retaining teeth by means of restorative treatment and daily oral care (Ekelund, 1991; Wårdh et al., 2000). Updated information is therefore needed for planning oral care to meet the needs of the long-term hospitalized elderly.

The combination of professional oral treatment and mucosal care is very useful for improvement of oral as well as general health in the institutional elderly. In conclusion, here we demonstrate the beneficial effects of mucosal care on infection control of pathogens such as mutans streptococci and *Candida* during oral professional care. However, the oral professional care is limited by the high costs of odontologic assistance, the oral and general health status of the elderly, the lack of time available for caregivers to carry out the tasks, etc., in the institutionalized elderly. The preventive attitude offered by repetition of oral professional care is based on nursing professionals and institutional resources. Moreover, there are limitations of this study in a small sample size, no group treated with only mucosal care, the influences to other oral pathogens, etc. Further investigation is warranted based upon these findings to evaluate whether mucosal oral care can support prevention of multiple oral pathogens in the expanded study plan.

Conflict of interest statement

None.

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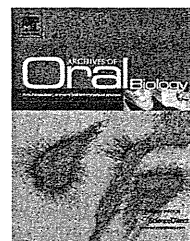
Inhibition of *Streptococcus mutans* adherence and biofilm formation using analogues of the SspB peptide

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ABSTRACT

Objective: Streptococcus gordonii is a pioneer colonizer of the enamel salivary pellicle that forms biofilm on the tooth surfaces. Recent reports show the surface protein analogue peptide {400 (T) of SspB 390–402 is substituted to K forming SspB (390–T400K–402)} from S. gordonii interacts strongly with salivary receptors to cariogenic bacteria, Streptococcus mutans. To characterize the analogue peptide biological activities, we investigated its binding and inhibiting effects, and the role of its amino acid moities.

Methods: We measured binding activity of analogue peptides to salivary components using the BIAcore assay; assayed inhibition activities of peptides for bacterial binding and growth on saliva-coated hydroxyapatite beads (s-HA); and describe the peptides interfering with biofilm formation of S. mutans on polystyrene surfaces.

Results: The SspB (390–T400K–402 and –401) peptides significantly bound with salivary components and inhibited the binding of S. mutans and S. gordonii to s-HA without bactericidal activity; but did not inhibit binding of Streptococcus mitis, a beneficial commensal. Further, the lack of D and E-L at position 390 and 401–402 in the peptide, and substituted peptide SspB (D390H– or D390K–T400K–402) did not bind to salivary components or inhibit binding of S. mutans. The SspB (390–T400K–402) peptide inhibited biofilm formation on salivary components-coated polystyrene surfaces in absence of conditioned planktonic cells.

Conclusions: We found constructing the peptide to include positions 390(D), 400(K) and 401(E), two surface positive and negative connective charges, and at least 12 amino acids are required to bind salivary components and inhibit the binding of S. mutans and S. gordonii.

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1. Introduction

Oral bacterial communities known as biofilms are characterized by species composition, surface or substratum composition, and the conditioning films coating the surfaces on which they form.[1,2] The interaction between oral streptococci and the salivary pellicle plays a large role in early colonization, aggregation and biofilm formation. *Streptococcus gordonii* colonizes the oral cavity very early in life[3] and interacts with other species such as *Porphyromonas gingivalis*, a periodontal pathogen, to form the predominant biofilm on the tooth surface.[4] *Streptococcus mutans* is the etiologic agent of human dental caries[2] and is shown to be able to colonize the mouth only after acquiring the enamel pellicle surfaces of the erupted tooth and to form part of the biofilm on tooth surfaces.[5] S. mutans and S. gordonii produce surface protein antigens (PAc, AgI/II, B, P1, SpaP, and MSL-1); and SspB (SspA), respectively, that have respective molecular masses of approximately 190[6–10] and

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180 kDa.[11,12] These proteins interact with salivary components that include lysozyme,[9,13] amylase,[9] 18,000 and 38,000 Da proline-rich proteins, and an agglutinin.[11]

We previously demonstrated the molecular mechanisms of the cell surface adhesion with the alanine-rich repeating region (residues 219–464, the A-region) of the SpaP (PAc) from S. mutans at the N-terminal region (residue 1–429) homologous to the SspB from S. gordonii.[5–12,9,13–17] The analogue peptide, position 400 T of SspB 390–402 was substituted with K forming SspB (390–T400K–402), had the highest binding activity to salivary components[17] and to the agglutinin (gp340/DNBT1) peptide (Scavenger Receptor Cystein-Rich domain Peptide 2; SRCRP2)[18] in comparison to various other SspB peptides and the PAc (365–377) peptide. This analogue peptide has two surface positive charges correlating with positively charged residues.[17] Recently, we reported the lysine position was important for binding between the SspB peptide and SRCRP2; and inhibited binding of S. mutans to saliva-coated hydroxyapatite (s-HA).[19] Therefore, the position of the charged amino acid residues in the peptide is essential for binding and inhibition.

Here we demonstrate inhibition of adherence of S. mutans and S. gordonii to surfaces previously treated with SspB (390–T400K–402) peptide, a truncated analogue peptide, compared to lysine alone using in vitro assays on s-HA, and biofilm formation on microtitre plates. Our data show the role of the analogue and truncated peptides and the connection to the surface's positive and negative charge results in the inhibition of the adherence. This provides important information about the initial phase of oral biofilm formation; and may yield preventive therapies for oral diseases.

2. Materials and methods

2.1. Bacterial strains and culture conditions

S. mutans MT8148, S. gordonii ATCC 10558 and S. mitis ATCC 6249 were grown in Brain Heart Infusion broth (BHI; Difco Laboratory, Detroit, MI) using 5% CO_2, 75% N_2 and 20% O_2 (GasPack CO_2, Becton Dickinson, Sparks, MD) at 37 °C prior to incubation with s-HA beads and in 96-well microtitre plates.

2.2. Peptide synthesis

The sequences of the analogue and truncated peptides are from a previous report[17] and were modified using the sequence of the SspB gene from S. gordonii M5[11] (Table 1). The peptides were synthesized using a stepwise solid-phase procedure at Scrum, Inc. (Tokyo, Japan). Purity was determined as greater than 95% using HPLC analysis. The peptides were dissolved in sterile de-mineralized water at 1 mg/ml; and aliquots were freeze-dried and stored at −20 °C. For each experiment, the freeze-dried peptides were re-dissolved to 1 mg/ml in sterile distilled-water (dH_2O).

2.3. Human saliva collection

Prior to saliva collection, the aim and details of the study were explained and consent was obtained from all subjects. Whole saliva from five human volunteers (27–44 years-old) was stimulated by chewing paraffin gum for 5 min; and the saliva was collected in an ice-chilled sterile bottle. After the saliva was clarified by centrifugation at 10,000 × g for 10 min at 4 °C, the supernatant without debris was taken and filter sterilized using a 0.45 μm filter (Millex-HV, Millipore, Cark, Co., Ireland); and used immediately to coat HA for the binding assay and the 96-well microtitre plates for the biofilm assay.

2.4. Bactericidal assay

S. mutans or S. gordonii was grown at 37 °C for 18 h using 5% CO_2, 75% N_2 and 20% O_2 (GasPack CO_2). The bacteria were harvested and washed with sterilized PBS three times; suspended in BHI; and adjusted to 5×10^7 CFU in a 1.5 ml bacterial suspension. Six hundred and fifty μM of SspB (390–T400K–402) peptide and 0.04% chlorhexidine (CHX) were, respectively, added into separate bacterial cultures. The mix suspensions were incubated for 16 or 22 h using 5% CO_2, 75% N_2 and 20% O_2 (GasPack CO_2). The bacterial concentration at 550 nm was measured using a spectrophotometer (Beckman Instruments, Inc., Fullerton, CA) and compared with control (no addition of peptide or CHX).

2.5. Inhibiting effects of the SspB (390–T400K–402) peptide to bind oral streptococci to s-HA

The s-HA binding assay was originally described by Liljemark et al.,[20] Koga et al.[21] and Arakawa et al.[22] with our modifications. To determine the effects of the SspB (390–T400K–402) peptide, truncated SspB (390–T400K–402) peptides, and various amino acids were used to examine S. mutans, S. gordonii and S. mitis adherence: 20 mg of HA (Sangi Co., Ltd., Tokyo, Japan) was equilibrated in phosphate buffered saline, pH7.4 (PBS); soaked in 1.5 ml of sterilized whole saliva; incubated for 60 min at room temperature; and washed twice with sterile PBS. s-HA was suspended and incubated for 60 min at 37 °C in 6.5, 32.5, 65.0, 650.0 and 3,250 μM of the test peptides or 68.5, 685.0, and 6,850.0 μM of lysine, threonine or aspartic acid solutions in PBS. Oral streptococci were grown at 37 °C for 18 h using 5% CO_2, 75% N_2 and 20% O_2 (GasPack CO_2) in BHI broth containing [methyl-^{3}H] thymidine (ICN Radiochemicals, Irvine, CA) at 10 μCi/ml having a specific activity of between 2.5×10^{-2} and 0.6×10^{-3} cpm/cell. The bacteria were harvested and washed with sterilized PBS three times; suspended in PBS; sonicated on ice for 10 s; and adjusted to 5×10^7 CFU in

Table 1	
Position	Sequence
SspB (T400K–401)	KE
SspB (399–T400K)	QK
SspB (399–T400K–401)	QKE
SspB (398–T400K–402)	YQKEL
SspB (391–T400K–402)	YQAKLAAYQKEL
SspB (390–399)	DYQAKLAAYQ
SspB (390–T400K)	DYQAKLAAYQK
SspB (390–T400K–401)	DYQAKLAAYQKE
SspB (390–T400K–402)	DYQAKLAAYQKEL
SspB (D390H–T400K–402)	HYQAKLAAYQKEL
SspB (D390K–T400K–402)	KYQAKLAAYQKEL

a 1.5-ml bacterial solution. The s-HA specimens were suspended in the [³H]-labelled bacterial solution and incubated with shaking for 90 min at 37 °C 5% CO_2, 75% N_2 and 20% O_2 (GasPack CO_2). Unattached cells were removed where the beads with bound [³H]-labelled bacteria were washed five times with sterilized PBS and transferred to a scintillation vial. After the addition of 10 ml of Ultimagold Scintillation Cocktail (Packard Co., Downers Grove, IL), the radioactivity was determined using a liquid scintillation counter (LSC-5000, Aloka Co., Ltd., Tokyo, Japan). In an assay without the peptide with an input of 7.5 × 10⁷ cells, 60% of the cells attached to the s-HA and showed radioactivity was from 36,500 to 42,500 cpm. Background values were less than 100 cpm.

2.6. Binding of analogue peptides to salivary components using the BIAcore Biosensor System

To confirm binding activities of the analogue peptides to salivary components, BIAcore Biosensor System (BIAcore 2000, BIAcore AB, Uppsala, Sweden) analysis for peptide binding was modified and performed as described previously.[17,20,15] We used a standard CM5 sensor chip with a carboxymethylated dextran-coated gold sensor chip activated with 70 μl of a solution containing 400 mM N-ethyl-N'-(3-diethylaminoprophyl) carbodiimide and 100 mM N-hydroxy-succinimide at a flow rate of 10 μl/min. Following activation, 70 μl of 1/4 diluted and sterilized whole saliva in 10 mM sodium acetate buffer (pH 5.0) was applied to the chip to immobilize it on the surface. Residual N-hydroxysuccinimide esters were then inactivated using 70 μl of 1 M ethanolamine hydrochloride. We used a flow rate of HBS-EP buffer saline (0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% surfactant P20) at 10 μl/min throughout the immobilization procedure. The peptide solutions at 0.625, 1.25 and 2.5 mM in PBS (pH 6.5) were exposed to the immobilized ligand(s) on the CM5 sensor chip (flow rate, 10 μl/min); and for the dissociation phase we injected HBS-EP at a rate of 10 μl/min. All binding experiments were conducted at 25 °C. At the end of each binding cycle, the surface of the sensor chip was regenerated using 50 mM glycine-NaOH (pH 9.5) for 60 s. Before re-using it, the returning to baseline of resonance unit was confirmed. The binding assays were performed less than three times using a single immobilized surface of the sensor chip because the reactivity was reduced by repeated use. Association (K_a) and dissociation (K_d) rate constants were determined using BIA-evaluation software (BIAcore AB).

2.7. Biofilm formation assay using microtitre plates

Biofilm formation by S. mutans was assayed using the method of Motegi et al.[23] The assay was performed as an in vitro experiment to study whether the inhibition effects by peptide on the adherence was useful for the prevention of biofilm formation which associated with development of dental caries in the condition including sucrose. The wells of a 96-well (flat bottom) micro titre plate (Sumitomo Bakelite, Tokyo, Japan) were coated with sterilized whole saliva for 30 min at 4 °C. To perform the inhibition assay using the SspB (390–T400K–402) peptide, 100 μl of the analogue peptide solution (650 μM in PBS) was added to the wells and incubated for 1 h at 4 °C. After

removing the peptide solution and washing three times with sterile PBS, 20 μl (4.0 × 10⁴ CFU) of a bacterial cell suspension was added to the wells with 160 μl tryptic soy broth without dextrose supplemented with 0.25% sucrose (TSBS). The plates were incubated at 37 °C for 1 h under 5% CO_2 in aerobic conditions; then the liquid medium including planktonic cells was removed by decantation; and the wells were rinsed twice with sterile PBS. Each well then received 200 μl fresh TSBS and the culture was incubated for 5, 8, 11 and 15 h at 37 °C aerobically with 5% CO_2. The liquid medium was removed and the wells were rinsed twice with sterile PBS. The plates were then air-dried and stained with 0.25% safranin for 15 min. After staining, the plates were rinsed with dH_2O to remove excess dye and air-dried. The biofilm mass was measured quantitatively using an ELISA microplate reader at A_{492} (Multiskan Bichromatic Laboratory Japan, Tokyo, Japan) where the biofilms were uniformly spread on the bottoms of the wells in the 96-well plates.[15]

2.8. Modeling of the secondary structure using surface charge

The A-region of SspB is thought to assume an α-helical structure.[11,23] Modeling of the secondary structures using the surface charge of the SspB (390–T400K–402), truncated SspB (390–T400K–402), SspB (D390H–T400K–402) and SspB (D390K–T400K–402) peptides were performed using a MOE (Molecular Operating Environment, Version 2006-08, Chemical Computing Group. Inc., Montreal, Canada) with the developing platform of PPh4Dock and the MMFF94 s force field subroutines used for energy evaluation.[24] The proteins and protein analogues were evaluated and analyzed based on an α-helical structure using the MOE system. An α-helical structure was constructed using a Phi-angle of −65° and Psi-angle of −39° with energy minimization of possible structures in these peptides; and was performed using atom size, bond stretch, angle bend, stretch-bend, out-of-plane, torsion, and charge.

3. Statistics

Comparisons of bacterial adherence and biofilm formation using peptide or amino acid treatment and non-treatment were performed using the ANOVA and Fisher's PLSD tests. Differences at $p = 0.05$ or less were considered to be statistically significant.
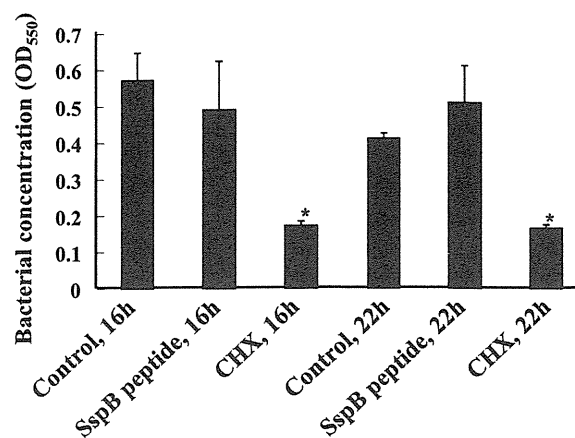
4. Results

4.1. Effects of SspB analogue peptide on bacterial binding to s-HA

The adherence of S. gordonii and S. mutans were significantly inhibited using 650 μM SspB (390–T400K–402) peptide; but not at 6.5 and 65 μM (Fig. 1A and B). Adherence of S. mitis was not inhibited by the peptides (Fig. 1C). This suggests the analogue peptide is a significant and specific inhibitor for the binding of S. gordonii and S. mutans to s-HA. The analogue peptide contains lysine which plays an important role in the binding of
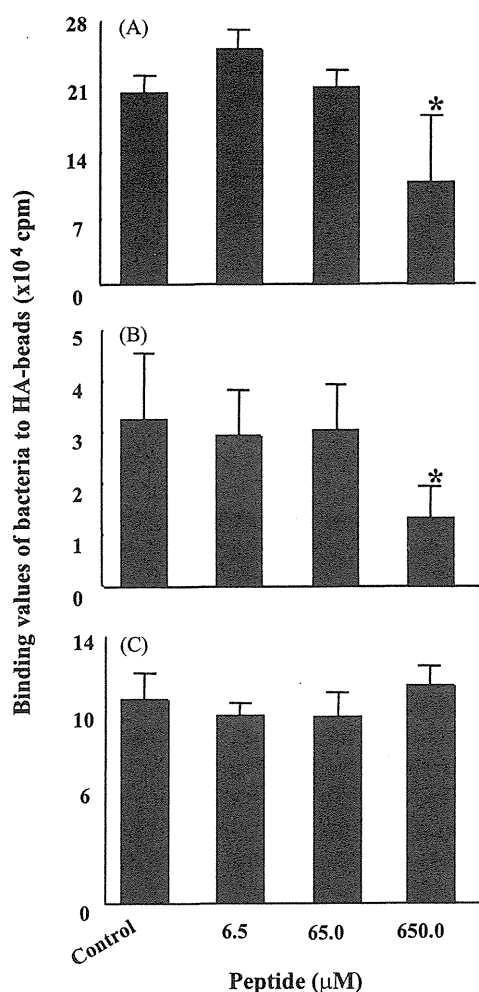
Fig. 1 – SspB peptide inhibits streptococcal adherence to s-HA. The adherence levels (cpm) of *S. gordonii* ATCC 10558 (A), *S. mutans* MT8148 (B) and *S. mitis* ATCC 6249 (C) to s-HA treated with 6.5, 65.0 and 650.0 μM of SspB (390–T400K–402) peptide are shown. The data are the means ± SD of three independent assays. Asterisks show significantly different relative levels of bacterial adherence (*p* < 0.05 vs. control: non-treated and saliva-coated hydroxyapatite).

Fig. 2 – Bacterial growth in the presence of the SspB peptide or CHX. To determine whether 650 μM the SspB peptide has cationic anti-microbial activity, SspB (390–T400K–402) peptide and a positive control, 0.04% CHX, were applied into *S. mutans* cultures grown in BHI. The mixed suspensions were incubated for 16 or 22 h; and the bacterial concentrations at 550 nm were measured using a spectrophotometer. The data are the mean ± SD of three independent assays. Asterisks show significantly different relative levels of bacterial growth (*p* < 0.05 vs. control: PBS-treated *S. mutans*).

the SspB peptide to salivary components where we constructed an analogue protein with two positive surface charges in connection with the positively charged residues. These peptides commonly adopt an amphipathic conformation in which positively charged and hydrophobic groups segregate onto opposing faces of an α-helix, a β-sheet, or some other tertiary structure and may have anti-microbial activities.[25] SspB (390–T400K–402) peptide has cationic amino acid and α-helix structures;[19] and, therefore, to determine whether the peptide has cationic anti-microbial activity we performed the bacteriocidal test. One mg/ml SspB (390–T400K–402) peptide and positive control, 0.04% chlorhexidine (CHX), were added to growing *S. mutans* cultures. CHX inhibited both 16 and 22 h growth of *S. mutans*; whereas the peptide did not show inhibition of growth (Fig. 2).

To determine the influence of the positive charged amino acid, lysine, in the binding of *S. mutans*, lysine alone was used in a competitive inhibition assay. At 685 μM lysine alone, higher than the molarity (650 μM) of the SspB (390–T400K–402) peptide, lysine alone did not show inhibition; whereas at an excess molarity of 6850 μM lysine alone significantly inhibited the binding activities (Fig. 3). Members of other similar amino acids such as glutamine and aspartic acid that are negatively charged and threonine, the original residue before substitution by lysine in SspB (390–402) did not inhibit the binding of *S. mutans* (data not shown). To determine the effects of changes in the secondary structure of the analogue peptide, we examined if the numbers of amino acids and two surface positive charges and their connection with the positive charged residues are required for the inhibition. We constructed various truncated peptides, substituted amino acids in the preparations, and made small peptides (Table 1). The dimer amino acid peptides (KE or QK) of lysine and glutamic acid or glutamine did not show significant inhibition as shown by SspB (390–T400K–402) peptide at the same molarities (Fig. 4). The trimer amino acid peptide (QKE) of lysine, glutamic acid and glutamine, and the pentamer amino acid peptide (YAKEL) containing lysine at its centre did not show significant inhibition. The central chain (amidohydrogen) of SspB has a positively charged aspartic acid that is partially exposed on the surface of the α-helical peptide structure determined in a previous crystallography analysis.[17] The authors suggest the two surface positive charges at residues 390(D) and 400(K) were important for the binding to the salivary components. To study the role of the positively charged residues for the inhibition activity, we constructed a peptide without aspartic acid and substituted a positive charge
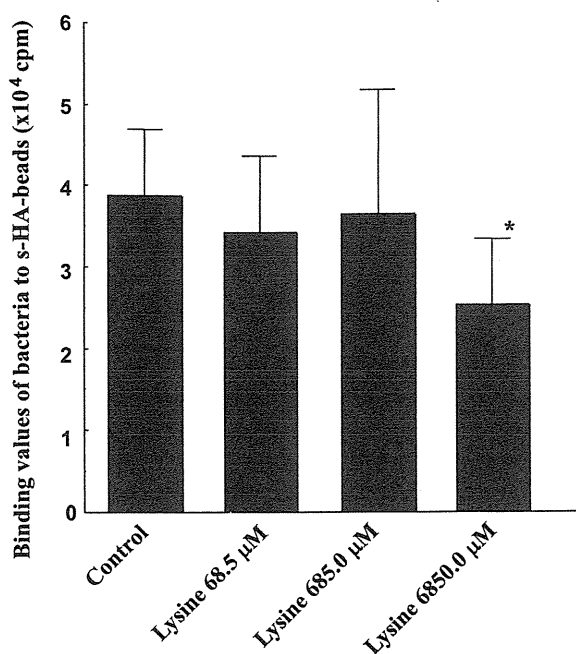
peptides lacking aspartic acid did not show significant inhibition (Fig. 4). Expression of the positive and negative charge at position 390 was required for the inhibition effects of the peptide in S. mutans adherence (Fig. 5). After removing leucine at position 402 (Table 1), the SspB (390–T400K–401) peptide was synthesized and used in the inhibition assay. The SspB (390–T400K–401) peptide showed significant inhibition at similar levels as the SspB (390–T400K–402) peptide. S. gordonii and S. mutans adherence had dose-dependent inhibition by the SspB (390–T400K–402) peptide; but not after removing more amino acids using the SspB (390–399) peptide (Fig. 6AB). S. mitis adherence was not inhibited by any of these peptides (Fig. 6C). Removing more amino acids from the SspB (390–T400K) peptide removed significant inhibition (Fig. 4). This shows the negative charged amino acid, glutamic acid, at position 401 was required for the inhibition effect (Fig. 5).

To further quantitatively confirm the binding activity of analogue and truncated peptides to salivary components, an adhesion binding assay was performed using the BIAcore Biosensor System. Peptides (1.25 mM) were applied to the sensor chip having the immobilized salivary components. The K_a and K_d were determined using the BIA-evaluation software (BIAcore). The K_a of peptides were lower than K_a levels (1×10^4–10^9 M^{-1}) seen with monoclonal antibodies to antigens; however, our values were significant (Table 2). There were no significant differences between SspB (390–T400K–402) and SspB (390–T400K–401) peptides in K_a and K_d. Other peptides and peptides at less than 650 µM were below the detectable K_a and K_d using the BIAcore assay. Taken together, the connection of the positively and negatively charged residues involving aspartic acid, lysine and glutamic acid at positions 390, 400 and 401; and the 12 amino acid sequence from positions 390 to 401 were required for binding and the inhibition activity of the SspB (390–T400K–402) peptide.
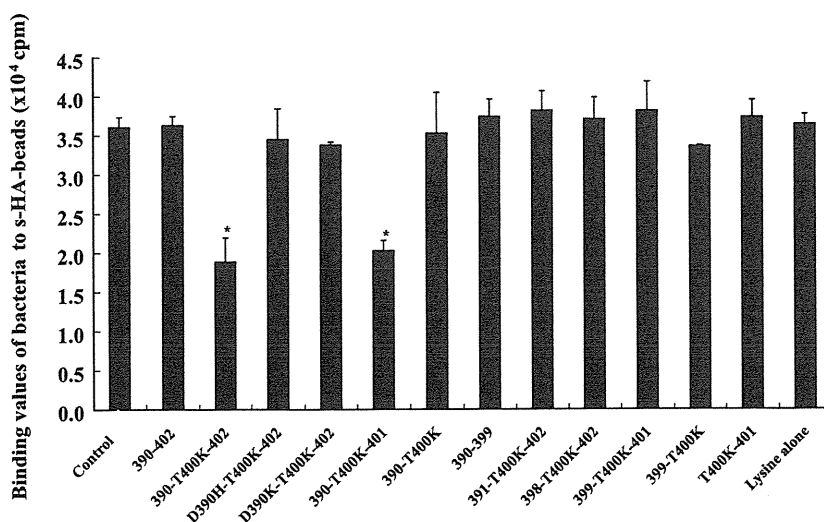

Fig. 3 – Inhibition using lysine alone for S. mutans adherence to s-HA. The adherence levels (cpm) of S. mutans MT8148 to s-HA treated with 68.5, 685.0 and 6850.0 µM lysine are shown. Control: binding values of S. mutans to non-treated s-HA. The data are the means ± SD of three independent assays. Asterisks show significantly different relative levels of bacterial adherence (p < 0.05 vs. control: non-treated s-HA).

amino acid at position 390 (Table 1). SspB (391–T400K–402) and SspB (D390H or D390K–T400K–402) peptides were synthesized and used in the inhibition assay. We found substituted

Fig. 4 – Inhibition using SspB truncated peptides for S. mutans adherence on s-HA. The adherence levels (CPM) of S. mutans MT8148 to s-HA treated using 650.0 µM lysine alone, SspB (T400K–401), SspB (399–T400K), SspB (399–T400K–402), SspB (398–T400K–402), SspB (391–T400K–402), SspB (390–399), SspB (390–T400K), SspB (390–T400K–401), SspB (390–T400K–402), SspB (D390H–T400K–402) and SspB (D390K–T400K–402) peptides are shown. Control: binding values of S. mutans to non-treated s-HA. The data are the means ± SD of three independent assays. Asterisks show significantly different relative levels of bacterial adherence (p < 0.05 vs. control: non-treated and saliva-coated hydroxyapatite).

SspB(D390K-T400K-402)   SspB(399-T400K)   SspB(390-T400K-401)

KYQAKLAAYQKEL   DYQAKLAAYQK   DYQAKLAAYQKE

SspB(D390H-T400K-402)   SspB(391-T400K-402)   SspB(390-T400K-402)
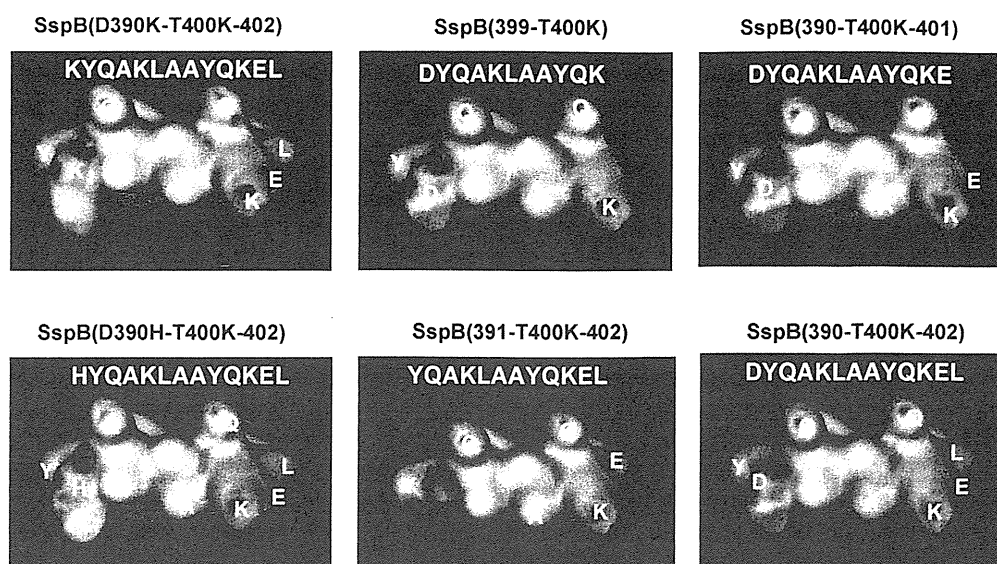
HYQAKLAAYQKEL   YQAKLAAYQKEL   DYQAKLAAYQKEL

Fig. 5 – Schematic computer model of the substituted peptides, SspB (390–T400K–402) and truncated SspB (390–T400K–402) peptides. Model of the secondary structure and surface charges (positive [blue] and negative [red]) in the peptides were constructed by using the MOE software with chemical computing graphics. Some of the amino acid residues on the surface of the peptides are indicated.
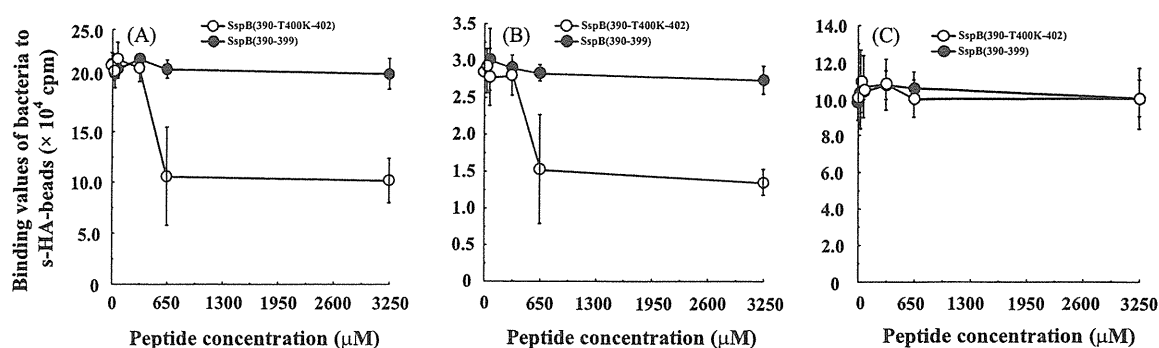

Fig. 6 – Inhibition using SspB (390–T400K–402) and SspB (390–T400K–401) peptides for streptococci adherence on s-HA. The adherence levels (cpm) of S. gordonii ATCC 10558 (A), S. mutans MT8148 (B) and S. mitis ATCC 6249 (C) to s-HA treated with 0.0, 32.5, 65.0, 325.0, 650.0 and 3,250.0 μM of SspB (390–T400K–402) or SspB (390–399) peptide are shown. The data are the means ± SD of three independent assays.

4.2. Effects of the SspB analogue peptide using the microtitre plate inhibition assay

We determined if SspB (390–T400K–402) inhibited adherence of S. mutans and influenced biofilm formation by S. mutans conditioned with 0.25% sucrose. The treatment with the

Table 2 – Association and dissociation rate constants of SspB peptides to immobilized salivary components.		
SspB peptide	Association rate constants $(K_a \times 10^4 \, M^{-1})$	Dissociation rate constants $(K_d \times 10^{-5} \, M)$
390–T400K–402	1.7 ± 0.9	8.7 ± 5.9
390–T400K–401	2.9 ± 1.1	3.8 ± 1.3

analogue peptide on the polystyrene plates before inoculation of S. mutans to the saliva-coated well did not inhibit biofilm formation at 6, 9, 12 and 16 h culture without withdrawing the planktonic cells (data not shown). However, after withdrawing the planktonic cells at 1 h, photographs shows binding at 5 and 8 h was inhibited by pre-treatment with 650 μM analogue peptide (Fig. 7A); and significant inhibition was confirmed using quantitative analysis in comparison to the control, a non-treated culture (Fig. 7B). At 650 μM analogue peptide, the biofilm formation after withdrawing planktonic cells at 1 h was significantly inhibited using S. mutans, when the plates were twice washed with sterile PBS, after the 5 and 8 h culture (Fig. 7C). The effect slowly diminished, where at 11 h the culture biofilm was not inhibited using pre-treatment with the SspB (390–T400K–402) peptide using no washing with PBS (Fig. 7B); and showed slight inhibition when twice washed
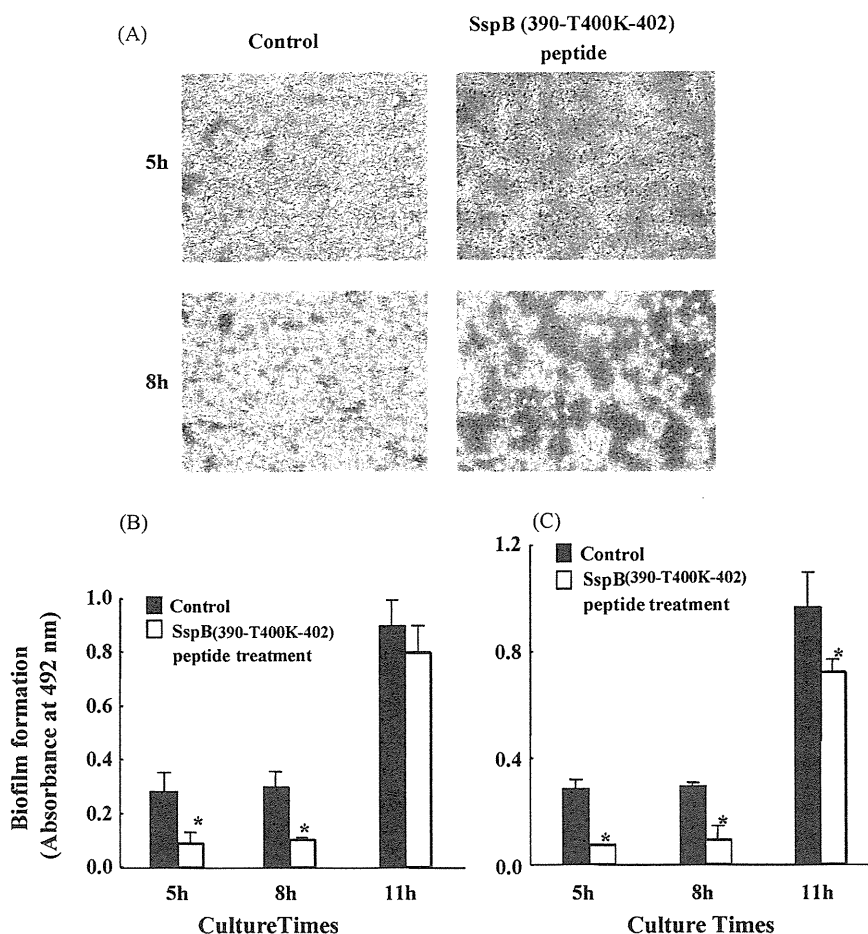
Fig. 7 – Inhibition using SspB peptides of S. mutans biofilm formation on polystyrene surfaces. The culture medium including planktonic cells of S. mutans MT8148 was removed from the polystyrene surface treated using 650 μM SspB (390–T400K–402) peptide after 1 h culture of S. mutans MT8148. Photographs of biofilms formed by S. mutans MT8148 on the polystyrene surface of a 96-well microtitre plate coated with salivary components and treated with SspB (390–T400K–402) peptide at 5 and 8 h culture (40×) (A). Formed biofilms were stained using safranin and the absorbance was measured at 492 nm (B). After removing the culture medium, the wells were rinsed a second time with sterile PBS (C). Fresh medium was added and they were cultured for 5, 8 and 11 h. The data are expressed as the means ± SD of triplicate assays. Asterisks show significantly different relative levels of biofilm formation ($p < 0.05$ vs. control: non-treated and the saliva-coated polystyrene surface).

with sterile PBS (Fig. 7C). By 15 h, there was no significant inhibition of adherence by pre-treatment with the peptide (data not shown).

5. Discussion

Controlling dental plaque bacteria is important in the prevention and treatment of oral diseases. Recently, various types of anti-microbial peptides against oral bacteria derived from various sources have been suggested.[26–30] However, the tested peptides shown here have no bactericidal activity and/or cell growth enhancement or growth inhibition (Fig. 2). The constructed analogue peptide described here exhibits a significant binding to salivary components and has a preferential adherence inhibition for S. mutans and S. goedonii on the salivary components-coated HA as compared to other

beneficial commensal streptococci such as S. mitis that were not inhibited. As S. gordonii and S. mutans likely compete for the same niche environment in the dental plaque biofilm, this capacity may allow S. gordonii to effectively win the interspecies competition with S. mutans.[31] Accordingly, S. gordonii and S. mutans may compete for salivary adhesion sites such as homologue SspB regions including SspB (390–402) and PAc (365–377).[17] Therefore, physical inhibition using the analogue peptide may potentially be useful in preventing the interaction between salivary components and the common adhesin area on S. mutans and S. gordonii surface proteins; and may play a role in preventing bacterial adherence and dental caries using a safe oral treatment without side-effects; whereas other peptides have direct effects such as bactericidal activity, killing both beneficial and pathogenic agents.

In previous studies, lysine inhibited whole saliva- and salivary agglutinin-mediated aggregation of both S. mutans

and S. sanguinis[32] as well as S. mutans adherence to an adhesin-promoting-coated hydroxyapatite.[33] Previous reports support our findings that in theory the lysine moiety has a role in the adherence inhibition of S. mutans and S. gordonii. Excess lysine alone inhibits S. mutans adherence; however at the same molarity, lysine alone does not inhibit adherence compared to the SspB (390–T400K–402) peptide, which shows significant inhibition (Fig. 3). Synthetically adding glutamine or glutamic acid, and glutamine and glutamic acid to the N or/and C-terminus at the lysine and other truncated peptides of the SspB (390–T400K–402), except for the SspB (390–T400K–401) peptide, did not increase binding to the salivary components and did not inhibit adherence (Fig. 4). This shows the leucine position 402 on the c-terminal side was not required for binding and inhibition. The responsible peptide adhering to the salivary receptors required the appropriate secondary structure with surface charges for adherence inhibition of S. mutans (Fig. 5). Recently, Daep et al. reported the SspB peptide (residues 1167–1193) containing positively charged amino acids at position 1182 or hydrophobic residues at position 1185 bound to P. gingivalis more efficiently than control peptides containing asparagine and valine at these positions.[34] The study suggested perturbation of the peptide secondary structure influences the adherence activity. Here we show a lack of aspartic acid at position 390 in the SspB (391–T400K–402) peptide did not inhibit the adherence of S. mutans to s-HA. Further, peptides substituted with positively charged amino acids at position 390 did not show significant inhibition. Aspartic acid at position 390 is a negatively charged amino acid showing negative and positive charged areas in the secondary structure of SspB (390–T400K–402) using chemical computing graphics (Fig. 5). The c-terminus neighbour of lysine at position 400 is glutamic acid having a negatively charged amino acid at position 401 in the SspB (390–T400K–402) peptide. The lack of glutamic acid at position 401 resulted in a marked loss of the inhibition activity for the peptide. Cheng et al.[35] suggests ion-pairing (positive and negative charged functional groups) interactions are important for protein stabilization. Therefore, we conclude the positive charges form durable interactions with negative charges in the analogue peptide. The structural and expressive difference in the surface protein charge in the active peptides may influence inhibition. Taken together, the two surface positive charges in connection with the negatively charged residues at position 390, 400 and 401, and the 12 amino acid sequence of 390–T400K–401 are required for the adherence inhibition of S. mutans. These new characteristic activities in amino acid sequence were found in addition to the required position of lysine previously observed by our laboratory.[19]

Biofilm formation at 5 and 8 h after culture re-start, S. mutans was inhibited with the peptide pre-treatment on saliva-coated polystyrene surfaces when the planktonic cells were removed after 1 h culture with the S. mutans. The biofilm formation at 5 and 8 h had log-phase biofilm growth.[36] We believe once the bacterial adherence is inhibited, biofilm formation could be immediately prevented. However, the inhibition effects were not shown with planktonic cells and were poor without planktonic cells in stationary phase biofilm growth (>11 h). Possibly, the planktonic cells adhered to polystyrene surfaces and was un-bound with the peptide

using synthesis of glucan in the medium containing sucrose because the synthesis of glucans is critical both for the adherence of the organisms to the tooth surfaces and for their accumulation and persistence.[37] Further, the planktonic cells reproduced increasing the developed biofilm where this may disturb the inhibition by the peptide during stationary phase biofilm growth. Therefore, biofilm formation in glycan inducing media including sucrose was limited to a short culture time (<8 h). However, if used regularly in routine home and clinically in the oral cavity, peptide therapy to prevent dental caries requires oral routine hygiene techniques to remove planktonic cells and the limitation of sugar diet intake.

After treatment using analogue SspB peptide, the commensal streptococci such as S. mitis which is the major bacteria in the oral cavity of healthy humans[38] may re-colonize immediately on the tooth surface to keep a healthy oral flora excluding S. mutans. Therefore, in conclusion, it may be possible to employ anti-adherence peptides in future routine therapies preventing oral infections. Thus as the oral cavity is readily accessible for regular local application to the tooth surface, oral hygiene with brushing and limitation of sugar diet intake may be particularly suitable for including this small peptide.

Acknowledgement

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多剤性耐性菌アシネトバクター&NOM-1の歯科医療における感染防止対策

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■はじめに

昨年発生した新型インフルエンザは、本年度8月になりWHO（世界保健機関）よりポストパンデミックが発表され、終息してきた。しかし、それにとって代わるようにこの夏、多剤耐性アシネトバクターやNDM-1産生多剤耐性菌の感染症が発生し、感染症の脅威は後を絶たたない（図1）。記憶に新しいSARS、増え続けているHIV感染者、150万人はいるとされるHBV、HCV感染者等、医療従事者にとって、感染症の問題はクリアしていかなければならない重要課題である。

歯科医療にとって、このような新規感染症は、直接歯科疾患にかかわらないとしても、治療の際の患者との近接、唾液や血液の飛び散りなどから病原体に曝されるリスクが高いため、脅威に感じる歯科医師も多いだろう。しかし、一般歯科医院で行う歯科治療において、標準予防策（スタンダートプレコーション）が施されていれば、それ程脅威になることは少ない。

問題は、スタンダートプレコーションが行われていない点にある。筆者が研究代表者を務める厚生労働科学研究班の事業の成果において、某県の歯科医師会所属歯科医師対象のアンケート調査では、スタンダードプレコーションの理解率は20%前後であり、これは回収率が約15%であることを考えると、全体はもっと低いことが予想される。

今回、緊急情報というタイトルで原稿を書くことになったが、この多剤耐性菌感染症の問題を契機に、今一度、各歯科医師がスタンダードプレコーションを導入する機会となることを望む次第である。

■アシネトバクターとは

土壌や河川水などの自然環境中に生息する環境菌で、健常人では普通無害である。がん末期、糖尿病などにより感染防御能力の低下した患者において尿路感染症、肺炎、敗血症、手術部位感染症の起因菌になり得る。欧米では、人工呼吸器関連肺炎の起因菌として10年前から警戒されるようになった。

アシネトバクター属菌は、グラム陰性桿菌で、湿潤環境を好むが乾燥にも強い（3週間生きる）。衣服、皮膚、人工呼吸器、流し、ドアノブなどの環境中に長期に生存する。*Acinetobacter lwoffii, Acinetobacter calcoaceticus, Acinetobacter baumanni* などの菌種が存在するが、現在耐性菌として問題に
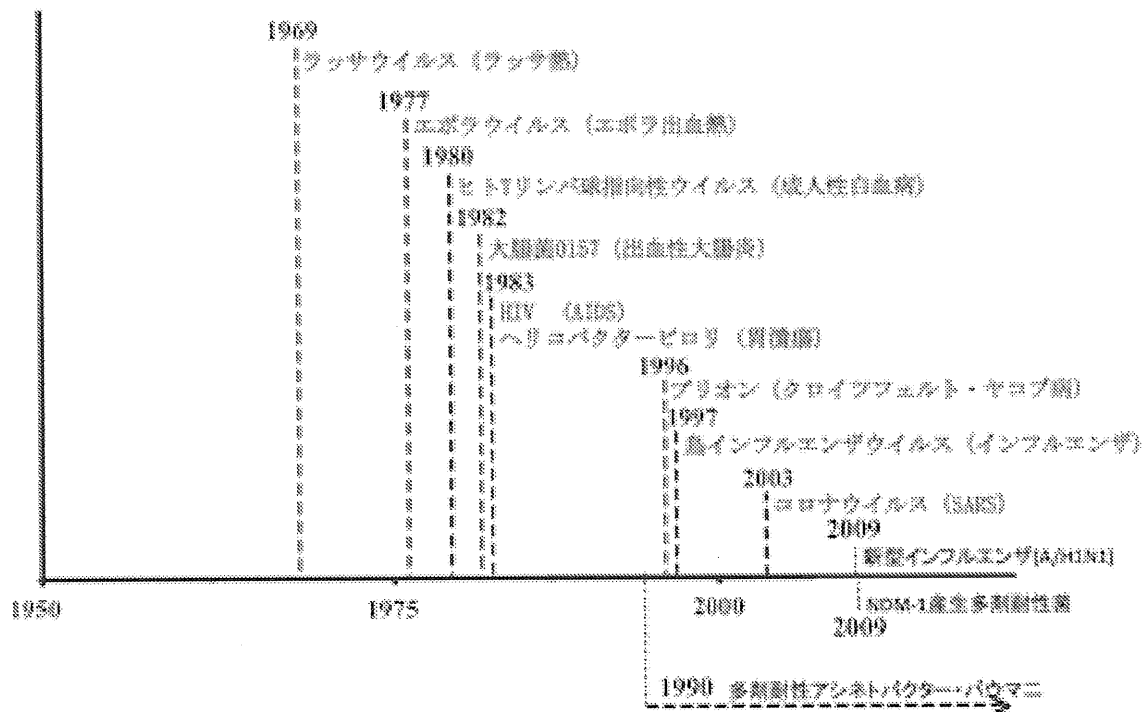
図❶　近年固定された主な病原体の年表

なっているのは *A. baumanni*（アシネトバ
クター・バウマニ）である。高齢者の口腔
からも日和見菌として検出された報告がある
（図 2 ）。

▓多剤耐性アシネトバクター

　日本の定義では、カルバペネム系、フルオ
ロキノン系、アミノグコシド系の抗菌薬に耐
性を示す株。その多くが *A. baumanni* であ
る。遺伝子断片でも取りこみやすく、変異し
やすい性質をもっているため、耐性遺伝子を
獲得しやすい。アシネトバクターの多剤耐性
化は、世界的にも最近20年くらいの間に急
激に進んでいる。最近、日本で検出される多
剤耐性アシネトバクターは、外国から流入し
てきた菌株と考えられることが多い。

　平成22年 9 月22日の Yahoo！ニュースで
は、某医療機関で、全入院患者の多剤耐性ア
シネトバクター・バウマニの保菌状態を調べ
た結果、昨年 1 月以降、感染した患者が合計
59人に上ったことが明らかになった。アシ
ネトバクターが多剤耐性化したからといって、
耐性化していないアシネトバクターと比べ病
原性が向上したわけではない。一般健常者に
は無害であり、例え口腔に入ったとしても、
感染症を引き起こすことはない。病院に入院
している防御能力が低下した患者や人工呼吸
器を装着した患者などに感染した場合、感染
症を引き起こす原因となる。その場合、抗生
物質による効果がないから、治療が困難にな
る。
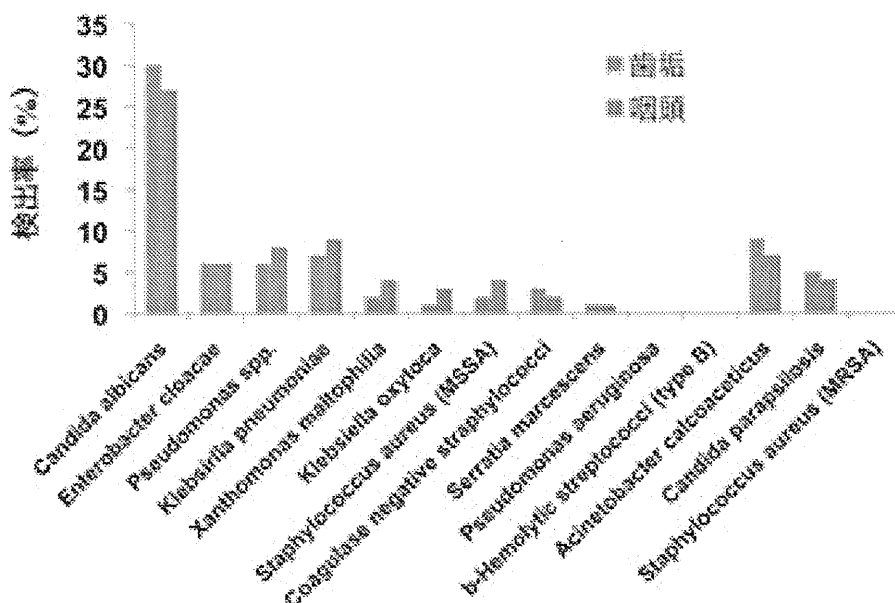
に囲まれた菌は、肺炎桿菌（*K. pneuminiae*）とアシネトバクター（*A. calcoaceticus*）である

図❷　自立高齢者（72±0.3歳）の口腔サンプルにおける日和見菌の検出率（Senpuku et al. Gerontology 2003: 49: 301-309. より引用改変）

　一般歯科医院の場合、感染防御力が低下した患者を治療するケースがなければ、それ程問題となることはない。しかし、その機会がないからといって完全に無視するわけにはいかない。いかなる感染症の患者が来院しても対応できるように、スタンダードプレコーションを確実に行い、患者と歯科医師の信頼関係を保つことが重要である。

NDM-1（ニューデリーメタロ -b- ラクタマーゼ）を産生する多剤耐性菌とは

　複数の抗菌薬に対する複数の耐性遺伝子を同時に保有しており、ほとんどの抗菌薬に耐性を示す。カルバペネムという切り札的抗菌薬を分解する NDM-1という酵素を産生する。NDM-1産生多剤耐性菌は、大腸菌、肺炎桿菌などの腸内細菌科の菌にみられる。日本で

は、2010年9月に第1例が報告された。

　大腸菌や肺炎桿菌は、一般の健常者の腸内に生息する常在菌である。肺炎桿菌(*Klebsiella pneumoniae*)は、高齢者の口腔でも分離される（図2）。

　アシネトバクターと同様に大腸菌や肺炎桿菌も感染防御能力の低下した患者において、院内感染症や日和見感染症の原因になる。健常者では、腸内、口腔内に留まっている限り、無害、無症状である。しかし、膀胱に感染すれば膀胱炎、肺に感染すれば肺炎、血中に感染すれば敗血症を発症し、耐性菌なので治療が困難になる。NDM-1を産生する菌が、非産生菌よりも病原性が高いということはない。

予防と対策
　両耐性菌とも同様に、手洗い、手指消毒を

図❸　某 A、某 C 県歯科医師会所属歯科医師に対するアン
　　　ケート調査の結果

他の多剤耐性菌

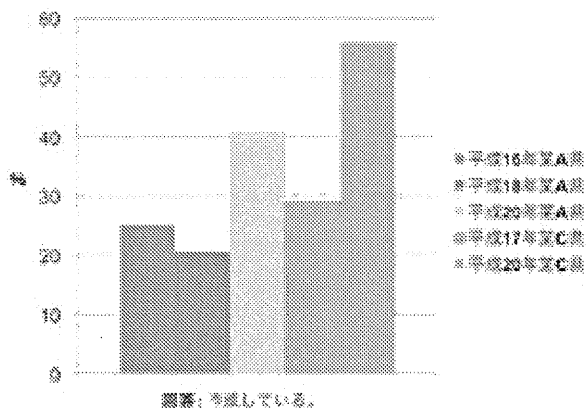　多剤耐性菌は、アシネトバクターや大腸菌だけでなく、MRSA などの黄色ブドウ球菌、腸球菌、肺炎球菌、緑膿菌などにもみられ、今後いかなる多剤耐性菌感染症が起こったとしても対応できる体制作りをしなくてはならない。

歯科医療における院内感染対策の導入についての研究成果

　歯科医療における院内感染対策の導入については、厚生労働科学研究班が平成16年から始め、今年で7年目を迎えようとしている。その間に、平成19年には医療法の一部が改正され、歯科診療所における院内感染制御体制の整備が求められるようになった。さらに、平成20年度の診療報酬改定では、歯科外来診療環境体制加算が新設された。歯科医師国家試験においても、スタンダードプレコーションが出題されるようになった。

　このような状況を踏まえ、多くの関係機関で研修会が開催され、また関連本も出版されるようになった。研究班の成果では、某 A 県か某 C 県歯科医師会所属歯科医師に対するアンケート調査結果において、平成16、17年〜平成20年にかけて、スタンダードプレコーションの理解率が2倍以上（図3）、自院による感染対策マニュアル作成や感染対策に関するスタッフへの教育率も約2倍上昇していることが明らかとなった（図4、5）。少しずつであるが、歯科医療において院内感染対策が導入される傾向にあると考えている。
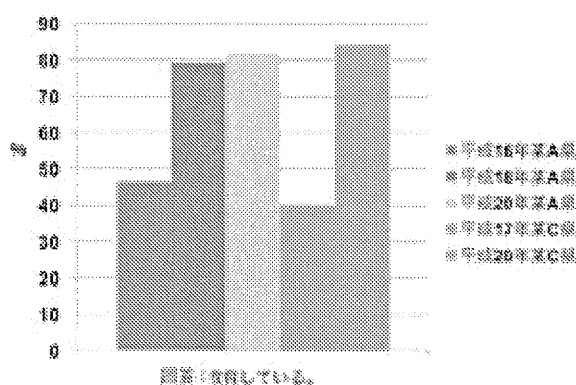
　しかし、口外バキュームの設置や患者ごとのタービンヘッドの交換は、若干の上昇に止

　徹底することを基本とし、流し台などの水回り環境、歯科ユニット、医療器具などの清掃、消毒を徹底する。現場スタッフによる環境の清掃・消毒に関して管理表を導入することが効果的である。次亜鉛素酸ナトリウム製剤(1w/v%)や消毒用エタノール（76〜81%）による消毒が効果的である。

　エプロン、マスク、帽子、紙コップ等、できる限りディスポーザブル化し、使用後廃棄をする。防護用メガネを使用する。歯科用器材は洗浄後、オートクレーブにて滅菌する。飛び散りのリスクを減らすために口外バキュームの設置。器具の整理整頓、患者ごとのタービンヘッドの交換、歯科ユニット間のパーティションの設置等、スタンダードプレコーションに必要な項目についてクリアしていく。

質問：感染対策マニュアルを作成していますか？

図❹　某A、某C県歯科医師会所属歯科医師に対する
　　　アンケート調査の結果

質問：感染対策に関しスタッフの教育をしていますか？

図❺　某A、某C県歯科医師会所属歯科医師に対するア
　　　ンケート調査の結果

まっている（図6）。これは、分析の結果、
一部の歯科医師に対する院内感染対策の導入
に関して一定の効果がみられるものの、地域、
年齢、収入格差に左右されているのが原因と
考えられ、院内感染対策達成はまだまだ不十
分のままであった。一律に院内感染対策を導
入していくためには、導入しやすい標準化さ
れた指標を作成し、それを普及するシステム
を構築する必要がある。

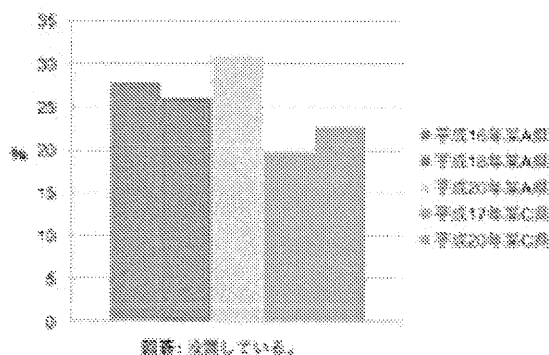質問：口外バキュームを設置していますか？

図❻　某A、某C県歯科医師会所属歯科医師に対す
　　　るアンケート調査の結果

▓おわりに

　多剤耐性菌が出現した理由は、近年の抗生
物質の乱用に起因しているのではないかと考
える。科学、医療の進歩とともに微生物も進
化しているのである。このイタチごっこは、
永年続くかもしれない。その進化に触発され
て、歯科医療における院内感染対策も成熟し
ていくことを期待している。

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3）国立感染症研究所IASRのホームページ：http://www.nih.go.jp/iasr
4）厚生労働省のホームページ：http://www.mhlw.go.jp/bunya/kenkou/kekkaku-kansenshou19
5）厚生労働科学研究費補助金（医療技術評価総合研究事業）：歯科医療における院内感染防止システムの開発；平成16年〜平成18年度総合研究報告書.
6）厚生労働科学研究費補助金（地域医療基盤開発推進研究事業）：歯科医療における院内感染対策の評価指標の開発と有効性の検証；平成19年〜平成21年度総合研究報告書.

SHORT COMMUNICATION

Progress of oral care and reduction of oral mucositis—a pilot study in a hematopoietic stem cell transplantation ward

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Hitomi Nishimoto · Yoshinobu Maeda ·
Mitsune Tanimoto · Shogo Takashiba

Abstract

Purpose Oral mucositis is a common symptomatic complication associated with hematopoietic stem cell transplantation (HCT). We use simple strategies aimed to reduce oral mucositis by keeping the oral cavity clean and moist. Here, we report on the progress of oral care and the changes in the degree of oral mucositis. The purpose of this pilot study is to evaluate the effects of our strategies on the prevalence and the severity of oral mucositis.

Methods Fifty-three consecutive patients from 2003 to 2006 administered with conventional allogeneic HCT were enrolled in this study. The degree of oral mucositis was evaluated daily in all patients. Our oral care program was divided into two periods: "examination and trial period (2003 and 2004)" and "intensive oral care period (2005 and 2006)." In the latter, an oral care regimen was carried out systematically by a multidisciplinary team.

Results Using our oral care strategies, the prevalence of ulcerative oral mucositis was decreased significantly. The rate was reduced from 76% (10 of 13) of patients with ulcerative oral mucositis in 2003 to only 20% (3 of 15) in 2006.

Conclusions Our pilot study suggests that oral mucositis in HCT patients can be alleviated by simple strategies aimed at keeping the oral cavity clean and moist.

Keywords Oral care · Supportive care · Oral mucositis · Hematopoietic stem cell transplantation

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Introduction

Oral mucositis is one of the most common symptomatic complications associated with high-dose chemotherapy, especially hematopoietic stem cell transplantation (HCT) [1, 2]. Severe mucositis is associated with not only intolerable pain but also the risk of systemic infection. Oral mucositis is a significant cause of suffering and morbidity in patients receiving myeloablative chemotherapy [3]. Effective interventions to alleviate this complication are needed [3].

Keeping the oral cavity clean is one of the important interventions because this prevents both mucositis itself and infection associated with oral mucositis. The Multinational Association for Supportive Care/International Society of Oral Oncology mucositis guidelines [4] and the National Cancer Center Network task force report [5] both recommend good oral hygiene in these patients.

Keeping the oral cavity moist may also be important. Oral dryness is caused by high-dose chemotherapy and total-body irradiation (TBI) performed as part of the conditioning regimen for HCT. Oral dryness not only results in discomfort

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but may also exacerbate oral mucositis. We have often seen the development of ulcerative mucositis on dry mucosa in contact with dry teeth clinically. One of the reasons may be that saliva is necessary to maintain oral mucosal health. Additionally, moisture in oral cavity may moderate irritation caused by mechanical contact between the teeth and oral mucosa.

We began attempts to implement oral care in our ward from 2003. Our strategy includes a multidisciplinary approach prior to and during cancer treatment aimed at reducing the oral microbial load and keeping the oral cavity moist. Here, we describe the effect of intensive oral care on the degree of oral mucositis in HCT recipients.

Materials and methods

Patients

Fifty-three consecutive patients administered conventional allogeneic HCT at Okayama University Hospital of Medicine and Dentistry between April 2003 and March 2007 (23 men, 30 women; mean age±SD, 34.3±11.8 y) were enrolled in this study. Patients administered autologous and reduced-intensity HCT (RIST) were excluded. Numbers of patients and diseases according to year are shown in Table 1.

The Ethics Committee of Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences approved this study.

HCT conditioning regimens

Most patients with related or unrelated donors received TBI at a dose of 12 Gy in six fractions followed by cyclophosphamide (CY) at a dose of 60 mg/kg once daily for 2 days. Alternatively, patients received a combination of busulphan (BU; 4 mg/kg/day×4 days) and CY (60 mg/kg/day×2 days). Patients with unrelated cord blood donors were treated with TBI at 12 Gy, CY (60 mg/kg/day×2 days) and cytarabine

(Ara-C; 6 g/m^2/day×2 days). Numbers of patients, sources of hematopoietic stem cells, and HCT protocols (conditioning regimen) according to year are shown in Tables 2 and 3.

General infection control

Fluoroquinolone for prophylaxis against bacterial infection and fluconazole for prophylaxis against fungal infection were administered orally. Prophylaxis against herpes virus infection with acyclovir was also given. Neutropenic fever was managed according to the guidelines of Hughes et al. [6].

Assessment of oral mucositis

The severity of oral mucositis in patients undergoing HCT was evaluated daily according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 3.0 [7]. The criteria for oral mucositis were as follows:

- Grade 1: Erythema of the mucosa
- Grade 2: Patchy ulcerations or pseudomembranes
- Grade 3: Confluent ulcerations or pseudomembranes; bleeding in response to minor trauma
- Grade 4: Tissue necrosis; significant spontaneous bleeding; life-threatening consequences
- Grade 5: Death

Assessments were performed as part of daily nursing care by nurses who were trained by dentists and dental hygienists. The consistency of these assessments was checked by the dental team at least once per week.

Progress of our oral care regimen

Implementation of our oral care program was divided into two periods: "examination and trial period (2003 and 2004)" and "intensive oral care period (2005 and 2006)." Throughout this study period, the core oral care providers consisting of an experienced dentist, dental hygienists, and nurses were the same.

Table 1 Diseases of patients

Diseases	Year				Total
	2003	2004	2005	2006	
Acute myelogenous leukemia	5	4	3	4	16
Acute lymphoblastic leukemia	5	3	1	2	11
Chronic myelogenous leukemia	1	0	1	0	2
Malignant lymphoma	1	4	6	4	15
Aplastic anemia	1	0	1	0	2
Myelodysplastic syndromes	0	0	2	5	7
Total	13	11	14	15	53

Table 2 Source of hematopoietic stem cells

Source	Year				Total
	2003	2004	2005	2006	
Related donors	7	4	2	6	19
Unrelated donors (without cord blood donors)	4	7	7	4	22
Unrelated cord blood donors	2	0	5	5	12
Total	13	11	14	15	53

Examination and trial period (2003 and 2004)

We provided oral care interventions when oral mucositis developed clinically in HCT patients. On the other hand, there was no consensus within our ward regarding the precise method of oral care, and sometimes some points were missed.

Intensive oral care period (2005 and 2006)

We provided preventive oral care interventions, keeping the oral cavity clean and moist. The core oral care providers educated all ward staff members including new personnel. The oral care regimen included:

1. All subjects were referred to dentists with experience in treating medically compromised patients, and necessary dental treatment aimed at reducing preexistent oral infection, and the oral microbial load was completed as much as possible before HCT.
2. All subjects were instructed regarding self management including performing meticulous oral hygiene geared to their individual needs. Staff members, including nurses and dental professionals performed oral hygiene measures

Table 3 Conditioning regimen of HCT

Conditioning regimens	Year				Total
	2003	2004	2005	2006	
With TBI					
CY/TBI	6	5	6	9	26
l-PAM/TBI	2	2	3	2	9
CA/TBI	1	0	0	0	1
CA/CY/TBI	0	1	3	2	6
CY/TBI/ATG	0	0	1	0	1
Without TBI					
BU/CY	3	3	1	1	8
CY/ALG	1	0	0	0	1
Flu/BU	0	0	0	1	1
Total	13	11	14	15	53

TBI Total-body irradiation, *CY* cyclophosphamide, *L-PAM* melphalan, *CA* cytarabine, *ATG* anti-thymocyte globulin, *BU* busulfan, *ALG* anti-lymphocyte globulin, *Flu* fludarabine

to patients in poor general condition. In patients with severe mucositis who could not tolerate tooth brushing, dental and mucosal debris was gently removed using saline-drenched gauzes, aimed at keeping the oral cavity as clean as possible.

3. Oral rinsing with saline was performed every 3 h during daytime. In addition, patients used a commercial saliva substitute, Oralbalance®, when they experienced oral dryness. Oral rinsing with chlorhexidine is not recommended in Japan. Oral rinsing with amphotericin B was indicated only when fungi were detected on the oral mucosa.

Statistical analysis

The frequencies of patients with oral ulcerative mucositis (NCI-CTCAE version 3.0≥2) during transplantation period for each year were analyzed statistically with Fischer's exact test. Mucositis frequencies of 2004, 2005, and 2006 were compared with that of 2003, and the period 2003–2004 and 2005–2006 were compared. P values were calculated using StatFlex statistical software (Artech, Osaka, Japan).

Results

Progress of oral care and oral mucositis
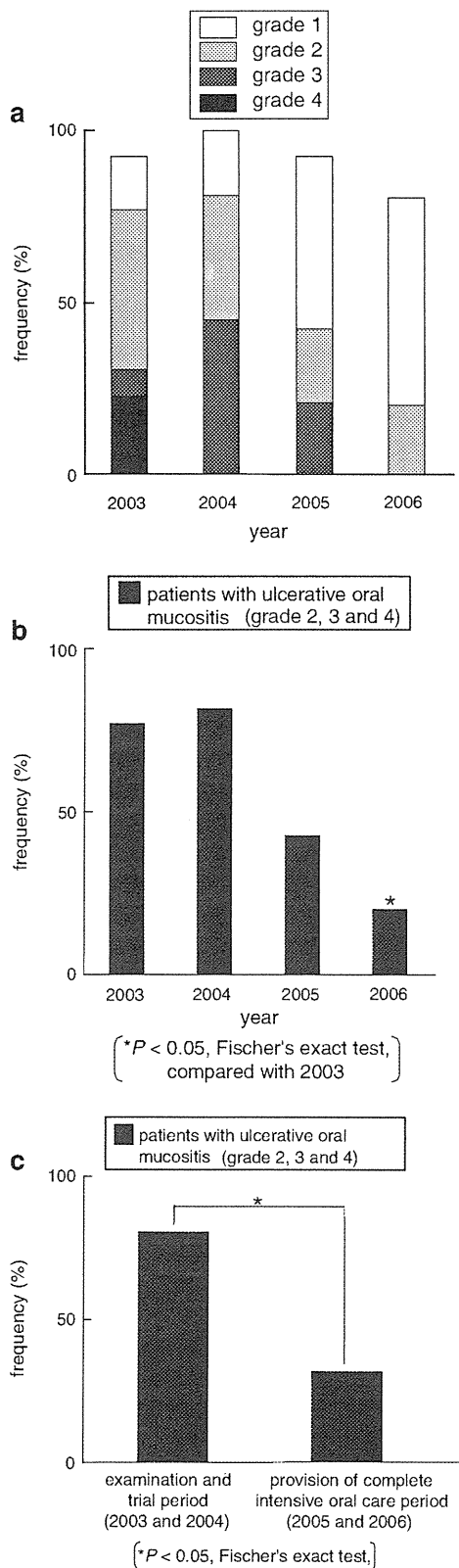
The frequencies of all grades of mucositis by year are shown in Fig. 1a. Subjects were categorized as follows: non-oral ulcer < Grade 1; oral ulcer carrier > Grade 2, since mucositis ≥ Grade 2 suggests disruption of the oral mucosal membrane barrier and formation of an infection route (Fig. 1b). With progress of oral care, the frequency of patients with ulcerative oral mucositis decreased significantly ($P \leq 0.05$, Fischer's exact test), whereas there were no significant changes relative to diseases or conditioning regimens (Tables 1 and 2); mucositis rate was reduced from 76% (10 of 13) of patients with ulcerative oral mucositis in 2003 to only 20% (3 of 15) in 2006. When the historical control group (2003+2004) was compared with the intensive oral care regimen group (2005+ 2006), a significant reduction in ulcerative mucositis was also observed (Fig. 1c; $P \leq 0.05$, Fischer's exact test).

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a

b

c

Discussion

Our oral care strategy aimed at keeping the oral cavity clean and moist reduced the degree of ulcerative oral mucositis in our ward. Borowski et al. [8] reported the superiority of intensive oral care in patients with and without TBI and in patients with good or poor oral hygiene; the observed risk of mucositis was reduced by 70% in each of these four subgroups in their study. Our results were very similar to those reported in this study. The ulcerative mucositis rate in our study was reduced from 76% (10 of 13) of patients in 2003 to only 20% (3 of 15) in 2006. Therefore, the rate of ulcerative mucositis in 2003 was reduced by 73.7% in 2006 by our intensive oral care regimen (Fig. 1b).

The Multinational Association for Supportive Care in Cancer/International Society of Oral Oncology mucositis guidelines recommend systematic oral care with brushing, flossing, bland rinses, and moisturizers [4]. This guideline recommends a multidisciplinary approach to oral care including nurses, physicians, dentists, dental hygienists, dieticians, pharmacists, and others, when relevant. Furthermore, dental examination and treatment are considered important prior to the start of cancer therapy [4]. The present pilot study supports these recommendations. Our oral care regimen included application of Oralbalance®, which has been shown to have an antimicrobial effect [9]. However, the use of additional antimicrobial agents may be indicated in patients who cannot continue tooth brushing. In our regimen, we used wet gauzes to clean the oral cavity in these patients, but this has been shown to be ineffective to remove dental plaque [10].

The shifts in some of the diagnoses (Table 1) and associated treatment regimens between the two periods evaluated may have had an impact on the outcomes. A prospective intervention study, including large numbers of subjects and controls, may provide more detailed information on optimal oral care measures and may demonstrate the significance of oral care in HCT patients to reduce oral mucositis and related outcomes including pain, fever and infection, length of hospital stay, and costs.

In conclusion, our results suggest that oral mucositis in HCT patients can be alleviated by intensive multidisciplinary oral care starting prior to HCT and aimed at keeping the oral cavity clean and moist in the immediate post-transplant phase.

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