Journal of Biotechnology 250 (2017) 29–44

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

The use of bacteriophages to biocontrol oral biofilms

Szymon P. Szafrański*, Andreas Winkel, Meike Stiesch*

Lower Saxony Centre for Biomedical Engineering, Implant Research and Development (NIFE), Stadtfelddamm 34, D-30625 Hannover, GermanyDepartment of Prosthetic Dentistry and Biomedical Materials Science, Hannover Medical School (MHH), Carl-Neuberg-Strasse 1, D-30625 Hannover, Germany

ARTICLE INFO

Article history:
Received 7 October 2016
Received in revised form 9 January 2017
Accepted 10 January 2017
Available online 17 January 2017

Keywords:
Bacteriophage
Biofilm
Lysin
Oral cavity
Periodontal disease
Phage therapy

ABSTRACT

Infections induced by oral biofilms include caries, as well as periodontal, and peri-implant disease, and may influence quality of life, systemic health, and expenditure. As bacterial biofilms are highly resistant and resilient to conventional antibacterial therapy, it has been difficult to combat these infections. An innovative alternative to the biocontrol of oral biofilms could be to use bacteriophages or phages, the viruses of bacteria, which are specific, non-toxic, self-proliferating, and can penetrate into biofilms. Phages for *Actinomyces naeslundii*, *Aggregatibacter actinomycetemcomitans*, *Enterococcus faecalis*, *Fusobacterium nucleatum*, *Lactobacillus* spp., *Neisseria* spp., *Streptococcus* spp., and *Veillonella* spp. have been isolated and characterised. Recombinant phage enzymes (lysins) have been shown to lyse *A. naeslundii* and *Streptococcus* spp. However, only a tiny fraction of available phages and their lysins have been explored so far. The unique properties of phages and their lysins make them promising but challenging antimicrobials. The genetics and biology of phages have to be further explored in order to determine the most effective way of applying them. Studying the effect of phages and lysins on multispecies biofilms should pave the way for microbiota engineering and microbiota-based therapy.

1. Introduction

In most habitats, including the human body, microorganisms reside in biofilms, *i.e.* surface-attached aggregates embedded in a matrix of extracellular polymeric substance. The biofilm matrix consists of polysaccharides, structural proteins, enzymes, DNA, lipids, and water. The biofilm protects its inhabitants from environmental challenges, *e.g.* phagocytosis, and allows long-term colonisation, and spatial organisation (Flemming et al., 2016). Physical and chemical gradients provide diverse niches for microorganisms. Biofilms are shelters for a dynamic community of interacting microbes. Members of mixed biofilms profit from synergistic interactions such as co-aggregation, and allow colonisation, sharing of extracellular enzymes, cross-feeding, and cross-protection. Competition between community members controls ecological succession and triggers segregation. The close distance between cells in the biofilm facilitates microbial communication (quorum sensing), *i.e.* synchronised, population-wide response to a changing environment (Sztajer et al., 2014). Interspecies interactions shape the overall activity of the biofilm and can positively or negatively impact human health (Peters et al., 2012).

There is usually ***homeostasis*** between the host and associated biofilms, *e.g.* in the oral cavity, gastrointestinal tract, or vagina. Commensal flora are beneficial, since they hinder colonisation of pathogens (at all body sites), provide nutrients to the host, and positively influence the immune system and developmental processes (mainly in the gut) (He et al., 2014; Ma et al., 2012; Sekirov et al., 2010). Certain environmental or genetic factors can however induce ***dysbiosis*** – microbial imbalance that harms the host body. Dysbiosis can develop gradually or rapidly and often leads to chronic destructive inflammation (Lamont and Hajishengallis, 2015). Opportunistic pathogens dysregulate the host immune defence and elevate the virulence of the whole community. As a result, the host tissue is damaged by autoimmunity and synergistic activities of microorganisms. Dysbiotic biofilms benefit from impaired host defence and nutrients released by damaged tissue. Health-associated commensals are outcompeted. The process escalates, since inflammation/tissue damage and dysbiosis reinforce each other.

Biofilm infections are persistent and therefore hard to prevent and cure (Donlan and Costerton, 2002). Biofilm matrix reduces the penetration of antimicrobials. Sessile cells grow more slowly and consequently are less susceptible to antibiotics that target metabolic processes. Localised gradients in biofilms provide

* Corresponding authors.
E-mail addresses: Szafranski.Szymon@mh-hannover.de (S.P. Szafrański), Stiesch.Meike@mh-hannover.de (M. Stiesch).

http://dx.doi.org/10.1016/j.jbiotec.2017.01.002

niches where cells can persist and initiate relapse of the disease. Additionally, antibiotic resistant strains can emerge during antibiotic therapy. Pathogenic biofilms can spread systematically and colonise body parts (including prosthetic devices) that are normally sterile, such as heart valves. In such a case, the therapeutic endpoint has to be complete eradication of the biofilm. Antibiotic treatment of dysbiotic biofilm also affects protective health-associated flora and can facilitate secondary infections, *e.g.* candidiasis. Alternatively, microbiota-targeted therapy can be applied to specifically eliminate opportunistic pathogens and re-establish homeostasis (Guo et al., 2015; Lemon et al., 2012).

Oral biofilms (Fig. 1) cause most prevalent polymicrobial infections. The oral microbiome is one of the best studied human-associated habitats (Dewhirst et al., 2010). Oral bacteria, archaea, viruses, fungi and protozoa can thrive in biofilms that are normally controlled by saliva flow, the host immune defence, and daily oral hygiene. Environmental factors, like carbohydrate-rich diet, smoking, chemotherapy or radiation treatments, and host genetics (*e.g.* lactoferrin gene polymorphism, Papillon–Lefèvre syndrome) can favour expansion of opportunistic pathogens that tip a balance from oral health towards dysbiosis. Dental caries, as well as periodontal, and peri-implant disease, are good examples of these.

In dental caries, acidogenic enamel-attached bacteria like streptococci metabolise dietary carbohydrates to organic acids. The frequent acidification favours demineralisation of enamel and selects for more aciduric species, like *Streptococcus mutans*, that further lower the pH. Demineralisation of enamel and degradation of organic matrix in dentin leads to cavity formation and expansion (Takahashi and Nyvad, 2011, 2016).

In periodontal disease, periodontopathogens like *Porphyromonas gingivalis,* synergistically disarm host defence systems and induce destructive inflammation of the tissue surrounding a tooth (Lamont and Hajishengallis, 2015; Teles et al., 2013). The mild form of the disease called gingivitis can progress to periodontitis that is characterised by the loss of alveolar bone supporting the tooth and, in the most severe form, can lead to tooth loss. During disease progression, proteolytic and immunogenic species expand, since inflamed gum is a rich source of proteins and peptides. The autoimmune reaction and dysbiotic biofilms are mutually reinforcing phenomena. Dental implants used to replace teeth are also readily colonised by periodontopathogens that can even quicker damage peri-implant tissue, and ultimately cause implant failure (Robitaille et al., 2015).

Dental caries and periodontitis affect 91% and 46% of adult western populations, respectively (Dye et al., 2015; Eke et al., 2015). Prevalences of 43% and 22% have been estimated for peri-implant mucositis (inflammation in the soft tissue surrounding a dental implant) and peri-implantitis (characterised by the loss of bone supporting the dental implant), respectively (Derks and Tomasi, 2015). The oral biofilm-associated diseases greatly influence quality of life, systemic health, and expenditure (Beikler and Flemmig, 2011; Hajishengallis, 2015). Good oral hygiene, low dietary carbohydrate intake and non-smoking favours but does not guarantee oral health. If disease develops, conventional therapy is applied. Dysbiotic biofilms are mechanically removed (using dental drills, ultrasonic instruments, periodontal scalers and curettes), treated with antiseptics, and sometimes with antibiotics. A conventional treatment is expensive and not always successful, therefore new prophylactics and adjuvant therapeutics are desirable (Flemmig and Beikler, 2011). Here, we discuss a concept of using bacteriophages to biocontrol oral biofilms.

1.1. Biology of bacteriophages

Bacteriophages, or short phages, are viruses that predate prokaryotes. In this review, we focus on tailed, double-stranded DNA phages that make up 96% of all phage isolates. Their taxonomy is as follows. Briefly, they are divided into three families: *Myoviridae*, encompassing large virions (phage particles) with a long contractile tail; *Siphoviridae*, including virions with a long flexible but non-contractile tail; and *Podoviridae*, embracing small virions with a short non-contractile tail (Ackermann, 2009). Tailed phages are obligatory parasites that are either strictly lytic or temperate (Hobbs and Abedon, 2016). All phages recognise receptors on the bacterial prey and inject DNA. Strictly lytic phages (also called virulent phages) use the host biosynthetic machinery to replicate, assemble to the phage particles, and finally lyse the host, liberating the progeny virions and completing a lytic cycle. Temperate phages can undergo either the lytic or the lysogenic cycle, depending on the state of the host cell. In lysogenic cycle, after injection, phage DNA is usually integrated within a host "chromosome" (or sometimes replicated as a plasmid) and stays dormant (as prophage) for many host (lysogen) generations. DNA damage can induce prophage to enter the lytic cycle at a stage of replication. The constant evolutionary arms race takes place between bacteria tuning the phage resistance mechanism – *e.g.* blocking phage binding, preventing phage DNA entry, or degrading phage DNA – and phages rapidly changing to bypass bacterial defence mechanisms (Labrie et al., 2010; Samson et al., 2013). On the other hand, phages can increase the fitness of their host by facilitating gene exchange within a bacterial population and transferring the new functional genes coding for metabolic enzymes, toxins, or adhesins (Brussow et al., 2004).

1.2. Bacteriophages as therapeutics

Almost from their discovery a century ago, phages have been studied as potential therapeutics. Medical use of phages has a long-standing tradition in Georgia, Poland and Russia (Abedon et al., 2011; Miedzybrodzki et al., 2012). Therapeutic phages awoke worldwide interest due to the emerging problem of antibiotic resistance and the appreciation of the role of the microbiome in the human health (Vandenheuvel et al., 2015). Half of prioritised approaches alternative to antibiotics are based on phages (Czaplewski et al., 2016), including wild type phages, engineered phages, and phage lytic enzymes, known as lysins. Phages possess unique properties that make them interesting but challenging candidates for therapeutics (Loc-Carrillo and Abedon, 2011; Lu and Koeris, 2011).

Phages have usually a narrow host range, so they are generally specific for a small set of strains of the same species. Some phages might reveal a wide host range, *e.g.* staphylococcal phage K, Sb-1, and Stau2 (Hsieh et al., 2011; Kvachadze et al., 2011; O'Flaherty et al., 2005). Cross-infection can sometimes occur, *e.g.* polyvalent phage K, SK311, U16, ϕ131, ϕ812 target multiple *Staphylococcus* species (O'Flaherty et al., 2005; Pantucek et al., 1998), but in most cases is limited to closely related species and rarely to higher taxonomic units. Therefore, in contrast to broad range antibiotics, phages should be able to eliminate pathogens without affecting indigenous flora. In fact, if a phage uses a bacterial virulence factor as receptor, it should target the "virulent" subpopulation only (Laanto et al., 2012). Similarly, interspecies bacterial interaction should be disrupted if a phage receptor is mediating it, *e.g.* as adhesin. This opens up the perspective of using phages as precise tools for microbiome engineering and microbiota-based therapy. If broader activity is required, phage isolates can be applied in cocktails (Chan et al., 2013). Alternatively, phages can be genetically engineered to broaden their host range (Ando et al., 2015).

Phages proliferate in infected bacteria. A strictly virulent phage produces usually more than 100 copies of itself during every successful infection. Prophylactic treatment could be based on increasing the primary dose as long as the infection is not cleared. Therapeutic phages preferably have a long lifetime, large burst size