

Vaccines and Photodynamic Therapies for Oral Microbial-Related Diseases

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Abstract: The mouth is a favorable habitat for a great variety of bacteria. Microbial composition of dental plaque is the usual cause of various oral diseases in humans, including dental caries, periodontal disease and halitosis. In general, oral antibacterial agents such as antibiotics are commonly used to treat oral bacterial infection. Traditional periodontal surgery is painful and time-consuming. In addition, bacterial resistance and toxicity of antibiotics have become a global pandemic and unavoidable. Recently, vaccines for dental caries and periodontal disease have been developed and applied. Moreover, the use of photodynamic therapy has become an alternative to antibiotic drugs. The purpose of this article is to highlight the advantages of vaccine therapy and photodynamic therapy for oral microbial-related diseases compared to treatments with antimicrobial agents and traditional periodontal surgery.

Keywords: Antimicrobial agent treatment, vaccine therapy, traditional periodontal surgery, photodynamic therapy.

ORAL BACTERIA ECOSYSTEM

The mouth is a favorable habitat for more than 300 bacterial species due to the presence of nutrients, epithelial debris, and secretions [1]. The distribution of the different microbes varies qualitatively and quantitatively according to the habitat [1]. *Streptococcus mutans*, *Streptococcus sobrinus*, *Streptococcus cricetus*, *Streptococcus rattus*, and *Streptococcus sanguis* are found in larger numbers on teeth, *Streptococcus oralis* and *Streptococcus sanguis* are primarily located on the mucosal surface whereas *Streptococcus salivarius* is found mostly on the tongue [2]. Dental plaque develops preferentially on surfaces such as the area between the teeth, the subgingival area, and the pits and fissures of the biting surfaces [1]. It contains a complex matrix including microbial extracellular products and salivary compounds [1]. The predominant organisms isolated from oral dental plaque are gram-positive, facultative bacteria, particularly *Actinomyces viscosus*, *Streptococcus sanguis* and *Streptococcus mutans* [3-5] and gram-negative facultative bacteria including *Actinobacillus actinomycetemcomitans*, *Capnocytophaga species* and *Eikenella corrodens* [5, 6]. Gram-negative, anaerobic bacteria such as *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Prevotella intermedia*, *Bacteroides forsythus*, and *Campylobacter rectus* are also isolated from dental plaque [3, 7].

DENTAL CARIES, PERIODONTAL DISEASE AND HALITOSIS

It is well established that some infectious disease such as caries and periodontal diseases are associated with the dental plaque [1, 3]. If oral streptococci gain entrance into wound sites created by dental manipulation or treatment and adhere to heart valves, they may initiate bacterial endocarditis [8]. Moreover, oral bacteria have been linked to premature birth, uterine infection, diabetes, and dementia [8].

Dental caries are characterized by localized, progressive, molecular disintegration of the tooth structure, which results in cavities or holes in the teeth [1]. They are associated with dental plaque of smooth coronal surfaces, pits, and fissures [1]. Caries may also appear on root surfaces that are exposed from gingival recession [1]. The complexity of the bacterial community in dental plaque makes it difficult to determine the single bacterial agent of caries.

However, more studies indicate that streptococci species (particularly *Streptococcus mutans* and *Streptococcus sobrinus*), *Lactobacillus* species and *Actinomyces* species are involved in the initiation and progression of caries [9-11]. These bacterial groups are able to rapidly damage carbohydrates such as sucrose, fructose, and glucose into acid, primarily lactic acid, and cause teeth to become sensitive to low pH [3,12,13]. Change of environmental pH will activate a shift in the proportions of the resident microbiota then prompt teeth caries [3, 12, 13]. At neutral pH, *Streptococcus mutans* and *Lactobacillus* are weakly competitive and constitute only a small percentage of the total plaque microbial community. The repeated consumption of fermentable carbohydrates may lead to frequent conditions of low pH in the plaque [3, 12, 13].

Periodontal diseases can be grouped into two major categories, gingivitis and periodontitis [3, 11]. Gingivitis is defined as an inflammation of the gums surrounding the teeth. It is associated with plaque accumulation around the gingival margin. In a healthy gingival crevice, the total number of microorganisms is small and the microbiota is dominated by facultative gram-positive bacteria [11]. However, there is an increase in the proportion of anaerobic gram-negative bacteria, although the facultative gram-positive bacteria still dominate in patients [1]. These gram-negative rods include *Fusobacterium nucleatum*, *Prevotella intermedia*, *Veillonella*, *Wolinella*, *Capnocytophaga*, and *Haemophilus* [1]. The predominant gram-positive bacteria are *Actinomyces naeslundii* genospecies 2, *Actinomyces naeslundii*, *Streptococcus sanguis*, *Streptococcus mitis*, and *Peptostreptococcus micros* [1]. Periodontitis involves the destruction of the connective tissue attachment and the adjacent alveolar bone [11]. It is a severe form of gingivitis with gingival crevice forming a periodontal pocket due to the apical migration of the junctional epithelium along the root surface [5]. It can cause progressive loss of the alveolar bone around the teeth and lead to the loosening and subsequent loss of teeth [11]. Several processes including plaque accumulation, release of bacterial substances, and host inflammatory response are involved in the induction and progression of periodontal tissue destruction [3, 14, 15]. Inflammatory responses, such as the release of lysosomal enzymes from phagocytes and the production of cytokines stimulating connective tissue cells to release metalloproteinases (including collagenases) or activating bone resorption, cause tissue damage [1]. Bacteria producing virulence factors in periodontal pockets are gram-negative rods include *Porphyromonas*, *Prevotella*, *Fusobacterium*, *Actinobacillus actinomycetemcomitans*, *Capnocytophaga*, and *Wolinella* [1]. Some species, including *Porphyromonas gingivalis*, *Prevotella intermedia*, *Bacteroides forsythus*, *Westralthachia recta*, *Eikenella corrodens*, *Treponema denticola*, and *Peptostreptococcus micro*, pre-

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dominate in periodontitis [1]. In addition, increasing evidence shows that patients suffering from periodontal disease may be at augmented risk for systemic disease [16].

Halitosis, is usually caused by oral bacteria on the tongue [17, 18]. These bacteria can degrade the sulfur-containing peptides and amino acids in saliva, gingival crevicular fluid, blood, food retained about the teeth and desquamated epithelial cells to sulfate compounds, especially methyl-mercaptan (CH_3SH) and hydrogen sulfide (H_2S) [19, 20]. It has been shown that anaerobic species, such as *Prevotella melaninogenica* and *Fusobacterium nucleatum*, colonize on the tongue prior to the eruption of teeth [21]. Their number, as well as the presence of other anaerobes such as *Treponema denticola* and *Selenomonas* species increases with the time of the primary eruption of teeth [22]. Other bacteria such as *Treponema denticola*, *Prevotella intermedia*, *Porphyromonas gingivalis*, *Bacteroides forsythus*, *Eubacterium* and subgingival species can also produce large amounts of sulfate compounds from methionine, cysteine or serum proteins [17, 18].

ANTIBACTERIAL AGENT TREATMENT

Antibacterial agents, non-antibiotic antimicrobials and antibiotics, are generally being used in therapeutic regimes for dental plaque related diseases [23]. They can be applied locally and systematically such as swallowed as pills, swished around teeth, or inserted into the pockets of advanced gum disease. Amoxicillin is a semi-synthetic penicillin with broad antimicrobial spectrum, and is used in periodontology to fight against some subgingival bacterial species such as *Peptostreptococcus micros* and *Actinobacillus actinomycetemcomitans* [24]. However, many bacteria isolated in subgingival plaque samples are resistant to amoxicillin [25]. Ciprofloxacin is effective in the treatment of periodontal superinfections by pseudomonas or staphylococci and *Actinobacillus actinomycetemcomitans* [24]. Azithromycin exerts bacteriostatic activity by blocking of bacterial protein synthesis in periodontal tissues, crevicular fluid and saliva [24], but it appears to promote the carriage of macrolide-resistant strains [26]. Clindamycin is a pyranoside antibiotic and has been tested in several clinical studies [24]. Nevertheless, resistance to clindamycin of certain *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* serotypes has been indicated [24]. Tetracycline is commonly used in dental practice as a prophylactic agent and for treatment of oral infections [27]. However, liver damage (fatty infiltration and cloudy swelling) in rats and dogs following exposure to tetracyclines has been described [28, 29]. It has also been shown that a variety of tetracycline resistance genes are present in the oral microflora of healthy adults [27]. Moreover, many side effects are associated with the oral use of tetracyclines, for instance, epigastric burning, nausea, vomiting, and diarrhea [30]. Metronidazole has been proved to be efficacious in treating acute ulcerative gingivitis, pericoronitis, and certain periapical infections [31]. However, toxicity studies of metronidazole in mice and rats were indicated [32]. Metronidazole is known to accumulate in patients with liver dysfunction and can cause peripheral neuropathy and central nervous system dysfunction [33]. Moreover, class D β -lactamase, ATP-binding cassette transporter and enolase were found to be significantly up-regulated in ampicillin-resistant *Fusobacterium nucleatum* [34]. Most non-antibiotic antimicrobials can reduce oral malodor. Chlorhexidine is often used as an active ingredient in mouthwash designed to kill dental plaque and other oral bacteria. But it has been shown that the outer membrane vesicles of *Porphyromonas gingivalis* promote bacterial resistance to chlorhexidine [35]. Cetylpyridinium chloride (CPC) is a cationic quaternary ammonium compound that is effective in preventing dental plaque and reducing gingivitis. However, CPC is toxic to rats, mice and rabbits when given orally [36]. Clinical signs of toxicity caused by CPC included weight loss, nasal discharge, chromodacryorrhoea, respiratory difficulty and eye irritation [36].

VACCINE THERAPY

As antibacterial agents can be rendered ineffective by resistance development in the target organisms, can be difficult to maintain at a therapeutic concentration in the oral cavity, and can be toxic to host, there is a need to develop alternative chemical approaches for treatment. Since both dental caries and periodontal disease have an infectious etiology, vaccine therapy has been proposed as a means to control them [1]. These vaccines were developed based on the identification of virulence factors that stimulate the induction of salivary immunoglobulin A antibody responses [37-40]. For example, cell-surface fibrillar proteins, which mediate adherence to the salivary pellicle, and glucosyltransferase enzymes, which synthesize adhesive glucans and allow microbial accumulation, are virulent components of *Streptococcus mutans*, and primary candidates for caries vaccines [36, 41-43]. The chitosan-coated poly(lactic-co-glycolic acid) microspheres are also potentially useful for antigen delivery in dental caries vaccination [44]. For periodontal disease vaccines, *Porphyromonas gingivalis* and *Aggregatibacter Actinobacillus* have been identified as antigenic targets [45, 46]. There is evidence of a potential mechanism involved in periodontitis vaccine-induced suppression of bone loss, which offers insight into the role of PGE₂ in periodontal destruction [47]. Recently, integrated proteomics has been used as an effective strategy for discovery and development of vaccines for important human pathogens [48]. For example, matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) is widely used for peptide/protein mass measurements and peptide mass fingerprinting. Shah H. *et al.* simply mixed a small amount of *Fusobacterium nucleatum* cell culture with α -cyano-4-hydroxycinnamic acid, and utilized MALDI-TOF MS to detect the masses from intact cells [49]. Although spectra were not provided, the cluster analysis of the MALDI-TOF profiles reflected the large intraspecies diversity among *Fusobacterium nucleatum*. The direct analysis of intact bacteria cells by MALDI-TOF can provide rapid and high-throughput MS profiling of cell surface molecules, and hence the possibility of rapid identification of oral pathogens.

TRADITIONAL PERIODONTAL SURGERY AND PDT THERAPY

Dental plaque that forms on teeth is the main cause of caries and periodontal diseases. If the plaque is not removed, toxins produced by bacteria in the plaque will destroy supporting tissues around the teeth [1]. Once the plaque becomes a rough deposit called calculus, and more calculus build up over time, the gums will pull away from the teeth [1]. Mature periodontal surgery is used to remove plaque and calculus. However, there are more anxiety and discomfort during the time consuming procedure. Moreover, following surgery, the treated area can become tender, sore or swollen. Patients may need antibiotics or antibacterial rinses to relieve post-surgical discomfort. Cleaning the surgical area may disturb normal healing. On the other hand, light alone or in combination with chemical compounds has been used for inducing therapeutic effect [50]. Moreover, photodynamic therapy (PDT) is a treatment modality for cancer and other diseases [51]. Thus, PDT could be a useful alternative to mechanical means as well as antibiotics in eliminating periopathogenic bacteria [52-57]. It involves two stages; first, a light-sensitive drug called a photosensitizer is applied to the area. Second, a light or laser is shone on that area. When the light is combined with the drug, phototoxic reactions induce the destruction of bacterial cells [58]. Toluidine blue O (TBO), azure B chloride, and methylene blue are effective photosensitizers for killing of *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, and *Fusobacterium nucleatum* following exposure to laser light [59]. Moreover, oral bacteria in multi-species biofilms can be killed by red light in the presence of TBO [60]. Furthermore, the use of a light-emitting diode and TBO promotes cellular death and prevents the formation of biofilms of *Streptococcus mutans* in a noninvasive way [61]. The anaerobic bacteria *Porphyromonas gingivalis*, *Fuso-*

Table 1. Comparison of Therapies for Oral Microbial-Related Diseases

Category	Antibacterial Agent Treatment	Vaccine Therapy	Traditional Periodontal Surgery	Photodynamic Therapy
Advantages	1. Common prescribed drugs 2. Easy manipulation 3. Systemic or topical delivery	1. Long-lasting effect 2. Systemic effect 3. Specific target to microbes 4. Systemic disease application 5. Proteomic tool	1. Mature technique 2. Specific target to infection site	1. No surgery 2. Short procedure 3. Specific target to disease lesion 4. Avoiding damage to adjacent host tissues 5. Avoiding resistance to target bacteria 6. Avoiding disruption of the normal microflora at other sites 7. No high level of photosensitizers requirement
Disadvantages	1. Multiple and high dose application 2. Systemic toxicity and drug resistance 3. Inefficient local treatment 4. Side effects	1. Lack of clinical trail 2. Preventive vaccine but not cured vaccine	1. Damage to adjacent host tissues 2. Painful, anxiety, discomfort, bleeding 3. Time-consuming 4. More attention after surgery 6. Long recovery time 7. Antibacterial agent requirement to relieve post-surgical discomfort	1. Unknown metabolism of photosensitizers 2. Special instrument requirement

bacterium nucleatum, and *Capnocytophaga gingivalis* can also be completely photo-inactivated by illumination in the presence of chlorin e6 and BLC 1010 photosensitizers [62].

CONCLUSION AND PERCEPTIVE

The great diversity of biological surfaces in the oral cavity provides many ecological sites for colonization by a variety of oral bacterial species [63]. Dental caries, periodontal diseases, and halitosis are oral infections caused by bacterial plaque accumulations. Infection from oral bacteria is also a potential contributing factor to a variety of clinically important systemic diseases. Here, we describe and compare the therapies for oral microbial-related diseases (Table 1). Antibacterial agents, non-antibiotics or antibiotics, are commonly prescribed drugs for periodontal disease and halitosis. Although they are also easy to manipulate, risks of drug resistance and toxicity to organisms have become serious problems [23]. Moreover, only higher levels and multi-dose of the local-applied antimicrobial can achieve the effect attained with systemic administration [23]. More and more vaccines against dental caries and periodontal diseases have been developed [23]. They can be applied to kill one specific oral microbe systematically. Additionally, the findings of associations between periodontitis and other systemic diseases could provide a rationale for the development of a vaccine against many diseases [16]. Vaccination of infants may establish long-lasting immunity against an ensuing colonization attempts by *Streptococcus mutans* [38]. However, a preventive vaccine may not be effective in patients that already have dental caries. Furthermore, progress towards practical vaccine development requires evaluation of candidate vaccines in clinical trials. Proteomic tools hold great promise in defining the full repertoire of candidate vaccine antigens expressed by a pathogen, from which the optimal antigens for a vaccine can be rationally selected. Coupling multi-dimensional protein identification technology with recently developed quantitative proteomic methods such as those using stable isotope labeling or the label-free approach is a powerful technique for high-throughput, highly efficient identification of potential drug targets for the treatment and control of oral diseases [64]. Improvements in proteomic technology will significantly enhance our understanding of antimicrobial resistance and the complications of antibiotic treatment. Traditional mechanical surgeries are maturely established and commonly used for caries and periodontal diseases. However, many patients due to suffering from the pain and time-consuming procedure do still not accept it. Because bacteria are sensitized to light through prior treatment with chemical photosen-

sitizers, lethal photosensitization to bacteria responsible for oral infection disease has been demonstrated. The advantages of this approach are avoiding resistance to target bacteria and damage to adjacent host tissues [58]. Moreover, because the chemical photosensitizers can be applied to the disease lesion, disruption of the normal microflora at other sites can be avoided [58]. Furthermore, the therapy can kill target organisms very rapidly, depending on the light energy dose and the power output used [58]. High concentrations of the photosensitizers, therefore, do not need to be maintained in the disease lesion for long time [58]. Overall, there are several advantages and few disadvantages to using vaccines and photodynamic therapy in place of antimicrobial agents and traditional periodontal surgery for the treatment of oral microbial-related diseases.

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ABBREVIATIONS

CH ₃ SH	= Methyl-mercaptan
CPC	= Cetylpyridinium chloride
H ₂ S	= Hydrogen sulfide
MALDI-TOF	= Matrix-assisted laser desorption ionization time-of-flight
MS	= Mass spectrometry
PDT	= Photodynamic therapy
TBO	= Toluidine blue O

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