A Comparative Study to Evaluate the Effectiveness of Two Commercially Available Disinfectants on Denture Base Acrylic Resin with an Organic Load

Shivakumar N Puranik MDS, Pavan Kumar MDS

Abstract
Background: The literature demonstrates that optimum disinfection can be obtained with the use of proper disinfectants for a proper period of time. Aim: To compare the efficacy of two disinfectants on denture base acrylic resin with an organic load. Materials and Methods: Seventy acrylic resin strips of size 48x2x8mm were made. Before each test the strips cleaned with chlorhexidine gluconate and two acrylic resin strips were selected and cultured for organism growth to ensure sterility. Six sterile acrylic resin strips were used for each disinfectant at each time. For each test two strips were used (two for Staphylococcus Aureus, two for E.Coli and two for Candida Albicans) in each test one strip used as control group (normal saline). The data were analyzed statistically using one way ANOVA and Duncan Multiple Range Test. Results: Among two disinfectants 2% Glutaraldehyde achieved complete disinfection in 2 minutes for S. Aureus, E. Coli and C. Albicans. Conclusion: It is necessary to use denture disinfectants after removal and before insertion of denture for any adjustment.

Keywords: Denture Disinfectants, 2% Glutaraldehyde, 5.25% Sodium Hypochlorite, Organic Load.

Received on: 05/01/10 Accepted on: 04/05/10

Introduction
Prostheses have been identified as a source of cross contamination between patient and dental personnel(1, 2). Although aseptic guidelines were established to minimize the potential for disease transmission by prostheses, many laboratories and dental offices have failed to follow these procedures(3). Dental personnel have an increased risk of infection through constant exposure to debris, plaque and saliva, which harbor pathogenic organisms and adhere to prostheses. Dental personnel also may transmit potentially harmful organisms to patients. These organisms pose an increased risk to elderly and medically compromised patients. Chemical disinfectants are a recommended method to prevent cross contamination when used after removal and before insertion of a prosthesis into the mouth.

Materials and Methods
Materials used in this study.
1. DPI heat cure acrylic denture base resin for making acrylic strips.
2. Cold Mould Seal, DPI Company India.
3. Acrylic strips [Figure 3]
4. Glutaraldehyde 2% (Cidex) Johnson and Johnson Company of India. [Figure 1]
5. 5.25% Sodium hypochlorite, Novo Company of India disinfectant. [Figure 1]
6. 0.9 Normal saline as a control group.
7. Chlorhexidine gluconate used for cold sterilization for acrylic strips before each test.
8. 0.2% sodium thiosulfate used as a neutralizer broth.
9. Deionized water to mix with disinfectant to dilute the solution.
10. Trypticase soy broth as a nutrient medium.
11. 10% inactivated horse serum as an organic load.
12. Suspension of albicans $10^5$ concentration.
13. Suspension of staphylococcus aureus $10^6$ concentration.
14. Suspension of E. coli $10^7$ concentration.

Instruments used:
1. Procelain Jar for mixing acrylic resin.
2. Metal die for preparing acrylic strips. [Figure 2]
3. Flask and clamp for curing.
4. Acrylic burs for trimming and polishing the strips.

Equipment used:
1. Acrylizer (Kavo) for curing the acrylic strips.

2. Hydraulic bench press
3. Water bath [Figure 4]
4. Incubator [Figure 5]
5. Centrifugator [Figure 6]
6. Biological oxygen demand incubator

B. Method employed
Preparatory procedures:
1. Seventy heat cured acrylic resin strips of polymethyl methacrylate resin of size 48x2x8 mm were made. All the fabricated resin strips are ensured for free of porosities, fins, cracks and depressions. The resin strips are finished and polished by following procedures used for complete dentures.
2. Before each test, the strips were cleaned and sterilized with chlororhexidine gluconate.
3. Two days after sterilisation, two acrylic resin strips were selected and cultured for organism growth to ensure sterility.
4. Six sterile acrylic resin strips are used to test each disinfectant efficacy and control solution, in each of four time periods.

5. Control group – one strip is used as a control for each organism. The control group selected is a 0.9 normal saline solution.

Microbiology lab procedures
1. The inoculum contained trypticase soy broth as the nutrient medium and 10% inactivated horse serum as the organic material. The serum is inactivated by heat treating at 56°C for 30 minutes in a water bath and diluted to 1:10 with nutrient broth.

2. Standardised suspension of C.albicans in the concentration of $10^5$, S.Aureus in the concentrations of $10^6$ and E.coli in the concentrations of $10^7$ organisms/ml are used in the inoculum.

3. To prepare stock culture, freeze dried organism are reconstituted and added to 9ml of sterile trypticase soy broth.

4. This broth is incubated at the appropriate organism temperature for 24 hours.

5. One ml of each organism suspension is transferred to 9ml of nutrient broth daily to maintain viability and were killed after 15 consecutive transfer.

6. Fresh solutions of disinfectants are prepared the day of the experiment with sterile deionized water in the ratio of 1:10.

Test Procedure
1. Each acrylic resin strip is immersed in 9ml of inoculum for 45 minutes.

2. Each strip is removed and immersed in 9ml of disinfectant and sterile saline solution for 2 minutes, 5 minutes and 10 minutes.

3. At the end of appropriate disinfectant time the strips are removed from the disinfectant and immediately immersed in neutralizer broth containing 0.02% sodium thiosulfate in trypticase soy broth.

4. The resin strip is incubated for 72 hours at 37°C for S. Aureus and E.coli and for 72 hours at 25°C for C. albicans.

5. Observation for turbidity is made over a period of 72 hours to determine the viability of the organisms.

6. At the end of 72 hours each test tube is centrifuged and 0.1ml aliquots are subcultured and plated on trypticase soy agar. The plates were incubated for 24 hours to observe for the presence of organisms.

7. All test procedures are repeated five times for each disinfectant with each organism in the 2min, 5min and 10minutes time period. The control is performed once in each of the time periods.

Results
The basic data presented in Table I Shows the comparison of effectiveness of disinfectants on S. Aureus, E. Coli and C. Albicans (+) indicates growth of the organism and (-) indicates no growth. 5.25% sodium hypochlorite is effective on S. Aureus E.coli and C.albicans at 5 min and 10 minutes disinfection time but ineffective at 2 minutes disinfection time. 2% Glutaraldehyde showed complete disinfection in 2 minutes for S.Aureus, E.Coli and C.Albicans.

All test procedures were repeated for 5 times for each disinfectant with each organisms in the 2,5 and 10 minutes time period. The control was performed once in each of the time period.
Discussion

Oral and other pathogenic microorganisms associated with local and systemic diseases have been cultured from contaminated dental prostheses. The potential pathogens include those tested in this study are C. albicans, S. aureus, and E.coli. Contaminated prostheses De paola (1983)[4] provide a source for cross contamination between patient and dental personnel when aseptic techniques are not followed. According to Kahn (1982)[1] often, short appointments scheduled for prostheses manipulation leave inadequate time for proper cleaning and disinfection of a prostheses. Organic material remaining on the prostheses before they are disinfected may inhibit the effectiveness of the disinfecting solution. Therefore, the selection of a disinfectant should be based on its biocidal effectiveness in the presence of organic material and on the time required for it to achieve disinfection.

This study stressed the importance of testing the disinfectants under conditions that simulate prostheses in the mouth. The strips were bathed in an EPA-designated organic material that reflected the minimal organic concentration present in the mouth combined with similar concentrations of disinfectant for short periods to simulate typical office procedures.

Sodium hypochlorite, used for disinfecting prostheses in many laboratory and clinical settings, is effective but has many limitations. Beaumont and McKinney tested S.aureus and E.coli and found that the biocidal activity of sodium hypochlorite decreased with increased concentrations of organic material. Speigelman and Giambrone compared sodium hypochlorite and chlorine dioxide by using various strengths of organic material and found chlorine dioxide to be less affected by the organic material than sodium hypochlorite. Similar results were found in this investigation. 2% Glutaraldehyde was more effective in killing the bacteria tested in the presence of a 10% organic load than sodium hypochlorite on acrylic resin. However, these results

<table>
<thead>
<tr>
<th>Disinfectants</th>
<th>S.aureus</th>
<th></th>
<th>E.coli</th>
<th></th>
<th>C.albicans</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 Minutes</td>
<td>5 Minutes</td>
<td>10 Minutes</td>
<td>2 Minutes</td>
<td>5 Minutes</td>
<td>10 Minutes</td>
</tr>
<tr>
<td>5.25% Sodium hypochlorite (Novo)</td>
<td>$a^+$</td>
<td>$b^-$</td>
<td>$b^-$</td>
<td>$a^+$</td>
<td>$b^-$</td>
<td>$b^-$</td>
</tr>
<tr>
<td>2% Glutaraldehyde (Cidex)</td>
<td>$b^-$</td>
<td>$b^-$</td>
<td>$b^-$</td>
<td>$b^-$</td>
<td>$b^-$</td>
<td>$b^-$</td>
</tr>
<tr>
<td>Control Saline</td>
<td>$a^+$</td>
<td>$a^+$</td>
<td>$a^+$</td>
<td>$a^+$</td>
<td>$a^+$</td>
<td>$a^+$</td>
</tr>
</tbody>
</table>

Table-1 Showing the comparison of microbial growth between disinfectants on trypticase Soya agar plates after 24 hours incubation

Note: $+$ = Growth, $-$ = No Growth
0.1 ml aliquots per microorganism were plated at each time period
One Way ANOVA: $P < 0.05$.
Duncan Multiple Range Test:
The same superscripts in the columns indicate no significant difference.
were not duplicated with the fungi. In 2 minutes, both disinfectants were effective in killing the C. albicans. It is important to note that 2% Glutaraldehyde reduced a greater number of fungi in 2 minutes and 5 minute compared with sodium hypochlorite. This suggests that 2% Glutaraldehyde rate of biocidal activity with C. albicans was initially faster. Since C. albicans is the major source of denture related disease in prostheses wearers, further studies to test disinfectants on this organism are needed.

Three factors affect the time required to completely disinfect a prosthesis. One factor is the concentration of the inoculum. For example, the s.aureus inoculum concentration of $10^7$ organisms/ml used in this study required twice as long to obtain complete disinfection compared with $10^6$ organisms/ml of concentration used in Spiegelman and Giannism study. The concentration of the disinfectant is an additional factor. The recommended ADA concentration of sodium hypochlorite, diluted 1:10, used in this study obtained complete disinfection within 10 minutes. Moore. TC, (1984)[5] on the other hand, reported that 30 minute were required when a weaker sodium hypochlorite concentration of 1.5:200 was used. The type of material and the amount of source exposed to a disinfectant are the third factor that affects the length of time required for complete disinfection.

It is evident that the concentration of the disinfectant on inoculum, the type and concentration of organic material used, and the materials disinfected will have an effect on the biocidal activity of the disinfectant. Additional studies needed to be conducted to determine the average concentration of oral organic matter adhering to prostheses, the concentration of microorganisms within the organic matter, and the mechanism by which microorganism adhere. The results of these studies should be used to determine their effects on complete dentures with the ADA – recommended concentrations of disinfectants.

**Limitation of the Study**

In this study three organisms were used to determine the efficacy of the disinfectants but their are many other pathogenic organism are present so this study is limited to some of the common organisms.

**Further Scope of the Study**

Further studies needed to be conducted to determine the average concentration of oral organic matter adhering to prostheses, the concentration of microorganisms within the organic matter and the mechanism by which microorganisms adhere.

**Summary and Conclusion**

From the results obtained the following conclusions were down.

1. 2% glutaraldehyde achieved disinfection for all three organism within 2 minutes.
2. 5.25% sodium hypochlorite achieved disinfection for all three organism within 5 minutes.

Thus recommending the use of denture disinfectants routinely to prevent transmission of diseases whenever prostheses is handled.

**Authors Affiliations:**

1. Shivakumar N Puranik MDS, Department of Prosthodontics, H.K.E Society’s S.N Institute of Dental Sciences and Research, Gulbarga, Karnataka, India.  
2. Dr. Pavan Kumar, Department of Prosthodontics, Al-Badar Dental College, Gulbarga, India.
References


5. William HN. The recovery and significance of non-oral opportunistic pathogenic bacteria in dental laboratory. JPD (1985; (54): 725-730.


7. Wakefield CW. Laboratory contamination of dental prostheses. JPD 9180; (44): 143-146.

Address of the Correspondence Author
Shivakumar N Puranik MDS
Department of Prosthodontics
H.K.E Society’s S.N Institute of Dental Sciences and Research,
Gulbarga, Karnataka, India.
E-mail ID: drshivakumarnpuranik@gmail.com
Mobile No. 9986353574