(Maeda *et al.*, 2005; Yoshida *et al.*, 2005). In this study, we expanded the application of the LAMP method to eight major periodontal pathogens – *Aggregatibacter (Actinobacillus) actinomycetemcomitans*, *Campylobacter rectus*, *Eikenella corrodens*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Treponema denticola* and *Tannerella forsythia* – and evaluated their sensitivity and specificity. Further, intact cells and boiled cells were tested as templates besides the extracted DNA template to examine the influence on LAMP sensitivity.

Materials and methods

Bacterial strains and culture conditions

The bacterial strains used in this study were as follows: *A. actinomycetemcomitans* Y4, *C. rectus* ATCC 33238, *E. corrodens* ATCC 23834, *F. nucleatum* ATCC 25586, *P. gingivalis* 381, *P. intermedia* ATCC 25611, *T. denticola* ATCC 35405, *T. forsythia* ATCC 43037. *Porphyromonas gingivalis*, *F. nucleatum* and *P. intermedia* were cultured in modified GAM broth (Nissui Seiyaku Inc., Tokyo, Japan) at 37 °C in an anaerobic box (Model ANX-1; Hirasawa Works, Tokyo, Japan) containing 80% N_2, 10% H_2 and 10% CO_2. *Aggregatibacter (Actinobacillus) actinomycetemcomitans*, *C. rectus* and *T. denticola* were cultivated according to the methods described previously (Kokeguchi *et al.*, 1989, 1991; Miyamoto *et al.*, 1991). Cultured *T. forsythia* was kindly provided by Dr T. Hino (Hiroshima University Graduate School of Biomedical Science). The cells were harvested by centrifugation at 10 000 **g** for 20 min at 4 °C, and cell numbers were determined using a Petroff–Hauser counting chamber and a phase-contrast microscope.

Preparation of the LAMP template

InstaGene Matrix (Bio-Rad Lab, CA) was used for DNA extraction from cultivated strains and clinical dental plaque samples according to the manufacturer's instructions. Briefly, plaque samples and cultivated bacteria were suspended in 1 mL of phosphate-buffered saline [PBS (−)] (Gibco BRL, MD) and were pelleted and resuspended in 100 μL of InstaGene Matrix. The suspension was incubated at 56 °C for 30 min and then at 100 °C for 8 min. After the incubation, the suspension was centrifuged and 2 μL of the resulting supernatant was used as a template for LAMP and conventional real-time PCR (Maeda *et al.*, 2003). To simplify the examination system, intact cells and boiled cells were tested as template besides the DNA extracted sample. Bacterial cells were suspended in 100 μL PBS (−), and 2 μL of the bacterial suspension was used for LAMP directly or after a boiling step for 5 min. To examine a detection limit of the LAMP method, templates were prepared from serial diluted cells of each periodontal pathogen.

Clinical samples

Sampling of subgingival plaque from periodontitis patients was performed at Okayama University Hospital of Medicine and Dentistry after obtaining informed consent. Samples were obtained from their periodontal pockets by inserting paper points (#45, Zipperer, Germany) as described previously (Nishimura *et al.*, 1990), and DNA templates were prepared by InstaGene Matrix. The DNA samples were used for clinical microbiological diagnosis by conventional real-time PCR (Maeda *et al.*, 2003). By real-time PCR, total bacterial counts, and numbers of *A. actinomycetemcomitans*, *P. intermeia* and *P. gingivalis* in the samples were quantified, and the remaining plaque DNA samples were stored at −30 °C. Ten plaque DNA samples including $> 10^6$ of total bacterial counts and various numbers of the three species were selected from the sample stocks for the LAMP analysis.

Primers for LAMP

Primers for LAMP were designed to target the 16S rRNA gene. Primer Explorer version 2 (Fujitsu, Tokyo, Japan) was used at the Net Laboratory website (http://www.netlaboratory.com/) to nominate the candidate primers. A set of four primers, a forward inner primer (FIP), a backward inner primer (BIP) and two outer primers (F3 and B3) (Notomi *et al.*, 2000), containing species-specific sequences of the 16S rRNA gene, was selected from the candidates for each pathogen. Before the selection, species-specific regions on the 16S rRNA gene were identified by alignment of the published sequence data from the GenBank at the National Center for Biotechnology Information website. For the alignment, 16S rRNA gene sequences of *Prevotella nigrescens*, *Porphyromonas endodontalis* and *Escherichia coli* were also compared with their eight sequences in this study. One or two loop primers (LF, LB) were designed for each target and used to accelerate the reactions (Nagamine *et al.*, 2002).

LAMP reaction

The LAMP reaction for each periodontal pathogen was carried out using a Loopamp DNA amplification kit (Eiken Chemical Co. Ltd, Tochigi, Japan) in a 25-μL volume. The reaction mixture contained 40 pmol each of FIP and BIP, 5 pmol each of F3 and B3 primers, 2 μL of template, 1 μL of *Bst* DNA polymerase (8 U) and 12.5 μL of reaction mixture prepared in the kit. For acceleration of the LAMP reaction, 20 pmol of loop primer (LB or each of LF and LB) was added to the reaction mixture. The reaction mixture was incubated at 62, 64 or 66 °C for 60 min. After the incubation, the reaction was terminated by heating the reaction mixture at 80 °C for 2 min.

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Detection of LAMP products

The LAMP product was detected by naked-eye inspection or agarose gel electrophoresis. For naked-eye detection, 1.0 μL of 10^{-1}-diluted SYBR Green I (Takara Bio Inc., Otsu, Japan) was added to the reaction mixture, and the color change was observed. For the electrophoretic analysis, 2 μL of reaction mixture was loaded on 2% agarose gel. The gel was stained with ethidium bromide (1 mg mL^{-1}) and assessed photographically under UV light (302 nm).

Real-time PCR

Real-time PCR was performed for quantitative detection of *A. actinomycetemcomitans*, *P. gingivalis* and *P. intermedia* as described previously (Maeda *et al.*, 2003). The GENEAMP® 5700 SEQUENCE DETECTION SYSTEM (PE Applied Biosystems) was used for monitoring the fluorescence from dsDNA-binding SYBR Green I. The PCR mixture contained 2 × SYBR Green PCR Master Mix (PE Applied Biosystems), 20 pmol of forward and reverse primer, and 2 μL of extracted DNA. Both forward and reverse primers were designed from the species-specific regions of 16S rRNA gene (Maeda *et al.*, 2003). The thermocycling program was 40 cycles of 95 °C for 15 s and 60 °C for 1 min with an initial cycle of 95 °C for 10 min. The data were analyzed using the GENEAMP 5700 SDS software (PE Applied Biosystems).

Results and discussion

Primer design and specificity of LAMP

Primer sets for the LAMP of eight periodontal pathogens are listed in Table 1. The primer sets were designed to include species-specific sequences of the 16S rRNA gene. Locations of the LAMP primers in the 16S rRNA gene sequence were shown in supplementary Fig. S1. Because species-specific sequences are conveniently identified, the 16S rRNA gene is now widely used as a target for molecular microbiological diagnosis. By targeting the 16S rRNA gene of a multicopy gene, high sensitivity would be expected. On the other hand, the sequence similarity of 16S rRNA gene among the closely related species sometimes causes nonspecific amplification. However, because LAMP was a highly specific method that required six distinct sequences for the amplification, the 16S rRNA gene was used in the current study.

Before the serial experiments, optimum reaction temperature was determined using the designed primers. The reactions of LAMP for each periodontal pathogen were performed under isothermal conditions at 62, 64 or 66 °C for 60 min using the DNA-extracted template from 10^3 cells of each target. LAMP was performed successfully under the three different thermal conditions. The LAMP product amplified at 64 °C exhibited a slightly larger amount of DNA as compared with other conditions in all tested strains (data not shown). Therefore, the series of experiments were performed at 64 °C.

The specificity of LAMP was examined using an extracted DNA template prepared from a cell mixture of all tested species (10^3 cells of each species) or a cell mixture of seven species (10^3 cells of each species) without the target. The LAMP products were electrophoretically detected and are shown in Fig. 1. LAMP reactions with the designed primer set for each periodontal pathogen successfully amplified the DNA by using the DNA-extracted template including all tested species. The LAMP products appeared as a ladder-like pattern on the agarose gel because of its characteristic structure (Notomi *et al.*, 2000); however, the primer sets did not amplify the DNA with the extracted DNA template prepared from the cell mixture excluding the target (Fig. 1). These results demonstrated the high specificity of the designed primer sets of LAMP for each periodontal pathogen.

Sensitivity of LAMP

For sensitivity testing, LAMP reaction was performed for 30 or 60 min using serial diluted templates prepared by InstaGene Matrix. The LAMP amplicons were detected by both agarose gel electrophoresis and naked-eye inspection. The results of electrophoretic detection for the 60-min LAMP are shown in Fig. 2. The detection limits of LAMP for the tested periodontal pathogens were less than one cell per tube except for *E. corrodens*. DNA amplification was occasionally seen in the seven species when the amount of template DNA was genome equivalent in 0.5 cell (Aa, Cr, Pg, Td and Tf in Fig. 2). By targeting the 16S rRNA of multicopy gene, high sensitivity was obtained. Ten target cells were required as template for the detection of *E. corrodens*. The LAMP conditions for the tested species were the same other than the primers. The lower sensitivity of LAMP for *E. corrodens* may be due to the primer design, and a new designed primer set would be needed for higher sensitivity. However, because the current sensitivity was not critically inferior to other PCR-based molecular techniques (Slots *et al.*, 1995; Harper-Owen *et al.*, 1999; Lyons *et al.*, 2000; Sakamoto *et al.*, 2001; Maeda *et al.*, 2003), the method is possibly applicable to clinical examination.

Compared with the 60-min LAMP, the sensitivity of the 30-min LAMP declined in all cases. Ten template cells were required for the detection of seven periodontal pathogens except for *E. corrodens*, and 100 cells were required for the detection *of E. corrodens* by the 30-min LAMP. The naked-eye inspection for each periodontal pathogen demonstrated equal detection limits to the electrophoretic analysis in both 30- and 60-min LAMP reactions (data not shown).

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Table 1. LAMP primers for the periodontal pathogens

Target species	Primer	Sequence
A. actinomycetemcomitans	FIP	5′-CCCCACGCTTTCGCACATCATACCGAAGGCGAAGGCAG-3′
	BIP	5′-AGATACCCTGGTAGTCCACGCTTTCGGGCACCAGGGCTAAAC-3′
	F3	5′-TGCGTAGAGATGTGGAGGAA-3′
	B3	5′-GGCGGTCGATTTATCACGT-3′
	LB	5′-AAACGGTGTCGATTTGGGGAT-3′
C. rectus	FIP	5′-CGTCATAGCCTTGGTAGGCCGTAGGATGAGGCTATATCGTAT-3′
	BIP	5′-AGTCACACTGGAACTGAGACGGGTTTCCCCCATTGAGC-3′
	F3	5′-AGTCGGGAAAGTTTTCGGTG-3′
	B3	5′-AAAGTGTCATCCTCCACGC-3′
	LB	5′-ACGGTCCAGACTCCTACGG-3′
E. corrodens	FIP	5′-GGTAGGCCTTTACCCCACCAACCAAGACCTCGCGTTATTCGA-3′
	BIP	5′-ACGATCAGTAGCGGGTCTGAGAAATTCCCCACTGCTGCTC-3′
	F3	5′-CTAATACCGCATACGTCCTA-3′
	B3	5′-GTTGCCCCCATTGTCCAA-3′
	LB	5′-TGAGACACGGCCGCTCCTA-3′
F. nucleatum	FIP	5′-TCCAATATTCCCCACTGCTGCCGCCACAAGGGGACTGAGA-3′
	BIP	5′-TCCAGCAATTCTGTGTGCACGAGTACCGTCATTTTTTTCTTC-3′
	F3	5′-AGGCGATGATGGGTAGCC-3′
	B3	5′-AGCCGTCACTTCTTCTGTTG-3′
	LF	5′-TCCCGTAGGAGTAAGGGCC-3′
	LB	5′-TCGGAATGTAAAGTGCTTTCAGTTG-3′
P. gingivalis	FIP	5′-CACCACGAATTCCGCCTGCCTGAGCGCTCAACGTTCAGCC-3′
	BIP	5′-ATCACGAGGAACTCCGATTGCGCGCCTTCGTGCTTCAGTG-3′
	F3	5′-GGTAAGTCAGCGGTGAAACC-3′
	B3	5′-GCGTGGACTACCAGGGTAT-3′
	LB	5′-GCAGCTTGCCATACTGCGA-3′
P. intermedia	FIP	5′-CGTTACCCGCACCAACAAGCTAATATGGCATCTGACGTGGAC-3′
	BIP	5′-GCCCACCAAGGCTCGATCAGGGACCGTGTCTCAGTTCCA-3′
	F3	5′-ACGGCCTAATACCCGATGTT-3′
	B3	5′-CTGCCTCCCGTAGGAGTT-3′
	LF	5′-CATCCTCCACCGATGAATCTTTG-3′
	LB	5′-TAGGGGTTCTGAGAGGAAGGT-3′
T. denticola	FIP	5′-CATCCTGAAGCGGAGCCGTAGTACCGAATGTGCTCATTTAC-3′
	BIP	5′-GCTGGTTGGTGAGGTAAAGGCCATCTCAGTCCCAATGTGTCC-3′
	F3	5′-CCCTGAAGATGGGGATAGCT-3′
	B3	5′-TGCCTCCCGTAGGAGTTTG-3′
	LB	5′-CACCAAGGCAACGATGGGTAT-3′
T. forsythia	FIP	5′-CCATCCGCAACCAATAAATCTCTAATACCTCATAAAACAGG-3′
	BIP	5′-TAAGCCATCGATGGTTAGGGCGTGTCTCAGTACCAGTGTG3′
	F3	5′-GATAACCCGGCGAAAGTCG-3′
	B3	5′-TGCCTCCCGTAGGAGTCT-3′
	LB	5′-GTTCTGAGAGGAAGGTCCCC-3′

Influence of the template preparation procedure

For further application of the LAMP method to clinical diagnosis, we attempted to simplify template preparation. Besides the extracted DNA template by InstaGene Matrix, intact cells and boiled cells were tested as templates to examine the influence of crude templates on sensitivity. The LAMP reaction was performed for 60 min and the amplicon was detected by agarose gel electrophoresis. The seven tested species except for *E. corrodens* demonstrated the same results; that the detection limits for boiled cells and intact cells were 10 and 100 cells per tube, respectively. The representative result of *A. actinomycetemcomitans* is shown in Fig. 3. For the detection of *E. corrodens*, 10 times more cells were required compared with other species. The results suggested the possibility of the direct use of the plaque sample for the LAMP assay. Because intact bacteria required 100 times the cell number for DNA amplification as compared with extracted DNA template, 1% of bacterial cells were estimated to be injured during the manipulation and incubation step at 64 °C.

The sensitivity of LAMP for the crude templates (10–100 cells per tube) was higher than that of culture

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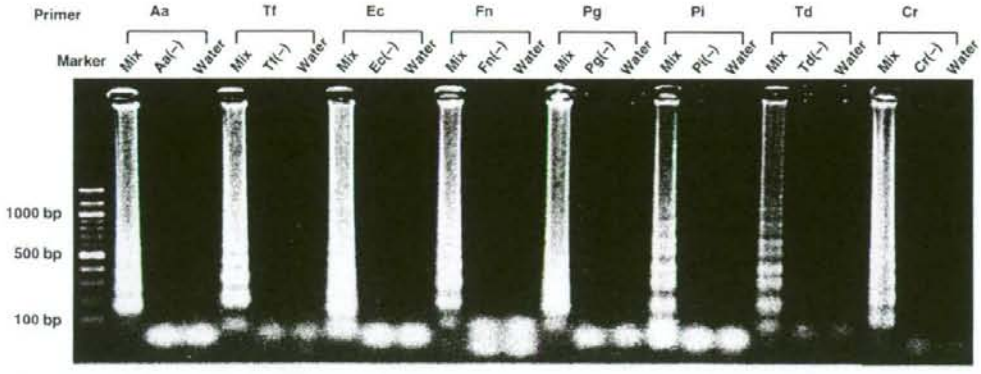

Fig. 1. Specificity of LAMP. The LAMP primer set was designed for each periodontal pathogen and the specificity was tested. Abbreviations Aa, Tf, Ec, Fn, Pg, Pi, Td and Cr represent *Aggregatibacter (Actinobacillus) actinomycetemcomitans*, *Tannerella forsythia*, *Eikenella corrodens*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Treponema denticola* and *Campylobacter rectus*, respectively. The LAMP product was electrophoretically detected on 2% agarose gel. DNA amplification was observed in all LAMP reactions for each periodontal pathogen with the DNA-extracted template including 10^3 cells of all tested species (lane Mix). No amplicon was seen when template DNA was prepared from the mixture of seven species (10^3 cells of each species) excluding the target species [lane (-) with the abbreviation of excluded species]. No amplicon was seen in the negative control of the water template.

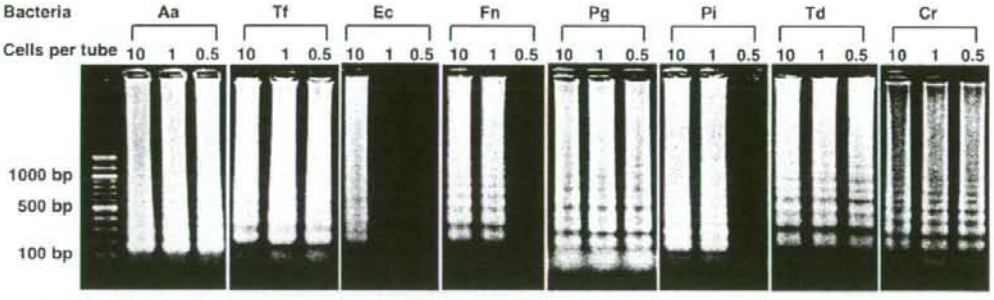

Fig. 2. Detection limit of LAMP. The DNA-extracted template was prepared from various cell numbers of each target, and LAMP was carried out at 64 °C for 60 min. The LAMP product was electrophoretically detected on 2% agarose gel. The target cell number equivalent to one reaction tube was shown on each lane. The detection limit of LAMP was less than one cell per tube except for *Eikenella corrodens*. The sensitivity tests were performed individually five times, and DNA amplification was occasionally seen when the amount of template DNA was equivalent to 0.5 cell. LAMP required 10 cells for the detection of *E. corrodens*. Abbreviations of the tested species correspond to those in Fig. 1.

methods (Lau *et al.*, 2004; Boutaga *et al.*, 2005), but was lower than the PCR method (Slots *et al.*, 1995; Harper-Owen *et al.*, 1999; Lyons *et al.*, 2000; Sakamoto *et al.*, 2001; Maeda *et al.*, 2003). The relationship between the presence of periodontal pathogens and the clinical aspects of periodontitis have been demonstrated (Socransky *et al.*, 1998; Tran & Rudney, 1999). Contrary to this, a few reports demonstrated the relationship between the quantities of periodontal pathogens and the clinical aspects (Socransky *et al.*, 1991; Kawada *et al.*, 2004). In addition, most periodontal pathogens are found in periodontally healthy subjects (Tran & Rudney, 1999) and the amounts of periodontal pathogens in periodontal pockets have been reported to vary a great deal (Socransky *et al.*, 1991; Kawada *et al.*, 2004). Therefore, the diagnostic cut-off of the quantities of periodontal pathogen cannot be defined so far. Eick & Pfister (2002) reported the cut-off of genome equivalents in 10^3–10^4 cells of periodontal pathogens using the microDent® kit (Hain Diagnostika, Germany) based on the PCR method. The utility of the kit was demonstrated in the report, but the definition of the cut-off seems to be unclear. In the current study, we cannot conclude that the crude templates are sufficient for the microbiological diagnosis of periodontitis. Simple DNA-purification methods using commercially

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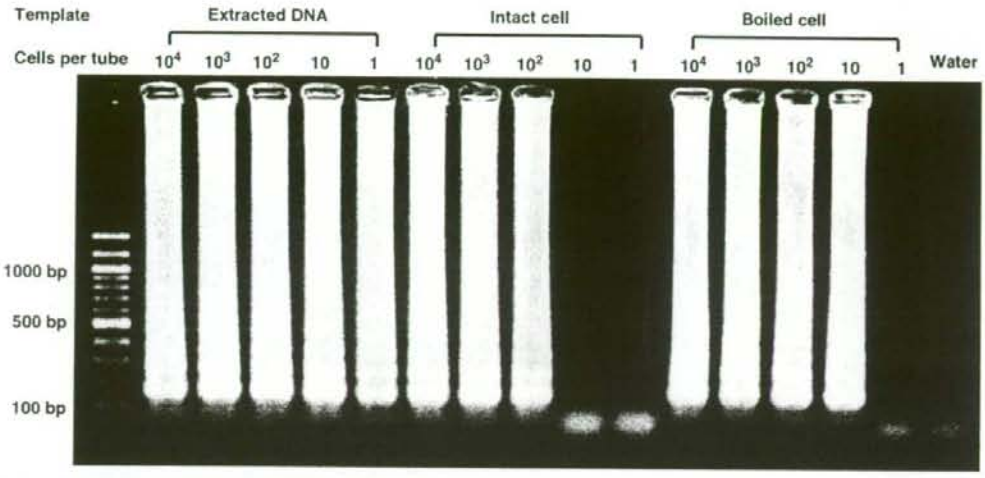

Fig. 3. Influence of the crude template on LAMP. Intact bacterial cells suspended in PBS (–) and boiled cells were tested as template besides the DNA-extracted template, and the influence on sensitivity was examined. Each LAMP was performed for 60 min, and the product was detected on 2% agarose gel. A representative result of *Aggregatibacter (Actinobacillus) actinomycetemcomitans* was demonstrated. The number on the gel denoted the target cell number equivalent to the amount of template in a reaction tube. The detection limit of LAMP using boiled cells and intact cells were 10 and 100 cells per tube, respectively.

available kits may give us a better result. However, it seems necessary to accumulate clinical data by quantitative, molecular-based microbiological methods before creating a guideline of microbiological diagnosis for periodontitis.

Examination of the clinical plaque sample

To evaluate the practicability, LAMP methods for *A. actinomycetemcomitans*, *P. gingivalis* and *P. intermedia* were applied to the clinical plaque samples. We have previously established a real-time PCR method for quantitative detection of *A. actinomycetemcomitans*, *P. gingivalis* and *P. intermedia* (Maeda *et al.*, 2003). The method is now applied to the clinical diagnosis of periodontitis in our hospital. DNA-extracted samples are being prepared from subgingival plaque, and the samples are stored at −30 °C after the examination. From the sample stock, five samples containing more than one cell $2\,\mu L^{-1}$ (one cell per reaction tube) of the target and five samples containing less than one cell $2\,\mu L^{-1}$ (less than the quantitative range of real-time PCR) of the target were selected for each LAMP of the three periodontal pathogens. The LAMP reaction was performed for 60 min, and the LAMP product was detected by agarose gel electrophoresis. A representative result of *A. actinomycetemcomitans* is shown in Fig. 4. The LAMP amplicon was seen in all cases when the real-time PCR detected more than one cell $2\,\mu L^{-1}$ of the target. One of the five was positive in the LAMP for *A. actinomycetemcomitans* and *P. gingivalis*, and four of the five were positive in the detection of *P. intermedia* when the cell number in the template was less than one cell. Because the cell number was less than one cell $2\,\mu L^{-1}$, it was out of quantitative range of the real-time PCR, and the accurate cell number in the clinical sample was unknown. However, these results demonstrated equal or higher sensitivity of LAMP as compared with real-time PCR. In addition, sensitivity was at equal level with the LAMP using the extracted DNA templates from cultivated bacteria. This result implied that the contamination of bacterial species other than the target or the contamination of human cells during the sampling step is not so critical as to influence the sensitivity. Although the crude template without the DNA-extraction step was not tested for clinical samples, these findings may suggest that a relatively high sensitivity would be expected. In the current study, the LAMP for *C. rectus*, *E. corrodens*, *F. nucleatum*, *T. denticala* and *T. forsythia* was not applied to clinical samples, and the applicability remains to be elucidated.

High sensitivity is one of the advantages of the LAMP method. However, the high sensitivity of LAMP sometimes causes a problem. We have recently established a LAMP method for the detection of *P. gingivalis* (Maeda *et al.*, 2005). Using two loop primers, the method demonstrated high sensitivity with a detection limit of 1–2 copy genes per tube. We applied the method for routine clinical examinations and had the problem of nonspecific amplification in the negative control of the water template (unpublished data). It

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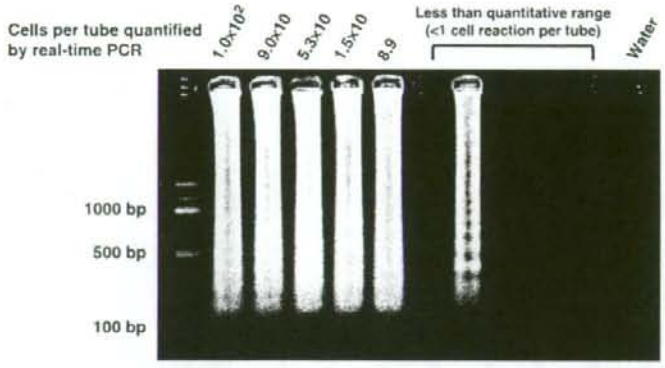

Fig. 4. Analysis of clinical plaque samples. The extracted DNA template was prepared from the subgingival plaque sample and was subjected to analysis by both real-time PCR and the LAMP method for *Aggregatibacter (Actinobacillus) actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia*. Before LAMP analysis, the target cell number in the reaction tube was quantified by real-time PCR, and an equal volume (2 μL) of the template was used for LAMP. Five samples containing numerous targets and five samples containing the targets under the quantitative detection limit of real-time PCR were selected for each LAMP. The LAMP products were detected from all samples that contained more targets than the quantitative detection limit of the real-time PCR. However, a positive result was obtained occasionally from samples under the quantitative range of the real-time PCR (one positive in the LAMP for *A. actinomycetemcomitans* and *P. gingivalis*, and four positives for *P. intermedia*). A representative result of *A. actinomycetemcomitans* was demonstrated.

is theoretically impossible to amplify DNA from water, and therefore a cause of the nonspecific amplification was considered to be contamination of *P. gingivalis* during the manipulations. Aerosol from the pipette might be the most probable cause. In the current study, a new primer set with only one loop primer was redesigned for *P. gingivalis*. Although the sensitivity declined a little, nonspecific amplification from the water sample has never been seen so far. Considering the high sensitivity of LAMP, scrupulous manipulations are required throughout the examinations.

The greatest advantage of the LAMP method is its simplicity and the rapidity attributed to continuous amplification under isothermal conditions within an hour (Notomi *et al.*, 2000). As expected, each LAMP method for the eight periodontal pathogens was accomplished in an hour, and naked-eye inspection was possible with high specificity and sensitivity. Further, the trial of the crude template suggests the applicability of the LAMP method without the DNA-extraction step. Considering these findings, the LAMP methods established in the current study are supposed to be powerful tools for the microbiological diagnosis of periodontitis, especially in places such as private clinics, bedsides or dental chair-sides.

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Supplementary material

The following supplementary material is available for this article:

Fig. S1. Location of the LAMP primers.

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1574-695X.2008.00417.x (This link will take you to the article abstract).

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SHORT COMMUNICATION

Antimicrobial effects of the saliva substitute, Oralbalance®, against microorganisms from oral mucosa in the hematopoietic cell transplantation period

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Abstract
Goals The commercially available saliva substitute Oralbalance® has been reported to alleviate symptoms of post-radiotherapy xerostomia in head and neck cancer patients. Oralbalance® may also be effective for xerostomia in patients undergoing hematopoietic cell transplantation (HCT) with high-dose chemotherapy and total-body irradiation. However, HCT patients are severely compromised, and saliva substitute must therefore not promote infection. This study was performed to determine the effects of Oralbalance® on microbial species identified during HCT.
Patients and methods Microbial identification of oral mucosa was performed in 28 patients undergoing HCT. The antimicrobial effects of Oralbalance® against bacteria and fungi detected in the HCT period were examined in vitro. Briefly, bacteria and fungi were spread on agar plates, and 0.1g of Oralbalance® gel was applied (about ϕ1cm). After incubation at 37°C for 24h, the presence of a

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transparent zone of inhibition around Oralbalance® was observed.

Main results Not only bacterial species constituting normal flora of the oral mucosa but also those not usually constituting normal flora, e.g., coagulase-negative *Staphylococcus*, were detected. A transparent zone was observed around Oralbalance® in all bacterial species examined. No transparent zone *was observed* for *Candida albicans*, but growth was inhibited in the area where Oralbalance® was applied.

Conclusions Oralbalance® does not facilitate increases in microorganisms in the HCT period. Oral care with Oralbalance® does not promote infection in patients undergoing HCT.

Keywords Hematopoietic cell transplantation · Xerostomia · Saliva substitute · Antimicrobial activity

Introduction

High-dose chemotherapy and total-body irradiation, which are performed as the conditioning regimen of hematopoietic cell transplantation (HCT), are associated with xerostomia. Xerostomia not only results in uncomfortable oral dryness but also may cause the oral mucositis induced by chemotherapy and/or irradiation to be more severe because patients with xerostomia lose one of the most important factors in protecting the oral mucosa, saliva, which contains many components of the innate and acquired defense systems and not only eliminates microorganisms from the oral cavity [1, 8] but also moderates mechanical contact between the teeth and oral mucosa. Indeed, we often see the development of ulcerative mucositis on mucosa in contact with dry teeth clinically. Oral care using saliva substitute may alleviate the symptoms induced by xerostomia.

Oralbalance®, which is a commercially available saliva substitute, has been reported to alleviate the symptoms of post-radiotherapy xerostomia in head and neck cancer patients [7, 9]. Therefore, this product may be effective in HCT patients. However, as these patients are in a markedly compromised condition throughout the period of HCT, saliva substitute must not promote infection.

Therefore, the present study was performed to investigate the effects of the saliva substitute, Oralbalance®, on microbial species identified during HCT.

Patients and methods

Identification of microorganisms from oral mucosa

A total of 28 patients undergoing HCT at Okayama University Hospital (male, 17; female, 11; 38.9 ± 16.6 years old) were enrolled in this study. Microbial samples were obtained from oral mucosal swabs. Culture and identification of microorganisms were performed at the Central Clinical Laboratory of Okayama University Hospital. Microbial samples from mucosal swabs were plated onto brain heart infusion agar plate and cultured in aerobic condition at 37°C. Identification of obtained colonies was performed by rapid ID 32 STREP API®, rapid ID 32 E API® or ID 32 GN API® identification kits (Japan bioMerieux, Tokyo, Japan) according to the manufacturer's instructions. Microbial identification was performed three times (first: day −7 ~ −1; second: day 0 ~ +7; third: day +8 ~ +14) for each patient (a total of 84 examinations in 28 patients).

Antimicrobial test of Oralbalance®

The antimicrobial effects of Oralbalance® against microbial species in the HCT period, with the exception of those detected only once throughout the total of 84 examinations of microorganisms, were examined in vitro. Antimicrobial tests were performed against the following standard strains: *Streptococcus sanguis* American Type Culture Collection (ATCC) 10556, *Streptococcus salivarius* Japan Collection of Microorganisms (JCM) 5707, *Neisseria mucosa* ATCC 19695, *Stomatococcus mucilaginosus* JCM 10910, *Staphylococcus epidermidis* National Institute of Technology and Innovation Biological Resource Center (NBRC) 12993, *Staphylococcus aureus* Food and Drug Administration 209, and *Candida albicans* NBRC 1385. Aliquots of these bacteria and fungi at concentrations of McFarland turbidity standard No. 0.5 were spread on brain heart infusion agar plates (Difco Laboratories, Detroit, MI, USA) or Sensitivity Disk Agar-N plates (Nissui Pharmaceutical, Tokyo, Japan). Then, 0.1g (about φ1cm) of Oralbalance® and an equal amount of Oralbalance® that had been pre-incubated at 90°C for 30min to denature the antimicrobial enzymes contained in the gel were applied separately to the same plates. Tetracycline disks for antimicrobial ability test (BD Sensi-Disk Tetracycline 30; BD Biosciences, Franklin Lakes, NJ, USA) or paper containing 100μg of amphotericin B (Invitrogen, Grand Island, NY, USA) were also applied to the plates as positive controls. After incubation at 37°C in air for 24h, bacterial and fungal growth on the plates was examined.

Results

Microorganisms identified on the oral mucosa during HCT

The microorganisms identified on the oral mucosa during HCT are shown in Table 1. No samples were obtained during 13 of the 84 examinations because of the patients' conditions. α- and γ-*Streptococcus* spp. (87.3% and

Table 1 Microorganisms identified from the oral mucosa and detection frequency during HCT

Microorganism	Detection frequency (%)	Number (/71)
Bacterial components of the normal flora		
α-*Streptococcus* spp.	87.3	62
γ-*Streptococcus* spp.	29.6	21
Neisseria spp.	43.7	31
Stomatococcus spp.	23.9	17
Bacteria not usually found in the normal flora		
Coagulase-negative *Staphylococcus* spp.	46.5	33
Staphylococcus aureus	2.8	2
Haemophilus influenzae	1.4	1
Enterococcus spp.	1.4	1
Stenotrophomonas maltophilia	1.4	1
Bacillus spp.	1.4	1
Fungi		
Candida albicans	5.6	4
Torulopsis glabrata	1.4	1

The microorganisms identified on the oral mucosa are shown. Microbial identification was performed three times (first: day −7 ~ −1; second: day 0 ~ +7; third: day +8 ~ +14) for each patient (total of 84 times for 28 patients). No samples were obtained during 13 of the 84 examinations because of the patients' conditions at these time points. Findings from 71 examinations are shown.

29.6%, respectively), *Neisseria* spp. (43.7%), and *Stomatococcus* spp. (23.9%), which are components of normal oral flora, were identified frequently. Coagulase-negative *Staphylococcus* spp. (CNS), which are not constituents of the normal flora, were also identified frequently (46.5%). The fungus, *C. albicans*, was identified at a frequency of 5.6%. *S. aureus*, *Haemophilus influenzae*, *Enterococcus* spp., *Stenotrophomonas maltophilia*, *Bacillus* spp., and *Torulopsis glabrata* were identified at low frequencies (1.4% ~ 2.8%).

Antimicrobial ability of Oralbalance®

The results of antimicrobial tests on Oralbalance® against *S. sanguis*, *S. salivarius*, *N. mucosa*, *S. mucilaginosus*, *S. epidermidis*, *S. aureus*, and *C. albicans* are shown in Fig. 1. The presence of a transparent zone of inhibition was observed around Oralbalance® for all bacterial species examined. No such transparent zone was observed around heated Oralbalance®. With regard to fungi, although there was no transparent zone on *C. albicans* cultures, growth was inhibited in the area where Oralbalance® had been applied.

Discussion

The commercially available saliva substitute, Oralbalance®, showed antimicrobial activity against the bacterial species

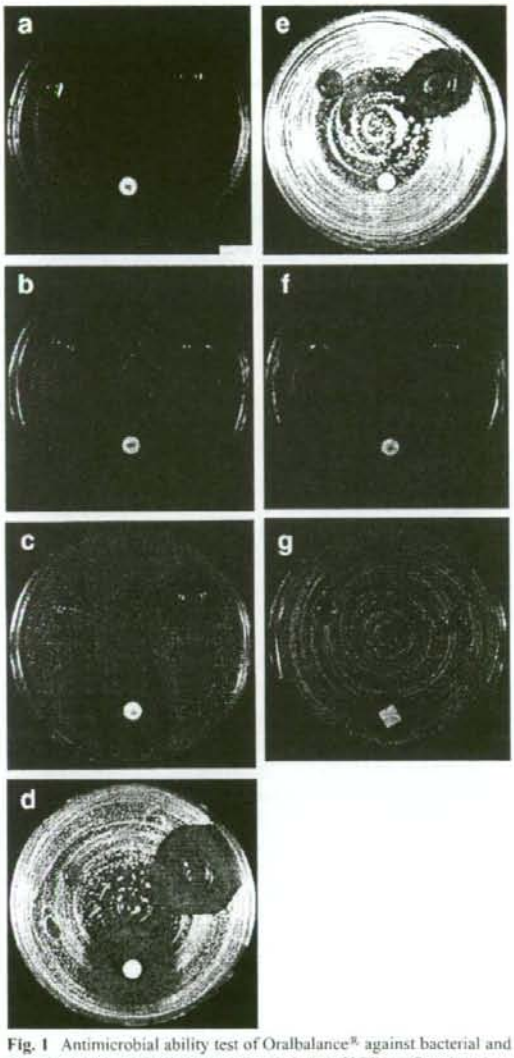

Fig. 1 Antimicrobial ability test of Oralbalance® against bacterial and fungal species isolated from patients during HCT. **a**: *Streptococcus sanguis*, **b**: *Streptococcus salivarius*, **c**: *Neisseria mucosa*, **d**: *Stomatococcus mucilaginosus*, **e**: *Staphylococcus epidermidis*, **f**: *Staphylococcus aureus*, and **g**: *Candida albicans*. Appearance of the entire plate surface; Oralbalance® was applied to the upper right portion of the plates. Heat-incubated Oralbalance® was applied to the upper left portion of the plates. Tetracycline disks (**a–f**) or paper containing amphotericin B (**g**) were applied to the lower part of the plates. There was a transparent zone of inhibition around Oralbalance® for all bacterial strains examined. Although there was no apparent transparent zone in *C. albicans* cultures, growth was inhibited in the area where Oralbalance® had been applied

detected during HCT. Against fungi, although there was no transparent zone observed on *C. albicans* cultures, growth was inhibited in the area where Oralbalance® had been applied in vitro. These result suggested that Oralbalance® would not contribute to the infection in patients undergoing HCT.

There have been some reports regarding the relationships between the bacteria that constitute the normal oral flora, e.g., *Streptococcus* species [6] and *Stomatococcus* species [2, 3], and bacteremia in neutropenic patients. In the present study, bacteria not usually seen in the normal flora in the oral mucosa, e.g., CNS, were also detected with high frequency during HCT, probably because bacterial substitution occurred due to the use of many antibiotics against infections in patients under neutropenic conditions. CNS is the bacterium isolated most frequently from blood cultures of febrile neutropenic patients [5]. The oral mucosa should be considered a potential source of organisms, including CNS, associated with bacteremia in immunocompromised patients [4]. In our in vitro studies, Oralbalance® did not facilitate an increase in such microorganisms related to bacteremia. The antibacterial effect of Oralbalance® is mainly due to antimicrobial enzymes of salivary origin, i.e., lactoperoxidase, lysozyme, and lactoferrin. Indeed, no transparent zone was observed around heat-incubated Oralbalance®. As Oralbalance® does not contain any antibiotics, it does not contribute to the appearance of antibiotic-resistant bacteria.

In conclusion, the saliva substitute, Oralbalance®, would not facilitate an increase in microorganisms during the HCT period.

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〈報　告〉

肝炎を中心とした医療関連感染に対する意識調査

佐藤　法仁[1,2]・渡辺　朱理[1]・苔口　進[1]

Investigation on Consciousness of Infection Related to the Medical Treatment Focusing on Hepatitis

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要　旨

歯科医療行為の多くは，観血的処置を伴い，患者からの血液や体液の暴露の危険性が絶えず存在する．このような状況下において，感染防止に関する知識と技能は必要不可欠であり，これは歯科臨床実習を受けている学生に対しても同様である．

今回，我々はより良い感染防止教育に寄与するため，歯科臨床実習を受けている歯学科学生109名(大学生)，歯科衛生士学校生161名(専門学校生)，合計270名に対して「肝炎を中心とした医療関連感染に対する意識調査」を行った．

その結果，「B型肝炎について」，「C型肝炎について」，「肝炎ウイルスについて」，「肝臓癌の原因のひとつであること」，「血液感染すること」，「唾液中に肝炎ウイルスが含まれていること」，「歯科医療で肝炎ウイルスに感染するリスクがあること」に関しては，歯学科学生，歯科衛生士学校生共に正しい知識を持つ者が多かったが，「肝炎ウイルスを持つ人の診療で使用したゴム手袋は76.9～81.4 v/v%エタノールで消毒すれば再利用してもよいか」では，歯科衛生士学校生72名(44.7%)が「再利用してもよい」と回答しており，ゴム手袋の適正使用に関する感染防止教育の必要性を認めた．

今後，医療関連感染原因微生物に対する正しい知識を有しているかの調査などを行い，感染防止のきめ細かい講義と実習を臨床実習前に徹底して行う必要があると考える．また，歯科臨床実習先である病院や歯科医院に対しても，最新かつ正確な感染防止情報を提供するシステムなどを構築する必要もあると考える．

Key words：歯科臨床実習生，肝炎，感染対策，意識調査

はじめに

現在，医療現場には患者あるいは環境中にも医療関連感染原因微生物が数多く存在し，医療従事者への暴露感染や院内への感染拡大などが問題となっている．そのため，医療従事者は各々の専門性だけではなく，感染防止に関する知識と技能が必要である．また，医療従事者を養成する教育機関では，学生に対して，臨床実習を行う前に感染防止に関する知識と技能の教授を行わなければならない．我々はこれまでに，歯学科学生，歯科衛生士学校生を対象に様々な医療関連感染原因微生物に対する意識調査を行い[1~3]，そこから得られた情報をもとに感染防止教育に活かしてきた．

B型および，特に肝臓癌などとの関係が深いC型肝炎は，針刺し事故や体液暴露による患者から医療従事者への感染事例だけではなく，医療従事者から患者への感染事故も報告されている[4]．

今回，我々は実際の歯科医療現場に出ている歯科臨床実習生を対象に肝炎に対する意識調査を行った．本調査で得られた結果を踏まえて，歯科臨床実習現場における

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より良い感染防止対策ならびに歯科臨床実習生の感染防止教育に寄与することを目的とした．

研究方法

1. 対象

対象は，歯科臨床実習を受けている5～6年次の歯学科学生(大学生)および2年次の歯科衛生士学校生(専門学校生)の合計283名である．

2. 調査方法

留置法により，無記名自記式質問紙調査にて実施した(2005年7月～2006年7月)．

3. 調査項目

調査項目は，9項目である．

質問内容は，質問1「B型肝炎という疾患を知っているか」(はい・いいえ)，質問2「C型肝炎という疾患を知っているか」(はい・いいえ)，質問3「肝炎ウイルスにより感染することを知っているか」，質問4「肝臓癌の原因のひとつであることを知っているか」(はい・いいえ)，質問5「血液感染をすることを知っているか」(はい・いいえ)，質問6「唾液中に肝炎ウイルスが含まれていることを知っているか」(はい・いいえ)，質問7「歯科医療で肝炎ウイルスに感染するリスクがあることを知っているか」(はい・いいえ)を調査した．また，感染防止に関する知識を質問する内容としては，質問8「肝炎ウイルスを持つ人が使用した歯ブラシの共用は避けるべきだと思いますか」(はい・いいえ)，質問9「肝炎ウイルスを持つ人の診療で使用したゴム手袋は，76.9～81.4 v/v%エタノール(以下，消毒用エタノール)で消毒すれば再利用してもよいと思いますか」(はい・いいえ)を調査した．

4. 倫理的配慮

調査実施前に，任意であること，個人情報保護および回答内容による個人の特定と評価を行わないこと，調査終了後は調査用紙ならびにデータを破棄することなどの点を説明した．

結　果

1. 回収率

全調査項目に回答しているものを有効とした．対象者のうち，有効回答総数は，270名(回収率95.4%，平均年齢22.0歳)であった．なお，各学生群の詳細は，歯学科学生109名(回収率95.4%，平均年齢24.5歳)，歯科衛生士学校生161名(回収率95.3%，平均年齢20.3歳)であった．

2. 回答結果

1) 肝炎に関する認知度について

肝炎に関する認知度については，歯学科学生および歯科衛生士学校生共に90%以上の認知度を有している結果を得た(表1)．

2) 感染防止に関する知識について

感染防止に関する知識については，質問8「肝炎ウイルスを持つ人が使用した歯ブラシの共用は避けるべきだと思いますか」では，歯学科学生と歯科衛生士学校生合わせて263名(97.4%)が「歯ブラシの共有を避けるべき」だと回答した．他方，質問9「肝炎ウイルスを持つ人の診療で使用したゴム手袋は消毒用エタノールで消毒

表1　歯学科学生および歯科衛生士学校生における医療関連感染に関する意識調査結果(質問1～7)

	歯学科学生(n=109)	歯科衛生士学校生(n=161)	合計(n=270)
	知っている人(%)	知っている人(%)	知っている人(%)
B型肝炎という疾患	105(96.3)	155(96.3)	260(96.3)
C型肝炎という疾患	105(96.3)	157(97.5)	262(97.0)
肝炎ウイルスにより感染する	105(96.3)	157(97.5)	262(97.0)
肝臓癌の原因のひとつ	102(93.6)	154(95.7)	256(94.8)
血液感染する	105(96.3)	156(96.9)	261(96.7)
唾液中に肝炎ウイルスが含まれる	103(94.5)	153(95.0)	256(94.8)
歯科医療での感染のリスクがある	102(93.6)	153(95.0)	255(94.4)

表2　歯学科学生および歯科衛生士学校生における医療関連感染に関する意識調査結果(質問8～9)

	歯学科学生(n=109)	歯科衛生士学校生(n=161)	合計(n=270)
	「はい」とした人(%)	「はい」とした人(%)	「はい」とした人(%)
歯ブラシの共有は避けるべきか	106(97.2)	157(97.5)	263(97.4)
ゴム手袋は消毒用エタノールで消毒すれば再利用してもよいか	14(12.8)	72(44.7)	86(31.9)

消毒用エタノール：76.9～81.4 v/v%エタノール

すれば再利用してもよいと思いますか」については，歯学科学生 14 名(12.8%)，歯科衛生士学校生 72 名(44.7%)，合計 86 名(31.9%)が「再利用してもよい」と回答した(表 2)．

考　察

今回の調査において，肝炎に関する認知度については，回答結果から歯学科学生および歯科衛生士学校生共に高かった．これは，臨床実習前の基礎科目(感染症学やウイルス学など)の講義や薬害肝炎などの社会問題などから肝炎に関する認知度が高かったと推測される．今後は，100%の認知度になるべく，臨床実習前の基礎科目の再教育などを徹底して講ずる必要があると考える．これは，歯科臨床実習は実際の患者を相手にしており，歯学科学生では患歯の切削，抜歯などの小外科処置や歯周外科での切開，縫合など歯科医師となんら変わらない歯科医療行為を指導医のもとで行っている．また，歯科衛生士学校生においても，歯科医師のアシスタント行為を行っており，患者から見れば歯学科学生と歯科衛生士学校生は歯科医療従事者の一員として認識されていると思われる．このような状況下においては，感染防止の基礎となる医療関連感染原因微生物に関する認知度の十分なる定着が求められる．また今後，認知度に限らず肝炎に関する正しい知識を有しているかの意識調査も行い，感染防止教育を充実したいと考える．

次に，感染防止に関する知識について，まず質問 8「肝炎ウイルスを持つ人が使用した歯ブラシの共用は避けるべきだと思いますか」では，歯学科学生と歯科衛生士学校生合わせて 263 名(97.4%)が「歯ブラシの共有を避けるべき」だと回答した．これは，肝炎ウイルスを持つ人が使用した歯ブラシでなくとも，個人的に口腔内で使用する「歯ブラシ」という衛生用品を他人と共有することに対して拒否感があるためだと推測される．逆に，7 名(2.6%)の学生は，「歯ブラシを共有してもよい」と回答している．歯ブラシの共有に関する理由は不明である．歯ブラシに付着した肝炎ウイルスを消毒する方法としては，次亜塩素酸ナトリウムなどによる消毒が必要となるが[5]，歯ブラシが安価であること，消毒に掛かる手間と費用，未消毒歯ブラシを誤使用する可能性などの問題点を考慮するならば，ディスポーザブルの歯ブラシを使用するべきだと考える．

次に，質問 9「肝炎ウイルスを持つ人の診療で使用したゴム手袋は消毒用エタノールで消毒すれば再利用してもよいと思いますか」では，歯学科学生 14 名(12.8%)，歯科衛生士学校生 72 名(44.7%)，合計 86 名(31.9%)が「再利用してもよい」と回答した．これは，まず肝炎ウイルスに対して消毒用エタノールでの消毒が有効ではないことなどの感染防止に関する知識の定着が不十分な点が考えられる．また，2001 年 8 月に日本歯科医師会から会員歯科医師に配布された，「一般歯科診療 C 型肝炎予防対策 Q & A」のマニュアルにおいて，オートクレーブを用いることのできないものに対して推奨されている消毒薬の中に消毒用エタノールでの消毒が含まれていることから[6]，この古い情報に基づいたまま診療に従事している歯科医師が歯科臨床実習生に対して消毒用エタノールでの消毒を指示している可能性なども考えられる．現在，B 型肝炎ウイルスに対しては消毒用エタノールでの消毒が不十分であることは立証されており，C 型肝炎ウイルスに対してもその消毒が十分であるかは不明なことから，厚生労働省でも消毒用エタノール以外の消毒法の情報提供を行っている[7,8]．

なお，他の調査研究においても患者ごとにゴム手袋を交換する者が少ないとの報告がなされており[9]，本調査結果も踏まえて，特にゴム手袋の適正使用に関する感染防止教育の重要性が必要であると考える．

他方，歯科医療現場の大多数を占める歯科医院や日本歯科医師会，感染防止関連学会に所属していない歯科医院などに対して，最新の情報などを逐次提供する感染防止情報システムを構築することや歯科衛生士の感染防止専門資格(仮称：「感染管理歯科衛生士(Infection Control Dental Hygienist: ICDH)」)[10]の新たな運用の施策などを講じることも必要だと考える．また現在，歯科医院の多くが経営的に苦しく，コストの掛かる感染防止をどこまで行えるかという問題も歯科では孕んでおり，最新かつ正確な感染防止の研究と同時に，「感染防止と医療経済」などの研究・教育も必要になると考える．これは，歯科臨床実習先のより良い感染防止の環境整備につながることにもなると考える．

今後，肝炎の感染防止教育の研究は，肝炎抗体検査，ワクチン接種などの意識調査や感染防止と医療経済などの研究を続けて行っていきたいと考える．

結　語

本調査では，歯科臨床実習生における B 型および C 型肝炎に関する認知度は高かったものの，ゴム手袋の再利用などの感染防止に関する知識は，特に歯科衛生士学校生 72 名(44.7%)が十分な知識を有していなかった．今後，肝炎に対する知識に関した意識調査や歯科衛生士学校生への更なる感染防止教育の必要性とともに歯科臨床実習先に対する最新の感染防止情報の提供，感染防止の専門資格の新たな運用などを講ずる必要があると考える．

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【原著】

感染防止と歯科医療受診行動 Ⅳ
～「感染予防対策」は患者が歯科医療施設を選択する際に重要なのか～

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[2]社会健康観研究会

(受付：平成20年11月18日)
(受理：平成20年12月 9日)

要　旨

関東および近畿地方在住の 812 名（平均年齢 43.6 歳）に対して、歯科医療施設が感染予防対策に対して高い意識を持っている点が受診選択の基準になるかどうかの意識調査を行った。

調査の結果、歯科医療施設を決める際に重要視する点では、「説明が丁寧」762 名（15.1%）、「待ち時間が短い」754 名（14.9%）、「噂が良い」741 名（14.7%）の 3 つが多い回答を得た。「感染防止がとられている」353 名（7.0%）は 14 項目中 6 番目に高い回答を得た。また、歯科医療施設スタッフの感染予防対策に対する意識の高さが歯科医療を受ける上で重要であるかに関しては、609 名（75.0%）が「重要である」と回答した。

本調査により、歯科医療施設を受診する際の選択基準として、「歯科医療施設およびスタッフの感染予防対策に対する意識が高い点」が重要な基準の一つとして影響を与えていることが示唆された。

キーワード：歯科医療、感染防止（感染制御）、意識調査、受診行動、歯科医院

目　的

歯科医療における感染予防対策のエビデンスは近年、徐々に蓄積されつつあるが[1]、患者側から見た歯科医療における感染予防対策の調査研究や感染予防対策が歯科医療施設を選択する際の要因調査などは未構築である。

今回、我々は「感染予防対策と医療に関する調査研究」のひとつとして、「患者が歯科医療施設（歯科医療）を選択する基準はどこにあり、感染予防対策は果たして選択基準に入っているのか」を調査した。本調査の結果は、歯科医療現場に求められる感染予防対策のあり方に寄与し、今後の歯科医療における感染予防対策の充実と向上につなげることを目的とした。

対象および方法

1. 調査方法

対象は、関東および近畿地方の歯科医療施設に通院している患者である。歯科医師または歯科衛生士が調査の趣旨を口頭にて説明し、了解を得られた患者を対象とした。

調査用紙は、無記名自記式質問紙を用い、2008年 1～4 月（4 ヵ月間）に行った。

2. 調査項目

調査項目は、2006 年に佐藤ら[2]が学生群を対象に実施した下記 3 つの調査項目を用いた。

質問 ①「あなたが歯科医療施設（歯科医院、病院）を探す際に用いる情報媒体はなんですか？」【複数回答可】(1. 歯科医療施設の広告看板、2. インターネットでの検索、3. 親類や友人などからの情報、4. その他（自由記述））

質問 ②「あなたが歯科医療施設を決める際に重要視する点はなんですか？」【複数回答可】

（1. 歯科医療施設が住んでいる所から近い、2. 親類や友人などからの噂が良い、3. 朝早く（7時）から開院している、4. 夜遅く（19時以降）まで開院している、5. 歯科医療施設の建物が大きく立派である、6. 歯科医療施設に駐車場がある、7. 歯科医療施設での待ち時間が短い、8. 歯科医師の説明が丁寧で理解しやすい、9. アシスタントが充実している、10. 器具などが清潔で感染予防対策がとられている、11. 歯科医師が有名大学出身である、12. 専門医・認定医資格を持つ歯科医師がいる、13. 自由診療の費用が安い、14. その他（自由記述））

質問 ③ 「あなたにとって、歯科医療施設のスタッフ（歯科医師、歯科衛生士、歯科技工士、歯科助手など）の HIV/AIDS や肝炎ウイルスなどの感染症に対する感染予防対策の意識の高さは、歯科治療を受ける上で重要ですか？」（1. 重要である、2. どちらかといえば重要である、3. どちらかといえば重要ではない、4. 重要ではない）

3. 倫理的配慮

調査実施前に、任意かつ、調査への参加・不参加や回答内容により治療への利益・不利益は生じないこと、個人情報保護から調査終了後は調査用紙とデータを破棄することを説明した。

結　果

1. 有効回答数

回答総数は、835 名（A 歯科医療施設 190 名、B 歯科医療施設 233 名、C 歯科医療施設 203 名、D 歯科医療施設 209 名）であった。

回答総数から全調査項目に回答しているものを有効とした結果、有効回答数は 812 名（A 歯科医療施設 185 名、B 歯科医療施設 229 名、C 歯科医療施設 196 名、D 歯科医療施設 202 名）、有効回収率は 97.2% であった。

2. 回答者のデータ

回答者の属性は、男性 481 名（59.2%）、女性 331 名（40.8%）であった。年代別では、10 代 40 名（4.9%）20 代 130 名（16.0%）、30 代 149 名（18.3%）、40 代 211 名（26.0%）、50 代 135 名（16.6%）、60 代 102 名（12.6%）、70 代 37 名（4.6%）、80 代 8 名（1.0%）であり、平均年齢は 43.6 歳であった。

3. 回答結果

図 1〜3 に示す結果が得られた。

質問 ① の回答は、「親類や友人などからの情報」707 名（50.4%）がもっとも多い回答を得た。「その他」は、「歯科医療施設に直接問い合わせる」、「親類、知人が勤務している歯科医療施設にする」などの回答が得られた。

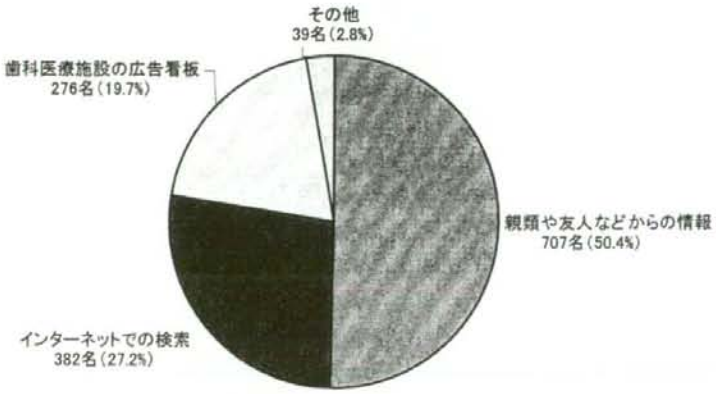

図 1 質問 ①「あなたが歯科医療施設を探す際に用いる情報媒体はなんですか？（複数回答）」
（N=812. TA=1,404）

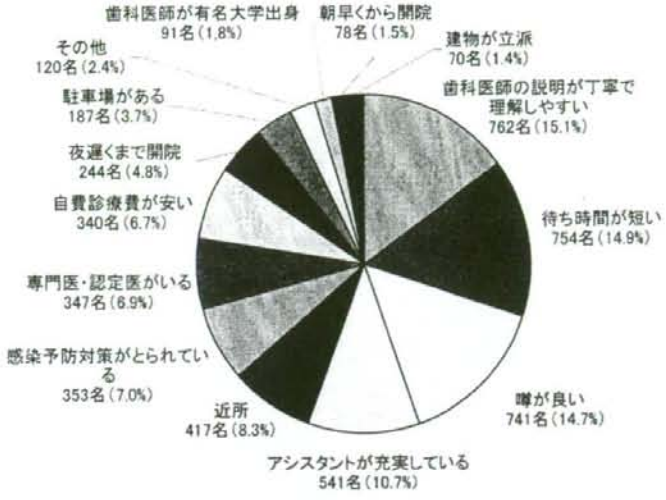

図 2 質問 ②「あなたが歯科医療施設を決める際に重要視する点はなんですか？（複数回答）」
（N=812, TA=5,045）

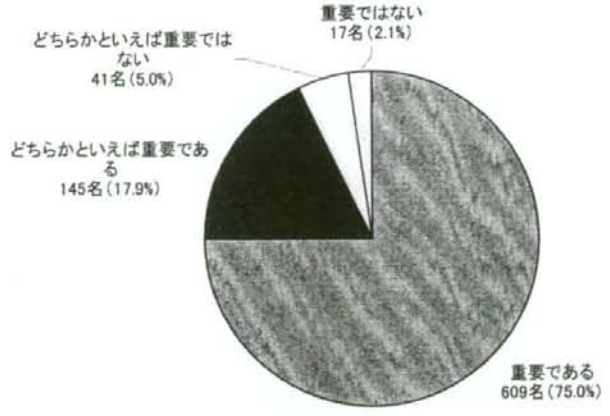

図 3 質問 ③「あなたにとって歯科医療施設のスタッフの感染予防対策に対する意識の高さは、歯科医療を受ける上で重要ですか？」（N=812）.

質問 ② の回答は、「歯科医師の説明が丁寧で理解しやすい」762 名（15.1%）、「待ち時間が短い」754 名（14.9%）、「噂が良い」741 名（14.7%）が多い回答を得た。「感染予防対策がとられている」353 名（7.0%）は、14 項目中 6 番目であった。「その他」では、「治療が痛くない」との回答が多く、「通りがかりの歯科医療施設にする」、「歯科医師やスタッフが眉目秀麗、容姿端麗である」、「親類、知人が勤務している歯科医療施設にする」などの回答が得られた。

質問③の回答は、「感染予防対策は重要である」609 名（75.0%）が大半を占めた。

また、年代別の回答結果は、図 4〜6 に示す結果が得られた。

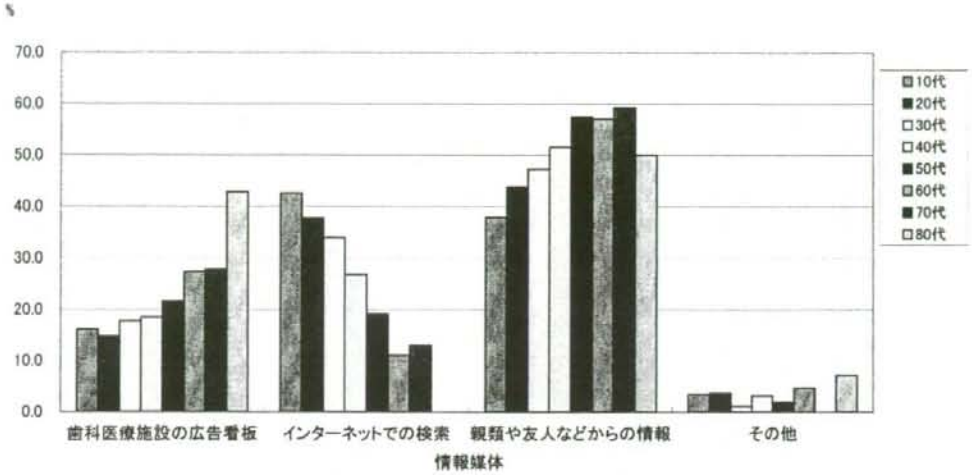

図 4　年齢別の歯科医療施設を探す際に用いる情報媒体（複数回答）（N=814, TA=1,404）

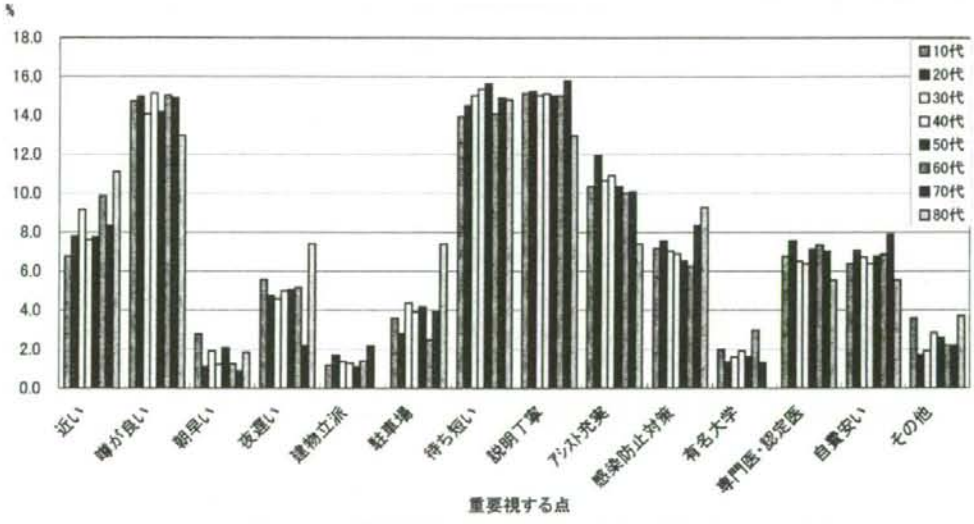

図 5　年齢別の歯科医療施設を決める際に重要視する点（複数回答）（N=812, TA=5,045）

考　察

質問 ① の回答結果について、「親類や友人などからの情報」707 名（50.4%）がもっとも多い回答を得たのは、情報媒体として容易、かつ、親類や友人という信頼できる人からの情報であるためだと推測される。2 番目に回答数が多い「インターネットでの検索」382 名（27.2%）は、10〜30 歳代で 40% 近い回答率を占めているが、年齢層が増すに連れて、その回答率は低下していた。これは、若い世代がインターネットを使用する環境に慣れている点が推測される。これは、佐藤ら[2]が行った学生を対象にした調査でも、日頃から電子メールやインターネットなどの情報ネットワークを頻繁に利用する学生は、その回答率が高かった点と共通していた。また、「歯科医療施設の広告看板」に関しては、10〜30 歳代で 16.2% であったが、60〜80 歳代では 32.7% とその比率が高かった。今回の調査結果では、60〜80 歳代はインターネットという室内での情報源よりも、外出先で目にする

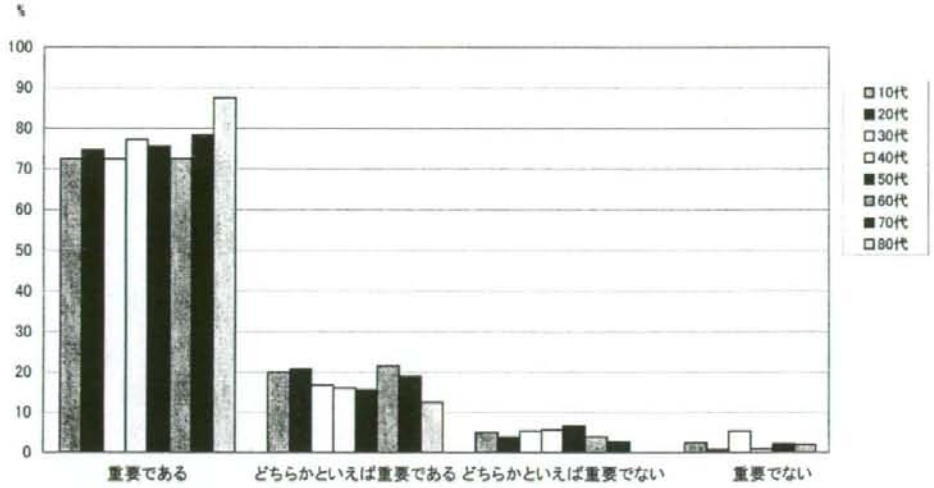

図6　年齢別の歯科医療施設のスタッフの感染予防対策に対する意識の高さは、歯科医療を受ける上で重要であるか(N=812)

広告看板の方が情報媒体として重きを置いているのかもしれない。

質問②の回答結果について、「説明が丁寧」762名（15.1%）、「待ち時間が短い」754名（14.9%）、「噂が良い」741名（14.7%）の3つが多くの回答を得た。これは、年代別回答でも同様であり、歯科医療施設の選択の重要な要因となっていると考える。

「感染予防対策がとられている」353名（7.0%）は、選択基準の14項目中6番目であり、「専門医・認定医がいる」347名（6.9%）、「自由診療の費用が安い」340名（6.7%）と近値であった。これは、患者が歯科医療施設を選択する上で、高度な専門技術とそれに見合う費用、そしてその医療に対する安全・安心を求めている結果ではないかと推測される。

今回の調査結果から、患者が歯科医療を受ける際の待ち時間を短くし、診療説明を丁寧に行えば、その患者はその歯科医療施設に対して好感を持ち、他人に良い噂を流すという連鎖をうみ出す。つまり、「噂を流す」という患者の行為が、他者の歯科医療施設を選択する基準として大きな要因を占めていると考えられる。その噂の中に、患者にとって安全・安心となる「感染予防対策」を加えることで、さらなるより良い連鎖をうみ出すと考える。これは、質問③の回答「感染予防対策は重要である」609名（75.0%）が示すように、患者が強く求める点でもある。

良い連鎖をうみ出すには、患者が求める点を把握し、実践することが重要である。改正医療法（平成19年4月1日施行）では、歯科医療従事者、実習生や職員などへの感染予防対策を講ずることや感染予防対策マニュアルの作成を記している[3]。また、2008年度診療報酬改定に伴い、「歯科外来診療環境体制加算」が新設され、「患者に安全・安心な歯科医療環境を提供できる」、「歯科医療機器の患者毎の交換、専用機器での洗浄・滅菌処理の徹底」、「感染症患者に対する歯科診療体制の常時確保」、「歯科用吸引装置等による飛散汚染物質の除去」、「院内感染防止対策などを実施している旨を院内掲示する」などの措置が常時講じられていれば初診時1回30点の保険請求が可能となる[4]など、徐々にではあるが歯科医療施設の感染予防対策が進められている。また、現在歯科医療の大多数を担う個人歯科医院において、感染予防対策の専門組織インフェクションコントールチーム（Infection Control Team：ICT）を設置するのは困難であるが、アメリカ疾患予防管理センター