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Antifungal agent effects on soft denture liners physical characteristics and ability to inhibit *Candida albicans*

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ABSTRACT

Objectives: Denture soft liners enhance comfort for patients with fungal denture syndrome by cushioning the tissue surface. Integration of them with antifungal agents further improves treatment outcomes. This study evaluates the drug release and antifungal efficacy of three antifungal agents - posaconazole, voriconazole, and chlorhexidine, incorporated into a resin-based denture soft liner (COE-Soft) at 1% concentration. The impact on antifungal activity, drug release, and the physical properties of the liner, including water sorption, color stability, and degree of conversion (DC), was assessed.

Methods: 240 samples were divided into two groups of 120 each. The samples were evaluated for antifungal activity against Candida albicans, drug release profiles, water sorption, color stability, and DC. Minimum inhibitory concentrations (MIC) were determined for each agent: posaconazole, voriconazole, and chlorhexidine.

Results: The MIC values were 0.8 µg/ml for posaconazole and voriconazole, and 25 µg/ml for chlorhexidine against Candida albicans. Chlorhexidine showed the highest release rate among the agents, followed by posaconazole and voriconazole. It improved the DC and color stability of the liner, while posaconazole and voriconazole decreased these properties and increased water sorption. Voriconazole exhibited the highest mean antifungal activity (35.73 ± 1.83), followed by posaconazole (21.8 ± 1.37) and chlorhexidine (13.73 ± 3.03).

Significance: Chlorhexidine enhances the physical properties of denture soft liners but has a higher MIC. Posaconazole and voriconazole exhibit stronger antifungal activity, though they compromise physical properties such as water sorption and color stability.

KEY WORDS

Degree of conversion;
Water sorption;
Chlorhexidine;
Voriconazole;
Posaconazole;
Soft denture liners

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Introduction

Resilient liners are denture soft liners that form a comfortable layer between the patient's denture base and the intaglio surface of the oral mucosa [1]. They act like shock absorbers, distributing oral mastication forces to the underlying tissues. Soft denture liners enhance the patient's comfort, especially for those with thin atrophic ridges and abused tissues due to prolonged misuse of hard dentures [2]. They are also used to manage patients with excessive residual ridge resorption, for immediate obturators for maxillofacial defects, and patients with xerostomia [2, 3]. Other uses of soft liners have evolved in the past few years for the management of carcinoma cases and to alter the transitional prostheses to a much definitive prosthesis [4, 5]. The primary issue with soft-lined dentures is the low bond strength between the liner and the hard base [6].

Soft liners were developed considering patient comfort. However, changes in the material that affect its color, solubility, absorption, or hardness negatively affect the denture's function. The durability of soft liners may be hampered by the development of these negative attributes. The soft-liner materials used for temporary purposes due to their limited clinical use include properties like low resilience, water sorption, color changes, and low bond strength between the denture base and the liner material, all of which may further lead to microbial contamination, thereby affecting the properties of the material [4]. Candida albicans is a common etiological agent responsible for the occurrence of denture-induced stomatitis. It manifests as an erythematous area of tissue, which outlines the overlying denture with a prevalence of about 66% among the geriatric age group [6-9].

Treatment includes application of antifungal agents over the affected mucosa or the intaglio surface of the denture [10-12]. Posaconazole and Voriconazole are new triazole antifungal agents best considered for the effective management of systemic candida infections, especially in treating recurrent candida infections [10, 11]. A long-term healing effect can be achieved by using the local drug conveyance mechanisms in which higher systemic doses are replaced by a steady release of their counterpart lower dose [10, 13]. Although using local drug delivery systems with a polymer as a base has produced promising results, it has been shown to impact denture lining materials, especially its physical properties. The incorporation of an antimicrobial chemical into a resin-based liner has been shown to affect polymerization, which relates to the degree of conversion (DC) of that material, altering its mechanical properties [13]. Fourier transform infrared (FTIR) spectroscopy has previously been employed to quantitatively assess the identification and tracking of setting reactions and polymerization in various dental materials [14, 15].

For the durability of the liner, analyzing the color stability aids in assessing the clinical properties of most dental materials, and such changes can be an indicator of aging or damage to materials. A material with a color change invariably reflects its property of water absorption [16-18]. Resin-based liners display a wide range of changes in properties when they are immersed in water [19]. With increased water sorption and solubility, there is a decline in the mechanical properties of

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the soft-liners, like hardness, fatigue limit, and transverse strength. Dimensional changes such as color change and separation from the denture base occur with bonding failure due to higher water sorption and hydrophilicity. Thus, discoloration and water sorption properties are of paramount importance to assess the functional properties of a soft liner [7, 20].

A room-temperature curing system was formed by P.D. Riggs et al.'s analysis of various distinct monomeric methacrylate powders [21]. The amount of product obtained from tetrahydrofurfuryl methacrylate-based compounds significantly exceeded that from other methacrylate monomers when 5.625% chlorhexidine diacetate was used. This appears to be caused by the polymer's channel formations. Additionally, it demonstrated how doping a polymer with chlorhexidine slowed down the polymerization process, increasing the amount of residual monomer and components with low molecular weight that leached out of the polymer [21]. Five acrylic denture-based resins and a denture-fixing resin were evaluated for color stability using accelerated aging settings. For 100 and 300 hours, five examples of each material were processed and aged. Color was measured before and after weathering, and the color difference (ΔE) was calculated for each sample at 100 and 300 hours. The color of the materials changed after 300 h. Lucitone Hy-pro and Acron demonstrated the lowest degree of color loss under accelerated aging circumstances and the lowest level of color stability among the compounds [22]. Through the use of an in vitro accelerated aging test, Anil et al. ascertained the color stability of two auto-polymerizing and three heat-polymerized soft denture lining materials [23]. Soft denture liners that have been auto polymerized have been shown to alter color over time following the National Bureau Standards, indicating their low color stability. The color stability of auto-polymerizing soft denture liners was variable. However, the color stability of soft denture liners made from heat-polymerization was comparable [23]. Recent research has focused on antifungal agents and their impact on denture liners. Some examples of recent works are mentioned here.

Neppelenbroek et al. studied how antifungal drugs' minimum inhibitory concentrations (MICs) affected the highest possible tensile force of temporary soft denture liners for the Candida albicans biofilm (SC5314) [24]. After 14 days, no significant difference ($p > 0.05$) was observed in the tensile strength of the materials between the groups modified with miconazole and itraconazole and the other groups, which had significantly reduced tensile strength. Miconazole and itraconazole were applied to both materials and after 7 and 14 days in water, the results showed very low elongation percentages than the other antifungal substances and control ($p < 0.0001$), which were comparable to each other ($p > 0.05$) [24].

A temporary denture relining material with silver-sodium-hydrogen-zirconium phosphate was studied by Chladek et al. for its antifungal and mechanical properties [25]. The sub-micrometer filler was found to improve antimicrobial resistance and preserve the material's key properties. Antifungal medications significantly aid the management of fungal infections related to denture liners. Denture liners are prone to fungus growth due to their perpetual contact with oral tissues and a wet environment. Fungal infections, such as candidiasis (oral thrush), can be prevented or treated in denture wearers by adding antifungal medications to denture liners. Antifungal medications aid in preventing fungal growth on denture liners. By adding these substances to the liners' composition, they lessen the conditions that stimulate the growth of fungi, effectively circumventing potential infections. Antifungal medications can aid in treating a fungal infection already shown on the denture liner or in the oral cavity. By directly targeting the fungi, their population can be controlled, and the accompanying discomfort and inflammation can be avoided. Antifungal medicines can extend the life of denture liners by inhibiting fungal colonization. In addition to posing health problems, fungal growth can cause the liner material to deteriorate, which compromises the material's integrity and performance. Antifungal medications help to preserve oral health by inhibiting the growth of fungi that may be linked to systemic problems. The incorporation of antifungal medications to denture liners helps to promote oral health, prevent and treat fungal infections, and increase the comfort and pleasure of denture users [26].

Polyenes and Azole antifungal medications are frequently utilized as the primary treatment for candida infections. Recently developed triazole antifungals (posaconazole and voriconazole) exhibit enhanced potency and wide activity when compared to older azoles. These drugs are commonly prescribed for systemic candidiasis in individuals with compromised immune systems and can potentially replace traditional azole therapies for various candidal infections. Posaconazole and Voriconazole have demonstrated superior efficacy in laboratory experiments against Candida species isolated from denture wearers.

This research seeks to assess and contrast the antifungal effectiveness and release profiles of these medications when incorporated into a Resin-based soft liner at different time intervals. It evaluates the extent of conversion, color stability, and water absorption characteristics of a resin-based soft liner modified with antifungal agents.

Materials and Methods

Ethical statement

This research has ethical approval from the XXXX, which met on 16.11.2016. All procedures were performed in compliance with relevant laws and institutional guidelines and have been approved by the appropriate institutional committee.

Minimum inhibitory concentration (mic) determination

The MIC was evaluated against Candida albicans by serially diluting Chlorhexidine diacetate, Voriconazole, and Posaconazole up to 10-9 in Brain Heart Infusion (BHI) media. From the stock cultures, 5 µl was added to 2 ml of the BHI broth. To this, 200 µl of the above culture suspension was then added. These tubes were incubated for about 24 h and observed for turbidity as an indicator of growth. The outcomes of the MIC are summarized in Table 1.

Table 1. Minimum inhibitory concentration (MIC) values for drugs against C. albicans.

Candida	100 µg/ml	50	25	12.5	6.25	3.12	1.6	0.8	0.4	0.2
Chlorhexidine	S	S	S	S	S	S	S	S	R	R
Voriconazole	S	S	S	S	S	S	S	S	R	R
Posaconazole	S	S	S	R	R	R	R	R	R	R

S-Sensitive, R-Resistant

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Preparation of storage solution

Potassium dihydrogen orthophosphate salt (6.8g) (0.2M) and 0.9 g of Sodium hydroxide pellets (0.2M) were weighed. Distilled water (1000 ml) was added gently to dissolve it, followed by adjusting the pH to 6.8 to mimic optimal oral salivary pH. This buffer solution was used as a storage media for the samples.

Sample preparation

A stainless-steel metal die of dimensions 10x3 mm (width×height) was fabricated per the ADA (American Dental Association) specifications [18]. In the present study, the resin-based resilient denture soft liner, Coe soft (GC, America), was utilized for its properties. The polymeric phase of the soft liner was weighed, and chlorhexidine diacetate was added in geometric distribution at 1% (1 mg of drug in 100 mg of powder) separately and stored in a plastic container. A similar procedure was applied to store Voriconazole and Posaconazole.

Of the 240 samples, 60 were assessed for antifungal activity and 60 for drug release, 40 were used for the estimation of DC, 40 were used for the estimation of color stability, and 40 for estimating water sorption (Table 2).

Table 2. National Bureau of Standards Units for Color Stability (NBS).

Color difference	NBS rating 13
Extremely slight	0.0-0.5
Slight	0.5-1.5
Perceivable	1.5-3.0
Marked	3.0-6.0
Extremely marked	6.0-12.0
Change to another color	>12.0

Agar disc diffusion test

Overnight incubation was performed on cultures prepared in 20 milliliters of Sabouraud agar culture media. C. albicans suspension was used to inoculate the Petri plates using the lawn culture method. Wells were formed in the plate after the inoculation period of five minutes. Five samples were positioned on top of each plate after five wells were made in the same way. The Petri plates were inverted after 15 minutes, put inside the chamber, and incubated for 48 h at 37° C. The sizes of the C. albicans growth inhibition zones were evaluated 48 h later. Every disk was measured three times, and the average diameter was computed and recorded. Every group's mean value was assessed and contrasted.

Estimation of drug release

The drug release was estimated by the absorbance in the UV-spectrophotometer. The wavelengths were adjusted to 254.5 nm, 256 nm, and 251 nm for Chlorhexidine diacetate, Voriconazole, and Posaconazole respectively. The absorbance values were recorded. The process was repeated at different time intervals i.e. 3rd day, 5th day, 7th day, 14th day, and 28th day.

Measurement of degree of conversion (dc)

FTIR spectrometer (IRTracer-100) was used to estimate the degree of conversion. The FTIR was programmed to include spectra ranges of 4000-5000 cm^{-1}, at 4 cm^{-1} resolution, for 32 scans co-addition. The FTIR spectra were initiated with a background spectrum without the use of a study sample to standardize the area for the next sample material to be scanned. This was followed by analyzing each freshly prepared soft-liner sample (dough stage) (40 samples) that was placed onto the surface tile for the spectroscopic measurements. The spectrum of both immediately prepared samples and samples after 24 h were obtained for the three drug-incorporated samples of 1% concentration (Chlorhexidine diacetate, Posaconazole, and Voriconazole).

The degree of conversion (DC%) was calculated by measuring the absorbance of aliphatic bands and aromatic bands in uncured and cured conditions at respective wavelengths. The DC% was measured and tabulated from the aliphatic C=C peak and against the carbonyl C=O peak as per the following formula [13].

$DC\% = \{1 - (a/b)\} \times 100$

Where a denotes [absorption of aliphatic/absorption of carbonyl] set, b denotes [absorption of aliphatic/absorption of carbonyl] unset, [Aliphatic cured = absorption peak at range 1614-1635 cm^{-1} of the set sample. Carbonyl cured = absorption peak at range 1720-1724 cm^{-1} of the set sample. [Aliphatic uncured = absorption peak at range 1614-1635 cm^{-1} of the unset sample] [Carbonyl uncured = absorption peak at range 1720-1724 cm^{-1} of the unset sample].

Estimation of color stability

The samples were immersed separately after a complete setting of time in 10 ml of the buffer storage media at 37 °C in transparent glass bottles, which were tilted gently three times daily throughout the period to simulate the clinical oral environment. Base color values before storage (after 24 h) and subsequently up to 30 days in the storage media were carried out using a Colorimeter. The color changes after 30 days; the ΔE*ab was measured from the three-color dimensions of L*, a*, and b* according to the following formula:1.

$$\Delta E*ab = \sqrt{l*1 - l*2\,2 + a*1 - a*2 + (b*1 - b*2)2}$$

In which (l*1, a*1, b*1) denotes the color change after 24 h of the complete set, (l*2, a*2, b*2) denotes the color change values after 30 days. Color changes (ΔE*ab) were converted to NBS units of color difference (National Bureau of Standards). The values of NBS units (Table 3) qualitatively denote the color change of the material and are calculated by the following formula,

$$\Delta E*ab(in\ NBS\ unit) = \Delta E*ab(in\ CIE) \times 0.92$$

Table 3. Post-hoc Bonferroni for antifungal activity.

Group	Groups	Mean Difference	P value
Control	CHX	-13.733	<0.001
	VOR	-35.733	<0.001
	POS	-21.8	<0.001
CHX	VOR	-22	<0.001
	POS	-8.067	<0.001
VOR	POS	13.933	<0.001

Estimation of water sorption

A total of 40 samples (30 for the drug-incorporated group and 10 for the control group) were prepared to estimate water sorption. Samples were placed in a vacuum desiccator at 37℃ ± 0.50℃ and were subjected to desorption. All

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samples were weighed in a digital analytical balance until a constant mass was obtained. The sample weights were considered stable when the differences between the averages of each evaluation period were lower than 0.0002 g after 24 hr. of desiccation. This initial weight of the sample disk is denoted by W1. After the initial mass was determined, each sample was immersed in 10 ml of storage media at 37℃. The subgroups were tested after 30 days. Accordingly, the samples were removed from the glass bottles, and excess water was removed using blotting paper. Each sample was then weighed, and this weight denotes the weight of the sample after absorption (W2) of storage media. Relative water sorption was obtained according to the revised ADA Specification No.1221 for denture base polymers measuring water and solubility in mg/mm2 in which sorption is presented by the following formula [18].

$$\text{Sorption (mg/mm}^2\text{)} = \frac{\text{W2-W1}}{\text{Surface area}}$$

Results and Discussions

Topical antiseptics

The efficient methods of denture cleaning and upkeep of Candida species are said to be the most frequent colonists of soft-liners [7]. Oral bacteria can colonize them due to their surface's porosity and roughness [8]. By adding antifungal medications to the soft liners, the material prevented Candida albicans from colonizing and penetrating [9]. This serves as the foundation for a microbiological investigation to validate the antifungal drug concentrations released from the soft liners and their antifungal efficacy. This study assessed the antifungal activity of the soft liner's integrated antifungal agents against candida albicans species using the agar disc diffusion test.

Spectrophotometry was used to assess the release of drugs from the specimens, a crucial component of the regional drug delivery system. The control group's agar culture test yielded no inhibitory zone, indicating that the soft-liner materials lack inherent antifungal properties. Goncalves et al.'s research on the release of zinc undecylenate from soft-liner material, which revealed that the material did not stop Candida biofilm growth, corroborated this data [27]. The groups treated with voriconazole, posaconazole, and chlorhexidine diacetate had the largest inhibitory zones. This implies that, compared to the other groups, the voriconazole group exhibited higher antifungal activity.

It was discovered that the medications voriconazole, posaconazole, and chlorhexidine diacetate were liberated from the soft liners and into the storage solution. Water needs to be absorbed into the resin for the medicine to become soluble and be released from the resin. Water channels in the polymer matrix grew as a result of absorption, facilitating the quick passage of water molecules [11]. The drug's solubility in water and its diffusion coefficient within the resin matrix directly account for the rate of the release of the drug. The hydrophilic nature of the resin is directly associated with the speed of drug release. A brief burst release of the medication is typically observed during its controlled release, which is then followed by a prolonged period of steady release. Because chlorhexidine diacetate has a higher potential to dissolve in storage media than other medicines, the specimens containing it showed the greatest mean cumulative drug release. Additionally, PMMA and PEMA soft liners containing chlorhexidine diacetate were shown to have an inhibiting impact on Candida albicans and their capacity to be released into a storage solution by Bertolini et al. This indicates that whereas voriconazole and posaconazole demonstrated modest release in the solution, chlorhexidine diacetate was better released in the storage buffer [33]. Every released concentration was higher than the MIC of the corresponding drug, indicating that the drug's released concentration is appropriate for suppressing candida activity. Because the drugs' diffusion gradient was initially much larger in the solution, there was a progressive increase in the cumulative release of the medications on days one and three, resulting in a much higher initial drug release for all groups. This was comparable to earlier research on medication release in denture base resin and soft liners [13].

The drug release chart indicates that the matrix is the most suitable model, as all medications exhibit non-fiction diffusion and zero-order kinetics. This suggests that the primary objective of a controlled-release medication delivery system is the consistent release of pharmaceuticals from the matrix. The efficacy of soft liners in combination with antifungal medications and drug release illustrates that the polymerization of the resin does not strongly impact the medication's efficacy and allows for delayed release. The use of low doses of antifungal medications in conjunction with soft liners not only reduces the likelihood of an adverse drug reaction but also possesses potent antifungal properties. Consequently, a drug delivery system was devised by blending these antifungal medications with resin-based denture soft-lining materials, thereby inhibiting C. albicans colonization and diminishing systemic side effects.

To determine the amount of medication to be added to the resin-based soft-liner material, the minimum inhibitory concentrations (MIC) of each drug versus Candida albicans were assessed. The MIC values for voriconazole, posaconazole, and chlorhexidine diacetate were determined to be 0.8 µg/ml, 0.8 µg/ml, and 25 µg/ml, respectively.

The mean distribution of the degree of conversion with the highest mean in the Chlorhexidine 1% group- (89.17 ±7.01) and the least was observed in the Posaconazole 1% group (20.97±8.6) (Figure 1). The characteristic aromatic carbonyl (C=O) stretching band was observed at 1,724 cm^{-1}, and the aliphatic C=C stretching band exhibited a peak at 1,614 cm^{-1} for both the control group and drug incorporated groups at cured and uncured state, and there was no shift to other bands in their respective absorbance peaks. The results suggest a substantial difference in absorbance values with respect to peak heights for each group observed at both 0 h and 24 h without shifting the characteristic wavelength band. This difference in absorbance and peak heights with no shift in the (C=C) or the (C=O) stretching absorption band signifies that due to the inherent properties of the drugs. There is only a chemical interaction (bonding) of the drug with the unpolymerized crosslink chains of the resin material without affecting the drug's chemical properties.

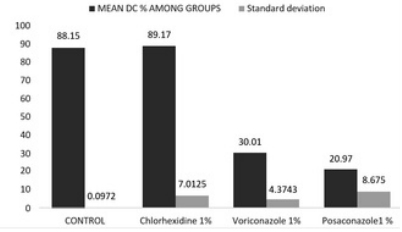

Figure 1. Mean distribution of Degree of conversion among groups.

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The figure shows the mean degree of conversion (DC) percentage among different groups. The control group exhibited the lowest degree of conversion, followed by chlorhexidine 1%, voriconazole 1%, and posaconazole 1%. Error bars representing standard deviation indicate the variability within each group.

The Mean distribution of the Color stability of groups at 30 days was the highest mean with the Control group (3.60±1.59); the least was seen in the Posaconazole 1% group (2.340±0.50) (Figure 2). The data was not statistically significant among the groups (p = 0.80). Figure 3 shows the mean distribution of the water sorption of groups at 30 days. The data shows the highest mean with the Posaconazole 1% group (0.03±0.01), and the least water sorption was seen in the Voriconazole 1 % group (0.002±0.02). The data was not statistically significant among the groups (p = 0.53).

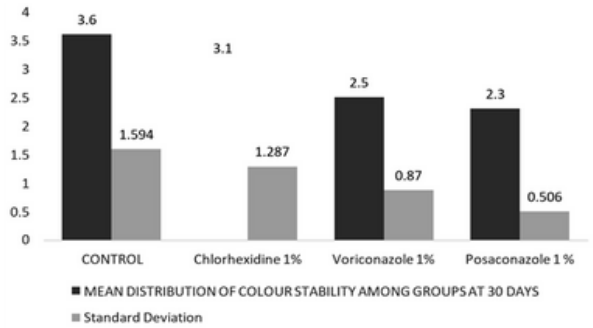

Figure 2. Mean distribution of color stability among groups at 30 days.

The figure shows the mean distribution of color stability among four groups after 30 days. The control group exhibited the highest color stability, followed by chlorhexidine 1%, voriconazole 1%, and posaconazole 1%. Error bars representing standard deviation indicate the variability within each group.

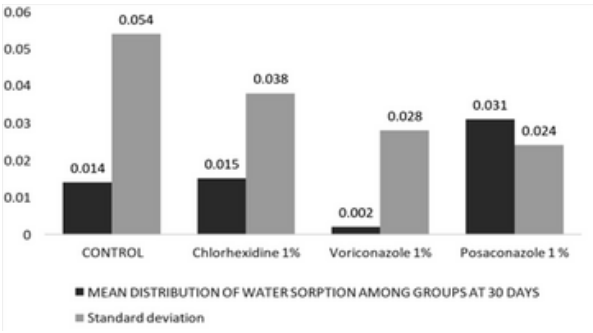

Figure 3. Mean distribution of Water sorption among groups at 30 days.

The figure illustrates the mean distribution of water sorption among four groups after 30 days. The control group exhibited the lowest water sorption, followed by chlorhexidine 1%, voriconazole 1%, and posaconazole 1%. Error bars representing standard deviation indicate the variability within each group.

A very weak correlation between color stability and degree of conversion was demonstrated (p = 0.05), whereas a strong positive correlation between color stability and water sorption (p = 0.03) was demonstrated in the Voriconazole group. A very weak correlation between the DC and water sorption was seen in the control group, which was not statistically significant (Table 4). A moderate correlation was demonstrated between DC and CS in the VOR 1% group, which was found to be statistically significant. However, the correlation between CS and WS in the VOR 1% group was found to be a negative, weak correlation and not statistically significant. A weak positive correlation was seen between the Degree of conversion and water sorption in the VOR 1% group, which was not statistically significant (Table 4). The data suggested that with a gradual increase in color change at different intervals of time, there is a decrease in the water sorption of the samples of the control group. A significant positive moderate correlation was seen between DC and Color stability in the group incorporated with 1% w/w Voriconazole indicating a correlation between degree of conversion and color stability after 30 days.

Table 4. Pearson's correlation between color stability, degree of conversion, and water sorption in the Control group and Voriconazole-1% group.

Control		**r value**	**p value**
Color stability	Degree of conversion	0.62	0.05*
	Water sorption	-0.23	0.51
Degree of conversion	Water sorption	0.39	0.25
Voriconazole 1%			
Color stability	Degree of conversion	0.17	0.63
	Water sorption	0.66	0.03*
Degree of conversion	Water sorption	0.06	0.87

*P>0.05 Not Significant, P<0.001 Highly Significance.

The inhibition zones in the agar disc diffusion test for each of the four specimen groups. The efficiency of soft liners combined with medications for antifungal activity and drug release demonstrates that the resin's polymerization does not negatively impact the medicine's effectiveness and permits delayed drug release (Figure 4). Low dosages of antifungal medications combined with soft liners minimize the possibility of an unpleasant drug reaction and provide strong antifungal properties. Consequently, these antifungal medicines were added to resin-based denture soft lining materials to establish a drug delivery system, as this lowers the risk of systemic side effects and prevents C. albicans from colonizing the material.

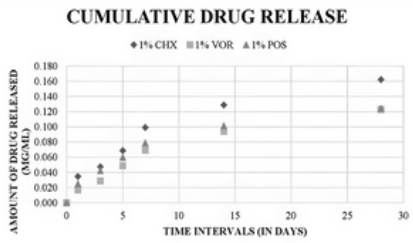

Figure 4. Zones of inhibition in the agar disc diffusion test for each of the four specimen groups.

The line graph illustrates the cumulative drug release over time for three different drug formulations: 1% CHX, 1% VOR, and 1% POS. The x-axis represents time intervals in days, and the y-axis represents the amount of drug released in mg/ml. The graph shows that the release of all three drugs increases over time, with 1% CHX showing the highest release followed by 1% VOR and 1% POS.

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The wavelength was changed to 254.5 nm to examine the specimens (group B) that contained chlorhexidine diacetate. The values for absorbance were then determined. In a similar vein, samples containing voriconazole (group C) and samples containing posaconazole (group D) were assigned wavelengths of 256 and 251 nm, respectively. Each sample's absorbance values were determined and entered into the table. For every specimen , the complete process was repeated at various intervals (i.e., 3rd day, 5th day, 7th day, 14th day, and 28th day). Each specimen's absorbance readings at every interval were found and tallied (Table 5). Since the release of other chemicals, such as polymethyl methacrylate and plasticizers, can increase absorbance, the test groups' changes in optical density were deducted from the control groups without drug use in proportion.

Table 5. Post-Hoc Bonferroni test for drug release.

Group	Groups	P-value (Day 1)	P-value (Day 3)	P-value (Day 5)	P-value (Day 7)	P-value (Day 14)	P-value (Day 28)
CHX	VOR	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	POS	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
VOR	POS	<0.001	<0.001	<0.001	<0.001	>0.05	>0.05

CHX – Chlorohexidine Diacetate, VOR- Voriconazole and POS- Posaconazole

*P> 0.05 – Not Significant, P< 0.001 – Highly Significant

The PCP DISSO V3 program was then used to individually record the final data for every medication. The program was then used to enter the slope and constant for each drug, which was found on the linear graph for chlorhexidine diacetate (33.01 & -0.1358), voriconazole (27.51 & -0.02227), and posaconazole (18.06 & -0.06710), respectively. The overall quantity of drugs released for every medication was determined by the software after it had evaluated the data (Figure 5).

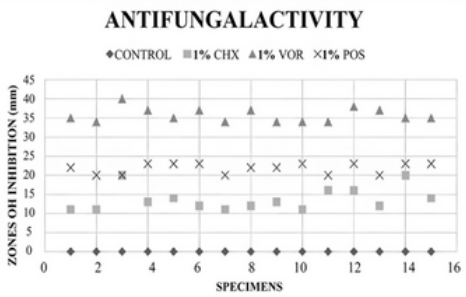

Figure 5. The cumulative drug release from each group of specimens at different intervals of time.

The bar graph illustrates the antifungal activity of four different groups of specimens. The x-axis represents the specimen number, and the y-axis represents the zones of inhibition in millimeters. The graph shows that 1% CHX and 1% VOR exhibit significantly higher antifungal activity compared to the control and 1% POS groups, indicating their effectiveness against the tested fungus.

Even after effective denture cleaning and maintenance, Candida species have been observed to be the most common invaders of soft-liners [18]. The roughness and porosity of their surface allow oral bacteria to colonize [19]. The incorporation of antifungal substances into the soft liners resulted in the material's ability to resist colonization and penetration by C. Albicans [20]. This serves as the foundation for microbiological research to confirm the antifungal medication concentrations produced by the soft liners, as well as their antifungal action. In this work, antifungal activity was assessed using an agar disc diffusion test to measure the efficacy of antifungal drugs included in the soft liner against C. albicans species. Posaconazole works by inhibiting CYP51, an essential membrane protein that is involved in the formation of ergosterol, the primary sterol found in fungal cell membranes, by acting as a 14-alpha-demethylas [21].

It was discovered that the medications voriconazole, posaconazole, and chlorhexidine diacetate were liberated from the soft liners and into the storage solution. Water needs to be incorporated into the resin for the medicine to become soluble and to escape from the resin. Water channels in the polymer matrix grew as a result of absorption, facilitating the quick passage of water molecules [22]. Depending on the drug's water solubility and its diffusion coefficient in the resin matrix, water would elute it gradually.

The drug on the specimens' exterior surface is most likely what caused the drug-incorporated discs to demonstrate an initial burst release. An essential component of the regional drug delivery system is the prolonged or controlled release of the pharmaceuticals, which were evenly distributed throughout the polymeric matrix due to the material's water absorption [23]. Because chlorhexidine diacetate has a higher potential to dissolve in storage media than other medicines, the specimens containing it showed the highest mean FFF cumulative drug release. Salim and colleagues demonstrated that the polymer impregnated with chlorhexidine shows better effectiveness against the formation of biofilms by C. albicans.

In this present study, a resin-based soft liner has been altered by impregnating/incorporating three anti-fungal agents, Chlorohexidine, Posaconazole, and Voriconazole, and evaluated and compared for the longevity of the material up to 30 days.The degree of conversion is the measured percentage of methacrylate double bonds that are converted into single bonds during the setting reaction. The degree of conversion affects the mechanical and physical properties of dental polymers, and therefore, it is of clinical performance to determine its biocompatibility [13]. FTIR is a valid technique for the analysis of other dental materials, such as composite resin monomers .The degree of

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conversion of resin-biomaterials depends on the chemical nature of the methacrylate monomer and the polymerization conditions to which it is subjected [27]. The concept of FTIR relies on the absorption and interaction of infrared radiation emitted from a heated material followed by measuring and analyzing a sample with a highly sensitive spectrometer [15].

We demonstrate that the incorporation of antifungal agents significantly influences the degree of conversion and the polymerization process. The decrease in the percentage of the degree of conversion in the drug-incorporated groups can be explained by the presence of the chemical structure of the drugs affecting the polymerization process. Although polymerization is a complex process, the inclusion of these medications could potentially modify the polymer composition to a greater extent and could also disrupt the process of polymerization chain propagation to a greater degree.

The earliest report of antifungal medicines being included in a liner date back more than 30 years. With such delivery systems, delayed local release of a smaller dose can replace high conventional systemic doses, resulting in a sustained therapeutic impact. It has been demonstrated that the use of local medication delivery systems utilizing polymers as bases affects the materials used to line dentures, particularly their physical characteristics, despite the encouraging results obtained from this approach. It has been demonstrated that adding an antibiotic to a resin-based liner affects the material's degree of conversion and polymerization, which may have an impact on the material's mechanical qualities [28]. Among all the groups, the group incorporated with 1% w/w Chlorohexidine exhibited a significantly higher percentage of DC. A statistically significant decrease in the percentage of the degree of conversion was observed in groups containing resin-based soft liners incorporated with Voriconazole and Posaconazole when compared to the drug-free control group. These results agree with the previous study, which showed a decrease in the percentage of the degree of conversion among the drug-incorporated groups [13].

Riggs et al. previously suggested that the incorporation of chlorohexidine diacetate salts decreased the property of polymerization significantly due to the protonation along with a shift in the peak height of the carbonyl bonds of chlorohexidine incorporated groups [21, 29]. However, the statistical analysis data states that the percentage of degree of conversion of groups incorporated with Chlorohexidine is insignificant when compared to the control groups. The lowest or lower percentage of the degree of conversion was observed among the groups incorporated with Posaconazole and Voriconazole drugs, respectively. This is explained due to the presence of unreacted and trapped molecules inside the cross-linked network, which could further lead to a limited mobility of the functional groups within the polymerized structure followed by termination of the reaction before the completion of the degree of conversion [13, 29-30]. Thus, the results of the degree of conversion study suggest that the selected antifungal agents, such as Posaconazole and Voriconazole, can be used for incorporation into resin-based soft liners as biocompatible carriers for local drug delivery mechanisms.

The distinct properties of each material most likely contributed to the notable color shift observed in the samples. This happens in the acrylic resin material because of increased water absorption and plasticizer solubilization. Shotwell showed that the primary disadvantages of plasticized acrylics are their substantial water absorption and dissolution as well as their gradual hardening because of plasticizer leaching. Such behavior in water may cause colorants to be absorbed and accumulate. Diffusion of stained solutions is facilitated by the inclusion of a plasticizer in the liner composition, which facilitates chain stretching of tiny organic molecules that are in the polymer. Additionally, staining agents could seep into the gaps left by the plasticizer's leaching. Comparing silicone-based liners to acrylic resin-based materials, which similarly had the lowest hardness after thermocycling, shows that silicone-based liners offer far greater color stability, absorption, and solubility. Globally, a significant proportion of patients still wear removable dentures, and even those who receive dental implants still need to wear dentures before receiving permanent prostheses.

A soft lining is necessary for comfortable usage of these, and the best material to utilize in this application over the long term has not yet been determined. The current findings indicate that a higher initial hardness causes a decrease in absorption, a decrease in solubility, and a decrease in color change as the material ages. Clinical necessity should be the basis for choosing liner materials, considering the anticipated outcomes and duration of usage.

Color stability

Color stability is directly proportional to the surface roughness of the liner material, which is of clinical relevance. Increased surface roughness favors the colonization of microorganisms, contributing to further tissue injury [31]. The color stability test was measured colorimetrically for all the groups over 30 days. The color change at this interval of time signifies the initial life span of the resilient liner (Coe-Soft), which is 3-4 months. The present study demonstrated the least color change among all drug-incorporated groups in comparison to the control group. This is in contrast to a study reported by Salim et al. where the drug-incorporated groups showed the highest color change (poor color stability) [13].

Clinically speaking, dental materials must be color-stable; a noticeable shift in hue signals aging or damage. Adding materials that increase a material's resistance to discoloration can improve a material's color stability and stop dental prostheses from discoloring. A prior study assessed the color stabilities of PMMA after nanosized particles of SiO_2, TiO_2, and ZrO_2 were added. The samples were then submerged in a range of discoloring liquids. It has been demonstrated that adding nano-ZrO_2 to PMMA increases its color stability, which offers a potential solution to the problem of denture base resin discoloration [32].

Herein, the statistical data for color stability of both control groups and drug-incorporated groups showed that all the groups demonstrated a gradual increase in color change after 30 days. However, the statistical data of color change among the drug-free and drug-incorporated groups was found to be statistically insignificant.

Water sorption

Denture soft liners show a wide range of changes in properties when submerged in water due to the loss of a plasticizer from their composition. They undergo two changes , which are

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leaching out of soluble plasticizers into water and the effective water imbibition by the polymer. The physical and mechanical properties of the materials undergo aging in the oral cavity [32]. An ideal denture soft liner is considered resistant to the imbibition of oral fluids from saliva, but as such, no material could entirely fulfill the criteria.

These differences in the water sorption of the respective groups could be explained because of an imbalance in the leaching out process and the imbibition process of the samples. These differences may also be attributed to benzyl salicylate and phthalate plasticizers in the liquid composition of Coe-Soft, which tend to decrease the water sorption due to their higher hydrophobicity and the potential to fill the micro-spaces present in the polymer [31]. Interim resilient liners are bound to have a higher concentration of leached-out plasticizers when compared to the other liner materials [33]. Also, other factors, such as precipitation of the water molecules inside the samples due to their low-density, lead to the formation of smaller entanglement of polymeric chains, thereby affecting its water adsorption [32, 33].

Rafał Brożek observed that after being stored for up to 28 days, the acrylic materials Villacryl Soft and Vertex Soft lost some of their elasticity. Still, the silicone materials Molloplast B and Mollosil retained their elasticity. It was discovered that these materials emitted several chemicals, such as dibutyl phthalate, methyl methacrylate, and EGDMA33. In general, the silicones released less of the different eluents and were stronger than the acrylics. The quantities eluted were always significantly below the allowed exposure levels [34].

Resin composites should ideally be extremely stable and impermeable to water. However, moisture can be absorbed by the polymer network, adding several percentage points to the material's overall weight [35]. Water absorption has several detrimental consequences for restorative materials, including color changes, mechanical property impairments, wear-resistance reductions, hydrolyzing, and chemical bond degradation at the resin–filler interface. Furthermore, the solubility of resin composites increases as a result of water absorption, which also causes the external movement of leftover monomers and ions. This can weaken the mechanical properties, decrease the volume of the materials, and cause discoloration by chemically breaking down the resin-filler bond of the resin matrix. Every sample in the current study exhibited notable variations in the three-color values before and after storage. This is related to the increased amount of water absorption in the samples, which is consistent with the study conducted by Riggs et al. [21]. With respective to the color change of the drug incorporated group, it is yet acceptable for short-term use as these values are within the ranges required for another permanent denture soft liners [34]. Within the limitations of the present study, the values for the parameters with the incorporation of drugs are within the acceptable ranges according to the revised ADA specification for dental polymers [18].

Extrapolating the study's findings to in vivo settings is challenging because it was conducted in vitro. More research must be done on the medication integration and the longevity of the resin-based liner. All the medications (CHX, VOR, and POS) added to the resin-based liner impacted the water sorption, color stability, and degree of conversion. Groups with CHX incorporation displayed a higher DC %, a greater color change, and a lower water sorption rate. Comparing VOR integrated groups to CHX and Control groups, a drop in the percentage of Degree of Conversion was observed. When compared to the CHX and POS groups with the least amount of water sorption, the VOR groups, however, displayed less color change after 30 days.

When compared to all the groups, the POS-integrated groups had the lowest percentage of the degree of conversion, the least amount of color change (at various intervals of time), and the highest amount of water sorption. Research has been done on the relationship between denture plaque, oral pathology, denture base resin surface topography, and other cutting-edge methods for preventing microbial adherence to denture surfaces. It takes innovative methods to stop candidiasis from adhering to the denture's intaglio surfaces because these surfaces cannot be sanded or smoothed. Comparing local drug delivery strategies with integration to conventional methods of preventing denture-induced stomatitis has shown to be an alternative strategy.

Conclusions

The study focused on the evaluation and comparison of the physical properties of a resin-based soft liner (COE-Soft) and the potential of the drugs CHX, VOR, and POS respectively in preventing the adhesion of Candida albicans to the dentures. The study is limited by the in-vitro design which may make it difficult to properly recreate the complex oral environment, which could have an impact on how broadly applicable the findings are. The antifungal drugs were evaluated at single concentrations, which may not represent the best or most efficient concentrations. Long-term performance under real-world circumstances cannot be reliably predicted by accelerated aging tests. Finding the best formulations by examining the effects of different antifungal drug concentrations and conducting prolonged aging tests may help further a better understanding of the liners' long-term stability and performance, conduct prolonged aging tests. Investigating a broader antifungal spectrum may help identify other possible options that could improve resin-based soft liners.

Statements & Declarations

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Conflict of Interest

The authors hereby declare no conflict of Interest.

Data Availability Statement

Available

Ethical Approval

This research has ethical approval from the Institution Ethical Committee of AME's Dental College, which met on 16.11.2016. All procedures were performed in compliance with relevant laws and institutional guidelines and have been approved by the appropriate institutional committee.

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Author Contributions

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.

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