ToothPhage™: Elimination of *P. gingivalis*, the etiological agent of periodontitis

Marleen van Smoorenburg, Master Infection & Immunity, Utrecht University

Abstract

***Porphyromonas gingivalis* is a Gram-negative bacterium present in the oral cavity and a major causative agent in the initiation and progression of severe forms of periodontitis. As a member of the oral microbiota comprising over 700 distinct bacterial species, this anaerobic bacterium can become highly destructive. It can elicit a severe immune response and when left untreated, result in alveolar bone resorption and loss of teeth. As current treatment of periodontitis is time-consuming and not fully effective, the goal of this project is to develop a formulation of bacteriophages as an easy-to-use at-home treatment for periodontitis. We hypothesize that ToothPhage™, a toothpaste containing bacteriophages specifically targeting pathogenic *P. gingivalis* strains, will eliminate the causative agent of periodontitis and consequently lead to restoration of the oral symbiotic microbiota.**

Introduction and problem definition

Oral microbial communities belong to the most complex microbiota in the human body. The oral cavity contains several habitats, all colonized by distinct microbial communities with a total of over 700 different bacterial species[1,2]. Usually there is homeostasis between the host and the microbiota however, certain genetic or environmental factors can induce a state of dysbiosis, in which the microbial imbalance is harmful to the host[3]. Dysbiosis in the oral microbiome is mainly responsible for two human diseases: dental caries (tooth decay) and periodontitis (gum disease). While Gram-positive streptococci are generally responsible for supragingival plaque (dental plaque above the gum line) leading to dental caries, Gram-negative anaerobic bacteria dominate subgingival plaque (below the gum line), responsible for periodontitis[2].

Periodontitis is an inflammatory disease destroying tissues surrounding the tooth, which can eventually lead to tooth loss. Periodontitis comprises various categories, related to disease severity. It is the second most common dental disease worldwide and its general incidence increases with age. The incidence of periodontitis in the Dutch population is estimated at 10% however, over the age of 50, up to 30% of people have a mild to severe form of periodontitis. Chronic periodontitis is the most common form of the disease in people over 36 years of age. The disease is clinically characterized by redness and swelling of the gingiva (gums), deepening of the pockets, attachment loss and alveolar bone resorption[4]. Risk factors are smoking, certain medication and bad oral hygiene.

Porphyromonas gingivalis, a Gram-negative anaerobic bacterium belonging to the phylum Bacteroidetes, is considered the etiological agent in (especially) the onset and progression of chronic periodontitis[5]. The biofilms initiated by the periodontopathogen, enhances virulence factors of other bacteria, elicits an immune response and can result in bone and soft tissue destruction[2]. Generally, the disease progression is slow to moderate however, patients might have periods of rapid progression. The symptoms can lead to speech- and eating problems, substantially influencing daily life and negatively affecting quality of life[4]. Current treatment comprises the mechanical or surgical removal of plaque by a dentist, frequently supplemented with systemic antibiotics and instructions on oral hygiene. Absence of treatment leads to an increased inflammatory reaction, increased alveolar bone resorption, and consequently to loss of teeth. Furthermore, periodontitis is associated with an increased risk on head and neck squamous cell carcinoma (HNSSC) specifically in the oral cavity, with

each additional millimetre of alveolar bone loss (ABL), associated with an >4-fold increased risk on HNSSC[6]. Besides, evidence is emerging that periodontitis is associated with an increased risk of developing coronary heart disease (CHD)[7].

Current treatment is suboptimal, patients need to see a dentist multiple times and the measures taken are often rough and aspecific. For example, the use of systemic antibiotics. Besides the target bacterium, antibiotics affect the microbiota throughout the body and negatively influences homeostasis. Working towards an antibiotic-free future, we are in need of specific treatments that take away the etiological agent of periodontitis. We think there is a need here for an at-home and easy-to-use application for the elimination of *P. gingivalis*, and consequent restoration of the symbiotic microbiota.

Aim

The aim of this project is to develop a formulation of bacteriophages for topical application in treatment of periodontitis.

Hypothesis

Application of *Porphyromonas gingivalis*-specific bacteriophages to the oral cavity of people suffering from periodontitis, will lead to elimination of *P. gingivalis*, one of the major etiological agents of periodontal disease, and consequently lead to restoration of the oral symbiotic microbiota.

Approach

Bacteriophages

We want to make use of *P. gingivalis*-specific bacteriophages to eliminate the main causative agent of periodontitis. The use of bacteriophages, endogenous killers of bacterial cells, has various advantages. Phages very specifically target a certain species, or even strain, thereby not disturbing the commensal flora[8]. Besides they are "auto-dosing", meaning that replication of phages at the site of infection leads to an increase in local titre. Additionally, use of phages is favourable compared to antibiotics regarding the quick and cheap development, the ability to lyse antibiotic-resistant strains, and that so far no side effects have been observed[8,9]. Challenges of the use of phages in general are that the causative agent should be known, fortunately in our case this has been resolved. Additionally, there is still a challenge in legislation surrounding this very promising new field of research in bacteriophage treatment that is gaining in popularity[8]. Nonetheless, we consider the use of phages to treat periodontitis a promising new application.

Application

Next, we considered the formulation of applying *P. gingivalis*-specific bacteriophages specifically to the oral cavity. Literature provided us with a semi solid formulation of bacteriophages that has proven to work in the treatment of acne. *Propionibacterium acnes*-specific phages formulated in an aqueous cream were capable of killing the *P. acnes* bacteria[9]. Similarly, the ToothPhage™ toothpaste will make use of a semi solid formulation but in this case to eliminate the etiological agent of periodontitis. The use of toothpaste is favourable for various reasons. First, it is an easy-to-use application that patients can use themselves at home on a daily basis. Second, it does not require any additional actions from the patient, apart from the normal twice a day teeth-cleaning routine now with ToothPhage™ instead of normal toothpaste. Third, by the mechanical procedure of brushing your teeth, the phages are easily dispersed throughout the mouth. Thereby, the phages are in close proximity to the niches containing the harmful bacteria, where they have to act. A challenge we will have to further examine is whether swallowing and consequently phages entering the digestive system and the rest of the body has

relevant adverse effects, general as well as specific on the internal microbiota (as the presence of *P. gingivalis* is not limited to the oral cavity).

For the isolation and characterization of phages and preparation of our semi solid formulation, we will refer to the protocols of Brown et al[9]. We will make use of the currently characterized phages of pathogenic *P. gingivalis* strains supplemented with phages isolated and typed from the human oral cavity of volunteers, to generate a cocktail of lytic phages able to lyse various *P. gingivalis* strains (free of genetic determinants that could potentially cause additional harm to the host)[3,9]. Besides, we will make use of the data collected by the National Institutes of Health (NIH) on the Integrative Human Microbiome Project (iHMP) and the Human Oral Microbiome Database (HOMD). Additionally, we will collaborate with companies specialised in the manufacturing of toothpaste, for optimal formulation of excipients.

After optimization and addressing possible safety hazards, such as the swallowing of a limited number of phages and its influence on internal microbiota, a pilot trial will be performed.

Clinical trial
Patients with various stages of periodontitis, officially diagnosed by qualified dentists, will be addressed to participate on a voluntary basis and under informed consent in this trial. Only patients that do not use (long-term) antibiotics and/or probiotics will be included in the study. The double-blind, randomized, placebo-controlled trial will be executed under supervision of qualified dentists. The placebo used is a toothpaste with the same constituents and excipients, apart from the addition of bacteriophages. Patients will use ToothPhage™ or the control toothpaste for fourteen days, twice a day, alternating with seven days of normal toothpaste for an initial period of three months. After three months, dentists will evaluate progress and determine whether the procedure will be continued for periods up to 6 months with a maximum of one year, or in the case of negative outcomes treatment will be aborted and regular treatment started. At the start of the ToothPhage™ treatment, after three months (at the dental visit) and at the end of treatment, oral swabs are taken to evaluate presence of *P. gingivalis* and determine treatment efficacy. Additionally, inflammation status and stage of periodontitis is assessed according to standardized criteria supplemented with possible side-effects, noted by either patient or dentist. Patients that need an antibiotic treatment during the follow-up of the study, will be excluded from the study.

Expected results
Elimination of the periodontopathogen *P. gingivalis* will lead to a reduction of inflammation by taking away the etiological agent and consequently decrease the incidence of periodontitis.

Future prospects
The results of the clinical trial will provide knowledge on the effectivity and efficiency of periodontitis treatment with ToothPhage™. With consent of the participants we will do a full analysis of the microbiome composition obtained from the oral swabs, additional to evaluating the presence of *P. gingivalis,* with the use of matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). We will compare the microbiome obtained at the start of treatment to the microbiome retrieved at various time points and study alterations in composition. For example to assess if and if so, which other strains (pathogenic or commensal) are recolonizing the oral cavity. Possibly this could result in the addition of various phages, targeting different pathogenic strains/species that recolonize the oral cavity and are implicated in the pathogenesis of periodontitis. Additionally, we will compare the oral microbiome to published data on oral microbiome composition

in healthy individuals. Next, in additional clinical trials we want to assess whether the supplementary use of probiotics, either formulated in the toothpaste or in an alternative form to deliver to the oral cavity, will speed-up recovery of the dysbiosis caused by *P. gingivalis* and help restoration of oral symbiotic microbiota. We also want to assess the possibility of using additional non-pathogenic *P. gingivalis* strains, not targeted by the phages, to replace the periodontitis-causing strain however, the possibility of these strains acquiring pathogenic capacity will have to be carefully examined.

Furthermore, we think our application might also be promising for future treatment of alveolar osteitis, inflammation of the alveolar bone that often occurs as a complication after tooth extraction, especially after extraction of mandibular third molars (wisdom teeth in the lower jaw). Currently the causes of alveolar osteitis are not fully understood however, bacteria colonizing the socket are a clear problem. Further elucidation of the pathogenic strains responsible for the serious infections after wisdom teeth extraction, could result in the use of different strain-specific bacteriophages formulated in toothpaste.

After a successful trial period and approved production of ToothPhage™ by respective authorities, we want to bring ToothPhage™ to the pharmacies for widespread at-home use in combatting gum disease. This would pave the way for our at-home, easy-to-use application ToothPhage™ reducing the incidence of periodontitis because as W.D. Miller, a student of Dr. Koch, and the scientist J.L. Williams already taught us in 1896: “A clean tooth never decays”.

References

1. Dewhirst, F. E. *et al.* The human oral microbiome. *J. Bacteriol.* **192,** 5002–5017 (2010).
2. Kuramitsu, H. K., He, X., Lux, R., Anderson, M. H. & Shi, W. Interspecies Interactions within Oral Microbial Communities. *Microbiol. Mol. Biol. Rev.* **71,** 653–670 (2007).
3. Szafrá Nski, S. P., Winkel, A. & Stiesch, M. The use of bacteriophages to biocontrol oral biofilms. *J. Biotechnol.* **250,** 29–44 (2017).
4. Teeuw, W. J. *et al.* Paradontitis en levenskwaliteit. *Ned Tijdschr Tandheelk* **16,** 199–201 (2011).
5. Mysak, J. *et al.* Porphyromonas gingivalis: Major periodontopathic pathogen overview. *J. Immunol. Res.* **2014,** (2014).
6. Tezal, M., Sullivan, M. A., Hyland, A., Marshall, J. R. & Stoler, D. Chronic Periodontitis and the Incidence of Head and Neck Squamous Cell Carcinoma. *Cancer Epidemiol Biomarkers Prev* **11,** 2406–2412 (2009).
7. Mathews, M. J., Mathews, E. H. & Mathews, G. E. Oral health and coronary heart disease. *BMC Oral Health* **16,** (2016).
8. Microbiome in Health and Disease. Artis microbiology lectures. (2018).
9. Brown, T. L., Petrovski, S., Dyson, Z. A., Seviour, R. & Tucci, J. The formulation of bacteriophage in a semi solid preparation for control of propionibacterium acnes growth. *PLoS One* **11,** e0151184 (2016).