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ORIGINAL ARTICLE

Disinfecting the acrylic resin plate using electrolyzed acid water and 2% glutaraldehyde: a comparative microbiological study

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Abstract Marked bactericidal activity of the electrolyzed acid water (EAW) and its less undesirable effects on biological tissues and the environment has been successfully utilized in dental practice. A review of literature reveals that not many studies have been performed to determine the disinfection effect of EAW on heat cured acrylic denture bases. The objective of the study is to determine the efficacy of EAW in disinfecting heat cured acrylic resin specimens as compared to the efficacy of disinfecting the specimens using 2% glutaraldehyde. Heat cured acrylic resin plates were immersed in Bacteria suspensions of *Staphylococcus aureus* and later disinfected using EAW which was produced using a custom made electrolyzing apparatus and 2% glutaraldehyde for different time intervals. The numbers of surviving bacteria were counted. As the control, the bacteria attached on the specimens were counted with out any disinfection treatment. The results obtained were statistically analyzed using ANOVA and post-hoc test. The disinfection potential of 2% glutaraldehyde was better than EAW when the specimens were disinfected for 1 and 3 min. Where as when the disinfection time was increased to 5 min there was no difference between EAW and 2% glutaraldehyde. However considering that the disinfecting time can be easily extended to 5 min, EAW can be effectively used for disinfecting the acrylic denture.

Keywords EAW · Denture base acrylic resin · 2% Glutaraldehyde · Disinfection

Introduction

Dental surgeons and para-dental staff frequently come in contact with the dentures during adjustment, repair or relining. These dentures may be contaminated with bacteria, viruses and fungi. Dental laboratory personnel are at risk for contracting infections from dental prosthesis that have not been disinfected. It as been established that infection of dental personal in the office and dental laboratory by transmission of micro organisms between dentist's offices and commercial laboratories does exist [1].

The potential for infection by transmission of micro organisms as prompted the American dental association to issue guidelines for cleaning, disinfecting, and handling impressions, dentures and other items transmitted to and from dental offices and dental laboratories [2].

Staphylococcus aureus are ubiquitous and form the most common cause of localized supportive lesions in human beings. There ability to develop resistance to penicillin and other antibiotics enhances their importance as a human pathogen, especially in the hospital environment [1, 3].

Chemical disinfectants, used after removal and before insertion of prosthesis in to the mouth, are a recommended method to prevent cross-contaminations. They include 2%

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glutaraldehyde, 5.25% sodium hypochlorite, chlorine dioxide, iodophors, alcohols and chlorhexidine [4–8].

It is reported that some of the chemical disinfectants caused problems such as color changes and surface roughness of the denture base resin surfaces [9–11].

It was also demonstrated that same denture cleaners and chemical disinfectants showed the possibilities to induce surface roughness and porosity on the tissue conditioners [12]. Recently, electrolyzed acid water (EAW) due to oxidation–reduction potential (ORP), pH and residual chlorine has attracted great attention because of its excellent bactericidal and virucidal activities and its less undesirable effects on biological tissues and the environment.

Its application has been investigated in medical [13–17], dental, animal husbandry, horticulture, restaurant and other areas where microbial growths are known to present potential health threats [18–20].

Since electrolyzed acid water (EAW) has generated a lot of interest in dentistry [21–24], its beneficial properties as to be employed for disinfecting the acrylic prosthesis. However it has not yet been well established by multicenter study to determine the efficacy of EAW in disinfecting the prosthesis, especially using a custom made device to generate EAW.

Hence this in-vitro study was undertaken to evaluate the disinfection capability of EAW as compared with 2% glutaraldehyde in disinfecting the heat cured acrylic resin prosthesis.

Methods

A MS metal die (30 × 30 × 3.0 mm) with a small handle were fabricated to be used as a template to prepare the acrylic specimens.

About 30 specimens were fabricated and they were reused after sterilization with autoclave.

In this study, the bacteria used were *Staphylococcus aureus* 25923 to determine the bactericidal activity. Bacteria suspensions were prepared to be 6.0×10^6/ml in brain heart infusion (BHI) media by using McFarland's standard 2, according to American Society of Microbiology. 50 ml of *Staphylococcus aureus* containing BHI media (6.0×10^6 bacteria /ml) was transferred in to 50 ml sterile sample carrying bottle. A total of 70 such sample bottles were made.

One acrylic specimen were entirely immersed in each of the 50 ml of bacteria-suspended solution (6×10^6/ml) and incubated at 37°C for 3 h in order to shift the bacteria to the surface of the plate (Fig. 1).

Two glass beakers were fused to each other with a grouch crucible (porous diaphragm) in between. Two copper electrodes were placed in two beakers respectively and were connected to the positive and negative ends of a 4.5 A and

Fig. 1 Heat polymerized acrylic specimens immersed in 50 ml of bacteria suspended solutions

Fig. 2 Custom made electrolyzing apparatus

6 V battery. This forms an electrolyzing apparatus (Fig. 2). 50 mg of A grade sodium chloride (NaCl) in every 100 ml of distilled water was mixed to form 0.05% sodium chloride aqueous solution. 0.05% sodium chloride aqueous solution was used as a common electrolyte in both the beakers. The electrolyte was electrolyzed in the electrolyzing apparatus for about 90 min. The electrolyte in the positive chamber was collected. The pH value was examined with a pH meter and was in the range of 2.3–2.4. The ORP were examined with a potentiometer and were found to be in the range of 1,010–1,030 mV. EAW thus obtained was transferred in to polyethylene terephthalate (PET) bottle and wrapped completely with a tinfoil. As the properties of EAW change with time by light or air-exposure, the EAW was supplied for experiment within 1-h storage.

50 ml of EAW was transferred into a sterile bottle. The acrylic specimen with the bacteria being attached to its surface was entirely immersed in the EAW (Fig. 3). Similarly another set of infected acrylic specimens were immersed in 2% glutaraldehyde (Fig. 4). After treatment the specimens were soaked in 50 ml of saline solution and placed in ultrasonic bath and vibrated for 30 s to dislodge the bacteria in to solution. 10 µl from the solution was added to the agar culturing media (Nutrient Agar, HIMEDIA) and incubated at 37°C for 24 h. The number of the

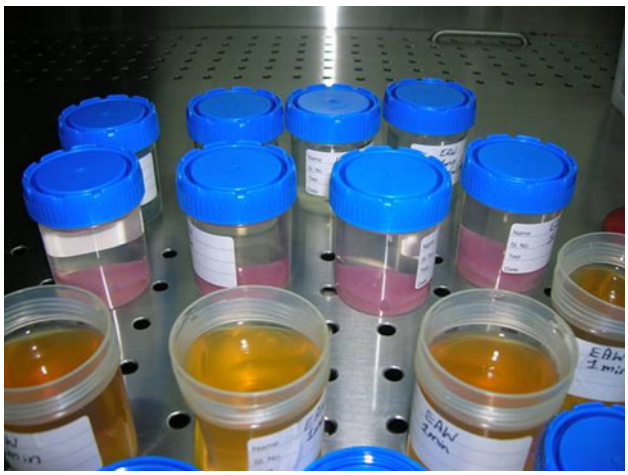

Fig. 3 Heat polymerized acrylic specimens with bacteria attached to its surface immersed in EAW

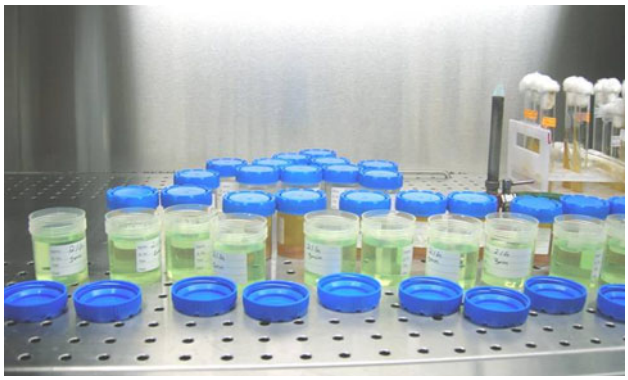

Fig. 4 Heat polymerized acrylic specimens with bacteria attached to its surface immersed in 2% glutaraldehyde

surviving bacteria in the media was counted and multiplied by 100. The above experiment was conducted in a laminar flow for biological safety.

All the experiments were repeated 10 times and the results were statistically compared by ANOVA and *t*-test.

Ten of the specimens infected with *Staphylococcus aureus* were soaked in 50 ml of normal saline and vibrated in an ultrasonic device for 30 s to dislodge the bacteria in to solution. 10 µl from the solution was added to the agar culturing media (Nutrient Agar, HIMEDIA) and incubation at 37°C for 24 h. confluent growth which was observed was considered to be having 6×10^6 cfu/ml. These observations were used as control for the study.

Among 30 specimens, 10 of the specimens infected with *Staphylococcus aureus* were soaked in 50 ml of EAW for 1 min, grouped as group 1 test specimens, 10 of the specimens infected with *Staphylococcus aureus* were soaked in 50 ml of EAW for 3 min, grouped as group 2 test specimens and other 10 of the specimens infected with *Staphylococcus aureus* were soaked in 50 ml of EAW for

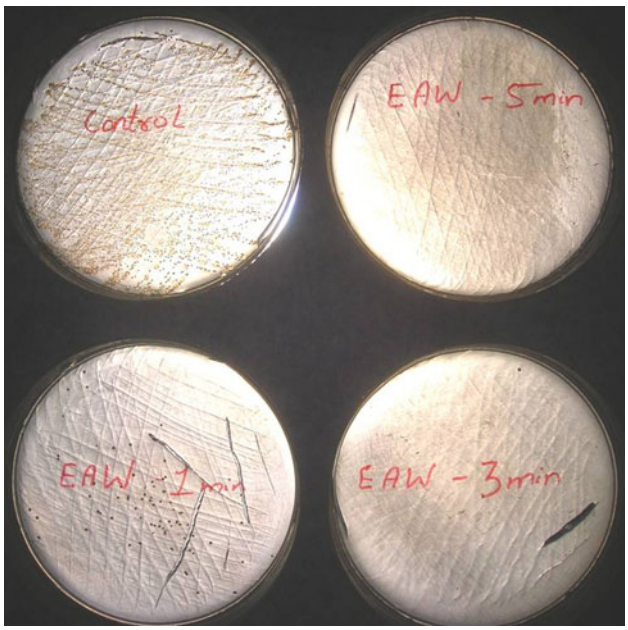

Fig. 5 Agar plate showing colony growth after treatment with EAW at 1, 3 and 5 min immersion time

5 min, grouped as group 3 test specimens. After treatment the specimens were soaked in 50 ml of saline solution and placed in ultrasonic bath and vibrated for 30 s to dislodge the bacteria in to solution. 10 µl from the solution was added to the agar culturing media (Nutrient Agar, HIMEDIA). After incubation at 37°C for 24 h the number of surviving *Staphylococcus aureus* were noted by counting the number of cfu (Fig. 5).

Among other 30 specimens 10 of the specimens infected with *Staphylococcus aureus* were soaked in 50 ml of 2% glutaraldehyde for 1 min, grouped as group 4 test specimens, 10 of the specimens infected with *Staphylococcus aureus* were soaked in 50 ml of 2% glutaraldehyde individually for 3 min, grouped as group 5 test specimens and last 10 of the specimens infected with *Staphylococcus aureus* were soaked in 50 ml of 2% glutaraldehyde for 5 min, grouped as group 6 test specimens. After treatment the specimens were soaked in 50 ml of saline solution and placed in ultrasonic bath and vibrated for 30 s to dislodge the bacteria in to solution. 10 µl from the solution was added to the agar culturing media (Nutrient Agar, HIMEDIA). After incubation at 37°C for 24 h the number of surviving *Staphylococcus aureus* were noted by counting the number of cfu (Fig. 6).

Results

Table 1 show the number of colony forming unit (cfu) developed from 10 control specimens and the test specimens which were divided into six groups.

Fig. 6 Agar plate showing colony growth after treatment with 2% glutaraldehyde at 1, 3 and 5 min immersion time

Control group: Confluent growth (100% growth) of cfu was observed among all the 10 specimens of the control group and hence 6×10^6 cfu/ml was considered as the number of colonies present in control group.

Group 1: Of the 10 specimens the minimum number of surviving bacteria was found to be 25×10^2 cfu/ml, and maximum number of surviving bacteria was found to be 200×10^2 cfu/ml.

Group 2: Of the 10 specimens no growth was observed in 2 specimens and a maximum growth among 1 specimen was found to be 600 cfu/ml.

Group 3: Growth was not observed in all the 10 specimens. Hence no analysis was carried for this group of specimen.

Group 4: Of the 10 specimens no growth was observed in two specimens and a maximum growth among 1 specimen was found to be 17×10 cfu/ml.

Group 5: Of the 10 specimens no growth was observed in six specimens and a maximum growth among 1 specimen was found to be 300 cfu/ml.

Group 6: Growth was not observed in all the 10 specimens. Hence no analysis was carried for this group of specimen.

Statistical Analysis

Table 2a shows the mean and SD for group 1, group 4 and control specimen.

For group 1 test specimen the mean number of surviving bacteria was found to be 67×10^2 cfu/ml and SD—5401.65.

For group 4 test specimens the mean number of surviving bacteria was found to be 710 cfu/ml and SD—699.92.

For control specimens the mean number of surviving bacteria was found to be 6×10^6 cfu/ml and SD—0.00.

Table 2b compares the statistical difference between the three groups i.e. control, group 1, and group 4. The ANOVA test showed P-value to be <0.05 which is significant. Though the level of significance can be determined by ANOVA test, it does not give us a comparison of individual group with the other group.

Table 2c shows the result of post-hoc test performed on the above three groups (control, group 1 and group 4 test specimens).

When group 1 was compared with control specimens, about 5993300.00 of bacteria i.e. 99.88% was eliminated which is statistically highly significant.

When group 4 was compared with control specimens, about 5999290.00 of bacteria i.e. 99.98% was eliminated which is statistically highly significant.

When group 1 was compared with group 4 test specimens, group 1 had 5990.00 i.e. 0.099% of surviving bacteria more than group 4 test specimens which is statistically significant ($P = 0.001$).

Bar graph (Fig. 7) show the comparison among the mean number of surviving bacteria of control group, group 1 test specimens and group 4 test specimens. From this graph we can infer that group 4 test specimens had least number of surviving bacteria.

Table 3a shows the mean and SD for group 2, group 5 and control specimen.

For group 2 test specimens the mean number of surviving bacteria was found to be 260.00 cfu/ml and SD—201.11.

For group 5 test specimens the mean number of surviving bacteria was found to be 90 cfu/ml and SD—128.67.

For control specimens the mean number of surviving bacteria was found to be 6×10^6 cfu/ml and SD—0.00.

Table 3b compares the statistical difference between the three groups i.e. control, group 2, and group 5. The ANOVA test showed P-value to be <0.05 which is significant. Though the level of significance can be determined by ANOVA test, it does not give us a comparison of individual group with the other group.

Table 3c shows the result of post-hoc test performed on the above three groups (control, group 2 and group 5 test specimens).

When group 2 was compared with control specimens, about 5999740.00 of bacteria i.e. 99.995% was eliminated which is statistically highly significant.

When group 5 was compared with control specimens, about 5999910.00 of bacteria i.e. 99.998% was eliminated which is statistically highly significant.

Table 1 Number of colony forming unit (cfu) developed from control specimens and test specimens

S. no	Control specimens	EAW			2% glutaraldehyde		
		Test specimens			Test specimens		
		Group 1 (1 min) (cfu/ml)	Group 2 (3 min) (cfu/ml)	Group 3 (5 min) (cfu/ml)	Group 4 (1 min) (cfu/ml)	Group 5 (3 min) (cfu/ml)	Group 6 (5 min) (cfu/ml)
1	Confluent growth 6×10^6 cfu/ml	100×10^2	6×10^2	No growth	10×10^2	2×10^2	No growth
2	Confluent growth 6×10^6 cfu/ml	200×10^2	3×10^2	No growth	1×10^2	No growth	No growth
3	Confluent growth 6×10^6 cfu/ml	25×10^2	2×10^2	No growth	No growth	No growth	No growth
4	Confluent growth 6×10^6 cfu/ml	50×10^2	10^2	No growth	17×10^2	3×10^2	No growth
5	Confluent growth 6×10^6 cfu/ml	30×10^2	No growth	No growth	2×10^2	No growth	No growth
6	Confluent growth 6×10^6 cfu/ml	35×10^2	3×10^2	No growth	15×10^2	10^2	No growth
7	Confluent growth 6×10^6 cfu/ml	30×10^2	4×10^2	No growth	10×10^2	No growth	No growth
8	Confluent growth 6×10^6 cfu/ml	50×10^2	2×10^2	No growth	15×10^2	3×10^2	No growth
9	Confluent growth 6×10^6 cfu/ml	100×10^2	5×10^2	No growth	No growth	No growth	No growth
10	Confluent growth 6×10^6 cfu/ml	50×10^2	No growth	No growth	1×10^2	No growth	No growth

cfu colony forming unit

When group 2 was compared with group 5 test specimens, group 2 had 170.00 i.e. 0.003% of surviving bacteria more than group 5 test specimens which is statistically significant ($P = 0.027$).

Bar graph (Fig. 8) show the comparison among the mean number of surviving bacteria of control group, group 2 test specimens and group 5 test specimens. From this graph we can infer that group 5 test specimens had least number of surviving bacteria.

Bar graph (Fig. 9) show the mean number of surviving bacteria in group 1, group 2, group 3, group 4, group 5 and group 6 test specimens. From this graph we can infer that there were no surviving bacteria in group 3 and group 6 test specimens at 5 min immersion time.

Discussion

Electrolyzed acid water has excellent bactericidal and virucidal potential and cause less undesirable effects on biological tissues and the environment due to its ORP, pH and residual chlorine [24]. These desirable properties of EAW make it suitable to be used for disinfecting denture base.

The desirable properties of EAW (ORP, pH and residual chlorine) can be obtained by electrolyzing NaCl dissolved in distilled water. When a small quantity of salt (NaCl) is added to the distilled water and than the water is divided into acid water and alkaline water by electrolysis via a diaphragm. The electrolysis is illustrated below.

$$NaCl \rightarrow Na^+ + Cl_2^-$$

At the anode site:

$$2Cl^- - 2e^- \rightarrow Cl_2$$

$$Cl_2 + H_2O \rightarrow HClO + H^+Cl^-$$

$$HClO \rightarrow H^+ + ClO^-$$

$$2H_2O - 2e^- \rightarrow O_2 + 4H^+$$

$$O_2 + H_2O - 2e^- \rightarrow O_3 + 2H^-$$

At the cathode site:

$$2H_2O + 2e^- \rightarrow H_2 + 2OH^-$$

$$2NaCl + 2OH^- \rightarrow 2NaOH + Cl^-$$

The EAW obtained from the anode site is characterized by a low pH (below 2.7) and a high ORP (above

Table 2 Statistical analysis between group 1, group 4 and control specimens

Test specimens	No. of specimens	Mean	SD	Minimum	Maximum
(a) cfu present					
Group 1	10	6700.00	5401.65	2500	20000
Group 4	10	710.00	699.92	0	1700
Control	10	6000000	0.00	6000000	6000000
Level of significance					
				Sig.	
(b) Comparison of statistical difference between three groups					
Between groups				0.000	
Within groups					

Test specimens		Mean difference (I–J)	Sig.
(I) Group	(J) Group		
(c) Results of post-hoc test			
Control	Group 1	5993300.00	0.000
	Group 4	5999290.00	0.000
Group 1	Group 4	5990.00	0.001
	Control	−5993300.0	0.000
Group 4	Group 1	−5990.00	0.001
	Control	−5999290.0	0.000

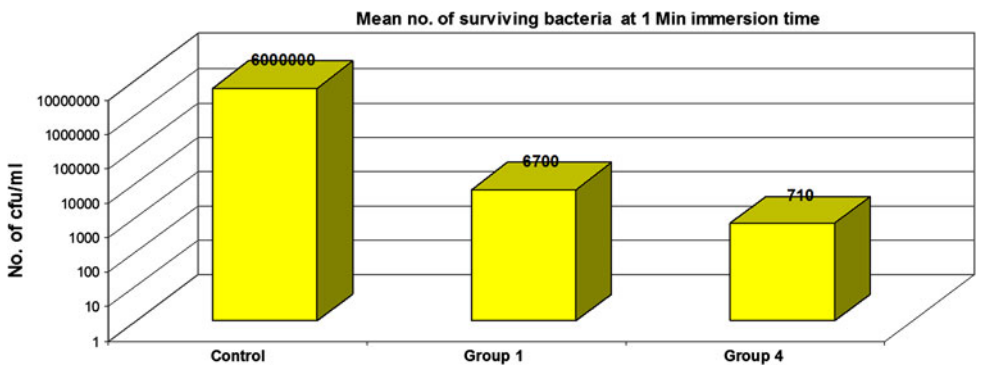

Fig. 7 Graph representing the mean number of surviving bacteria in control specimens, group 1 and group 4 test specimens

11.00 mV), and it includes active oxygen and chlorine (Cl_2).

Generally, many pathogenic bacteria grow at a slightly above neutral Ph-7 [3]. Some microorganisms such as lactobacilli, the yeast *Candida* and pathogenic fungi grow well at a low pH with a range from 5.0 to 5.5 [3]. Since the pH of EAW is 2.7, most microorganisms cannot survive at this very acidic value [3, 24].

Apart from the low pH of EAW the chlorine present in EAW is also responsible for disinfecting action. The antibacterial effect of Cl_2 and Cl_2 compounds is dependent on the formation of hypochlorous acid (HClO) with water. Cl_2 compounds are greatly reduced by the presence and amount of organic substances. Also it has been considered that Cl_2 reach with hydrogen ions of $–NH_2$ and –OH groups of substances such as proteins, ATP, DNA, RNA and NADH in bacteria.

The ORP, pH and residual chlorine of EAW are interrelated to one another, and do not change as independent variables. Therefore, the disinfection mechanism is by a synergistic effect created by oxidation of hypochlorous acid in addition to pH and ORP, which makes the membrane potentials of organelles exceed the stabilizing limit and inhibits the energy metabolism and breathing, and all these work to kill microorganisms synergistically.

Glutaraldehyde which is widely used as a disinfectant has many disadvantages as it causes surface pitting, difficulty in disposing and hazardous to patients and medical personnel [6, 14, 15].

Table 3 Statistical analysis between group 2, group 5 and control specimens

Test specimens	No. of specimens	Mean	SD	Minimum	Maximum
(a) cfu present					
Group 2	10	260.00	201.11	0	600
Group 5	10	90.00	128.67	0	300
Control	10	6000000	0.00	6000000	6000000
Level of significance					
					Sig.
(b) Comparison of statistical difference between three groups					
Between groups					0.000
Within groups					

Test specimens		Mean difference (I–J)	Sig.
(I) Group	(J) Group		
(c) Results of post-hoc test (multiple comparisons)			
Control	Group 2	5999740.00	0.000
	Group 5	5999910.00	0.000
Group 2	Group 5	170.00	0.027
	Control	−5999740.0	0.000
Group 5	Group 2	−170.00	0.027
	Control	−5999910.0	0.000

Dependable variable: cfu

Tukey: HSD

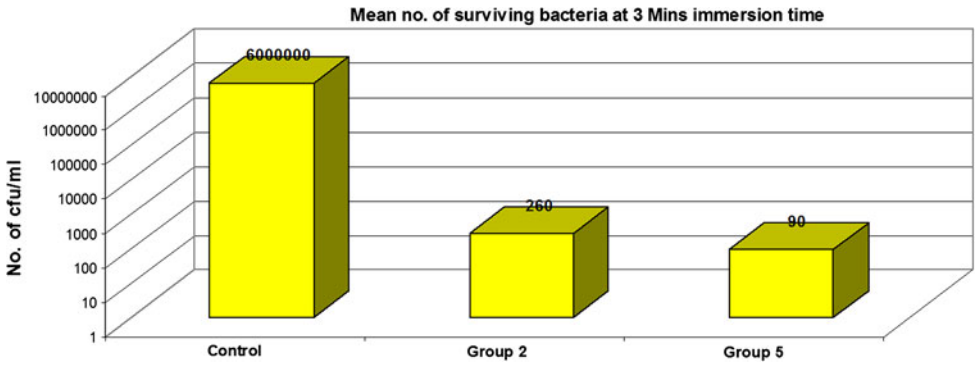

Fig. 8 Graph representing the mean number of surviving bacteria in control specimens, group 2 and group 5 test specimens

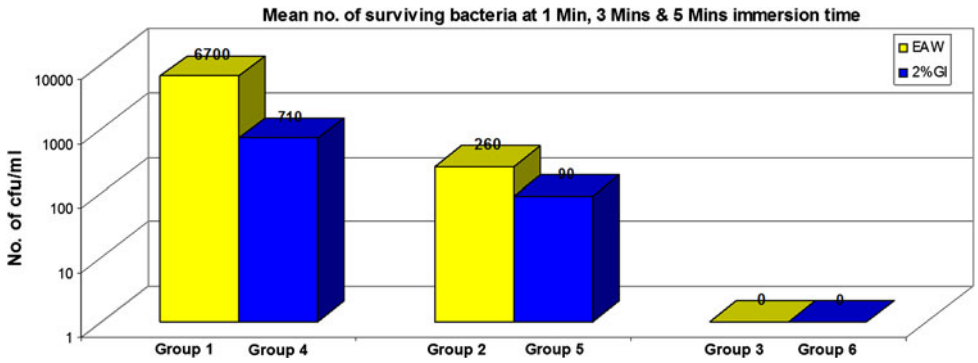

Fig. 9 Graph representing the mean number of surviving bacteria in group 1, group 2, group 3, group 4, group 5 and group 6 test specimens

When compared to the control group the disinfection potential of 2% glutaraldehyde was better than EAW when the specimens were disinfected for 1 and 3 min. Where as when the disinfection time was increased to 5 min there was no difference between EAW and 2% glutaraldehyde.

However considering that the disinfecting time can be easily extended to 5 min, EAW can be effectively used for disinfecting the acrylic denture.

The use of EAW for disinfection of the denture has several advantages when compared to the conventional denture disinfections. It costs very little and takes a reasonably short time for disinfection treatment causing no measurable adverse effects. Further more, the EAW has a unique feature that it is neutralized to be plain water with time and hence there is little fear of drainage contamination while discharge of the waste denture cleaner and disinfectant consisting of chemicals may have a risk of environmental contamination through the drainage water. Owing to these advantages, it can be said that at 5 min immersion time EAW is equal to 2% glutaraldehyde.

One of the problems is that the bactericidal activity of the EAW may be rapidly reduced by the presence of protein or organic substances [24]. To over come this, before disinfecting the denture with EAW it has to be cleaned with tap water so that it is free from organic substances. On the other hand it is known that the durability of the bactericidal activity of EAW is less. EAW in a closed and shaded polyethylene terephthalate bottle, the storage life can be prolonged to 40 days and further prolonged to 65 days in the refrigerator.

Further study can be under taken to determine the difference in the bactericidal and virucidal effect of EAW as it is with different mode of production.

One of the disadvantages of recommending EAW to patient is that it is not readily available from the store and even when it is available its shelf life is less than 40 days.

However the simple equipment to generate EAW used in the study costs one thousand rupees only. However if the apparatus is made commercially available the cost may be further reduced and it may be possible for us to recommend the EAW for the dental staff and patient to disinfect the denture base. At this point of time it may not be feasible for us to recommend all the patient to use EAW, it is certainly possible to set up a small apparatus in the prosthodontics department or clinic to generate EAW to disinfect the dentures that come in contact with the dentist or co-dental staff for adjustment, repair, relining and before delivering to the patients.

Conclusions

Based on the results, statistical analysis of the results and within the limitations of this In-vitro study the following conclusions can be drawn.

(a) The efficacy of EAW in eliminating the bacterial colonies on heat cured acrylic specimens raised from 99.88 to 99.995% as the immersion time was raised from 1 to 3 min, and complete disinfection was achieved at 5 min immersion time.
(b) The efficacy of 2% glutaraldehyde in eliminating the bacterial colonies on heat cured acrylic specimens raised from 99.988 to 99.998% as the immersion time rose from 1 to 3 min, and complete disinfection was achieved at 5 min immersion time.
(c) At 1 min immersion time, the efficacy of 2% glutaraldehyde in eliminating the bacterial colonies on heat cured acrylic resin specimens is 0.099% more than the efficacy of EAW at 1 min immersion time.
(d) At 3 min immersion time, the efficacy of 2% glutaraldehyde in eliminating the bacterial colonies on heat cured acrylic resin specimens is 0.003% more than the efficacy of EAW at 3 min immersion time.
(e) As a disinfectant, EAW is as efficient as commercially available 2% glutaraldehyde at 5 min immersion time.

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