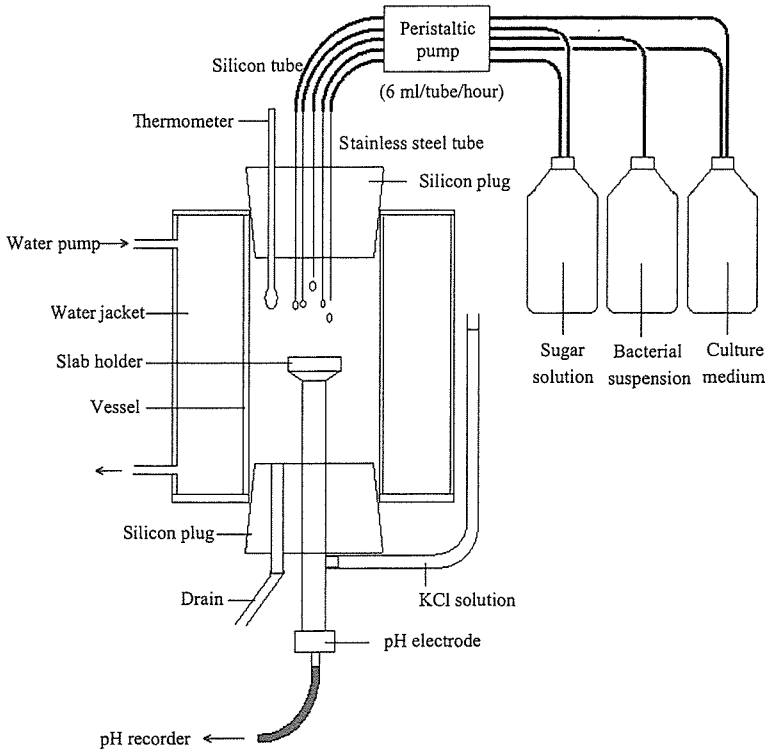

Fig. 1 Cross-sectional appearance of the artificial mouth system

flat electrode and enamel slabs were photographed. The biofilm formed on each enamel slab and flat bulb was carefully removed with a silicon scraper before being immersed in a 0.5 N NaOH solution to resolve the WIG on ice. This was followed by centrifugation (6,000 rpm, 20 min) to separate the bacterial cells from the WIG. Bacterial cells were re-suspended in PBS and the turbidity at 500 nm was measured. In addition, the amount of WIG in the NaOH solution was determined colorimetrically at 492 nm by the phenol-sulfuric acid method. Both cell turbidity and the amount of WIG were used as indicators of the amount of biofilm.

Vicker's hardness values of the enamel slabs were then determined after the experiment and compared with those before the experiment. The differences in hardness (ΔH) were used to infer the degree of demineralization.

4) Statistical analysis

Student's *t*-test was used to compare differences in the mean value of the degree of enamel demineralization and the amount of artificial biofilm in the control and experimental groups.

Results

1. Effect of XF on artificial biofilm formation by *S. mutans* in culture system

S. mutans MT8148 was cultured in HI broth containing 1% sucrose with 0-5% XF in a glass test tube. The amounts of cells in the total adherent (loose plus firm) fraction were significantly inhibited by XF in a dose-dependent manner. The inhibitory rates for 1%, 2.5%, and 5% XF were 68%, 83%, and 86%, respectively (Fig. 2). Figure 3 shows the amounts of WIG and water-soluble glucan (WSG) in the presence of 1% sucrose and each concentration of XF. The amounts of WIG and WSG in each adherent fraction were significantly inhibited by XF in a dose-dependent manner. XF alone contributed minimally to the development of artificial biofilm formation in terms of bacterial cells and WIG.

2. Effect of XF on artificial biofilm formation, biofilm pH environment, and enamel demineralization in AMS

Two identical artificial mouths were operated simultaneously under the same conditions except for sugar supplementation. Changes in pH values beneath the artifi-

cial biofilm are shown in Fig. 4. The pH values of the control (2% sucrose) began to decrease after 8 hr of running the AMS, reaching pH 5.5, which is thought to be a critical pH for enamel demineralization after 13 hr and pH 4.6 after 20 hr. Conversely, the pH values in the experiment (2% XF) remained unchanged for 20 hr. Table 1 shows the changes in Vicker's hardness values (ΔH) of the enamel slabs before and after the experiments with the amount of artificial biofilm indicated by the turbidity of the bacterial cell suspension and the amount of WIG produced. The amount of artificial biofilm and change of microhardness were considerably lower in the case of XF alone.

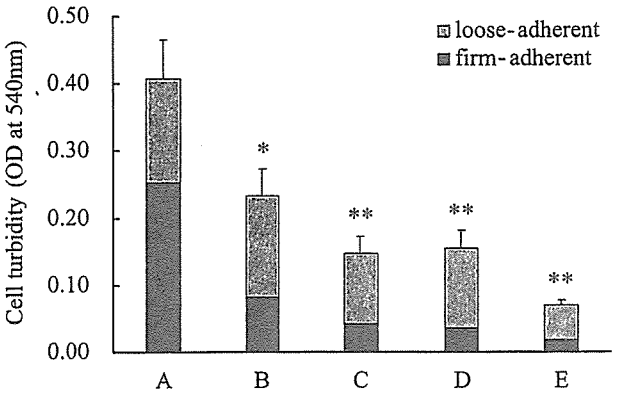

Fig. 2 Amounts of bacterial cells in firm- and loose-adherent fractions in a culture system of *S. mutans* MT8148 in the presence of sugars
A : 1% sucrose, B : 1% sucrose + 1% XF, C : 1% sucrose + 2.5% XF, D : 1% sucrose + 5% XF, E : 1% XF.
* : $p < 0.05$, ** : $p < 0.01$ (Compared with A)

Figure 5 shows the change in pH values of AMS in the presence of 1% sucrose (control) and 1% sucrose plus 1% XF (experiment). The decrease in the pH of the experiment was slightly delayed compared to that observed in the control. The pH values beneath the biofilm on the electrode decreased to 5.5 after 14 hr and 17 hr of AMS operation in the control and experiment, respectively. As shown in Table 2, no significant differences were observed in the amount of biofilm and ΔH from the enamel slabs in the control and experiment.

Figure 6 shows the changes in the pH values underneath the artificial biofilm in the AMS in the presence of 1% sucrose (control) and 1% sucrose plus 2.5% XF (experiment). In the control, pH values began to decrease after 8 hr, reaching 5.5 after 16 hr and 4.7 after 22 hr of operating the AMS. However, the pH values in the experiment did not exhibit any apparent decreases, even after 22 hr. The amount of biofilm produced in terms of bacterial cell turbidity in the experiment was significantly less than that of the control (Table 3). Similarly, no WIG could be detected on the enamel slabs in the ex-

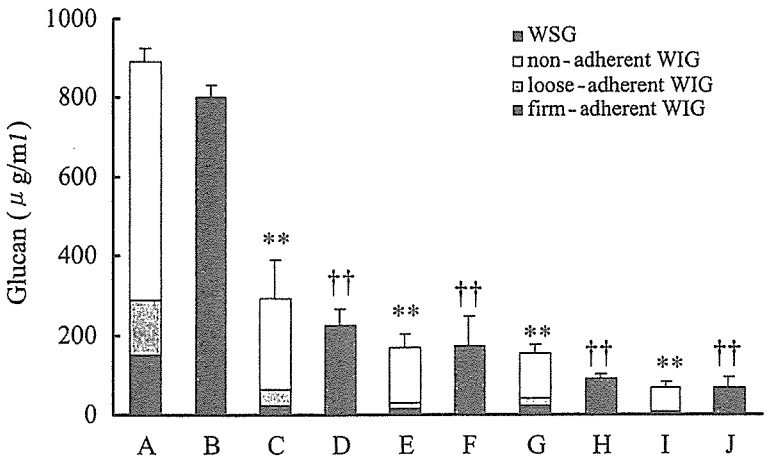

Fig. 3 Amounts of glucans in each adherent fraction in a culture system of *S. mutans* MT8148 in the presence of sugars
A and B : 1% sucrose, C and D : 1% sucrose + 1% XF, E and F : 1% sucrose + 2.5% XF, G and H : 1% sucrose + 5% XF, I and J : 1% XF
** : $p < 0.01$ (Compared with A), †† : $p < 0.01$ (Compared with B)

Table 1 Effect of XF on enamel demineralization and the amount of artificial biofilm formed on bovine enamel slabs by *S. mutans* MT8148 in the artificial mouth system

	ΔH [a]	OD_{500} [b]	WIG (μg/mm²) [c]
Control (2% sucrose)	252.7 ± 5.1	0.009 ± 0.002	2.503 ± 0.360
Experiment (2% XF)	8.2 ± 4.2 [d]	ND	0.239 ± 0.183 [d]

[a] : Differences in microhardness of 4 enamel slabs before and after experiments (Mean ± SD)
[b] : Bacterial cell turbidity at 500 nm per square mm of 4 enamel slabs (Mean ± SD)
[c] : Amount of total WIG per square mm of 4 enamel slabs (Mean ± SD)
[d] : $p < 0.01$ (Compared with the control group)
ND : Not detected

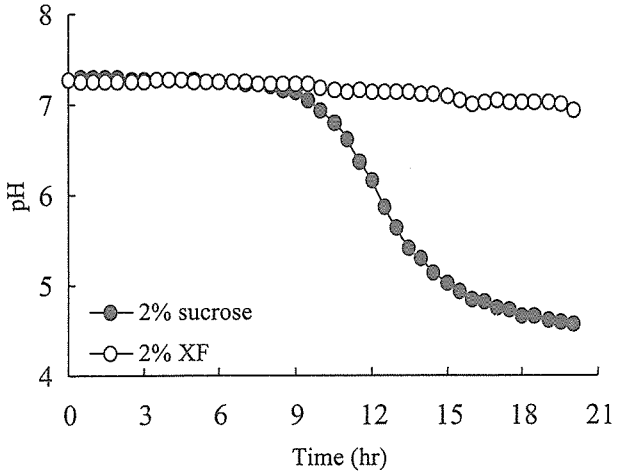

Fig. 4 Changes in pH underneath the artificial biofilm formed by 2% sucrose or 2% XF

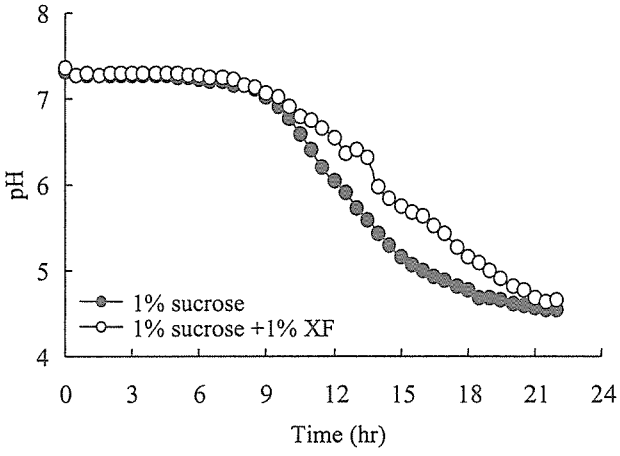

Fig. 5 Changes in pH underneath the artificial biofilm formed by 1% sucrose or 1% sucrose + 1% XF

periment, and the value for ΔH in the experiment was significantly less than that of the control.

Discussion

Adherence of *S. mutans* MT8148 cells and WIG to a glass surface were inhibited by XF in a dose-dependent manner in this study. WSG production was inhibited by XF in a similar manner. Imai et al. [11] reported that 83% of WIG adherence by *Streptococcus sobrinus* 6715 in the presence of 5 mM sucrose was inhibited by 10 mM XF in a culture system. Takeuchi[15] reported that 10 mM XF inhibited both the production and adherence of WIG by crude glucosyltransferase from *S. sobrinus* 6715 in the presence of 5 mM sucrose by 86% and 94%, respectively. Kishi[16] reported that XF effectively inhibited purified GTF from *S. sobrinus* MT3791 and that XF competitively inhibited the sucrose degrading activity of GTF, inhibiting the activity of glucan production. Togashi[17] reported that purified GTF from *S. mutans* MT8148 was competitively inhibited by XF.

Under the culture conditions used in this study, 43% of *S. mutans* MT8148 cells and 33% of WIG were recovered in the total adherent (loose and firm adherent) fraction. Imai[18] reported that the adherence ability of mutans streptococci to a smooth surface was dependent on the ability to produce WIG from sucrose, and that a positive correlation existed between adherent WIG and adherent bacterial cells. Consequently, bacterial cells and WIG were both suitable for use as adherence parameters of *S. mutans*.

In vivo test systems such as animal experiments[19], human plaque pH tests[20], and human intraoral cariogenicity tests (ICT)[21] have been used to assess both the cari-

Table 2 Effect of XF on enamel demineralization and the amount of artificial biofilm formed on bovine enamel slabs by *S. mutans* MT8148 in the artificial mouth system

	ΔH [a)]	OD_{500} [b)]	WIG (μg/mm²) [c)]
Control (1% sucrose)	245.8 ± 8.4	0.014 ± 0.005	4.527 ± 0.991
Experiment (1% sucrose + 1% XF)	244.2 ± 11.3	0.014 ± 0.002	4.183 ± 0.627

a) : Differences in microhardness of 4 enamel slabs before and after experiments (Mean ± SD)
b) : Bacterial cell turbidity at 500 nm per square mm of 4 enamel slabs (Mean ± SD)
c) : Amount of total WIG per square mm of 4 enamel slabs (Mean ± SD)

Table 3 Effect of XF on enamel demineralization and the amount of artificial biofilm formed on bovine enamel slabs by *S. mutans* MT8148 in the artificial mouth system

	ΔH [a)]	OD_{500} [b)]	WIG (μg/mm²) [c)]
Control (1% sucrose)	242.7 ± 5.4	0.009 ± 0.002	1.850 ± 0.692
Experiment (1% sucrose + 2.5% XF)	11.1 ± 3.0 [d)]	0.0005 ± 0.0001 [d)]	ND

a) : Differences in microhardness of 4 enamel slabs before and after experiments (Mean ± SD)
b) : Bacterial cell turbidity at 500 nm per square mm of 4 enamel slabs (Mean ± SD)
c) : Amount of total WIG per square mm of 4 enamel slabs (Mean ± SD)
d) : $p < 0.01$ (Compared with the control group)
ND : Not detected

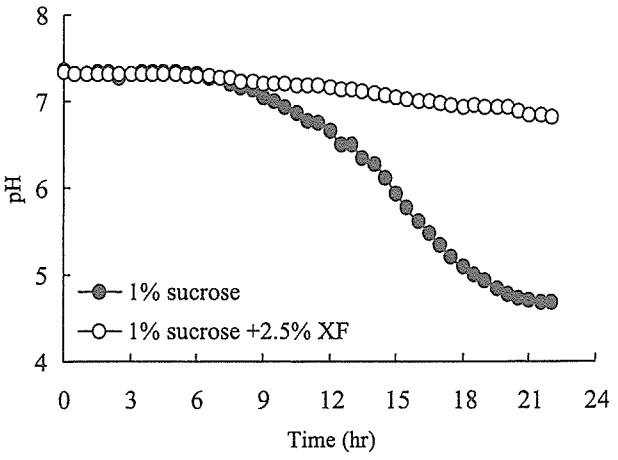

Fig. 6 Changes in pH underneath the artificial biofilm formed by 1% sucrose or 1% sucrose + 2.5% XF

ogenic potential and inhibition of sugar substitutes or enzyme inhibitors. However, *in vivo* test systems have had several associated intrinsic time and economic problems, so that *in vitro* test systems, especially artificial mouth systems, have been developed[22-32]. We improved an AMS originally developed by Hinoide et al.[31]. The characteristics of this system made it possible to assess the important parameters responsible for causing dental caries such as biofilm formation, the pH underneath biofilm, and enamel demineralization.

As mentioned previously, XF was not used as a substrate for GTF and it was found to inhibit GTF activity and the production of WIG. In ICT in human subjects, 2% XF did not inhibit enamel demineralization in the presence of 2% sucrose[33]. However, enamel demineralization by 2% XF alone was significantly lower than that observed when exposed to 2% sucrose, indicating that XF appears to be a low-cariogenic sugar. It is not known whether or not XF produces the same results in the AMS as those in ICT. As shown in Fig. 4 and Table 1, 2% sucrose caused marked decreases in pH, the production of large amounts of biofilm, and considerable enamel demineralization. These results indicate that the use of the AMS facilitated the assessment of the cariogenicity of sucrose. Conversely, 2% XF alone did not decrease the pH in the AMS, and the amount of biofilm and enamel demineralization were considerably low, suggesting that XF might be a low- or non-cariogenic sugar. These results corroborated previous findings obtained by ICT. Using *S. sobrinus* 6715, Hinoide et al.[31] re-

ported that xylitol, a non-cariogenic sugar alcohol, did not decrease the pH of their AMS, the prototype of the system used in our study, over a period of 24 hr.

The capacity of 1% and 2.5% XF to differentially inhibit the cariogenicity of 1% sucrose was interesting. While pH values under conditions of the simultaneous addition of 1% XF and 1% sucrose were slightly delayed compared with 1% sucrose alone in AMS, 1% XF did not suppress the formation of biofilm and enamel demineralization (Fig. 5 and Table 2). There was also a discrepancy between the results obtained in the culture system and those from the AMS. In the culture system, 1% XF significantly inhibited the adherence of *S. mutans* cells and WIG to glass surfaces in the presence of 1% sucrose (Figs. 2 and 3). This discrepancy might be ascribed to the differences in experimental conditions between the static conditions of the culture system and the flowing system of the AMS. However, 2.5% XF significantly inhibited the formation of biofilm on enamel slabs, pH decrease underneath the biofilm, and enamel demineralization (Fig. 5 and Table 3). These results suggested that an appropriate concentration of XF could inhibit, in part, the cariogenicity of sucrose.

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人工口腔装置におけるスクロースのう蝕原性に及ぼす二糖類キシロシルフルクトシドの影響について

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今井　　奨*　　西沢　俊樹*　　花田　信弘*

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概要：甘味二糖類キシロシルフルクトシド（XF）が歯面へのミュータンスレンサ球菌の付着や，非水溶性グルカンの合成を効果的に阻害することが報告されている．本研究は *in vitro* の実験系と，う蝕誘発過程に重要なバイオフィルム形成，バイオフィルム直下の pH，エナメル質脱灰度を同時に検討できる人工口腔装置による評価系を用いて，XF のう蝕誘発性およびスクロースのう蝕誘発性に及ぼす XF の抑制効果を検討した．ガラス試験管に 1% スクロースと 1～5% XF をそれぞれ含む培地に，*S. mutans* MT8148 を植菌し，培養を行った．その結果，XF はガラス管壁付着性の非水溶性グルカン量と菌体量を濃度依存的に抑制した．また人工口腔装置において，1% XF は 1% スクロース存在下における人工バイオフィルム形成やエナメル質脱灰度は抑制しなかったが，2.5% XF は人工バイオフィルム直下の pH をほとんど低下させず，人工バイオフィルム形成量，エナメル質脱灰度を有意に低下させた．以上のことから，XF は濃度によってスクロースのう蝕誘発性を部分的に抑制することが示唆された．

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索引用語：キシロシルフルクトシド，人工口腔装置，う蝕誘発性，バイオフィルム形成，ミュータンスレンサ球菌

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Original article

Relationship of cariogenic bacteria levels with periodontal status and root surface caries in elderly Japanese

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Relationship of cariogenic bacteria levels with periodontal status and root surface caries in elderly Japanese

Objective: The relationship of the levels of cariogenic bacterial species with periodontal status and decayed root surfaces was investigated in elderly Japanese subjects.
Methods: Three hundred and sixty-eight individuals (each 75 years old) were examined for periodontal status (pocket depth, attachment loss), root surface caries and salivary levels of mutans streptococci (MS) and lactobacilli (LB).
Results: Values >4 mm of attachment loss (rAL4) and for average attachment loss (aAL) of sites measured were significantly higher in subjects with LB than those without. Multiple regression analysis also showed a correlation between aAL and rAL4 values with the presence of LB (aAL $p = 0.003$; rAL4 $p = 0.002$). Further, multiple regression analysis of interacting factors regarding decayed root surfaces showed that LB carriers had a greater incidence of decayed root surface caries ($p = 0.003$), while MS and LB levels were correlated to the number of decayed root surfaces (LB $p = 0.010$; MS $p = 0.026$).
Conclusion: Our results indicate that considerable attachment loss elevates the possibility of having LB, thus increasing the risk of root surface caries. It was also found that LB and MS measurements may be useful indicators of decayed root surfaces in elderly individuals with attachment loss.

Keywords: elderly, periodontal status, attachment loss, root caries, mutans streptococci, lactobacilli.

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Introduction

Dental caries is a considerable health problem for elderly individuals, as the condition has been shown to be significantly associated with tooth loss[1,2]. In addition, epidemiological surveys have found that an increased number of *Streptococcus mutans* in children is associated with a higher incidence of decayed, missing, and filled teeth (DMFT), i.e. caries experiences[3–7]. Several species of bacteria, including *S. mutans, Streptococcus sobrinus* and lactobacilli, as well as yeast, are considered to be pathogens related to dental caries incidence in elderly individuals, although definitive findings are yet to be reported[8–12].

Periodontitis is another major factor associated with tooth loss[13,14]. In Japan, a majority of the elderly population suffers from periodontitis[15], which often induces gingival recession, resulting in an increase in susceptibility to root surface caries by causing exposure of the root surface. Furthermore, gingival recession changes the oral condition, which may cause ecological changes, resulting in microbiological changes and attachment of cariogenic bacteria to supragingival plaque[16,17]. Therefore, the levels of cariogenic bacterial species in the oral cavity of elderly patients with periodontal disease must be carefully monitored.

There have been a number of studies that analysed the relationship between dental caries and cariogenic bacteria, however, detailed findings regarding the relationship between periodontal status and cariogenic bacteria as a result of ecological changes have not been reported. The aim of

the present study was to analyse the relationship of cariogenic bacterial species with periodontal status and root surface caries in a large cohort of age-matched elderly subjects.

Material and methods

Subjects

Initially, questionnaires were sent to all 4542 known 75-year-old residents (born in 1927) living in Niigata City, Japan. After categorising the returned questionnaires by gender, 600 people, with approximately the same number of males and females, were asked to participate in the study. From the replies, a total of 368 subjects (194 males, 174 females) were enrolled in the study, which was conducted in June 2003. All were functionally independent and dentate. Each of the participants agreed to undergo medical and dental examinations, and signed informed consent forms regarding the protocol, which was approved by the Ethics Committee of Niigata University School of Dentistry.

Questionnaire

Data regarding lifestyle-related dental caries were obtained by questionnaire before the oral examinations were performed. The lifestyle parameters examined were eating between meals (none, less that once per day, or once or more per day) and toothbrushing (once or more per day, less than once per day).

Oral examination

Oral examination for dental caries was performed according to the WHO oral examination procedures[18]. A total of four examiners were used, who were calibrated by results of examinations of 18 volunteer patients at the University Hospital before and during the survey, with interexaminer reliability also assessed. The kappa scores were calculated using five categories (sound; filled; decayed; filled, decayed and bridge abutment; special crown or veneer/implant) and the values between each pair of examiners ranged from 0.84 to 0.97.

For the subjects, root surfaces with gingival recession were recorded if the surface was clinically visible beyond the cemento–enamel junction. Root decay was defined as when a lesion was detected on an exposed root surface that felt soft or leathery when probed. For a single incidence of decay or for a filling that affected both the crown and root, the likely site of origin of the lesion was recorded as decayed or filled. For indicators of root surface caries status, the numbers of root surface decayed teeth (RDT), root surface filled teeth (RFT) and root surface decayed and filled teeth (RDFT) were used. The periodontal examination included assessment of probing pocket depth (PPD) and clinical attachment level (CAL)[19] at six sites (mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual, disto-lingual) around each tooth. Probing was performed using a pressure constant probe (Vivacare TPS Probe, Schaan, Liechtenstein) with a probing force of 20 g. The periodontal examinations were performed by four trained and calibrated dentists under sufficient illumination using artificial light. As determined by the calibration examinations of the 18 volunteers, the per cent agreement (within plus or minus 1 mm) ranged from 85.5% to 100% for PPD and 70.0% to 100% for CAL, while the kappa values (within plus or minus 1 mm) ranged from 0.77 to 1.00 for PPD and 0.62 to 1.00 for CAL. To estimate periodontal status, the following indicators were utilised: average pocket depth (aPD) of sites measured, average attachment loss (aAL) of sites measured, rate of sites with >4 mm of pocket depth (rPD4), and rate of sites with >4 mm of attachment loss (rAL4).

Saliva collection

To obtain whole saliva samples, the subjects were asked to chew a piece of paraffin wax for 3 min to stimulate the salivary gland, after which they tilted the head forward and expectorated all of the saliva into a graduated pre-weighted conical tube for 3 min.

Microbiological processing

The subjects were asked to not brush their teeth at least 2 hours prior to the sampling. A sterile cotton stick was immersed in the collected whole saliva sample for 10 s, then placed in transport fluid (0.4% agar, 0.15% thioglycolate/phosphate and taken to Bio Medical Laboratory (Tokyo, Japan) for measurements of mutans streptococci (MS) and lactobacilli (LB). Duplicate saliva samples of 25 µl at appropriate dilutions were poured onto modified mitis salivarius culture plates containing 0.2 U/ml of bacitracin (MMSB) agar[20] for culturing of MS, or Rogosa selective lactobacillus (SL) agar for culturing LB, using an EDDY JT spiral plating system (IUL, S.A., Barcelona, Spain). The MMSB plates were incubated for 2 days at 37°C in 5% CO_2 in N_2, and the SL plates were incubated aerobically for

3 days at 37°C. Colonies on MMSB agar with a morphology typical of MS and those on SL agar typical of LB were counted to determine the numbers of colony-forming units (CFU).

Statistical analysis

A chi-square test was used to compare the rates of subjects with decayed root surfaces among groups. Comparisons of RDT and periodontal indicators among groups were performed by a Mann–Whitney *U*-test or Kruskal–Wallis test. Correlations between two variables were tested by Spearman rank correlation. For evaluation of the relationships between caries and bacteria species, linear multiple regression analysis was performed, with caries status as a dependent variable, while the independent variables were gender, frequency of eating between meals (none, less than once per day, once or more per day), toothbrushing (once or more per day, less than once per day), stimulated saliva flow (ml in 3 min) and level of bacteria (CFU/ml). Differences at the 0.05 level were considered statistically significant. An analysis of the relationship between periodontal status and carcinogenic bacterial species was performed in subjects who did not have untreated dental caries, including root surface caries, to eliminate the influence of carious lesions.

Results

Periodontal status and root surface caries were examined in all the subjects. The distribution of subjects by the four types of periodontal status is presented in Table 1. More than 90% had aPD values in a range of 1.0–2.9 (1.0–1.9, 41.0%; 2.0–2.9, 52.4%), while about 75% of the subjects had a value for aAL ranging from 2.0 to 3.9 (2.0–2.9, 34.5%; 3.0–3.9, 40.8%). As for rAL4, 70% of the subjects had a value between 0 and 50 (0–25, 39.9%; 26–50, 29.3%) and than 80% had an rPD4 value between 0 and 25. The average values for RDT, RFT and RDFT with regard to root surface caries were 0.3, 3.6 and 4.0 respectively. The presence of root surface caries was examined in the 356 subjects who had gingival recession and untreated caries on root surfaces was found in 23.0% of those individuals (Table 1). In addition, more than 80% of the subjects had one or more roots with a decayed lesion or filling.

The distribution of subjects based on MS and LB levels is shown in Table 2. Detectable levels of MS and LB were found in 88.2% and 82.6%, respectively, while high numbers ($>10^5$ CFU/ml saliva) of MS and LB were found in 31.5% and 17.7% respectively.

Table 3 shows the relationship between periodontal status and untreated root surface caries. Subjects with root surface caries had significantly higher values for aAL ($p = 0.023$), rAL4 ($p = 0.027$) and rPD4 ($p = 0.027$). Significant relation-

Table 2 Distribution of subjects by bacteria species level.

	ND	$<10^5$	10^5–10^6	$>10^6$
MS	62 (17.4)	231 (64.9)	50 (14.0)	13 (3.7)
LB	42 (11.8)	202 (56.7)	99 (27.8)	13 (3.7)

Values are given as number (%).
MS, mutans streptococci; LB, lactobacilli.

Table 1 Distribution of subjects by periodontal status and root surface caries.

	1.0–1.9	2.0–2.9	3.0–3.9	4.0–
(1) Periodontal status				
aPD	151 (41.0)	193 (52.4)	22 (6.0)	2 (0.5)
aAL	12 (3.3)	127 (34.5)	150 (40.8)	77 (20.9)
	0–25	6–50	51–75	76–100
rPD4	319 (86.7)	43 (11.7)	5 (1.4)	1 (0.3)
rAL4	147 (39.9)	108 (29.3)	73 (19.8)	38 (10.3)
	0	1–5	6–10	11–
(2)Root surface caries				
RDT	274 (77.0)	81 (22.8)	1 (0.3)	0 (0)
RFT	92 (25.8)	170 (47.8)	77 (21.6)	17 (4.8)
RDFT	70 (19.7)	180 (50.6)	86 (24.2)	20 (5.6)

Values are given as number (%).
aPD, average pocket depth; aAL, average attachment loss; rAL4, rate of sites with >4 mm of attachment loss; rPD4, rate of sites with >4 mm of pocket depth; RDT, root surface decayed teeth; RFT, root surface filled teeth; RDFT, root surface decayed and filled teeth.

Table 3 Relationship between periodontal status and decayed root surface incidence.

	n	*Median*	*25%*	*75%*	*p-value**
aPD					
Caries	90	2.11	1.89	2.58	0.053
Non-caries	265	2.07	1.82	2.40	
All	355	2.08	1.83	2.42	
rPD4					
Caries	90	7.52	3.96	17.31	0.027
Non-caries	265	5.77	1.85	13.65	
All	355	6.32	2.12	14.78	
aAL					
Caries	90	3.49	2.76	4.07	0.027
Non-caries	265	3.20	2.59	3.76	
All	355	3.22	2.61	3.80	
rAL4					
Caries	90	43.61	17.06	61.82	0.027
Non-caries	265	31.68	13.99	53.70	
All	355	33.33	14.17	55.52	

*p-value based on Mann–Whitney U-test.
aPD, average pocket depth; aAL, average attachment loss; rAL4, rate of sites with >4 mm of attachment loss; rPD4, rate of sites with >4 mm of pocket depth.

ships, with regard to correlation coefficients, were found between the aAL value and the number of root surfaces with decay ($p = 0.037$).

The relationship between cariogenic bacterial species and periodontal status were analysed in the 276 subjects who did not have untreated dental caries, including root surface caries (Table 4). Those who had a detectable level of LB (LB carriers) had significantly higher values for aAL ($p = 0.014$) and rAL4 ($p = 0.014$), whereas no significant difference in pocket depth was found between LB carriers and non-carriers. Periodontal status did not show a correlation with the presence of MS. To evaluate the contribution of LB in predicting aAL and rAL values, stepwise multiple regression analyses were performed (Table 5). It was shown that aAL ($p = 0.003$) and rAL4 ($p = 0.002$) had significant correlations with the presence of LB.

Table 6 shows the relationship between cariogenic bacterial species and decayed root surfaces. LB carriers had a significantly higher number of decayed root surfaces than non-carriers, and subjects with both LB and MS showed highest rates of root surface decay occurrence and RDT. Subjects with LB and without MS had higher rates of root surface decay and RDT than those without LB and with MS. In addition, RDT was positively correlated with MS ($r = 0.115$, $p = 0.030$) and LB ($r = 0.183$, $p = 0.001$) levels.

Table 4 Relationship between oral bacteria and periodontal status.

	n	*Median*	*25%*	*75%*	*p-value**
LB					
aPD					
Non-carrier	55	1.94	1.75	2.38	0.113
Carrier	221	2.08	1.83	2.41	
All	276	2.05	1.82	2.40	
rPD4					
Non-carrier	55	3.17	1.72	12.78	0.209
Carrier	221	6.37	1.85	13.65	
All	276	5.56	1.80	13.43	
aAL					
Non-carrier	55	2.97	2.43	3.56	0.014
Carrier	221	3.28	2.67	3.81	
All	276	3.20	2.58	3.75	
rAL4					
Non-carrier	55	25.07	7.69	44.44	0.014
Carrier	221	34.85	14.29	55.21	
All	276	31.63	13.27	53.69	
MS					
aPD					
Non-carrier	38	1.93	1.79	2.40	0.343
Carrier	238	2.07	1.82	2.41	
All	276	2.05	1.82	2.40	
rPD4					
Non-carrier	38	5.28	1.67	10.28	0.532
Carrier	238	5.56	1.84	14.48	
All	276	5.56	1.80	13.43	
aAL					
Non-carrier	38	2.91	2.41	3.77	0.124
Carrier	238	3.23	2.65	3.75	
All	276	3.20	2.58	3.75	
rAL4					
Non-carrier	38	19.52	7.31	48.63	0.086
Carrier	238	33.33	14.00	53.70	
All	276	31.63	13.27	53.69	

*p-value based on Mann–Whitney U-test.
aPD, average pocket depth; aAL, average attachment loss; rAL4, rate of sites with >4 mm of attachment loss; rPD4, rate of sites with >4 mm of pocket depth; MS, mutans streptococci; LB, lactobacilli.

The relationship of RDT, lifestyle and saliva flow are shown in Table 7. RDT did not have a significant relationship with toothbrushing, eating between meals or saliva flow.

Results of stepwise multiple regression analyses are presented in Table 8. As MS and LB levels were significantly correlated ($p < 0.001$), these were introduced separately into regression models. The rate of decayed root surfaces was significantly correlated with the presence of LB ($p = 0.030$). Further, the number of instances of untreated root caries was significantly correlated with MS ($p = 0.026$) and LB ($p = 0.010$) levels.

Table 5 Effects of various parameters on the detection of LB.

Variable	*β*	*T*	*p-value*
Model 1, $r^2 = 0.45$*			
aAL	0.193	2.987	0.003
Toothbrushing	−0.026	−0.399	0.691
Eating between meals	−0.080	−1.238	0.217
Saliva flow	−0.029	−0.457	0.648
Model 2, $r^2 = 0.47$*			
rAL4	0.199	3.072	0.002
Toothbrushing	−0.025	−0.386	0.699
Eating between meals	−0.076	−1.186	0.237
Saliva flow	−0.035	−0.537	0.592

aAL, average attachment loss; rAL4, rate of sites with >4 mm of attachment loss; LB, lactobacilli.
*$p < 0.05$.

Discussion

In the present study, elderly subjects with LB had significantly greater levels of attachment loss than those without LB. It was previously reported that salivary bacterial levels of LB were correlated with bacterial levels in dental plaque[21]. In addition, it is considered that elderly individuals with greater levels of attachment loss have greater levels of LB in dental plaque than those with low levels of attachment loss.

The components of bacterial flora are thought to be influenced by ecological changes and living habits[16]. Gingival recession leads to exposure of the root surfaces to oral bacteria and an aerobic condition on the root surface, which is not suitable for anaerobic bacterial species that occupy the major part of bacterial flora in periodontal pockets. In addition, gingival recession was shown to neutralise the pH level on the root surface by a salivary buffering action and changed nutritional conditions[22]. These ecological conditions may be necessary for the growth of LB in the oral cavity, while LB levels are not correlated with the extent of pocket depth, because the ecological condition, such as O_2 concentration, pH and nutrition in the periodontal pockets, is not conducive for LB growth.

Table 6 Relationship between cariogenic bacterial species and decayed root surface: (1) rate of root surface caries incidence and mean number of RDT in Carrier and non-carrier of both cariogenic bacterial species; (2) correlation coefficients between RDT and MS level, and LB level.

(1)	*n*	*Rate of root surface caries, n (%)*	*p-value**	*RDT (mean ± SD)*	*p-value†*
(a) Each bacterial species					
MS					
Non-carrier	42	8 (19.0)	0.138	0.2 ± 0.5	0.168
Carrier	314	82 (26.1)		0.4 ± 0.8	
LB					
Non-carrier	62	6 (9.7)	0.001	0.2 ± 0.7	0.003
Carrier	294	84 (28.6)		0.4 ± 0.8	

LB	*MS*	*n*	*Rate of decayed root surface, number (%)*	*p-value**	*RDT (mean ± SD)*	*p-value‡*
(b) Association of LB and MS with decayed root surface incidence and RDT						
Non-carrier	Non-carrier	12	1 (8.3)	0.017	0.08 ± 0.28	0.015
Non-carrier	Carrier	50	5 (10.0)		0.15 ± 0.62	
Carrier	Non-carrier	30	7 (23.3)		0.22 ± 0.49	
Carrier	Carrier	264	77 (29.1)		0.41 ± 0.82	

(2)	*Correlation coefficients*	*p-value§*
MS	0.115	0.030
LB	0.183	0.001

RDT, root surface decayed teeth; MS, mutans streptococci; LB, lactobacilli.
*p-value based on chi square test.
†p-value based on Mann–Whitney U-test.
‡p-value based on Kruskal–Wallis test.
§p-value based on Spearman rank correlation.

Table 7 Relationship between RDT and life style, and saliva flow.

	RDT (mean ± SD)	*p-value*
(1) Life style		
Eating between meals		
None (122)	0.35 ± 0.69	0.769*
Lesser than once per day (189)	0.33 ± 0.79	
Once or more per day (44)	0.36 ± 0.87	
Toothbrushing		
Toothbrush and dental floss (334)	0.34 ± 0.76	0.295†
Toothbrush only (18)	0.50 ± 0.86	
(2) Saliva flow		
Correlation coefficients		−0.033
p-value		0.532‡

RDT, root surface decayed teeth.
Missing data were excluded.
*p-value based on Kruskal–Wallis test.
†p-value based on Mann–Whitney U-test.
‡p-value based on Spearman rank correlation.

Table 8 Effects of various parameters on root surface decay incidence and number of root surface decay.

Variable	*β*	*T*	*p-value*
Root surface decay incidence			
LB carrier	0.157	2.953	0.003
Toothbrushing	0.056	1.058	0.291
Eating between meals	0.020	0.379	0.705
Saliva flow	−0.083	−1.558	0.120
$r^2 = 0.035$*			
Number of root surface decay			
Model 1			
MS level	0.120	2.239	0.026
Toothbrushing	0.045	0.845	0.399
Eating between meals	0.067	1.244	0.215
Saliva flow	−0.084	−1.577	0.116
$r^2 = 0.028$*			
Model 2			
LB level	0.140	2.608	0.010
Toothbrushing	0.046	0.850	0.395
Eating between meals	0.068	1.265	0.207
Saliva flow	−0.075	−1.399	0.163
$r^2 = 0.033$*			

LB, lactobacilli; MS, mutans streptococci.
*$p < 0.05$.

Lactobacilli, a uric acid-associated bacterium, is thought to be a predominant cause of root surface decay[23]. In the present study, a significant difference in the number of decayed root surfaces was seen between subjects with and without LB, while the number of decayed root surfaces was correlated to LB level. These results suggest that LB is associated with the occurrence of decayed root surfaces and confirms the results of Loesche, who reported that salivary levels of LB were associated with root surface caries[12]. Our results also suggest that the presence of LB is a risk factor for root caries in elderly individuals with attachment loss.

Salivary levels of MS and LB have been shown to be correlated to root caries incidence[24–26]. In the present study, surface decay was found in about 23% of the subjects with LB and without MS. It could be considered that LB is able to contribute to the incidence of root surface decay with little or no help from MS in elderly individuals with gingival recession. In addition, the rates of root decay caries and RDT in elderly with both LB and MS were higher than that in those with LB and without MS, suggesting that the presence of MS may also contribute to the incidence of root surface decay in collaboration with LB. MS synthesises glucan and plays a significant role as a pathogenic coloniser in the development of oral biofilm, leading to the development of dental caries. LB is known to attach to tooth surfaces by incorporating into biofilm that includes MS, thus causing caries. Although salivary MS levels did not show a significant relationship with periodontal status, MS should be treated, particularly in elderly patients with attachment loss, as its presence increases the risk of root surface caries.

These results indicate that attachment loss is associated with the salivary level of LB, which is correlated with the number of root caries. These findings may explain why the extent of attachment loss is correlated with root caries incidence in elderly individuals. The results also suggest that elderly individuals with considerable attachment loss must be treated, especially regarding the level of salivary LB, whilst MS level must also be monitored carefully, as this is associated with an increase in the risk of root surface caries in collaboration with LB. Thus, measurements of LB and MS in saliva may be useful for the determination of oral health in elderly individuals.

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Post-operative Infection by Pathogenic Micro-organisms in the Oral Cavity of Patients with Prostatic Carcinoma

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The aim of this study was to analyse the change in the oral cavity microflora of 14 patients who had undergone a radical prostatectomy for prostatic carcinoma. The detection of micro-organisms in the oral cavity was compared before and after the surgical procedure. Post-operative infection, defined as those patients who had increased *Candida* species counts and/or pathogenic bacteria only at the post-operative examination, was observed in 10 patients. Six patients showed increased *Candida* species counts at the post-operative examination compared with the pre-operative examination. In five patients, pathogenic bacterial species were detected at the post-operative examination but not at the pre-operative examination. One patient had detectable pathogenic bacterial species only at the post-operative examination along with increased *Candida* species counts. Our findings suggest that pre-operative oral hygiene to remove bacterial and *Candida* species from patients who are scheduled for surgical procedures is important for satisfactory clinical outcomes.

KEY WORDS: PROSTATIC CARCINOMA; OPPORTUNISTIC INFECTION; ORAL CAVITY; SURGERY; *CANDIDA* SPECIES

Introduction

In recent years, a new concept in micro-organism infection – biofilm infection – has been proposed.[1] Oral micro-organisms that attach to tooth or oral tissue surfaces aggregate in a hydrated extracellular polymeric substance (EPS) of their own synthesis to form biofilms, in which the micro-organisms colonize and coat the surface of the tooth or oral tissues.[2] Biofilms constitute a protected mode of growth that allows micro-organisms to survive in hostile environments, such as those containing antibiotics and when under attack from the immune system.[2] As a result, biofilms can be the cause of many persistent and chronic infections.[2] Persistent oral infection has been thought to be the cause of infection and chronic inflammatory disease of various organs via periodontal tissue, oral membranes, the tonsils, the airway and the oesophagus.[3–5]

Oral biofilms in elderly people harbour opportunistic pathogens (such as *Enterobacter* species, *Klebsiella pneumoniae*, *Pseudomonas*

aeruginosa, Serratia marcescens and *Candida* species, as well as commensal bacterial species including Gram-positive streptococci) causing dental caries and periodontal disease.[3,4] Several reports have suggested a relationship between decreased immunity and opportunistic infection of the oral cavity.[5-7]

Colonization of the oral cavity by pathogenic bacteria increases the risk of systemic disease, such as pneumonia and bacteraemia,[1,8] and infections can occur at non-operated sites in immunocompromised people after surgery.[9-12] Infection of the oral cavity could, therefore, be expected to occur after surgery at other sites within the body.

We performed this pilot study to investigate the pathogenic infection of the oral cavity in patients with prostatic carcinoma before and after they underwent a radical prostatectomy.

Patients and methods

PATIENTS

Subjects were patients with prostatic carcinoma who underwent radical prostatectomy at Okayama University Hospital (Okayama, Japan) between July 2002 and January 2004. Prior to the study, the study aims, design and procedures were explained, and informed consent was obtained from each patient. Ethical approval was not required for this study. As surgical prophylaxis, 1.5 g of ampicillin sulbactam or 1.0 g of cefazolin was given immediately before surgery, and the same antimicrobial agent at the same dosage was administered after surgery if the procedure had taken longer than 240 min. Ampicillin sulbactam or cefazolin, at the same dosage, was administered on the night of the surgical procedure and twice daily for 2 days post-operatively. Patients were starved overnight prior to the operation and they resumed normal eating and drinking from the day after the operation. None of the patients had undergone radiotherapy or chemotherapy.

BACTERIAL EXAMINATION

Supragingival plaque samples were collected from the postero-anterior buccal surface of the upper right second premolar and first molar using a cotton swab (Seedswab No. 1; Eiken, Tokyo, Japan) at 1 day before and 3 days after surgery. Sampling was performed by a doctor from the Urology Section (Okayama University Hospital) who had undergone training in the plaque sampling technique. Plaque samples were placed into transport fluid (0.4% agar and 0.15% thioglycollate/phosphate buffered saline) and transported to the Bio Medical Laboratory (Tokyo, Japan) for analysis to detect the following bacterial species: *Acinetobacter* species, *Citrobacter diversus, Citrobacter freundii, Enterobacter agglomerans, Enterobacter cloacae, Escherichia coli, Haemophilus parainfluenzae, Klebsiella oxytoca, K. pneumoniae,* methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-sensitive *Staphylococcus aureus* (MSSA), *P. aeruginosa, Proteus mirabilis, S. marcescens, Streptococcus agalactiae* and *Stenotrophomonas maltophilia.* Each plaque sample was placed directly onto chocolate, OPA Staphylococcus and Drigalski agar plates (Nippon Becton Dickinson, Kobe, Japan) using a stick. The plates were incubated in an atmosphere of 5% CO_2 in H_2 at 37 °C for 24 – 48 h. Representative microbial colonies from each plate were Gram stained and isolated by identification of their characteristic appearance, as well as by haemolytic, catalytic and oxidase reaction.[13] Isolates were suspended in 1 ml of 0.5% saline, gently shaken and tested in microbial detection kits (VITEK; BioMérieux Vitek Japan, Tokyo, Japan).[3,5] With regard to

Candida species, quantitative analysis was performed by using fresh plaque samples from the swab, which were gently shaken in 3 ml of phosphate buffered saline for 5 min. The plaque samples were inoculated onto sabouraud dextrose agar plates using the EDDY JET spiral plating system (IUL, S.A. Torrent, Spain) and incubated for 48 h at 35 °C to count *Candida* species colonies, which were identified by their characteristic morphological appearance and colour.

STATISTICAL ANALYSIS

The χ^2 test was used to assess statistical significance when comparing numbers for different categories. Differences at the $P < 0.05$ level were considered statistically significant. SPSS for Windows (Version 10.0, Chicago, IL, USA) was used for all statistical analyses.

Results

PATIENT CHARACTERISTICS

The clinical and surgical characteristics of the 14 patients enrolled in this study are shown in Table 1. The average age of the patients was 65.5 years (50 – 59 years, $n = 3$; 60 – 69 years, $n = 6$; 70 – 79 years, $n = 5$). Seven patients had complications (hypertension, $n = 5$; diabetes mellitus, $n = 3$; angina pectoris, $n = 1$; and pulmonary emphysema, $n = 1$). Radical prostatectomy was performed via an open ($n = 10$) or laparoscopic ($n = 4$) operation. The duration

TABLE 1:
Clinical and surgical characteristics of patients with prostatic carcinoma who underwent radical prostatectomy ($n = 14$) during this pilot study

Patient number	Age (years)	Complication(s)	Operative method	Operation duration (min)
1	63	None	Open	265
2	74	None	Laparoscopy	270
3	64	None	Open	250
4	68	Hypertension Angina pectoris	Open	300
5	67	None	Laparoscopy	355
6	70	Diabetes mellitus	Open	255
7	59	Hypertension Diabetes mellitus	Laparoscopy	380
8	74	Hypertension Pulmonary emphysema	Open	175
9	50	None	Laparoscopy	380
10	72	None	Open	375
11	73	Hypertension	Open	255
12	66	Hypertension	Open	245
13	63	Diabetes mellitus	Open	245
14	54	None	Open	220

of surgery was between 175 and 380 min (mean, 284 min).

ORAL MICROFLORA PRE- AND POST-SURGERY

Micro-organisms detected in the oral cavities of patients in this study are shown in Table 2. The detection rate of *Candida* species at the pre- and post-operative examinations was 35.7% (five of 14) and 57.1% (eight of 14), respectively. After surgery, six patients (42.9%) showed a logarithmic increase in *Candida* species counts (measured as colony-forming units [CFU]). Before surgery, three patients (21.4%) possessed pathogenic bacterial species in oral cavities. Of 11 patients who did not have pathogenic bacterial species in their oral cavities at the pre-operative examination, five patients had pathogenic bacteria (*E. cloacae*, $n = 2$; *P. aeruginosa*, $n = 1$; *Acinetobacter* species, $n = 1$; *C. freundii*, $n = 1$; *K. pneumoniae*, $n = 1$ and coagulase-negative *Staphylococcus* species, $n = 1$) at the post-operative examination. Of these five patients, four did not show increased CFU counts of *Candida* species at the post-operative examination. In one patient (patient 8), *E. cloacae* and *K. pneumoniae* were detected only at the post-operative examination along with increased *Candida* species counts.

POST-OPERATIVE INFECTION AND THE RELATIONSHIP WITH AGE, COMPLICATIONS AND DURATION OF SURGERY

As shown in Table 3, the distribution of patients who demonstrated increased *Candida* species counts, or who had detectable pathogenic bacterial species only at the post-operative examination, was investigated with regard to relationship to age, presence of complication(s) and duration of surgery. No significant differences were observed between the two groups within each category.

Discussion

Candida species and pathogenic bacterial species were detected more frequently at the post-operative examination than at the pre-operative examination. These micro-organisms have been reported to cause opportunistic infections.[14 - 18] Decreased immunity may result in infection by these micro-organisms, and surgical procedures are thought to increase the risk of infection by decreasing immunity.[9,10] Radical prostatectomy may decrease the immune function of patients, resulting in a change in their oral microflora; in addition, long-term administration of antibiotics may also cause opportunistic infections. In this study, each post-operative examination was performed 3 days after the operation in order to minimize the effects of prophylactic antibiotics. Infection was not considered to be a direct result of the surgery because of the distance between the operation site and the oral cavity.

Cross-sectional studies have reported that opportunistic infections of the oral cavity occur in people who seem to have a decreased immune function. *Candida* species levels were higher in the oral cavities of critically ill patients than in women who were considered to be healthy.[19] Smith *et al.*[20] reported that coagulase-negative *Staphylococcus* species emerged in many debilitated elderly patients and in those with oral Crohn's disease. The nutritional status has also been reported to be related to the detection of MRSA.[7] Senpuku *et al.*[5] found that several pathogenic micro-organisms were isolated at a significantly higher rate in functionally dependent elderly people with heart disease than in those that were functionally independent. A longitudinal

TABLE 2:
The bacteria detected and the levels of detection for *Candida* species at pre- and post-operative examination of patients with prostatic carcinoma who underwent radical prostatectomy (n = 14)

Patient number	Pathogenic bacteria		Colony-forming units of *Candida* species	
	Pre-operative	Post-operative	Pre-operative	Post-operative
1	ND	ND	ND	305
2	ND	ND	ND	13 902
3	ND	ND	ND	ND
4	ND	*Citrobacter freundii* *Acinetobacter* species	26 258	2134
5	ND	ND	ND	ND
6	ND	*Enterobacter cloacae*	ND	ND
7	ND	*Pseudomonas aeruginosa*	8780	ND
8	ND	*Enterobacter cloacae* *Klebsiella pneumoniae*	671	70 122
9	*Serratia marcescens* *Pseudomonas aeruginosa*	*Serratia marcescens*	ND	3110
10	ND	ND	488	34 091
11	ND	ND	ND	ND
12	ND	Coagulase-negative *Staphylococcus* species	ND	ND
13	*Klebsiella pneumoniae* *Enterobacter cloacae*	*Klebsiella pneumoniae*	ND	143 293
14	*Enterobacter cloacae*	*Enterobacter cloacae*	61	61

ND, none detected.

TABLE 3:
Distribution of patients with prostatic carcinoma who had pathogenic bacteria only at the post-operative examination and/or increased *Candida* species counts after radical prostatectomy (n = 10) according to age, presence of complications and duration of surgery

Category (n = 14)	No. of patients with pathogenic bacteria/increased *Candida* species post-surgery (%) (n = 10)
Age (years)	
≤ 69 (n = 9)	6 (66.7)
≥ 70 (n = 5)	4 (80.0)
Complications	
No (n = 7)	4 (57.1)
Yes (n = 7)	6 (85.7)
Operation duration (min)	
< 300 (n = 9)	6 (66.7)
≥ 300 (n = 5)	4 (80.0)

None of the parameters reached statistical significance.

study investigating the relationship between oral micro-organisms and general health has not been performed. This is the first report to examine the change in oral cavity microflora before and after surgery involving organs that are not in the oropharyngeal region. Our study provides novel information about the influence of surgery on oral microflora.

Increased *Candida* species counts were not observed at the post-operative examination in the majority of patients who demonstrated detectable pathogenic bacterial species at the post-operative examination. One patient had detectable levels of *E. cloacae* and *K. pneumoniae* at the post-operative examination along with increased *Candida* species counts, which suggested that the optimal condition for *Candida* species growth was probably different to that preferred by pathogenic bacterial species. The growth of oral microflora is likely to be dependent on the condition of the oral cavity.

Micro-organisms detected at the post-operative examination in this study have been reported to cause bacteraemia and several diseases in other organs via transmission through the bloodstream.[21,22] In the case of patients with periodontal disease, these micro-organisms in the oral cavity can invade in the bloodstream by gingival bleeding, and a relationship between septicaemia and periodontitis has been suggested.[23 - 25] Micro-organisms such as *Candida* species, *P. aeruginosa*, *Acinetobacter* species, *K. pneumoniae*, and *C. freundii* in the oral cavity might cause pneumonia by aspiration.[26] Furthermore, micro-organisms detected in this study have been reported to cause nosocomial infection.[27,28] An antiseptic decontamination of the dental plaque with a 0.2% chlorhexidine gel decreased dental bacterial colonization and reduced the incidence of nosocomial infection in intensive care unit patients exposed to

mechanical ventilation.[29] Considerable attention needs to be paid to oral biofilms and the oral hygiene of patients prior to surgery, even when the surgical site is some distance from the oral cavity. Our study suggests that good oral care of patients who are scheduled for surgery is important for satisfactory post-operative management.

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Conflicts of interest

No conflicts of interest were declared in relation to this article.

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