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Original

A pilot study to evaluate the adhesion of oral microorganisms to temporary soft lining materials

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Abstract: The aim of the study was to compare the adhesion of oral microorganisms to different types of soft liner and acrylic resin surfaces. Three different soft lining materials were applied to cavities formed on the fitting surfaces of prostheses in 17 patients. On days 1, 7 and 14, the specimens were taken out and immediately processed for bacteriological evaluation. The numbers of adhering microorganisms were calculated and the specimens were compared among each other and also with a control group (acrylic resin). Data were analyzed by two-way ANOVA and least squares differences at a significance level of $P < 0.05$. Among the four materials tested the total number of oral microorganisms adhering to Softliner material was the greatest after each of the time periods tested. Higher numbers of oral bacteria and *Candida* were shown to adhere to soft lining materials than to acrylic resin. Microbial coverage increased continuously with time and the differences between days 1 and 14 were statistically significant ($P < 0.05$). Temporary soft lining materials are not resistant to adhesion and possible surface damage caused by oral bacteria, and therefore their use should be limited to short-term periods. (J. Oral Sci. 50, 1-8, 2008)

Keywords: oral microorganism; bacteria; soft lining materials; adhesion.

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Introduction

Adhesion of microorganisms to denture surfaces is a prerequisite for their colonization (1-5). The formation of plaque on the surface of dentures is a common problem among denture wearers and can lead to stomatitis (6,7). Denture stomatitis, generally known as "denture sore mouth", is a term used to describe certain pathological changes in the oral mucosa of denture-bearing tissues (8,9). These changes are characterized by erythema and are found under complete or partial dentures in both jaws, but more frequently in the maxilla (9). There are different opinions regarding the etiology of this inflammatory condition. Some controversy exists as to whether the predominant cause is trauma or infection (10). Some researchers have highlighted *Candida albicans* as a major factor in the development of this problem (9,11,12), while other reports point out the significance of other microorganisms (13-15).

Soft lining materials are frequently used to coat dentures on a permanent or semi-permanent basis (16,17). Because of their viscoelastic properties, they act as shock absorbers and reduce and distribute the stress on denture-bearing tissue (17,18). These materials interact with oral microbes and their surface texture makes efficient mechanical cleaning difficult. Furthermore, chemical disinfection solutions are not recommended because they effect the physical properties of the soft lining materials (19-21). Microbial accumulation on these soft materials is therefore a potentially serious problem (16,22).

Although the microbial properties of soft denture base materials have been evaluated in numerous laboratory studies (23-27), only a few are representative of clinical conditions (16). The aim of the present study was to

evaluate the adhesion of oral microorganisms to three different temporary soft denture lining materials applied to maxillary complete dentures *in vivo*.

Materials and Methods

Seventeen edentulous volunteers, 8 males and 9 females, each with a healthy palatal mucosa and a good general health status, participated in the study. The subjects ranged in age from 55 to 75 years.

The materials used in the present study are listed in Table 1. The codes given in Table 1 are used for identification of the materials in the text. The materials were stored, handled and mixed in accordance with the manufacturers' directions. The experimental protocol used for the study was approved by the Gazi University ethical review comitte (No: 2003/84).

For the study, three acrylic-based complete dentures were made for each of the 17 patients by one prosthodontist and one technician. Three aluminum disks (12 mm in diameter and 2 mm in depth) were placed on the right, left and posterior left sides of the fitting surfaces of the maxillary dentures at the laboratory stage. The right posterior sides of the fitting surfaces of the dentures were left as a control group (Vertex acrylic resin denture base material). After adjustment of the dentures in the mouth, the aluminum disks were removed and the dentures were disinfected with a spray disinfectant (Mikrozid, Schülke & Mayr, Schleswig-Holstein, Germany) (Fig. 1). Then silicone- and acrylic-based soft liners were applied randomly to the empty places and the curing process was completed in the patient's mouth. The first denture was used for 24 h, the second for 7 days, and the third for 14 days. During this time the patient carried out ordinary hygiene habits, but avoided brushing the inner denture surface and the use of chemical detergents.

Two hundred four test specimens were removed for microbiological analysis using sterile scalpels and forceps, and the acrylic specimens were cut out with a sterile bur. To avoid undue accumulation of food debris at meal times, 192 test specimens were removed and immersed in distilled

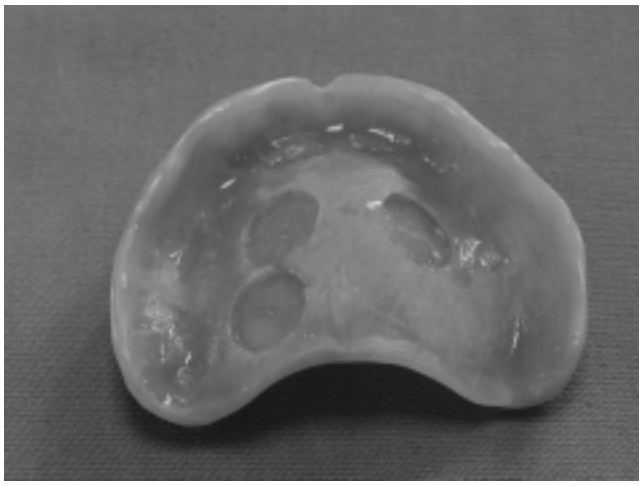

Fig. 1 The fitting surface of the prosthesis.

Table1 Materials used in the study

Brand name	Composition	Type	Code	Manufacturer
UFİ GEL P	Polydimethylsiloxane A silicone	Cold cured	UP	Voco, Germany
SOFTLİNER	The mixture of different polysiloxanes A silicone	Cold cured	SL	Promedica, GmbH
COE SOFT	Powder: polyethyl methacrylate Liquid: aromatic ester, ethyl alcohol	Cold cured	CS	GC America Inc., USA
VERTEX	Polymethylmethacrylate	Heat polimerized	VX	Dentimex BV, Holland

water and thereafter each sample was transferred to a vial containing 1 ml of BHI (brain heart infusion broth, Oxoid). Twelve test specimens were examined in a scanning electron microscope (JSM-84, JEOL, Tokyo, Japan).

Microbiological examinations

All the samples were immediately processed for bacteriological evaluation. In order to separate microorganisms from the denture surfaces and to achieve homogeneous dispersion, sample tubes were vortexed for 30 s. Then 0.01 ml of each suspension was inoculated onto blood agar and EMB (eosin methylene blue) agar plates. The plates were incubated aerobically for 48 h at 37°C, then examined and isolated colonies were evaluated according to their morphology, pigment formation, Gram staining characteristics, and catalase and oxidase tests (28,29). Also wet mount preparations were used to determine the *Candida* species. Differentiated colonies were counted and subcultured onto blood agar plates. Following a 24-h incubation at 37°C, sub-cultured strains were identified using conventional biochemical tests (30). A Mini API (BioMerieux, Marcy I'Etoile, France) identification system was used in some cases that could not be identified by conventional methods. *C. albicans* was identified definitively by a germ tube test (31).

Adhering microorganisms were quantified based on colony counts at the first isolation, and the results were subjected to two-way ANOVA and LSD (least squares differences) statistical analysis.

Scanning electron microscopy

Twelve test specimens were removed from the fitting surfaces of the dentures of one patient after 1, 7 and 14 days of wear, washed with distilled water, and fixed with 2% glutaraldehyde at 4°C for 48 h. Each specimen was dehydrated in increasing concentrations of ethanol, then all specimens were air-dried, sputter-coated (BioRad Polaran Division SEM Coating System, JEOL, Tokyo, Japan) with a layer of gold to a thickness of 15-20 nm, and examined using a scanning electron microscope.

Results

Total numbers of aerobic and facultative anaerobic bacteria adhering to Ufi gel P (UP), Softliner (SL), Coe Soft (CS) and Vertex (VX) were analysed. *C. albicans*, *Candida* spp., and Gram-positive and negative cocci and rods were also identified and evaluated.

The adhesion of total microorganisms was the greatest with SL material after each of the time periods, and the results of comparisons with other materials tested are shown in Fig. 2. Statistical analysis revealed no significant differences among materials and time periods (F = 0.088, $P = 0.0997$), and that the interactions among the materials after each of the three time periods were not significant. The results of two-way comparisons revealed significant differences between SL and VX materials ($P < 0.05$) whereas no significant differences were evident among SL, UP, CS or UP, CS, VX materials ($P > 0.05$). Microbial coverage increased continuously with time and the differences between 1 and 14 days of use were statistically significant.

Among the four materials tested, the total number of Gram-positive cocci adhering to SL was greatest after each of the time periods, decreasing in the order shown in Fig. 3. No significant difference was found between the materials after these time periods (F = 0.092, $P = 0.997$). Furthermore, the interactions among SL, UP, CS and VX materials were not statistically different (F = 1.494, $P = 0.214$) and also the differences among the time periods were

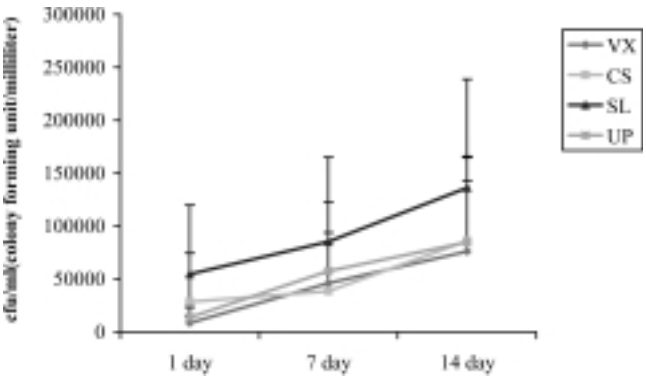

Fig. 2 Mean numbers of total microorganisms adhering to four denture base materials after various time periods (n = 480).

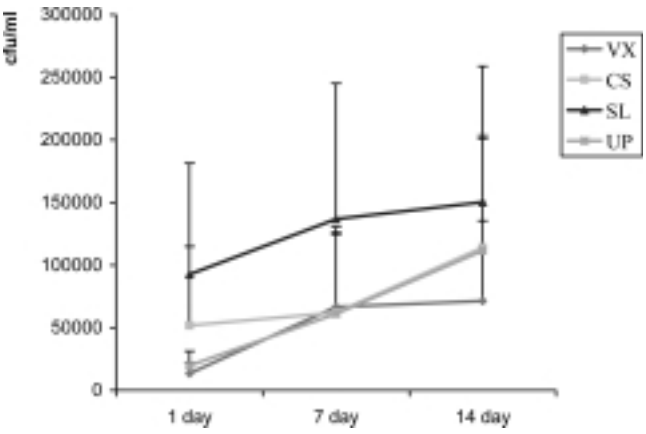

Fig. 3 Mean numbers of Gram-positive cocci adhering to four denture base materials after various time periods (n = 256).

not significant (F = 2.058, $P = 0.128$).

Adhesion of Gram-negative cocci was greatest with VX material at 7 and 14 days of use, and the results of comparisons with other materials are shown in Fig. 4. Statistical analysis demonstrated significant differences among the materials in terms of microorganism adhesion after 1, 7 and 14 days (F = 4.525, $P = 0.000$). Two-way comparisons between the materials after 1 and 7 days revealed no significant differences (F = 0.281, $P = 0.839$ / F = 1.194, $P = 0.320$), but after a 14-day period significant differences in these interactions were found (F = 4.881, $P = 0.004$).

Adhesion of Gram-positive rods was greatest with SL material after 14 days of use, and comparisons with the other materials are shown in Fig. 5. Statistical analysis revealed no significant difference among the materials and time periods (F = 0.378, $P = 0.893$). Furthermore, two-way comparisons between the materials demonstrated no significant differences (F = 0.306, $P = 0.821$). However, significant differences were found between the time periods (F = 4.426, $P = 0.012$). Tukey HSD and Scheffé analysis revealed that the interactions between 1 and 14 days in terms of microbial adhesion were significantly different ($P < 0.05$).

Among the materials tested, the total number of Gram-negative rods adhering to SL was greatest after 1, 7 and 14 days of use, decreasing in the order shown in Fig. 6. The numbers of bacteria on the surfaces of VX material were lower than on the other materials. No significant differences were found between materials and time periods (F = 0.740, $P = 0.617$). Two-way comparisons showed that the differences between the materials (F = 1.153, $P = 0.327$), and between the time periods (F = 1.056, $P = 0.348$) were not significantly different.

Adhesion of *C. albicans* to the test materials was seen in only three patients after a 1- day period and in four patients after 7 and 14 days. Among the materials tested, the total number of *C. albicans* adhering to CS after 14 days was the greatest, decreasing in the order shown in Fig. 7. The numbers of *C. albicans* on the surfaces of VX material were lower than on the other materials after each of the time periods. Statistical analysis revealed no significant differences between materials and time periods (F = 0.239, $P = 0.963$). Two-way comparisons demonstrated no significant differences between the materials (F =

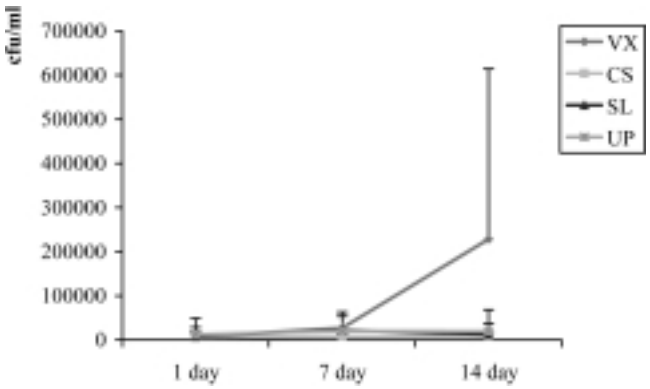

Fig. 4 Mean numbers of Gram-negative cocci adhering to four denture base materials after various time periods (n = 16).

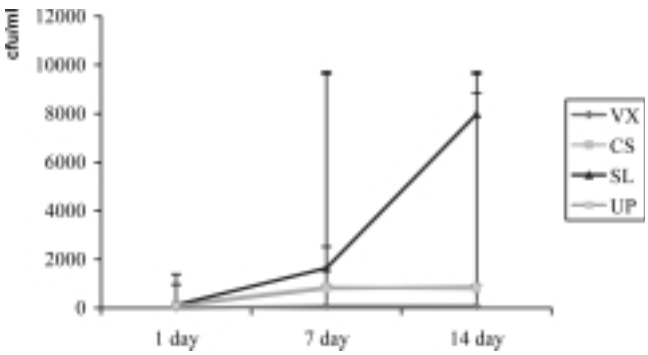

Fig. 6 Mean numbers of Gram-negative rods adhering to four denture base materials after various time periods (n = 128).

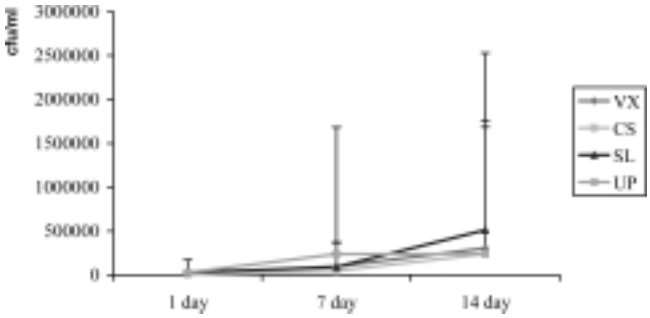

Fig. 5 Mean numbers of Gram-positive rods adhering to four denture base materials after various time periods (n=16).

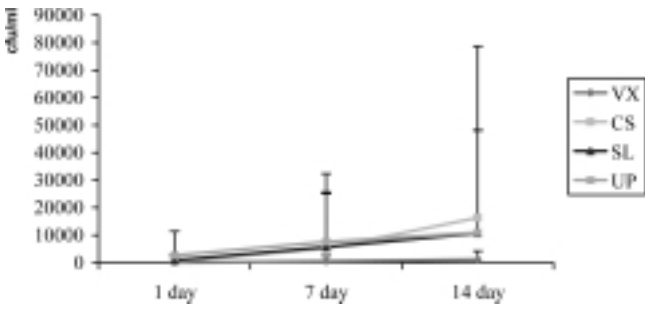

Fig. 7 Mean numbers of *C. albicans* adhering to four denture base materials after various time periods (n = 16).

0.698, $P = 0.555$) or between the time periods (F = 1.840, $P = 0.162$).

Adhesion of *Candida* spp. was seen in two patients after 1 day and in three patients after 7 and 14 days. Numbers of adhering *Candida* after each of the time periods were greatest for SL material, and comparisons are shown in Fig. 8. No significant interactions between materials and time periods were found (F = 0.004, $P = 1.000$), and there was no significant difference between the time periods. Two-way comparisons between the materials revealed significant differences (F = 2.746, $P = 0.044$). Also the interactions between SL and the other materials were statistically significant ($P < 0.05$).

Scanning electron micrographs of adherent bacteria on the each of the specimens after 14 days of use are shown in Figs. 9 - 12. No striking differences in the types of adhering bacteria were evident among the materials. The adhering bacteria reflected the variety of oral flora at the early stage. At the last stage (2 weeks), confluent sheets of cocci and rods were prevalent, but bacteria never covered the entire surface. Microbial coverage appeared to increase continuously with time.

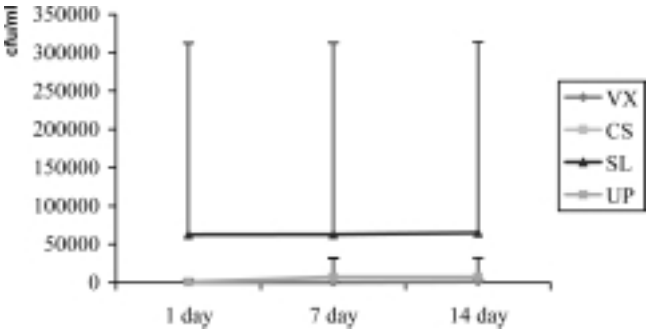

Fig. 8 Mean numbers of *Candida* spp. adhering to four denture base materials after various time periods (n = 16).

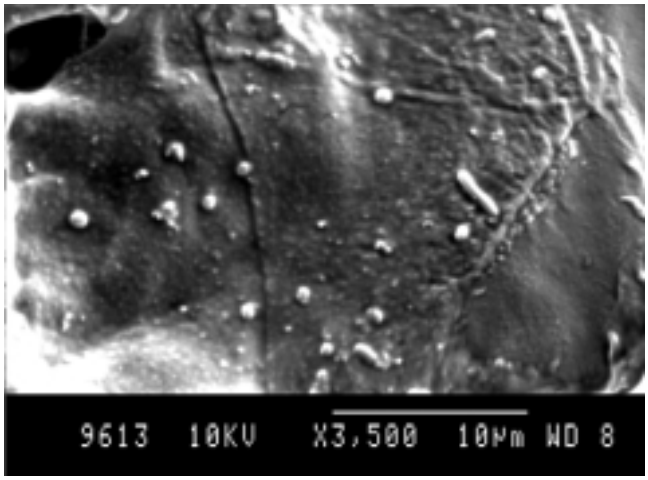

Fig. 9 SEM of bacteria adhering to UP after 14 days.

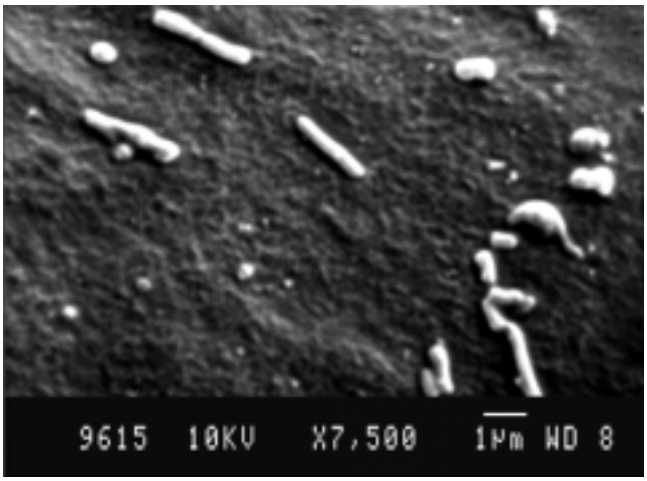

Fig. 10 SEM of bacteria adhering to SL after 14 days.

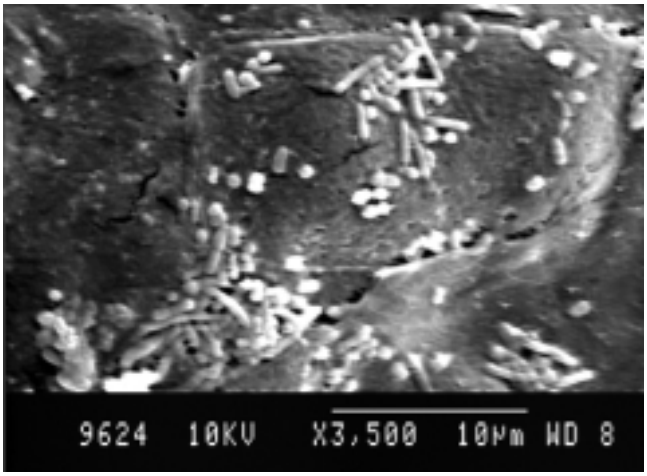

Fig. 11 SEM of bacteria adhering to CS after 14 days.

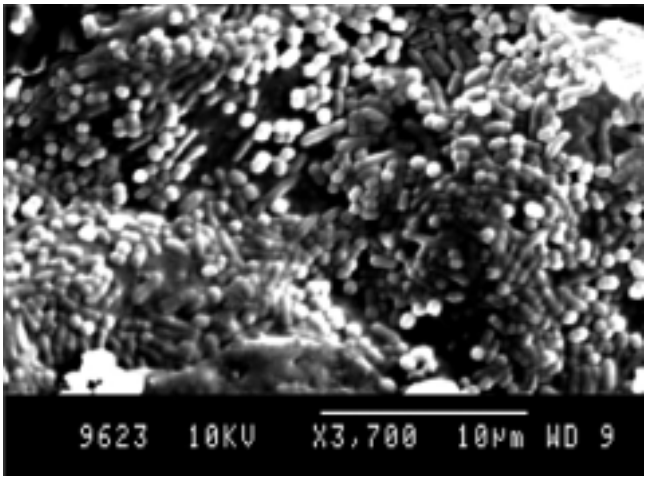

Fig. 12 SEM of bacteria adhering to VX after 14 days.

Discussion

Although soft lining materials are widely used as dynamic impression materials, and are essential adjuncts in the prosthodontic treatment and management of traumatized oral mucosa, they have certain physical and microbiological disadvantages. One of the most serious problems is surface colonization and infection by *C. albicans* and other microorganisms, which may result in denture stomatitis (32). In addition, it has recently been pointed out that continuous swallowing or aspiration of microorganisms from denture plaque exposes patients, particularly the immunocompromised or medicated elderly, to further infection (7).

Bacterial colonization of prostheses is an inevitable consequence of their being in almost continuous contact with bacteria-containing saliva. Since bacterial adhesion is affected by the surface characteristics of the appliance, such as its roughness, surface free energy, surface tension, hydrophobicity and affinity for absorption of salivary components, some workers have tried to modify these characteristics to reduce the propensity of bacteria to adhere (33).

Bacterial colonization may reduce the intraoral life of soft lining materials (26). Fungal growth deteriorates the surface quality of the material and may cause irritation of the oral tissues through a combination of surface roughness and concentration of exotoxins and metabolic products of the fungal colonies (34). Masella et al. (35) suggested the use of a 1/750 concentration of benzalkonium chloride as an effective antifungal agent. Yılmaz et al. (27) stated that some disinfectant solutions significantly reduced bacteria on the surface of resilient denture lining materials. However, it is difficult to control plaque on these soft lining materials because most chemical cleansers are reported to cause their deterioration to some extent (19,20). Rodriques-Garcia (34) indicated that when considering practical plaque control on resilient lining materials, the choise of denture cleansers depends on many factors including composition and expected time of service. Yılmaz et al. (21) evaluated the effects of different disinfectants on the physical properties of temporary soft denture materials, and found that application of 5.25% and 2% sodium hypochlorite solution, 5% deconex or 3.5% savlex solution effected some of the physical properties of soft liners. Furukawa et al. (36) stated that chlorine dioxide was inadequate for denture liners at the recommended 3-minute disinfection time, and that increasing the time of disinfection did not significantly reduce the numbers of microorganisms. Therefore the importance of denture plaque control should be recognized (7). Awareness of the susceptibility of these materials to microbial adhesion is an important factor in their chose and use (23).

Since the growth of *C. albicans* and other microorganisms on soft lining materials is of clinical importance, in the present study microbial adhesion to commercial soft lining materials was tested *in vivo*, and higher numbers of bacteria and *Candida* spp. were shown to adhere to soft lining materials than to acrylic resin. This finding corroborates previous studies indicating that soft lining materials for dentures are susceptible to microorganism adhesion (12,16,22,37), and suggests that soft liners are more susceptible in this respect than acrylic resin (16,37).

Among the three brands of soft lining materials we tested, no significant difference was seen, but the number of total bacteria on the surfaces of SL was higher than on the others. Significant differences were evident between SL and VX after each of the three time periods. Furthermore, Gram-positive and negative rods were isolated predominantly from SL, whereas Gram-negative rods were isolated predominantly from VX. The differences in the adhesion of bacteria between the materials could be related to the differences in their chemical composition. Surface porosity and texture and biological and physical/chemical affinity between the materials and microbial cells may also be important factors (16,38).

The present results confirm the observation of Okita et al. (16) that microbial adhesion increases with time. This is not unexpected, since the chemical and physical properties of these materials change with time *in vivo*. The results suggest that appropriate control of denture plaque is essential for the clinical use of soft lining materials, and that beyond functional, esthetic and economic considerations, selection of material for denture bases should consider the extent of plaque formation.

The present results confirm the observation of Makila and Hopsu-Havu (37) that soft-lined mandibular dentures have a much greater tendency than conventional maxillary dentures to harbor yeast. Thus inadequate cleaning of soft material is a significant reason for the higher frequency of yeast isolation from this type of surface.

In healthy denture wearers, the number of yeast species such as *Candida* is low (39). In the present study, *C. albicans* was seen in only four patients and *Candida* spp. was seen in only three. This may have been because only patients with healthy mucosa were selected. In these patients, *Candida* adhesion increased with time and adhesion was low on acrylic resin surfaces.

The present study demonstrated rapid and extensive microbial colonization on the palatal surfaces of temporary soft lining materials carried by volunteers *in vivo*. These results suggest that clinical use of these three types of soft

lining materials on existing prostheses should be restricted to short time periods. Considering that temporary soft liners are often applied to mucosa susceptible to pathosis, it is important to exercise care with their clinical use.

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