Molecular Interaction of the Analogous Peptide SspB (390-T400K-402) derived from *Streptococcus gordonii* Surface Protein Peptide with Periodontal Bacteria

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Abstract

The initial stages of dental biofilm formation involved the adherence of early colonizing organisms such as *Streptococcus gordonii* on the saliva-coated tooth surface. The surface protein SspB of *S. gordonii* is known to play a role in adherence to salivary protein and mediates coaggregation with other bacteria. We previously demonstrated that the analogous SspB peptide, SspB (390-T400K-402), effectively bound to salivary agglutinin peptide SRCRP2 (scavenger receptor cysteine-rich domain peptide 2). To investigate molecular interaction of supra- and sub-gingival dental plaque biofilm formation among streptococci, periodontal bacteria, and salivary agglutinin as a unit, we examined the binding activity of the SspB (390-T400K-402) peptide to periodontal bacteria. The binding activity of SspB (390-T400K-402) was detected by ELISA. The SspB (390-T400K-402) peptide bound to *P. gingivalis* ATCC 33277, *F. nucleatum* ATCC 23726, and *F. nucleatum* #20. This binding was reduced by the presence of whole saliva; however, the presence of SRCRP2 enhanced the binding of the SspB peptide to these bacteria. These findings suggest that the binding ability of *S. gordonii* to these periodontal bacteria may provide, either directly (by attachment to *P. gingivalis* Mfa1) or indirectly (by attachment to *F. nucleatum*), a support system for the colonization and the biofilm formation of periodontal bacteria.

Introduction

Dental plaque is a complex biofilm that accumulates through the colonization of large number of different species of bacteria (1, 2). Early biofilm is formed by attachment and colonization of a variety of streptococci and is dependent on both the species involved and the surface composition (3-6). Later biofilm inhabitants such as *Porphyromonas gingivalis* are capable of binding to the antecedent organisms, and these attachment mechanisms are thought to driven the temporal and spatial development of pathogenic plaque (1, 7-10). *In vitro*, *P. gingivalis* adheres avidly to sessile *Streptococcus gordonii* and, once attached, rapidly forms a

biofilm comprising towering microcolonies separated by fluid-filled channels (11).

Adherence of *P. gingivalis* to *S. gordonii* is multimodal and involves a number of distinct adhesin and receptor pair on the surfaces of both organisms. These molecules include the major fimbriae and a 35-kDa protein of *P. gingivalis* and the Ssp proteins of *S. gordonii* (12, 13). The Ssp proteins (SspA and SspB, 1,500 amino acid residues), encoded by tandemly arranged genes (*sspA* and *sspB*), are members of the antigen I/II family of major streptococci surface proteins and are multifunctional adhesins (1, 8). The binding domain of the SspB to *P. gingivalis*, designated BAR (SspB Adherence Region), has been mapped to the C terminus within amino acid residues 1167 to 1250 (14). *P. gingivalis* pre-incubated with synthetic BAR peptide (1167-1193) has

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been shown to inhibit adherence of *P. gingivalis* to *S. gordonii*, and thereby inhibiting biofilm formation of *P. gingivalis* on the established *S. gordonii* biofilm using a dual-species open-flow biofilm culture model (15). For *in vivo* study, *S. gordonii*-colonized mice that were subsequently infected with *P. gingivalis* in the presence of BAR peptide showed levels of alveolar bone resorption similar to those of sham-infected mice; hence, the BAR peptide inhibited colonization of *P. gingivalis* on the murine oral cavity by preventing its interaction to the *S. gordonii* (16). Further, in the N-terminal region of Ssp (amino acid residues 1 to 429), the cell surface adhesins SspB and SspA showed extensive homology (63 and 60% identity, respectively, with PAc, which is surface protein antigen produced by *Streptococcus mutans*) (17). This region consists of three long and two incomplete repeating sequences likely such as the A region of PAc (17). Each repeating sequence of the N-terminal region of Ssp contains the homologous to the amino acid sequence ³⁶⁵TYEAALKQYEADL³⁷⁷ of PAc (365-377), which is an important region for the initial attachment of *S. mutans* to the tooth surface (18,19). We previously demonstrated the molecular mechanisms of the surface proteins of *S. gordonii* for binding to salivary components, peptides homologous to PAc (365-377) in the N-terminal region of SspB (20). The specific residues within sequences SspB (283-295) and SspB (201-213) of SspB contribute to bind to salivary components (20). In addition, the analogous peptide SspB (390-T400K-402) peptide was the highest binding activity to the salivary components (20). This peptide had two surface positive charges at residues 390(D) and 400(K) in connection with the positively charged residues. The positively charged amino acid residue, i.e. lysine, is essential for binding activity on the peptide surface, the positively charged α -helical structure, to the negatively charged salivary components (21). More recently, Okuda *et al.* (22) showed the SspB (390-T400K-402) peptides significantly bound with salivary components and inhibited the binding of *S. mutans* and *S. gordonii* to saliva coated hydroxyapatite without bactericidal activity; but did not inhibit binding of *Streptococcus mitis*, a beneficial commensal.

In the present study, we evaluated the binding activity of the analogous peptide SspB (390-T400K-402) peptide to periodontal bacteria and the influence of salivary components to the binding activity in order to investigate molecular interaction of supra- and sub-gingival dental plaque biofilm formation among streptococci, periodontal

bacteria, and salivary agglutinin as a unit. An understanding of these molecules and the periodontal bacteria is important for elucidating the mechanisms of supra- and sub-gingival dental plaque biofilm formation.

Materials and Methods

Bacterial strains and culture conditions

The bacterial strains used in this study were *Fusobacterium nucleatum* strains #20 (clinical isolate and laboratory strain in National Institute of Infectious Diseases) and ATCC 23726, *P. gingivalis* strains W83, W50 and ATCC 33277, and *Aggregatibacter actinomycetemcomitans* strains ATCC 29522, ATCC 29523 and Y4. *P. gingivalis* strains were grown Trypticase soy broth (Becton, Dickinson and Company; BD, Franklin Lakes, NJ, USA) supplemented with 5 μ g/ml of hemin (Sigma Chemical Co., St. Louis, Mo.) and 0.5 μ g/ml of menadione (Wako Pure Chemical Industries, Osaka, Japan) under anaerobic conditions (10% CO₂, 10% H₂, and 80% N₂) at 37°C for 48 h. *F. nucleatum* and *A. actinomycetemcomitans* strains were grown anaerobically in Brain Heart Infusion (BD) at 37°C for 24 h.

Peptide synthesis

The peptide analogous to the SspB peptide, which change of T at position 400 to K in SspB (390-402), resulting in the SspB (390-T400K-402) peptide (DYQAKLAAYQKEL) and SRCRP2 (QGRVEVLYRGSWGTVTC) on salivary agglutinin/gp340/DMBT1, an innate immunity scavenger receptor glycoprotein, were synthesized by Asahi Techno Glass Co. Inc. (Tokyo, Japan).

Human saliva collection

This study was approved by the Nihon University Institutional Review Board (approved number: EC16-031). The saliva samples from two healthy human subjects (28 and 34 years old, males) were also collected after stimulation by biting paraffin gum and placed into ice-chilled sterile bottles over a period of 5 min. The samples were then clarified by centrifugation at 10,000 $\times$ g for 10 min, filter sterilized, and used immediately for the peptide binding assay.

Peptide binding assay

The binding activity of the SspB (390-T400K-402) peptide to periodontal bacteria were detected by an enzyme-linked immunosorbent assay (ELISA) technique. Briefly, 50 μ l of

whole periodontal bacterial cell (*F. nucleatum* strains #20 and ATCC 23726, *P. gingivalis* strains W83, W50 and ATCC 33277, and *A. actinomycetemcomitans* strains ATCC 29522, ATCC 29523 and Y4) suspension in phosphate buffered saline (PBS) (each 1×10^8 cells) were used to coat each well of a 96-well plate at 37°C for 60 min. After washing three times with PBS, wells were blocked with 200 µl of 3% bovine serum albumin in PBS at 4°C overnight. Biotin-labeled SspB (390-T400K-402) peptide (50 µg/ml) in 50 µl of PBS was applied to the wells and incubated at 37°C for 60 min. The wells were then washed three times with PBS and further incubated for 60 min at 37°C with 1/1000 diluted streptavidin-conjugated alkaline phosphatase (Invitrogen, Corp., Carlsbad, Calif, USA). Subsequently, SspB (390-T400K-402) adhered to periodontal bacteria was detected by chromogenic development using para-nitrophenyl phosphate as the alkaline phosphatase substrate. After development, absorbance at 405 nm was measured.

To prepare sonic extracts of these bacteria, washed cells were suspended in PBS (30 mg/ml, wet weight) and disrupted using sonication (Sonifier 250D, Branson Ultrasonics. Co., Danbury, CT, USA) for 10 min at 100 W on ice. The binding activity of the peptide to the sonic extracts of periodontal bacteria was carried out as described above with slightly modified. Briefly, 25 µg/ml or serial two-fold dilutions of sonic extracts per well in 50 µl of 50 mM carbonate buffer (pH 9.6) were used to coat each well at 4°C overnight. After washing with PBS containing 0.1% Tween 20 (PBST) and blocking, the wells were applied with 50 µl of whole saliva, 200 µg/ml of SRCRP2 peptide in 50 µl of PBS or PBS (Control), and incubated for 60 min at 4°C (filter-sterilized, clarified human whole saliva) or 37°C (SRCRP2). Biotin-labeled SspB (390-T400K-402) peptide (50 µg/ml) in 50 µl of PBS was applied to the wells and incubated at 37°C for 60 min. Following application of the peptide apply was described above.

Competitive ELISA with biotin-labeled and non-labeled SspB (390-T400K-402) peptide was carried out as described above with some of application. 25 µg/ml or serial two-fold dilutions of sonic extracts were coated each well. After blocking, 100 µg/ml of non-labeled SspB (390-T400K-402) peptide per well in 50 µl of PBS or PBS were treated with each well and incubated at 37°C for 60 min. After three times of washing with PBST, 10 µg/ml of biotin-labeled SspB (390-T400K-402) peptide in 50 µl of PBS was apply to each well and further incubated at 37°C for 60 min. Following steps

were described above. All of these experiments were performed individually at least three times.

Statistical Analyses

Data were indicated as means $\pm$ standard deviations (SD). Graph Pad Prism version 5.0 d for Mac OS X (GraphPad Software, San Diego, CA) was used to assess significance. The statistical significance of differences between two groups was determined by unpaired *t*-test. For comparisons between multiple groups, one-way analysis of variance (ANOVA) and Tukey's test were used. *P*-values less than 0.01 or 0.05 were considered to be statistically significant using two-tailed comparisons. All experiments were repeated and analyzed independently.

Results

Binding of SspB (390-T400K-402) peptide to periodontopathic bacterial cell

In order to examine the binding activity of the biotin-labeled SspB (390-T400K-402) peptide to periodontopathic bacterial cell, *P. gingivalis* strains W83, W50, and ATCC 33277, *F. nucleatum* strains #20, and ATCC 23726, and *A. actinomycetemcomitans* strains ATCC 29522, ATCC 29523, and Y4 were tested (Fig. 1). *P. gingivalis* strain ATCC 33277 showed high binding activity to this peptide. Two of *F. nucleatum* strains also showed moderate binding activity. On the other hand, *P. gingivalis* strains W50 and W83, and all of *A. actinomycetemcomitans* strains exhibited poor binding activity of the peptide. We found that SspB (390-T400K-402) possess binding ability to *P. gingivalis* strain ATCC 33277, and *F. nucleatum* strains #20 and ATCC 23726; therefore, these bacteria was used for further studies. To clarify the binding activity of the SspB (390-T400K-402) peptide to these periodontal bacteria, we tested the binding activity of this peptide with sonic extracts of these bacteria (Fig. 2). The SspB (390-T400K-402) peptide significantly response to sonic extract of *P. gingivalis* strain ATCC 33277, and *F. nucleatum* strains #20 and ATCC 23726. Further, we examined the binding activity of the peptide on various concentration of sonic extract of these periodontal bacteria (Fig. 3). The results indicated that the binding of this peptide to these bacteria increased in a concentration-dependent manner. We found that SspB (390-T400K-402) possess binding ability to *P. gingivalis* strain ATCC 33277, and *F. nucleatum* strains #20 and ATCC 23726; therefore, these bacteria was used for further studies.

Fig. 1. Binding response of biotin-labeled SspB (390-T400K-402) peptide to periodontal bacteria. Whole cells of periodontal bacteria were coated to 96-well ELISA plate, and reacted with biotin-labeled SspB (390-T400K-402) peptide. Data were indicated as means $\pm$ SD. Fn, *F. nucleatum*; Pg, *P. gingivalis*; Aa, *A. actinomycetemcomitans* (** P <0.01, * P <0.05).

Fig. 2. Binding response of biotin-labeled SspB (390-T400K-402) peptide to sonic extracts of the periodontal bacteria. 25 μ g/ml of the sonic extracts of these bacteria were coated to 96-well ELISA plate, and reacted with biotin-labeled SspB (390-T400K-402) peptide. Data were indicated as means $\pm$ SD. Fn, *F. nucleatum*; Pg, *P. gingivalis*; Aa, *A. actinomycetemcomitans* (** P <0.01, * P <0.05).

Salivary components affect the binding of SspB (390-T400K-402) peptide to periodontal bacteria

We previously demonstrated that the SspB (390-T400K-402) peptide had the highest binding activity to salivary components as compared with the other peptides derived

Fig. 3. Binding response of biotin-labeled SspB (390-T400K-402) peptide to serial dilution of sonic extracts of the periodontal bacteria. Serial diluted sonic extracts (3.2 μ g/ml, 6.3 μ g/ml, 12.5 μ g/ml and 25 μ g/ml) of these bacteria were coated to 96-well ELISA plate, and reacted with biotin-labeled SspB (390-T400K-402) peptide. Data were indicated as means $\pm$ SD. Fn, *F. nucleatum*; Pg, *P. gingivalis* (vs. 3.2 μ g/ml, ** P <0.01; * P <0.05).

from *S. gordonii* SspB and *Streptococcus sobrinus* PAg(20). Thus, we hypothesized that when the extract of the periodontal bacteria was treated with whole saliva, the binding activity of the SspB (390-T400K-402) peptide may be increase. In order to assess this hypothesis, the sonic extracts of these bacteria treated with saliva were subjected (Fig. 4). The results showed the binding activity of the peptide was significantly low level in all of bacterial extracts treated with whole saliva compared to non-treated extracts. The SspB (390-T400K-402) peptide was also able to induce the highest level of binding to the SRCRP2 peptide, which is a member of SRCR superfamily and known for a bacterial binding feature with a multivalent character(23). Thus we investigated the binding activity of the SspB (390-T400K-402) peptide to SRCRP2 treated extraction of periodontal bacteria (Fig. 5). When the sonic extracts of these bacteria were reacted with SRCRP2 peptide, the binding activity of the SspB (390-T400K-402) peptide was significantly higher than that of non-treated extraction.

Discussion

Dental plaque is complex and dynamic, and the initial colonizers comprise predominantly gram-positive commensal bacteria(2, 24, 25). *S. gordonii* is an early colonizer of the salivary pellicle and its ability to bind to salivary proteins

(a) Pg : ATCC 33277

(b) Fn : #20

(c) Fn : ATCC 23726

Fig. 4. Analysis of the effect of whole salivary components to SspB (390-T400K-402) peptide binding. Serial diluted sonic extracts of these bacteria were coated to 96-well ELISA plate. The wells were treated with whole saliva (■) or PBS (▨). Data were indicated as means $\pm$ SD. (** P <0.01; * P <0.05).

and glycoproteins are important in oral biofilm (dental plaque) development (26,27). In addition, *S. gordonii* plays an important role in *P. gingivalis* colonization of later biofilm (28-30).

In this study, we utilized biotin-labeled SspB (390-T400K-402) peptide in our experiments to investigate molecular interaction of supra- and sub-gingival dental plaque biofilm formation among streptococci, periodontal bacteria, and salivary agglutinin as a unit. We synthesized the peptide with a single N-terminal biotin label and assessed preliminary the effect of labeling of biotin in N-terminal of SspB (390-T400K-402) to binding activity of the periodontal bacteria. As the result, the labeling of biotin in N-terminal of

Fig. 5. Analysis of the effect of SRCRP2 peptide to SspB (390-T400K-402) peptide binding. 25 µg/ml of the sonic extracts of these bacteria were coated to 96-well ELISA plate. The wells were treated with 200 µg/ml of SRCRP2 peptide (■) or PBS (▨). Data were indicated as means $\pm$ SD. Fn, *F. nucleatum*; Pg, *P. gingivalis* (** P <0.01; * P <0.05).

SspB (390-T400K-402) was no effect to the peptide binding activity (data not shown).

In this study, we found that the analogous peptide SspB (390-T400K-402) have abilities to bind to periodontal bacteria, *P. gingivalis*, *F. nucleatum*, and *A. actinomycetem-comitans* (Figs. 1 and 2). In these bacteria, *P. gingivalis* strain ATCC 33277 showed the highest binding activity to SspB (390-T400K-402). *P. gingivalis* strain ATCC 33277 expressed two distinct fimbriae molecules, major fimbriae encoded by *fimA* (31) and minor fimbriae encoded by *mfal* (32,33). Previous reports indicated that Mfal of *P. gingivalis* showed to mediate adherence to surface protein SspB of *S. gordonii* (29,30), and reported that the Mfal-SspB mediated interaction is necessary for biofilm development. The SspB (390-T400K-402) peptide was not detected to bind to other *P. gingivalis* strains W50 and W83. These two strains express type IV fimbriae (31), while the minor fimbriae have not been determined. These and our present results suggested that the analogous peptide SspB (390-T400K-402) bind to Mfal, and that critical residues for binding the peptide to Mfal are involved within the synthetic peptide SspB (390-T400K-402) adhesion epitope. At the same time, we found that the analogous peptide SspB (390-T400K-402) also bind to other periodontopathic bacterium, *F. nucleatum* strains #20 and ATCC 23726 (Figs. 1 and

2). These results suggested that *S. gordonii* interact with *F. nucleatum* via SspB mediated adhesion. *F. nucleatum* is considered an important bacterium in the development of complex dental plaque biofilms: a "bridge bacterium", it mediates multiple coaggregation interactions with other oral bacteria (34). A general view concerning the formation of dental plaque suggests that *F. nucleatum* initially colonizes the tooth and gingival surfaces to form the substrate onto which later colonizer including periodontopathic bacteria adhere via coaggregation or receptor-interaction based mechanisms. In addition, our findings suggest that the ability of *S. gordonii* to bind to these periodontal bacteria may provide a support system for the colonization and the biofilm formation of periodontal bacteria either directly (by attachment to *P. gingivalis* Mfal) or indirectly (by attachment to *F. nucleatum*).

S. gordonii is an early colonizer and has binding ability to salivary pellicle through Ssp protein (1). We previously reported that the analogous peptide SspB (390-T400K-402) strongly bind to the high-molecular-weight protein complex of salivary components and the agglutinin peptide SRCRP2 (20). While, major fimbriae of *P. gingivalis* (Type I fimbriae) can bind to salivary acidic proline-rich protein1 and statherin through protein-protein interactions (35). *F. nucleatum* also bind to salivary proline-rich glycoprotein (1). These bacteria have the ability to bind salivary proteins, however the attachment domains of salivary protein were different between *S. gordonii* and the periodontal bacteria. Furthermore, recent reports and our results have shown that SspB mediates adhesion of periodontal bacteria. Therefore, we hypothesized that interaction between SspB and salivary components may important role for development of supra- and sub-gingival biofilm formation. We investigated the binding ability of analogous peptide SspB (390-T400K-402) and the periodontal bacteria in the presence of salivary protein. However, the binding activity was decreased in salivary proteins components existence condition (Fig. 4). Stinson *et al.* reported that human whole saliva and human submandibular-sublingual saliva (HSMSL) inhibit adherence of *P. gingivalis* to *S. gordonii*; and inhibition activity by HSMSL was associated with a 43-kDa protein (36). Histatins and lysozyme can also inhibit coaggregation between *P. gingivalis* and oral streptococci (24). As for *F. nucleatum*, it has strong binding ability for fibronectin, which is a glycoprotein found in saliva (34). These findings, taking the above results (Fig. 4), suggest

that binding epitopes of SspB (390-T400K-402) to periodontal bacteria were competitively masked by these salivary components. On the other hand, when the SRCRP2 peptide was present, the binding activity was increased (Fig. 5). Recent report indicated that *Actinomyces* species, which predominate in early dental plaque biofilm, can be interacted to salivary gp340 (37). They also suggested that salivary gp340 has the high bacterial cell binding capacity and salivary gp340 recognizes different bacterial receptors according to whether gp340 is present in the fluid phase or surface bound. These suggest that the periodontal bacteria may bind to gp340 involving SRCRP2 domain. However we did not obtained the evidence to this suggestion. More research is required to address this phenomenon.

In conclusion, the abilities of the analogous peptide SspB (390-T400K-402) derived from *S. gordonii* to bind to the periodontal bacteria were demonstrated. Although further investigation is necessary to understand the *S. gordonii* binding mechanisms to the periodontal bacteria in detail, since our findings were generated by the analogous peptide from this organism. However our finding suggested that *S. gordonii* may be able to bind to the periodontal bacteria, subsequently the organism may play an important role of the development of late colonized bacterial biofilm as well as early colonized biofilm. The specificity of *S. gordonii* adherence with periodontal bacteria may represent a mechanism which contributes to colonization of the oral biofilm and may also be relevant in the infection and colonization of the periodontopathic bacteria.

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References

1. Kolenbrander PE, London J: Adherence today, here tomorrow: oral bacterial adherence. J Bacteriol, 175: 3247-3252, 1993.
2. Marsh PD: Microbial ecology of dental plaque and its significance in health and disease. Adv Dent Res, 8: 263-271, 1994.
3. Bowden GH: Microbiology of root surface caries in humans. J

- Dent Res, 69: 1205-1210, 1990.
4. Gibbons RJ: Role of adhesion in microbial colonization of host tissues: a contribution of oral microbiology. J Dent Res, 75: 866-870, 1996.
 5. McEldowney S, Fletcher M: Adhesion of bacteria from mixed cell suspension to solid surfaces. Arch Microbiol, 148: 57-62, 1987.
 6. Pratt-Terpstra IH, Weerkamp AH, Busscher HJ: The effects of pellicle formation on streptococcal adhesion to human enamel and artificial substrata with various surface free-energies. J Dent Res, 68: 463-467, 1989.
 7. Lamont RJ, Hersey SG, Rosan B: Characterization of the adherence of *Porphyromonas gingivalis* to oral streptococci. Oral Microbiol Immunol, 7: 193-197, 1992.
 8. Rosan B, Lamont RJ: Dental plaque formation. Microbes Infect, 2: 1599-1607, 2000.
 9. Slots J, Gibbons RJ: Attachment of *Bacteroides melaninogenicus* subsp. *asaccharolyticus* to oral surfaces and its possible role in colonization of the mouth and of periodontal pockets. Infect Immun, 19: 254-264, 1978.
 10. Stinson MW, Safulko K, Levine MJ: Adherence of *Porphyromonas (Bacteroides) gingivalis* to *Streptococcus sanguis* in vitro. Infect Immun, 59: 102-108, 1991.
 11. Cook GS, Costerton JW, Lamont RJ: Biofilm formation by *Porphyromonas gingivalis* and *Streptococcus gordonii*. J Periodontal Res, 33: 323-327, 1998.
 12. Lamont RJ, Bevan CA, Gil S, Persson RE, Rosan B: Involvement of *Porphyromonas gingivalis* fimbriae in adherence to *Streptococcus gordonii*. Oral Microbiol Immunol, 8: 272-276, 1993.
 13. Lamont RJ, Hsiao GW, Gil S: Identification of a molecule of *Porphyromonas gingivalis* that binds to *Streptococcus gordonii*. Microb Pathog, 17: 355-360, 1994.
 14. Brooks W, Demuth DR, Gil S, Lamont RJ: Identification of a *Streptococcus gordonii* SspB domain that mediates adhesion to *Porphyromonas gingivalis*. Infect Immun, 65: 3753-3755, 1997.
 15. Daep CA, James DM, Lamont RJ, Demuth DR: Structural characterization of peptide-mediated inhibition of *Porphyromonas gingivalis* biofilm formation. Infect Immun, 76: 5756-5762, 2006.
 16. Daep CA, Novak EA, Lamont RJ, Demuth DR: Structural dissection and *in vivo* effectiveness of a peptide inhibitor of *Porphyromonas gingivalis* adherence to *Streptococcus gordonii*. Infect Immun, 79: 67-74, 2011.
 17. Demuth DR, Lammey MS, Huck M, Lally ET, Malamud D: Comparison of *Streptococcus mutans* and *Streptococcus sanguis* receptors for human salivary agglutinin. Microb Pathog, 9: 199-211, 1990.
 18. Oho T, Yu H, Yamashita Y, Koga T: Binding of salivary glycoprotein-secretory immunoglobulin A complex to the surface protein antigen of *Streptococcus mutans*. Infect Immun, 66: 115-121, 1998.
 19. Russell MW, Lehner T: Characterisation of antigens extracted from cells and culture fluids of *Streptococcus mutans* serotype c. Arch Oral Biol, 23: 7-15, 1978.
 20. Hamada T, Kawashima M, Watanabe H, Tagami J, Senpuku H: Molecular interactions of surface protein peptides of *Streptococcus gordonii* with human salivary components. Infect Immun, 72: 4819-4826, 2004.
 21. Koba H, Okuda K, Watanabe H, Tagami J, Senpuku H: Role of lysine in interaction between surface protein peptides of *Streptococcus gordonii* and agglutinin peptide. Oral Microbiol Immunol, 24: 162-169, 2009.
 22. Okuda K, Hanada N, Usui Y, Takeuchi H, Koba H, Nakao R, Watanabe H, Senpuku H: Inhibition of *Streptococcus mutans* adherence and biofilm formation using analogues of the SspB peptide. Arch Oral Biol, 55: 754-762, 2010.
 23. Bikker FJ, Ligtenberg AJ, Nazmi K, Veerman EC, van't Hof W, Bolscher JG, Poustka A, Nieuw Amerongen AV, Mollenhauer J: Identification of the bacteria-binding peptide domain on salivary agglutinin (gp-340/DMBT1), a member of the scavenger receptor cysteine-rich superfamily. J Biol Chem, 277: 32109-32115, 2002.
 24. Lamont RJ, Jenkinson HF: Subgingival colonization by *Porphyromonas gingivalis*. Oral Microbiol Immunol, 15: 341-349, 2000.
 25. Marsh PD: Host defenses and microbial homeostasis: role of microbial interactions. J Dent Res, 68: 1567-1575, 1989.
 26. Carlsson J, Grahnen H, Jonsson G: Lactobacilli and streptococci in the mouth of children. Caries Res, 9: 333-339, 1975.
 27. Van Houte J, Gibbons RJ, Banghart SB: Adherence as a determinant of the presence of *Streptococcus salivarius* and *Streptococcus sanguis* on the human tooth surface. Arch Oral Biol, 15: 1025-1034, 1970.
 28. Chung WO, Demuth DR, Lamont RJ: Identification of a *Porphyromonas gingivalis* receptor for the *Streptococcus gordonii* SspB protein. Infect Immun, 68: 6758-6762, 2000.
 29. Lamont RJ, El-Sabaeny A, Park Y, Cook GS, Costerton JW: Role of the *Streptococcus gordonii* SspB protein in the development of *Porphyromonas gingivalis* biofilms on streptococcal substrates. Microbiology, 148: 1627-1636, 2002.
 30. Park Y, Simionato MR, Sekiya K, Murakami Y, James D, Chen W, Hackett M, Yoshimura F, Demuth DR, Lamont RJ: Short fimbriae of *Porphyromonas gingivalis* and their role in coadhesion with *Streptococcus gordonii*. Infect Immun, 73: 3983-3989, 2005.
 31. Amano A, Nakagawa I, Okahashi N, Hamada N: Variations of *Porphyromonas gingivalis* fimbriae in relation to microbial pathogenesis. J Periodontal Res, 39: 136-142, 2004.
 32. Hamada N, Sojar HT, Cho MI, Genco RJ: Isolation and characterization of a minor fimbria from *Porphyromonas gingivalis*. Infect Immun, 64: 4788-4794, 1996.

33. Ogawa T, Yasuda K, Yamada K, Mori H, Ochiai K, Hasegawa M: Immunochemical characterisation and epitope mapping of a novel fimbrial protein (Pg-II fimbria) of *Porphyromonas gingivalis*. FEMS Immunol Med Microbiol, 11: 247-255, 1995.
34. Bolstad AI, Jensen HB, Bakken V: Taxonomy, biology, and periodontal aspects of *Fusobacterium nucleatum*. Clin Microbiol Rev, 9: 55-71, 1996.
35. Amano A, Sojar HT, Lee JY, Sharma A, Levine MJ, Genco RJ: Salivary receptors for recombinant fimbriin of *Porphyromonas gingivalis*. Infect Immun, 62: 3372-3380, 1994.
36. Stinson MW, Haraszthy GG, Zhang XL, Levine MJ: Inhibition of *Porhyromonas gingivalis* adhesion to *Streptococcus gordonii* by human submandibular-sublingual saliva. Infect Immun, 60: 2598-2604, 1992.
37. Loimaranta V, Jakubovics NS, Hytonen J, Finne J, Jenkinson HF, Stromberg N. Fluid- or surface-phase human salivary scavenger protein gp340 exposes different bacterial recognition properties. Infect Immun, 73: 2245-2252, 2005.