

Amount of Residual Disinfectant after Disinfection of Different Denture Base Resin Materials

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Abstract

To evaluate the residual disinfectant remained after disinfection of different denture base materials. Sixty samples are taken (n=30) each of heat cure and Self cure denture base resins and immersed in three different commonly available disinfectant solutions for 1 day and 7 days. Residual disinfectant remained is measured with UV spectrophotometer.

Aim: To evaluate the residual disinfectant remained after disinfection of different denture base resin materials.

Materials & methodology: Sixty samples are taken (n=30) each of heat cure and Self cure denture base resin materials. Samples are sub divided into 3 sub-groups based on disinfectants. Three most commonly used disinfectants are chosen, which are 2% glutaraldehyde, 2% chlorhexidine, 1% sodium hypochlorite. The specimens were produced in molds prepared by the investment of stainless-steel dies of 30 mm diameter and 3.0 mm width. With an ISO standardization number ISO/DIS 1567. Specimens are placed in each 100 ml of three different disinfection solutions. Specimens are stored for 1 day and 7 days. The testing was done with UV-Visible spectrophotometer.

Results: It was shown that more leaching is seen with sodium hypochlorite, after one day than after days followed by chlorhexidine and glutaraldehyde.

Conclusion: The amount of residual disinfectant was found more in Self cure resins when compared to heat cure resins. And it was found more after 1 day than 7 days when immersed in disinfectant solutions. The amount residual disinfectant was found to be more with sodium hypochlorite followed by chlorhexidine and least with glutaraldehyde.

Keywords: Sodium hypochlorite; Chlorhexidine; Glutaraldehyde; Disinfection; Acrylic resins; Dentures; Denture disinfection methods.

Introduction

Microbial growth on the denture surface results from the adherence of microbial cells enhanced by surface roughness, and from adhesive interactions between *Candida* species and oral bacteria¹. They can induce a chronic inflammatory response in the oral mucosa, described as denture stomatitis, which is the most common infectious disease affecting the oral mucosa and is highly prevalent in denture wearers². Materials for prostheses, such as acrylic resins, represent a perfect support for biofilm formation. *C.albicans* has been found

on both hard and soft denture base acrylic resins *in vitro* and *in vivo*³. The chemical and physical characteristics of the surface of these materials support biofilm formation through reversible and then irreversible adhesion to the surface⁴.

To prevent the transmission of disease and cross-infection, infection control procedures such as the disinfection of dental prostheses by immersion in a chemical solution must be performed before and after clinical procedures⁵. The most appropriate chemicals for disinfection by immersion of dentures are chlorine compounds, aldehydes and per acetic acid. It has been observed that 10 min of immersion in 1% sodium hypochlorite, or 2% glutaraldehyde has proved to be effective in reducing the number of microorganisms on the dentures⁶.

The studies demonstrate that the usual operating procedures of the prosthodontic laboratory are a possible source of cross-contamination between patients, technicians, and dental personnel⁷. Chemical disinfection of prosthetic materials has been an effective procedure to avoid cross-contamination among dentists, patients, assistants and technicians in the dental prosthetic laboratory⁸. However acrylic resins adsorb water and consequently disinfecting solutions. These solutions can later be released in the saliva, as demonstrated by Mähönen et al., who observed the presence of disinfecting solutions in saliva after denture disinfection procedures using 2% glutaraldehyde⁹.

This study is aimed to determine the residual disinfectant with three different disinfectant materials between self cure and heat cure denture base resins after 1 day and 7 days of immersion.

Materials and Methodology

Sample Size: Sixty samples are taken (n=30) each of heat cure and Self cure denture base resin materials. Samples are sub divided into 3 sub-groups based on disinfectants.

Three most commonly used disinfectants are chosen, which are 2% Glutaraldehyde, 2% Chlorhexidine, 1% Sodium hypochlorite. The study was conducted to compare the residual disinfectant of three disinfectant systems on the surfaces of heat cure and Self cure denture base resins after 1 day and 7 days.

The specimens were produced in molds prepared by the investment of stainless steel dies of 30 mm diameter and 3.0 mm width. With ISO standardization number ISO/DIS 1567. Wax patterns are prepared of similar dimension, using molten wax without any air bubbles and having a plane surface (FIG- 1). Dewaxing was done in a conventional manner, the liquid/powder ratio of the polymer dough for all materials was mixed according to the manufacturers' instructions inserted into the molds, and packed. Heat cure specimens are polymerized with compression moulding technique following long curing cycle. Samples with voids and depressions are discarded. The selected samples were polished with 600 grit silicon carbide paper in order to remove the surface irregularities to make the specimen smooth and flat (FIG- 1).



Figure 1: Specimen Smooth and Flat

All the specimens were placed in each 100ml of disinfectant solution, to make the samples standardized series dilutions were done which results in equal amount of disinfecting for each sample (FIG- 2).



Figure 2: 100 ml of disinfectant solution

All the samples were stored in disinfecting solution for 1 day and evaluated followed by storing the samples for 7 days and evaluation was done using UV-Visible Spectrophotometer (FIG- 3). The samples don't have any control group and no testing was done prior.



Figure 3: A-UV-Visible Spectrophotometer and B- Disinfectant placed in cuvette.

Each material has its own wavelength to produce an absorption spectrum which helps in measurement of concentration gradient. Studies recommended that for Chlorhexidine testing was done at 255nm wavelength, for Sodium hypochlorite at 492nm wavelength and for Glutaraldehyde at 380nm. So, these wavelengths were used in this study to obtain absolute levels of concentration gradient. Each specimen was placed in standard concentration of solution with a standardized

wavelength. A beam with a determined wavelength passes through a solution in a cuvette. The sample in the cuvette absorbs this Ultra violet light. The transmittance of light is an indication of the concentration of the analyte in the sample. If there is no absorption of the light passing through the solution, the transmittance is 100%. The relation of absorbance to concentration is given by Lambert-Beer's law. All the sample were tested and amount of absorbance was measured.

After testing each sample and cuvette used for placing samples were cleaned with distilled water to remove the remaining media. All the samples were tested for 1 day and 7 days and residual disinfectant was measured with concentration gradient and the absorption spectrum.

Results

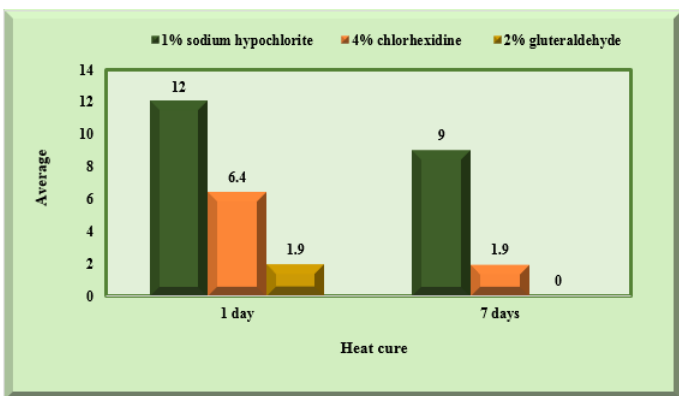
Table 1: Statistical data of residual disinfectant after 1 day and 7 days between heat cure and Self cure denture base resins with different disinfectant solutions using ANOVA test.

Time period	Materials	Disinfectants	N	Mean	Median	S.D	P value
7 days	Heat cure	1% sodium hypochlorite	10	9	11.5	1.764	<0.01
		2% chlorhexidine	10	1.9	6	1.075	
		2% gluteraldehyde	10	0	0	0	
	Self cure	1% sodium hypochlorite	10	17.1	17	1.792	<0.01
		2% chlorhexidine	10	9.6	9.5	1.897	
		2% gluteraldehyde	10	1.9	2	0.876	
1 day	Heat cure	1% sodium hypochlorite	10	12	11.5	1.764	<0.01
		2% chlorhexidine	10	6.4	6	1.075	
		2% gluteraldehyde	10	1.9	2	0.738	
	Self cure	1% sodium hypochlorite	10	46.3	47.5	3.713	<0.01
		2% chlorhexidine	10	17.4	17.5	1.792	
		2% gluteraldehyde	10	3.7	3.5	0.823	

The above table depicts the amount mean residual disinfectant between three different disinfecting solutions between Self cure and heat cure denture base resins after 1 day and 7 days.

- Heat cured resins showed the least residual content than Self cure resin.
- Amount of residual disinfectant was more at 1 day than at the 7 days.

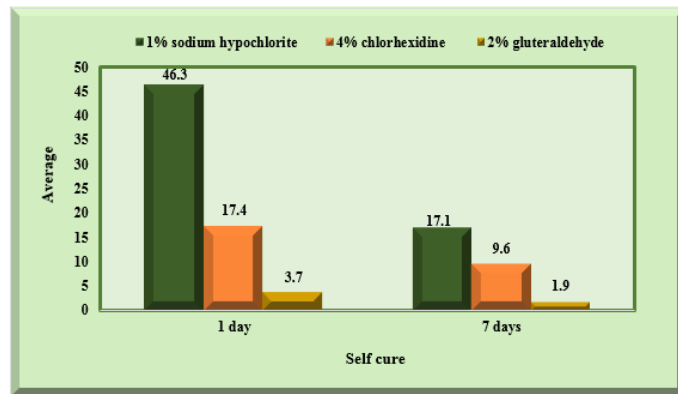
Graph 1: Statistical data of residual disinfectant after 1 day and 7 days between heat cure denture base resins with different disinfectant solutions.



The above graph depicts the amount mean residual disinfectant between three different disinfecting solutions between heat cure denture base resins after 1 day and 7 days.

- Less amount of residual disinfectant after 7 days than after 1 day.
- The descending order of residual content is Sodium hypochlorite followed by Chlorhexidine and least with Glutaraldehyde.

Graph 2: Statistical data of residual disinfectant after 1 day and 7 days between heat cure denture base resins with different disinfectant solutions.



The above graph depicts the amount mean residual disinfectant between three different disinfecting solutions between Self cure denture base resins after 1 day and 7 days.

- Less amount of residual disinfectant after 7 days than after 1 day
- The descending order of residual content is Sodium hypochlorite followed by Chlorhexidine and least with Glutaraldehyde.

Discussion

The greatest reduction in microbial contamination of the internal aspects of acrylic resin has been reported by immersion in 1% sodium hypochlorite and 2% glutaraldehyde^{4,5}. Moreover, the porosity of resins allows them to adsorb water and certain components of disinfection solutions could penetrate in them, being later released to the saliva as some components of the resins are released, which are then swallowed^{11,12}. There is concern that denture immersion in chemical solutions used for cleaning and disinfection could lead to the absorption of these solutions with subsequent release in the saliva. It is necessary to establish an appropriate protocol to eliminate the residues of disinfectant after disinfection⁴.

Methods to measure the residual disinfectant were:

1. Cyclic voltammetry
2. UV-Visible Spectrophotometer
3. Toxicity tests
4. Quantofix (chemical test strip)

5. HPLC (High Performance Liquid Chromatography)
6. HPLC with spectrophotometer
7. Chemical methods with dye indicator
8. Flow injection analysis etc.

Out of these UV-Visible Spectrophotometer was chosen in this study because of simplicity in procedure, accuracy in measurement and common availability.

The potential of sodium hypochlorite solutions to cause allergic reactions is frequently reported in the medical literature, but is really scarce in dental literature¹³. An *in vitro* study of sodium hypochlorite in cell culture concluded that a 5% concentration is highly toxic for clinical use during root canal irrigation whereas the same disinfectant at 0.5% showed cytopathological effects in some cells¹⁴.

Kaufman and Keila verified in their study the ability of sodium hypochlorite solutions to cause allergic reactions after contact with intact skin and some patients that may be hypersensitive to these solutions. Thus, remnants of this disinfectant in dentures can cause allergic reactions in patients with hypersensitivity to sodium hypochlorite¹³.

The greater the toxicity of a disinfectant, washing longer periods should be used for devices and/or medical and dental items, to reduce the level of disinfectant below the dose that cells are damaged. Washing and rinsing cycles can provide adequate reduction of remnants below toxic levels for a specific disinfectant, but these procedures may not be effective for others substances¹⁵. It is essential to introduce in oral rehabilitation some dis-infection procedures effectively proven and practical, but without damage the patient's health neither the physical and mechanical properties of dentures⁴.

Shen et al studied the effect of glutaraldehyde base disinfectants (alkaline, phenol buffered) on surface morphology of denture base resins and reported that no apparent surface change was observed with the regular

alkaline formulation. However, the disinfectant with phenolic buffer caused surface pitting of the material after 10 minutes of immersion, and softening and swelling of the surface after 2 hours of immersion¹⁶. Similar results were obtained by Ma et al, who observed that the phenolic-based disinfectant was the only solution that caused surface damage to five resins after 30 minutes of immersion^{6,17}.

Inflammatory reactions in the oral mucosa are commonly observed in patients that use their dentures continuously. Several compounds including residual monomer, methyl methacrylate and other additives, such as hydroquinone, benzoyl peroxide, N,N-dimethyl p- toluidine, formaldehyde (formed from residual monomer) are released from the acrylic polymers, spread into saliva and come into contact with the oral mucosa, causing flushing and a burning sensation in the adjacent areas¹⁸. Chemical disinfectants that have become absorbed into the resin can also be released and cause similar irritation to the oral mucosa. In order to choose the correct disinfectant, factors to be considered include cost, risk of toxicity to the patient or dental professional, potential instrument damage, stability, antimicrobial activity, and ability to inactivate the microorganisms rapidly¹⁹.

Testing was done after immersing the samples for 1day which is equivalent to 1440 minutes (equal to 144 days of normal immersion protocol of 10minutes a day) and immersion of 7 days was equivalent to 10080 minutes (equal to 1008 days of normal immersion protocol of 10minutes a day). So, this study helps in estimation of long exposure of disinfectants in relation with the denture base resins.

The results in this study showed that self-cure resins have high amount of residual disinfectant may be because of internal configuration of polymer chain, which can help in adsorption of fluids more when compared with heat cure

denture base resins (as the polymer chains were compactly packed).

In this study more amount of residual disinfectant was observed with Sodium hypochlorite might be because of its bleaching action which can help in creation of micropores results in entry of fluids into the denture base resin. Release of chlorhexidine can be due to degree of water sorption which in turn related to the hydrophilicity of denture base resins. Glutaraldehyde release can be due to oxygen methyl methacrylate copolymer is thermally unstable.

I.A. Orsi et al^{4,9} evaluated the release of Glutaraldehyde and Sodium hypochlorite from heat-polymerized acrylic resins subjected to disinfection followed by chemical and mechanical polishing. High performance liquid chromatography (HPLC) and cyclic voltammetry was used to detect and quantify the Glutaraldehyde released after each period. The findings of this showed that chemically polished specimens from the 3 resin brands did not release glutaraldehyde after different periods of immersion, while glutaraldehyde release was observed from the mechanically polished specimens.

The biological factors that provide the sterilizing properties can also be the responsible for the allergenic properties. Therefore, not using glutaraldehyde as a sterilizing agent because of its toxicity. It is necessary to establish a protocol to eliminate the residues of disinfectant after disinfection. The toxicity of glutaraldehyde depends on the contact period and its concentration. Its cytotoxic effect increases with the increase of the exposure time²⁰. There is concern that denture immersion in chemical solutions used for cleaning and disinfection could lead to the absorption of these solutions with subsequent release in the saliva.

So, it is necessary to remove all the residual disinfectant before immersion of the prosthesis, thorough washing in a

running can help in the removal and minimize the immersion time period as exposure time plays a key role in toxicity of disinfectant. The time period of immersion should be 5-10 minutes only. Longer the immersion time, greater the toxicity which results in harmful effects to the normal physiologic state. Acrylic resin should be immersed in hot water (50°C) before insertion to decrease the cytotoxicity of denture base resins.

Conclusion

From the study following conclusions can be drawn:

- The amount of residual disinfectant was found more in Self cure resins when compared to heat cure resins.
- And it was found more after 1 day than 7 days when immersed in disinfectant solutions.
- The amount residual disinfectant was found to be more with sodium hypochlorite followed by chlorhexidine and least with glutaraldehyde.
- It is necessary to establish a protocol to eliminate the residues of disinfectant after disinfection.

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