

Comparative Study to Assess the Effectiveness of Various Disinfectants on two Microorganisms and the effect of same on Flexural Strength of Acrylic Denture Base Resin - An In Vitro Study

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ABSTRACT

Background: To evaluate and compare the effectiveness of various disinfectants on *Candida albicans* (*C.albicans*) and *Staphylococcus aureus* (*Staph.aureus*) inoculated on acrylic denture base resin and effect of disinfectants on flexural strength of denture base resin.

Materials and Methods: A total of 130 acrylic denture base resin specimens were fabricated and processed according to manufacturer instructions. 82 sterile specimens were used for microbiological study. 2 specimens were cultured for organism growth to ensure sterility. 40 sterile specimens each were inoculated by immersing in Sabouraud & Nutrient broth containing microorganisms for 45 minutes each. Then the specimens were immersed in chlorhexidine, glutaraldehyde & distilled water (control) for 4 & 8 minutes. Then the specimens were neutralized. After neutralization the specimens were cultured onto Sabouraud's broth for *C.albicans* and Nutrient broth for *Staph.aureus* incubated for 72 h and observed for turbidity. At the end of 72 h subculture were made onto Sabouraud's dextrose agar media for *C.albicans*, Blood agar media for *Staph.aureus* and incubated for 48 h to observe growth.

For flexural strength testing, 8 specimens each was immersed in the above mentioned disinfectants and distilled water for 8 & 16 minutes. Each of which was then subjected to 3 point flexural load in Lloyd's Universal testing machine. The peak load was recorded and flexural strength values were calculated.

Results: The microbiological study revealed that both disinfectants were equally effective at 4 minutes against *C.albicans* & *Staph.aureus* microorganisms. Flexural strength test revealed no significant difference between test and control groups.

Conclusion: Chlorhexidine and Glutaraldehyde disinfectants are equally effective against *C.albicans* and *Staph.aureus* microorganisms. Heat polymerized acrylic denture base resin did not demonstrate any significant change in flexural strength between control and test specimens.

Key Words: Chlorhexidine, Glutaraldehyde, *C.albicans*, *Staph.aureus*, Flexural strength.

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Introduction

Dental prostheses used by patients exposed to oral microbial flora which includes bacteria, viruses and fungi. If disinfection procedures is not practiced, a cycle of cross contamination may occur and thereby expose dental personnel and patients to various infections.¹⁻²

To eliminate cross contamination all prostheses and dental appliances shall be properly disinfected by a method that is practical, easy and satisfactory.³⁻⁵ Immersion of denture in a suitable disinfecting solution for an adequate length of time to achieve disinfection or sterilization is a convenient and inexpensive method.⁵⁻⁶

Commonly used chemicals for disinfecting prostheses are glutaraldehyde, sodium hypochlorite, chlorine-dioxide, chlorhexidine etc.⁷

Various studies have been conducted to check the efficacy of disinfectants. Bell JA. et al. compared the biocidal effectiveness of chlorine dioxide and 5.25% sodium hypochlorite on acrylic resin strips inoculated with *Staphylococcus aureus*, *Candida albicans* and *Escherichia coli*.⁸ Brace & Plummer studied effectiveness of a 4% chlorhexidine scrub for 15 seconds followed by a 3-minute contact time with a chlorine dioxide solution.⁹

A denture exposed to disinfectants routinely has shown that immersion in certain disinfectant solution can affect the strength and the structure of denture base resin.⁶ The assessment of transverse strength of acrylic resins reported as a reliable method to estimate resin behavior under different experimental conditions.

Materials and Methods

Preparation of Acrylic Resin Specimen

A metal mold with three plates was fabricated of which middle plate had windows measuring 65x13x3 mm for flexural strength as per ADA specification no 12, with a one degree flare on all

four walls to facilitate easy removal of wax pattern. This was done to get uniform size of wax pattern, to make acrylic test specimens.¹⁰ Wax (modeling wax, Hindustan Dental Products) was melted and poured into the openings in metal mold. The numbering of wax pattern done, after it hardened. The wax patterns were flaked according to conventional technique and dewaxing was carried out. Acrylic resin(DPI Heat-Cure Improved ; DPI India, India) packed, trial done before final closure. After bench curing for 30 minutes, polymerization was carried out according to the manufacturer's instruction. The flask is opened after cooled to room temperature. The specimens were recovered and finishing and polishing was done using conventional method. Total of 130 specimens were fabricated. 82 specimens were used for microbiological study and remaining 48 specimens were used for flexural strength testing.

Evaluating the efficacy of various disinfectants on micorganisms:

82 acrylic specimens used for evaluating efficacy of disinfectants. Before testing the specimens cleaned, packed in sterile pouch and sterilized in ethylene oxide sterilizer.^{1,11} 80 specimens for evaluating the efficacy of disinfectants and 2 specimens cultured for organism growth to check sterility. 80 specimens divided into various groups, 40 specimens each used for testing *C.albicans* and *Staph.aureus*.

Effects of Glutaraldehyde and Chlorhexidine disinfectants on *C. albicans* & *Staph.aureus*⁹

Preparation of the inoculum:

The standard strain of *Candida albicans* and *Staphylococcus aureus* was subcultured into Sabouraud's dextrose broth & nutrient broth respectively in individual McCartney bottle (total of 80 McCartney bottles). They were incubated at

37°C for 6 hours to get a turbidity equivalent to 10⁶ organisms/ml.

Turbidity was compared to McFarland turbidity standard, adjusted to contain 10⁶ organisms/ml. Sterile horse serum was added to final concentration of 10% to simulate organic matter.

Exposure of specimens to Candida albicans and Staphylococcus aureus:

40 sterile specimens each were dipped in the above prepared inoculum for 45 minutes.

Exposure to disinfectant solutions:

At the end of 45 minutes, various groups of specimens were immersed in separate McCartney bottles containing chlorhexidine and glutaraldehyde.

Exposure to distilled water (Control):

At the end of 45 minutes, control group specimens were immersed in separate McCartney bottle containing distilled water.

Exposure to Neutralizing broth:

At the end of 4 and 8 minutes, acrylic specimens were immersed in neutralizing broth containing 0.02% Sodium thiosulphate in trypticase soy broth. This was done to neutralize the effect of disinfectant.

Inoculation into broth:

Acrylic specimens exposed to *C.albicans* were transferred to Sabouraud's dextrose broth & specimens exposed to *Staph.aureus* were transferred to nutrient broth incubated at room temperature for 72 h. Broth was observed for turbidity.

Inoculation onto media:

The specimens exposed to *C.albicans* and *Staph.aureus* were subcultured onto Sabouraud's

dextrose agar plate and blood agar plate. The plates were then incubated at 37°C for 48 hours to observe the growth of microorganisms.

The growths of *C.albicans* & *Staph.aureus* microorganisms were observed visually and were recorded.

Evaluating effects of immersion in chlorhexidine and glutaraldehyde on flexural strength of denture base acrylic resin.

The specimens were stored in distilled water, kept in incubator at 37°C for 50 ± 1 hour before testing.

The specimens were divided into various groups to immerse in chlorhexidine, glutaraldehyde and distilled water for 8 and 16 minutes respectively.

Although it was found from the microbiological results that chlorhexidine and glutaraldehyde disinfectants were effective in 4 minutes. When patient visits a dental clinic/hospital for repair, adjustments or for new denture the prosthesis will be subjected to disinfection twice, hence flexural strength test was done for 8 and 16 minutes.

After disinfection, the acrylic specimens removed from disinfectant solutions, thoroughly washed in running water dried with absorbent paper.³

Flexural Strength Testing:

The flexural strength was determined using a 3-point bend testing device in a Lloyd's Universal Testing Machine. The specimens were loaded until fracture occurred. The peak load (fracture load) was recorded in chart recorded.

Statistical Analysis

The effects of disinfectants on *Candida albicans* and *Staphylococcus aureus* were analyzed using contingency coefficient, Analysis of variance-Two way (General Linear Model), The cross tab procedure (Contingency table analysis), descriptive statistics. All the statistical calculations were done using SPSS (Statistical Package for the Social Sciences).

Results

This study was done to evaluate and compare the effectiveness of disinfectants on *C.albicans* and

disinfectants on flexural strength of denture base acrylic resin. The results are shown in table 3. No significant difference existed in the mean flexural

Table 1: Incidence of Candida albicans in test and control groups

| Duration | Disinfectants | CANDIDA ALBICANS | | | | Total | |
|--|-----------------|------------------|---------|-----------|---------|-----------|---------|
| | | Absent | | Present | | Frequency | Percent |
| 4 minutes | | Frequency | Percent | Frequency | Percent | | |
| | Chlorhexidine | 8 | 100.0 | - | - | 8 | 100.0 |
| | Chlorhexidine | 8 | 100.0 | - | - | 8 | 100.0 |
| | Distilled water | - | | 4 | 100.0 | 4 | 100.0 |
| | Total | 16 | 100.0 | 4 | 100.0 | 20 | 100.0 |
| Contingency coefficient =.707; P<.000 (HS) | | | | | | | |
| 8 minutes | Chlorhexidine | 8 | 100.0 | - | - | 8 | 100.0 |
| | Chlorhexidine | 8 | 100.0 | - | - | 8 | 100.0 |
| | Distilled water | - | | 4 | 100.0 | 4 | 100.0 |
| | Total | 16 | 100.0 | 4 | 100.0 | 20 | 100.0 |
| Contingency coefficient =.707; P<.000 (HS) | | | | | | | |

Table 2: Incidence of Staphylococcus aureus in test and control groups

| Duration | Disinfectants | STAPHYLOCOCCUS AUREUS | | | | Total | |
|--|-----------------|-----------------------|---------|-----------|---------|-----------|---------|
| | | Absent | | Present | | Frequency | Percent |
| 4 minutes | | Frequency | Percent | Frequency | Percent | | |
| | Chlorhexidine | 8 | 100.0 | - | - | 8 | 100.0 |
| | Chlorhexidine | 8 | 100.0 | - | - | 8 | 100.0 |
| | Distilled water | - | | 4 | 100.0 | 4 | 100.0 |
| | Total | 16 | 100.0 | 4 | 100.0 | 20 | 100.0 |
| Contingency coefficient =.707; P<.000 (HS) | | | | | | | |
| 8 minutes | Chlorhexidine | 8 | 100.0 | - | - | 8 | 100.0 |
| | Chlorhexidine | 8 | 100.0 | - | - | 8 | 100.0 |
| | Distilled water | - | | 4 | 100.0 | 4 | 100.0 |
| | Total | 16 | 100.0 | 4 | 100.0 | 20 | 100.0 |
| Contingency coefficient =.707; P<.000 (HS) | | | | | | | |

Staph.aureus. The results are shown in Table 1 and 2. Both disinfectants were equally effective. The study also evaluated and compared the effects of

strength values of different disinfectants. The respective mean flexural strength values of

control, glutaraldehyde and chlorhexidine groups are 72.67, 72.39 and 72.46, which is statistically insignificant.

Discussion:

Cross Contamination

When a patient visits a dental office to be treated with new dentures or to have an adjustment, repair or relines the denture has to undergo a series

To minimize cross infection, prosthesis soaked in a solution that destroys or remove organisms. Disinfectant solution suggested as a method for effectively cleaning dentures.

Microorganisms

Oral and non-oral pathogenic organisms associated with local and systemic diseases, cultured from contaminated prostheses. The potential pathogens include *Candida albicans*,

Table 3: Univariate Analysis of Variance Descriptive statistics

| Duration | | Mean | Std. deviation | N |
|----------------|-------|----------|----------------|----|
| Control | 8min | 72.58681 | 1.34244 | 8 |
| | 16min | 72.76875 | 1.24506 | 8 |
| | Total | 72.67778 | 1.25429 | 16 |
| Glutaraldehyde | 8min | 72.53183 | 1.43310 | 8 |
| | 16min | 72.26036 | 1.19028 | 8 |
| | Total | 72.39609 | 1.28032 | 16 |
| Chlorhexidine | 8min | 72.50074 | 1.45204 | 8 |
| | 16min | 72.43104 | 1.19027 | 8 |
| | Total | 72.46589 | 1.28311 | 16 |
| Total | 8min | 72.53979 | 1.34780 | 24 |
| | 16min | 72.48672 | 1.17505 | 24 |
| | TOTAL | 72.51325 | 1.25114 | 48 |

of procedures. The procedures may include try in, trimming, finishing, polishing etc.,⁶ Likewise the prostheses in contact with oral tissues, saliva or blood when removed from patient mouth at various stages contaminated by pathogenic organisms, which transmitted through direct contact or through aerosol produced during trimming, finishing or polishing procedure. Opportunistic microorganisms with varying levels of pathogenicity may be spread and disseminated in the air leading to cross infection and exposing dental professional and patients to various diseases.^{4,12} It is futile to eliminate the associated microorganisms from the mouth, if the oral tissues are inoculated repeatedly by contaminated prosthesis.¹³

Staphylococcus aureus, *Escherichia coli* etc.,⁸ The presence of *Candida albicans* as well as the cohabitation of different *Candida* species is more frequent in denture related stomatitis.¹⁴ *C. albicans* was isolated in 86% of the patients with atrophic denture stomatitis and *Staph. aureus* was isolated in a similar percentage.¹⁵

Kulak et al. studied the microbial flora in denture plaque of patients with denture stomatitis. They showed the presence of *C. albicans* at a level 100 times greater than in healthy mucosa.¹⁶ Glass et al. demonstrated that patient's dentures harbour numerous pathogenic and opportunistic bacteria and fungi. *Staph. aureus* species were the dominant cocci present and fungal isolates were primarily *C. albicans*

As per these studies *Candida albicans* and *Staphylococcus aureus* were chosen for the present study.

Disinfectants

Glutaraldehyde is bactericidal, viricidal, fungicidal, sporocidal, pseudomonacidal and tuberculocidal and is recommended for removable prosthetic dentures. Chlorhexidine a widely used antiseptic and disinfectant acts by destroying cell membrane and precipitating the cell cytoplasm. It has a broad-spectrum efficacy and is much less irritating to tissue than other products.^{4,5}

Glutaraldehyde and povidone iodine killed all contaminating microorganisms within 1 h, while the 1:5 dilution of sodium hypochlorite solution was equally effective after 24 h. Two percent glutaraldehyde was the most effective disinfectant with the least adverse effects on the physical properties of the set cast.¹⁷

Considering the above mentioned advantages, glutaraldehyde and chlorhexidine disinfectants were selected for the study.

Even the recent studies have shown that above selected disinfectants were effective, in one of the study the disinfection of acrylic resin specimens contaminated in vitro by *Candida albicans*, *Streptococcus mutans*, *S. aureus*, *Escherichia coli*, or *Bacillus subtilis*. The results showed that 1% sodium hypochlorite, 2% glutaraldehyde, and 2% chlorhexidine digluconate were most effective against the analyzed microorganisms.¹⁸

Chlorhexidine was effective in disinfection of dentures contaminated with azole resistant *Candida albicans*.¹⁹ Another study showed that 1 min time of exposure with Sodium hypochlorite was able reduce *S. aureus* and *S. viridans* followed by 0.525% sodium hypochlorite and 2% glutaraldehyde for 10 min.²⁰

In the present study the efficacy of chlorhexidine and glutaraldehyde disinfectants were tested at 4 & 8 min. time intervals against *C. albicans* and

Staph. aureus and distilled water as control. It was found that there was no growth of microorganisms in test group after 4 min. immersion. This suggests that chlorhexidine and glutaraldehyde are equally effective.

The control group showed visible growth for the tested microorganisms.

Flexural Strength

Flexural strength, transverse strength, or modulus of rupture, as this property is variously called, is essentially a strength of a bar supported at each end or thin disc supported along a lower support circle, under a static load.²¹

The longevity of a dental prosthesis depends on the physical properties of the denture base resin. Denture base polymers may fail clinically due to flexural fatigue. The assessment of transverse strength of acrylic resins has been reported to be a reliable method to estimate resin behaviour under different experimental conditions.⁴

It is desirable that the disinfection process should not involve any physical, mechanical or chemical changes in the prostheses. Since a new denture disinfected before delivery to the patient and denture disinfected before and after adjustment.

The denture could be exposed to certain disinfectants often and immersion in certain disinfectant solution can affect the strength and the structure of denture base resin.⁶

Hence decided to test the effects of chlorhexidine and glutaraldehyde disinfectants on the flexural properties of denture base resin.

The present study compared mean flexural strength recorded for heat-polymerized Acrylic denture base resin specimens subjected to immersion in disinfectants and distilled water (control) for 8 and 16 minutes.

The statistical analysis of test results revealed no significant difference among the mean flexural strength values between control and test specimens after 8 and 16 minute-immersion time

intervals. Since flexural strength depends on the bulk of the material, an insignificant change in flexural strength indicates that the bulk of the material remained intact from the influence of the disinfectant agent.

The recent study using 1%, 2%, 5.25% sodium hypochlorite, 2% glutaraldehyde, 4% chlorhexidine gluconate showed that all disinfectant solutions promoted a statistically significant decrease in hardness, where as with surface roughness, the materials tested showed a statistically significant increase, but these results could not be considered as clinically significant.²²

Heat-polymerized acrylic resins either mechanically or chemically polished, did not demonstrate significant changes in transverse strength during immersion in the disinfecting solution tested, regardless of time of immersion.²³

The microhardness values of the acrylic denture base resins were affected by the thermal cycling and disinfection (Microwave, Efferdent, chlorhexidine and hypochlorite) procedures, but values were within acceptable clinical limits for the acrylic resins.²⁴

The present study demonstrated that both chlorhexidine and glutaraldehyde are effective disinfectants against *C.albicans* and *Staph.aureus* microorganisms, with least effect on flexural strength of denture base resin.

Further studies needed to test efficacy of disinfectants on different microorganisms and various properties of denture base resin.

Conclusion

The following conclusions were made:

1. Glutaraldehyde (Cidex) and Chlorhexidine (Microshield) disinfectants were equally effective against acrylic specimens inoculated with *Candida albicans* and *Staphylococcus aureus* microorganisms after 4 minutes of immersion.

2. Heat polymerized denture base acrylic resin did not show any significant changes in flexural strength after immersion in disinfectants for 8 and 16 minutes between control and test specimens.
3. There was no significant change in flexural strength of denture base acrylic resin between Glutaraldehyde (Cidex) and Chlorhexidine (Microshield) disinfectants after immersion for 8 and 16 minutes.

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