

BIOFILM FORMATION ON COMPLETE DENTURE LINERS

ABSTRACT

AIM: To clinically evaluate biofilm growth on 4 liners in complete denture base surfaces of 20 geriatric patients. **MATERIAL AND METHODS:** Patients received new complete maxillary dentures prepared with 4 chambers (10x10x2 mm) in the tissue surface of acrylic denture base. Each of the 4 chambers was randomly filled with the following denture liners: Eversoft (M1); Kooliner (M2); GC Reline Extra Soft (M3); Elite Soft Relining (M4). Patients were randomly separated into 2 treatment groups: T1- sanitization with soft brush and dentifrice; T2- similar to T1 with daily immersion in cleansing chemical solution (Ortoform). Patients had 8 follow-up sessions over a 3-month period. The internal denture surface was stained with a dental plaque dye at each of the follow-up visits. Standardized photographs were taken, and biofilm growth was scored. Data were tabulated and submitted to Analysis of Variance. Means were compared by Tukey ($p < 0.05$) and T tests. **RESULTS:** Kooliner (M2) means were significantly different from the others for both groups T1 and T2. Treatment 1 promoted higher biofilm growth scores than treatment 2. The highest score after treatment 1 was Kooliner (M2) and the lowest was Elite Soft Relining (M4). As for treatment 2, Eversoft (M1) was statistically different from Elite Soft Relining (M4). Again, Kooliner (M2) presented the highest score and Elite Soft Relining (M4) the lowest. Kooliner (M2) was statistically different from both GC Reline Extra Soft (M3) and Elite Soft Relining (M4). **CONCLUSION:** Of the materials and treatments studied, the best clinical selection for lower biofilm growth scores would be Elite Soft Relining (M4) with treatment 2.

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KEYWORDS

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INTRODUCTION

Heat-activated acrylic resin has been the most common material for fabrication of denture bases since the 1930's. However, this material is not ideal since it is rigid, while the patient mucosa is not. Consequently, resilient materials have appeared on the market to relined acrylic denture bases. These materials were created to compensate for the deficiencies of acrylic resin by increasing adaptation and retention of complete dentures in patients with limited supporting structures, reduced ridge height¹. Nevertheless, the need for monitoring and frequency of needing replacement of soft liners are of the main problems for the clinical use of these materials. These liners fail for many reasons, such as hardening (loss of plasticizer), odor absorption, bacterial and fungal growth, color alterations, dislocation of denture base and even the fact that absorption and solubility are accompanied by volumetric alteration^{2,3}.

High levels of denture stomatitis in patients with complete and partial removable dentures lead to microorganism accumulation on the denture base, which is worrisome as well as a risk to oral health. Budtz-Jorgensen et al.⁴ and Bergendal⁵ found this type of inflammation in 50% of patients examined.

Minimal alteration of the internal denture surface so as to maintain intimate contact between mucosa and prosthesis. The aforementioned precautions are not as

efficient as a polishing process in preventing microorganism adhesion. Moore et al.⁶ suggested that dentures should not only be free of stains and deposits, but they should also be relatively free of microorganisms. It would be pointless to eliminate microorganisms associated with the mouth if the oral tissues continued to be repeatedly inoculated by a contaminated denture.

Complete and partial removable dentures may be cleansed by either mechanical or chemical methods. According to Sesma et al.⁷, the most commonly used method is mechanical cleaning with a dental brush and dentifrice or soap. Alternatively, chemical cleaning utilizes denture immersion in chemical products. Results of chemical cleaning are sometimes similar or superior to mechanical cleaning; however, the greatest benefit of chemical cleaning is its convenience for handicapped, disabled or geriatric patients who sometimes cannot adequately brush their dentures.

Quality of denture hygiene and efficiency of patient's cleaning method should be routinely evaluated by the dentist. Use of dyes or biofilm indicators has clinically proven to be efficient and practical, especially when associated with a standardized plaque index allowing for rapid comparisons with earlier records. According to Budtz-Jorgensen & Theilade⁸ and Jeganathan et al.⁹, biofilm quantity is much more important to oral health

than the type of microorganisms found within the biofilm.

Resilient materials are increasingly available to provide short term modification and/or tissue conditioning of denture bases. These materials combined with an acrylic resin are functional and comfortable for patients with complete or partial removable dentures. The importance of maintaining oral health in individuals who require this type of prosthetic rehabilitation emphasizes the need for studies of lining materials with available hygiene methods. A lack of clinical studies evaluating the efficiency of hygiene methods of complete dentures in general, and the association of acrylic resin/soft liners in this investigation, aims to evaluate biofilm growth on complete upper denture base surfaces with liners in vivo, since the only place to accurately evaluate plaque accumulation is in the mouth¹⁰.

MATERIAL AND METHODS

This study utilized the materials specified in Table 1.

For the present study, twenty patients aged 65 or older each having been treated with a complete maxillary denture as well as good oral and systemic health were chosen. The project was first evaluated and approved by the Ethical Research Committee at the Piracicaba School of Dentistry of the University of Campinas under protocol nº 026/2001.

Patients received new complete

dentures. Maxillary dentures contained 4 cavities (10x10x2 mm) in the internal base surface. Cavities were obtained by positioning 4 portions of polymerized silicone per condensation reaction with the plaster cast before polymerization the acrylic resin. After polymerization, deflasking, finishing and polishing, the four cavities were randomly filled with the following: Eversoft (M1), Kooliner (M2), GC Reline Extra Soft (M3) and Elite Soft Relining (M4), and the denture was placed in the patient's mouth.

Patients were randomly separated into 2 treatment groups: Patients in T1 cleaned their dentures with Kolynos extra-soft children's toothbrush (Kolynos do Brasil, São Bernardo do Campo – SP, Brazil) and Colgate Triple Action dentifrice (Colgate – Palmolive, São Paulo – SP, Brazil); hygiene for T2 was similar to that of T1 but also included daily immersion in a cleansing chemical solution (Ortoform, F&A Laboratório Farmacêutico Ltda, São Paulo – SP, Brasil).

Eight follow-up sessions were realized over a three-month period (0h, 24h, 1, 2, 3 and 4 weeks, 2 and 3 months). At each follow-up session, dentures were treated with a dye for biofilm quantification and standardized photographs were taken. Dentures were removed from the patient's mouth, washed in running water, dried with an air syringe, and then coated with 2% malaquita green plaque dye (Farmadoctor, Curitiba – PR, Brasil). After

one minute denture was washed and dried again. Afterwards, each denture were

photographed with slide film for later analysis and quantification of biofilm formation.

Table 1. Material, commercial name, manufacturer, composition, lot and origin.

MATERIAL	COMMERCIAL NAME/ MANUFACTURER	COMPOSITION	LOT	ORIGIN
Soft Liner	Elite Soft Relining / Zhermack S.p.A.	Poly vinyl siloxane	K25	Badia Polesine, Italy
Soft Liner – powder	Ever Soft / Myerson– Austenal Inc.	Poly ethyl metacrilate	081033	Chicago, USA
Soft Liner – liquid	Ever Soft / Myerson – Austenal Inc.	Di butilic ptalate Ethilic acetate	081044	Chicago, USA
Soft Liner – sealer	Ever Soft / Myerson– Austenal Inc.	Ethilic alcohol Metil etil cetone	081050	Chicago, USA
Soft Liner	GC Reline Soft / GC Dental Products Corp.	Poly vinyl siloxane	0005081	Tokyo, Japan
Hard Liner	Kooliner / GC Dental Products Corp.	Metacrilate	L062900A	Alsip, USA
Chemical Cleaner	Ortoform / F&A Laboratorio Farmacéutico Ltda.	Sodium perborate	0003	São Paulo, Brazil
Plaque Dye	Verde malaquita / Farmadoctor	Poteolytic enzyme Malaquita green 2%		Curitiba, Brazil

The plaque index published by Tarbet¹¹, Ambjornsen et al.^{12,13}, Budtz-Jorgensen & Thylstrup¹⁴, Pietrokovski et al.¹⁵ and Keng & Lim¹⁶ was utilized to quantify biofilm formation. Scores are as follows: 0 – absence of plaque; 1 – light plaque (until 25% of surface covered with plaque); 2 – moderate plaque (26-50% of surface covered with plaque); 3 – severe plaque (51-75% of surface covered with plaque); 4 – very severe plaque (76-100% of surface covered with plaque).

The study was double blind with the same previously trained technician performing all data collection. Photographs of each material were projected to fill a 100x100cm screen. A vertical and horizontal grid marked the screen surface forming 10x10cm squares

so that the dyed surface in each square represented 1% of the biofilm formed.

Results were computed and submitted to an Analysis of Variance after application of Tukey and T tests at $p < 0.05$.

RESULTS

To quantify results, material scores from the 8 follow-up sessions were averaged for each patient. This step allowed the evaluations to be represented by a parametric measure of position. A T-test (Table 2) was applied to the means to assess the influence of the two treatments on the materials tested.

Differences were noted between all material groups in both treatments. Average

T1 values were greater than those of T2 for all materials. M2 presented the greatest discrepancy between the two treatments and was the only material that had a statistically

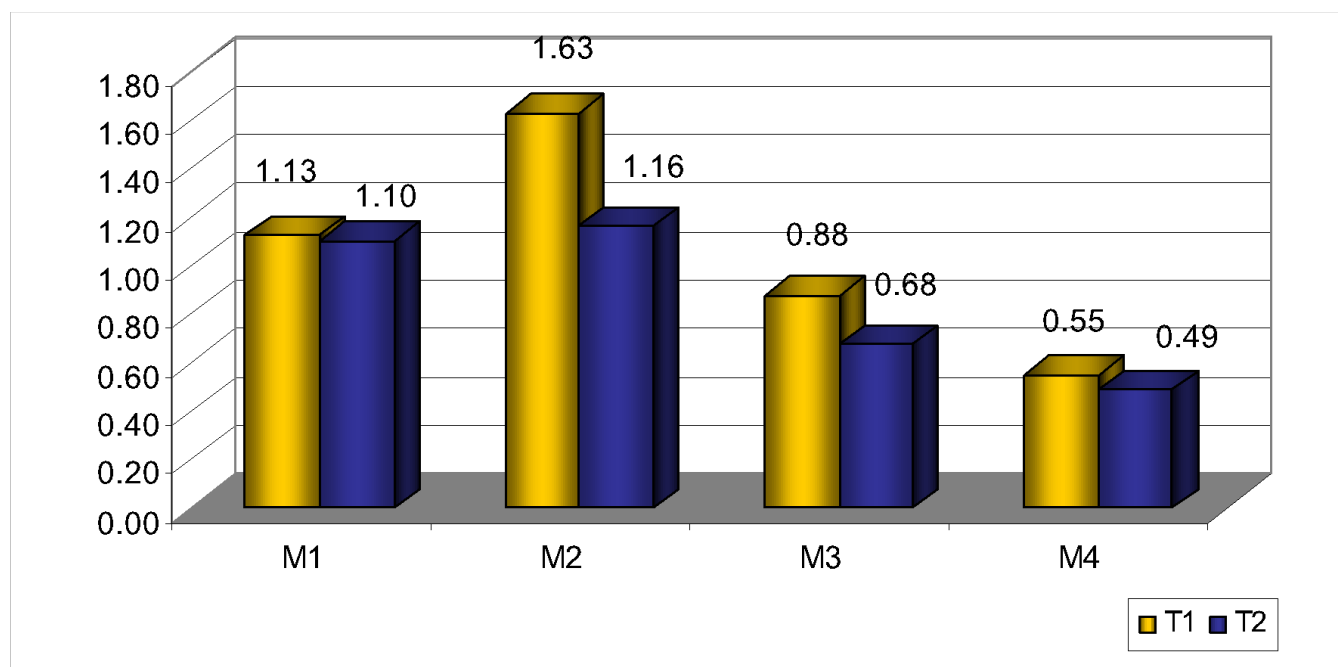
significant difference among scores within the same group.

Material means after treatments 1 and 2 are graphically represented in Figure 1.

Table 2. T- test results for each material and treatment.

MATERIAL	M1		M2		M3		M4	
	T1	T2	T1	T2	T1	T2	T1	T2
Treatment								
Mean	1.125	1.1	1.625	1.1625	0.875	0.675	0.55	0.4875
Variance	0.1771	0.2875	0.1840	0.1703	0.1146	0.0632	0.0736	0.0849
Observations	10	10	10	10	10	10	10	10
GroupVariance	0.2323		0.17717		0.0889		0.0792	
Hypothesis of mean difference	0		0		0		0	
gl	18		18		18		18	
T stat	0.1159		2.4569		1.5		0.4964	
P (T<=t) two-way	0.9089	ns	0.0244	*	0.1509	ns	0.6256	ns
Two-way t-test	2.1009		2.1009		2.1009		2.1009	

Figure 1. Material means after treatments 1 and 2 related to mean scores.



The four materials presented different means when submitted to the same treatment. To verify if these differences were significant, a randomized design was adopted considering the materials M1, M2, M3 and M4 as causes of material variation at a 5% significance level and was separately applied within T1 and T2

treatments.

Significant differences among the T1 materials could be seen in the Analysis of Variance. A Tukey Test verified a significant difference between M2 and the other materials

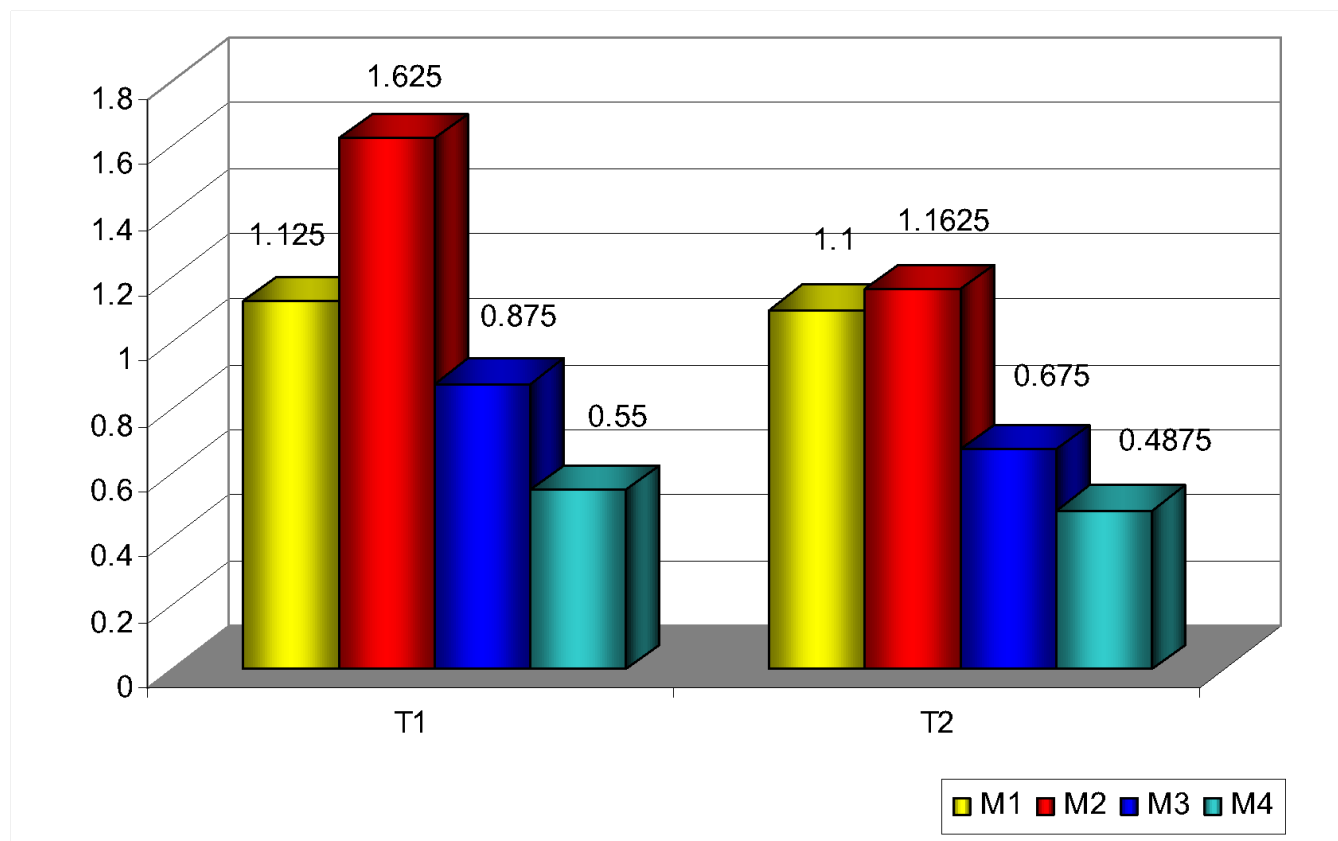
(M1, M3 and M4), as well as a significant difference between M1 and M4.

The analysis of variance was also significant for treatment T2. ANOVA indicated a difference among the materials, which was verified in the multiple comparison Tukey

Test. There was a significant difference between M1 and M4 and also between M2 with M3 and M4.

Figure 2 graphically represents mean material scores per treatment.

Figure 2. Mean scores of material per treatment.



DISCUSSION

From the moment that it is reported that most chemical cleansers cause greater or lesser deterioration of resilient materials, it becomes difficult to control biofilm formation on these materials. Material inhibition or collaboration with microorganism growth on dentures should be a primary concern and a twofold object of study: the first is of basic interest – which liner components affect

microorganism growth? The second is of clinical interest – if the soft liner has an anti-fungal effect, with what potency can the same liner help control and/or inhibit biofilm formation?¹⁷

The present study was *in vivo* and not *in vitro*. Manufacturers generally keep the formulation of their materials proprietary chemical compositions of the materials under study. This study focused its attention on the

quantity of biofilm accumulation on the materials investigated. Any differences among materials were associated with the affect of hygiene on biofilm accumulation.

Analysis and comparison of the two hygiene protocols indicated greater biofilm formation after treatment 1 that solely employed manual brushing with an extra-soft toothbrush and dentifrice. The lower values presented by treatment 2 proved the association of manual brushing and chemical cleansing to be effective. Wright et al.¹⁸ observed a significantly reduced ($p < 0.02$) prevalence of yeast in patients that cleaned their dentures by immersion in a type of alkaline peroxide compared with those that used brushing with soap or paste alone for denture cleaning.

Mechanical brushing is not always enough to completely clean the surface of certain materials used to manufacture denture bases⁸. Chemical cleaners help clean areas that brushing cannot efficiently reach and cannot be polished such as the internal surface of a denture. Chemical cleaners are also very useful for patients with physical or other difficulties that IMPEDEM effective and efficient mechanical brushing of their prosthesis. Furthermore, according to Tamamoto et al.¹⁹, chemical cleansing action, using an enzymatic cleanser lyses microorganisms destroying their cell walls or through protein and polysaccharide lyses, destroying the products

through which the microorganisms adhere to the resin surface. The chemical cleanser used in this study is composed of proteolytic enzymes.

Another factor that supports the results of this study was utilization of a surface glaze or varnish sealant that is present in two of the materials under study (M1 and M4) but not in the other two (M2 and M3). These substances modify the surface structure by sealing microporosities in the polymerized lining materials. Sealing the surface of the liner and the interface formed between a lining material and acrylic resin in the denture base also plays an important role in reducing biofilm accumulation.

According to Quirynen & Bollen²⁰, surface roughness as well as free energy of the solid substrate surface that is being treated plays an important role in bacterial adhesion. A rougher surface helps protect bacteria from being dislodged from the surface to which they adhere. The above authors also cited that hydrophobic surfaces accumulate 10x less biofilm than hydrophilic surfaces. Also, substrates possessing low surface free energy are less capable to retain biofilm since the biofilm mass frequently decreases between 6 and 9 days.

When each material was analyzed separately, comparison of the two treatments revealed a significant statistical difference only within the material 2 groups. In other words,

hygiene technique was only significant for material 2. Average biofilm accumulation on material 2 after treatment 2 was similar to that of the other materials in the same treatment. What caused a statistical difference was the high mean obtained by material 2 in the treatment 1 group. This led to the conclusion that manual brushing alone is not as good as that associated with chemical cleaning.

Surface characteristics of material 2 probably permitted much more effective microbe adhesion since no sealing agent was applied after material application. Furthermore, material 2 is an autopolymerizing acrylic resin and as such is a rigid lining material. This substance when used for denture bases presents greater surface roughness and a greater number of internal and external micro pores. Since mechanical brushing does not penetrate the denture, it is not able to eliminate or inhibit microorganism adhesion sufficiently enough to diminish biofilm accumulation, thus chemical cleaning in treatment 2 had an additional benefit over brushing alone.

Observing the 4 materials within each treatment, it was found that in treatment 1, material 2 was statistically different from the others, as was material 1 different from material 4. As for treatment 2, a statistical difference occurred between means of materials 1 and 4 and between material 2 with materials 3 and 4. This analysis confirms

earlier interpretations, since the greatest means of biofilm accumulation were found for materials whose basic composition was acrylic resin, and one of these materials did not even have its surface sealed after installation.

CONCLUSION

Lastly, results showed that manual cleaning alone as much as that associated with chemical cleaning are effective hygiene techniques of complete dentures with liners; however, chemical cleaning promoted more satisfactory results in materials prone to greater microorganism growth. After evaluating biofilm accumulation on lining materials, the best indication would be a silicone-based material with a sealer to be applied to surfaces after installation. Adequate hygiene of this material consists of mechanical cleansing and chemical cleansing.

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