ORIGINAL RESEARCH

An *In-Vitro* Assessment of Surface Roughness, Tensile Bond Strength and Antifungal Activity of Grape Seed Extract-modified Soft Liner

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Abstract

Aim: This study was conducted to evaluate the grape seed extract (GSE)-modified soft liner regarding surface roughness, tensile bond strength to the denture base material, and the antifungal activity.

Materials and methods: The GSE powder was blended with the soft liner powder in ratios of 5 and 10% w/w, and three groups were employed: I, control; II, 5% w/w GSE-modified soft liner; III, 10% w/w GSE-modified soft liner. Evaluation parameters included surface roughness, tensile bond strength to the denture base material, and the antifungal activity. Changes in surface topography were evaluated by scanning electron microscopy. The statistical analysis was performed using the one-way ANOVA followed by the Tukey's test ($\alpha = 0.05$).

Results: The 5% w/w GSE-modified soft liner showed a significant increase in surface roughness, while both ratios (5 and 10% w/w) of the modified-soft liner exhibited significant increase in tensile bond strength and antifungal activity ($p < 0.05$).

Conclusion: The GSE of 10% w/w considerably enhanced the antifungal activity and tensile bond strength of the modified soft liner to the denture base material without compromising its surface roughness.

Clinical significance: The 10% w/w GSE-modified soft liner may be a promising formulation with antifungal activity. It could inhibit fungal adherence and development of fungi-induced lesions or exacerbation of existing ones.

Keywords: Antifungal activity, Grape seed extract, Soft liner, Surface roughness, Tensile bond strength.

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Introduction

Complete dentures are frequently constructed from rigid acrylic resins that have many favorable properties, including satisfactory physicomechanical properties capable of withstanding biting forces, as a consequence resist fracture and distortion.[1] Nevertheless, this rigidity may cause patient discomfort, mucosal lesions, or exacerbation of existing lesions.[2] Several causes have been correlated to the denture-induced stomatitis such as allergy to the denture base material, poor oral hygiene, fungal infection, trauma from occlusion, hematological disorders, and occlusion trauma.[3] Accordingly, resilient denture liners have been developed for restoration of inflamed supporting tissues, severe bone resorption, and providing more stability to the prosthesis.[4] These materials are designed to absorb part of the masticatory forces during function reducing energy transmitted to the underlying tissues.[5] Despite these advantages, soft liners still have certain drawbacks like hardening due to loss of plasticizer, colonization of microorganisms, particularly *Candida albicans*, porosity, poor tear strength, and the failure of bond to denture base.[6] The composition, surface roughness, and micromorphology of tissue-relining materials are important factors to be kept in mind, considering that roughness provides more retention of residues, microorganisms, and pigments that may compromise the longevity of the material.[7]

Preceding studies[8,9] reported that incorporation of antifungal agents into soft liners to compensate for these problems could affect their structural properties and bond strength. Maintaining a good bond of the liner to the denture base ensures the longevity of the liner in service.[10]

Natural products are important sources to be considered for getting chemically standardized extracts for medical applications.[11] Recently, an interest on the grape seed extract (GSE) (*Vitis vinifera*) as an antimicrobial and antifungal alternative is noticed. This extract is rich in naturally occurring polyphenolic compounds and consists of free monomeric flavanols, i.e., the proanthocyanidins (PAs).[12] Proanthocyanidins are a combination of monomers, oligomers, and polymers of flavan-3-ols (known as catechins). They are extensively used as natural antioxidants and free radical scavengers and have been verified to be used safely in many clinical situations.[13] It has been reported that PAs increased collagen synthesis and hastened

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the conversion of the soluble collagen to the insoluble collagen during development.[14] Proanthocyanidin-treated collagen matrices were proven to be nontoxic and resisted enzyme digestion both *in vitro* and *in vivo*.[15] Furthermore, the previous *in vitro* study has suggested that GSE could be a possible agent for the treatment of chronic periodontitis. Its antioxidant properties could make it effective against microorganisms causing periodontitis. Also, it has been considered to reduce collagen degradation and thus could have the potential to hold up the progression of periodontitis.[16]

Although the antioxidant and antimicrobial properties of GSE have been relatively assessed with a reported optimistic impact in the restorative dentistry and periodontology, the application of PAs in the field of dentistry as an active substance is still fairly limited and has few publications up till now. Based on the suggested antimicrobial activity of GSE and the drawbacks accompanied by the denture soft liners, the aim of this study was to assess grape seed extract-modified acrylic soft liner regarding; surface roughness, tensile bond strength to the denture base material and the antifungal activity. The null hypothesis was that GSE would neither improve the antifungal activity of the soft liner nor influence other evaluated properties.

Materials and Methods

A commercial self-cured acrylic soft liner (Acrostone; Acrostone Dental Factory, under the exclusive license of England, Egypt), the heat-cured polymethyl methacrylate (PMMA) denture base material (IQ-15; IMICRYL, Turkey), and the grape seed extract (Nutra Manufacturing, Greenville, South Carolina, USA) were used in the study. The GSE powder was added to the soft liner powder with weight ratios (w/w) of 5 and 10%, and the unmodified soft liner was used as a control. Powders were hand mixed using the glass slab and the stainless steel spatula for 10 minutes to achieve a homogeneous blend, and three assigned groups were considered as follows:

Group I: Unmodified soft liner (control)
Group II: 5% w/w GSE-modified soft liner
Group III: 10% w/w GSE-modified soft liner

Surface Roughness

A total of 15 disc-shaped specimens, 5 specimens for each group, were prepared in a stainless steel mold of 10 mm diameter and 2 mm thickness. Powder and liquid were proportioned and mixed according to the manufacturer's instructions. The mixture was then poured into the mold that was placed over a glass slab; another glass slab was placed over the filled mold and polymerized in accordance with the manufacturer's guidelines. The specimens were removed from the mold and stored in distilled water at 37°C for 48 hours prior to testing. A profilometer (Surftest SJ210, Mitutoyo Corp., Kawasaki, Japan) was used to measure surface roughness according to the ISO 4287-1997. Each specimen was scanned five times, and the mean roughness parameter (R_a) was calculated in µm. The tracing length was 8 mm, at a scanning speed of 0.5 mm/seconds. The resolution of the recorded data was 0.01 µm.[17]

Tensile Bond Strength to Denture Base Material

A total of 15 dumbbell-shaped specimens (5 for each group) of 50 mm length, 12 mm diameter at the thickest section and 7 mm at the thinnest section, were prepared in a splited stainless steel mold. The mold was positioned vertically resting on a glass slab; base plate wax was softened and poured into the mold. The wax was pressed at the top of the upper compartment of the mold with another glass slab and a weight of 1 kg over it to expel the excess material until the wax was leveled with the edge of the mold. Upon cooling to room temperature, the wax pattern was carefully removed. The wax specimens were embedded in a dental stone in a dental flask followed by immersion of the flask in boiling water for 5 minutes. The flask was opened and the mold cavity was rinsed with boiled water for elimination of the wax remnants.

Mixing of the polymer and monomer of Acrostone heat-cured PMMA resins was performed in a glass jar with the recommended ratio of 3:1 (by volume) in line with the manufacturer's instructions. Packing of the dough in the mold cavity, trial closure, curing, deflasking, and minor finishing and polishing according to the manufacturer's recommendations were done. The specimens were cut into two equal halves, in which 3 mm were removed from the thin middle section using a water-cooled diamond edge saw. The sectioned specimens were secured back into the mold used for preparation of the wax specimens. Finally, the powders of assigned groups were mixed with the liquid and used for relining the sectioned specimens and polymerized according to the manufacturer's instructions. The specimens were stored in distilled water at 37°C for 48 hours prior to testing.[18]

Tensile load was applied to the specimens using the Universal Testing Machine (Model 2006, Instron Corp, 5500 R, England) at a cross-head speed of 5 mm/minute. Tensile stress (S) was calculated by the following equation and expressed in MPa:[18]

$$S = F / D$$

where F is the maximum force and D is the cross-sectional area of the strained specimen.

Antifungal Activity

A total of 15 disc-shaped specimens of 8 mm diameter and 2 mm thickness were prepared (5 specimens each group). The antifungal activity was investigated using the agar diffusion test. The *C. albicans* was cultured from clinical samples and kept overnight in a specific culture media at 37°C. A base layer containing 15 mL of agar mixed with 100 µL of inoculum was prepared in a sterilized petri dish (100 mm diameter) at pH of 7.5. After the solidification of the culture medium, discs of the different assigned groups were transferred to the plates and incubated at 37°C for 48 hours. The positive control was included in each plate. Such control was composed of a sterile cellulose paper (8 mm) that is impregnated with fluconazole (5 µg/disk) as an antifungal agent. The diameters of the inhibition zones surrounding the specimens were measured in mm at three different points, and the average value was considered to be the mean inhibition zone value (mm).

Data were collected from the tests and analyzed using the one-way ANOVA; groups were subsequently compared using the Tukey's test at the level of significance $p \leq 0.05$.

Scanning Electron Microscopy

The scanning electron microscope (SEM) (JSM 6300, JEOL, Japan) was used to examine the surface morphological changes of randomly selected specimens of each studied group; three specimens were examined for each group. Specimens were first sputtered with gold for better image resolution and to avoid electrostatic charging prior to the analysis, which done at a magnification of 10,000×.

Results

Surface roughness means (µm) of the studied groups are shown in Table 1. The soft liner modified with 5% w/w GSE exhibited significantly higher surface roughness (4.07 ± 0.57) than the

unmodified one (3.3 ± 0.52) ($p < 0.05$). On increasing the ratio of modification to 10% w/w, the surface roughness decreased (3.28 ± 0.26) to be comparable to that of the control group with no significant difference detected between them (groups I and III) ($p > 0.05$).

Mean tensile bond strengths (MPa) to the denture base material are shown in Table 1. Both 5 and 10% w/w modified soft liners significantly increased the bond strength to 1.01 ± 0.032 and 1.22 ± 0.11, respectively ($p < 0.05$). Additionally, a significant difference was recognized between group II and group III ($p < 0.05$).

Inhibition zones means (mm) of the studied groups are shown in Table 1. Increasing the percentage of the extract used to modify the liner to 10% w/w significantly increased the antifungal activity (7.7 ± 0.82) than did group II, which also caused a significant increase (2.7 ± 0.032) compared to the unmodified liner ($p < 0.05$).

A graphical presentation of surface roughness, tensile bond strength and antifungal activity results is shown in Figure 2.

Table 1: Means, standard deviations, and the Turkey's analysis of the studied properties of the GSE-modified soft liner

	Surface roughness (μm)	*Tensile bond strength (MPa)*	*Antifungal activity (mm)*
Group	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*
I (control)	3.3 ± 0.52^{b}	0.87 ± 0.03^{c}	0^{c}
II (5% w/w GSE-modified soft liner)	4.07 ± 0.57^{a}	1.01 ± 0.032^{b}	2.7 ± 0.032^{b}
III (10% w/w GSE-modified soft liner)	3.28 ± 0.26^{b}	1.22 ± 0.11^{a}	7.7 ± 0.82^{a}
p value	0.03	<0.0001	0.0001

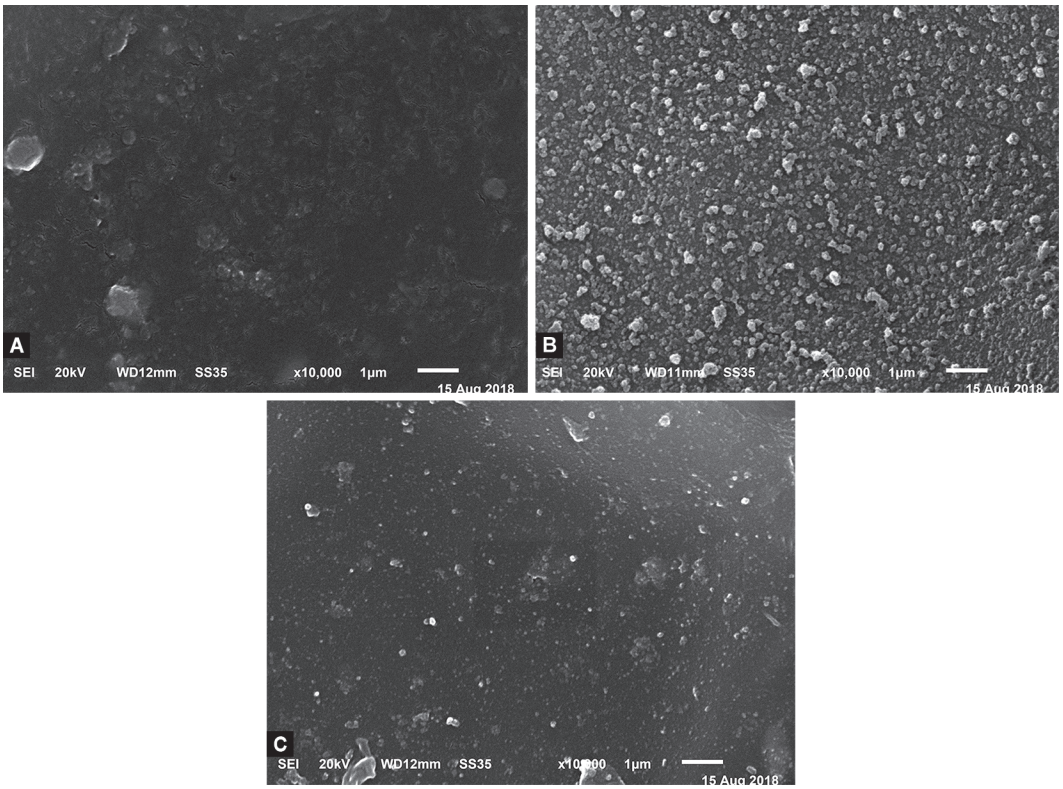
The values with different superscript letters within the same column are significantly different at $p < 0.05$. (GSE, grape seed extract)

SEM

Scanning electron micrographs of the assigned groups are shown in Figures 1A to C. In Figure 1A, the polymer matrix with the characteristic polymer interconnections can be seen on the surface of the control specimen with much smoother surface compared to that of group II. In Figure 1B (group II), specimens showed widely dispersed GSE particles within the polymer matrix with a predominantly rough surface. The SE micrographs of group III specimens (Fig. 1C) showed less surface irregularities and roughness indicating a more uniform distribution of the extract within the surface, fairly minimizing the pores within the polymeric matrix and forming a more even surface layer.

Discussion

The surface roughness of a relining material is of significance as it, directly or indirectly, influences the retention of microorganisms, staining, plaque accumulation, as well as oral tissue health and patient comfort.[19,20] The inclusion of antifungal agents into soft lining materials has been shown to be effective through extending their longevity and reducing the biofilm accumulation. The composition of soft liners should be kept in mind, particularly those with antifungal activity accompanied by maintained oral hygiene, which may counteract the slightly increased surface roughness.[21]



Figs 1A to C: Scanning electron micrographs showing the surface of: (A) Unmodified soft liner specimen (control); (B) The 5% w/w GSE-modified soft liner specimen; (C) The 10% w/w GSE-modified soft liner specimen

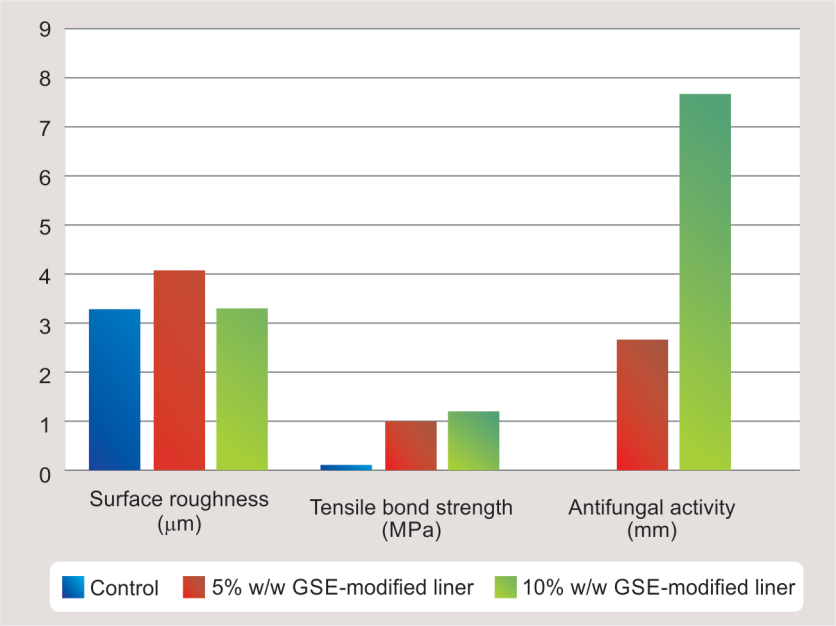


Fig 2: Graphical presentation of the surface roughness, tensile bond strength and antifungal activity results. (GSE, grape seed extract)

In the present study, the null hypothesis was rejected, as the soft liner modified with 5% w/w has significantly higher surface roughness compared to the unmodified liner. It was recognized that GSE particles scattered within the polymer matrix in such manner increased the surface roughness of the 5% w/w GSE-modified soft liner. Other contributing factors that may increase the material's roughness are the particle size, distribution, and concentration of the inclusions (in which higher concentration may cause more uniform distribution within the smaller spaces between the matrix particles as in 10% w/w compared to 5% w/w GSE modification).[8,21] These findings are in agreement with the study[22] carried out by Bueno et al., which concluded that surface roughness of soft liners may be increased by antifungal agents such as itraconazole. Scanning electron micrographs are consistent with the results of the surface roughness and confirmed the greater surface roughness of the 5% w/w GSE-modified soft liner.

Regarding the tensile bond strength, acrylic soft liners are known to have excellent adhesion to the denture base resins compared to silicone-based liners. The adhesion failure may lead to the formation of an area where maintaining hygiene is difficult, which may affect the prosthesis longevity.[23,24] The GSE contains monomeric flavanols, and these monomeric compounds may cause more dissolution of the denture-fitting surface, which may facilitate the diffusion of monomers from the lining material to the denture base forming an interwoven network and thus increasing the bond strength.[25,26] Moreover, several studies[27,28] confirmed that PA is a naturally occurring cross-linking agent and it can link different monomeric matrices, so it may link the matrix of the liner to the matrix of the denture base, thereby promoting adhesion. On the other hand, this finding is in disagreement with another study,[29] which revealed that the decrease in the bond strength of soft liners to the denture base material could be attributed to the cross-linking ability of the liner. The high cross-linking of the resins counteracts the diffusion process of the liner monomers into the denture base and adversely affects bond strength. Also, several factors should be taken into account when explaining the bond strength results. Among these factors is the rate of diffusion, which may be influenced by the cross-linking ability of the modified liner and time available for diffusion.[30,31] Accordingly, a recent study[32] suggested dichloromethane as an effective treatment to increase the bond strength between cross-linked resins and denture base resins through surface dissolution and the roughness creation for increasing the surface area for bonding and mechanical retention.

Candida albicans is considered the most prevalent oral fungus, causing denture stomatitis.[33] In recent decades, there has been an increasing focus on the natural antimicrobial products for both oral hygiene and the prosthesis targeting the prevention of the prosthetic stomatitis.[34] A recent study[35] by Simonetti et al. verified that GSE exhibited high antifungal activity against various strains of *Candida* both *in vivo* and *in vitro*. In the current study, both concentrations of GSE used to modify the soft liner showed antifungal activity that was more obvious in the 10% w/w GSE-modified soft liner. The results are in harmony with other studies[36,37] which confirmed that GSE can be used as an alternative against *Candida* and that results may vary according to the type of the phenolic compounds of the extract. Also, the fungi species and the method of cultivation should be considered as influencing factors to the results. Polyphenolic compounds of GSE proposed to have the potential to cause cell wall damage of C. albicans and induced cell apoptosis by destruction of mitochondria, and this may explain the strong antifungal activity of the GSE.[38,39] The findings are in line with another study[40] which concluded the possibility of using GSE against fungal infections instead of different chemicals which may have side effects.

Conclusion

Based on the results and within the limitations of this *in vitro* study, the 5% w/w modified acrylic soft liner exhibited antifungal activity with improved adhesion to the denture base material; however, it adversely affected the surface roughness. This increase in roughness would permit the accumulation of microorganisms and debris. Conversely, GSE of 10% w/w considerably enhanced the antifungal activity and tensile bond strength of the modified soft liner to the denture base material without compromising its surface roughness.

Recommendations

As a natural antifungal product, GSE might be used to generate clinically effective dental formulations to maintain oral health particularly for patients with removable prosthodontics. An assessment of the long-term antifungal activity of the modified liner is proposed. Further supporting studies to evaluate biocompatibility of the modified liner with the oral tissues and other properties, such as water sorption and resilience, should be considered.

Compliance with Ethical Standard

Ethical Approval

All procedures performed in studies were in accordance with the ethical standards of the institutional research committee. The study was approved by the institutional review board ST/54/2018.

Consent for Publication

The author has approved the manuscript and agrees with submission. I confirm that this manuscript is my original unpublished work and has not been published or under consideration somewhere else.

Availability of Data

All data presented or analyzed during this study are included in this article.

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