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COMPARISON OF THE EFFECT OF MICROWAVE RADIATION IN THE DISINFECTION COMPLETE DENTURES CONTAMINATED WITH TWO TYPES OF BACTERIA *S.AUREUS* AND *P.AERUGINOSA* (IN VITRO) WITH MECHANICAL METHODS AND CHEMICAL SOLUTIONS OF GLUTARALDEHYDE

ZAHRA KHALILI[*1]

[1]College of Dentistry, Qazvin University of Medical Sciences, Shahid Bahonar, Qazvin, Iran

***Corresponding Author:Zahra khalili: Email: ahadzara99@yahoo.com; Tel +989123813075**

ABSTRACT

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Introduction and goal

various pathogens colonized in oral cavity that cause systemic disease such as pneumonia, endocarditis, and chronic obstructive pulmonary disease. Denture contamination with pathogenic microorganisms considered as a potential source of contamination between patients and dental staff. The study aimed to compare the effect of microwave radiation in the disinfection complete dentures contaminated with *S.aureus* and *P.aeruginosa* in vitro using mechanical methods and chemical one by Chemical Solutions of Glutaraldehyde

Methods:

72 dentures with standard techniques prepared and disinfected in an autoclave (121˚C, for 20 minutes) was performed and 30 samples were considered for each bacteria, which 6 samples were in each subgroup assigned. 6 denture was used as a negative control (n =6) (Group 1). The rest of them, individually with *S.aureus* and *P.aeruginosa* inoculated (for 48 hours, were kept in the incubator 37c ○) and then were sterilized by one of the following methods. Chemical disinfection using Chemical Solutions of Glutaraldehyde (water-soluble 37˚C for 15 minutes) (n = 6) (Group 2), mechanical disinfection by brushing dentures for 5 minutes (n =6) (Group 3), microwave irradiation in W 650for 3 minutes (n =6) (group 4) and the positive control group did

not receive any disinfection method (n = 6) (group 5). µl 25 suspension in dilutions 3-10 to 6-10 were cultured in Nutrient Agar. Colonies after incubation (37 c ○ for 48 hours) were counted (cfu / ml). To assess the long-term disinfection, containers include Nutrient Broth and dentures in 37 c ○ were kept for 7 days and its opacities were investigated. Data were analyzed using one-way ANOVA and T-test.

Results:

There was no evidence of growth in 48 hours and no opacities in containers Nutrient Broth samples after 7 days of incubation dentures by microwave radiation disinfection (650 W, 3 minutes andChemical Solutions of Glutaraldehydeand statistically in comparing with positive control group was statistically significant (P=0) in dentures disinfection by mechanical methods (brushing) microbial growth after 48 hours, the opacities was observed in all the dishes created Nutrient Broth and statistically was not significant in comparison to the positive control (P>0.05).

Conclusion:

microwave radiation (650 W, 3 minutes) and Chemical Solutions of Glutaraldehyde were completely sterile in the short term and long term against *S.aureus and P.aeruginosa.*

Keywords: microwave, glutaraldehyde, disinfection, *S.aureus, P.aeruginosa* and *Candida albicans*

1- INTRUDUCTION

Mouth and tooth health status is not only a reflection of the health status of society but alsois effective on the quality of life, social and individual ability. (1) Plaque and denture poor health cause Stowe Matatus denture (2 and 3) and can also be used as a potential source of infection, in addition to cause bad breath and even caries and periodontal problems in people who are remaining teeth. Dentures can be the location of the microorganisms that cause bacterial endocarditis, pneumonia, stomach and intestinal infections are lightweight and chronic obstructive pulmonary disease (3). To prevent cross-infection, prosthesis should bedisinfected thoroughly before sending to the laboratory or try on patient's mouth (4). Various methods have been proposed for disinfecting dentures to prevent cross-infection (5). Disinfect dentures can be done entirely by glutaraldehyde. In addition to a broad antimicrobial effect, using glutaraldehyde has other important benefits as disinfectant also.This material enables it easily penetrate on the blood and exudate of the device.The chemicals are useful to clean the blood from suction chamber

unfortunately, if this material is not used properly, they can cause damaging the metal instruments. For example, if molding instruments that are nickel-covered and steel mills taking a long time in a solution of glutaraldehyde 2 - 2.3 percent, it may cause discoloration or corrosion and can also penetrate through to acrylic resin and denture discoloration (6).

To prevent the side effects of chemical solutions on the denture, microwave proposed as a simple, effective and cheap method. Neppelenbroek et al (7) examined disinfecting effects of microwave radiation (6 minutes and 650 watts) on three types of ester resins and causing complete sterilization dentures contaminated with four types of microorganisms. Further studies by Silva (8) showed that microwave radiation (6 minutes and 650 watts) causing complete sterilization dentures contaminated with *Staphylococcus aureus* and *Candida albicans* however, the dimensional changes of denture and denture reline materials. Ribeiro (9) showed a decrease radiation prevent mechanical damage to the denture.

Studies also Mima (10) on different time showed that only 3 min radiation lead to inactivation of all species evaluated. Considering the importance and fact that few studies exist regarding the most effective and easiest way to disinfect dentures, this study aimed to determine the most effective method of full dentures disinfect infected with two types of bacteria *S.aureus, P.aeruginosa* and fungus *Candida albicans* compared with mechanical and chemical methods for in vitro in the short term and long term was conducted.

2-MATERIALS AND METHODS

Laboratory study (in vitro) was performed. The study population consisted of 108 full denture lower jaw identical to the standard method that made of tough heat acrylic resin denture base. After disinfection in an autoclave (121c ○, for 20 minutes) they were randomly assigned to each group of bacterial and fungal and divided into groups: 6 denture negative controls, 6 denture positive controls, 6 denture to mechanical methods (brushing), 6 denture for chemical methods and 6 denture to sterilize by microwave. In this study, microorganisms infecting dentures, including *P.aeruginosa* (ATCC = 27853) and *S.aureus* (ATCC = 25923) were selected. By choosing these species were recommended for study based on data Disinfectants and Antiseptic book in which gram negative for *P.aeruginosa* and gram positive for *S.aureus* as a marker microorganisms of pathogenic agents (8).

First standard strains of *P.aeruginosa* (ATCC = 27853), *S.aureus* (ATCC = 25923) and *Candida albicans* (ATCC = 10231) was cultured on Nutrient Agar medium and incubated for 24 hours. Then new strain was prepared at a concentration of 5.0 McFarland suspension. The resulting leachate microbial must contain 108 × 3cfu/ml microbes. The visual comparison was done in an environment with enough light. Nutrient Broth was used for the acclaimed a suspension. Then all dentures and glass containers that had been previously autoclaved, Nutrient broth was placed environments with sterile forceps into sterile glass containers containing 150 ml. (6 denture for negative controls, 6 dentures for positive controls, 6 denture for sterilizing Chemical Solutions of Glutaraldehyde and 6 denture for sterilizing by microwave)Nutrient Broth was added to infect dentures 1.5 ml bacterial suspension with a concentration of 0.5 McFarland glass containers. Glass containers containing dentures were incubated for 48 h at 37 ° C. After incubation, dentures beside the flame from the outside glass were sterile under the hood in sterile plates containing Whatman filter papers until their excess water taken. In this stage positive and negative control groups were counted to determine the number of microorganisms on the surface of infected dentures. In the previous step of disinfection the number of colonies was compared with each other after decontamination methods. Positive and negative control samples was placed after drying in sterile glass containers containing 150 ml of sterile saline and vortex for 1 minute and nine minutes after the break, again this was repeated practice to separate microorganisms sticking to dentures. Then for Kant colonies 25 micro-liters of normal saline removed by pipette for inside glass containers and cultured on agar with pipettes loop and after incubation at 37 ° C for 48 hours, the number of colonies was counted. It has build agar primarily by the size of distilled water in the flask casting powder, then boiled, and then sterilized in an autoclave molding plate and then the samples were transferred to it. Dentures placed in normal saline and in a glass container containing 150 ml of Nutrient Broth for a week and was incubated at 37 ° C and the opacities was determined after 48 hours by counting the number of clones (colony count) and was multiplied by the number of colonies obtained in a milliliter. After culturing microorganisms, samples were purged by one of the following methods:

Mechanical methods:

For this purpose all surfaces was struck each of denture separately using a soft toothbrush for 5 minutes with sterile water, for each sample new toothbrush was used and after brushing samples placed in sterile saline in a vessel containing distilled water for 5 minutes. 150 Ml and the dilution was prepared and vertex then the value of λ25 in Nutrient Agar were cultured and incubated for 24 hours and the colony count. Then samples incubated in nutrient broth placed and for 7 days.

Chemical methodsusing Solutions of Glutaraldehyde:

in sterilization group through immersion in a solution of 2% glutaraldehyde [Behsad 2%, Behsa pharmaceutical company (Arak - Iran) denture, samples were placed in sterile containers containing glutaraldehyde for 10 minutes. dentures were incubated in vitro with volume 200 cc and equivalent concentrations 106 Cfu / ml to 5.0 McFarland for 48 h at 37 ° C and after incubation, the dentures were dried with filter paper and 6 samples contaminated with any bacteria putinto glutaraldehyde container for 10 minutes according to manufacturer's instructions and after ten minutes dentures were removed andput in 150 ml sterile saline and vortexes and dilution was prepared and in Nutrient Agar value 25 λ cultured and incubated for 48 hours and then become colony counts. The samples placed in nutrient broth and incubated for 7 days are.

Disinfection methods using the microwave radiation:

In disinfection group physical methods using microwave radiation for 3 minutes with the power of 650 watts of samples after drying was done, the glass containers containing 150 ml of distilled water was placed in the microwave for 3 minutes with the power of 650 watts. To prevent reduction of energy every single time, a sample was placed right in the center of the microwave. After incubation, culture environment was counted for 48 h at 37 ° C, the number of colonies in culture environment (cfu / ml). In the next step, a new culture of samples was conducted after incubation at 37 ° C for 7 days to evaluate the long-term effect of disinfection and opacities were investigated (Cfu / ml). In this study, for each experimental group, the colonies of microorganisms presented on average. In order to determine significant differences between the groups one-way analysis of variance (ANOVA)was used. Average of groups compare mutually independent t-testwas used. For ease, speed

and accuracy of statistical analysis, statistical software SPSS21 and Excel was used.

3-RESULTS

According to the results of the colony counts microorganisms *Psedumonas aeruginosa, Staphylococcus aureus and Candida albicans* completely sterilized in test groups after 48 hours of incubation and glutaraldehyde chemical sterilization and using microwaves dentures and any colony *Psedumonas aeruginosa* and *Staphylococcus aureus* in cultivation environment observed. While the mechanical method, was not able to complete disinfection of dentures and control of bacteria not complete occurred and in culture environment *Psedumonas aeruginosa* average was 103 × 1.5 cfu / ml. In culture environment *Staphylococcus aureus* average was 103 × 1.96 cfu / ml. However, infection rate in this method was less than the positive control group but was not statistically significant difference between these two methods ($p>0.05$). The absence of bacterial colonies in culture environment than negative control and large colonies on positive control showed the accuracy of tests.

The results of the colony counts fungus *Candida albicans* in the experimental groups after 48 hours of incubation showed that glutaraldehyde chemical disinfection method and using microwave to sterilize the full dentures and no colony of fungus in vitro was observed. Mechanical methods were not able to complete disinfection and medium dentures average 102 × 0.8 cfu /ml seen Tables 1 and 2.

Table 1: *P.aeruginosa* colony count in the experimental groups after 48 hours of incubation in terms of cfu / ml

Number Plate / Method	1	2	3	4	5	6	Average
Positive control	$10^6\times32.1$	$10^6\times48.1$	$10^6\times43.1$	$10^6\times22.1$	$10^6\times53.1$	$10^6\times18.1$	$10^6\times36.1$
Negative control	0	0	0	0	0	0	0
Mechanical (brushing)	$10^3\times7.1$	$10^3\times2.1$	$10^3\times4.1$	$10^3\times2$	$10^3\times3.1$	$10^3\times8.1$	$10^3\times5.1$
Microwave	0	0	0	0	0	0	0
Chemical(glutaraldehyde)	0	0	0	0	0	0	0

Table 2: *S.aureus* colony count in the experimental groups after 48 hours of incubation in terms of cfu / ml cfu / ml

Number Plate / Method	1	2	3	4	5	6	Average
Positive control	9.22×10^5	9.48×10^5	11.27×10^5	8.8×10^5	7.92×10^5	11.44×10^5	9.76×10^5
Negative control	0	0	0	0	0	0	0
Mechanical (brushing)	2.2×10^3	1.92×10^3	2.04×10^3	2.2×10^3	1.88×10^3	1.52×10^3	1.96×10^3
Microwave	0	0	0	0	0	0	0
Chemical (glutaraldehyde)	0	0	0	0	0	0	0

The results of the opacities of the culture of the colony *P.aeruginosa, S.aureus* and *Candida albicans* in the experimental groups after one week showed that chemical disinfection glutaraldehyde and use the microwave to sterilize the dentures

completely even after a week, no opacities in the culture was detected. As mechanical procedure, the opacities almost the same as the positive control after a week were. No opacities and the colony in culture environment for negative control and high opacities in positive control showed the accuracy of tests. Therefore, we can say that chemical disinfection methods, glutaraldehyde solution and microwave are useful methods for the long term Tables 3 and 4.

Table 3: Evaluation of opacities in culture *P.aeruginosa* in the experimental groups after one week of incubation

Number Plate / Method	1	2	3	4	5	6
Positive control	10^7<Opacities	10^7<Opacities	10^7<Opacities	10^7<Opacities	10^7<Opacities	10^7cfu/ml<Opacities
Negative control	Lack of opacities	Lack of opacities	Lack of opacities	Lack of opacities	Lack of opacities	Lack of opacities
Mechanical (brushing)	10^{11}<Opacities	10^{11}<Opacities	10^{11}<Opacities	10^{11}<Opacities	10^{11}<Opacities	10^{11} <Opacities cfu/ml
Microwave	Lack of opacities	Lack of opacities	Lack of opacities	Lack of opacities	Lack of opacities	Lack of opacities
glutaraldehyde Tablets	Lack of opacities	Lack of opacities	Lack of opacities	Lack of opacities	Lack of opacities	Lack of opacities

Table 4: testing opacities in culture *S.aureus* in the experimental groups after one week of incubation

Number Plate / Method	1	2	3	4	5	6
Positive control	10^7<Opacities	10^7<Opacities	10^7<Opacities	10^7<Opacities	10^7<Opacities	10^7 cfu/ml<Opacities
Negative control	Lack of opacities	Lack of opacities	Lack of opacities	Lack of opacities	Lack of opacities	Lack of opacities
Mechanical	6.2×10^9	4.44×10^9	5.92×10^9	6.72×10^9	4.96×10^9	5 cfu/ml/12×10^9
chemical (Glutaraldehyde)	Lack of opacities	Lack of opacities	Lack of opacities	Lack of opacities	Lack of opacities	Lack of opacities
Microwave	Lack of opacities	Lack of opacities	Lack of opacities	Lack of opacities	Lack of opacities	Lack of opacities

The infection with *P.aeruginosa* in mechanical method compared with positive control after 48 hours was low and this difference was not significant (p>0.5).

Mechanical method in group P.a infection rates in the short term (48 hours) less than long-term (7 days) and this difference was statistically significant (p <0.5).

In the method using the microwave and glutaraldehyde no P.aeruginosa infection was observed after 48 hours and it is also significant statistically (p <0.5)

The opacities in mechanical method compared with positive controls after 7 days, and no significant difference was the same (0.05 <p).

In the method using the microwave and glutaraldehyde tablets no resentment was observed after 7 days and it was not statistically significant (p <0.5).

The infection with *S.aureus* in mechanical method was lower compared with positive control after 48 hours, but this difference was not significant (p>0.5).

In mechanical methods in S.a group infection rates in the short term (48 hours) under long-term (7 days) and this difference was statistically significant. (p <0.5)

In the method using the microwave and glutaraldehyde no S.aureus infection was observed after 48 hours and it is also significant statistically (p <0.5)

The opacities in mechanical method compared with positive controls after 7 days are the same and no difference was statistically significant (0.05 <p).

In the method using the microwave and glutaraldehyde no opacities was observed after 7 days and it is also significant statistically (p <0.5) Tables 5 and 6.

Table 5: One-way analysis of variance (ANOVA) Method of disinfection in *P.aeruginosa*

Groups		P value
Positive control 48 hours	Mechanical 48 hours	1.000
	Other methods	0.000
Positive control 7 days	Mechanical 7 days	1.000
	Other methods	0.000
Mechanical 48 hours	Mechanical 7 days	0.000

Table 6: One-way analysis of variance (ANOVA) Method of disinfection in S.aureus

Groups		P value
Positive control 48 hours	Mechanical 48 hours	1.000
	Other methods	0.000
Positive control 7 days	Mechanical 7 days	1.000
	Other methods	0.000
Mechanical 48 hours	Mechanical 7 days	0.000

4-DISCUSSION

The results of the opacities in the culture environment of the fungus Candida albicans colonies in the experimental groups after one week of incubation showed that using the microwave to sterilize dentures made that perfectly even after a week, no opacities in the culture environment was detected. In disinfection procedure with Chemical Solutions of Glutaraldehydeand mechanical method, opacities in culture environment after one week was about the same as a positive control. Lack of opacity and the presence of fungal colonies in culture environment to high opacities in the negative control and a positive control showed the accuracy of tests. Therefore, we can say that disinfection in chemical method and microwave method is useful for the long term.

In this study, the results indicate that microwave with high power 650 W for 3 minutes can kill bacteria on the surface of S.aureus and P.aeruginosa in dentures of dental powerfully and it can be used as an effective method for sterilization of denture

and prevent the transmission of infection in patients with denture applied. Microwave energy (650 w and 3 minutes) considered as an effective method of disinfection of microorganisms such as P.aeruginosa and S.aureus.

Silva (16) and Neppelenbroek (6) said that using microwave radiation were able to complete disinfection of dental dentures, except that the exposure time of 6 minutes and had power 650 W. Many studies have shown that the application of microwave with power 650 W for 3 minutes is enough to complete disinfection of dental dentures against Candida albicans and other pathogenic microorganisms such as P.aerginosa, B.subtilis and S.aureus. In this case, studies Mima (7), Ribeiro (8) and Dantas (9) cited. In addition study Dantas et al showed that the disinfection with microwave (3 minutes, 650 W) coincident of denture base with the conventional method improved (9).Microwave radiation for 6 minutes with 650 W power could have adverse effects on the physical and mechanical properties of acrylic resin (13). So should reduce the microwave radiation to disinfect without the other harmful effects on acrylic resin obtained. Radiation 2 minutes heat to disable Candida albicans provides sufficient. But it is not enough for the bacteria. In addition, the fungal cells are larger than bacterial cells (17).

5-CONCLUSION

In this study, 2% glutaraldehyde for 10 minutes not only was completely disinfect dental dentures but also it's effects of disinfection remainsmore than a week after its use.But glutaraldehyde 2% have side effects, such as headache - irritation of eyes, nose and throat and dry cough, rash, dizziness, nausea and sensitivity (18). Also limit access to chemicals; lack of adequate information in patient limit it's use.Both methods of chemical disinfection (2% glutaraldehyde for 10 minutes) and microwave (for 3 minutes and with power w 650) were suitable for disinfection and control of P.aeruginosa and S.aureus bacteria and Candida in dental dentures that have long-term stability.Although use of glutaraldehyde has side effects, sousing microwave for 3 minuteswith antiseptic power 650 wis suggested for complete dentures dental procedures.It is suggested to determine the exact effects of disinfection by microwave radiation stability, denture cleaning tablets and glutaraldehyde, in vivo may also be performed this experiment is suggested more studies on the effectiveness of this method on short-term and long-term physical and mechanical properties as

possible. It is suggested strains isolated from bacteria and fungi used.

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