J Oral Res
oral and craniofacial sciences

Article

Tensile strength evaluation of acrylic after ozonated water and microwave disinfection.

Evaluación de la resistencia a la tracción del acrílico después de la desinfección con agua ozonizada y microondas.

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Receipt : 07/01/2019 Revised: 07/20/2020
Acceptance: 09/15/2020

Abstract: Background: There is a need for safe disinfection methods without a detrimental effect on the acrylic denture base. **Aim:** the purpose of the current study was to evaluate the effect of ozonated water and two protocols of microwave disinfection on tensile bond strength of high-impact acrylic. **Material and methods:** Eighty samples were divided into four major groups: one control and three experimental groups, 20 specimens each. The experimental groups each received either disinfection by ozonated water, microwave 850W/1min, or microwave 650W/6min. Further division of the major groups into two sub-groups depending on the number of disinfection cycles and total immersion time in distilled water: One cycle and one day *versus* seven cycles and seven days. The control group specimens were immersed in distilled water without any disinfection for either one day or seven days. Tensile strength testing was performed on the specimens from the eight groups. The data were analyzed by SPSS version 21#. The ANOVA and Tukey-HSD multiple comparison tests were used for comparison for groups. **Results:** A non-significant difference in the tensile strength was observed in the groups subjected to one cycle of ozone or microwave disinfection at 850W/1min. There was a significant decrease in tensile strength for the specimens subjected to one cycle of 650W/6min microwaving. The number of testing cycles and storage time in distilled water had a significant impact on the tensile strength of the acrylic in all tested groups, by decreasing it. **Conclusion:** Ozone disinfection and microwaving at 850W for one minute did not affect the tensile strength of the acrylic, but repeated disinfection over seven days at one cycle per day impaired the tensile strength of the acrylic resin.

Keywords: Ozone; microwaves; acrylic resins; disinfection; denture bases; tensile strength.

Resumen: Antecedentes: Existe la necesidad de métodos de desinfección seguros sin un efecto perjudicial sobre la base de prótesis acrílica. **Objetivo:** el objetivo del presente estudio fue evaluar el efecto del agua ozonizada y dos protocolos de desinfección por microondas sobre la resistencia a la tracción del acrílico de alto impacto. **Material y Métodos:** Ochenta muestras se dividieron en cuatro grandes grupos: un grupo control y tres grupos experimentales, 20 especímenes cada uno. Cada grupo experimental recibió desinfección con agua ozonizada, microondas de 850 W/1 min o microondas de 650 W/6 min. Los

Cite as:
Aziz HK.
Tensile strength evaluation of acrylic after ozonated water and microwave disinfection.
J Oral Res 2020; 9(3):212-219.
Doi:10.17126/joralres.2020.038

grupos principales se dividieron en dos subgrupos según el número de ciclos de desinfección y el tiempo total de inmersión en agua destilada: un ciclo y un día *versus* siete ciclos y siete días. Las muestras del grupo de control se sumergieron en agua destilada sin ninguna desinfección durante uno o siete días, respectivamente. Se realizaron pruebas de resistencia a la tracción en las muestras de los ocho grupos. Los datos fueron analizados por SPSS versión 21. Se utilizaron las pruebas de comparación múltiple ANOVA y Tukey-HSD para la comparación entre grupos.. **Resultados:** Se observó una diferencia no significativa en la resistencia a la tracción en los grupos sometidos a un ciclo de desinfección con ozono o microondas a 850 W/1 min. Hubo una disminución significativa en la resistencia a la tracción para las muestras sometidas a un ciclo de microondas de 650 W/6 min. El número de ciclos de prueba junto al tiempo de almacenamiento en agua destilada tuvieron un impacto significativo en la resistencia a la tracción del acrílico en todos los grupos probados, al disminuirla. **Conclusión:** La desinfección con ozono y el microondas a 850 W durante un minuto no afectaron la resistencia a la tracción del acrílico, pero la desinfección repetida durante siete días en un ciclo por día afectó la resistencia a la tracción de la resina acrílica.

Palabra Clave: *Ozono; microondas; resinas acrílicas; desinfección; bases para dentadura; resistencia a la tracción.*

INTRODUCTION.

Denture cleaning is a vital part of oral hygiene since dentures are a favorable environment for bacterial and fungal pathogens such as *streptococci*, *Candida*, and other microorganisms including respiratory pathogens.[1] The method of decontamination needs to be carried out in a way that inactivates microorganisms without having a detrimental influence on the acrylic resins, while preventing cross-contamination and maintaining a healthy oral mucosa.

Denture disinfection can be achieved through various ways, such as soaking on a chemical solution and through microwave radiation. There are many problems associated with chemical cleansers such as the adverse effects of the cleansers and difficulty in managing prostheses health. Bleaching can lead to discoloration of prostheses resin and corrode the metal framework. To overcome that, using microwaves instead of chemical solutions is an option as it requires no special storage, has no expiration date and does not induce anti-fungal resistance.[2-5]

Therefore, the use of this technique is recommended. The microwave oven power and the exposition period should be adjusted in order to produce adequate disinfection without any adverse effects. Many researches succeeded in the use 650W for 6 min.[6,11] Also, using 650 W power for 6 minutes produced prosthesis with no alteration in water sorption and solubility. In the case of the duration of the treatment cycle, higher exposure times can deleteriously affect the physical and mechanical properties of acrylic resins.[12] Therefore, trials should be done to minimize the exposure time needed to achieve disinfection without unwanted effects. An exposure period of one minute has been found to be effective against *Candida albicans*.[6,13] Fortes *et al.*[2] studied the effects of microwave irradiation exposure for 1 minute at high power on the hardness and flexural strength that is safe for disinfection of complete dentures maintaining the physical-mechanical properties of the acrylic resin without damage.

On the other hand, ozone in the gaseous and aqueous phase had a powerful antimicrobial agent against bacteria, fungi, protozoa, and viruses.[14] The utilization of ozone as a denture cleaner is one of the successful techniques employed for the sterilization of prostheses.[15] Research revealed that the oral Gram-positive and Gram-negative bacteria as well as *Candida albicans* were killed by immersion in ozonated water for one hour.[16] Another study by Arita *et al.*,[17] showed that the use of ozonated water resulted in a decrease in *Candida albicans* counts Nakhaei *et al.*,[18] evaluated the effect of ozone and two common denture cleansers on the surface hardness and bond strength of a silicone-based soft liner to the acrylic denture base material. A few studies have reported on the effects of ozone on the mechanical properties of the denture, so the present research was carried to study the effects of ozonated water compared to the microwaving on the tensile strength of heat-cured resins in two periods of exposure (at 1 day and 7 days). The null hypothesis was there would be no differences among three different disinfection methods: ozone, microwaving at 850W for 1min, and microwaving at 650W for 6min, on the tensile strength of the acrylic resin.

MATERIALS AND METHODS.

The heat-cured denture base resin (high impact acrylic, Vertex-Dental, Netherlands) was used for fabrication of eighty samples that were distributed into four treatment groups according to disinfection type: The control group, in which specimens were immersed in distilled water; the second group, in which specimens were subjected to microwaving at 850W for 1 minute; the third group, in which specimens were subjected to microwave disinfection at 650W for 6 minutes; and the fourth group, in which the specimens were disinfected by ozonated water.

Further, each treatment group was subdivided into two sub-groups with different periods of immersion post disinfection: for one day or for seven days (each group N=10) (Table 1). The wax patterns (Polywax, Bilkim chemical company, Turkey) were constructed in the shape of a flat dumbbell 80mm, 9mm, 3mm, length, width, thickness respectively[19] using a metallic template of the same dimensions. They were used for the fabrication of acrylic specimens for all groups used to measure tensile strength in this study. The mould was fabricated by filling the lower part of the flask with dental stone, then wax patterns were positioned in the stone after coating with a separating medium, then the upper part was placed over the lower part and filling with stone was completed (Figure 1).

When the stone setting was achieved, the two parts of the flask were opened and the wax was eliminated from it. The proportion of acrylic mixing was 10g:4.4ml according to the manufacturer's instructions, waiting until the dough stage was reached, and then it was manipulated into the mould.

The curing of the mixture was done conventionally using a short cycle at 74°C for 90 minutes, then the temperature was increased to boiling point for 30 minutes. The deflasking was done after cooling for 30 minutes. The specimens were then removed from the stone mould, smoothed, and polished in a regular way while continuously cooling with water to avoid overheating that may lead to the distortion of the specimens. Each sample was re-measured to check there was no change in dimensions using digital vernier calipers to ensure the previously mentioned specification dimensions.[20, 21]

Microwave disinfection: All the prepared specimens of the second group and third groups were exposed to microwave radiation in order to be disinfected. The specimens were soaked in a glass cup containing 150ml of distilled water and inserted inside a microwave oven (Samsung TDS, Samsung, Korea) which was set at 850W for 1minute[2] the second groupand at power 650W for 6 min for the third group.[9] Each cycle was done by immersion of the sample in distilled water and microwaving as described. Specimens were then removed and stored in distilled water for one day.

Each subsequent day the specimens were placed in the microwave for one more cycle of disinfection, as described. As such for the seven days group, the specimens were subjected to seven cycles of microwaving, once per day. Specimens were stored in distilled water in between cycles.

Ozone disinfection

For the groups of immersion in ozonated water, the specimens were immersed in ozone rich water (4mg/L) that was prepared by an ozone generator (Hyper Medozon comfort, Herrmann Apparateau GmbH, Germany). For the ozone-1 specimens were exposed to the ozonated water for one cycle of 60 minutes in the ozonated water, and then immersed and stored in distilled water[22] for 1 day.

While for the ozone-7 group specimens were exposed to the ozonated water for one cycle of 60 minutes ozonated water each day for seven days (specimens were stored in distilled water in between cycles). For the control groups, the specimens were stored in distilled water for one day (control-1), or for seven days for the control-7 group.

Testing Procedures

The acrylic specimens for all groups were prepared to measure the tensile strength. The test was carried out on a WDW200E Universal Testing Machine (Tensile Strength Testing Machine). Each specimen was positioned on a bending fixture, consisting of two parallel supports 50mm apart, the load was applied with a crosshead speed 5mm/min until fracture occurred (Figure 2) and the force was registered in Newton.[23]

The values of tensile strength were maintained from the equation:

Tensile strength = F/A 19.

F = force at failure (Newton).

A= minimum cross-sectional area (mm).

Data were analyzed by using Statistical Package for Social Science (SPSS) version #21(Chicago, Illinois, USA). Means and standard deviation with the ANOVA-test and Tukey-HSD multiple comparison test were applied to get significant differences at a significance level ($p<0.05$).

RESULTS.

Descriptive statistics of tensile strength values are shown in Table 2 and Figure 3. The tensile strength of specimens of the ozonated water groups showed the highest mean values, while the lowest mean values of tensile strength were observed for the 650W/6min microwave groups (both one day and seven days). The comparison of the tensile strength mean values according to the disinfection cycles showed a significant difference among all study groups as shown in Table 2.

Moreover, the comparison among the groups according to the disinfection types per ANOVA-test corroborates the significant differences in the tensile strength observed among the different groups (Table 2).

Further analysis was done by Tukey HSD multiple comparison test, which showed a significant difference between the 650W/6min microwave group and the other disinfection groups: the 850W/1minmicrowave groups, the ozone groups, and the control groups. In addition, comparison of the tensile strength mean values of the control group and the 850W/1min microwave group revealed a non-significant difference.

There was also a non-significant difference between the control group and ozonated group, and between the 850 W/1min microwave groups and ozone groups as shown in Table 3.

Figure 1. The wax pattern inside the mould.

Figure 2. The specimen during test.

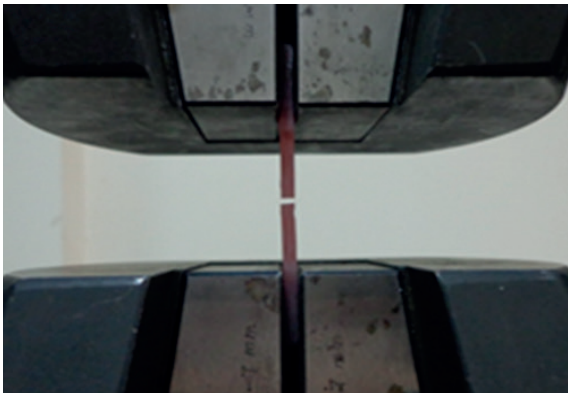

Figure 3. Tensile strength mean values (N/mm^2) for all groups.

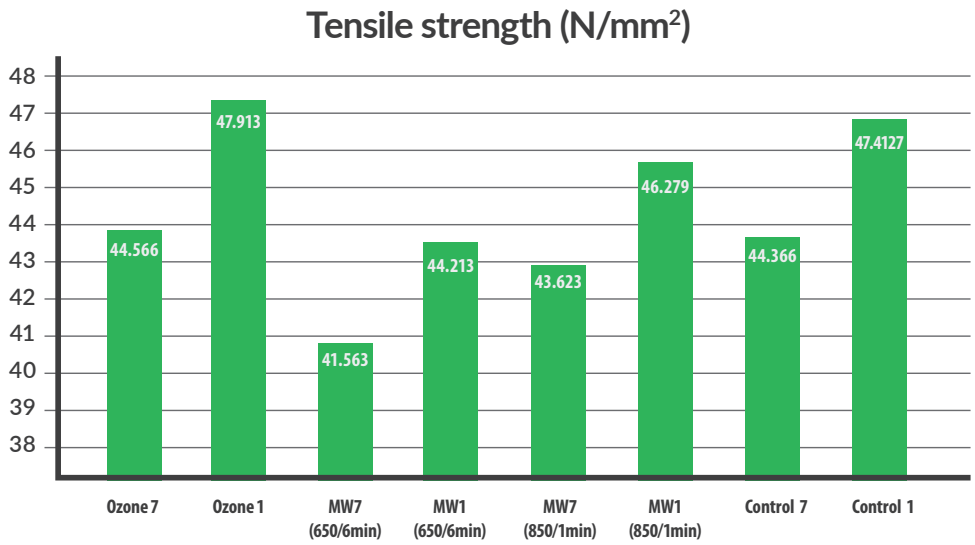

Table 1. Distribution of study groups.

Study groups	Disinfection type	Disinfection time
Control	Control 1	1 day
	Control 7	7 days
Microwave(850W/1min)	MW1(850W /1min)	1 minute for 1 day
	MW7(850W /1min)	1 minute for 7 days
Microwave(650W /6min)	MW1(650W /6min)	6 minute for 1 day
	MW7(650W /6min)	6 minute for 7 days
Ozone	Ozone 1	60 minute for 1 day
	Ozone 7	60 minute/day for 7 days

Table 2. Descriptive Statistics of studied groups .

Study Groups	N	Mean	Standard deviation		**p-value
Control	Control 1	10	47.4127	2.2642	0.000
	Control 7	10	44.3660	1.2848	
Microwave (850W /1min)	MW1(850W /1min)	10	46.2790	1.0168	0.001
	MW7(850W /1min)	10	43.6230	0.9550	
Microwave (650W /6min)	MW1(650W /6min)	10	44.2130	0.9646	0.001
	MW7(650W /6min)	10	41.5630	0.9435	
Ozone	Ozone 1	10	47.9130	1.4066	0.000
	Ozone 7	10	44.5660	1.1093	
**F*-test	25.646				
p-value	0.000 S				

*: ANOVA-Test. **: Tukey HSD multiple comparisons test.

Table 3. Tukey HSD multiple comparisons test for tensile strength according to disinfection type.

Between groups		*p*-value
Control 1	MW1(850W /1min)	0.533
	MW1(650W /6min)	0.000
	Ozone 1	0.989
MW1(850W /1min)	MW1(650W /6min)	0.016
	Ozone 1	0.115
MW1(650W /6min)	Ozone 1	0.000
Control 7	MW 7(850W /1min)	0.908
	MW 7(650W /6min)	0.000
	Ozone 7	1.000
MW 7(850W /1min)	MW 7 (650W /6min)	0.017
	Ozone 7	0.744
MW7(650W /6min)	Ozone 7	0.000

p-value >0.05: Non-significant. *p*-value >0.05: Significant. **MW:** microwave.

DISCUSSION.

This study was carried out in order to assess the influence of microwaving and ozone disinfection on the tensile strength of high-impact acrylic. The tensile strength is *"the resistance of the material to a tensile or stretching force where the maximum force that indicates the maximum stress to which a material can be subjected to failure"*.[24]

Disinfection by microwaving is an efficacious technique for cleansing a prosthesis from contamination.[9]Numerous protocols have been used for microwave denture sterilization including the use of different power and time, but different studies have indicated a need for reducing the time of microwave exposure in order to reduce the side effects over a long period of use.[2,9] So in the present study, the microwave oven was used at 850W for 1min because this energy and time are capable of sterilizing the denture from *Candida albicans* contamination.[6,13]

The findings showed that the disinfection of specimens in the microwave at 850W for 1min had a non-significant effect on the tensile strength. This is in agreement with Fortes *et al*.,[6] who found the protocol of microwaving at 850 W for 1min had no effect on the flexural strength of the acrylic.[2] We also used 650W for 6min, as 650W has been reported to inactivate *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and spore forms of *Bacillus subtilis* and it is an effective method for acrylic resin disinfection.[25] In the current study, this protocol minimized the strength in comparison to other groups, in agreement with Konchada *et al*.,[7] who reported a decrease in flexural strength values among three different acrylic resins.

This can be attributed to the increased exposure time to the microwave irradiation leading to additional polymerization and loss of the residual monomer into water, and which reduced the plasticizing effect of H_2O absorption on the resin.[9,26] It is also in agreement with other studies that concluded the microwave oven caused a reduction in transverse strength that weakened the dentures and breaks during clinical use, and as such it was not an acceptable method for the sterilization of dentures.[27] The immersion period of one week and repeated cycles significantly lowered the strength in comparison to one day only.

This is in agreement with studies that reported that the use of repeated cycles of microwaving leads to a change in the features of any polymer,[7,14] such as flexural strength. Also, it coincided with the study that concluded the elastic properties decreased after 36 disinfection cycles.[28] This may be due to disarrangement in the polymer chains as a result of multiple cycles of exposure. Moreover, the resilience of the samples was altered after frequent cycles.[29]

On the other hand, this study has backed the use of ozone as a method of denture disinfection because the ozone has been suggested for cleansing the surface of the removable partial denture as a disinfectant for acrylic appliances.[30] The selection dose of ozonated water was 4mg/L according to previous reports.[16,17]

There was no change in tensile strength after the exposure to ozonated water once, in accordance with previous studies.[18,31] Regarding the effects of exposure period, tensile strength decreased with the increase of the ozonated water immersion period.

This result is in agreement with that reported by Ekren *et al*.,[22] who found that prolonged exposure to ozone lead to a significant decrease in tensile strength; other studies have shown the mechanical properties of the resins were impaired as a result of storage in water for a long period.[32] The use of water may affect the mechanical properties of the acrylic resins and the prolonged exposure to water reduces the strength of the denture, which could be attributed to the brittleness of the resin and weakness of its tensile strength, as well as a loss of monomer by exudation and a decrease in mechanical properties when the denture base resin is conditioned for long periods.[33]

The null hypothesis was accepted concerning disinfection with the ozone, microwaving at 850W/1min, but it was rejected in the part related to the use of microwave at 650W/6min for the sterilization of acrylic.

CONCLUSION.

The use of microwave at 850W for one minute or ozonated water did not change the tensile strength of resin but microwave disinfection of the acrylic at 650W for 6 minutes reduced the tensile strength. On the other hand, the long immersion period (7 days) also decreased the strength.

The effects of ozone on other physical and mechanical properties of the acrylic should be assessed in order to confirm the safe use of this disinfection method without any undesirable effects.

Conflict of interests: None.
Ethics approval: None.
Funding: Self-financed.
Authors' contributions: The author conducted the work and wrote the manuscript.
Acknowledgements: None.

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