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Chitosan- A Biological Alternative to Chemical Denture Cleansers

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ABSTRACT

Statement of the problem: Although different chemical based and enzyme based denture cleansers are available, the use of a biocompatible, nontoxic naturally abundant biological material with good antimicrobial properties has never been investigated.

Purpose: The purpose of the study was to compare and evaluate the efficacy of different concentrations of low molecular weight chitosan and commercially available denture cleanser on the candidal biofilm formed on the dentures.

Materials and method: Polymethyl methacrylate acrylic discs (n=20) were fabricated and then coated with artificial saliva. Candidal biofilm was developed on the surface of the discs and was allowed to mature for 72 hours. The specimens were then treated with commercial denture cleanser, 1% LMW chitosan, 3% LMW chitosan. Distilled water served as control. One sample from each group was subjected SEM analysis to view the candidal biofilm and rest of the samples were subjected to sonication to separate the adhered microorganisms and the colony forming units were counted. The results obtained were subjected to One ANOVA and Tukey's post hoc analysis.

Results: All the denture cleansers used have significant anticandidal activity when compared with the control group. Within the experimental groups, highest anticandidal activity was shown by 3% LMW chitosan followed by 1% LMW chitosan and the lowest was shown by the commercial denture cleanser group.

Conclusion: LMW chitosan is a promising material as a denture cleanser with excellent antifungal properties. 3% LMW chitosan showed the highest antifungal activity when compared with 1%.

INTRODUCTION

Conventional removable partial and complete dentures are the most economic and common alternative to fixed restorations in the rehabilitation of missing teeth. Good hygiene maintenance is a significant factor that determines the prognosis of these prosthesis. Also, it is of paramount importance in sustaining the general health of denture wearers. Continuous wearing of dentures can lead to an increased prevalence of streptococcus mutans, lactobacillus, staphylococcus and yeasts in the oral cavity.These organisms form a biofilm over the dentures which cannot be easily removed. Hence along with mechanical methods, the use of immersion cleansers is recommended especially in elderly who cannot adequately maintain hygiene due to disease, dementia or poor dexterity.[1]

There are different types of cleansers available commercially which includes alkaline hypochlorites, alkaline peroxides, disinfectants, enzymes, dilute organic and inorganic acid.[2] Unfortunately most of these cleansers contain chemicals which can adversely affect the patient's health and also the physical properties of the dentures base resin for example, Persulfate is an ingredient present in most of the commercial cleansers. FDA in 2008 issued reports on the risks of persulfate. Even residual traces of this soaking solution can trigger a Type IV hypersensitivity reaction. Hypochlorite is also a mild to moderate tissue irritant. Severe irritation can occur in prolonged contact or in large quantities and dilute acids may alter the normal pH of the oral cavity and also the gastrointestinal system.[3] Also, there are numerous studies on the detrimental effects of these chemical cleansers on the physical properties of denture base resins such as the surface roughness, colour, hardness and flexural strength.[4] Hence, it is high time to switch over to a biological alternative that is effective, non deleterious to denture base resins and safe for patient use.

Chitosan, a biomaterial derived from partial deacetylation of chitin has excellent antifungal and antibacterial properties. Chitin is a naturally abundant and renewable polymer derived from the shells of crustaceans, arthropods and fungal cell wall. This is obtained as a by-product of fishing industry. The biocompatibility, biodegradability and lack of toxicity of chitosan along with the antimicrobial properties has led to its usage in various fields such as medicines, cosmetics, food, agriculture etc.[5] Also, chitosan-based materials have been explored extensively for a wide range of dental applications such as in oral drug delivery, enamel repair and remineralisation, prevention of caries, modification of restorative materials, as a scaffold for alveolar bone and periodontal complex healing and so on.[6] But, the use of chitosan as a denture cleanser has never been investigated.

Hence this study aimed at evaluating the efficacy of Low Molecular Weight chitosan and compare it with commercially available denture cleanser on the candidal biofilm formed on the dentures.

AIM

1) To evaluate the efficacy of Low Molecular Weight chitosan on the candidal biofilm formed on the dentures.
2) To compare efficacy of chitosan with a commercially available denture cleanser.
3)

METHODOLOGY

Twenty specimens of heat cured Polymethyl methacrylate resin of 10mm x10 mm x 2 mm were fabricated according to manufacturer's instructions (Fig 1). Before developing the biofilm, the specimens were ultrasonically cleaned to remove any contaminants from the surfaces and then coated with artificial saliva. (Fig 2).

Fig. 1: Acrylic specimens

Fig. 2: Acrylic specimens coated with artificial saliva

For biofilm development, *C albicans* was cultured in Sabaroud Dextrose Broth at 37^{o}C for 24 hours. The optical density of the culture was standardised to 0.38 which correspond to an inoculum of 1x10 ^{7} CFU/ml (Fig.3). Nutrient media was prepared for the growth of these organisms.(Fig 4)

Fig. 3: Candidal culture

Fig. 4: Nutrient broth

Twenty sterile plastic containers were then taken with 5 ml of nutrient broth each. Acrylic discs were suspended in each of these containers and 1 ml of the candidal culture was transferred to each of these containers. These containers were incubated at 37^{o}C for 72 hours to allow the candidal biofilm to mature. The nutrient media was changed to a fresh media every 24 hours. After 72 hours the candidal broth was decanted aseptically and the acrylic discs were washed with 0.9 % saline (Fig 5). These samples were then randomly assigned to 4 groups of 5 specimens each. (Table 1, Fig 5,6)

Group A: Acrylic specimens were immersed in 3 ml of distilled water overnight which served as control.

Group B: Acrylic specimens were immersed overnight in 3 ml of a commercial alkaline peroxide based denture cleanser, Clinsodent.

Group C: Acrylic specimens were immersed overnight in 3 ml of cleanser solution containing 1% Low molecular weight chitosan.

Group D: Acrylic specimens were immersed overnight in 3 ml of cleanser solution containing 3% Low molecular weight chitosan.

Table 1: Groups and the tests done

Groups	Sample size	Incubation	Test
Group A	5	Immersion overnight	Distilled water (Control)
Group B	5		Clinsodent Commercial cleanser
Group C	5		1% Low molecular weight chitosan
Group D	5		3% Low molecular weight chitosan

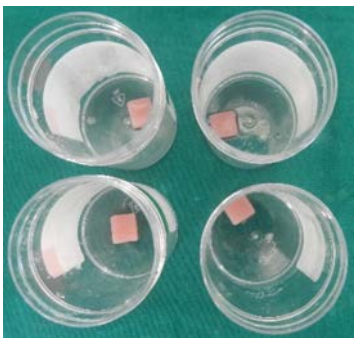
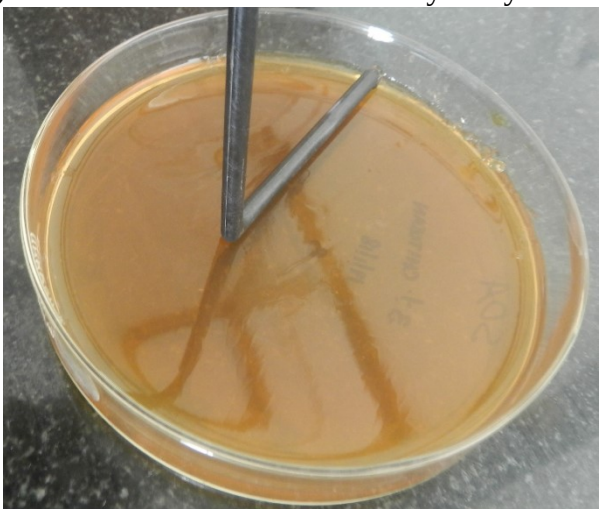


Fig. 5: Acrylic samples with candidal biofilm

Fig. 6: Samples immersed in different cleansers

After 10 hours of overnight immersion the acrylic specimens were washed with 0.9% saline. One sample from each group were then dried completely and then coated with gold and the biofilm features were visualised with SEM at 1500x in a high vacuum mode at 15kv.The remaining four samples from each group were subjected to sonication at 7 W for 30 seconds in 0.9% saline to disrupt the residual biofilm adhered to the surface. 100 microliters of this sonicated solution was cultured in Saboraud Dextrose Agar using spread plate technique (Figures 7a,7b) and was incubated at 37^{o} C for 24 hrs and the colony forming units were counted using a digital colony counter. (Figure 8) The results were then statistically analysed using one way ANOVA and Tukey's post hoc test.



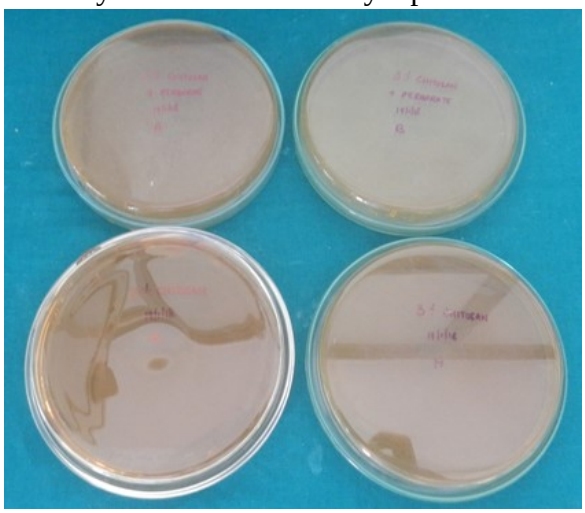


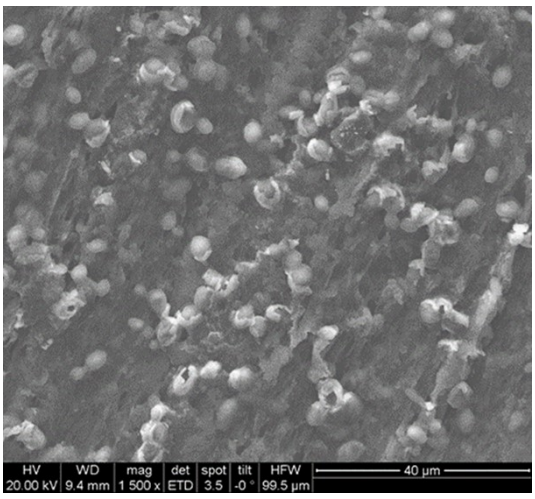
Fig. 7a,7b: Candida culture using spread plate technique

Fig. 8. Digital colony counter

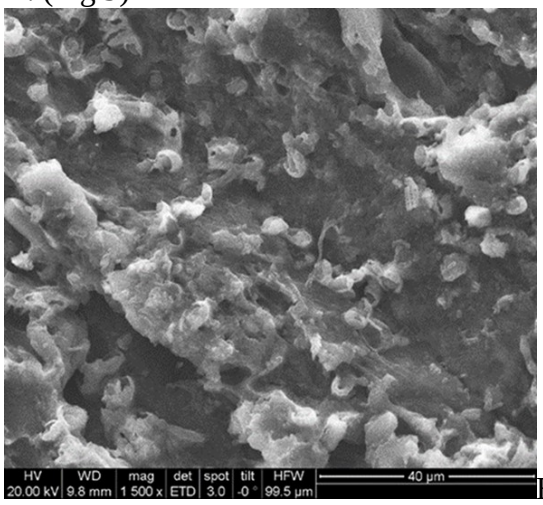
RESULTS

SEM analysis

Scanning electron microscopy revealed a well defined candidal biofilm on the surface of acrylic specimens immersed in distilled water. Scattered and lysed candida cells were seen in the commercial cleanser group. Only few cells could be seen in 1% LMW chitosan group whereas in 3% LMW chitosan, the number of cells seen were almost nil. (Fig 9)



A



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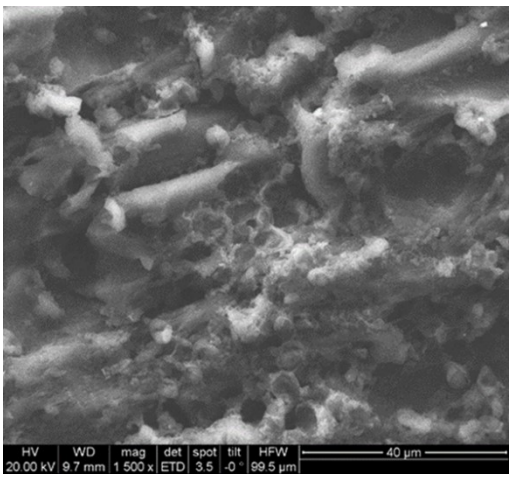

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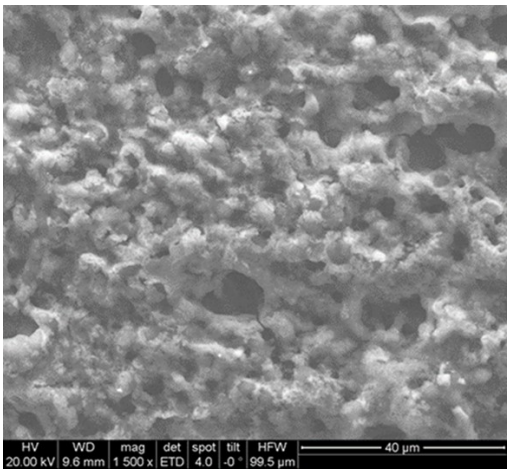


D

Fig. 9. SEM images: A. Control, B. Commercial cleanser, C.1% LMW chitosan, D. 3 % LMW chitosan

Culture study

Figure 10 shows the candida albicans colony forming units on the culture plates in different groups.

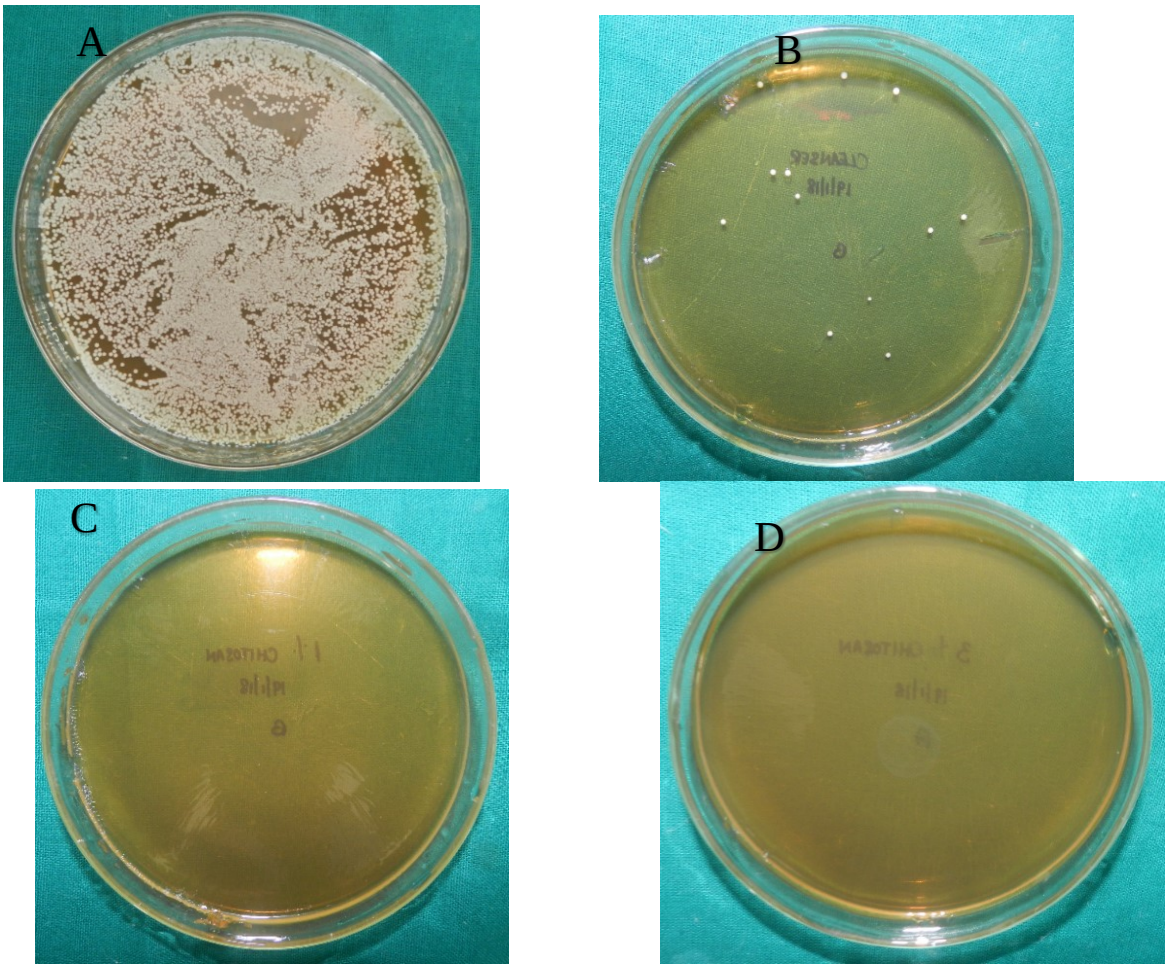


Fig. 10: Culture plates A. Positive control B. Cleanser C. 1% LMW chitosan D. 3 % LMW chitosan

The colony count after treatment with different cleansers were summarised in table 2**.**

Table 2: Candida albicans viable cell count after treatment with different cleansers

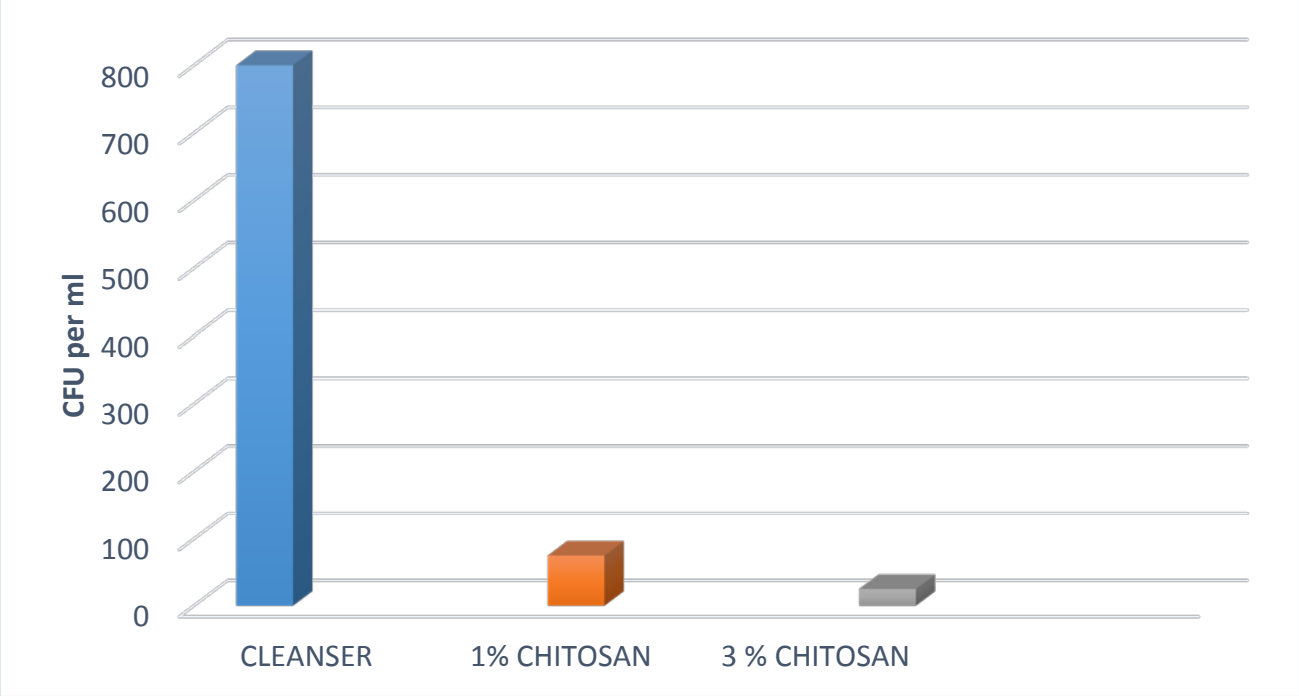
Samples	CFU per ml			
	I	II	III	IV
Distilled water	100000	120000	90000	200000
Commercial denture cleanser	1200	800	400	800
1% LMW chitosan	100	0	0	200
3% LMW chitosan	0	100	0	0

These results were then statistically analysed using one way ANOVA and the results showed a statistically significant difference between the groups (table 3) which indicates that all the cleanser groups showed significant antifungal activity.

Table 3: Difference in the mean values of CFU count in between the study groups using One-Way ANOVA.

Groups	N	Mean	Std. Deviation	F value	Sig value
Distilled Water	4	127500.00	49916.597	25.984	0.001
1% LMW chitosan	4	75.00	95.743		
3% LMW chitosan	4	25.00	50.000		
Commercial Denture Cleansers	4	800.00	326.599		

To compare the difference in between the experimental groups, Tukey HSD post hoc tests were done and the mean difference was plotted in graph 1.



Graph 1: Distribution of mean values of CFU per ml among the experimental groups.

DISCUSSION

Oral cavity has an ecological microbial population group predominated by bacteria and yeast coexisting in a relationship of commensalism. Denture wearing and indeficient denture hygiene maintenance are the predisposing factors for increase in the number of microorganisms, thereby acting as a potential source of infection. Baena-Monroy et al. reported the presence of Candida albicans on the internal surface of complete dentures.[7] Candida albicans adhesion to resin materials is promoted by oral environment temperature and the acquired pellicle formed over the dentures. It was found that Candida species (65.5%) were more than Strep. mutans and Staph. aureus adhered on the dentures.[8,9]

Candida albicans is a well-known etiologic factor for denture stomatitis. This inflammatory disease affects approximately 60% of denture wearers and causes inflammation of the oral mucosa. There are different types of cleansers available commercially which includes alkaline hypochlorites, alkaline peroxides, disinfectants, enzymes, dilute organic and inorganic acid. Unfortunately, most of these cleansers contain chemicals which can adversely affect the patient's health and also the physical properties of the dentures base resin. This has led to a greater inclination towards use of natural materials. Chitin is the second most abundant polymer in nature after cellulose and has a safe biological profile for patients. Chitosan is an amino polysaccharide with antifungal effects derived from chitin by alkaline deacetylation. It has a cationic polysaccharide with a positive charge that can react with the negatively charged cell walls of microorganisms and can damage the targeted cells, causing loss of cell membrane. It prevents the development of fungal diseases by preventing formation and maturation of biofilm and preventing the attachment of C. albicans to human mucosal cells.[10]

So, the present study aimed to compare and evaluate the efficacy of different concentrations of low molecular weight chitosan and a commercial denture cleanser on the candidal biofilm formed on the dentures. Polymethyl methacrylate acrylic discs (n=20) were fabricated and then coated with artificial saliva. Candidal biofilm was developed on the surface of the discs and was allowed to mature for 72 hours. The specimens were then treated with commercial denture cleanser, 1% LMW chitosan, 3% LMW chitosan. Distilled water served as control. One sample from each group was subjected SEM analysis to view the candidal biofilm and rest of the samples were subjected to sonication to separate the adhered microorganisms and the colony forming units were counted. The results obtained were subjected to One ANOVA and Tukey's post hoc analysis.

The results showed that all the denture cleansers resulted in significant reduction in the mean colony forming units of candida. When the antifungal efficacy in between the experimental groups were compared, it was seen that the mean colony forming units of candida in the chitosan groups was significantly less when compared with the commercial denture cleanser group which implies that antifungal activity of chitosan was superior to the commercial cleanser solution. In between the different concentrations of chitosan used, 3 % showed excellent results, in which the mean candidal colony forming units after immersion, was significantly low when compared with 1%. This may be attributed to the higher concentration of chitosan present in the cleansers.

CONCLUSION

Within the constraints of the study, it can be concluded that LMW chitosan is a promising material as denture cleanser as it showed superior antifungal properties when compared with commercial cleanser.

Limitations of the study:

In this study, the antifungal activity of the cleansers was evaluated in invitro conditions. Intra-oral conditions like the cleansing action of saliva and presence of other oral microbes are not simulated.

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