.1	<i>Siphoviridae</i>	Unpublished
EFDG1	Lytic	KP339049.1	<i>Myoviridae</i>	(128)
EFLK1	Lytic	KR049063.1	<i>Myoviridae</i>	(129)
Q69				(125)
Phi4D			<i>Myoviridae</i>	(130)
IME_EF3	Lytic	KF728385	<i>Siphoviridae</i>	(131)
EFRM31	Lytic	GU815339	<i>Siphoviridae</i>	(132)
EFRM42	Lytic		<i>Siphoviridae</i>	(132)
EFRM54	Lytic		<i>Siphoviridae</i>	(132)
PhiFL1A	Lysogenic	GQ478081	<i>Siphoviridae</i>	(133)
PhiFL1B	Lysogenic	GQ478082	<i>Siphoviridae</i>	(133)
PhiFL1C	Lysogenic	GQ478083	<i>Siphoviridae</i>	(133)
PhiFL2A	Lysogenic	GQ478084	<i>Siphoviridae</i>	(133)
PhiFL2B	Lysogenic	GQ478085	<i>Siphoviridae</i>	(133)
PhiFL3A	Lysogenic	GQ478086	<i>Siphoviridae</i>	(133)
PhiFL3B	Lysogenic	GQ478087	<i>Siphoviridae</i>	(133)
PhiFL4A	Lysogenic	GQ478088	<i>Siphoviridae</i>	(133)
EFC-1	Lysogenic	KJ608188	<i>Siphoviridae</i>	(134)
Phi EF11	Lysogenic	GQ452243	<i>Siphoviridae</i>	(135)
vB_EfaS_GEC_EfS_3			<i>Siphoviridae</i>	(136)
Phi FC1	Lysogenic			(137)
F4		EF653454		(138)

count and protect the mice from a lethal challenge of *E. faecalis*, with a survival rate of 60 or 80% (123). Similarly, *in vivo* therapeutic potential of ϕ EF24C evaluated in a sepsis BALB/c mouse model proved to be effective at a low concentration with no host sensitivity and no change in mouse lethality following a single or repeated phage exposure (124). In addition, the Q69 phage eradicated an *E. faecalis* strain mainly responsible for biogenic amines (BA) tyramine accumulation in food, which is considered as a toxicological hazard (125).

Phage therapy against *E. faecalis* biofilms

Apart from planktonic bacteria eradication, a more challenging and relevant part of *E. faecalis* infections is eliminating its biofilms. So far, among the *E. faecalis* described phages (Table 2), only EFDG1 was tested on *E. faecalis* biofilms (128). EFDG1, isolated from sewage water, was very efficient in nearly eliminating a 2-week-old *E. faecalis* biofilm of $\sim 100 \mu\text{M}$ thickness. Evaluating the biofilm biomass showed a fivefold reduction within 7 days in the phage-treated samples compared with the

untreated biofilms, which were stable and showed no reduction. Bacterial viable counts from the biofilm supported this notion by showing a five log reduction compared with the untreated biofilms. Scanning electron microscopy revealed the destruction of the treated biofilm which looks like clumps of distributed bacteria in comparison with the intact, untreated biofilm (Fig. 2).

The activity of EFDG1 was further tested in post-treated root canal infections (128) using an *ex vivo* two-chamber bacterial leakage model of human teeth (139). Measurements of bacterial leakage from the root apex showed that the obturated root canals subjected to EFDG1 irrigation resulted in dramatic reduction of eight logs in bacterial leakage compared with the conventional sample. Confocal microscopy images of horizontal root sections demonstrated that live bacteria were evident in the dentinal tubules of the control group, whereas dead bacteria were seen in the phage-treated teeth (128).

These results indicate that phage therapy might be a worthy additive solution in combatting *E. faecalis* biofilms in root canals where all other anti-infective and aseptic

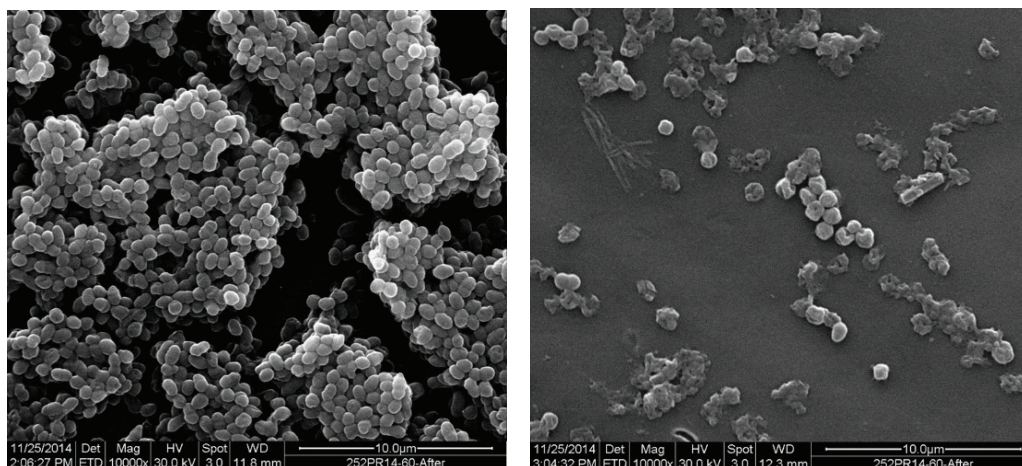


Fig. 2. Scanning electron microscopy images of untreated and treated biofilms with the phage EFDG1. The image on the left shows a well-developed biofilm, while the right image is one with EFDG1 phage treatment. Both the biofilms are 2 weeks old.

technique strategies, including the current use of increased apical preparation sizes, and inclusion of chlorhexidine in combination with sodium hypochlorite, fail (66, 140, 141).

Future perspectives

A lot is known about the importance of phages in nature (142, 143); however, the phage–bacteria interaction in the oral microbiome still needs to be explored. Moreover, using phages to remove specific bacteria from the microbiome will allow us to study the role of their host in the microbiome and identify keystone pathogens in various infections. Thus, the use of phages will be beneficial both in gaining knowledge about oral pathogens and in removing them. Understanding the oral microbiome with the help of phages can potentially lead to the development of ‘microbiome engineering’ to prevent infections. Using ‘good’ bacteria as a probiotic (118) and phages against the pathogens might be a new avenue yet to be explored in oral health. However, the inadequate number of phages which can specifically target oral bacteria raise the need for the isolation and characterization of more phages against oral pathogens, for example, the ones responsible for root canal infections.

In conclusion, considering all the available positive outcomes from the usage of phages against not only *E. faecalis* but also other bacteria in biofilms, phage therapy appears to be a tool against infectious biofilms. In the future, phages such as EFDG1 and other phages of *E. faecalis* like phiEF24C, IME-EF1, and EFLK1 can be used either as cocktails or as combinations with antibiotics to combat VRE *E. faecalis* in dental biofilms. In root canal treatments, although alternative antibacterial irrigants (such as chlorhexidine and sodium hypochlorite) were shown to be effective, they still do not prevent recurrent *E. faecalis* infections. Consequently, combinations of anti

E. faecalis phages and antibacterial agents can benefit the host by reducing the chances of recurrent infections.

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