Eur. J. Prosthodont. Rest. Dent., Vol.16, No. 2, pp 50-55
Printed in Great Britain

Colonization and Deterioration of Soft Denture Lining Materials *in vivo*

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Abstract - *Colonization of denture lining materials by microorganisms including Candida albicans can result in deterioration of the material, as observed by a previous in vitro study by the authors. The current in vivo study monitored the microbial colonisation and penetration of five types of denture liners and their topography over six months. There was no significant difference in the microbial colonisation on the denture liners and no penetration observed, indicating a slower rate of deterioration of materials in vivo. However, the surface roughness of all materials increased during use, which might alter susceptibility to subsequent microbial colonisation.*

KEY WORDS: Denture soft lining materials, *Candida albicans*, colonization, surface roughness.

INTRODUCTION

Resilient denture lining materials are used for a variety of reasons. They reduce the traumatic effect that a denture may have on patients with thin atrophic mucosa; or with normal mucosa but with a resorbed ridge, sharp alveolar ridge crest, deep anatomic undercuts, bony protuberances, bruxomania, or where the oral mucosa exhibits a reduced tolerance to the load applied by the denture, and in obturators for acquired and congenital cleft palate[1-5]. Currently, there are two main types of these materials; silicone elastomers and soft acrylic compounds, represented in a variety of commercial forms.

One of the most serious problems associated with soft denture lining materials is the growth of *Candida albicans* and related *Candida* species, on and within these materials. This fungal colonisation has been reported to destroy the surface quality of materials and may cause irritation to the oral tissues due to increased surface roughness and concentration of yeast metabolic products[6].

The hyphae of *C.albicans* more readily colonise soft denture liner materials than the acrylic resin denture base[7, 8]. Indeed the majority of studies report that denture lining materials support the growth of Candida[9-11]. Over time, in-use liners will experience wear with the use of denture cleansers being shown to change the surface texture and roughness of liners[12, 13]. The extent of roughening and rate of wear of soft denture liner materials occurs to varying degrees[7, 9, 10,, 14, 15] but increased roughness has been shown to raise the susceptibility of the liner to microbial colonisation and deterioration[16-18].

This *in vivo* study continues from a previous *in vitro* investigation[16] by the authors to monitor the microbiological colonisation and penetration of denture soft liners over a six month usage period, and to determine any accompanying changes in surface topography.

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MATERIALS AND METHODS

Selection of Subjects

Sixteen edentulous adult patients (10 male and 6 female) between the ages of 60 to 80 years were selected randomly for the study. Each had been wearing maxillary and mandibular complete dentures and attended the University Dental Hospital of Manchester seeking replacement complete upper and lower dentures. Each patient selected for the study was supplied with a new complete upper and lower denture. Ethical approval was obtained for this study by the Manchester Local Research Ethics Committee and by the University Ethics Committee. The project was explained to each patient via a patient information sheet and informed consent was given. There were three exclusion criteria: (1) patients should not have a local or systemic fungal infection or be taking medication (e.g. antibiotics) which may induce one; (2) patients would not have been involved in a research project in the preceding six months and (3) patients would not have a soft denture liner in their new dentures, except those placed for the purpose of this study.

Application of Soft Liner Materials to the Lower Dentures

Five soft denture lining material types were used for this study (Table 1). Before processing the lower dentures, five square indentations (1 cm^2 and 2 mm depth) were created on the wax pattern in the lingual flanges, with three squares placed on one side of the denture and two on the other side. The dentures were then polymerised, trimmed and polished.

The five soft lining materials were applied into the square indentations created in the lingual flanges of the lower denture (Fig. 1) and were polymerised according to the manufacturers recommendations using the conventional

Table 1. *Denture lining materials used in the study*

Brand	*Material Type*	*Composition*
Molloplast-B	Heat curing silicone	Polydimethylsiloxane (polymer) and benzyl peroxide (initiator)
Permaflex	As above	As above
Flexor	As above	As above
Luci-Soft	As above	As above
Eversoft	Chair-side curing soft liner	Polyethylmethacylate (powder) and Dibutyl phthalate, Ethyl acetate, Ethyl Alcohol (liquid) and Methyl ethyl ketone (sealer liquid)

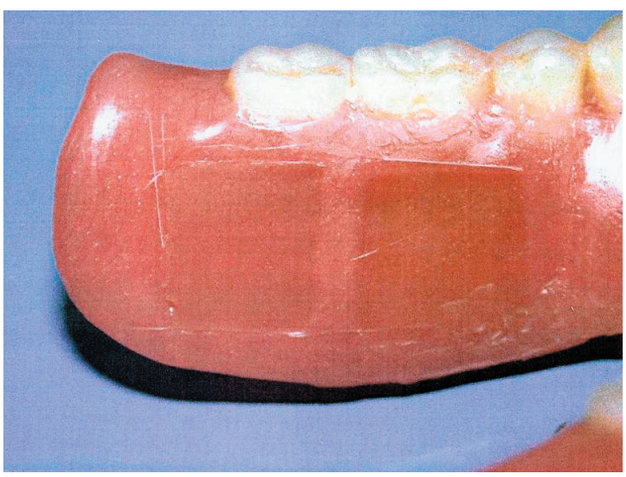

Figure 1. *The position of soft lining materials on the lingual flange of a lower denture*

flasking technique (flasked dental stone moulds). A wet polymerisation technique was used for the heat cured lining types. Eversoft was polymerised by heating the dental flask in a water bath at 100 ºC for 15 minutes, again as recommended by the manufacturers. After polymerisation, the flasks were re-opened to remove the lower denture and surplus lining material was trimmed from the denture.

Patient Instructions

Patients were instructed to use normal hygiene procedures with the exception that they must avoid using any mechanical devices (e.g. brushes) or any commercial denture cleansers on the lingual flanges where the soft liners were placed. Patients who did not routinely wear their dentures at night were instructed to soak them in water only. Patients returned to the clinic on three occasions with a full oral health assessment made each time.

Oral *Candida* Carriage

Oral *Candida* carriage was determined for each patient prior to the first insertion of the new dentures. Two techniques were employed: oral rinse and microbial swab[19, 20]. For the oral rinse method, each patient was requested to rinse his/her mouth thoroughly with 5 ml of sterile distilled water for one minute after removal of their old dentures. The patient was then expected to expectorate the rinse into a sterile 25 ml container. For the second method, two swabs were taken from each patient, the first from the dorsum of the tongue and the second from the lower alveolar ridge mucosa in the posterior region. A sterile swab was moistened in sterile phosphate buffered saline (PBS) and rolled gently for 10 seconds over the area. The swab was then placed into a sterile bottle containing 1 ml of sterile PBS.

For both methods, the colony forming units (CFU)/ml of yeast (presumptive *Candida*) were determined by serial dilution spreading onto Sabouraud agar. Plates were incubated at 37 ºC for 48 hours. Prior to counting, presumptive yeast colonies were Gram stained and their identification confirmed via light microscopy. The germ tube test was used to determine whether the presumptive *Candida* colonies were *C. albicans*.

Determination of the amount of oral microorganisms on denture lining materials

The amount of oral *Candida* and total microorganisms retained on the surface of the liner materials was measured after 1, 3 and 6 months. The mandibular dentures were removed from the mouth and each lining material was swabbed prior to its removal from the denture to avoid disturbing the plaque biofilm. Sterile swabs were moistened in sterile PBS and rolled gently five times over the upper third of each liner material. The swab was then placed into a sterile bottle containing 1 ml PBS. Serial dilutions (up to 10^{-5}) were made and plated onto Sabouraud agar and 7% defibrinated horse blood-Brain Heart Infusion (BHI) agar. All plates were incubated at 37 ºC for 48 hours, aerobically for Sabouraud plates and anerobic and CO_2 for blood-BHI plates. Counts and preliminary identification of predominant colony types of yeasts and total microorganisms were made using Gram staining and light microscopy. Data were analyzed using one way ANOVA at the 95% confidence level and the T-test for pairwise comparisons.

Surface Roughness Determination

At each sampling period (1, 3 and 6 months), the portion of liner material that had previously been swabbed (10 x 3 x 2 mm) was removed from the denture using a sharp scalpel and placed into a sterile container with 4% gluteraldehyde in PBS for at least 2 hours. Voids left in the denture following removal of the strip were immediately filled with a conventional chairside reline material and polished, care being taken not to damage the remaining soft liners. Subsequently the average roughness, Ra, of the materials was determined using a Perthometer S8P (Feinpruf-Perthen GmbH, Gottingen, Germany). The topography of the liner materials had previously been recorded after processing in order to establish a baseline. Statistical analysis of the data was performed as above.

Penetration of oral microorganisms through Denture Liner Materials

Immediately after measuring the surface roughness, test strips were placed into an ethyl alcohol series to dehydrate them, as previously described[21]. Test pieces were manually sectioned using a scarp scalpel blade so that three sections (approximately 1 mm thick) could be obtained from each test piece. Sections were stained using 0.03% acridine orange and viewed using epifluorescence microscopy (x 1000). Any microbial penetration into the material was noted and recorded as described previously[16].

RESULTS

Oral *Candida* Carriage

Yeasts (presumably *Candida*) were isolated from 15 of the 16 patients (93%) who participated in the study. Yeasts were isolated from both the tongue and alveolar ridge swabs and oral rinse samples. Mean carriage values obtained from swabs taken from the dorsum surface of the tongue were 271 ± 436 CFU per swab, while those taken from the mucosa covering the posterior area of the alveolar ridge were 109 ± 134 CFU per swab. Values obtained by the oral rinse method were 74 ± 98 CFU per ml (Table 2). Patients 6, 7 and 14 with the highest counts of oral yeast complained of discomfort underneath their mandibular dentures. Germ tube tests (n = 48) of the tongue, alveolar ridge and expectorate samples were positive for the 15 patients, indicating the presence of *Candida albicans*.

Determination of the quantity of oral microorganisms on denture lining materials

Yeast counts obtained from denture liners increased over time ($P<0.05$). However, there was no significant difference ($P>0.05$) in the amount of oral yeasts isolated from the liner material types for each time period investigated. Due to the similarity in counts from all the materials, data are presented as the mean number of yeasts isolated from all the materials after 1, 3 and 6 months (Table 3). The highest counts were obtained from Eversoft after 6 months with 6.55 ± 9.33 x 10^5 CFU per swab.

Similar trends were observed for other oral microorganisms (Table 3). In addition, there was no significant difference ($P>0.05$) in counts between anaerobic and CO_2 incubation and hence results are presented as mean total microbial counts from all material types. Again the highest counts, although not significant, were obtained from Eversoft after 6 months with 3.01 ± 0.35 x 10^7 CFU per swab.

Table 2. *Carriage of Candida in patients at the start of the study*

Patient	*Microbial Swab (CFU per swab)*		*Oral Rinse CFU per ml*
	Tongue	*Alveolar ridge*	
1	20	10	10
2	40	30	30
3	50	40	40
4	0	0	0
5	20	10	10
6	1100	290	270
7	1200	300	250
8	20	10	10
9	310	320	70
10	20	10	10
11	50	20	30
12	40	50	40
13	20	20	20
14	1080	280	280
15	320	310	80
16	50	40	30

Table 3. *The number of oral yeasts and microorganisms isolated from all denture lining materials after 1, 3 and 6 months in vivo wear.*

Months	*Mean CFU per swab from all materials ± Standard deviation*	
	Yeast (x 10^5)	*Total microorganisms (x 10^7)*
1	0.056 ± 0.008	1.15 ± 0.025
3	2.24 ± 0.013	2.28 ± 0.004
6	5.29 ± 0.707	2.79 ± 0.117

The predominant type of oral microorganism isolated from the denture liners were α-haemolytic streptococci (Gram positive cocci, catalase negative), although β-haemolytic and presumptive (non-haemolytic) streptococci were also cultured.

Surface Roughness Determination

There was no significant difference (P = 0.85) in the average roughness (Ra) between the liner materials immediately after processing (Table 4). However, over time there was a significant difference in the Ra values between the denture liners (P < 0.001) with Eversoft being the roughest. A significant difference (P < 0.002) was determined in the mean roughness for Eversoft after only one month of wear compared with the post-processed value. However there was no significant difference for the four silicone based liner materials (Molloplast-B, Flexor, Permaflex, Luci-Soft) for the same duration period (P = 0.83, 0.65, 0.62, 0.71 respectively). A significant difference was also recorded in the Ra values of Permaflex and Flexor after 6 months compared to the post-processed value (P < 0.01 and P < 0.05 respectively).

Penetration of oral microorganisms through Denture Liner Materials

Visual examination of the soft liners removed from the lower dentures showed that a marked layer of plaque had formed on the surface of the materials. However, microscopic examination of the soft liner sections showed no evidence of microbial penetration of any liner type for all time periods investigated.

DISCUSSION

Yeast species are commensals of the oral cavity in both the dentate and edentulous with the majority of the yeasts present being *Candida*, in particular *C.albicans*[22]. This study showed that 93% of the denture-wearing participants were *Candida* carriers, with *C. albicans* detected in all cases. This is in agreement with previous studies who demonstrated that yeasts were found in 85 - 90%[19, 23-25] of denture wearers tested, with *C. albicans* being the predominant species isolated, although carriage rates under 40% have also been reported in edentulous patients[27, 28]. Differences may be attributed to the population sampled, the sensitivity of the sampling and isolation technique used and the time of sampling[22]. This can be evidenced by the different carriage values obtained from the swab method compared to oral rinsing.

The presence of yeast on soft lining materials has been noted previously[7-10, 29] contributing towards the destruction of their surface quality and irritating the oral tissues[6].

In this study, the accumulation of oral yeast and other microorganisms on five types of soft lining material applied to the lingual flanges of mandibular dentures was assessed. Not surprisingly in the absence of denture hygiene on the materials, plaque built up significantly over the six month period. Eversoft had the highest value of retained yeast and total microorganisms, although the difference was not significant with Ra values over twice as high as the other liner materials after 6 months. This is a chair-side curing soft acrylic liner as opposed to the other materials used in the study, which were all heat cured silicone. In a previous *in vitro* study[16] we showed that Eversoft exhibited the least penetration by Candida when compared with the same silicone liners. However, in that short six week study, materials were processed against glass and exposed to a pure culture of *C. albicans*, making comparison to clinical use difficult.

Total microbial counts on blood-BHI plates were much higher than yeast counts on Sabouraud agar. Although yeasts are larger in size than other oral microorganisms, they are usually significantly lower in number. Not surprisingly, streptococci were the predominant type of oral microorganism isolated from liner materials which have been shown to facilitate and consolidate the subsequent attachment of *C.albicans*[30].

Microscopic examination of sections of soft lining materials removed from the lower dentures did not show any microbial penetration of lining materials over the six month period. Using the same technique of sectioning, we have demonstrated *in vitro* penetration of the same liners by *C. albicans* in only six weeks[16]. However, *in vivo*, it appears that the presence of other microorganisms, salivary flow rates and shear forces within the oral cavity significantly retards this process. The location of soft liners is also an important factor as other *in vivo* studies have observed microbial penetration into soft lining materials when the materials were applied as a lining to the denture fitting surface[7, 9]. Although soft liners are traditionally placed on the fitting surface of lower dentures, this study and others[11] applied the liner strips in a non-functional condition on the lower dentures.

Surface properties of soft liners are clinically important since they influence the microbial adherence and accumulation of plaque, which may then lead to the deterioration of the material[7, 31]. In general, silicone elastomers and other soft lining types replicate the surface texture of the material

Table 4. Roughness values (Ra) of five soft denture liners after 1, 3 and 6 months *in vivo* wear.

	Mean Ra (μm) ± standard deviation after:			
Materials	*Processing*	*1 month*	*3 months*	*6 months*
Molloplast-B	2.42±0.06	2.42±0.06	2.45±0.06	2.59±0.17
Permaflex	2.40±0.04	2.41±0.05	2.44±0.06	2.67±0.15
Flexor	2.40±0.08	2.40±0.08	2.43±0.09	2.62±0.19
Luci-Soft	2.40±0.04	2.41±0.06	2.45±0.06	2.57±0.20
Eversoft	2.41±0.05	2.53±0.09	4.62±1.25	6.84±2.64

against which they are processed[17] with smoother silicone surfaces following processing against vacuum-mixed stone compared with hand-mixed stone[18], while samples processed against glass showed the smoothest surfaces[16,17]. This increase in roughness greatly facilitates initial attachment of microorganisms[17, 18] and plaque maturation.

There was no significant difference in the Ra values between the five soft lining materials directly after processing (baseline samples). This may be due to the fact that all the materials were processed in the same manner against vacuum-mixed dental stone[32]. After 6 months wear, a rise in Ra values was recorded for all materials with Eversoft showing significant increases in surface roughness for each test period. As previously mentioned, Eversoft is an acrylic liner containing ethanol (ethyl alcohol) and a plasticizer (dibutyl phthalate). These constituents are known to diffuse out of the liner materials easily[33-36] and may account for the increased roughness during use. The remaining materials studied were silicone liners, processed using both heat curing and the application of pressure to provide greater cross linking thus creating a denser material[33]. This can make the materials less prone to changes in surface texture as evidenced by minimal changes in Ra values during the six month study period. Several studies have reported an increase in surface roughness of soft lining materials over extended clinical use in the mouth. Of the soft liners evaluated in this study, Molloplast-B has been investigated many times in the literature with the material only showing changes in surface texture late on in clinical use[15]. Figures range from 83% of Molloplast-B liners showing no noticeable wear after six years[9] to roughening of the liner surface observed after nine years in use[14]. Overall wear is inevitable to some extent particularly for the more flexible materials. In addition the extent of oral hygiene and denture care and the patient condition will affect the longevity of materials.

In conclusion, the majority of patients who participated in this study were colonised by *C. albicans* prior to the placement of new dentures. There was no significant difference in the retention of oral yeasts and other microorganisms on the denture liners materials studied over a six month period with no microbial penetration of liners observed during this period. The surface roughness of materials increased, particularly Eversoft, over a six month period which has implications for both microbial colonisation and cleanability of materials.

MANUFACTURERS' DETAILS

- Molloplast-B, Detax, GmbH & Co., Germany
- Permaflex, Kohler, Medizintechnik, Danningen, Germany
- Flexor, Schutz-Dental Gmblt, Rosbach, Germany
- Luci-Soft, Dentsply Trubyte, Dentsply International Inc., UK
- Eversoft, Austenal Inc., USA
- Chairside reline material, Rebase, Toshiba, Japan
- Sabouraud and Brain-Heart Infusion agar, Oxoid Ltd., UK

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