

## Review

# Biological plaque control: novel therapeutic approach to periodontal disease

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**Abstract:** Despite its important role in the control of periodontal disease, mechanical plaque control is not properly practiced by most individuals. Therefore, adjunctive chemical plaque control using chlorhexidine and antibiotics has also been suggested as an additional therapeutic strategy to augment mechanical plaque control. However, the additional effects of adjunctive antibiotic therapy are small, and topical chlorhexidine therapy is not without side effects. Given current limitations, new approaches for the control of biofilm are required. The new therapeutic approaches discussed in this review are divided into two categories: probiotics and vaccines. Probiotics is an interesting new field of periodontology research that aims to achieve biological plaque control by eliminating pathogenic bacteria. In addition, passive immunization using egg yolk antibody raised against periodontal pathogens may be an effective approach for the treatment of periodontitis. Further study to evaluate the possible effects of these biological plaque control methods against periodontal disease is warranted. (*J Oral Sci* 54, 1-5, 2012)

**Keywords:** periodontal disease; biological plaque control; probiotics; passive immunization.

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## Introduction

Periodontal disease is highly prevalent and can affect up to 90% of the world population. Since the 1960s when Løe et al. (1) established the essential role of dental plaque as the etiological agent responsible for periodontal disease, the control of biofilm accumulation on teeth has been the key to periodontal disease prevention. Tooth brushing and the use of dental floss and other devices to remove bacterial plaque from the teeth are the most common ways of removing biofilm. Despite its important role in the control of periodontal disease, mechanical plaque control is not properly practiced by most individuals. A systemic review of the effectiveness of self-performed mechanical plaque removal in subjects with periodontal disease concluded that it had limitations (2). Therefore, adjunctive use of chemical plaque control might be beneficial.

Meta-analyses have indicated that oral care products containing chlorhexidine exert anti-plaque effects. Chlorhexidine is a cationic biguanide with a broad spectrum of antibacterial activity, encompassing Gram-positive and Gram-negative bacteria, yeasts and some viruses (3). However, topical chlorhexidine therapy is not without its problems, and hypersensitivity reactions to the agent have been reported. Hypersensitivity to chlorhexidine is rare, but its potential to cause anaphylactic shock is probably underestimated. IgE antibodies against chlorhexidine have been detected in the majority of sera from a small group of predominantly Japanese individuals showing anaphylactic-type adverse reactions to chlorhexidine. In Japan, therefore, the use of chlorhexidine at a concentration effective for oral care has been

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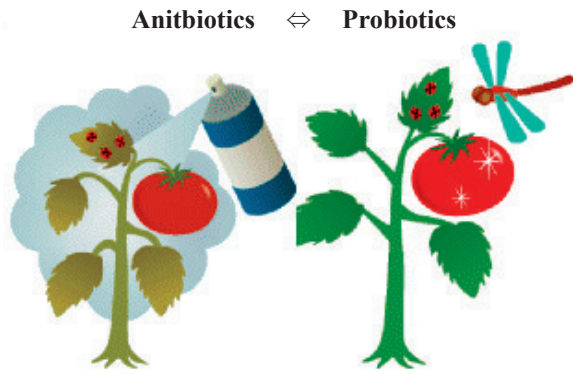


Fig. 1 Concept of probiotics.

banned.

Adjunctive use of antibiotics has also been suggested as an additional therapeutic strategy to augment mechanical plaque control. However, a systematic review has questioned the clinical significance of adjunctive antibiotic therapy because the magnitude of any additional effects is small (4). Furthermore, the emergence of antimicrobial resistance is currently posing a major global challenge, with an increasing number of strains, including commensal and pathogenic oral bacteria, becoming resistant to commonly used antibiotics. Given current limitations, new approaches for the control of biofilm are clearly required. The new therapeutic approaches that will be discussed in this review are divided into two categories: probiotics and vaccines.

## Probiotics

The term probiotic was originally proposed in 1965 as an antonym to the term antibiotic (Fig. 1), and probiotics are currently defined by the World Health Organization as live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host (5). The concept of probiotics dates back to the early 20th century, when Nobel laureate Elie Metchnikoff reported that the consumption of Bulgarian yogurt was beneficial to health. The first probiotic bacteria studied were lactic acid bacteria (6), and three main modes of action have been proposed to contribute to the effects of probiotics: 1) production of antimicrobial substances against pathogens, 2) competitive exclusion mechanisms, and 3) modulation of host defense systems. Studies have shown that lactic acid bacteria can produce different antimicrobial components, such as organic acids, bacteriocins, and low-molecular-weight antimicrobial substances, and that probiotics can also activate and modulate the host immune system. These probiotic actions, however,

have been demonstrated entirely on the basis of studies of the lower gastrointestinal tract, and probiotic actions within the oral microflora are not clear. Lactobacilli are indigenous bacteria colonizing the oral cavity and digestive tract, and a large body of evidence indicates that exogenous lactobacilli play a positive role in the prevention and treatment of gastrointestinal disorders. Since the mouth is the upper part of the gastrointestinal tract, at least some probiotic actions are presumed to occur in the oral ecosystem (7). Lactobacilli comprise approximately 1% of the culturable oral microflora, and several studies have shown that lactobacilli reduce the colonization of caries-associated mutans streptococci (8-10). In addition, most of the oral *Lactobacillus* strains isolated from periodontally healthy and diseased subjects have been reported to exert antimicrobial activity against periodontopathic bacteria, such as *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Prevotella intermedia* (11).

Lactobacilli can produce different antimicrobial components, and are generally considered to be cariogenic bacteria because they ferment sugars and reduce pH. Among the *Lactobacillus* species, *L. salivarius* TI2711 (LS1), isolated from saliva of a healthy human volunteer, was highly susceptible to both acidity and lactic acid. The acid-susceptibility of LS1 also suggested that this strain could be used as a non-cariogenic probiotic for maintaining a healthy ecosystem for the oral microflora, thereby preventing the colonization of periodontopathic bacteria.

An *in vitro* study has shown that coculture of these bacteria with LS1 resulted in a dramatic reduction of their numbers at around 12 h of coculture and almost complete eradication after 24 h. Lactic acid released from LS1 is thought to be a major bactericidal factor because addition of 100 mM lactic acid to a single culture of *P. gingivalis* killed all the bacteria within 6 h (12).

To examine the effects of probiotics on periodontal disease, LS1 was administered to volunteers orally (13). The subjects were divided into a placebo group and two experimental groups. The experimental groups received either  $2 \times 10^8$  CFU or  $2 \times 10^7$  CFU LS1 daily for 12 weeks. At 0, 4, and 12 weeks after LS1 intake and 4 weeks after the termination of LS1 intake (16 weeks), subgingival plaque was collected from the subjects to count the numbers of *P. gingivalis*, *Tannerella forsythensis*, and total bacteria using real-time PCR. A significant reduction of the bacterial count was observed for *P. gingivalis*, but not for *T. forsythensis* (13). The significant reduction of the *P. gingivalis* count was evident in the experimental groups at both 4 and 12 weeks, but not at 4 weeks after

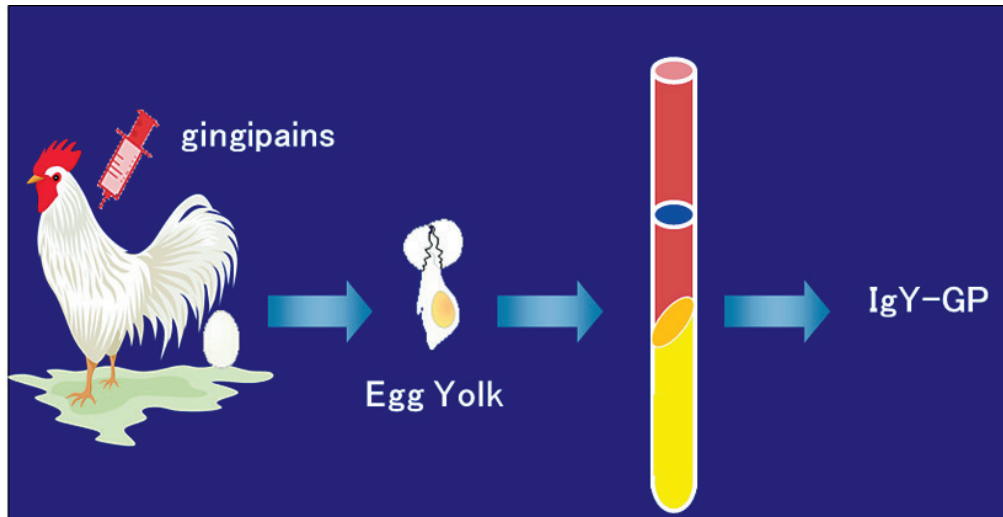


Fig. 2 Preparation of egg yolk antibody against gingipains.

terminating the intake of LS1. In these subgingival plaque samples, the *L. salivarius* count increased after administration and decreased at 4 weeks after withdrawal of LS1 intake. No significant change was observed in the placebo group. These results indicate that LS1 is transmitted into subgingival plaque and reduces the count of *P. gingivalis*. In subgingival sites, lactobacilli were rarely detected in the periodontally healthy group or the periodontitis group, indicating that the subgingival regions are not the usual habitat of lactobacilli. A variety of anatomical and physiological factors, as well as synergistic and antagonistic interactions among microbes, may influence the colonization of lactobacilli in subgingival sites.

Despite the obvious antimicrobial effects of probiotics, they can also act on a wide variety of cells to modulate the immune system towards anti-inflammatory actions. Only a few studies have attempted to determine whether immunomodulation by probiotics can also be applied to the oral environment. Twetman et al. (14) reported the effects of probiotics on gingival inflammation and the production of inflammatory cytokines. During the two weeks of intervention, the gingival crevicular fluid volume decreased significantly in the probiotic group, whereas no significant changes were observed in the placebo group. The levels of TNF- $\alpha$  and IL-8 also decreased in the probiotic group. However, these effects were temporary and the levels returned to the baseline after the probiotics had been withdrawn. It is known that probiotics can regulate the expression of phagocytosis receptors in the neutrophils of healthy individuals (15) and enhance natural killer cell activity (16). They have also been shown to modulate the immune response via

adaptive immunity (17). However, the exact regulatory systems responsible are still unclear.

### Vaccines

*P. gingivalis*, a Gram-negative anaerobe, has been implicated as an important periodontal pathogen in terms of its virulence. Several virulence factors have been reported to contribute to the pathogenicity of *P. gingivalis*, including lipopolysaccharide (LPS), fimbriae, hemagglutinin, hemolysin, and the Arg-X- (Rgp) and Lys-X-specific (Kgp) cysteine proteinases (gingipains) (18). Emerging evidence suggests that inhibition of these virulence factors may protect the host against periodontal pathogens (19). Gingipains are present in large quantities on the cell surface of *P. gingivalis*, and play a major role in the pathogenesis of periodontitis by dysregulating the host defense mechanisms and degrading various host proteins (20). Gingipains also play a role in bacterial housekeeping, including uptake of amino acids from host proteins, acquisition of iron from erythrocytes, and maturation of fimbriae. Therefore, inhibition of gingipain by vaccination might reduce the periodontitis caused by *P. gingivalis* infection. Active and passive immunization approaches have been developed for immunotherapy against periodontitis.

Active immunization is induced by exposure to foreign antigens. Lymphocytes are activated to produce antibodies against antigens. However, a systemic review of preclinical studies has concluded that because of the insufficient quantity and quality of animal trials, there is still no adequate evidence for any beneficial effects of active immunization against periodontal pathogens (21).

Protective immunity can also be obtained through

passive immunization, which is achieved by transfer of specific antibodies against the target antigens. In most infectious diseases, side effects of passive immunization may also arise from a single injection of antiserum from an immune donor. In contrast, effective immunization against periodontitis may be achieved by local application of a specific antibody to the gingival area; intravenous injections of antibodies are not necessary.

The advantages of specific antibodies derived from hen egg yolk for passive immunization include low cost, biosafety, and easy preparation in large quantities using eggs from immunized hens (22) (Fig. 2). A clinical trial using egg yolk antibody against gingipains (IgY-GP) examined five patients with chronic periodontitis who had high levels of *P. gingivalis* in their subgingival flora (23). IgY-GP-containing ointment was administered directly into the periodontal pocket. Before administration of IgY-GP at the baseline, scaling and root planing (SRP) was performed on all surfaces of the tested teeth. *P. gingivalis* levels in the pockets of all the treated teeth were expressed as a percentage of total bacteria determined using real-time PCR. SRP combined with the use of IgY-GP reduced the probing depth, bleeding on probing, and levels of *P. gingivalis* at 4 weeks as compared with SRP only. An additional study also investigated the effect of IgY-GP on periodontitis in 42 patients after scaling and root planing employing a double-blind placebo-controlled approach (24). Subjects with untreated periodontitis were randomly assigned to receive full-mouth scaling and root planing along with oral administration of IgY-GP tablet or a placebo tablet. Clinical measurements were recorded at the baseline and at 4 and 12 weeks after therapy. The deepest pocket was selected, and samples were collected to determine the number of *P. gingivalis* cells using the PCR-Invader method. A significant improvement in mean probing depth was noted in the IgY-GP group at 12 weeks after therapy. Parallel to the clinical changes, the number of *P. gingivalis* cells in subgingival plaque from the deepest pocket was significantly reduced by IgY-GP administration. These results indicated that daily administration of IgY-GP, in conjunction with scaling and root planing, in patients with periodontitis produced significantly better clinical and microbiological results than the use of a placebo. Thus, passive immunization using IgY-GP may be an effective method for the treatment of periodontitis.

### Concluding remarks

To summarize, biological plaque control may exert beneficial effects by preventing the growth of *P. gingivalis* in subgingival sites. In the field of probiotics,

Metchnikoff's concept is also applicable to the promotion of periodontal health. Probiotics is an interesting new field of periodontology research aimed at the elimination of pathogenic bacteria in dental plaque. In addition, passive immunization using egg yolk antibody against periodontal pathogens may be an effective treatment for periodontitis. However, the effects of these two approaches on periodontal disease and their use in combination with periodontal treatment are not fully understood. Further studies are needed to determine the mechanisms of action of probiotics and passive immunization, as well as clarifying further details of the complex interplay that occurs in the development of biofilms.

Nevertheless, observations obtained so far suggest that further studies to evaluate the possible effects of biological plaque control on periodontal disease are warranted.

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