

Effect of Chemical and Microwave Disinfection on the Surface Microhardness of Acrylic Resin Denture Teeth

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Abstract

Purpose: The purpose of this study was to evaluate the effect of simulated disinfections (2% glutaraldehyde, 1% sodium hypochlorite, and microwave energy) on the surface hardness of Trilux, Biocler, Biotone, New Ace, and Magister commercial artificial teeth.

Materials and Methods: Specimens (n = 10) were made with the teeth included individually in circular blocks of acrylic resin, leaving the labial surface exposed. Cycles of simulated chemical disinfection were accomplished with the specimens immersed in the solutions at room temperature for 10 minutes, followed by tap water washing for 30 seconds and storage in distilled water at room temperature for 7 days until the next disinfection. Simulated disinfection by microwave energy was carried out in a domestic oven with 1300 W at a potency of 50% for 3 minutes with the specimens individually immersed in 150 ml of distilled water. Control (no disinfection) and the experimental groups (first and third disinfection cycles) were submitted to Knoop hardness measurements with indentations at the center of the labial tooth surface. Data were submitted to repeated measure two-way ANOVA and Tukey's test ($\alpha = 0.05$).

Results: Biocler, Magister, and Trilux showed lower surface microhardness when submitted to microwave. Lower microhardness for Biotone was promoted by hypochlorite, while no significant difference was shown for New Ace. The third disinfection cycle significantly decreased the tooth surface hardness only for microwave.

Conclusions: Different disinfection methods promoted different effects on the microhardness of different types of artificial teeth. Surface microhardness of the teeth was less affected by the simulated chemical disinfections when compared to microwaved specimens.

Posterior tooth wear in functional complete dentures causes occlusal prematurities, loss of occlusal vertical dimension,¹ loss of masticatory efficiency, altered tooth relationships, increased horizontal stresses causing associated sequelae,² and stresses on the oral mucosa and underlying bone, adversely influencing esthetics.³ The basic material commonly used in the manufacture of artificial teeth is acrylic resin (poly methylmethacrylate copolymer) in which the molecules are bonded by covalent bonds.⁴ Modified acrylic resins for teeth have been developed using the interpenetration of polymer networks (IPN), such as Trubyte Bioform IPN (Dentsply International), as well as teeth made from microfilled composite resin, such as SR-Orthosit-PE (Ivoclar, Schaan, Liechtenstein).^{1,5} The SLM tooth resin (Sustained Life Material, Dentsply, York, PA) constitutes an improvement that incorporates cross-linking in the polymer networks with polyethylene particles of high molecular weight to increase the lubricant effect.² Each modified high-strength resin tooth has been introduced with claims to have better resistance to abrasion and wear when compared to the conventional acrylic resin tooth.^{1,6}

The most common method for cleaning removable prostheses is toothbrushing with tap water and soap or toothpaste. Thus, irregularities and porosities present on the denture base surface play a major role in retaining microorganisms, difficult to clean by conventional toothbrushing methods.⁷ Insufficient hygienic care seems also to be a significant predisposing condition for candidiasis in denture wearers.⁸ The denture base is a significant environment for microbial adherence and an infection source because niches on the resin surface protect entrapped microorganisms.⁹ In addition, glazing of the denture surface does not prevent bacterial colonization.¹⁰ A microorganism commonly associated with dentures is *Candida albicans*.¹¹

Disinfection methods have been suggested in the classic literature by some authors to prevent cross contamination caused by pathogenic agents. These include glutaraldehyde, sodium hypochlorite, iodoform, carbon dioxide, chlorhexidine, or alcoholic solutions.¹²⁻¹⁶ Chemical disinfection presents disadvantages in clinical use, having a bleaching effect on the prosthesis¹⁷ and tarnishing and corrosive effects on metal.¹⁸ Depending on the duration of immersion and the types of disinfectants used, some solutions can cause changes in the mechanical properties of acrylic resins.¹³ Dental prostheses must not only be disinfected on the surfaces but also on the interior material, because it can also be a source of contaminating microorganisms.¹⁵

Irradiation by microwave energy has been used as an alternative to chemical disinfection and can be considered as a practical procedure for disinfecting complete dentures as an adjunct to treatment of oral candidiasis.¹⁹ In addition, the evidence that microwave disinfection is an efficient method to kill microorganisms has been shown by classic works^{17,18,20,21} and in more recent studies.²²⁻²⁵ Nonetheless, the number of times that the prosthesis can be safely disinfected by microwaving is still uncertain, and it is difficult to predict what effects microwaves have on the denture base and artificial teeth over the long term.

Based on these considerations, this study aimed to evaluate the effect of chemical (2% glutaraldehyde, 1% sodium hypochlorite) and microwave disinfections on the surface hardness of acrylic resin teeth of different commercial brands (Trilux, Biocler, Biotone, New Ace, and Magister) used in dental prostheses. The hypothesis verified in this in vitro study was that different disinfection methods would cause different effects on the surface hardness of acrylic resin teeth of different commercial brands.

Materials and methods

Five commercial brands of artificial teeth were used in the study: Trilux (Ruthinium; Pirassununga, Brazil) with Ormocer nanotechnology, triple pressing, and double crosslinkage; Biocler (DentBras, Pirassununga, Brazil) with double pressing and cross-linkage; Biotone (Dentsply, Petropolis, Brazil) with high density of cross-linkage; New Ace (Yamahachi Dental Co., Gamagori, Japan) with composite resin surface; and Magister (Heraeus-Kulzer, Sao Paulo, Brazil) with four injected and compressed layers.

Specimens (n = 10) were made with lower central and lateral incisors for the following experimental protocols: (1) control specimens (C) without simulated disinfection procedure; (2) simulated chemical disinfection by one cycle (G1 and H1) and three cycles (G3 and H3); and (3) simulated microwave disinfections by one cycle (M1) and three cycles (M3). PVC cylinders (Tigre, Sao Paulo, Brazil) (6 mm high, 20 mm diameter) were filled with liquefied wax. Teeth were individually fixed in the wax of each cylinder with the labial surface facing up. After wax hardening, the cylinders were conventionally included in plastic flasks (Vipi Dental Products, Pirassununga, Brazil) with



Figure 1 Schematic drawing of the tooth included in acrylic resin and location of the Knoop hardness test (large arrow).

type II dental plaster (Asfer Chemical Industry, Sao Caetano do Sul, Brazil).

After dental plaster setting, the flask was heated in a microwave oven for 3 minutes, and the melted wax removed from the PVC cylinder. Vip Wave acrylic resin (Vip), proportioned according to the manufacturer's instructions was placed into the plaster mold and the flask pressed by the conventional procedure. The acrylic resin was polymerized by microwave energy in a domestic oven (Eletrolux: Manaus, Brazil) with a potency of 1300 W following manufacturer's instructions (10% potency for 20 minutes, 0% potency for 4 minutes, 30% potency for 5 minutes). After resin polymerization, the flask was cooled at room temperature, the specimens were deflasked, and no grinding or polishing procedure was performed on the labial tooth surface. Specimens were manufactured using this method to simulate the same condition experienced by the artificial tooth during the denture process. The labial surface of the lower central and lateral incisors is an almost flat area, allowing measurement of the microhardness values in a surface that did not need to be flattened by grinding and polished again. A schematic drawing of the tooth included in acrylic resin and location of the indentation for the microhardness test is shown in Figure 1.

Ten specimens of each commercial brand were made for control and each simulated disinfection type and stored in distilled water at room temperature for 24 hours. After water storage, each simulated chemical disinfection was accomplished leaving the specimens immersed in 2% glutaraldehyde^{13,16} (Rioquimica Pharmaceutical Industry, Sao Jose do Rio Preto, Brazil) for G1 and G3 cycles, and 1% sodium hypochlorite^{12,16} (Asfer Chemical Industry) for H1 and H3 cycles at room temperature for 10 minutes. For G1 and H1, after disinfection, the specimens were washed in tap water for 30 seconds, dried with air jets and stored in distilled water at room temperature for 7 days. For G3 and H3, the specimens were washed in tap water for 30 seconds, dried with air jets and stored in distilled water at room temperature for 7 days until the next disinfection cycle. After the last cycle, the specimens remained in distilled water for 7 days.

Simulated microwave disinfections¹⁷ for M1 and M3 cycles were accomplished in a domestic oven (Eletrolux) with 1300 W at a potency of 50% for 3 minutes with the specimens individually immersed in 150 ml of distilled water.²⁶ The interval

Table 1 Repeated measure two-way ANOVA

Variation	df	Sum of squares	Mean square	F	р
Disinfection (Di)	3	211.494875	70.498292	29 <i>.</i> 69	<0.0001
Tooth (To)	4	2663.108600	665.777150	280.43	< 0.0001
Di × To	12	89.558000	7.463167	3.14	0.0004
Residue A	180	427.336500	2.374092	1.39	0.0139
Cycle (Cy)	1	9.455625	9.455625	5.54	0.0197
Cy × Di	3	12 <i>.</i> 935075	4.311692	2.52	0.0591
Cy × To	4	6.222500	1.555625	0.91	0.4589
$Cy \times Di \times To$	12	14.031300	1.169275	0.68	0.7650
Residue B	180	307.430500	1.707947		
Total	399	3741.572975			

Note: Variation Coefficient = 5.57%.

 Table 2
 Means of Knoop microhardness for disinfection and tooth interaction

		Disinfection							
Tooth	Control		Glutaraldehyde		Hypochlorite		Microwave		
Biocler	20.05	d,AB	20.49	c,A	19.82	c,AB	18.67	c,B	
Biotone	24.54	b,A	23.13	b,AB	22.30	b,B	23.04	b,AB	
Magister	27.13	a,A	27.66	a,A	26.54	a,A	24.68	ab,B	
New Ace Trilux	26.11 22.22	ab,A c,A	26.72 22.42	a,A b,A	26.21 21.80	a,A b,A	25.15 19.82	a,A c,B	

Note: Means followed by different lowercase letter in each column and different uppercase letters in the row differ significantly by the Tukey's test (p < 0.05).

between the simulated microwave disinfection cycles for M3 was 7 days, in which the specimens were stored in distilled water at room temperature. After M1 cycle and after the last cycle for M3, the specimens remained stored in distilled water at room temperature for 7 days.

Knoop hardness measurements were made using a hardness indenter (HMV-2000; Shimadzu Corporation, Tokyo, Japan). Indentations were made under a load of 50 gf for 10 seconds for the procedures (control [after specimen deflasking] and after the first and third simulated disinfection cycles). Three indentations were made on the labial surface of the teeth, and the arithmetic mean was considered as the Knoop hardness number of each tooth. A single calibrated examiner performed all tests. Data were submitted to repeated measure two-way ANOVA considering the factors disinfection and tooth and their interaction. The differences were submitted to Tukey's test at $\alpha = 0.05$.

Results

Repeated measure two-way ANOVA (Table 1) revealed statistically significant differences for the variables disinfection, tooth, and cycle. The interaction between disinfection and tooth was significant.

Table 2 compares the Knoop microhardness values for disinfection and tooth interaction. For control: Magister showed hardness significantly higher than Biotone, Trilux, and Biocler;

 Table 3
 Knoop microhardness means for cycle and disinfection interaction

Cycle		Disinfection							
	Control		Glutaraldehyde		Hypochlorite		Microwave		
1 3	24.01 24.01	a,A a,A	24.14 24.03	a,A a,A	23.44 23.23	a,AB a,A	22.73 21.81	a,B b,B	

Note: Means followed by different lowercase letter in a column and different uppercase letters in a row differ significantly by the Tukey's test (p < 0.05).

New Ace presented hardness significantly higher than Trilux and Biocler; Biotone showed hardness significantly higher than Trilux and Biocler; Trilux revealed hardness significantly higher than Biocler. For glutaraldehyde and hypochlorite: Magister and New Ace showed hardness significantly higher than Biotone, Trilux, and Biocler; Trilux and Biotone presented hardness significantly higher than Biocler. For microwave: New Ace showed hardness significantly higher than Biotone, Trilux, and Biocler; Magister revealed hardness significantly higher than Trilux and Biocler; Biotone showed hardness significantly higher than Trilux and Biocler. The comparison among disinfections showed that for Biocler, the surface microhardness promoted by the glutaraldehyde was significantly higher than showed by the microwave, while control and hypochlorite presented intermediate values. For Biotone, control showed hardness significantly higher than hypochlorite, while glutaraldehyde and microwave showed intermediate values. For Magister and Trilux, control, glutaraldehyde and hypochorite revealed hardness statistically higher than microwave. For New Ace, there was no significant difference among groups (p > 0.05).

Table 3 shows the Knoop microhardness values for cycle and disinfection interaction. In the first disinfection cycle, microwave disinfection led to tooth surface hardnesses significantly lower than control and glutaraldehyde, while hypochlorite showed an intermediate value (p < 0.05). In the third disinfection cycle, microwaving showed hardness significantly lower than control, glutaraldehyde, and hypochlorite (p < 0.05). When the cycles were compared, the results showed that the third disinfection significantly decreased the tooth surface hardness only for microwave (p < 0.05).

Discussion

A material's microhardness is determined by standardized tests that promote the indentation on the material surface with a device called an indenter. Although suitable for brittle materials, some authors have used Vickers indentation for analyzing the surface hardness of artificial teeth, claiming that the method is appropriate for evaluating the microhardness of rigid polymers.^{27,28} However, the method considered to be most adequate for the microhardness study of polymeric materials is the Knoop hardness test, because the greater diagonal of the indentation diamond point remains free of dimensional changes, and the elastic recovery and dimensional changes occur along the shorter diagonal. As a consequence, the Knoop hardness value is independent of the material ductility.⁴

An important improvement in the manufacturing of plastic teeth occurred in the 1950s with the development of cross-linking agents, which are responsible for the formation of bridges between macromolecules of rectilinear 3D chains. These, in turn, improve mechanical resistance and decrease the solubility and water sorption by the acrylic resin denture tooth.⁴

Repeated measure two-way ANOVA found statistically significant differences for disinfection, tooth, cycle, and interaction between disinfection and tooth (Table 1). The hypothesis verified in this in vitro study that different disinfection methods could cause different effects on the hardness of different commercial brands of acrylic resin teeth was partially confirmed.

Table 2 shows the Knoop microhardness values for disinfection and tooth interaction. The results revealed that the microhardness of the artificial teeth was partially influenced by different disinfection methods. It may be postulated that the different disinfection methods promoted different effects on the surface hardness of the teeth due to manufacturing characteristics, such as pressing and cross-linkage agent; however, another important result is that a greater decrease in the surface microhardness was caused by microwave disinfection. In other words, a greater negative influence occurred on the surface microhardness of the microwaved teeth, mainly when three-cycle microwave disinfection was used. It is possible to assume that the microwave procedure promoted a higher softening of the teeth, when compared to the chemical procedures. Conversely, previous findings showed that two cycles of microwave disinfection had no effect on the hardness of most acrylic resin denture teeth when the specimens were previously immersed in water for 90 days.28

In general, the New Ace and Magister teeth presented the highest microhardness values. It is possible that these results occurred because Magister has four injected and compressed layers, and New Ace has two layers, both promoting higher surface hardness. In contrast, however, no statistically significant difference in hardness of two types of denture teeth was shown, because the tooth surface softened after 90-day immersion in water regardless of the disinfecting solution.²⁷ Water can interfere in the mechanical properties of acrylic polymer-based materials, producing a plasticizing or softening effect by diffusion into the polymer chains,²⁹⁻³² a condition that can promote a considerable decrease in the microhardness of resin-based teeth.

In general, the results of this study suggest that water storage at room temperature during disinfection cycles (1 day for one-cycle disinfection and 14 days for three-cycle disinfection) may have softened the surface of teeth and exerted a different influence on the hardness values when associated with disinfection procedures; however, when conventional acrylic resin, IPN resin, and Isosit teeth were soaked in distilled water for 7 days at 37°C, the initial and final microhardness values were almost equal, indicating lack of softening in the resin-based teeth surface.¹ The difference of the water temperature used in each and the influence exerted by the association with the disinfection procedures in this study could be factors causing these conflicting results.

Temperature affects the rate at which polymer-based materials absorb water, since the diffusion coefficient is increased by a factor of two different temperatures, and the equilibrium absorption value does not change.³³ Thus, a higher level of water can be absorbed when the temperature is higher. In addition, microwave irradiation promotes vibration of the water molecules, resulting in friction and increase of temperature that can facilitate the plasticizing effect caused by the water on the tooth surface. Since the microhardness of the teeth submitted to three-cycle microwave disinfection was statistically lower when compared to chemical disinfection (Table 3), it is possible to assume that water, high temperature (55°C in each microwave cycle), and number of cycles are factors causing the decrease in the hardness of microwave deeth. In addition, the third disinfection cycle significantly decreased the tooth surface hardness only for microwave when compared to the first cycle of disinfection (p < 0.05).

Differences in the chemical composition can cause changes in the surface microhardness values of different resin teeth. It is also possible that different components of cross-linkage existing in different commercial types of teeth may be a factor responsible for the results of this investigation. Cross-linkage is employed to increase the mechanical properties of materials based on acrylic resin.^{4,34} The addition of cross-linking agents decreases the solubility with increasing concentration and directly affects the water sorption of the denture base resin, suggesting that this occurrence is due to the chemical nature of the polymer versus that of the water molecule.³⁵ By analogy, it is possible to infer that component types and different amounts of cross-linkage agents can also change the microhardness of teeth made by different manufacturers.

Magister has four injected and compressed layers. New Ace has a surface of composite resin. Biotone and Biocler have double pressing and high density of cross-linkage. Trilux is characterized by triple pressing and double cross-linkage. It has been argued that cross-linkage is a descriptive term of the composition of the tooth, and the manufacturers do not indicate the number or exact type of the covalent links present in the polymeric structure. For this reason, it is probable that these commercial teeth have been differently influenced by the different disinfection procedures. This fact suggests that the tooth's pressing number is equally or more important than the cross-linkage, mainly due to absence of residual monomer. In agreement, differences in the cross-linking nature of the acrylic resin matrix and hardness of each layer of the polished cross-sectioned teeth have been observed for different commercial brands of artificial teeth.36

It may be postulated that the different disinfection procedures promoted different effects on the surface hardness of the teeth due to differences in the manufacturing characteristics, such as pressing and cross-linkage agent; however, another important result of this study is that simulated microwave disinfection caused a greater decrease in microhardness than chemical procedures did. In other words, a more negative influence occurs on the surface microhardness of microwaved teeth, mainly when three-cycle microwave disinfection was used. On the contrary, according to a previous study using a different protocol, long-term microwave disinfection (seven daily cycles at 650 W for 6 minutes) and immersion (7 days) in 4% chlorhexidine gluconate, 1% sodium hypochlorite, and distilled water decreased the hardness of acrylic resin denture teeth.³⁷ The clinical implication of this study should be that the commercial tooth type could determine the dentist's prescription in relation to the chemical or microwave procedures for denture disinfections. The clinical frequency of the disinfection should be considered for the security of the artificial tooth in the long term because dentures with larger biofilm areas required longer irradiation exposure to be disinfected,²⁵ and different types of disinfectant, concentrations, and periods of immersion can influence the disinfecting effect on the acrylic resin.¹⁶

For this reason, manufacturers and dentists should consider the hardness of the denture tooth when submitted to oral conditions involving the effects of thermal, chemical, and mechanical events. On the other hand, dental practitioners should advise patients on the oral toxicity related to glutaraldehyde solution. After disinfection, the residual disinfectant should be completely removed from dentures by toothbrushing procedures followed by immersion in water (at least one night). In addition, a safe frequency for denture disinfection should also be strongly recommended for the patients.

Based on the complexity of the effect promoted by the chemical and microwave disinfections on the surface hardness of different commercial types of artificial teeth, it would be convenient to establish future studies with the purpose of verifying other variables that may affect this parameter. These studies may verify the correlation between surface hardness and tooth wear, since wear resistance is considered the most important physical property of artificial teeth, providing the ability of these teeth to maintain a stable occlusal relationship over time.³⁸

The limitations of this in vitro study include the fact that the long-term effect on hardness was not considered. Hardness of the labial surface compared to the internal layers after tooth polishing and surface color of the tooth after disinfection procedures should also be topics for further investigations. Another interesting finding to be investigated is that the hardness and elastic modulus of artificial denture teeth showed a positive correlation; however, the results have not shown any correlation between material properties and wear resistance.³⁹

Conclusions

Considering the limitations of this in vitro study and based on the results that have been statistically analyzed and discussed, the following conclusions can be drawn:

- 1. Different disinfection procedures promoted different effects on the microhardness of different types of artificial teeth.
- Surface microhardness of the teeth was less affected by the simulated chemical disinfections when compared to microwaved specimens.

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