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COMMENTARY

Modification of phage for increased antibacterial effect towards dental biofilm

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Introduction of new genetic engineering techniques has enabled a more precise modification of bacteriophage genomes in basic science and engineering. Pires et al. (1), in a review paper, discuss advances in genetically engineered phages over the last decade. The present commentary focuses on the aspect of modification of phage for enhanced antibacterial activity in dental biofilms.

Biofilms consist of bacteria transferred from a free-swimming (planktonic state) to a multitude of bacterial cells encased in a self-produced polysaccharide matrix of hydrated extracellular polymeric substances (2). Their structure is complex being filled with pillar-formed mature macrocolonies surrounded by fluid-filled channels (3). These structured microbial communities are characterized by reduced metabolic activity, particularly in the inner layers. Another important feature is their association with chronic infections such as *Pseudomonas aeruginosa* infection in cystic fibrosis, *Staphylococcus epidermidis* and *S. aureus* infections, urinary tract infection, periodontal disease, and root canal infection. It is well known that biofilm infections can be difficult to eradicate with antimicrobials. Thus, a 100- to 1,000-fold increase in antimicrobial tolerance to biofilms compared to planktonic cells has been reported (4). This effect is often related to the biofilm matrix that can limit diffusion of molecules and particles, or to reduced bacterial metabolism (3, 5, 6).

After their discovery in the early 20th century, bacteriophages were considered to have a great potential as antibacterial agents. Due to poorly controlled clinical trials and inconsistent results, this potential has still to be realized (1). The discovery of penicillin in 1928 and the arrival of the antibiotic era also reduced the interest for phage therapy, at least in the West, while its use continued in Eastern Europe and the former Soviet Union (7). In recent years, the increase in multidrug-resistant bacteria has renewed the interest for using phages as antimicrobial agents, recently also in the oral cavity (8). Gene engineering has made it possible to modify these bacterial viruses so that they can precisely control and detect bacteria and serve as new sources of antibacterials (1). They are also being developed as vehicles for drug delivery and vaccines and for assembly of new materials.

To increase the efficiency of phage therapy against biofilms, Lu and Collins (9) engineered a T7 phage to express the biofilm-degrading enzyme dispersin B (DspB). Interestingly, the *dsB* gene from *Aggregatibacter actinomycetemcomitans* was cloned downstream of the T7select415-1 *10B* capsid gene under the control of the T7φ 10 promoter. The phage created was efficient against *Escherichia coli* TG1 biofilms and reduced biofilm cell counts by ~4.5 orders after treatment for 24 h. This reduction was ~2 orders of magnitude more than that achieved by the wild-type nonenzymatic phage. In future work, this technology might involve other enzymes that could target the heterogeneous extracellular composition of dental biofilms to improve their eradication.

Also, the T7 phage has been engineered to encode an enzymatic interference with quorum sensing (10). This is a bacterial cell–cell communication system involved in biofilm formation (11, 12). Here, the engineered phage T7aiiA was created by cloning the acyl-homoserine lactone lactonase (AHL-lactonase) gene *aiiA* from *Bacillus anthracis* into the T7select415-1 phage vector (9, 10). The quorum-quenching enzyme inactivates acyl-homoserine lactone (AHL), which is a quorum-sensing molecule, by hydrolyzing its lactone bonds (13). In order to test the effect of quorum-sensing phage T7aiiA on biofilm formation, *E. coli* and *P. aeruginosa* were mixed together to form biofilms in the presence of the engineered or wild-type phage for 4 and 8 h (10). Interestingly, phage T7aiiA

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reduced the biofilm biomass by 74.9 and 65.9% after 4 and 8 h, respectively. In contrast, the control T7 phage gave only a 23.8 and 31.7% reduction, respectively, in comparison with no phage.

Quorum sensing is critical for virulence and biofilm formation for oral pathogens. The ability to interfere with bacterial quorum sensing could provide a sophisticated means for manipulating the composition of pathogenic biofilms and possibly eradicate oral infection.

It should be noted that the oral cavity is not a foreign area to phages as many of them have active roles in shaping the ecology of oral bacterial communities acting both as commensals and pathogens exceeding the number of bacteria in human gums ($>$35:1) (reviewed in (14)). Santiago-Rodriguez et al. (15), using RNA sequencing, found that reads homologous to siphoviruses that infect the phylum Firmicutes were among the most prevalent transcriptome reads both in periodontal health and disease. However, the expression of some genes from the lytic phage module was significantly higher in subjects with biofilm-induced periodontal disease, indicating that periodontitis favors the expression of lytic phages.

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There is no conflict of interest in the present study for the author.

References

1. Pires DP, Cleto S, Sillankorva S, Azeredo J, Lu TK. Genetically engineered phages: a review of advances over the last decade. Microbiol Mol Biol Rev 2016; 80: 523–43.
2. Flemming HC, Wingender J. The biofilm matrix. Nat Rev Microbiol 2010; 8: 623–33. doi: http://dx.doi.org/10.1038/nrmicro2415
3. Olsen I. Biofilm-specific antibiotic tolerance and resistance. Eur J Clin Microbiol Infect Dis 2015; 34: 877–86. doi: http://dx.doi.org/10.1007/s10096-015-2323-z
4. Ceri H, Olson ME, Stremick C, Read RR, Morck D, Buret A. The Calgary Biofilm Device: new technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. J Clin Microbiol 1999; 37: 1771–6.
5. O'Toole G, Kaplan HB, Kolter R. Biofilm formation as microbial development. Annu Rev Microbiol 2000; 54: 49–79. doi: http://dx.doi.org/10.1146/annurev.micro.54.1.49
6. Watnick P, Kolter R. Biofilm, city of microbes. J Bacteriol 2000; 182: 2675–9. doi: http://dx.doi.org/10.1128/JB.182.10.2675-2679.2000
7. Olsen I, Handal T, Løkken P. Bacteria-killing viruses, Stalinists and 'superbugs'. Tidsskr Nor Laegeforen 2001; 121: 3197–200. [Article in Norwegian, abstract in English].
8. Khalifa L, Shlezinger M, Beyth S, Houri-Haddad Y, Coppenhagen-Glazer S, Beyth N, et al. Phage therapy against *Enterococcus faecalis* in dental root canals. J Oral Microbiol 2016; 8: 32157. doi: http://dx.doi.org/10.3402/jom.v8.32157
9. Lu TK, Collins JJ. Dispersing biofilms with engineered enzymatic bacteriophage. Proc Natl Acad Sci USA 2007; 104: 11197–202.doi http://dx.doi.org/10.1073/pnas.0704624104
10. Pei R, Lamas-Samanamud GR. Inhibition of biofilm formation by T7 bacteriophages producing quorum-quenching enzymes. Appl Environ Microbiol 2014; 80: 5340–8. doi: http://dx.doi.org/10.1128/AEM.01434-14
11. Davies DG, Parsek MR, Pearson JP, Iglewski BH, Costerton JW, Greenberg EP. The involvement of cell-cell signals in the development of bacterial biofilm. Science 1998; 280: 295–8. doi: http://dx.doi.org/10.1126/science.280.5361.295
12. Sakaguri Y, Kolter R. Quorum-sensing regulation of the biofilm matrix genes (pel) of *Pseudomonas aeruginosa*. J Bacteriol 2007; 189: 5383–6. doi: http://dx.doi.org/10.1128/JB.00137-07
13. Dong YH, Wang LH, Zhang HB, Zhang LH. Quenching quorum-sensing-dependent bacterial infection by an N-acyl homoserine lactonase. Nature 2001; 411: 813–7. doi: http://dx.doi.org/10.1038/35081101
14. Edlund A, Santiago-Rodriguez TM, Boehm TK, Pride DT. Bacteriophage and their potential roles in the human oral cavity. J Oral Microbiol 2015; 7: 27423. doi: http://dx.doi.org/10.3402/jom.v7.27423
15. Santiago-Rodriguez TM, Naidu M, Abeles SR, Boehm TK, Ly M, Pride DT. Transcriptome analysis of bacteriophage communities in periodontal health and disease. BMC Genomics 2015; 16: 549. doi: http://dx.doi.org/10.1186/s12864-015-1781-0