

**INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN  
CHEMISTRY AND PHARMACEUTICAL SCIENCES**

(p-ISSN: 2348-5213: e-ISSN: 2348-5221)

[www.ijcrcps.com](http://www.ijcrcps.com)

Coden: IJCROO(USA)

Volume 4, Issue 12 - 2017

DOI: 10.22192/ijcrcps



IJCRCPS

**Research Article**

DOI: <http://dx.doi.org/10.22192/ijcrcps.2017.04.12.001>

**An *In vitro* Comparative Evaluation of Three Disinfectants  
on Heat Cure Acrylic Resin Specimens Contaminated  
with Standard and Clinical Strains of *S.mutans*  
Microorganism**

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**Abstract**

**Background and objectives:** Dental prosthesis, due to contact with tools, people and different places, have a higher risk of contamination, while preventing and disinfecting them is very important. The aim of this study was to evaluate the effect of antimicrobial solutions of 0.5% sodium hypochlorite, 100% vinegar, and 0.2% chlorhexidine on heat cure acrylic resin specimens contaminated with standard and clinical strains of *S.mutans* microorganism.

**Materials and methods:** A total of 60 acrylic sheets were made with dimensions of 17x6x1mm in one shape and one size and the same thickness. First, a new colony of *S. mutans* standard strain was prepared. Then, the specimens were immersed in the microorganism suspension to form an *experimental biofilm-related* *Candida* infection. The specimens were divided into four subgroups of 15 and each group was immersed in sterile containers containing 0.5% sodium hypochlorite, 0.2% chlorhexidine, 100% vinegar, and water. A total of five samples were isolated from each group and were turbid in an interval time of 30min, 2hrs, and 4hrs, respectively. *Data were analyzed using SPSS Ver 19.0.*

**Results:** All three disinfectant solutions resulted in complete elimination of *S.mutans* micro-organism provided that the immersion time lasts about thirty minutes. The long term effect of vinegar after 2hrs reduced to 80% and the effect of hypochlorite after 4hrs significantly reduced to 40%.

**Conclusion:** Among the three denture cleansers used in the present study, all were considered to be effective in cleaning and removing bacteria from denture at specific time interval.

**Keywords:** Microorganism, *S.mutans*, Disinfectant, Denture,

## Introduction

Dental prosthesis, due to contact with tools, people and different places, have high probability of contamination, and *disinfection* and sterilization of them are very important. The *prosthesis* becomes colonized with various *microorganisms* which can produce diseases such as pneumonia, conjunctivitis, and meningitis.<sup>1</sup>

*Biofilms* are the predominant growth state of many *microorganisms*. Dental *biofilm* pathogenicity in the *oral cavity* is magnified by specific *biofilm*. The connection and colonization of various microorganisms on the denture surface causes biofilm production in the oral cavity.<sup>2</sup> *Denture-related stomatitis* is a chronic biofilm-mediated disease and the *most common* form of oral candidiasis affecting two in every three complete denture wearers.<sup>3,4</sup> Denture plaque containing *Candida* could cause not only oral candidiasis, like oral thrush or denture-induced stomatitis, but also caries, root caries and periodontitis of abutment teeth. These *plaque* deposits can lead to serious problems, such as systemic infections with *candida* fungi, pneumonia, and even heart disease.<sup>5</sup> It is essential to pay attention to the condition of oral mucosal membrane and denture hygiene habits. Denture sanitization is an important element in the treatment of denture stomatitis and should be emphasized to the patients. The 6-month incidence of denture stomatitis can be significantly reduced by educating nursing home caregivers about oral health care. The incidence of denture stomatitis and the duration of denture wear are highly correlated. Dentists can help prevent this condition by instructing patients to take their dentures out of their mouth for 6-8 hours each day. Mechanical plaque control and appropriate denture-wearing habits are the most important measures in preventing and treating the disease.<sup>6</sup>

*Studies of microbial biofilms have led to realize that chemical antimicrobial agents play a significant role in the removal of microorganisms and microbial plaques.*<sup>10</sup> The efficacy of a *disinfectant* depends on *sufficient* length of treatment. The sufficient immersion time is effective to inactivate most oral microorganisms. There are various chemical disinfectants such as sodium hypochlorite, chlorhexidine, glutaraldehyde, alcohol, hydrogen peroxide, iodophors, and phenols which *despite their success some researches have cited drawbacks.*<sup>11-15</sup>

Recent scientific investigations clearly demonstrate the antimicrobial properties of household materials such as vinegar to disinfect acrylic resins and oral prostheses. The acetic acid in undiluted vinegar may be used effectively for cleaning dentures, and, unlike bleach solutions, vinegar residues left on dentures were not

associated with mucosal damage.<sup>16,17</sup> Chlorhexidine is a potent antiseptic agent. It reduces plaque formation and inhibits the growth of Gram-positive cariogenic microorganisms including *S. mutans*. It binds to negatively charged constituent parts including bacterial cell walls, salivary pellicle and mucosa, giving it high substantivity.<sup>16,17</sup> At low concentrations (<1%), chlorhexidine is bacteriostatic by interfering with cell wall transmission leading to leakage of intracellular components. At high concentrations (>1%), it is bactericidal by causing precipitation of the intracellular cytoplasm. It also inhibits the action of glycosyltransferase, thus preventing adhesion of bacteria to the tooth surface.<sup>16,17</sup> Alkaline hypochlorite solutions have also shown good results for disinfecting the denture. Antibacterial and antifungal properties of this solution can inhibit the growth of *bacteria*.<sup>9,18</sup> Sodium hypochlorite(NaOCl), also called as liquid *bleach*, is a powerful oxidizing agent that is *widely used* as a *disinfecting and bleaching agent*.<sup>19</sup> Sodium Hypochlorite 5% is a broad-spectrum disinfectant that has an effect on a large number of aerobic and anaerobic bacteria.<sup>20,21</sup> Various studies have shown that *sodium hypochlorite* reduce the adhesion of all *Candida albicans* strains. This solution is *inexpensive and readily available and has a wide spectrum of antimicrobial*.<sup>22</sup> However, these solutions have not only bad taste, but, most importantly, may damage denture materials, depending on the immersion time and concentration.<sup>13,14,19</sup> For example, they may whiten acrylic resins and cause corrosion to metal components.<sup>23,24,25</sup> Other studies have shown that immersion in solutions such as 1% sodium hypochlorite and 4% chlorhexidine can reduce the hardness of acrylic resins depending on the duration of immersion and the concentration of the solution.<sup>26,27</sup> A variety of *denture disinfection methods* have been studied. Further researches are needed to develop towards developing *solution cleansers*. The aim of this study was to evaluate the effect of antimicrobial solutions of 0.5% sodium hypochlorite, 100% vinegar, and 0.2% chlorhexidine on heat cure acrylic resin specimens contaminated with standard and clinical strains of *S. mutans* microorganism.

## Materials and Methods

The present study was an *in vitro experimental study* conducted in the *Department of Prosthetics*, School of Dental Medicine and Laboratory of Microbiology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences in 2016. This study was carried out to evaluate the efficacy of three disinfectants of 0.5% sodium hypochlorite (Pakshoma,Iran,Tehran), 100% vinegar (Melas Gostar,Iran,Tehran) and chlorhexidine 0.2% (Iran Naju,Iran,Tehran) on on Akropars heat cure acrylic resin (Marlik Corporation, Iran-Tehran), which has been contaminated with *S. mutans*. In order to make an acrylic sample the plaster (Pars dental, Tehran, Iran) was

invested in the lower part of the flask and a glass slab was placed in the lower part on the plaster. Following the hardening of the plaster and application of biofilm on the edges of the slab, a metallic mold with dimensions  $1 \times 6 \times 17$  mm was placed on the glass surface and the upper half of the plaster was invested all around the flask. After hardening of the second stage of plaster, while the glass slab was in place, the third stage was invested and sealed in the flask and placed under pressure until the plaster became hard. In this way, the acrylic samples were enclosed in the lower and upper parts of the glass slabs. The purpose of this kind of *investing* the cast and a waxed denture in a *flask* was to obtain completely polished acrylic resin *samples* without finishing process. Then; the mold was filled with acrylic molding paste. After *acrylic performing on Stage 3*, samples were placed in the sintering machine (Kavo Ewl type 5518) at  $70^{\circ}\text{C}$  for 9 hours. A total of 64 acrylic sheets were made with the dimension of  $1 \times 6 \times 17$  mm at the same shape, size, and thickness. To protect acrylic sheets from dehydration, they were immersed in a sterile container containing sterilized distilled water and then were exposed to a temperature of about  $121^{\circ}\text{C}$  for 15 minutes and pressure of one atmosphere autoclave. The specimens were kept in a refrigerator until use. At the outset, to ensure the sterilization of the specimens, two acrylic sheets as a negative control were exposed to 2ml of liquid *medium* of brain heart infusion (*BHI*) *broth* (Merck KGaA, Darmstadt, Germany) for 24 h at  $37^{\circ}\text{C}$ . Following incubation for 24 hrs at  $37^{\circ}\text{C}$ , the samples were examined for turbidity. To validate the accuracy of the results, the samples were cultured in solid-culture media of Blood Agar.

In this study, the *biofilm formed by the S. mutans* (ATCC1683) reference strain, retrieved from the Iran Scientific and Industrial Research Center in the form of lyophilized vials. After breaking the vial, standard strains were transferred to the TSB liquid medium and incubated for 24 hours at  $37^{\circ}\text{C}$ . Afterwards, they were cultured on a solid medium for 24 hours.

Then and there, Suspension with concentration of 0.5 McFarland units ( $1.5 \times 108$  cells / mL) was prepared using colony and saline. In the next step, the remaining 62 samples were immersed in the microorganism suspension to form an *experimental biofilm*. After 24 hours, the specimens were removed from the suspension and washed with sterile distilled water. Then, they were placed on sterilized dry gauze pad. To ensure the contamination, two samples were transferred to the liquid *medium* of brain heart infusion (*BHI*) *broth* (Merck KGaA, Darmstadt, Germany) for 24 h at  $37^{\circ}\text{C}$  as positive control and their turbidity were examined. The rest of the specimens were divided into four subgroups of 15 and each group was immersed in sterile containers containing 0.5% sodium hypochlorite, 0.2% chlorhexidine, 100% vinegar, and water. At 30 minutes, 2 hours and 4 hours, 5 samples were

separated from each subgroup and washed with distilled water and were placed on sterilized dry gauze pad. Each sample was placed in test tubes containing 10cc BHI medium and the lid of test tube was closed. The test tubes were incubated at  $37^{\circ}\text{C}$  for 24 hours and then were examined for growth by observing *turbidity*. In order to ensure the results of the liquid medium, all of the BHI culture media were also sampled into a Blood Agar medium.

As a final point, the results of *S. mutans* growth in an interval time of 30 minutes, 2 hours, and 4 hours in 0.5% sodium hypochlorite disinfectants, 0.2% chlorhexidine, and 100% vinegar were examined. Data were analyzed using IBM SPSS 19.0 software (IBM, Armonk, NY, USA).

## Results

Statistical analysis showed that after 30 minutes immersion of the samples in each of the three *disinfectants* solutions, all of them were able to completely eliminate the growth of *S. mutans*. Similarly, the results of two hours immersion were consistent with the results of 30 minutes immersion, with the exception of vinegar, whose effect has fallen by 20% (downtrend pattern) and reached to 80%. The results of the four-hour period were similar to those of two previous periods, with the difference that the hypochlorite effect is reduced to 40%.

## Discussion

Despite the great advice on brushing as an effective way of removing denture biofilms, this method is more dependent on the denture's owner's skill and may have limited effects in some cases.<sup>28</sup> A practical way of compensating for this is the simultaneous use of brushing and immersion in solutions. This method is recommended as *denture-cleaning methods*.<sup>29,30</sup>

Alkaline hypochlorite solutions have revealed favorable results for denture hygiene. They exhibit *fungicidal* and *bactericidal* properties in the presence of organic matrix of the biofilm and can remove stains.<sup>30,31</sup> Various studies have recommended dilution of sodium hypochlorite (*Bleach*) for decontamination.<sup>32,33</sup> Sodium hypochlorite has not only *bad taste*, but, most importantly, may damage denture materials, depending on the immersion *time* and *concentration*.<sup>33</sup> It has been shown that immersion in denture cleanser solution, sodium hypochlorite, can affect the flexural strength and structure of acrylic resins.<sup>34,35,36</sup> Several studies have reported the antimicrobial activity and denture biofilm removal capacity of 1% - 5.25% sodium hypochlorite.<sup>37,38,39</sup> However, there is no clear consensus on *time-concentration* to achieve the highest efficacy and *minimal* adverse effects on dentures.

Chlorhexidine is a broad-spectrum biocide effective against Gram-positive bacteria and fungi. Over the years, chlorhexidine has been used in the dental practice as an excellent antiplaque antigingivitis, and antistomatitis agent. Chlorhexidine not only exhibits special property of substantivity, it also possesses a broad antimicrobial spectrum which makes its use in wide variety of oral disorders. Nearly, all disciplines of dentistry use this material in different formulations like mouth wash, gel, spray, varnish, and restorative material.<sup>46,47</sup> Vinegar is mainly a dilute aqueous solution of acetic acid and this is reflected in its physical and chemical properties. The acid part of vinegar is what gives it its sour taste and its antiseptic (germ killing) properties as well as its cleaning properties.<sup>48,49,50</sup>

In the present study the *Streptococcus mutans* was selected. *Streptococcus mutans* is a Gram-positive organism that is the primary causative agent in the formation of dental cavities in humans. This organism is facultatively anaerobic, Gram-positive coccus-shaped bacterium commonly found in the human oral cavity and is a substantial contributor to tooth decay.<sup>51,52</sup>

The present study showed that after half an hour, all solutions were able to eliminate all colonies, while after two hours, the vinegar had an effect of 80% and after 4 hours the hypochlorite effect was 40%.

Lavanya et al., 2015, in India examined the effectiveness of sodium hypochlorite 1%, chlorhexidine 2%, vinegar 100%, and sodium perborate 3.8% for disinfection of acrylic resin specimens contaminated with *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* microorganisms. They used 150 standard acrylic resin samples, of which 30 were considered as controls. Finally, each of the subgroups of study had 10 samples. The results showed that the four disinfectants had a good effect on bacterial biofilms. Chlorhexidine and sodium perborate had only a complete disinfectant effect on *Candida albicans* than two other bacteria; this finding is consistent with the results of the present study. Similarly, vinegar was effective at inhibiting the growth of *Escherichia coli* rather than two other bacteria.

Silva et al., 2015, in Brazil examined antimicrobial action of sodium hypochlorite (0.25% and 0.50%) and 10% castor oil solution for denture cleaning. In this study, 320 samples of acrylic resins were infected with *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Bacillus subtilis*, *Escherichia coli*, *Streptococcus mutans*, *Enterococcus faecalis*, and *Candida glabrata*, and then each group (10 samples) were placed in disinfectant solution for 20 minutes. Moreover, a group was placed as a negative control in the saline solution. The bacterial colonies were incubated at 37 °C for 24 hours on a solid culture medium. The results showed that sodium hypochlorite (0.25% and 0.50%) completely eliminated all detectable microorganisms. The castor oil solution eliminated *B. subtilis* and reduced counts

for other strains.<sup>27</sup> The results of Silva et al.'s study were inconsistent with the results of present study. Williams and Lewis suggested that surface roughness favored colonization by the microorganisms, contributing indirectly to tissue injury.<sup>53</sup> Sodium hypochlorite as a disinfectant has the following advantages: It can easily store and transport when it is produced on-site. Dosage is simple. Transportation and storage of sodium hypochlorite are safe. Sodium hypochlorite is as effective as chlorine gas for disinfection. Sodium hypochlorite produces residual disinfectant.<sup>54,55</sup> This material was proposed by Rodrigues et al. as the most effective method for disinfection of prostheses made of acrylic resin, provided that it has 2% active chlorine and 30 minutes immersion time.<sup>56</sup> Chau et al. confirmed that immersion for more than 10 minutes in sodium hypochlorite guarantees the disinfection of the surface of the material. However, its disadvantages include corrosion and rusting on the surface of metals, causative effects on the surface of the skin and other cells, and the destruction of linen clothes.<sup>58</sup>

Nascimento et al., 2003, reported that 100% white vinegar has effective antimicrobial activity against *E. coli* and *S. aureus* for acrylic resins.<sup>48</sup> Vinegar and other acetic acid solutions have become very popular due to the toxicity of chlorine and other disinfectants.<sup>48,59</sup> Sodium perborate tablets are used for removing dental debris and preventing breakage of instruments and can be efficient in removing biofilm.<sup>60,61</sup> Rudd et al., 1984, stated that immersion of hypochlorite in 5 minutes with concentration of 5.25% eliminates all microorganisms and 1% concentration in 10-15 minutes can eliminate strains of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*.<sup>37</sup> Other studies showed that immersion of 1.5% concentration in 20 minutes and 2% in 5 minutes could be effective against *Candida albicans*.<sup>64,65</sup> Furthermore, the concentration of hypochlorite can influence the physical properties of denture acrylic resin.<sup>34,35</sup> Paranhos et al. 2013, studied the surface roughness and flexural strength of an acrylic resin submitted to simulated overnight immersion in denture cleansers. They used the solution of 0.5% and 0.25% sodium hypochlorite for 20 minutes. The results showed changes in color and increase of surface roughness of acrylic resin, but different concentrations of hypochlorite did not have much clinical difference. Additionally, 20 min daily soaking had impact of denture cleaning method and overnight storage condition on denture biofilm.<sup>34</sup>

## Conclusion

All three disinfectant solutions resulted in complete elimination of *S. mutan* micro-organism provided that the immersion time lasts about thirty minutes. The long term effect of vinegar after 2hrs reduced to 80% and the effect of hypochlorite after 4hrs significantly reduced to 40%.

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DOI: 10.22192/ijcrcps.2017.04.12.001	

How to cite this article:

Assadollah Ahmadzadeh, Effat Abasi Montazeri, Sara Mogharabi, Maryam Jarahzadeh. (2017). An *In vitro* Comparative Evaluation of Three Disinfectants on Heat Cure Acrylic Resin Specimens Contaminated with Standard and Clinical Strains of *S.mutans* Microorganism. Int. J. Curr. Res. Chem. Pharm. Sci. 4(12): 1-7.  
DOI: <http://dx.doi.org/10.22192/ijcrcps.2017.04.12.001>