ORIGINAL RESEARCH

Curcumin Containing Soft Liner as an Alternative Treatment Modality for Oral Candidiasis

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Abstract

Aim and objective: Recently, herbal treatment has been validated to be a safe and effective alternative to antimicrobial drugs since it has minimal or no complications. Consequently, this study was conducted to evaluate the antifungal activity of soft liner/curcumin formulation considering its surface roughness, and tensile bond strength to the denture base.

Materials and methods: Ethanolic extract of curcumin was prepared in a concentration of 10%. The curcumin solution was blended with the soft liner liquid in ratios of 10 and 20 v/v%. Two experimental groups were considered besides the control (unmodified liner). A total number of 90 specimens was prepared for evaluating antifungal activity, surface roughness, and tensile bond strength of the modified soft liner to the denture base resin. ANOVA and Tukey *post hoc* tests were used for the statistical analysis at a level of significance of 0.05.

Results: 20 v/v% curcumin-modified soft liner exhibited a significant decrease in surface roughness compared with the unmodified liner. Both experimental ratios of curcumin; 10 and 20 v/v%, significantly enhanced both tensile bond strength of the liner to the denture base and antifungal activity.

Conclusion: Curcumin has the qualities of a natural potent antifungal agent against oral candidiasis paired with denture soft liners. Moreover, its positive impact on surface roughness reduction and the increase in adhesion potential of the soft liner to the denture base has been verified.

Clinical significance: The incorporation of curcumin into the denture soft liner seems to be a better alternative for oral candidiasis treatment to the incorporation of conventional antimicrobial drug regarding its antifungal activity, adhesion to the denture base, and surface roughness.

Keywords: Antifungal activity, Curcumin, Oral candidiasis, Soft liner, Surface roughness.

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Introduction

Denture stomatitis (DS) or oral candidiasis is the most common oral lesion observed in 65% of patients wearing removable dentures. It is most related to the use of removable dentures and identified as inflammation of soft tissues covered by dentures.[1,2] Its main etiological factor is infection by *Candida* species, especially *Candida albicans*. Other local factors related to the denture are also correlated to this pathological condition as the presence of biofilm,[3,4] local trauma caused by ill-fitting dentures,[5] xerostomia,[6] dentures continuous wear, and changes in salivary pH.[5]

Numerous treatment modalities are used for DS and may include topical antifungal and systemic therapy, oral hygiene care, cleaning and disinfection of dentures, old dentures replacement.[7] However, re-infection of the treated oral mucosa may occur after treatment, and is attributed to the survival of *Candida* species due to insufficient concentration of the antifungal agent on the denture surfaces.[8] Furthermore, the occurrence of drug-resistant fungi and the toxicity of existing drugs[9] are also related to the treatment of DS. Unpleasant taste and the need for frequent use of DS treatment led to patient discomfort and lowering his compliance.[10] Therefore, it is essential to adopt other treatment modalities for DS by using a natural antimicrobial agent.

As ill-fitting dentures can also cause DS, resilient denture liners may be considered a long-term solution, providing a cushion-like effect to the injured oral mucosa.[11] Meanwhile, these materials are easily degradable and liable to microbial colonization, which may promote DS.[12]

Peeling of the resilient material from the denture base has been reported as the cause of clinical failure of the resilient liner to adequately perform its function of recovering the tissues injured by trauma.[13]

To solve this problem, the incorporation of antimicrobial agents into these resilient denture liners has been adopted[14] and is effective and viable both in *in vitro* and *in vivo* studies.[14–16]

Curcumin is derived from the underground stems of *Curcuma longa*. It is an active ingredient of turmeric with yellow coloration. It is used as a spice and as a medicinal herb for treatment modalities.[17] It is characterized by the antioxidant and antimicrobial properties of

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its naturally occurring phenolic compounds. Turmeric extract may inhibit fungal growth by morphology alteration, plasma membrane disruption, and mitochondrial destruction.[18] They also have anti-inflammatory,[19–21] anticancer,[19,20,22] hepatoprotective, anti-allergic, wound healing, antispasmodic,[23] and anti-HIV properties.[23,24] Despite these therapeutic advantages, the physical properties of polymeric materials like resilient liners, may be affected by the incorporation of drugs into these materials.

Thus, this study aimed to evaluate the effect of the addition of natural antimicrobial agents (curcumin) to a resilient liner regarding its surface roughness as well as its tensile bond strength to the denture base resin and its antifungal activity. The hypothesis investigated in this study was that the addition of curcumin to a resilient denture liner would result in changes in the surface roughness as well as its tensile bond strength to a denture base acrylic resin.

Materials and Methods

A resilient acrylic denture relining material; Trusoft (Bosworth Co, Midland, TX, USA), heat-cured polymethyl methacrylate (PMMA) denture base material (IQ-15; IMICRYL, Turkey), and curcumin powder (Sigma Chemical Company, St. Louis, MO, USA) were utilized for preparing the specimens of this study. Each one of the two concentrations of ethanolic extract of curcumin (10 and 20 v/v%) were prepared by dissolving 10 and 20 g of curcumin powder in 100 mL of pure ethanol, respectively, and the solutions were placed on the shaker for 48 hours. Then, the extracts were filtered through the Whatman filter paper. The filtration solutions were incubated at 37°C for 48 hours.

The ethanolic extracts of curcumin were then blended with the liquid of the soft liner in ratios of 10 and 20 v/v% and the mixtures were kept on the stirrer for 24 hours. The soft liner powder was mixed separately with the two experimental formulations of the liquid and the unmodified liquid; served as a control. Accordingly, the following three assigned groups were considered in the study;
Group I: Control (unmodified monomer).
Group II: 10 v/v% curcumin-modified soft liner.
Group III: 20 v/v% curcumin-modified soft liner.

A total number of 90 specimens were prepared for estimation of surface roughness, antifungal activity, and tensile bond strength to the denture base. For each test, 30 specimens were specified, 10 specimens for each group. For surface roughness and antifungal activity tests, powder and liquid of each group were mixed according to the manufacturer's instructions. The mix was poured in a stainless-steel mold rested on a glass slab and another slab was placed over the mix and maintained under pressure till curing. Careful removal of the specimen from the mold and smoothening of the edges was performed. Specimens were kept in distilled water at 37°C for 48 hours before testing.

Surface Roughness

The 30 specimens assigned for evaluation of surface roughness were prepared in a stainless-steel mold of 10 mm diameter and 2 mm thickness. The assessment was performed using a surface roughness tester; a profilometer (Surftest SJ-301; Mitutoyo Corporation, Kanagawa, Japan) standardized with a 0.8-mm cut-off and speed of 0.5 mm/second, resulting in 4.0 mm distance. Five measurements were recorded for each specimen and the average surface roughness (Ra) was calculated and expressed in µm.

Tensile Bond Strength to the Denture Base

Thirty dumbbell-shaped heat-cured acrylic resin specimens of 50 mm length, 12 mm diameter at the thickest section, and 7 mm at the thinnest section were prepared. A split stainless-steel mold was located vertically resting on a glass slab; filled with softened base plate wax. Another glass slab with a weight of 1 kg over it was placed over the top of the upper compartment to expel the excess wax until the wax was leveled with the mold frame. Careful removal of the wax on cooling to the room temperature was applied. Wax specimens were flasked and then wax elimination was performed. The powder and liquid of the heat-cured acrylic resin were mixed, packed, cured, finished, and polished according to the manufacturer's instructions. Each specimen was sectioned into two halves by removal of 3 mm from the middle section using a water-cooled diamond saw. The two sections of each specimen were secured back into the mold and relined by the soft liner. For each group of a soft liner, the powder was mixed with the liquid and used for relining the sectioned specimens. The specimens were stored in distilled water at 37°C for 48 hours before testing.

Specimens were subjected to tensile load using the Universal Testing Machine (Model 2006, Instron Corp, 5500 R, England) at an across-head speed of 5 mm/minute. Tensile strength (*S*) was calculated by the subsequent equation and expressed in MPa:

$$S = F / D$$

where *F* (*N*) is the maximum force and *D* (mm^2) is the cross-sectional area of the specimen.

Antifungal Activity

Agar diffusion test was used for the assessment of antifungal activity. In this test, representative disc-shaped specimens from the tested groups of 8 mm diameter and 2 mm thickness were prepared. The fungal strain *C. albicans* used in this study was kindly provided from the Microbiology and Immunology Department, Faculty of Pharmacy, Mansoura University, Egypt. A Sabouraud dextrose agar was used to incubate *C. albicans* that were kept in the culture media overnight at 37°C. Specimens of each group were placed in a 100-mm Petri dish containing a solidified base layer of 15 mL agar mixed with 100 µL inoculums at pH 7.5. The specimens-containing Petri dishes were incubated at 37°C for 48 hours. A sterile cellulose paper (8 mm) was impregnated with the prepared curcumin solution and fluconazole antifungal agent (5 µg/disk) that served as controls. The diameters of the inhibition zones surrounding each specimen were measured in mm at three different points, and the mean value was the mean inhibition zone (mm).

Fourier Transformation Infrared Spectroscopy (FTIR)

The ethanolic extract of curcumin was investigated for its chemical structure using a spectrometer (Nicolet iS10, USA). The representative sample of the extract was mixed with KBr and examined for detecting chemical groups. The Fourier transformation infrared spectroscopy (FTIR) data were recorded in the range of 500–4,000 cm^{-1}.

Data were collected from the different tests and analyzed using one-way ANOVA followed by the Tukey test at $p \leq 0.05$.

Results

Means and standard deviations of surface roughness (µm) are shown in Table 1. The unmodified soft liner group (group I)

Table 1: ANOVA and *post hoc* test results of the studied parameters

Groups	*Surface roughness (µm)*	*Tensile bond strength (MPa)*	*Antifungal activity (mm)*
Group I: unmodified soft liner	3.98 ± 0.58^{a}	0.40 ± 0.024^{b}	0^{e}
Group II: 10 v/v% curcumin-modified soft liner	$3.3 \pm 0.52^{a,b}$	0.52 ± 0.024^{a}	6 ± 0.79^{d}
Group III: 20 v/v% curcumin-modified soft liner	2.53 ± 0.48^{b}	0.55 ± 0.058^{a}	8.2 ± 0.57^{c}
Curcumin solution	–	–	12 ± 0.49^{b}
Fluconazole	–	–	20.3 ± 1.2^{a}
p value	0.0027	0.0001	0.0001

Means with the same superscript letters are not significantly different at $p \leq 0.05$

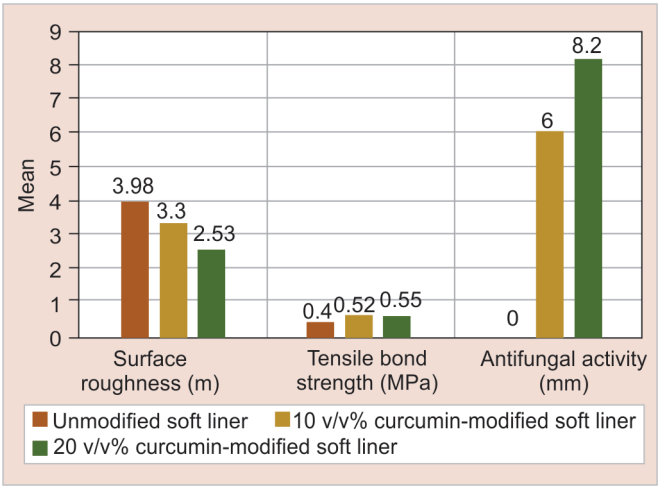

Fig. 1: A graphical presentation of the assessed parameters results

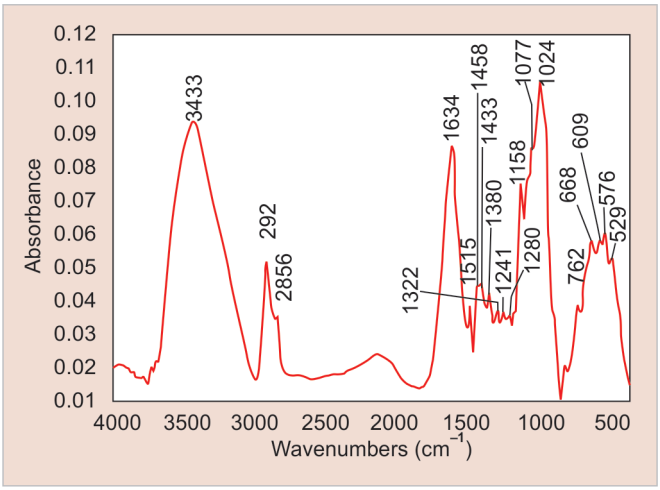

Fig. 2: FTIR spectra of the ethanolic extract of curcumin

exhibited the highest mean value (3.98 ± 0.58) while group III; 20 v/v% curcumin-modified soft liner had the lowest value (2.53 ± 0.48). ANOVA indicated a significant difference among groups ($p \leq 0.05$). *Post hoc* test revealed that there was a significant difference between group I; the unmodified soft liner and group III; 20 v/v% curcumin-modified soft liner. On the other hand, group II; 10 v/v% curcumin-modified soft liner was not significantly different from both groups I and III.

For the tensile bond strength (MPa), the results are presented in Table 1. The highest mean value (0.55 ± 0.058) belonged to group III; 20 v/v% curcumin-modified soft liner while the lowest value (0.40 ± 0.024) was for group I; the unmodified soft liner. A significant difference was detected by ANOVA ($p \leq 0.05$). Tukey test showed that both 10 and 20 v/v% curcumin-modified-soft liner were significantly different from the control group. Alternatively, no significant distinction was identified between groups II; 10 v/v% curcumin-modified soft liner and III; 20 v/v% curcumin-modified soft liner.

Antifungal activity (mm) results are displayed in Table 1. The prepared curcumin solution (10%) exhibited high antifungal activity (12 ± 0.49). On incorporation of curcumin into the soft liner, group III; 20 v/v% curcumin-modified soft liner, demonstrated the highest mean value (8.2 ± 0.57) compared with both the control group; the unmodified soft liner, which did not exhibit antifungal activity, and group II; 10 v/v% curcumin-modified soft liner, that showed a lower value (6 ± 0.79). ANOVA noted significance among groups ($p \leq 0.05$). *Post hoc* test illustrated that all tested groups were significantly different from each other.

A graphical presentation of surface roughness, antifungal activity, and tensile bond strength to the denture base is shown in Figure 1. The FTIR spectra of the ethanolic extract of curcumin are presented in Figure 2. Curcumin's IR spectra showed stretching vibrations at 1,634 cm^{-1}, which are mostly due to the overlapping stretching vibrations of alkenes (C=C) and carbonyls (C=O). Curcumin infrared spectra revealed stretching vibrations at 3,433 and 3,050 cm^{-1} attributable to O–H and C–H groups. Both $-CH_3-$ and $-CH_2$ asymmetric stretching was noted at 2,959 and 2,924 cm^{-1}, respectively. The C=C aromatic stretching vibrations at 1,589 cm^{-1} and the high-intensity band at 1,515 cm^{-1} were attributed to benzene ring bending vibration. Additionally, both CH_2 and CH_3 bending were noted at 1,433 and 1,380 cm^{-1}, respectively. The bending vibration of the (C–O) phenolic band was related to a significant intense band at 1,158 cm^{-1}.[25,26]

Discussion

Herbal treatment is a very trustworthy alternative to antimicrobial agents' drugs since it has little to no adverse effects. As a result, medicinal plant extracts are becoming more prevalent around the world, and extensive research is being conducted on this topic regularly to ensure its biological safety.[27] Accordingly, this investigation was based on the coupling of curcumin as a natural herbal remedy into resilient denture liner with the evaluation of the antifungal activity of the new formulation and other important properties as the surface roughness and bond strength to the denture base resin.

The null hypothesis of this study was completely rejected as all parameters investigated were influenced. The relining material should have a smooth surface to facilitate cleaning, counteract the biofilm formation, and the subsequent oral mucosa inflammation.[28] Although different *in vitro* investigations recommended 0.2 µm as

the threshold of roughness values, most materials tested were not found to accomplish this criterion, particularly those supplied as powder and liquid. When the powder and liquid are mixed, the air bubbles may be incorporated and trapped near the surface causing higher surface roughness values, and this may explain the higher surface roughness value of the unmodified liner.[29,30]

Curcumin typically includes 0.76% alkaloid, 0.45% saponin, 1.08% tannin, 0.03% sterol, 0.82% phytic acid, 0.40% flavonoid, and 0.08% phenol in its composition, and both flavonoids and phenolic compounds are known to have a plasticizing effect.[31] The inclusion of an extra plasticizer into the resilient liner beside the plasticizer originally involved in its composition could increase the viscoelastic behavior of these plasticized resins. This enables the material flow over time with a corresponding occlusion of the porosities and irregularities within the liner surface, hence, generating lower surface roughness values.[32,33] This plasticizing impact seems to be stronger in 20 v/v% curcumin-modified soft liner. The results may be inconsistent with Aref,[34] who concluded that the grape seed extract; a compound containing flavonoids and phenols, increased the surface roughness of the soft liner when incorporated into it at a lower concentration (5 wt%) and decreased it insignificantly at its higher concentration (10 wt%). Although acrylic resin-based relining materials have great adhesion to denture base resins, the deterioration of adhesion between the denture base and liner results in the formation of difficult-to-clean areas, which can lead to both fungal and bacterial adhesion and proliferation. This proliferation may risk the prosthesis' durability and increase its surface roughness.[35,36]

The results of the tensile bond strength to the denture base resin seem to be very consistent with the roughness findings. The presence of the flavonoids and phenolic compounds with their plasticizing effect and their ability to cause viscoelastic behavior and continuous flow of the plasticized resins may clear up the results. This flow may allow the relining material to diffuse into the denture base resin, with a corresponding interwoven network formation.[32,33]

Furthermore, flavonoids may soften the denture fitting surface, thus enhancing diffusion, and cross-linking between the denture resin and the relining resin.[37–40] The results are in accordance with another study[34] revealing that modification of the soft liner with 10% w/w grape seed extract considerably increased its tensile bond strength to the denture base owing to grape seed flavonoid content. However, another study[41] conflicts with our findings in terms of bond strength as it concluded that the incorporation of various antimicrobial agents in different concentrations into a resilient liner did not change its bond strength to the denture base resin.

Fungal colonization, particularly *C. albicans*, is the most prevalent issue associated with the use of denture soft lining material, and alongside plaque accumulation, inflammation, and infection of the denture bearing area will occur.[42] In this investigation, a soft liner with antifungal activity was developed by formulating curcumin/soft lining material. Both tested concentrations of curcumin (10 and 20 v/v%) were able to make antifungal activity to the soft liner, however, this effect appeared to be concentration-dependent. The ethanolic extract of turmeric was chosen as it contains a variety of chemical components such as phenolic compounds and derivatives, weak acid esters, fatty acid esters, terpenes, and others. It has been verified that crude extracts contain a vast number of distinct chemical compounds; accordingly, they can affect various target sites in the fungus.[43]

Antifungal activity of curcumin is dependent on different strategies as morphology alteration and collapsing of the hyphae, disrupting the plasma membrane, mitochondrial destruction, lack of cytoplasm, folding of the nuclear membrane, and thickened cell wall.[17] Curcumin was evidenced to be a more effective antifungal agent than fluconazole by a previous study.[44] The study concluded that curcumin significantly reduced *Candida* species (isolated from AIDS patients) adhesion to buccal epithelial cells, suggesting that curcumin is a very promising compound for therapeutic use in patients with immuno-compromised diseases.

Alalwan et al. revealed that curcumin influenced immature morphological forms (yeast and germlings) more favorably and actively supported cell aggregation. The analysis disclosed that curcumin has anti-adhesive properties and induces transcription of genes involved in biofilm formation processes. Curcumin and associated polyphenols may thus be formulated for use in oral healthcare to supplement existing preventive strategies for *Candida* biofilms on denture surfaces.[45] The results are in harmony with the outcome determined by Murugesh et al., who reported that curcumin's antifungal properties are undeniable. At lower concentrations, it has static effects and at higher concentrations, it exhibits fungicidal effects.[46] Another sustaining study[47] indicated that methanol extract of turmeric demonstrated antifungal activity against *Cryptococcus neoformans* and *C. albicans* with minimum inhibitory concentration values of 128 and 256 µg/mL, correspondingly. Additionally, one of the major complications during therapies against chronic asthma is oropharyngeal candidiasis, and curcumin was detected as a prospective target for the treatment of candidiasis with anti-inflammatory activity.[48] This study has more than one limitation which is the use of a limited number of concentrations of curcumin besides the lack of investigating the impact of curcumin addition on soft liner resilience as well as surface hardness. One of the future perspectives of this study is to compare the effect of curcumin incorporation into soft liners to those of other types of medicinal herbs.

Conclusion

Based on the results and within the restrictions of this study, this investigation supported that curcumin is a very powerful natural herb that can develop an antifungal activity when coupled with different dental or medicinal materials. Resilient liner/curcumin at a certain concentration of 10 v/v% is a very hopeful formulation of denture soft liner with enhanced adhesion to the denture base, potential antifungal activity together with a decrease in surface roughness.

Ethical Approval

All procedures performed in studies were following the ethical standards of the institutional and/or national research committee.

Consent for Publication

The authors have approved the manuscript and agree with the submission. The authors confirm that this manuscript is our original unpublished work and has not been published or under consideration for publication somewhere else.

Availability of Data

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

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