Routine oral hygiene is important for the control of opportunistic pathogens in the oral cavity of institutionalized elderly individuals. We evaluated the effects of routine oral care on opportunistic pathogens at various time points after admission to a nursing home. Twenty-five elderly subjects living in the nursing home (mean age: 86.0 ± 10.4 years) participated in the study. Caregivers and dental hygienists cleaned the teeth, dentures, tongue, and mucosa after each meal using both routine and professional oral care techniques. Opportunistic pathogens were collected from the teeth, tongue, and mucosal surfaces using a cotton swab; and the species of microbes were determined and the numbers were counted following cultivation on selective agar. Regular oral care including professional oral care was found to be effective for reducing infections by many kinds of opportunistic pathogens on the teeth surfaces and the oral environment without food residue during a long-term study (6 months). Further, this care after 1 month significantly reduced infections by opportunistic pathogens on mucosal surfaces in subjects without dentures; however, this was not observed in those with dentures. Our data shows the importance of regular oral care in cleaning hard and soft surfaces of the oral cavity improves the oral health of the institutionalized elderly.

Key words: oral biofilm, denture, oral care, opportunistic pathogens, institutionalized elderly

Introduction

In Japan, the number of elderly people is steadily increasing where those over the age of 65 will account for approximately 25% of the population by 2025. Accordingly, the number of bedridden elderly requiring systemic care in residential and nursing homes will also increase. Reports show institutionalized elderly individuals have poorer oral health than those who live independently at home. Further, the oral cavity is thought to be a potential reservoir of opportunistic pathogens that are risk factors for pneumonia in the elderly. Reports show a higher prevalence of nosocomial and Gram-negative enteric bacilli pathogens in institutionalized elderly patients with severe pneumonia. El-Solh et al. reported respiratory pathogens colonizing dental plague were implicated in the infections of the lower respiratory tract in institutionalized elderly subjects. There are possible links with poor hygiene and host-defense problems to an increased incidence of pneumonia in institutionalized elderly patients. Therefore, oral hygiene is considered to be important to control opportunistic pathogens on teeth and mucosal surfaces; and some studies indicate oral hygiene for hospitalized elderly patients reduces the risk of nosocomial pneumonia. Thus, regular dental care may be effective in reducing the numbers of dental and respiratory bacteria for the elderly residents in long-term care facilities. Although the effects of oral care...
have been reported, few studies have surveyed the different opportunistic pathogens in institutionalized elderly subjects between, before and after receiving regular dental care provided by care-givers and dental hygienists. In this study, we isolated opportunistic pathogens from the teeth, tongue, and mucosal surfaces from elderly subjects requiring systemic care at various time points before and after regular and professional oral care. The purpose of this study was to evaluate the effects of oral care on opportunistic pathogen populations in institutional elderly people.

Materials and Methods

Subjects
Beginning 2 weeks after entering a Toshima-ward, Tokyo nursing home, 25 residents (mean age: 86.0 ± 10.4 years; 6 males, 19 females) who required long-term nursing care participated in this study. This nursing home was new (established May, 2004) with a capacity of 62. The study was conducted from October 2004 to May 2005 where the subjects were randomly selected from the residents of the same floor using a random-numbers table and held blind from the investigators. The subjects were in two groups; those requiring little care, i.e. not bedridden or confined to bed (n = 8); and those requiring intensive care, i.e. confined to their bed (n = 17). Prior consent was obtained from all subjects. The study was approved by the Ethics Committee of the Tokyo Medical and Dental University and performed according to the rules of the Helsinki Declaration. Dental examinations to determine the presence of dental caries, periodontal pocket depths, dental calculus, remaining food residues, and other typical oral conditions were performed using artificial white light by trained dentists before the study and during routine professional oral care. Four dentists assessed the subject’s dental and periodontal condition using six measurements points for each tooth: mesiobuccal, buccal, distobuccal mesiolingual, lingual, and distolingual.

Oral care
At the initial examination, all patients had a routine dental and medical examination. Oral care techniques by dental hygienists and care givers were standardized before beginning oral care. For daily oral care, subjects who were able used the sink facilities in their rooms and performed standard oral hygiene three times a day by themselves; and cleaning status was confirmed by the care givers examining the oral cavity. Whereas the other subjects were performed by care givers three times a day and assisted in oral cleaning with tooth brushing, brushing of denture surfaces and oral rinsing with tap water. The patients cleaned their teeth, dentures, tongue, and mucosa surfaces after each meal using routine oral care techniques. Further, for 20 minutes twice per month, dental hygienists provided professional care such as removing oral calculus with a scaler, dental brushing of teeth surfaces, mucosal cleaning with a sponge brush, denture cleaning, and oral washing with 0.5% povidone-iodine solution (Isodine-Gargle, Meiji seika, Tokyo, Japan) in addition to the daily oral care. The routine oral care including professional care was performed for 6 months. Daily oral care without professional care was performed from entering the institution to the first sampling. No antibiotic therapy was administered during the 2 weeks before the start of this study and during the 6 month study period; and none of the subjects suffered from severe infections or systemic diseases. There was no information about antibiotic therapy for the subjects before entering the institution. The percentage of subjects who retained their own teeth was 36% (9/25). None of the subjects dropped-out of the study.

Bacterial sampling
Supragingival plaque samples were collected from the posteroanterior buccal surface of the upper right second premolar, the buccal surfaces of the upper right second premolar and first molar using a cotton swab (Seedswab No. 1, Eiken Chemical Co., Ltd., Tokyo); and transferred to 1 ml of reduced transport fluid (0.4% agar, 0.15% thioglycollate/phosphate-buffered saline) in sterile bottles. For edentulous subjects who used complete dentures (n = 12), the samples were collected from the same regions of the upper right second premolar and first molar of the complete dentures. For edentulous subjects not using dentures, plaque samples were not collected. Subjects using partial denture (n = 2) and not having any of the above mentioned teeth were sampled from the opposite side or other remaining teeth. Samples were also collected by swabbing five times from the center of the tongue and right buccal surface of the oral mucosa. All samples were taken before professional care by a dental hygienist. After placement in transport fluid, the samples were immediately transported to the Biomedical Laboratory (BML, Tokyo, Japan) for analysis to detect opportunistic pathogenic bacteria.
Identification of bacteria and fungi
The isolated bacteria and fungi from the plaque, the tongue, and mucosal surfaces were identified using culture procedures. The samples were pour plated on chocolate agar, blood agar, OPA staphylococcus, and Drigalski agar plates; and were incubated in an atmosphere of 5% CO₂ at 37°C for 24-48 hours. Representative colonies from each plate were isolated and analyzed using Gram stain, hemolysis, and oxidase reactions. The colonies were suspended in 1 ml 0.5% saline; gently shaken; and tested using microbial identification kits (VITEK; BioMerieux Vitek Japan, Tokyo, Japan). The bacteria and fungi detected in the samples are shown in Table 1.

Statistical procedures
All data were analyzed using the Statistical Package for Social Science (SPSS) version 11.5. The proportion of elderly subjects in the two groups was compared using the Chi-square and Wilcoxon signed-rank tests for equal and unequal variations. A p-value of less than 0.05 was considered to be significant.

Results
Samples were not taken from the teeth, tongue and mucosal surfaces of two elderly subjects respectively at 4 and 6 months after the beginning of oral care owing to poor health. One month after starting oral care, the numbers of opportunistic pathogens on the teeth, tongue, and oral mucosal surfaces decreased in 6 of 21 (28.6%), 11 of 25 (44.0%), and 10 of 25 (40.0%) of the subjects, respectively, in comparison with their numbers at the beginning of the study (Fig.1). The proportion of subjects with decreasing opportunistic pathogens on teeth surfaces was significant at 4 (12/19, 63.2%, p = 0.027) and 6 (13/19, 68.4%, p = 0.011) months as compared to after 1 month (Fig. 1). In contrast, the proportion of subjects with decreased numbers (13/23, 56.5%) on the mucosa surfaces after 4 months were greater but were not significantly higher than at 1 month (10/25, 40.0%) after the beginning of oral care (Fig.1). The proportion of subjects with decreasing numbers on the tongue after 4 months (6/23, 26.1%) was fewer and those after 6 months (13/23, 56.5%) increased as compared to those after 1 month (11/25, 44.0%); but none were significantly different. Candida albicans tended to remain on all surface areas at varying sampling times after professional care but other opportunistic pathogens did not.

The infection or accumulation of multiple species of opportunistic pathogens is a risk factor for respiratory tract infections in institutionalized elderly subjects. Therefore, detection of opportunistic pathogens was performed using a qualitative analysis to isolate multiple species. The proportion of elderly subjects where more than four species and strains of opportunistic pathogens were isolated was 10/21 (47.6%), 11/25 (44.0%) or 11/25 (44.0%) on the teeth, tongue and mucosal surfaces, respectively, before the beginning of professional care. To evaluate the effects of oral care in the elderly subjects, the proportion of subjects with

![Fig. 1. The effects of routine oral care on the numbers of opportunistic pathogens compared to the numbers on initial examination.](image)

The strains and species of opportunistic pathogens were counted in each sample.
more than four species and strains of opportunistic pathogens was employed as an indicator. On teeth surfaces the numbers significantly decreased after 4 (2/19; 10.5%, \( p = 0.012 \)) and 6 months (1/19; 5.3%, \( p = 0.003 \)) in comparison to before the beginning of professional care (Fig. 2). On mucosal surfaces the opportunistic pathogens decreased after 4 and 6 months (3/23; 13.0% and 4/23; 17.4%) but not significantly. And there were no significant differences for the tongue surface. The comparison was confirmed using the mean±SD of opportunistic pathogens numbers as analyzed using the Wilcoxon signed-rank test (see below) on the teeth surfaces. The opportunistic pathogen numbers (2.4±0.8) at 6 months after professional oral care was significantly lower than before the start of professional oral care (3.2±1.5) on the teeth surfaces (\( p = 0.028 \)). However, there were no significant differences in the other comparisons. Therefore, long-term professional oral care is significant to effectively decrease opportunistic pathogen numbers on teeth surfaces in comparison to short-term professional oral care.

The proportion of elderly subjects having decreased numbers of opportunistic pathogens on mucosal surfaces was significantly lower in patients with dentures (2/13, 15.4%) than in those without dentures (8/12, 66.7%) at 1 month (\( p = 0.013 \)) but not at 4 and 6 months after the beginning of oral care (Fig. 3C). However, there were no significant differences for the teeth and tongue surfaces between subjects with and without dentures at various time points (Fig. 3A and B). The number of subjects with decreasing num-

Fig. 2. The effects of routine oral care on the reduction of more than four types of opportunistic pathogens.

The number of subjects with more than four species and strains of opportunistic pathogens detected on the teeth, tongue, and oral mucosal surfaces at 0, 1, 4, and 6 months are shown. Asterisks denote significant difference in the chi-square test (\( p < 0.05 \), 0 month versus 1, 4 or 6 months for each sample).

Fig. 3. The effects of routine oral care in subjects with and without dentures.

The number of subjects with decreasing numbers of opportunistic pathogens on the teeth (A), tongue (B), and mucosal (C) surfaces with and without dentures at 1, 4, and 6 months are shown. The number of subjects with dentures were 13, 12 and 12 for teeth, 13, 11 and 12 for the tongue, and 13, 12 and 12 for mucosa at 1, 4 and 6 months, respectively, after beginning professional care. The numbers of opportunistic pathogens were 2.7±1.5 and 3.8±1.3 for teeth, 3.7±0.9 and 3.5±1.3 for the tongue, and 3.1±0.9 and 3.4±1.1 on oral mucosal surfaces of elderly subjects with and without dentures, respectively, before the beginning of the study. Asterisks denote significant difference in the chi-square test (\( p < 0.05 \), with dentures vs. without dentures at 1, 4 or 6 months for each sample).
bers of opportunistic pathogens in elderly subjects with food residue (2/7, 28.6%) was significantly lower than those in subjects without food residue (11/12, 91.7%) on the teeth surfaces at 6 months ($p = 0.010$) (Fig. 4A). However, there were no significant differences among the patients with and without food residues on the tongue and mucosa at each sampling period after the beginning of oral care (Fig. 4B, C). Moreover, there were no significant differences between the two groups (non-bedridden and bedridden) in all data (data not shown) and among other dental and periodontal parameters.

**Discussion**

We investigated the effects of routine oral hygiene using professional care in institutional elderly subjects determining the numbers of opportunistic pathogens in samples taken from the teeth, tongue, and mucosal surfaces. Our data show routine oral hygiene with professional care was effective in reducing infections by a number of different opportunistic pathogens on teeth surfaces when food residues were removed from patients during long-term care. In addition, short-term treatment of 1 month showed a significant reduction of opportunistic pathogens on the mucosal surfaces between elderly subjects with and without dentures. Dentures may be reservoirs of opportunistic pathogens as well as the teeth surfaces $^{17,18}$, and may be a risk factor for opportunistic infection by many kinds of microorganisms in the institutionalized elderly. Therefore, dentures may disturb the effects of oral care on opportunistic pathogen infections in the short-term (1 month) on the oral mucosa. Consequently, long-term (4 and 6 months) oral care is necessary for decreasing the opportunistic pathogens in oral mucosa of elderly individuals with dentures.

It is important to consider the influence of oral health on elderly subjects living communally in the same institution, as well as communication between caregivers and those subjects. Accumulating evidence suggests community and health-care associated infections have a unique epidemiology; and the pathogens involved and outcomes may be related with nosocomial processes $^{19-21}$. Further, transfers of microorganisms between the elderly or from caregivers and dental hygienists operating in the facility may have an influence on opportunistic infections in the oral cavity. Therefore, it may be possible that community- and care-associated infections were decreased or
abridged by controlling opportunistic pathogens in this long-term care study.

The oral biofilm is produced by the sequential attachment of bacteria; and is dependent on the various bacteria and the composition of the hard tissues involved\(^{22-24}\). Microorganisms become attached to and accumulate on surfaces of the oral cavity\(^{25}\). And the biofilm is known to be able to evade antimicrobial challenges from antibiotics or host immune defenses using multiple mechanisms\(^{26-28}\) where antimicrobial agents fail to fully penetrate the bacterial cells that compose the biofilm\(^{29}\). Further, the bacterial community may increase on hard tissue surfaces presenting considerable hygiene and host-defense problems for elderly individuals\(^{30}\). Here our data shows remaining food residues made cleaning difficult to remove opportunistic pathogens from teeth surfaces in long-term routine care (Fig. 4A). Such food residues provide nutrition for microbial growth as well as a colonization site for biofilm formation. In a previous report on special oral care, the number of streptococci recovered shortly after treatment was reduced\(^{30,31}\). Using oral professionals to remove the biofilm and calculus and mouth washing with 0.5% povidone-iodine solution cleared the tooth and mucosal surfaces; and this then allowed re-establishment and growth by commensal bacteria such as streptococci that replace infections as opportunistic pathogens\(^{31}\). Professional oral care may be useful in elderly patients to prevent respiratory infections; however, routine oral care without professional care does not show a significant effect on the microbiological community of the oral cavity\(^{32}\). Therefore, we considered routine oral care with professional oral care cleaned the teeth and mucosal surfaces after which beneficial commensal bacteria re-established and grew dependent on the amount of the remaining food residues in the oral cavities of the elderly subjects. Thus, routine oral care over a long term that completely cleans the oral cavity may be necessary to remove biofilm and re-establish microbiological flora with the commensal bacteria.

In conclusion, routine oral cleaning along with professional care was able to control infection with many types of opportunistic pathogens on teeth and denture surfaces using long-term care of the institutionalized elderly but was not of value in short-term care; however, on the tongue and mucosal surfaces opportunistic pathogens were removed during the short term. Our data indicates the important role of routine oral care in cleaning hard and soft surfaces of the oral cavity where this improves the oral health for institutionalized elderly individuals.

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