Original Article

Detection of the Release of Chlorhexidine from Cured Denture Resins Discs: Subsequently Deducing the Ability of Denture Resin as a Drug Carrier

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Abstract

Introduction: The impact of Candida-associated denture stomatitis has been vastly discussed in the literature, starting from simple routine oral hygiene practices to the use of the denture itself as a drug delivery unit. Since candidiasis tends to keep recurring, the use of antifungal drugs in the denture or liner provides the benefit of placing the patient under oral drug intake for a long period of time. This *in-vitro* study aimed to assess the release of chlorhexidine from the acrylic resin discs made from two resins, heat cure and light cure. The study also intended to assess the presence of the drug release and its sufficient effectiveness to be reproduced *in-vivo* in the treatment of oral candidiasis. **Methods:** Standard chlorhexidine solution, Chlorhexidine treated heat cure, and light cure resin disc soaked solutions were subjected to test under high-performance liquid chromatography (HPLC) and scanning electron microscopy for the release of chlorhexidine. **Results:** The HPLC analysis of heat cure resin demonstrated that the area and height of chlorhexidine release were comparable to those of the standard chlorhexidine solution. This implies the local release of chlorhexidine *in-vitro*. **Conclusion:** In this study, it was found that chlorhexidine release in heat cure discs is less when compared to the standard stock solution; nonetheless, it is good enough to reach the minimum inhibition concentration of chlorhexidine to be effective against candidiasis. Although this study was limited in nature, the results raised hope for further evaluation of dentures as a drug delivery system.

Keywords: Acrylic Resin, Candidiasis, Chlorhexidine, High-performance Liquid Chromatography

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Introduction

Oral candidiasis is the most common infection affecting the oral mucosa in complete denture wearers (1,2). Candida-associated denture stomatitis is characterized by general inflammation of palatal mucosa covered by the denture. Candida albicans is considered the principal pathogen in the development of Candida-associated denture stomatitis. Despite the use of several effective antimycotics for the treatment of oral candidiasis, the failure of therapy is not uncommon owing to the unique environment of the oral cavity where the flushing effect of saliva and the cleansing action of the oral musculature tends to reduce the drug concentration to sub-therapeutic levels (3).

To overcome these drawbacks of mouth rinses, studies were conducted regarding the use of polymerized acrylic resins as carriers for medications (4,5). In a similar vein, soft liners and tissue conditioners placed in dentures have been used as carriers for antifungal drugs in the treatment of denture stomatitis (6). The advantages of this method of drug delivery include a less emphasized need for patient compliance, reduced application frequency and simultaneous treatment of injured denture-bearing tissues, apart from treating Candidal infection. Although

polymethylmethacrylate resin surfaces could represent an important predisposing factor for micro-organism colonization, other factors, such as good fracture resistance, satisfactory optical properties, acceptable aesthetics, easy manipulation, reasonable cost and insertion in the oral cavity, justify their use (7).

Acrylic resins have the innate ability to absorb water and other chemical compounds from the oral environment and also release them into the surrounding environment (8). Due to the polymeric nature, continuous exposure of polymethylmethacrylate resin surfaces to antifungal agents may permit their absorption and release after treatment interruption, which could be detected as a residual effect (9). In light of the aforementioned issues, the present study aimed to verify the usage of pre-polymerized discs soaked with chlorhexidine mouth rinses as a local drug delivery system.

Materials and Methods

1. Preparation of Discs

Specimen discs of heat cure resin made from Pyrex Heat Cure Resin material (Pyrax Polymars, Uttarakhand, India) measuring 40 mm in diameter (Figure 1). Thereafter, these 40-mm discs were cut into smaller discs 10 mm in size, and 10-mm circles were drawn on the discs using a geometric compass (Figure 2). Diamond-coated abrasive disc trimmer on a micromotor handpiece was used to cut these according to the drawn circles. Therefore, 10-mm discs were obtained for the study (Figure 3). Photosil Polyvinylsiloxane impression material (Dental Products of India, Tamil Nadu, India) was manipulated and pressed onto a rectangular plastic container. On this putty base, the cut 10-mm heat-cured discs were placed gently after applying Vaseline on the discs before the silicone base started to set (Figure 4).

Once the Polyvinylsiloxane was completely set, the discs were removed, thereby providing space for preparing light cure discs (Figure 5). Mylar strips were placed on the mold space. Light cure resin (Heliomolar flow Ivoclar Vivadent Schaan, Liechtenstein) was gently filled in the mold space without incorporating any air bubbles and cured layer by layer using a light cure unit. Following that, these light-cured discs were removed using a sharp excavator. These were then polished using sandpaper starting from 320-800 fine grit (Figure 6).

2. Treatment Simulation

Specimens were randomly assigned to two groups, each containing five heat cure discs and five light cure discs. Specimens were individually immersed in test tubes containing Asep RC chlorhexidine gluconate solution of 2% w/v (Figures 7,8). This solution was selected as a standard for High-performance liquid chromatography (HPLC) analysis since it did not have any colouring agents. To simulate the clinical condition, the heat cure and light cure discs were soaked for 10 h in the standard chlorhexidine solution, removed, washed, dried, and placed in 2 ml of distilled water in test tubes, each separately (Figures 7,8).

Figure 1. Heat cure acrylic discs

Figure 2. 10-mm circles drawn

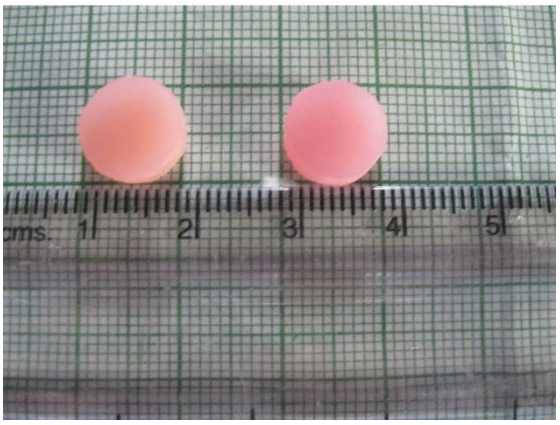


Figure 3. 10-mm discs

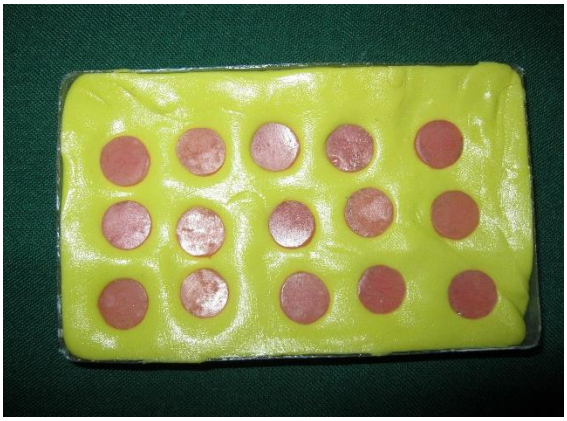


Figure 4. 10-mm heat cure disc pressed on silicone material

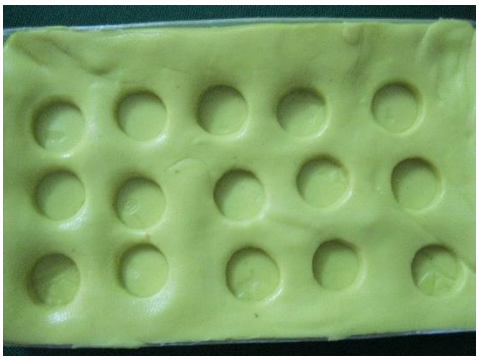


Figure 5. Mould space for light cure resin

Figure 6. 10-mm light cure discs

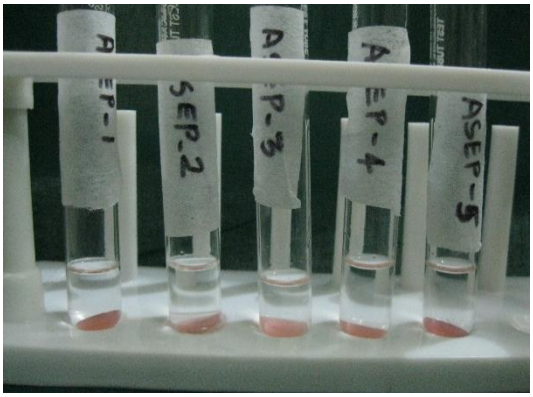


Figure 7. Soaked heat cure discs

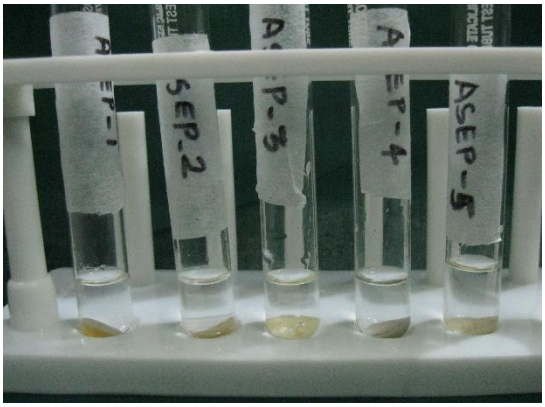


Figure 8. soaked light cure discs

3. Instrumentation

a. *High-Performance Liquid Chromatography*

The analysis was carried out on an Isocratic HPLC-apparatus, which had the following components: HPLC Schimazu model LC 20 AT; HPLC injector type Rheodyne 7125 (USA); HPLC-UV-Vis detector type GBC (Australia), model SPD 20A. The HPLC apparatus was operated under the following working conditions:

Eluent: acetonitrile / 0.01M phosphate buffer (25:75%); Eluent flow rate: 1.0 ml/min; Injection volume: 20 µl; Column: BDS-C18 (25 cm x 4.6 mm, particle size 5 µm); Detector: UV – VIS Spectrophotometer (λ : 210 nm,

Range: 1.0); integrator chart speed: 0.5 cm/min, attenuation:8

b. *Preparation of Stock Standard and Working Standard Solution*

Stock solutions: 1mg/mL chlorhexidine in methanol diluent.

Solvents: A: DI H2O/ 0.1% formic acid

B: 97% Acetonitrile/ 3% DI H2O/ 0.1% formic acid

Working solutions: A 100µL aliquot of the stock was diluted to 0.1mg/mL using 900µL

50% solvent A/ 50% solvent B mixture diluent.

Standard stock solution: A 1000µg/ml stock standard solution was prepared for each sample by dissolving 10 mg of the sample in 10.0 ml HPLC-water. These prepared solutions were kept in a refrigerator.

c. *Preparation of Storage Solution*

After 1 h of immersion of discs in the distilled water, 150 µL of an aqueous solution of the corresponding internal standard (160nmol/mL) was added and mixed. Finally, 20 µL of this mixture was injected onto the HPLC column according to the aforementioned conditions. Therefore, the storage solution was subjected to HPLC analysis and compared with the standard.

d. *Scanning Electron Microscopy*

After soaking in chlorhexidine, the specimens were visualized with scanning electron microscopy magnifications starting from 50, 200, 500,750, and 1000 to determine if there was a deposition of drugs on the acrylic resin surface.

Results

Retention time comparison (Figure 9)

The release of chlorhexidine is tabulated for both heat cure and cold cure resin discs (Table I and Table II). The chromatogram of chlorhexidine which is the standard to which the samples were compared demonstrated seven peaks of release, with the major retention time in 2.48 and 4.35-min intervals (Figure10). In comparison with this standard solution, the sample of the retention time of heat cure averaged 2.45 and 2.83 mins for peaks one and two, respectively (Figure11). In comparison with the standard, the chromatography results of retention time of light cure averaged 2.47 and 2.87 mins for peaks one and two, respectively (Figure 12).

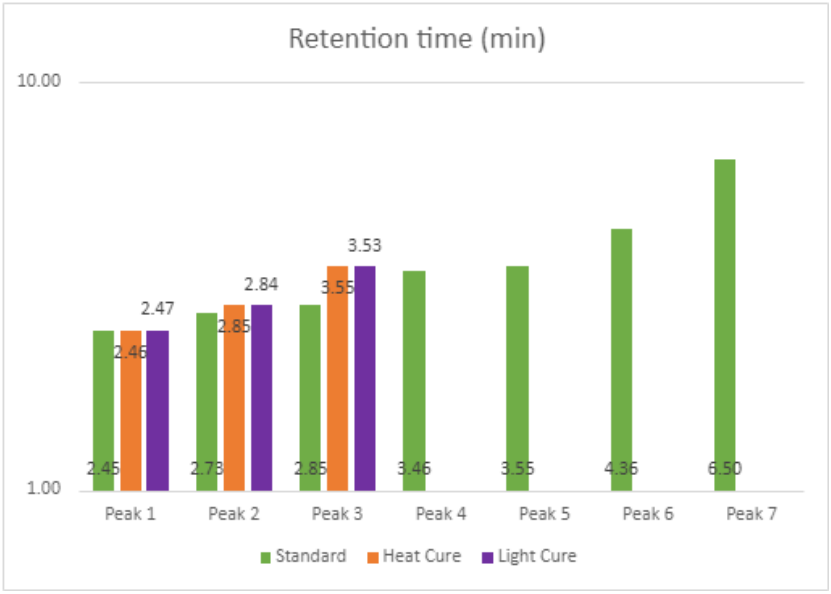


Figure 9. High-performance liquid chromatography analysis-retention time comparison

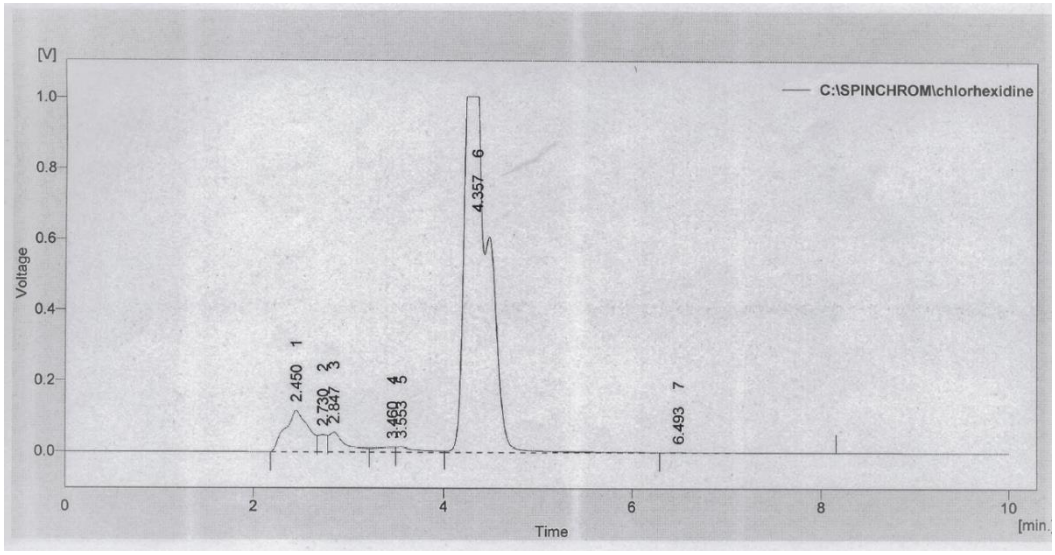


Figure 10. Chromatogram of Chlorhexidine standard solution

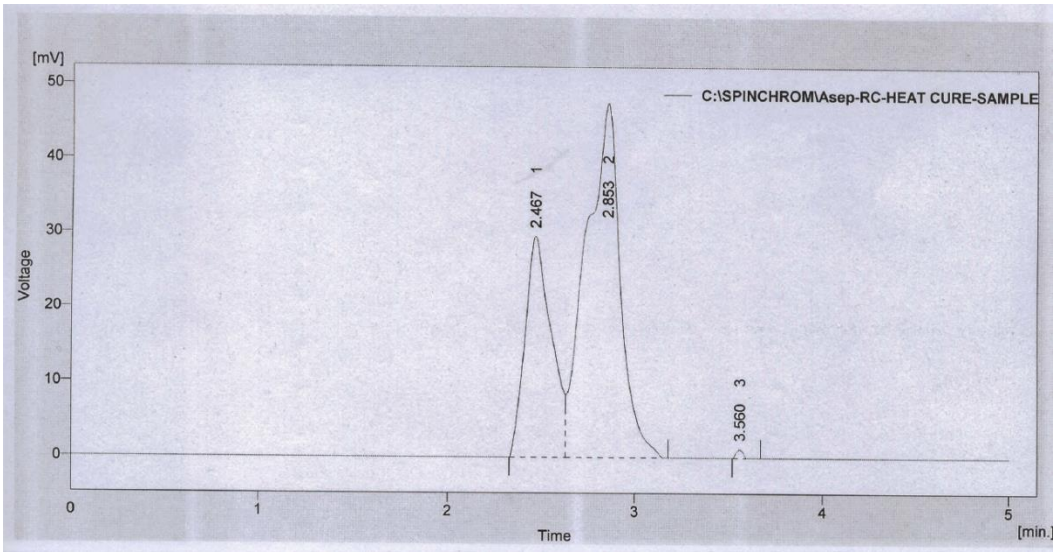


Figure 11. Chromatogram of samples from solutions in which heat cure resin was soaked

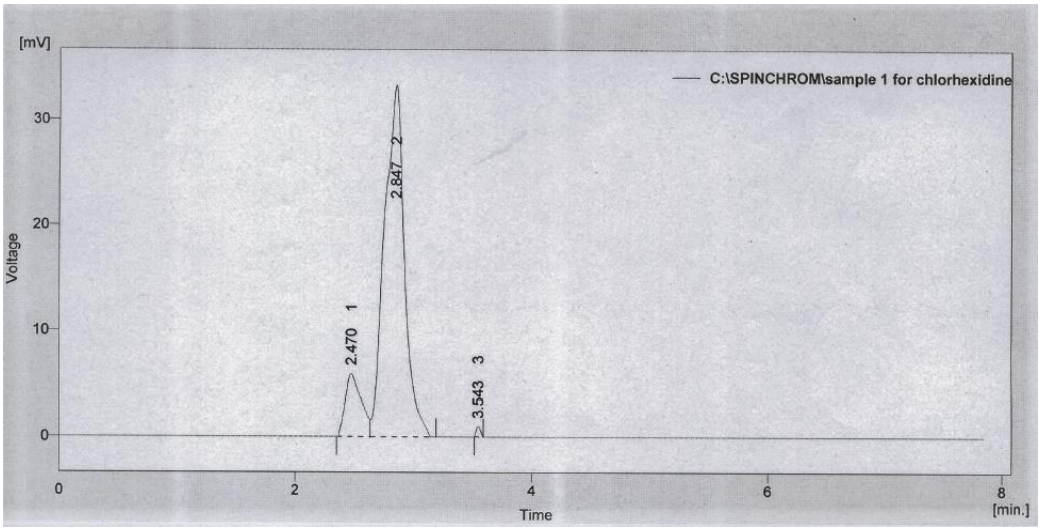


Figure 12. Chromatogram of samples from solutions in which light cure resin was soaked

Peak area comparison (Figure 13)

The areas of the peak were compared for the retention time. The areas of release of chlorhexidine standard solution for the retention times of 2.45, 2.84, and 3.55 mins were 1963.39, 687.729, and 226.56 mV.s, respectively. In the case of heat cure discs, the area was 287.32 mV.s for the first peak (retention time 2.46 min), 606.89 mV.s for the second peak (retention time: 2.85 min), and 2.97 mV.s for the third peak (retention time: 3.56 min). The analysis revealed that the second peak area attained by the heat cure disc was comparable to the second peak of the standard solution; nonetheless, the third peak was well below the standard. In the case of light cure discs, the area was only 55.10 in 2.47 retention time, 390.88 in 2.84 retention time, and 2.51 in 3.53 retention time, which is very much lower than the one observed in standard solution.

Height comparison (Figure 14)

The height obtained with the standard solution was 116.17 mV and 55.86 mV. The height of the first peak was 29.58 mV and that of the second peak was 47.60 mV for the heat cure discs, whereas the height of the first peak was 5.89 mV and that of the second peak was 33.29 mV for the light cure discs. The results indicated that the retention time and the area of the second peak of the heat cure disc were comparable to the standard. The comparison between heat and light cure discs indicated that heat cure resin attained better results.

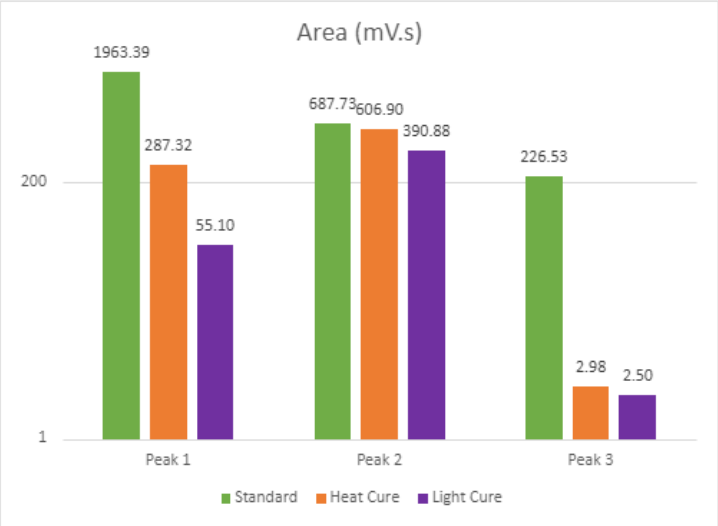


Figure 13. High-performance liquid chromatography analysis–Peak Area comparison

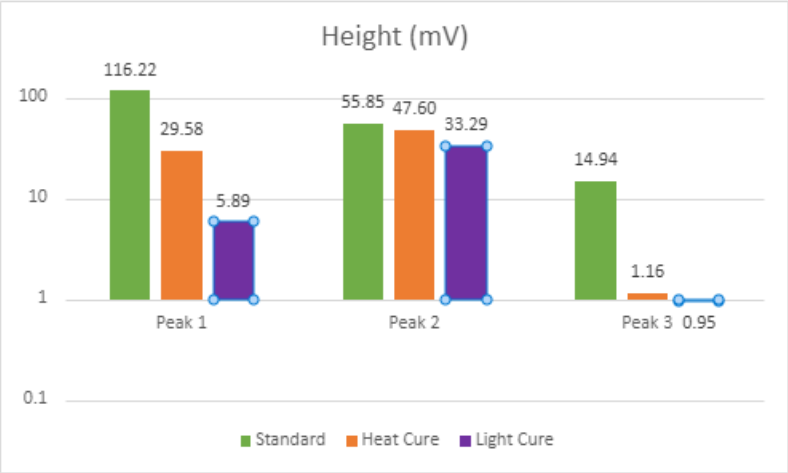


Figure 14. High-performance liquid chromatography analysis – Height comparison

Table I. Chromatogram result for heat cure samples

	Peak 1			Peak 2			Peak 3		
Sample No	Retention time (min)	Area mV.s	Height mV	Retention time (min)	Area mV.s	Height mV	Retention time (min)	Area mV.s	Height mV
1	2.46	287.30	29.60	2.85	606.80	47.60	3.56	2.98	1.15
2	2.47	287.40	29.80	2.83	607.20	47.80	3.58	3.04	1.10
3	2.45	287.28	29.50	2.86	606.70	47.50	3.51	2.96	1.18
4	2.47	287.34	29.40	2.84	606.88	47.48	3.55	3.00	1.18
5	2.46	287.26	29.60	2.85	606.90	47.60	3.55	2.90	1.20

Table II. Chromatogram results for light cure samples

	Peak 1			Peak 2			Peak 3		
Sample No	Retention Time (min)	Area mV.s	Height mV	Retention Time (min)	Area mV.s	Height mV	Retention Time (min)	Area mV.s	Height mV
1	2.47	55.08	5.88	2.84	390.48	33.36	3.54	2.51	0.95
2	2.48	55.00	5.92	2.82	391.04	33.40	3.56	2.53	0.92
3	2.47	55.28	5.84	2.80	391.28	33.16	3.52	2.48	0.98
4	2.46	54.98	5.94	2.88	389.48	32.98	3.50	2.52	0.96
5	2.46	55.14	5.88	2.84	392.14	33.56	3.54	2.48	0.94

Discussion

Denture stomatitis and poor oral hygiene cause plaque accumulation and lead to a decrease in the pH level of the oral cavity, producing an acidic environment which favors Candidal growth (10). Low levels of pH can facilitate the adhesion and proliferation of Candida yeast. A pH equal to 3 is optimal not only for the adhesion of

yeasts but also for the enzymatic activity of the proteinases which, in conjunction with the lipases, are the most important factors contributing to the virulence of Candida due to their cytotoxic and cytolytic effects(11). In particular, this pathogen is capable of causing persistent and intractable infections in immunocompromised, diabetic, and differently-abled patients, as well as those with a local disturbance in their oral flora, such as individuals who have been subjected to long term antibiotic therapy.

The treatment of Candida albicans-associated denture stomatitis has been evolving from a simple emphasis on maintaining good oral hygiene to the use of an advanced system of slow drug delivery. Lal et al. investigated the use of Chlorhexidine gluconate in the form of Peridex, both as a mouth rinse and denture soaks, in the treatment of denture stomatitis. It was revealed that chlorhexidine eliminated Candida albicans on the acrylic resin denture surface and significantly reduced palatal inflammation. Nevertheless, several weeks after the termination of Peridex treatment, Candida albicans recolonized on the denture surface and palatal inflammation recurred (12).

Chlorhexidine is a cationic chlorophenyl bisbiguanide that binds to negatively charged surfaces. It has a broad spectrum of antimicrobial activity, including against Candida albicans (13). Chlorhexidine digluconate has a bimodal action on Candida since this compound ruptures the cell membrane of yeasts, demonstrating a fungicidal effect even in low concentrations (14). A notable feature of Chlorhexidine is that it adheres to salivary glycoproteins in plaque and is slowly released over time. Following a one-min rinse with Chlorhexidine,30% of the mouthwash was retained in the oral cavity for 24 h. As oppose to Nystatin, Chlorhexidine has a very stable chemical structure and is not inactivated by heat, light or acid.

Although these acrylic resins are a major habitat in which the microorganism can flourish well, they can leach out chemicals; consequently, various studies were conducted to use them as a drug delivery system. It has been established that methacrylate-based polymers absorb up to 30% water depending on the osmolarity of the external solution or the formulation of the particular polymer (15). It has been established that due to the porous nature of the acrylic surface, water goes into it by a slow diffusion process and tries to bring out the chemicals absorbed by the resin (16). Diffusion-based release of residual monomer occurs with the absorption of water into the spaces between the polymer chain (17).

There are various methods to detect the release of chemicals from the resin. The present study was performed using HPLC which has the additional advantage of being non-destructive and facilitates sample recovery. It was mandatory to obtain pure samples for standardization; therefore, the Asep RC solution was used since it contained only 2% Chlorhexidine without colouring agents. This study was undertaken to assess the absorption and release of chlorhexidine from acrylic resin discs immersed in chlorhexidine overnight, simulating the clinical conditions.

The release of Chlorhexidine was assessed employing HPLC by comparing the chromatography obtained for a standard against the samples. The data obtained from this analysis suggested that the height and area of the peak decrease with time and the number of peaks also reduces. It signifies that the release of Chlorhexidine is comparable to the standard in the second peak, suggesting its antifungal effect in accordance with the study conducted by Anibal, P.C. et al. Chlorhexidine exhibits extended activity following exposure in the oral environment and allows a wide spacing of doses.

The release of Chlorhexidine from the resin discs to distilled water indicated that these discs can be used as drug delivery systems. This finding is in agreement with previously reported studies that employed polymers for delivering Chlorhexidine. In the two discs used, the heat cure discs were demonstrated to have a comparably larger area and height for both peaks than the light cure discs, thereby providing better release of Chlorhexidine. Therefore, this may be of great help in the treatment of oral candidiasis. The minimum inhibitory concentration of Chlorhexidine required for achieving its antifungal property is 7.81 µg/ml. Mouthwashes contain a higher concentration of Chlorhexidine, thereby eradicating the fungus within only 60 sec of exposure (18). Moreover, the chlorhexidine mouthwashes also offer the benefit of effectively cleaning one's denture as well as treating oral candidiasis (19).

Scanning electron microscopy gives us the advantage of viewing the topographical, morphological, and composition information. The amorphous matrix of polymethylmethacrylate can be visualized from the resin discs before treatment. The irregularities observed on the surface in the post-treatment photographs can be attributed to either deposition of chlorhexidine or the distilled water in which they have been soaked (figure 15). Further studies are necessary to verify the nature of the deposition particles on the resin discs.

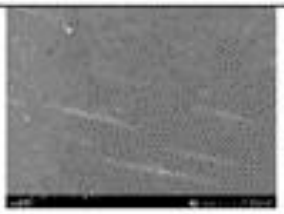
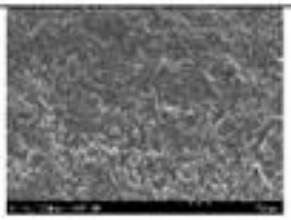
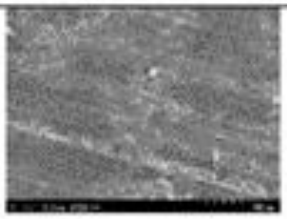
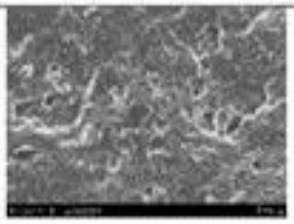
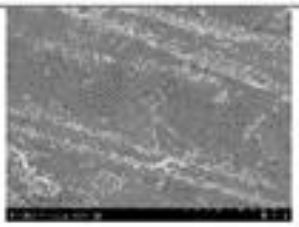
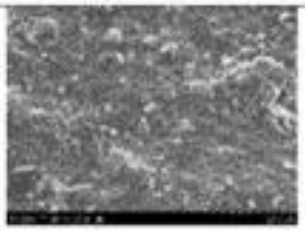
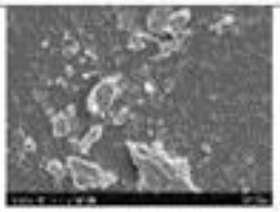
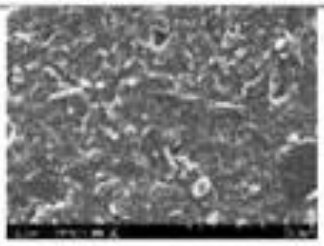
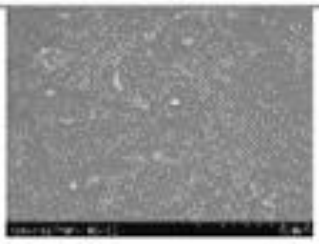
SEM magnification	Heat cure	Light cure
50		
200		
500		
750		
1000		

Figure 15. Images of Scanning Electron Microscope

The technique was selected due to the as tedious steps were not required to weigh chlorhexidine powder and mix them with acrylic resin powder in proper proportions. The fear of chlorhexidine affecting polymerization was avoided by the addition of chlorhexidine in already cured discs (20). Immersing appliances and testing for efficacy mimic the real-life use of mouthwashes (21). HPLC testing technique is simpler and faster. It is also specific, validated, and reliable and can be used for quality control.

However, the present study suffers from some limitations. Firstly, since this is an *in-vitro* study, the obtained results cannot be directly extrapolated to the *in-*

vivo environment. Therefore, the subsequent step would be to implement the same idea in *in-vivo* tests; nonetheless, the collection of saliva samples from patients has always been perplexing and challenging. There have been always concerns over the collection of samples since it requires a lot of pre-planning as to which hour in the day these samples should be collected, whether they should be stimulated or unstimulated, and it needs a higher degree of compliance from participating subjects.

Various antimicrobial agents like nystatin, amphotericin, tea tree oil etc have been tested either by directly mixing with the resin or by soaking the resin in these agents. With development in nanoparticles chemistry use of them have become beneficial and ease in incorporation. Similar studies have been conducted by mixing acrylic resin with silver nanoparticles acting as an effective antimicrobial agent (22). These results are encouraging enough to proceed further and one day the prosthesis will be certainly used as a drug delivery system.

Conclusion

In this study, it has been observed that chlorhexidine release in heat cure discs is less when compared to that in standard stock solution; nonetheless, it is good enough to reach the minimum inhibition concentration of chlorhexidine to be effective against candidiasis.

Conflicts of Interest

The authors declare that they have no conflict of interest regarding the publication of this study.

Acknowledgments

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