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A STUDY TO COMPARE ADHERED ORAL FLORA TO SOFT LINER AND CONVENTIONAL DENTURE BASE SURFACE IN COMPLETE DENTURE PATIENTS

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ABSTRACT

Background: Denture base materials are subjected to sorption, a process of absorption and adsorption of liquids depending on the environmental conditions, also their fitting surface is subjected to microbial adhesion due to surface irregularities. Soft silicon liners are usually used with removable appliances to decrease load concentration on the hard and soft tissues. Silicon liners also undergo fluid sorption and microbial adhesion.

Aim: This study was carried out to assess the effect of standardizing the oral hygiene conditions on the microbial load difference between conventional acrylic denture bases and silicon soft relining material (Mucopren soft) used to reline mandibular complete dentures in atrophied mandibles.

Methods: Conventional complete dentures were constructed for 25 patients with flat atrophic mandibular ridges to be used for two weeks, salivary swabs were collected to evaluate oral flora attached on the fitting surface of the denture base and the buccal vestibule, soft liner was applied for all patients mandibular denture surfaces and the same approach was repeated after two weeks of the liner use.

Results: Microbial load was found to be significantly higher in the fitting surfaces of the silicon soft liner, than in the conventional acrylic base fitting surfaces.

Conclusion: Even under controlled oral conditions, Mucopren soft silicon liner incorporates greater microbial load than acrylic material thus, subjecting patients to numerous infections.

KEY WORDS: Soft liner, oral flora, denture base materials, biofilm.

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INTRODUCTION

Oral cavity of the edentulous patients contains numerous species of inhabitant microorganisms including bacteria, viruses, and fungi integrated into dental plaque. They are able to form thin biofilms resistant to mechanical removal or pharmaceutical treatment [1]. Bacterial colonization of prosthesis is an inevitable sequelae of their being in almost continuous contact with microorganisms containing saliva. Attachment of microorganisms to prosthetic appliances is affected by surface characters of the device, as roughness, free energy, surface tension, hydrophobicity and affinity for salivary components absorption [2]. Adhesion of microorganisms, such as streptococci, is relevant to be evaluated as they are one of the early colonizers and constitute a crucial integrant of oral biofilm [3, 4].

It has been pointed out that constant swallowing and aspiration of microorganisms from plaque adhered to dentures exposes patients in general and particularly those elderly, medicated or immunocompromised to further infections thus endangering their lives [5].

Conventional heat-polymerized polymethyl-methacrylate (PMMA) resins have been broadly used in partial and complete removable prostheses, attributed to their acceptable esthetics, good thermal conductivity, low permeability to oral fluids, color stability and facility of processing, handling and repair [6-8]. Dentures used for elongated intervals are difficult to be relined as microorganisms secrete methyl mercaptan, leading to liner separation even following the primer dissolution. Since bacteria penetrate to approximately 3mm deep [9].

Soft liners have been broadly utilized in dentistry to reshape prostheses surfaces in proximity with soft tissues of the oral cavity [10]. The utilization of a soft liner can improve both masticatory and oral comfort for patients presenting a reduced thickness of the oral muco-periosteum [11]. Liners are noninvasive and comparatively affordable if compared to new denture construction [12, 13].

Soft liners are utilized in various removable prosthesis to distribute functional loads uniformly on the prosthetic bearing tissues. They are endorsed in cases of irregularly resorbed bone, bony undercuts, atrophied thin mucosa, atrophied bony ridges, immediate prosthesis, healing following implant placement, cleft palate and for patients with bruxism or xerostomia. The resilient lining materials can be sorted as plasticized acrylic resins or silicone elastomers. The silicone elastomers, consisting of dimethyl-siloxane polymers, with a chemical composition devoid of leachable plasticizers, retain their elastic properties for prolonged periods, thus the term permanent liners [14]. Resilient liners are intended to be elastic, absorb energy, and act on the cushion effect [15]. Resilient reline materials are also sorted as short- or long-term types. Long-term resilient denture liner materials sustain their resilience up to 30 days or more and could be utilized for up to 1 year, while short-term liners are indorsed for application for up to 30 days [16].

Most of the presently available liners have various drawbacks, including color stability, long-term resiliency, abrasion resistance, bond strength and porosity which affects its microbial load [14]. The attachment of micro-organisms on the surface of acrylic resin and denture liners is determined by the surface topography and the constitution of these biomaterials [17]. Rough surfaces and changes in hardness adversely affects the material's serviceability [18].

Microorganisms originally adhere to the surface of the liners, then they infiltrate within the material. Several studies have proven that using soft lining materials can escalate fungal and bacterial growth which is reinforced by environmental conditions under the denture, as well as the material structure [19]. This disadvantage shows restricted possibilities of conventional cleaners commonly used by patients. Denture disinfectant agents such as chlorhexidine gluconate, sodium hypochlorite, hydrogen peroxide and others were outlined to induce unfavorable changes to the physical and chemical properties of soft liners [20, 21].

Bacterial colonization may reduce the intraoral life of soft liners, with deterioration of its surface quality and irritation of the mucosal tissues by exotoxins and metabolic products [22]. In general, an optimized denture base material should exhibit minimal susceptibility to bacterial and fungal adhesion while maintaining the desired physical properties [23].

Mucopren soft liner material showed lower roughness values and a smoother surface than the acrylic resin- based material, thereby making it preferred when selecting more appropriate material, due to its tendency to promote less biofilm build-up[24].

There are two hypotheses tested, the first was that: candida would be the highest microbial strain isolated from both material specimens,

The second was that: there would be no difference in the microbial load between the conventional acrylic denture bases and Mucopren soft liner after standardizing the oral conditions by Betadine mouth wash.

AIM

This study was carried out to evaluate the difference in microbial load of conventional acrylic denture bases and that of silicon soft relining material Mucopren soft, after using Betadine mouth wash to exclude the variability of personal hygiene and standardizing the oral conditions.

MATERIALS AND METHODS

This study was conducted in Faculty of Oral and Dental Medicine, Ahram Canadian University. The residents of this clinical study constituted of 25 completely edentulous patients who had atrophic mandibles. All patients have been treated with complete dentures then undergone soft liner application on the mandibular dentures. All patients agreed with the trial protocol and signed a voluntary consent agreement.

Age of the patients ranged between 45 and 66 years with a mean age of 54 years, patients were free from any systemic disease that may affect the salivary flow or soft tissue of the oral cavity, any infectious disease, or history of antibiotic therapy for the last 3 months. Heavy smoker patients or patients with para-functional habits were excluded. The experimental protocol used in this study was approved by National Research Center Ethics Committee (No.: 19 025).

Complete dentures construction: Mandibular and maxillary primary impressions were conducted with irreversible hydrocolloid impression material (Chromopan-lascod B.A. sestoflorentino Firenze, Italy). Definitive impressions were conducted with zinc oxide and eugenol impression material (Cavex outline B.V., Holland). Impressions were boxed and poured in dental stone (Zeus dental stone hard type, Italy) to obtain definitive casts on which occlusion blocks were assembled. Following maxillary occlusion rim adjustment a maxillary face bow record (Whip Mix #8645 quick mount. Louisville, K.Y., USA) was performed to mount the maxillary cast on a semi-adjustable articulator (Whip Mix #8500 Semi-adjustable articulator, Louisville, K.Y., USA). Centric occluding relations were recorded using wax wafer technique to mount the mandibular cast. Artificial teeth were set utilizing cross-linked acrylic resin teeth (Acrylic teeth, Cosmo MEA, Dentsply-USA) then dentures were processed.

Soft liner application: mandibular denture bases were cleaned and dried, then trimmed to allow for the desired thickness of the soft liner (Mucopren soft, Kettenbach GmbH & Co. KG, Germany) which ranged from 2 – 3 mm. Thin coat of Mucopren adhesive was brushed onto the roughened surface, left for 30 seconds followed by applying a second coat and allow them to dry in air for 90 seconds. Delivery syringes were used to apply the Mucopren soft uniformly on the fitting surface of the dentures and placed in the patient's mouth immediately. Various functional movements were performed to mold the liner material. Following denture removal with the lining from the mouth, it was immersed in mild temperature water (45-50 °C) for 5 minutes. Scissors, a scalpel or bur were utilized to detach the excess material, polishing disks were used for finishing the soft liner. Finally, thin coat of Mucopren silicone sealer was applied to the trimmed surface of the soft liner. All patients were given the same post-insertion instructions and a standardized oral and denture hygiene protocol throughout the study with avoidance of all factors affecting liner roughness.

Microbiological evaluation: Sterile cotton swabs were utilized to collect saliva from the fitting surface of the mandibular dentures and the buccal vestibule after two weeks of denture use (25), for both acrylic denture base and silicon liner. Samples were taken in the fore noon hours between 10 and 12 O'clock allowing patients to use the dentures couple of hours prior to collecting the swabs.

Sample collection(26): Each patient used Betadine mouth wash 1% concentration with 20 ml. gargling for 30 sec.(27), Approximately an area of a 1cm. square dimension was swabbed for 30 seconds for each of the vestibular tissues and denture fitting surfaces. A rotating motion with the swab held firmly and laterally against the denture for three times with the same swab to ensure proper adherence of the plaque. The same procedure was applied for the vestibular tissue as shown in Figure (1). After the soft lining material was applied to the denture base by two weeks, the denture and tissues were swabbed as mentioned before. Swabs were immediately transferred to commercially available sterile swab holder and placed in ice box for less than 2 hours before plating.

Fig. (1) Swabbing the vestibular tissues

Samples preparation & bacterial counting (28): Quantification of the bacterial colonies was done by the number of *Colony Forming Units* per ml. (CFUs) which was calculated by the following method: Swab tip of each sample was cut by sterile scissor and placed in 9 ml. of sterile 1% buffered peptone water and thoroughly mixed for 30 sec. using vortex to make culture homogenate (initial dilutions). This dilution was serially diluted into further decimal (tenth fold) dilutions till fourth dilution. For total bacterial count, plate count agar petri dishes were inoculated in duplicate by spreading with 0.1ml. from each dilution. Incubation was performed for 72 hours at 30°C. Plates with fewer than 3 colonies were considered negative for enumeration (plates that contained 3 to 30 colonies only are to be mentioned). Total bacterial count (TBC) was calculated as follows according to the ISO protocol (29):

TBC = Colonies count at last positive plate X 10 X dilution (10^n).

Bacterial identification (30): Different 5 colonies shapes from positive plates were picked and each isolate was transferred to separate sterile buffered peptone water 1% test tube and incubated at 30°C. For 12 hours, for enrichment. Loopful is taken from the peptone test tubes and spread by streaking over specific media surface (EMB. Eosin Methylene Blue, Rose Bengal Agar and blood agar media) to identify the isolated micro-organisms. Then, the isolated colonies were evaluated and identified according to their morphology, Gram staining and biochemical tests as mentioned in the respective protocols [31-33]. Bacterial strains identification was conducted at Serology and Bacteriology departments of Animal health research institute in Doki - Cairo.

All complete dentures were made in the same prosthodontic laboratory, by the same technician performing standardized finishing and polishing procedures. All trimming, soft liner application were done by one clinician, and sampling procedures were done by one clinician.

Statistical analysis of the microbial count was conducted by using Student- t test (two samples) to detect the difference in the count means. Statistical analysis of the obtained results was conducted using Statgraphics Centurion 18 program (Statgraphics Technologies, Inc.). Significance was set at $p < 0.05$.

RESULTS

Candida, Gram –Ve, Gram +Ve and aerobic bacteria were isolated and identified as shown in Table (1) and Figure (2a, 2b), in which the isolated microbial strains from all patients under inspection were recorded.

It clearly appears that, Candida albicans was the highest frequency isolated strain in 60% of inspected specimens. While, Staphylococcus epidermis and Klebsiella pneumonia isolated frequencies were 48% each.

While, Staphylococcus aureus & Klebsiella oxytoca were isolated from 32% each. Average of aerobic and facultative anaerobic total microbial counts in acrylic denture bases were less than those obtained in Mucopren soft liner 3.24 & 3.91 log. / CFU., respectively. While, the minimum bacterial count in acrylic denture bases and Mucopren soft liner were 2.6 & 2.78 log. /CFU., respectively. On the other hand, the maximum bacterial count in acrylic denture bases and Mucopren soft liner were 4.26 & 5.38 log./CFU., respectively, as shown in Table (2).

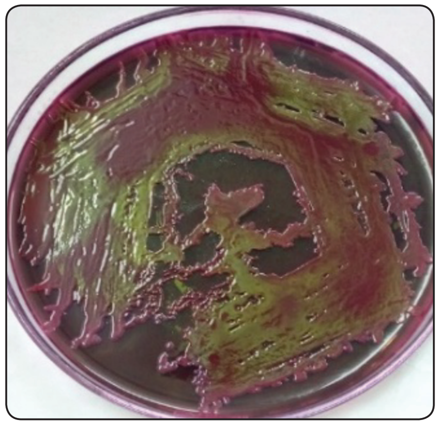

Fig. (2a) Klebsiella pneumonia on EMB

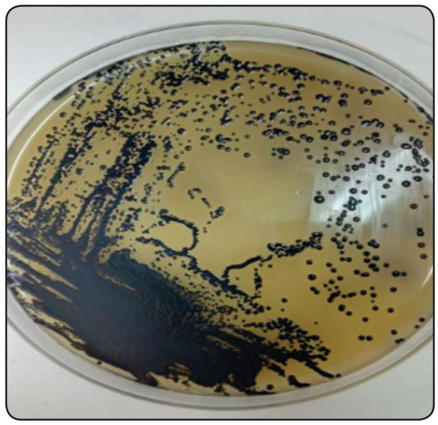

Fig. (2b) Staphylococcus epidermis on Paird Parker

TABLE (1) Isolated microbial strains

Microbial strains	No. of +ve patients		No. of -ve patients	
	No.	Frequency	No.	Frequency
Staphylococcus Aureus	8	32%	17	68%
Staphylococcus epidermis	12	48%	13	52%
Klebsiella pneumonia	12	48%	13	52%
Klebsiella oxytoca	8	32%	17	68%
Candida albicans	14	60%	11	40%

TABLE (2) Microbial logarithmic minimum & maximum & mean counts

Type of material	Min	Max	Mean± SD	P-value
Acrylic denture base	2.60	4.26	3.24 ± 0.55	0.0069
Mucopren soft liner	2.78	5.38	3.91 ± 1.11	

Statistical analysis of the obtained results was conducted using Statgraphics Centurion 18 program (Statgraphics Technologies, Inc.) significance was set at $p < 0.05$, and null hypothesis of no difference between microbial load means was rejected, which means that there is a difference between total microbial count in acrylic denture bases and Mucopren soft lining material. Thus, the total microbial count in Mucopren soft lining material is overhead those with acrylic denture bases. Otherwise stated, the soft liner applied to the denture base surface increased the microbial load of the removable prosthesis even under the same standardized oral conditions.

DISCUSSION

Based on the current results, the study's first hypothesis was accepted as the microbial outcomes did in fact reveal the predominance of candida amongst all other microbial strains. The second hypothesis however was rejected as the acrylic base showed significantly lower microbial counts when compared to the Mucopren soft liner.

In the current study soft liner was used to prevent further bone resorption of the mandibular ridge.

The most appropriate testing environment for the microbial oral load is intra oral not the oral conditions simulations. The inter-individual variability in personal hygiene and microbial counts is very important to consider. In order to control this variability, the selected participants of this study undergone mouth wash with Betadine to exclude the personal hygiene factor effect on research results and to quantify the oral bacterial load under controlled environment. This point was not investigated in any previous studies concerning microbial load. In this study, a significant quantity of microorganisms was detected on the surfaces of both the denture base resin and the soft liner.

Though the frequency of isolation of gram negative bacilli was low at 24hrs, it increased significantly at 1-week from both the denture polished surface and tissues surface [25]. This study timing is less than the two weeks' time used in this study, which proves that two weeks after denture use is enough to produce reliable results.

Several situations increase liners' roughness, a favoring factor for microbial accumulation thus increasing microbial load on the soft liner surface. This situations that increase soft liner roughness include cleaning solutions, immersion time, concentration of the solutions, increase in temperature as in microwave use, brushing with toothpaste and the use of organic solvents[18]. There is still no consensus on whether roughness is reduced when a surface sealant is applied [34]. Meanwhile other studies reported that sealed soft liners showed less microorganism growth and biofilm formation in comparison to unsealed ones [35, 36].

Irregularities of the prosthesis surfaces allow the attached microorganisms to survive longer, since they are protected from the removal forces originating from oral hygiene habits. In addition, the superficial roughness increases the available area for the adhesion of microorganisms [3, 16, 22, 37-39].

Soft liners are known to have a superior surface roughness to that of acrylic resins and so, when exposed to the oral environment, they are potentially more prone to microbial adhesion and biofilm formation [38]. All those studies were in agreement with the results of the current study. On the contrary a study on the surface roughness of acrylic material compared to Mucopren soft liner concluded that, the mean surface roughness value was higher for the acrylic resin- based material (Trusoft) than for Mucopren, at all the time intervals [24]. Another study conducted on different soft lining materials concluded that, at baseline, Mucopren soft presented the lowest roughness value, and Dentuflex the highest. However, at 3, 6 and 12 months, no difference was observed among Dentuflex, Mucopren soft and Ufi Gel SC materials [34].

In the current study increase of microbial load in the soft liner material may be attributed to interaction with oral flora, and difficulty of mechanical cleaning efficiently due to its surface texture.

The microorganisms carry adhesives that bind to complementary receptors on the surface stereochemically, as it was stated in some other studies[38,40]. The electrochemical reaction that occurs between C.albicans and resilient liners may be the reason for the increased adhesion on the Mucopren surface than on the acrylic surface, also it may be the reason of the increased Candidal percentage than other organisms in both materials.

On the other hand, the decreased hydrophobicity of the soft liner than acrylic bases may have also contributed to difference in microbial load.

Methacrylate resilient liners surfaces are rougher than silicone liners surfaces attributable to their chemical structure, residual monomer content, polymerization method, monomers' volatility, and mixing technique , thus Candida albicans adhesion on denture base acrylics was significantly less than those of silicon liners [15, 41, 42].

In this study, Candida was found to constitute the highest percentage of the microbial flora detected on the surfaces of both acrylic bases and soft liner. A study found that, following six months of utilization under clinical conditions, fungal colonies were detected on one-third of silicone linings. Nevertheless, no failures attributed to fungal colonization were recorded. After one year, fungal colonies were noted on 44% of the linings, and a sum of 31% of the noted failures were attributed to fungal colonization. Nonetheless, the severity of colonization varied significantly depending on the type of material utilized [39]. These findings were compatible with the outcomes of in vitro study, which revealed that none of the available soft lining materials were resistant to colonization by pathogenic fungi [43].

However, in vitro inhibition of fungal growth was documented in a few studies of Molloplast-B material [44, 45]. Another study concluded that, silicone rubber-based soft lining materials, enhance the growth of fungi such as Candida albicans in the presence of saliva [46].

In the present study aerobic microorganisms (Staphylococcui & Klebsiella) were isolated with a mean count of 3.24 log./CFU. from acrylic base and 3.91 log./CFU. from Mucopren soft which was in agreement with the results of a study that shows total aerobic count of 3.83 log./CFU. from ProBase Hot soft liner and 4.16 log./CFU. from Vertex Soft materials [47] . Another study stated that there was a frequency of 64.9 % aerobic bacteria isolated from acrylic denture bases [48]. Also it was found in another study that the total bacterial count was 6. 3 log./CFU. in acrylic bases and 6.45 log. /CFU. in soft liner bases after 14 days of use [49]. Another study done on acrylic microbial load concluded that aerobic bacterial load was found to be 3.34-6.39 log./CFU.[50] , these two last researches found higher counts than what was resulted in the present study which means that the influence of standardizing the oral conditions before collecting the samples decreases the microbial load in general, but it had no effect on the difference between the two materials.

Limitations of this study include investigating only one type of soft liner and one type of acrylic denture base, also include short period of denture use before testing the microbial load difference.

CONCLUSION

Candida was found to have the highest percentage in both acrylic and Mucopren liner. Mucopren soft silicon denture liner, exhibited higher microbial load in comparison to conventional heat-polymerized acrylic resin commonly used in denture bases, regarding Candida, total aerobes, total facultative anaerobes, staphylococcus and Klebsiella. This difference in microbial load was resulted even after standardization of the oral conditions before collecting the samples.

The application of Mucopren soft liner to a hard denture base, may lead to a greater risk of oral and systemic infections for patients, highlighting a greater need for oral hygiene protocol application.

RECOMMENDATIONS

Based on the results of this study, it is better to use Mucopren soft liner with strict oral hygiene measures for limited period of time. Further investigations are recommended on the surface roughness of Mucopren and its correlation with its microbial load.

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