

An in Vitro Study to Evaluate the Growth of Candida Albicans on Three Different Forms of Denture Adhesives

Abstract:

Statement of Problem: Dentists are pivotal in guiding patients towards selecting the most suitable denture adhesives from the diverse range of commercially available adhesives.

Purpose: The purpose of this study is to comprehensively evaluate and analyse the influence exerted by three distinct forms of denture adhesives on the growth and proliferation of *Candida albicans*.

Material and Methods: Growth of *Candida albicans* was tested on Poligrip powder, cream, and strip form of adhesives. 160 wax patterns were processed into denture base resin specimens using heat-cure techniques. *Candida albicans* suspension was prepared and inoculated onto the specimens. Four groups of specimens were treated with different forms of Poligrip denture adhesives while one group served as control. Specimens were incubated for various periods, and pH was measured. Colony counting was conducted to assess candidal growth on the specimens. One-way ANOVA and Tukey's post hoc test were performed to determine the significance among mean values.

Results: At both 6 and 24-hour intervals, the strips and powder forms exhibited more favourable pH levels and fewer colony forming units (CFUs), while at 48 and 120-hour intervals, the strips form showed the most favourable pH and the lowest number of CFUs. Specifically, the strips form demonstrated the lowest colony count, followed by the powder form, then the cream group, indicating that the control group had the highest *Candida albicans* growth.

Conclusions: The strip form of denture adhesive showed least number of colony forming units and showed better antifungal property as compared to other forms.

Key-words: Growth of *Candida Albicans*, Different forms of Adhesives, Better form of Denture Adhesive

Introduction:

The presence of *Candida albicans* as a colonizing agent on denture surfaces contributes to the development of chronic denture stomatitis. This condition manifests as persistent inflammation and redness in the mucosal regions covered by the denture, creating discomfort and potential complications for affected individuals. The chronic nature of denture stomatitis shows the importance of addressing *Candida* colonization as a preventive measure to mitigate the risk of this common and impactful disorder among denture wearers. Furthermore, the impact of *Candida albicans* colonization extends beyond denture stomatitis to include conditions like angular cheilitis. This ailment involves inflammation and

fissuring at the corners of the mouth, adding another dimension to the spectrum of denture-related issues associated with *Candida* overgrowth. The interplay between *Candida* colonization and these oral conditions emphasizes the need for a comprehensive understanding of the risk factors

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involved and the implementation of effective preventive measures in the care of denture wearers.



Fig. 1

Materials and Methodology:

A sum of 160 acrylic resin Specimens (32*8*4 mm) were fabricated and separated into 4 groups, each containing 40 specimens. The initial group (Group 1) served as control, receiving no adhesive treatment. The remaining groups were treated with different forms of Poligrip denture adhesives: Powder (Group 2), Cream (Group 3), and Strip (Group 4). Standard strains of *Candida* were then added to each subgroup, and then specimens from each subgroup were placed in incubation for specified time periods, Subgroup A was incubated for 6 hours, subgroup B for 24 hours, subgroup C for 48 hours and subgroup D for 120 hours. Consequently, based on the duration of incubation, each group was further subdivided into four subgroups.

Fabrication of Heat Cure Denture Base Resin Specimens:

160 wax patterns were placed into the Hanau dental flask, followed by flasking, and packing procedures utilizing DPI heat-cure. Afterward, the specimens underwent polymerization, in a water bath at 74°C for 8 hours, followed by boiling at 100°C. Subsequently, the flasks were left to cool at room temperature before being opened. Upon deflasking, all samples were submerged in distilled water at 37°C for 12 hours to facilitate the release of residual monomers.

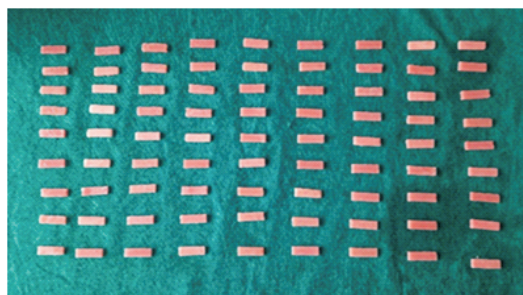


Fig. 2

Preparation Of Candidal Suspension:

A suspension of *C. Albicans* was utilized in this study. *Candida* was cultured on sabouraud dextrose agar (SDA) at

37°C for 48 hours under aerobic conditions and the inoculum of *candida* was prepared in sabouraud dextrose broth (SDB) by incubating at 37°C for 48 hours with vigorous agitation.



Fig. 3



Fig. 4

Addition of Denture Adhesives:

The acrylic resin specimens underwent sterilization and were subsequently categorized into four groups, each comprising of 40 specimens. The initial group of 40 specimens served as the control (Group 1), where no adhesive was applied. In the other three groups, consisting of powder (Group 2), cream (Group 3), and strip (Group 4) forms of Poligrip denture adhesives, were added accordingly (figures 5,6,7). The acrylic resin specimens were positioned at the flat bottom of tissue culture bottles. 50 micro litres of candidal suspension were introduced to tissue culture bottles and inoculated at 37°C. The specimens underwent a one-hour polymerization process to facilitate the inoculation of *candida* on them. Following this incubation period of one hour., 2ml of SD broth was dispersed in all culture bottles. Then based on incubation period (i.e. 6H,24H,48H,120H). each group was subdivided into 4 subgroups. Specimens from each subgroup were then placed in incubation at 37°C for 6 hours (A), 24 hours (B), 48 hours (C), and 120 hours (D).



Fig. 5



Fig. 6



Fig. 7

Ph Measurement:

The pH measurement of all samples was done with the help of pH meter at specific incubating period of each specimen (fig 8).



Fig. 8

Counting of Colonies:

After measuring the pH, solution in each culture bottle was utilized to create inoculum on agar plate using inoculating loop. Later colony forming units (per millimeter) were counted with the help of colony counter.

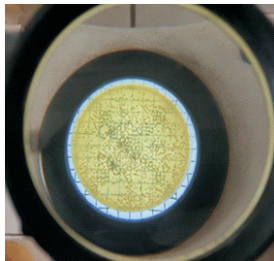


Fig. 9

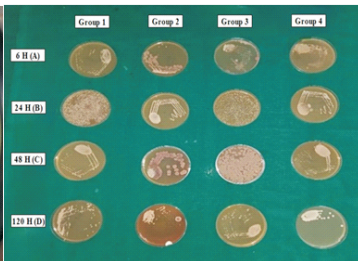


Fig. 10

Statistical Analysis:

Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp. was used to perform statistical analysis of the study.

Descriptive Statistics:

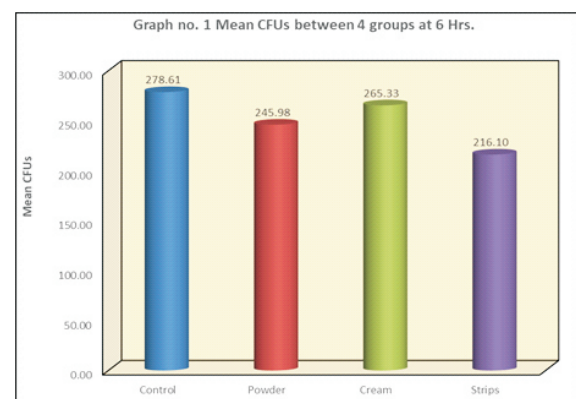
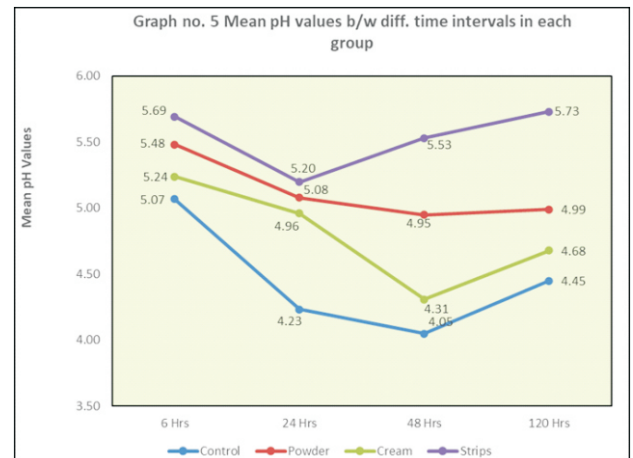
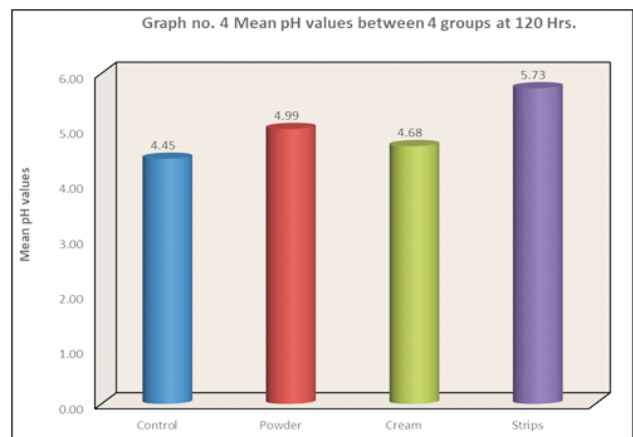
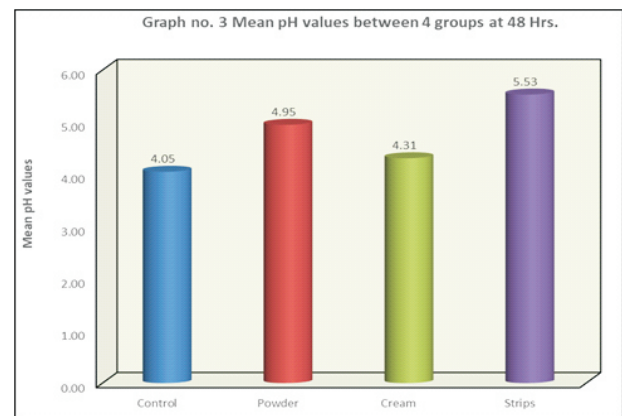
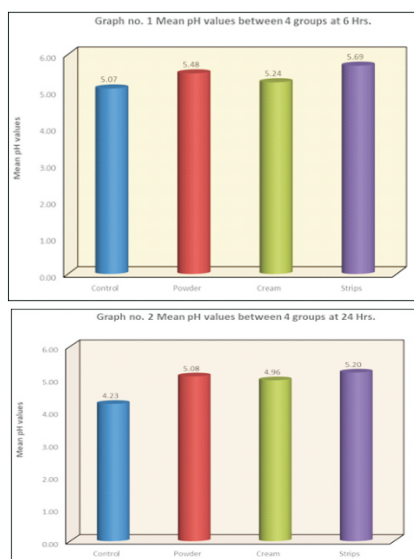
Descriptive analysis includes expression of pH values & CFUs in terms of Mean & Standard deviation for each group.

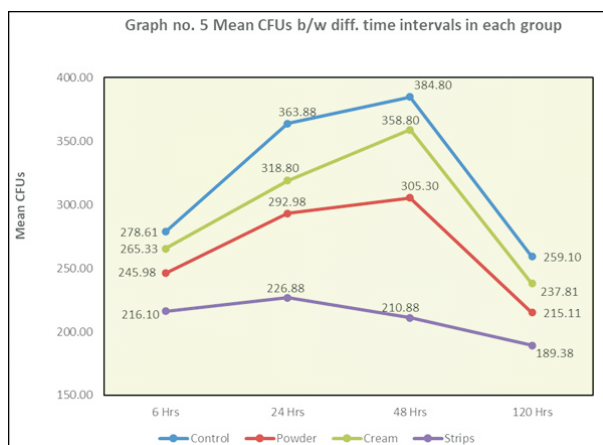
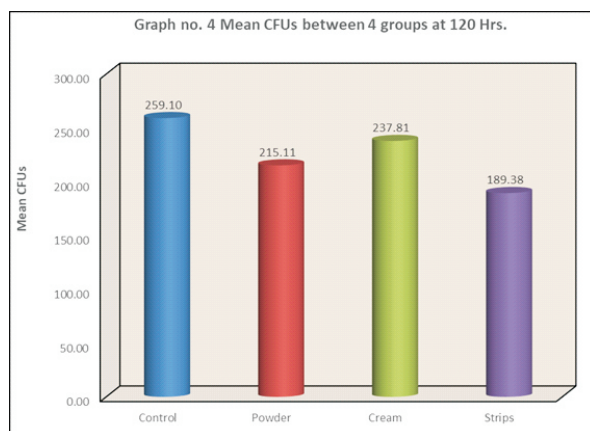
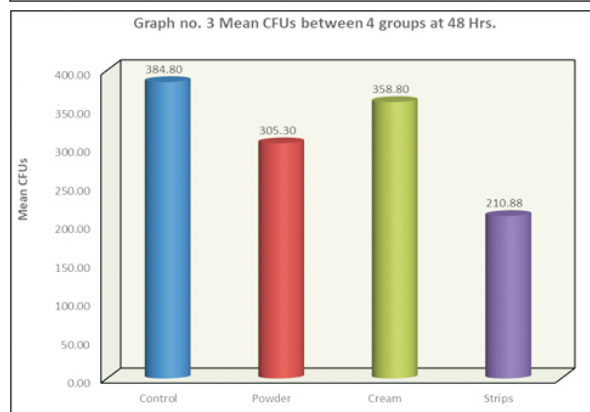
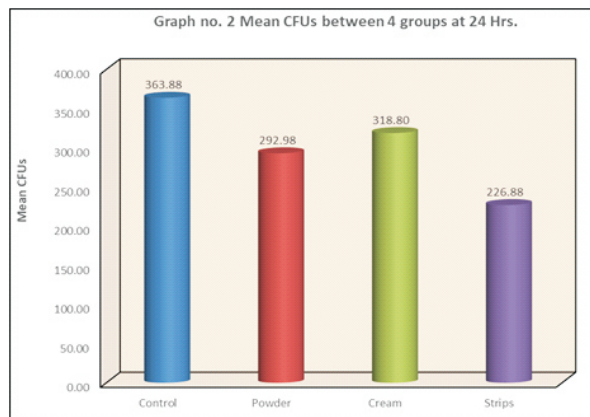
Inferential Statistics:

One-way ANOVA Test followed by Tukey's post hoc Test was used to compare the mean pH values & CFUs between 4 groups at different time intervals.

Repeated Measures of ANOVA Test followed by Bonferroni's post hoc test was used to compare the mean pH values & CFUs between different time intervals in each group. The level of significance was set at $P < 0.05$ in this study.

Results:





Discussion:

The findings revealed that after incubation of 6 hours, there was a decline in the medium's pH, possibly due to acid generated by candidal organisms during their early growth stage. Specimens treated with adhesive in strip (Group 4) and powder (Group 2) forms showed optimal pH with lesser colonies, while cream (Group 3) and the control group (Group 1) exhibited lower pH levels and increased colony-forming units (CFUs). After 24 hours, the pH decline and rise in colony count could be attributed to yeast cells entering the logarithmic growth phase. Strips (Group 4) and powder (Group 3) displayed a more favourable pH and reduced colony count. After incubation of 48 hours, a decline in pH with rise in candida colonies was observed in specimens with control (Group 1), powder (Group 2) and cream (Group 3) forms respectively. However, specimens with strip form (Group 4), showed increase in pH and decline in the number of CFUs.

After incubation of 120 hours, a rise in pH and decline in CFUs was observed in all the groups. It could be due to release of soluble microbicidal components from adhesives. Lowest number of colonies were observed in strip form (Group 4). The study's findings led to rejection of the null hypothesis, while accepting alternate hypothesis suggesting a significant difference.

Patients with complete or partial edentulism commonly utilize different types of denture adhesives. Limited research has been carried out on various forms of denture adhesives in the literature. Therefore, this study was conducted to evaluate the antifungal impact of different denture adhesive forms on *C. albicans*. out of other forms, strip form showed better antifungal effect compared to powder and cream forms. The inclusion of various forms of denture adhesives broadened the scope of this research, providing valuable insights into the potential impact of these materials on oral health and the prevention of candidal infections in denture wearers.

Conclusion:

With the limitations of this in vitro study, the following conclusions can be drawn:

1. Control group exhibited greater number of colony forming units of *C. albicans* compared to other forms of denture adhesives.
2. Strip and powder form of denture adhesives maintained more favourable pH and resulted in less no. of colony forming units after 6 and 24 hours of incubation compared to control and cream group.
3. Strip form of denture adhesive exhibited lowest count of colony forming units and sustained a favourable pH after 48 and 120 hours of incubation in contrast to other groups.

4. Strip form of denture adhesive has shown better antifungal property followed by powder, cream, and control group.

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