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Original Research Article

Evaluation and comparison of the antimicrobial efficacy of three commercially available denture cleansing agents against different microorganisms: An in vitro study

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ABSTRACT

Introduction: Heat cured poly methyl methacrylate denture base acrylic resins are most commonly used material for fabrication of complete dentures. In certain situations, denture hygiene is compromised both due to the constraints of the denture material and/or non-compliance from the patients owing to lack of manual dexterity. This leads to formation of microbial biofilm on the intaglio surface of the denture and if left untreated, it can cause denture stomatitis. Denture cleansing tablets are of great help in such cases.
Aim: The primary goal of this in vitro experiment was to assess and compare the effects of commercially available and common household denture cleansing tablets viz. Polident, Fittydent and Clinsodent against Staphylococcus aureus, Streptococcus mutans and Candida albicans
Materials and Methods: A total of 100 heat polymerized acrylic resin samples were divided into 4 groups of 25 each to evaluate the antimicrobial effectiveness of commercially available denture cleansing agents.
Results: The results of this study showed that there was significant reduction in colony forming units in three commercially available denture cleansing agents (Clinsodent, Fittydent and Polident denture cleansing tablets) as compared to distilled water.
Conclusion: All three commercially available denture cleansing agents used in the study were capable of reducing the colony forming units per ml of the heat cure acrylic resin denture base samples. The order of efficacy was Clinsodent tablets > Fittydent tablets > Polident tablets > Distilled water.

1. Introduction

The synthetic acrylic resins have been used since long as they have adequate physical, mechanical, and cosmetic properties; nonetheless, they are prone to microbial adhesion, which can result in denture stomatitis. By keeping plaque masses in touch with the oral mucosa for long periods of time, the denture is termed a 'plaque applicator.' Good denture hygiene is the most critical part of controlling this interaction.[1,2]

Denture hygiene is crucial to avoid odour, poor aesthetics, plaque/calculus deposition, and subsequent negative consequences on the mucosa., exogenous discoloration and removal mucin[2] and thus is of paramount importance to prevent the oral diseases amongst edentulous patients.[3]

When compared to non-denture-wearing people, denture wearers have a higher prevalence of mutans streptococci, lactobacilli, staphylococci, and yeasts in the oral cavity.[4] Neglected dentures can even produce harmful tissue

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changes.[5]

Dentures can be cleaned chemically, mechanically or a combination of two. Chemical methods are classified according to their composition and mechanism. Immersion of complete dentures in alkaline peroxide is a simple hygiene method. When these peroxides are dissolved in water, they become alkaline hydrogen peroxide, which decomposes and releases small oxygen bubbles with the mechanical action of detaching the biofilm from the denture surface. A good denture cleaner should thus meet a number of criteria, including the capacity to remove both organic and inorganic deposits, as well as any accompanying stains.

The present study was planned with following objectives:

1. To evaluate the antimicrobial effectiveness of Polident, Fittydent, Clinsodent denture cleansing tablets against Staphylococcus aureus, Streptococcus mutans and Candida albicans.
2. To compare the antimicrobial effectiveness of three commercially available denture cleansing agents viz. Polident, Fittydent and Clinsodent denture cleansing tablets against Staphylococcus aureus, Streptococcus mutans and Candida albicans.

The null hypothesis was that there is no difference in the antimicrobial efficacy of different denture cleansing agents.

2. Materials and Methods

The study was conducted at Department of Prosthodontics, VSPM Dental College and Research Centre, Nagpur and Department of Microbiology, NKP Salve Institute of Medical Sciences, Nagpur.

Metal dies were fabricated to prepare molds for the fabrication of the heat cured acrylic resin samples. Gypsum molds were prepared with preformed brass metal dies, these were then invested in lower half of flask.[6,7] The mold cavities thus obtained were used for the preparation of heat polymerized acrylic resin samples. A total of 100 samples, each of the dimension 40mm × 20mm and thickness 3mm ± 0.1mm were fabricated.

The cured specimens were carefully removed, finished and polished. The finished and polished samples were stored in distilled water for 24 hours at 37^0C to remove any residual monomer present.[8–10]

The heat cured resin samples were randomly picked and serially numbered with an acrylic bur.

In 100 ml of distilled water, 2.8 grammes of commercially available nutritional agar powder was dissolved and carefully mixed. In an autoclave, the combined solution was sterilised for 20 minutes at 15 pounds of pressure. 10 ml of sterile defibrinated sheep blood was added and stirred thoroughly. The prepared blood agar was poured into Petri dish (circular plates) and stored in refrigerator.[8]

In an unmodified domestic microwave, this beaker was put on the rotatable plate. (Morphy Richards MWO20MS)[11] and irradiated at 650 W for 3 minutes.

A swab was obtained from the surface of the polymethylmethacrylate resin specimens using sterile swab stick by wiping the surface starting from one end to other. This swab was then inoculated on blood agar plate and lawn culture was made. The plates were incubated in an incubator for 24 hours at 37 0 C. After 24 hours, plates were examined for presence of any growth and were confirmed to be sterile.[12]

Diluting the original material in serial dilution tubes, followed by plating the resulting serial dilutions onto blood agar plates, was used for the plate count procedure. Counting values between 30 to 300 CFU per agar plate are common.

Pure cultures of Staphylococcus aureus, Streptococcus mutans and Candida albicans were grown on blood agar culture media and incubated at 37^0C for 24 hours.[1] After 24 hours colonies of Staphylococcus aureus, Streptococcus mutans and Candida albicans were taken using the inoculating loop and dispersed in a test tube containing 2 ml saline solution. This tube was vortexed vigorously for 1 minute for even dispersion of colonies and then allow for a 9-minute stand time before a quick vortex to suspend any organisms present.

2.1. Three test tubes were taken as follows.

TEST TUBE A: Containing 2 ml of saline solution with colonies of Staphylococcus aureus, Streptococcus mutans and Candida albicans evenly dispersed and density was adjusted to 0.5 McFarland Standard (1.5x108 CFU)

TEST TUBE B: Containing 1.8 ml of saline solution. 0.2 ml solution from test tube A was transferred to test tube B. This test tube now had a dilution of 10 times than original solution of test tube A.

TEST TUBE C: Containing 1.8 ml of saline solution, 0.2 ml solution from test tube B was transferred to test tube C. This test tube now had a dilution of 102 times than original solution of test tube A.

All the prepared serial dilution solution were streaked separately on blood agar culture media. Total bacterial cell count for cultures from test tube A and B were > 300 CFU Total bacterial cell count for culture from test tube C was between 100 – 200 CFU.

The samples were divided into 4 groups, each group consisting of 25 samples. In group A the samples (n=25) were immersed in Polident denture cleansing solution made according to manufacturer's instructions. Contaminated samples were immersed in a container with 250 ml of warm distilled water[13] and one Polident denture cleansing tablet for 3 minutes. One sample was immersed at a time.

In group B - The samples (n=25) were immersed in Clinsodent denture cleansing solution made according to

manufacturer"s instructions. Contaminated samples were immersed in a container with 250 ml of warm distilled water and one Clinsodent denture cleansing tablet for 30 minutes. One sample was immersed at a time.

In group C In this group the samples (n=25) were immersed in Fittydent denture cleansing solution made according to manufacturer"s instructions. Contaminated samples were immersed in a container with 250 ml of warm distilled water and one Fittydent denture cleansing tablet for 30 minutes. One sample was immersed at a time.

In group D the contaminated samples (n=25) were immersed in 250 ml of distilled, sterilized water for 15 minutes.

After immersion of the polymethylmethacrylate resin specimens in the denture cleansing solutions for the appropriate time (according to manufacturer"s instructions), the samples were rinsed, excess water was removed and a swab was obtained from the surface of the specimen using sterile swab stick [14] by wiping the surface starting from one end to other. The swab was inoculated on blood agar plate and lawn culture was made. The plates were then incubated in an incubator for 24 hours at 37 ^{0} C. [3]

Every colony is assumed to be distinct and created by a single live microbial cell when calculating CFUs. The total colony counts in CFU acquired from incubated agar plates, as well as the dilution factor employed, may then be combined to determine the original number of microorganisms in the sample as CFU per ml. The formula was used to compute colony forming units per millilitre. [15,16]

3. Results

In Group A (Samples treated with Polident denture cleansing solution), colony forming units per ml (CFU/ml) was 17.6± 5.87 before treatment and after treatment was significantly reduced to 1.04 ± 1.33 which was statistically significant with p value < 0.0001. (Table 1)

In Group B (Samples treated with Clinsodent denture cleansing solution), colony forming units per ml (CFU/ml) was 17.6± 5.87 at the baseline and then after treatment it was significantly reduced to 0.48 ± 0.87 which was statistically significant with p value < 0.0001. (Table 2)

In Group C (Samples treated with Fittydent denture cleansing solution), colony forming units per ml (CFU/ml) was significantly reduced to 0.6 ± 1.0 after treatment from baseline value of 17.6± 5.87 which was statistically significant with p value < 0.0001. (Table 3, Figure 1)

Colony forming units per ml (CFU/ml) in Group D (Samples treated with Distilled Water) before treatment was 17.6 ± 5.87 and after treatment was significantly reduced to 12.12 ± 4.88 with p value < 0.0001.

Mean change in colony forming units after treating samples by distilled water was 5.48, 16.56 by Polident denture cleansing solution, 17.0 by Fittydent denture cleansing solution and 17.12 by Clinsodent denture cleansing solution. (Table 4)

After applying Tukey"s multiple comparison test to compare percentage reduction in CFU/ml amongst distilled water and three commercially available denture cleansing agents (Clinsodent, Fittydent and Polident denture cleansing tablets); Clinsodent denture cleansing tablets were most effective with percentage change of 97.27%, followed by Fittydent tablets with 96.59%, then Polident tablets with 94.09% and lastly distilled water with percentage change of 31.14%. (Table 5) From these observations it is concluded that the Clinsodent denture cleansing tablets were more effective against Staphylococcus aureus, Streptococcus mutans and Candida albicans spp.

Clinsodent tablets > Fittydent tablets > Polident tablets > Distilled water. (Figure 1)

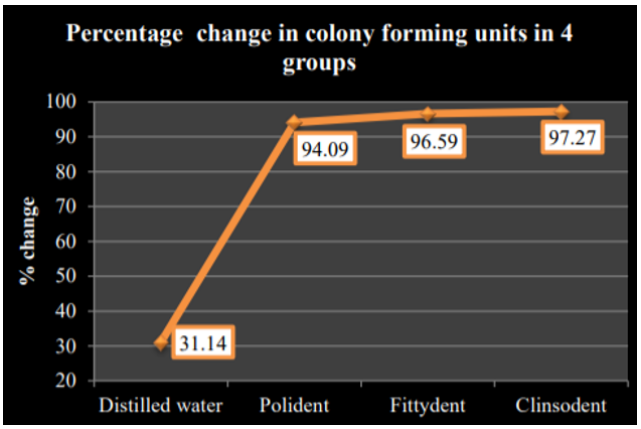

Figure 1: % change in CFU in 4 groups

4. Discussion

Any material placed in the oral cavity is quickly exposed to an organic film of glycoproteins from saliva, which forms a biofilm on the surface of the denture material, to which bacterial colonies like Staphylococcus aureus, Streptococcus mutans, and opportunistic organisms like Candida albicans attach.

The microbial plaque that accumulates on the denture's tissue-fitting surface is probably the most clinically significant. Denture stomatitis, inflammatory hyperplasia, and chronic candidacies are examples of soft tissue alterations.

Chethan et al [13] found that using chemical cleaners to clean dentures reduced the number of bacteria by a substantial amount, suggesting that this is a good strategy for cleaning dentures in geriatric patients. Jorgensen [15] found that immersion of dentures daily in alkaline hypochlorite's and denture plaque was significantly reduced when a weak solution of Chlorhexidine gluconate 0.2 percent was administered, although long-term usage of Chlorhexidine gluconate produces extensive staining of the

Table 1: Colony forming units per ml (10^4) in Polident tablets: before and after treatment

	Before treatment	After treatment
Mean	17.6	1.041
SD	5.87	1.33‘
% change	94.09%	
t value	14.7513	
p value	<0.0001, highly significant	

Table 2: Colony forming units per ml (10^4) in Clinsodent tablets: before and after treatment

	Before treatment	After treatment
Mean	17.6	0.48
SD	5.87	0.87
% change	92.27%	
t value	14.7835	
p value	<0.0001, highly significant	

Table 3: Colony forming units per ml (10^4) in Fittydent tablets: before and after treatment

	Before treatment	After treatment
Mean	17.6	0.6
SD	5.87	1.0
% change	96.59%	
t value	15.3492	
p value	<0.0001, highly significant	

Table 4: Mean change in colony forming units per ml (10^4) after treatment in 4 groups

	Polydent tablets	Clinsodent tablets	Fittydent tablets	Distilled water
Mean	16.56	17.12	17.0	5.48
SD	5.61	5.79	5.53	2.66
% Change	94.09%	97.27%	96.59%	31.14%
F Statistics		31.74		
P value				

Table 5: Post hoc multiple comparison

	Polydent	Fittydent	Clinsodent
Distilled water	11.08* (<0.0001)+ Highly Significant	11.52 (<0.0001) Highly Significant	11.64 (<0.0001) Highly Significant
Polident	—	0.44 (1.000) Not significant	0.56 (1.000) Not significant
Fittydent	—	—	0.12 (1.000) Not significant

(*indicates mean difference and + indicated p-value)

acrylic dentures.

In the present study, heat cured acrylic resin samples were used. Polident (Group A), Clinsodent (Group B), Fittydent (Group C) denture cleansing tablets and Distilled water (Group D) were used to evaluate and compare their antimicrobial effectiveness against Staphylococcus aureus, Streptococcus mutans and Candida albicans. According to the manufacturer“s recommendations the present protocol comprised soaking the heat polymerized polymethylmethacrylate resin specimens for 30 minutes in both Clinsodent and Fittydent denture cleansing solutions and 3 mins in Polident denture cleansing solution.

These sterilized polymethylmethacrylate resin specimens were then contaminated by 2 ml of saline solution containing colonies of Staphylococcus aureus, Streptococcus mutans and Candida albicans evenly dispersed and density adjusted to 0.5 McFarland Standard (1.5x108 CFU).[7] Quantification of the colonies was done by the number of colony forming units per ml (CFUs)

The CFU/ml of the samples of Group A after being treated was reduced by 94.09% (p<0.0001) which was found to be highly significant reduction statistically. The CFU/ml of the samples of Group B after being treated was reduced by 97.27% (p< 0.0001) which was found to be highly significant reduction statistically. The CFU/ml of the

samples after being treated was reduced by 96.59%, ($p< 0.0001$) which was found to be highly significant reduction statistically. The CFU/ml of the samples of Group D after being treated was found to have reduced by 31.14% which was statistically insignificant.

5. Limitations of the Study

Being an in-vitro study, the oral environment was not simulated completely. A clinical trial can be undertaken which would be more significant. The antimicrobial effectiveness of denture cleansing agents can be evaluated against other microorganisms present in the oral cavity (E.Coli, Klebsiella, Fusobacterium etc.)

Although all the samples were visually checked for any irregularities and porosities before testing, micro porosities and irregularities present in the samples may play an important role in reducing the efficiency of denture cleansing agents. It is difficult to simulate the oral conditions appropriately as the study was carried out under laboratory conditions.

The order of efficacy was Clinsodent tablets > Fittydent tablets > Polident tablets > Distilled water.

6. Conclusion

All three commercially available denture cleansing agents used in the study were capable of reducing the colony forming units per ml of the heat cure acrylic resin denture base samples. There was significantly more reduction in CFU/ml after the use of three commercially available denture cleansing agents (Clinsodent, Fittydent and Polident denture cleansing tablets) as compared to distilled water.

7. Source of Funding

None.

8. Conflict of Interest

None.

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