



Estimation of the autoclave and different surface treatments on microleakage between soft liner and heat-cured acrylic

Estimativa da autoclavagem e diferentes tratamentos de superfície na microinfiltração entre o *soft liner* e o acrílico termopolimerizável

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ABSTRACT

Objective: The present study was oriented to estimate the effect of different surface treatments on the microleakage between the soft liner and acrylic with and without the use of autoclave as disinfection method. **Material and Methods:** Sixty samples were split into two groups: the autoclaved groups and non-autoclaved groups. Each one subdivided into three groups: first one without any treatments as a control group; in the second group surface of the samples were treated with CO₂ laser (10.6 nm wavelength for 15 seconds), and in the third group the surface was treated with sandblasting (250 μm Al₂O₃). All the samples exposed to thermocycling, then the microleakage test was evaluated by gauging dye penetration depth between the soft liner and acrylic disc using a digital microscope. Data analyzed statistically by One-way ANOVA and Tukey's post-hoc tests. In addition, t-test was used for comparison between two groups (P-value ≤ 0.05). **Results:** The maximum mean values for the microleakage were observed in the untreated group (control) followed by the group treated by CO₂ laser and the lowest mean value of microleakage was related to the third group for both non-autoclaved and autoclaved groups with significant differences among them. In addition, depending on the use of autoclave, there was non-significant in all studied groups. **Conclusions:** There was a decrease in the microleakage when the surface treated with CO₂ laser and sandblast. The use of autoclave did not badly change the microleakage between the soft liner and denture base.

KEYWORDS

Microleakage; Denture Liners; Autoclave; CO₂ laser; Sandblast.

RESUMO

Objetivo: O presente estudo teve como objetivo estimar o efeito de diferentes tratamentos de superfície na microinfiltração entre o soft liner e o acrílico usando ou não a autoclave como método de desinfecção. **Material e Métodos:** Sessenta amostras foram divididas em dois grupos: grupo com uso da autoclave e grupo sem uso da autoclave. Cada um subdividido em três grupos: o primeiro sem nenhum tratamento como grupo controle; no segundo grupo, a superfície das amostras foi tratada com laser de CO₂ (comprimento de onda de 10,6 nm por 15 segundos) e, no terceiro grupo, a superfície foi tratada com jateamento (250 μm Al₂O₃). Todas as amostras foram expostas à termociclagem, em seguida o teste de microinfiltração foi realizado medindo-se a profundidade de penetração do corante entre o soft liner e o disco de acrílico em microscópio digital. Os dados foram analisados estatisticamente por One-way ANOVA e testes post-hoc de Tukey. Além disso, o teste t foi usado para comparação entre dois grupos (P-valor ≤ 0,05). **Resultados:** Os valores médios máximos de microinfiltração foram observados no grupo não tratado (controle) seguido pelo grupo tratado com laser de CO₂ e o valor médio mínimo de microinfiltração foi relacionado ao terceiro grupo para ambos os grupos não autoclavado e autoclavado com diferenças significativas entre eles. Além disso, dependendo do uso de autoclave, não houve significância em todos os grupos estudados. **Conclusão:** Houve diminuição da microinfiltração quando a superfície foi tratada com laser de CO₂ e jateamento. O uso de autoclave não alterou a microinfiltração entre o *soft liner* e a base da prótese.

PALAVRAS-CHAVE

Microinfiltração; Reembasadores de dentadura; Autoclave; Laser CO₂; Jateamento.

INTRODUCTION

The soft liners have been widely utilized for prosthetic denture as a cushion on the tissue surface for removable dentures and maxillofacial prosthesis [1,2] due to viscoelastic properties [3]. They belong to the resilient materials indicated for relining the inner surfaces of denture base to withstand the stress-bearing area [4]. Liners are used when there are unfavorable sharp bothering edges, failure of the denture fitness inveterately [5]. As well as, they are also used for prostheses fracture and cleft palate [6], tissue conditioning during implant healing [7]. Soft liners are noninvasive and relatively more economical when compared to make a new denture [8] for both two liner categories: short or long-products because they remain more than one year for long-type denture liner and up to one month for short-type liner product [9]. The patient prefers resilient liners over hard ones because they improve comfort [10]. The main problem associated with soft liner is the lack of bonding to the resin materials [11,12]. The debonding of soft liners from the denture base can create a potential surface for bacterial growth, plaque accumulation, and calculus formation [13]. The failure of adhesion may be attributed to the microleakage between them [14]. The microleakage defined as a major factor that is clinically undetected able to elapse of microorganisms from the oral cavity into the prosthesis, the success of the material depends on the ability to prevent microleakage [15]. The major output of the good adhesion strength is preventing microleakage [16]. Various surface pretreatments have been done to enhance the bond strength and evaluate microleakage between the liner and denture base [17]. Some of these are roughening the bonding surface by airborne-particle abrasion, laser, or by the monomer [11,18].

On the other hand, the main interest of denture use is disinfection and controlling the contamination particularly in the prosthodontics

branch [19]. Therefore, the indication of the autoclave as a sterilization method because of its good destroying of bacteria, fungi, viruses, and any reason that result in contamination of liquids and equipment in use [20]. But some studies were reported that the specimens were suffered from the internal stress formation and shrinkage after heat postpolymerization, so to overcome this problem the specimens were left in the autoclaves for cooling to reduce shrinkage and internal stress formation [21,22].

So, the aims of this in-vitro study were oriented to assess the effect of airborne particle abrasion with aluminum oxide and laser treatment on microleakage between soft liner and denture base. The null hypothesis consisted of two parts: the first part was the surface treatment with aluminum oxide, and laser treatment should not affect the microleakage between the liner and resin. The second part was microleakage between soft liner and denture base remain unchanged statistically after sterilization by autoclave.

MATERIALS AND METHODS

In this study sixty polymethylmethacrylate denture base resin (Heat-cured denture base resin, vertex, Netherlands) were supplied in a disc shape (30 mm in diameter and 2 mm in thickness) [23], these specimens were heat cured in a water bath according to the manufacturer's directions which were processed by a short curing cycle (74 °C for 1.5 hours then boiling for 30 minutes), then they were extracted from the flask and all flashes were trimmed and the accurate measurements of each specimen and verified by a caliper device (Renfert, Germany) from different points. The 60 specimens divided into three major groups depending on the surface treatments that were applied to each group as follow (Table I):

First group: 20 specimens did not receive any surface treatment (control group).

Second group: 20 specimens in this group were subjected to laser machine (CO₂ laser, CMA-1080 kll, Yue Ming Laser Technology Co., Ltd, China) at power 6-15 Watts, 85-212 J/cm² energy [24] at 12.5 mm distance from exposed area to laser beam [25]. An aluminum metal plate mesh was made (the measurement of this metal plate mesh was equal to the dimension of the acrylic specimen) CO₂ laser emitting radiation with continuous plus with a wavelength of 10.6 nm for 15 seconds on the treated surface [26]. The operator wore the specified eyeglass to protect the eye against the hazards of the CO₂ laser beam.

Third group: 20 specimens were roughened by sandblasting machine (No.90003-6327, China) by airborne particle abrasion with 250 μm Al₂O₃ on the treated surface of a disc shape acrylic denture base resin at a pressure of 0.62 Mpa for 30 seconds [17] a special holder was custom made to fix the tip of the nozzle and the specimen during the sandblast procedure without affecting the constant distance between the treated surface of the specimens and the tip of the nozzle.

Table 1 - Specimens grouping according to surface treatment type

Study groups		Treatment type
Non-autoclaved groups	Control	Without any treatment
	Laser	Surface treatment with CO ₂ laser for 15 seconds
	Sandblast	Surface treatment with 250 μm Al ₂ O ₃ for 30 seconds
Autoclaved groups	Control	Without any treatment
	Laser	Surface treatment with CO ₂ laser for 15 seconds
	Sandblast	Surface treatment with 250 μm Al ₂ O ₃ for 30 seconds

The specimens of acrylic cleaned in an ultrasonic device then removed and left for complete dryness. After that, the 2 mm of heat-cured soft liner material (Vertex™ Soft, Netherlands) was applied on the treated surface of the acrylic by the aid of the mold followed by curing the soft liner according

to the manufacturer's instructions that was polymerized by water bath at 70 °C for an hour and a half, then the temperature was raised to 100 °C for 30 minutes. In the end, we obtained disc specimens that had 30 mm in diameter and 4mm in thickness (2 mm for acrylic resin and 2 mm for soft liner). That was adapted by placing the heat cured acrylic specimen in the mold taking 2 mm of mold thickness, and the other 2 mm thickness was filled with soft liner material that was applied with aid of the split mold that was placed between two plates to extrude excessive material after a complete setting the specimen was removed from the mold [27]. Then all samples put in thermocycling (Julabo Labortechnik, GMBH, Germany) for subjecting to (5 ± 1 °C-55±1 °C, 500 cycles) for 60 seconds in an isolated container [28]. Then the autoclaved groups were exposed to the sterilization by autoclave (HICLAVE HV-25 L, HIRAYAMA, Japan) monitored as follow: 121 °C temperature, 15 pound/inch² pressure, and 15 minutes as immersion time, the diameter and thickness of each specimen was rechecking by vernier to exclude any distorted specimen [19]. In addition, the non-autoclaved groups remained without autoclave disinfection. After thermocycling and sterilization, the specimens of each group soaked in dye pigment (methylene blue) that was placed in an incubator for one week [27]. After the storage period was completed, they washed them with water and put aside to dry. Then 4 lines were delineated on the specimens to divide the diameter into 4 equal pieces by using a separating disc in a slow speed handpiece (Marathon, Korea) with continuous water cooling to avoid overheating of the specimens, then the specimen split into 8 pieces in equal measurements (Figure1), so each specimen had 8 readings for microleakage value that was done by measuring the amount of penetration of methylene blue dye between the acrylic disc and soft liner. The measurement done by a digital microscope (Dino-Lite, Taiwan) at a magnification of (40X) [29], the microscope

had a camera that has captured images for each specimen, and the penetration of the dye evaluated in millimeter. Eight readings were recorded for each specimen, and the average of them was maintained to get one microleakage value for each specimen.

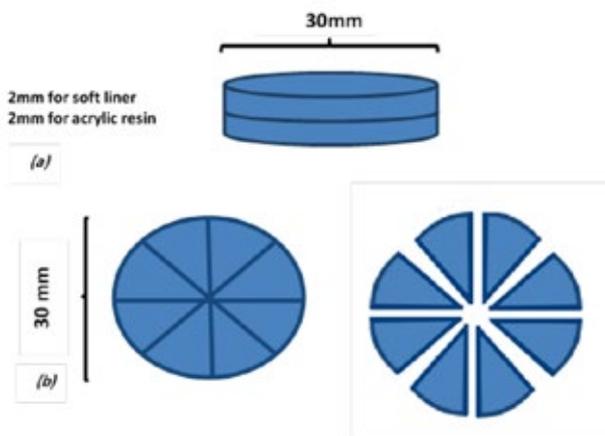


Figure 1 - The disc-shaped of the specimen for microleakage test (a); the specimen split into 8 pieces (b).

Scanning electronmicroscopy (SEM):

The changes in surface morphology of acrylic resin samples after surface treatments and before soft liner application were evaluated for all groups by using the a scanning electron microscope (SEM) (S 50, FEI Company, Netherlands). The SEM the images were taken at two magnifications: X 1000, X 2000 [30,31].

Statistically, the microleakage data of each group were analyzed by One-way analysis of variance (ANOVA) and Tukey HSD multiple comparison tests to determine the significant differences between the different surface treatments. The independent T-test was used for analyzing the difference between the autoclaved and non-autoclaved groups.

RESULTS

The descriptive statistics of microleakage with and without autoclave were presented in Table II, the maximum mean values for the

microleakage were observed in the untreated group(control) followed by the group treated by CO₂ laser and the lowest mean value of microleakage was related to the group treated by sandblasting with 250 μm Al₂O₃ for both groups without autoclave and the groups subjected to autoclave (Figure 2). One-way analysis of variance produced significant differences among all studied groups with and without autoclave (Table III). Further analysis to compare all groups using the Tukey-HSD test were showed significant differences among the tested groups (Table IV). The results of analyzing the autoclaved groups and non-autoclaved groups by using the independent t-test indicated a statistically non-significant difference between the microleakage values for all groups with and without autoclave (Table V). Figure 3 showed the microleakage which represented by dye penetration of the control groups was maximum penetration of the pigment for both groups autoclave and non- autoclaved groups (Figure 3, a and b). While, the dye breakthrough with less amount than control groups for the treatments with laser (Figure 3, c and d), and lest amount of dye was shown in sandblast with and without autoclave disinfection groups (Figure 3, e and f).

SEM analysis showed the surface topography of each surface treatment for autoclave and nonautoclave groups. There was a visual reduction in surface roughness in the control group and a uniform surface without any visual surface topography change (Figure 4, a and b; Figure 5, a and b). For laser groups the SEM examination showed that the rough surface with many holes formations. Melted areas in the resin polymer and many cavities (Figure 4, c and d; Figure 5, c and d). While there was clearly rough surface on acrylic surface with scratches and appearance of micro porous and irregularities with sharp edge rims in sandblast groups (Figure 4, e and f; Figure 5, e and f).

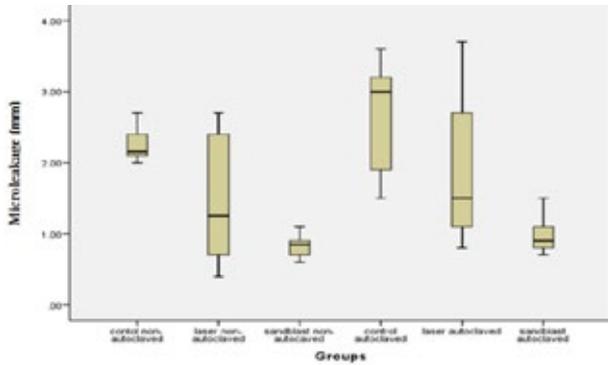


Figure 2 - Box plot of the microleakage for all tested groups.

Table II - Descriptive statistics of microleakage (mm) for all groups

Study groups	N	Mean	Std. Dev.	95% Confidence Interval for Mean		Min. value	Max. value	
				Lower Bound	Upper Bound			
Non-autoclaved groups	Control	10	2.25	0.2369	2.081	2.419	2.0	2.7
	Laser	10	1.53	0.9031	0.884	2.176	0.4	2.7
	Sandblast	10	0.84	0.1713	0.717	0.963	0.6	1.1
Autoclaved groups	Control	10	1.54	0.7890	1.245	1.835	0.4	2.7
	Laser	10	2.71	0.7202	2.194	3.225	1.50	3.60
	Sandblast	10	1.84	0.9720	1.144	2.535	0.80	3.70

Table III - One-way ANOVA for comparison the microleakage between groups according different surface treatments in autoclaved and non-autoclaved groups

Study groups	Sum of Squares	Df	Mean Square	F-value	P-value	
Non-autoclaved groups	Between Groups	9.942	2	4.971	16.550	0.000*
	Within Groups	8.110	27	0.300		
	Total	18.052	29			
Autoclaved groups	Between Groups	15.138	2	7.569	14.837	0.000*
	Within Groups	13.774	27	0.510		
	Total	28.912	29			

*; significant at P-value ≤ 0.05

Table IV - Tukey HSD-test for Multiple comparison between different treatment groups control, laser, and sandblast) in autoclaved and non-autoclaved groups

Study groups	Mean Square	F-value	P-value	
Non-autoclaved groups	Control Laser	0.7200	0.2451	0.018*
	Control Sandblast	1.4100	0.2451	0.000*
	Laser Sandblast	0.7200	0.2451	0.018*
Autoclaved groups	Control Laser	0.6900	0.2451	0.024*
	Control Sandblast	1.4100	0.2451	0.000*
	Laser Sandblast	0.6900	0.2451	0.024*

*; significant at P-value ≤ 0.05

Table V - Independent T-test for comparison between autoclaved and non-autoclaved groups in control, laser, and sandblast groups

Study groups	F-value	P-value
Non-autoclaved Control group vs Autoclaved Control group	1.919	0.071ns
Non-autoclaved Laser Group vs Autoclaved Laser group	0.739	0.470ns
Non-autoclaved Sandblast group vs Autoclaved Sandblast group	1.326	0.201ns

ns; non-significant at P-value > 0.05

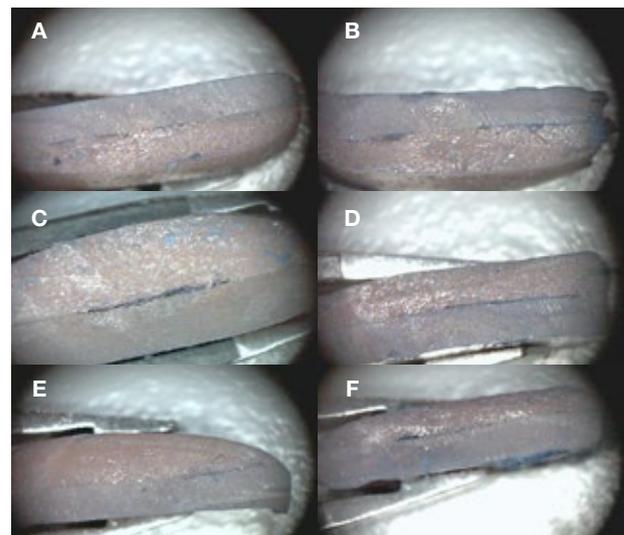


Figure 3 - (a-f).Microleakage for control groups nonautoclave(a) autoclave (b); Laser groups nonautoclave (c)autoclave (d);sandblast groups nonautoclave (e) autoclave (f).

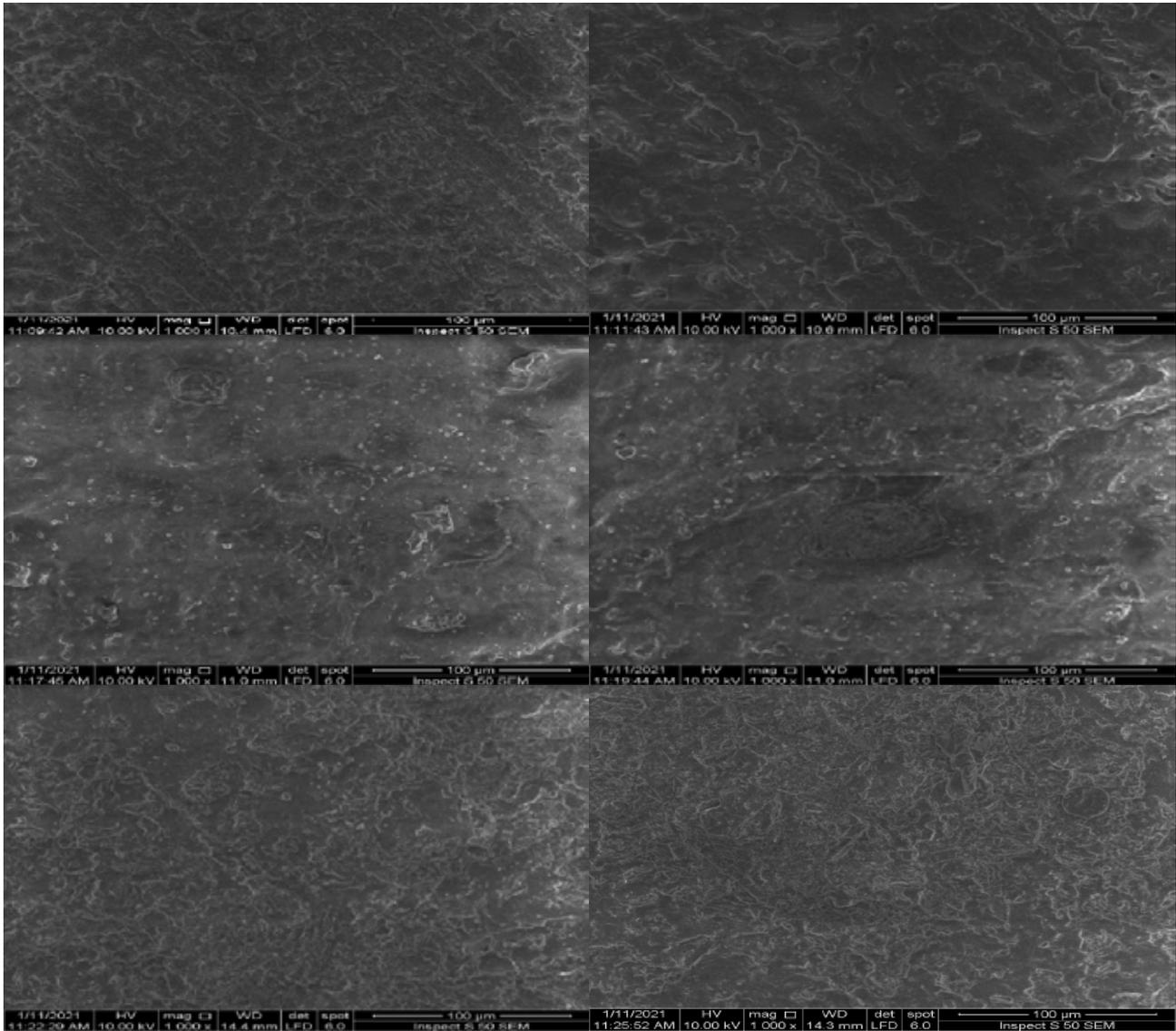


Figure 4 - (a-f). SEM for control groups nonautoclave(a) autoclave (b); Laser groups nonautoclave (c)autoclave (d);sandblast groups nonautoclave (e) autoclave (f) at X1000 amagnification.

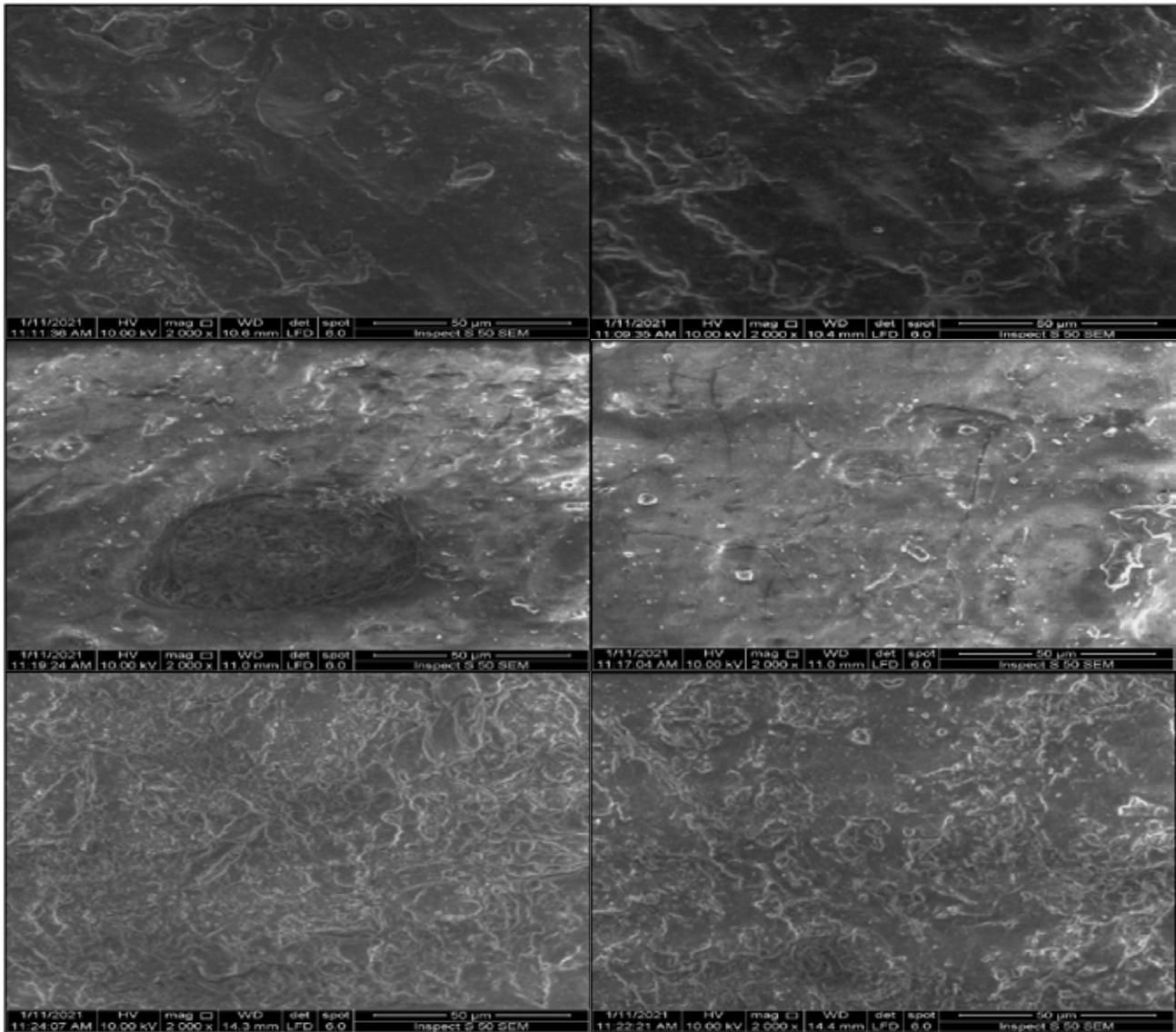


Figure 5 - (a-f).SEM for control groups nonautoclave(a) autoclave (b); Laser groups nonautoclave (c)autoclave (d);sandblast groups nonautoclave (e) autoclave (f) at X 2000 amagnification.

DISCUSSION

Soft liner materials have several important problems including loss of softness, colonization by candida Albicans, low tear strength, porosity [14 ,32] , but the loss of bond between the liner and the denture surface beneath it that produces microleakage which is considered the major one [33]. Therefore, the prevention of the leak between them is essential to increase the period of denture use [34]. To solve this perplexing

problem the investigators have focused on methods to alter the denture base resin surface which is including the chemical and mechanical such as laser treatment [35]. According to the result of this study, pretreatment of the denture base resin with airborne particle abrasion by sandblasting with 250 µm Al₂O₃ and CO₂ laser decreased the microleakage of soft liner material to acrylic resin denture base in comparison to the control group. So, the first part of the hypothesis was rejected. This result coincided with previous

research that found the sandblasting of the denture surface with $50\ \mu\text{m}$ Al_2O_3 reduced the degree of microleakage by improving the bond strength between a silicon-based resilient lining material and denture base [12]. Also agreed with another study that found that roughening the denture base by sandblasting reduced microleakage for acrylic-based linings [27]. It was assumed that airborne particle abrasion of acrylic resin improved the bond strength of resilient lining material to the denture base, through the production of irregularities that could facilitate mechanical interlocking [36,37]. As well as, it believed that the roughening of the acrylic surface was affected the bond strength with soft lining material in a positive way [38]. Nakhaei et al. stated that airborne abraded group exhibited significantly higher bond strength than the control group [39]. The same result gained from Storer [40] and Uzumez et al. [36]. The enhanced bond strength in the result of Nakhaei could be attributed to the larger size of alumina particles $110\ \mu\text{m}$ that created larger pits and depressions, thereby, the resilient lining material could penetrate into them more easily [39]. The presence of definite cavities on surfaces hit by alumina oxide particles indicated this. On contrary, these larger particles were more easily removed from the surface of the liner and the acrylic resin, leaving fewer residual particles that exhibited less interference with the bonding procedure compared with smaller particles this was explained why the microleakage of sandblasting group in this study was reduced more than other treatment and control group. Adversely, Amin et al., concluded that the application of aluminum oxide was lowered the bond strength between the acrylic and liner which increased the microleakage[41]. The same result by Korkmaz and other researchers in 2013, who revealed the Al_2O_3 produced a weak bond between polyamide and silicon lining material[42]. On the other hand, the laser attracted attention as an alternative technique for the preparation of the surface of the acrylic resin

before placement of the soft liner that results in suitable surface pits and roughness which in turn increase soft liner bonding [43]. In this study reduced the microleakage by treatment with the CO_2 laser than the untreated surface this in agreement with studies that indicated that surface treatment with laser can be an effective technique in increasing the bond strength of liner to acrylic resin [23, 39]. A study indicated that Er, Cr:YSGG laser improved the adhesion of the resin to the silicon[42]. Researchers found that ND: YAG laser treatment of PMMA increased the surface roughness and irradiated specimens exhibited higher bond strength to the resilient lining material, although the difference was not statistically significant [36]. Also, other research showed there was an improvement in the peel strength by surface treated with Al_2O_3 or CO_2 laser, one of the factors that might affect the outcomes in laser group in different studies is the rate of scanning of the laser tip and the distance of the tip to the surface [26], the scan rate could result in different cavities on the surface of acrylic resin. The use of the laser in the focus or defocus mode might also affect the ability to create pits by the laser beams [44]. In this study in the comparison between Al_2O_3 group and CO_2 laser group, the Al_2O_3 had decreased value of microleakage in comparison to CO_2 laser this could be explained by the roughened acrylic denture base by laser might have pits in discontinuities manner that didn't lead to enough penetration of the liner into them that created gaps and oral fluids accumulation [11 ,45]. In addition, an irregular surface might produce contact angle and surface tension which resulted in decreases in the penetration of material into the irregularities on the acrylic. If the logic is applied to the penetration of liners into the irregularities produced by CO_2 laser, increasing the viscosity of resilient liners for a given contact angle and surface tension reduces the penetration of the liners this might be explained the microleakage of the group treated by CO_2 laser lower than the group treated by sandblasting [26].

These explanation were supported by SEM observations that were revealed for Control groups, the SEM analysis clearly showed the specimens obtained the visual homogenous appearance with smooth topography than different treatments groups without changes in topography compared before and after autoclave disinfection [46]. While, SEM observation findings for laser groups explained by the discharge of laser energy promoted the surface changes such as the material removal due to the punctate nature of the laser induced micro explosions, resulting in the formation of voids, and fusing and melting of the most superficial layer followed by solidification to a smooth blister-like surface [47]. On the other hand, sandblast groups were showed irregularities compared to the untreated specimen; also, remnants of the alumina particles were viewed on the surface that producing micro-retentive areas in which more wettability may occur on the surface, which may cause better flow due to the roughness obtained through them [48].

On the other hand, the obtained result of present study was revealed that the use of autoclave as a disinfection technique did not change the microleakage statistically so the second part of hypothesis was accepted. The design of autoclave depended on the pressure that was maintained by heating liquids above their boiling point for sterilization but the liquid water did not heat above 100 °C in an open vessel, further heating result in boiling but does not raise the temperature of the liquid water. As the container was heated, the pressure raised as a result of the constant volume of the container [49]. This explained the non-significant difference in microleakage between groups with sterilization by autoclave and groups without sterilization by autoclave, that was supported by the SEM images which indicated there was no alteration in morphology of the surface before and after autoclave disinfection for all groups.

One of the advantages of the present study compared with similar studies was the concomitant evaluation of the effect of different surface treatments with and without sterilization by autoclave on microleakage of soft lining material to acrylic resin denture base.

The limitation related to present research was the evaluation of microleakage should be done with more time of aging and autoclave sterilization. The condition of this research was in-vitro which might different from in-vivo conditions including fluctuation in the saliva pH, different percentages of the salivary ions, presence of immunoglobulins, and serum markers in the saliva those factors could affect microleakage.

This study opens new scope for further research such as evaluation of the effect of other types of surface treatments for acrylic denture base on the microleakage between different types of soft liner and acrylic denture base resin and microscopic estimation after surface treatment of the soft liner bonded to acrylic resin.

CONCLUSIONS

Within the limitations of the present study, it was concluded that the sandblast treated group was showed a better reduction in microleakage than the laser-treated group in comparison to the untreated group. As well as, the use of autoclave for disinfection did not change the microleakage between the soft liner and acrylic resin.

Acknowledgments

Non

Funding

This study was self-funded.

Conflict of interest

The authors have no conflict of interest related for this study.

Regulatory Statement

Non

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Date submitted: 2020 Nov 05

Accept submission: 2021 Jan 21