ORIGINAL RESEARCH

An *In Vitro* Assessment of Physicomechanical Properties of Heat-cured Denture Base Resin Disinfected by Ozonized Water

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Abstract

Aim: This study investigates the influence of ozonized water disinfection on flexural strength, surface roughness, and surface microhardness of heat-cured denture base material [(polymethyl methacrylate (PMMA)].

Materials and methods: A total number of 90 specimens were prepared from heat-cured denture base material. In the control group ($n = 30$), 10 specimens from each test were immersed only in distilled water at 37°C for 48 hours before testing. For the two experimental groups ($n = 60$), 10 specimens of each group in each test were immersed in 2% chlorhexidine for 10 minutes and another 10 specimens were immersed in ozonized water with a concentration of 10 mg/L for 30 minutes. In the flexural strength test, specimens were subjected to three-point loading at a crosshead speed of 5 mm/minute of a universal testing machine. Hardness measurements using Vickers microhardness tester and roughness measurements by the Surftest analyzer were performed. Measurements of flexural strength, surface roughness (R_a, μm), and hardness (kg/mm^2) were analyzed using one-way analysis of variance (ANOVA) and Tukey least significant difference (LSD) test ($\alpha = 0.05$).

Results: Flexural strength values of ozonized water-disinfected specimens were insignificantly decreased. However, the use of ozonized water disinfection significantly increased roughness values. At the same time, microhardness values significantly decreased.

Conclusion: The use of ozonized water in disinfecting heat-cured denture base resin did not exhibit a deleterious effect on its strength nor surface roughness. Thus, it may be a much more safe disinfection method rather than chlorhexidine chemical disinfectant.

Clinical significance: Disinfection of heat-cured PMMA denture base resin using ozonized water may be a more valuable hygienic method compared to chlorhexidine, the most common chemical disinfectant.

Keywords: Flexural strength, Microhardness, Ozone, Polymethyl methacrylate, Surface roughness.

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Introduction

Most of the denture users cannot maintain their dentures clean and almost have bad oral hygiene. Unclean dentures are the main causative factor in diseases of oral mucosal tissues. A considerable relationship of poor denture cleanliness and denture stomatitis was reported in denture wearers. Maintaining good oral hygiene of edentulous patients and cleanliness of their dentures are essential for better health, especially in old ones.[1]

Mechanical and chemical methods are in general recommended for dentures users to get rid of dental biofilms from their dentures. However, mechanical cleaning methods are unsatisfactory for decreasing the microorganisms on dentures.[2] Using water and a toothbrush in cleaning the dentures is the most common manual method.[3] Nevertheless, toothbrushes are unsuccessful against the activity of microbes in biofilms of dentures and can only remove large particles.[4] A suitable cleaning method implicates the toothbrushing technique to get rid of food debris, which may influence the surface of the denture that encourages the formation of plaque.[5]

On the contrary, soaking of the dentures in chemical disinfectant solutions[6] was proved to be a helpful method to reduce the number of microorganisms, while some chemical agents used are shown to destroy both acrylic resin[7] and metallic materials. Recent advances recommended microwaving, ultraviolet C (UVC) light, and ozonized water as powerful methods in monitoring infection. Ozonized water was recommended to be functional in diminishing the *Candida albicans* count on the denture surface.[8]

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Ozone (O_3) is a strong oxidizing agent and one of the allotropic forms of oxygen. It oxidizes amino acids and damages the proteins existent inside the cell membranes of microorganisms; consequently, ozone may have excellent antimicrobial activity.[9] It presents greater bactericidal properties when compared to chloride and has the benefit of presenting lower toxicity. Its activity is related to the interference with bacterial growth and the ability of viral inactivation. Ozonized sunflower seed oil proved to have a positive effect in the treatment of herpetic gingivostomatitis.[10]

Baysan et al. reported that the exposition of *Streptococcus mutans* and *S. sobrinus* for 10 seconds to ozonized water significantly reduced their number *in vitro*. A period of 30 minutes of exposition to ozonized water was reported to be sufficient to inactivate *Staphylococcus aureus*.[11] Murakami et al. and Oizumi et al. revealed that ozone could be useful in dentures' disinfection.[12,13] *C. albicans* is the most frequently isolated species from human infections (80–90%) and is responsible for 75% of the neonatal infections.[14]

Moreover, oral candidiasis may be considered a potential risk of the development of systemic diseases, especially among immune-compromised patients. Since the use of antifungal therapy may lead to the appearance of resistant isolates, new alternatives are necessary.[15] Still limited studies linked the ozone disinfection of the dentures to different properties of the denture base materials. Accordingly, this study aimed to assess the influence of ozonized water as an effective denture disinfectant versus chlorhexidine on the flexural strength, surface roughness, and surface microhardness. The research hypothesis was that the ozone disinfection of heat-cured acrylic resin would affect its physicomechanical properties.

Materials and Methods

A commercial heat-cured polymethyl methacrylate (PMMA) denture base material (Acrostone; Acrostone Dental factory, under exclusive license of England, Egypt) was used for the preparation of the specimens tested for flexural strength, surface roughness, and surface microhardness. The disinfectant solutions used in this study were 2% chlorhexidine (Endox, GCI, China) and ozonized water with a concentration of 10 mg/L.

Specimens Preparation

A custom-made stainless steel metal mold having five rectangular cavities of dimensions 64 mm (length) × 10 mm (width) × 3.3 mm (height) in the middle part was used for flexural strength testing. Another mold having five disc-shaped cavities of dimensions 15 mm (diameter) × 4 mm (thickness) was used for both hardness and roughness testing. Each mold was positioned horizontally resting on the lower piece; and each mold cavity was coated with a thin layer of white petroleum jelly, and the base plate wax (Cavex, Haarlem, Holland) was softened and poured into the mold. The upper piece was used to press the wax. A weight of 1 kg was placed over it to expel excess wax. Upon solidification and cooling to room temperature, the wax patterns were carefully removed.

Type III dental stone (Herodent; Vigodent SA Ind. Com., Rio de Janeiro, RJ, Brazil) was used for flasking of the wax specimens. The flask was immersed in boiling water for 5 minutes for wax elimination. The flask was opened and the mold cavity was rinsed with boiled water to eliminate the wax remnants. The powder and liquid were mixed according to the manufacturer's instructions in a prescribed ratio (3:1 by volume) in a ceramic jar for 1 minute. On reaching the dough stage within 20 minutes, the paste was kneaded properly and packed into the mold space of the customized mold. Trial closure was done at 1500 Psi, flash removed, and the final closure was done at 3500 Psi under hydraulic bench press (Carlo De Giorgi S.R.L., Italy) for 30 minutes. Processing was done at 100°C as recommended by the manufacturer. After polymerization, the flasks were allowed to bench cool at room temperature for 30 minutes and 15 minutes under running water. The specimens were removed, carefully finished with 400-grit silicon carbide paper (Norton; Saint-Gobain Abrasives, Brazil), and polished on a wet rag wheel with slurry pumice.

Disinfection Methods

A total of 90 specimens were fabricated. In the control group ($n = 30$), 10 specimens in each test were immersed only in distilled water at 37°C for 48 hours before testing. For the two experimental groups ($n = 60$), 10 specimens in each test were immersed in 2% chlorhexidine for 10 minutes and the other 10 specimens were immersed in ozonized water with a concentration of 10 mg/L for 30 minutes. The ozonized water was produced by placing 250 mL of autoclaved distilled water in the system with a glass tube coupled to the ozone generator. Then the ozone was bubbled through the water for 20 minutes, thus producing a concentration of 10 mg/L/minute from ozone.[16] The ozone generator used was Humazone PM generator (Humares GmbH, Kamlst, Germany).

Flexural Strength

Before flexural strength testing, the length, width, and thickness of each specimen were measured with a digital vernier caliper (Mitutoyo, Kawasaki, Japan). Specimens of each group were exposed to three-point loading at a crosshead speed of 5 mm/minute in a universal testing machine (Model LRX-plus; Lloyd Instruments Ltd., Fareham, UK). The flexural testing device consisted of a central loading plunger and two polished cylindrical supports. The distance between the centers of the supports was 50 mm. The load was applied perpendicular to the center of the specimen until the fracture of the specimen occurred. The data were recorded and the flexural strength was calculated by computer software (Nexygen 4.6; Lloyd Instruments Ltd. 2002, UK) associated with the machine using the formula:

$$FS = 3FL / 2bd^2$$

where FS is the flexural strength (MPa), F is the load or force at the point of fracture (N), L is the span of the specimen between the supports (mm), b is the width (mm), and d is the thickness (mm).

Surface Roughness

Surface roughness values were measured by a profilometer (SurftestSJ210; Mitutoyo Corp., Kawasaki, Japan). The stylus moved across the specimen and three readings were recorded for each specimen and the mean roughness (R_a) of the specimen was calculated. The tracing length was 2.5 mm and the cutoff value was 0.8 mm, at 0.5 mm/second. The resolution of the recorded data was 0.01 μm.

Surface Microhardness

Surface hardness (kg/mm^2) was measured using a microhardness tester (Micromet II; Buehler, Lake Bluff, IL, USA) with a load of 200 g for 15 seconds. Three indentations, equally spaced over a circle, were carried out and averaged for each specimen.[8]

Collected data were analyzed using statistical analysis software (SPSS 12.0; SPSS, Chicago, Illinois). One-way analysis of variance (ANOVA) was conducted on flexural strength (MPa), hardness (kg/mm^2), and surface roughness (μm) data, followed by the Tukey (LSD) test for *post hoc* comparisons ($\alpha = 0.05$).

Results

Table 1 shows the mean average flexural strength values of the ozonized water, chlorhexidine disinfected specimens and control specimens. The average flexural strength is 69.01 MPa for the control group and decreased insignificantly to be 67.93 and 65.59 MPa for ozonized water and chlorhexidine disinfected groups, respectively ($p = 0.4$).

The results of surface roughness are presented in Table 1. One-way ANOVA for the surface roughness values identified significant differences among the studied groups ($p < 0.0001$). The LSD test showed that the surface roughness value of the control group (1.37 µm) was significantly increased to be 2.10 and 1.51 µm for chlorhexidine and ozonized water-disinfected PMMA specimens, respectively.

Table 1 shows the mean microhardness values of the three groups. In comparison with the microhardness value of the control group (22.98), chlorhexidine and ozonized water-disinfected groups demonstrated lower microhardness values (19.97 and 19.30) respectively. One-way ANOVA test showed a significant difference in microhardness values of the three groups ($p = 0.02$). The LSD test showed a significant difference in microhardness values of both chlorhexidine and ozonized water-disinfected groups in comparison with that of the control group. Meanwhile, no significant difference was observed in microhardness values between the two types of disinfection.

A graphical presentation of flexural strength (MPa), surface roughness (µm), and surface microhardness (kg/mm^2) results is shown in Figure 1.

Table 1: Mean and standard deviation of studied physicomechanical properties

	Flexural strength (MPa)	*Surface roughness (µm)*	*Surface microhardness (kg/mm^2)*
Group	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*
Control	69.01 ± 6.1	1.37 ± 0.09^c	22.98 ± 0.09^a
Chlorhexidine disinfection	65.59 ± 2.04	2.10 ± 0.09^a	19.97 ± 0.09^b
Ozonized water disinfection	67.93 ± 2.43	1.51 ± 0.11^b	19.30 ± 0.11^b
p value	0.4	<0.0001	0.02

The letters a,b,c express the significant differences between the different groups

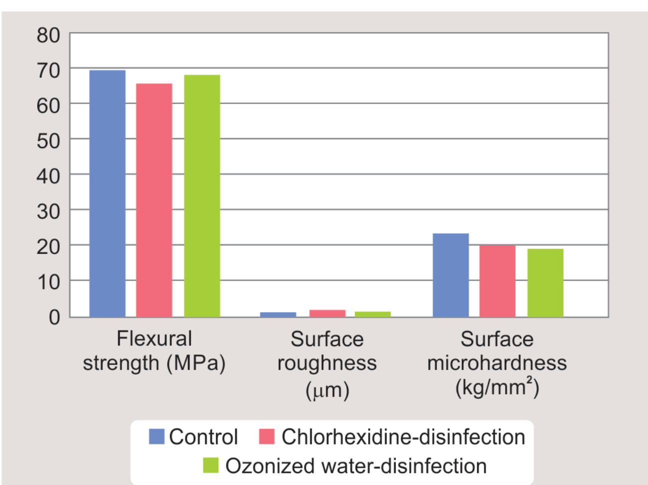

Fig. 1: Graphical presentation of the flexural strength, surface roughness, and surface microhardness results

Discussion

Based on the results of this study, the research hypothesis was partially accepted. This is because the disinfection of heat-cured acrylic resin specimens using ozonized water significantly increased their roughness values. At the same time, their microhardness values were significantly decreased. Whereas the flexural strength values were insignificantly decreased.

Denture care is crucial to avoid malodor, poor esthetics, and the growth of plaque/calculus and biofilms. Numerous methods for denture cleaning are clinically used to overcome these problems and are mostly divided into mechanical and chemical methods.[8]

Ozone is considered as a substitution disinfectant in the field of dentistry due to its powerful antimicrobial impact without developing any kind of drug resistance.[17] According to Bezirtzoglou et al. the use of O_3 for a brief period had a bacteriostatic effect, while ozonation for a period of more than 30 minutes had a bactericidal effect. They also revealed that ozonized water has a limited half-life, and the remaining ozone could be present in water for a maximum period of 8 hours, so it would be difficult to generate and store ozone for long periods.[18] Huth et al. assessed the biocompatibility of gaseous and aqueous forms of ozone concerning the recognized antimicrobials. They concluded that aqueous ozone form is less cytotoxic than gaseous ozone or recognized antimicrobials (chlorhexidine digluconate 2% and 0.2%; sodium hypochlorite 5.25% and 2.25%; hydrogen peroxide 3%) under the majority of situations. Moreover, the aqueous form of ozone is considered more biologically biocompatible for oral application.[19]

Immersion of heat-cured acrylic resin specimens in 2% chlorhexidine for 10 minutes significantly increased their surface roughness. This surface change was attributed to the slow dissolving action of the disinfectant on the matrix phase and the resultant exposure of the polymer beads.[20] This finding was in agreement with Carvalho et al. who reported surface pitting and formation of polymer beads in acrylic resin denture base exposed to glutaraldehyde for 10 minutes.[21]

Conversely, Shen et al. studied the influence of glutaraldehyde-based disinfectants (alkaline, phenol buffered) on the surface morphology of denture base resins and no obvious surface change was detected with the consistent alkaline formulation. Nevertheless, the disinfectant with phenolic buffer produced surface pitting of the material after10 minutes of immersion, and softening and swelling of the surface after 2 hours of immersion.[20] In addition, ozone disinfection of heat-cured acrylic resin significantly increased the surface roughness to a less extent than chlorhexidine. This might be explained by the possible retention of surface and subsurface oxide-related substances during ozone application, with the resultant increase in surface roughness.[22]

Both types of disinfection; chlorhexidine and ozonized water significantly decreased the surface microhardness of heat-cured acrylic resin. The polymerization process of conventional PMMA resin occurs by free addition, thus resulting in the presence of free radicals as well as partially cross-linked polymer chains containing high levels of residual monomer. This is believed to have an adverse effect on the hardness of the resin due to the diffusion of the monomer from the polymer and simultaneous water sorption by diffusion of whether chlorhexidine or ozonized water into the resin. This produces a plasticizing effect, which decreases the interchain forces allowing easy distortion and a significant reduction in the hardness of PMMA acrylic resin following immersion in both disinfectants.[23] Moreover, some reports suggested that the use of ozonized water might result in the conversion of the oxygen to free oxygen radicals, which may cause a chemical softening of the resin.[24]

Although chlorhexidine disinfection decreased the flexural strength of acrylic resin specimens to a greater extent than did ozonized water concerning the control group, yet the difference was nonsignificant.

This finding might be linked to the fact that the strength of a denture polymer at a given time after immersion in any media is affected by the relative amount of various molecules like unreacted monomer, plasticizer, and initiator present. If a component that leaches out applies a less plasticizing affect than the disinfectant solution molecule, then the strength of denture polymer should decrease. On the contrary, if the component that leaches out applies a more strong plasticizing effect than a water molecule, then the strength of denture polymer should increase. Accordingly, both chlorhexidine and ozonized water disinfectants seemed to have a more profound plasticizing effect than the constituents that leached out of acrylic resin.[25]

The current study results are contradictory to that of Vallittu et al. who stated that the pendant MMA attached to PMMA might remain as such and might form complexes with chemicals of denture disinfectants of chlorhexidine or ozonized water that might increase the degree of cross-linking with the corresponding increase in strength.[25]

Conclusion

Within the limitation of this study, the following results were obtained:

- Flexural strength values of ozonized water-disinfected heat-cured acrylic specimens were insignificantly decreased; compared to non-disinfected specimens.
- Even though the use of ozonized water in disinfecting heat-cured acrylic resin specimens significantly increased their roughness values, this effect was much more pronounced in the case of chlorhexidine disinfection.
- Surface microhardness values of ozonized water-disinfected specimens were significantly decreased in comparison to those of chlorhexidine.
- Although the use of ozonized water in disinfecting heat-cured acrylic resin had adversely affected not only its roughness but also its microhardness, it may be considered as a more tolerable method for disinfection rather than chlorhexidine disinfectant.
- The use of ozonized water in disinfecting heat-cured denture base resin did not exhibit a deleterious effect on its strength nor surface roughness. Thus, it may be a much more safe disinfection method rather than chlorhexidine chemical disinfectant.

Recommendations

Further supporting studies to assess the surface texture of disinfected specimens should be well thoughtout. Thus, a more comprehensive understanding of how the acrylic resins are altered by these disinfection methods could thereby be achieved. It is necessary to evaluate the heat-cured acrylic resin disinfected by ozonized water regarding other properties as water sorption and solubility, dimensional stability, color stability, and bond strength with relining materials. As well, the long-term effect of ozonized water on heat-cured denture base resins should be considered in these supplementary research.

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Compliance with Ethical Standard

Ethical Approval

All procedures performed in studies were in accordance with the ethical standards of the institutional and/or national research committee. The study was approved by institutional review board 04021018.

Consent for Publication

The authors have approved the manuscript and agree with the submission. We confirm that this manuscript is our original unpublished work and has not been published or considered for publication somewhere else.

Availability of Data

All data presented or analyzed during this study are included in this article.

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