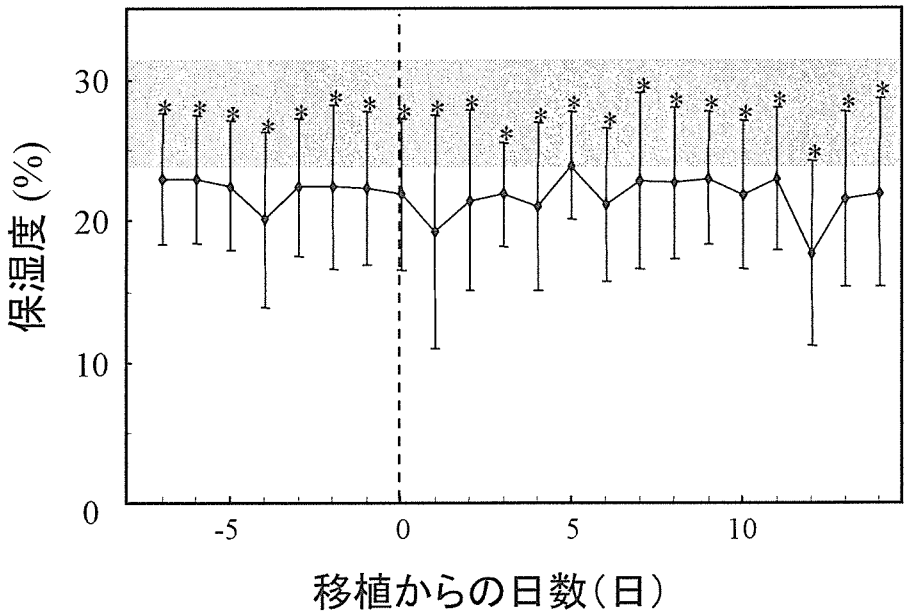

図2. 造血幹細胞移植期の患者の口腔粘膜の保湿度の推移

対象とした患者数は36名。グラフは，“対象および方法”の項に記載した方法を用いて，患者群の口腔粘膜の保湿度の平均±標準偏差の推移を示す。有意差はStudent-*t*検定を用いて，健常対象(N=62)の口腔粘膜の保湿度(27.3 ± 3.5 %)と比較して判定した。なお，図のグレーゾーンは，健常対象の口腔粘膜の保湿度の平均±標準偏差の領域を示す。*: $p<0.05$。

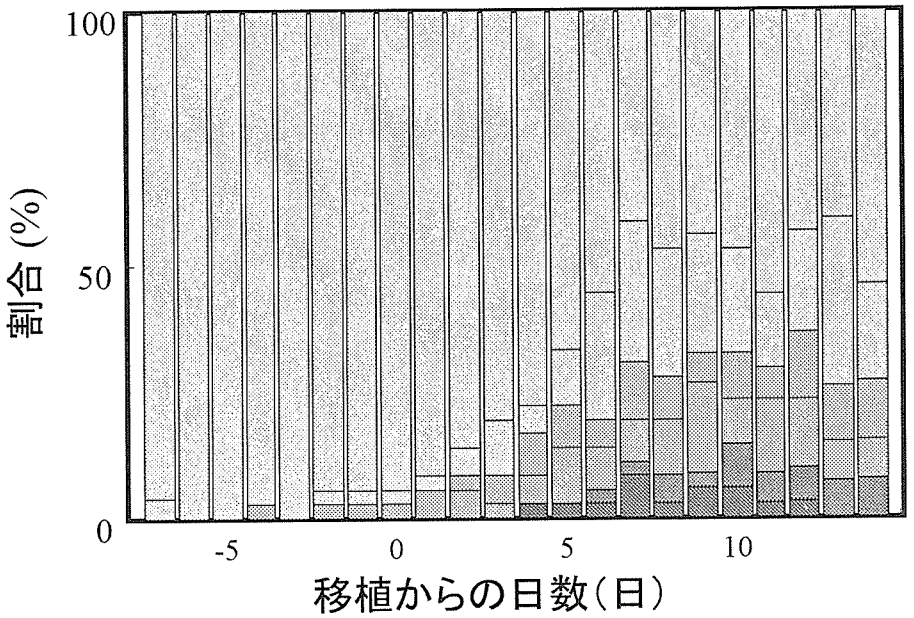

図3. 造血幹細胞移植患者の口腔粘膜痛の推移

対象とした患者数は36名。グラフは，Wongらのペインスコアを基準として，造血幹細胞移植期の患者群における口腔粘膜痛の程度の推移を示す。□：スコア 0，痛みがまったくない；□：スコア 1，わずかに痛みがある；▨：スコア 2，軽度の痛みがあり，少し辛い；▨：スコア 3，中等度の痛みがあり，辛い；▨：スコア 4，かなりの痛みがあり，とても辛い；▨：スコア 5，強い痛みがあり，とても耐えられない

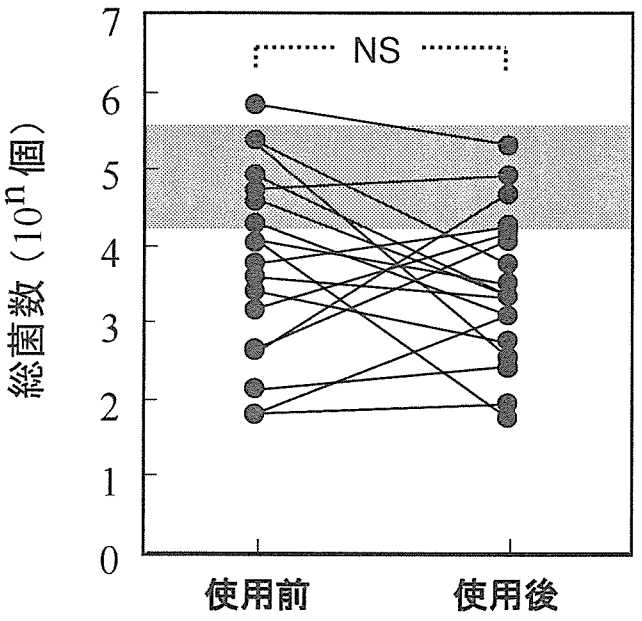

図4. 唾液代替剤（オーラルバランス®）の使用前後における頬粘膜上の総菌数の変化

対象患者（18名）の唾液代替剤（オーラルバランス®）の使用前後における頬粘膜上の総菌数の変化。ウィルコクソン符号順位検定を用いて使用前後の総菌数の有意差を検定した。なお，図のグレーゾーンは健常者（N=10）の頬粘膜上から検出された総菌数の領域を示す。NS：有意差なし。

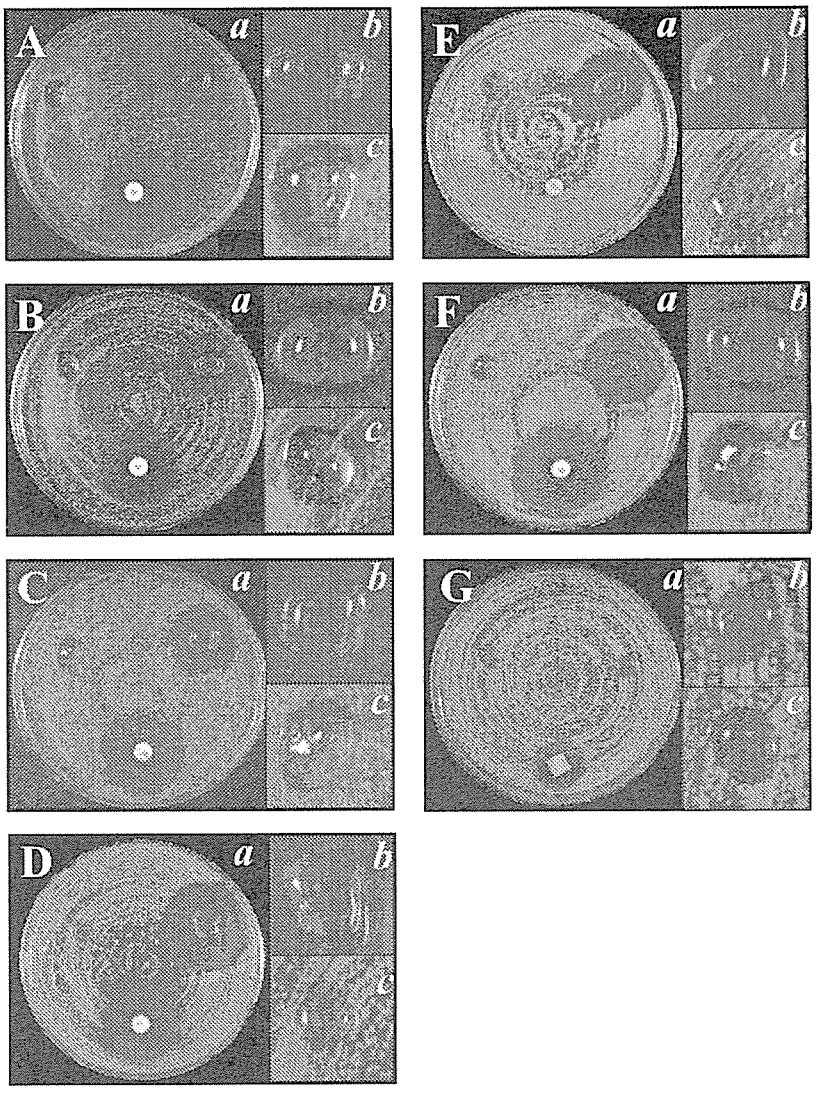

図5. 唾液代替剤(オーラルバランス®)の抗菌性の検討

A〜G:　唾液代替剤の増殖阻止効果を調べた細菌や真菌。　A, *Streptococcus sanguis* ATCC10556; B, *Streptococcus salivarius* JCM5705; C, *Neisseria mucosa* ATCC19695; D, *Stomatococcus mucilaginosus* JCM10910; E, *Staphylococcus epidermidis* NBRC12993; F, *Staphylococcus aureus* FDA209; G, *Candida albicans* NBRC1385。

a:"対象および方法"の項に記載した方法を用いて行った細菌や真菌の増殖阻止円。上部左側は，煮沸して抗菌性を失活させた唾液代替剤オーラルバランス®。上部右側は，唾液代替剤オーラルバランス®。下部は，陽性対照。b:唾液代替剤オーラルバランス®による阻止円の拡大写真。C:煮沸して抗菌性を失活させた唾液代替剤オーラルバランス®による阻止円の拡大写真。なお，実験の陽性対照として，A-Fには薬剤感受性試験用ディスクーテトラサイクリンを用い，Gにはメトロニダゾールを用いた。

II. 研究成果の刊行に関する一覧表

別紙５

研究成果の刊行に関する一覧表

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III. 研究成果の刊行物・別刷り

The Journal of International Medical Research
2006; 34: 95 – 102

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* *Enterobacter cloacae*	8780	ND
8	ND	*Klebsiella pneumoniae* *Serratia marcescens*	671	70 122
9	*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