Original Article

Tensile Strength and Elastic Modulus of Gutta-percha Cones Disinfected with Sodium Hypochlorite at Different Immersion Times: An *In Vitro* Comparative Study

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Abstract

Aim: The tensile strength and modulus of elasticity of gutta-percha cones can be chemically altered due to disinfectant solutions. Therefore, the aim of the present study was to compare tensile strength and elastic modulus of gutta-percha cones subjected to sodium hypochlorite (NaOCl) disinfection at different times. **Materials and Methods:** This *in vitro* and longitudinal experimental study consisted of 45 gutta-percha cones, divided equally into three groups: Group 1 (disinfection with 2.5% NaOCl), Group 2 (disinfection with 5.25% NaOCl), and control group. All groups were subdivided according to immersion times for 1, 5, and 10 minutes. Tensile strength and elastic modulus were measured with a universal testing machine. For comparing more than two independent groups, parametric analysis of variance test with Sheffe's post hoc was used and for multivariate analysis, and multivariate analysis of variance test based on Pillai's Trace was used. In all statistical analysis, a significance level $P \leq 0.05$ was considered. **Results:** When comparing the tensile strength of gutta-percha cones, no significant differences were observed after being immersed at 1, 5, and 10 minutes in NaOCl 2.5% ($P = 0.715$) and 5.25% ($P = 0.585$). Regarding the elastic modulus, a significant decrease ($P < 0.05$) was observed in those that were immersed in NaOCl 2.5% and 5.25% for 1, 5, and 10 minutes. Furthermore, increased NaOCl concentration significantly reduced the elastic modulus ($P < 0.001$). However, there were no significant differences in tensile strength ($P > 0.05$) and elastic modulus ($P > 0.05$), when evaluating the interaction between NaOCl concentration and time. **Conclusion:** Increasing NaOCl concentration significantly reduced the modulus of elasticity without affecting the tensile strength of gutta-percha cones, regardless of immersion time. Furthermore, the interaction of time and NaOCl concentration did not significantly affect the tensile strength and elastic modulus.

Keywords: *Disinfection, elastic modulus, gutta-percha, sodium hypochlorite, tensile strength*

Introduction

The most commonly used filling materials inside the root canal are gutta-percha cones due to properties such as not irritating periapical tissues, sealing lateral and apical canal,[1] hindering bacterial growth, among others; and although these are generally sterile, 5%–19%

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of gutta-percha packages may be contaminated.[2-4] In addition, once removed from their sealed packaging, there is a possibility of contamination by incorrect handling or by aerosols from the clinical environment.[5]

Because conventional sterilization methods use humid or dry heat, they cannot be applied to gutta-percha cones because they can irreversibly alter their morphological structure due to their thermoplastic characteristics.[6,7] Therefore, the disinfection of cones must be performed by immersion in antimicrobial chemical agents.[8]

Among the chemical agents used in dentistry, we have sodium hypochlorite (NaOCl), which has the capacity to disinfect gutta percha cones by having a broad-spectrum antibacterial effect,[9-11] through the chloramination mechanism. This consists in forming chloramines that will interfere in the cellular metabolism of bacteria inactivating their essential enzymes by means of oxidation,[12] proving to be effective in concentrations of 0.5%–5.25% and in different time intervals such as 45 seconds, 1, 5, 10, 15, 20, and 30 minutes.[13-15] However, it has been reported that NaOCl, being a solution with high oxidative power, can cause structural alterations in multiple dental materials.[16]

Some reports revealed that topographical changes occur in gutta-percha cones when immersed in NaOCl for disinfection, forming chloride crystals that produce morphological alteration of the surface and cause irregularities that affect the filling of interface between the cone and the wall of dentinal canal.[17-19] If we add to this, a poor adaptation of the gutta-percha cone to the apical stop, and the risk of microleakage due to lack of sealing of the root canal increases considerably.[20,21] On the other hand, higher concentrations of NaOCl distort the saturated trans polyisoprene chemical bonds of the gutta-percha cone causing a reduction of the polymeric component, causing zinc oxide to become the main component thus producing alterations in its mechanical properties such as flexibility,[22-26] tensile strength, elastic modulus, and elongation percentage, being these directly related to wax and resins, gutta-percha, and zinc oxide, respectively.[27,28]

The importance of the present study lies in performing an analysis on mechanical properties of gutta-percha cones after disinfection with NaOCl at different concentrations and time intervals and demonstrating how this solution can alter the mentioned properties, revealing the effects after immersion of this oxidizing agent, increasing evidence on the decontamination of gutta-percha cones and helping to choose a possible disinfection protocol that can be used in an adequate way in daily clinical practice.

Therefore, aim of the present study is to compare the tensile strength and elastic modulus of gutta-percha cones disinfected with 2.5% and 5.25% NaOCl for 1, 5, and 10 minutes.

Materials and Methods

Type of study and delimitation

The present *in vitro*, longitudinal, and prospective experimental study was conducted at the Universidad Nacional Federico Villarreal and at the Certified High Technology Laboratory (ISO/IEC Standard: 17025), Lima, Peru, from January to March 2022, with approval letter No. 041-2021-COVID-FO-UNFV. This study considered the CRIS Guidelines (Checklist for Reporting *In-vitro* Studies).[29]

Sample calculation and selection

Forty-five gutta-percha cones were selected and equally distributed in three groups. The sample size per group was 15 gutta-percha cones and these were subdivided into groups of 5 ($n = 5$), according to immersion time (1, 5, and 10 minutes). Sample was calculated based on data obtained in a previous pilot study, where a formula for multivariate analysis of variance (MANOVA) was applied in the statistical software G*Power version 3.1.9.7 considering a significance level (α) = 0.05 and a statistical power ($1 - \beta$) = 0.80, with an effect size (V) = 0.994, with 9 groups and 2 response variables. The study units were distributed in a simple random manner without replacement [Figure 1].

Variables

Variables included in the study were tensile strength, elastic modulus, NaOCl concentration, and immersion time.

Sample characteristics and preparation

Gutta-percha cones No. 30 taper 0.02 (VDW-Zipperer, München, Germany) were used for the present study (synthetic gutta-percha: 21%, zinc oxide: 69% and barium sulfate plus stearic acid plus polyethylene glycol: 10%). First, 7.5% NaOCl (Daryza, Daryza S.A.C, Lima, Peru) was dissolved at different concentrations (2.5% and 5.25%), then the solutions were placed in test tubes labeled according to concentration and immersion times, while the control group was immersed in distilled water. Subsequently, the gutta-percha cones were immersed in these tubes and after the immersion time (1, 5, or 10 minutes), and they were washed in 5 mL of distilled water for 1 minute and allowed to dry in sterile gauze.

Preparation of NAOCL concentrations

The dissolution of 7.5% NaOCl at different concentrations (2.5% and 5.25%) was performed with

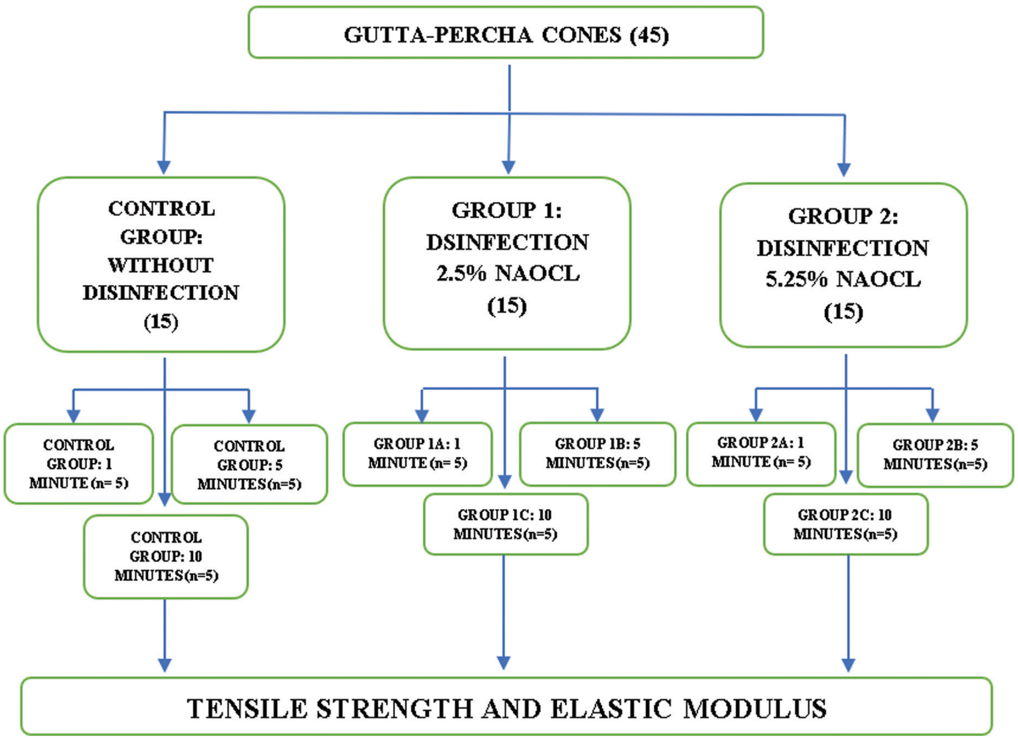

Figure 1: Assignment of study units according to groups formed to perform mechanical tests

the chemical equation $I_v * I_c = F_v * F_c$, where I_v is the initial volume; I_c is the initial concentration; F_v is the final volume, and F_c is the final concentration.

I_v*7.5% = 60 mL * 2.5% (60 mL is the container content)

I_v = 20 mL (is the extracted hypochlorite concentrate)

It is filled with 40 mL of distilled water to reach the 60 mL of the container.

On the other hand,

7.5% * I_v = 5.25% * 60 mL (60 mL is the container content)

I_v = 42 mL (NaOCl 7.5%)

It is filled with 18 mL of distilled water to reach the 60 mL of the container.

Tensile strength test

The tensile strength of gutta-percha cones was performed with a universal testing machine (CMT-5L Liangong, Shandong, China) based on the International Organization for Standardization (ISO) 527-1:2019-12 standard. The cone ends were inserted into the holders of universal testing machine, and then the load was applied at a crosshead speed of 1 mm/min, until the maximum tensile failure was obtained [Figure 2]. While the test was performed, the formative process of the stress/strain diagram could be observed on a computer interfaced with the testing machine, which yielded two important consecutive zones (elastic zone and plastic zone) [Figure 3].

Statistical analysis

The data collected were recorded in a Microsoft Excel 2019® file and subsequently imported for statistical analysis by the SPSS program (*Statistical Package for the Social Sciences* Inc., IBM, NY) version 28.0. For the descriptive analysis, measures of central tendency and dispersion, such as mean and standard deviation, were used. For hypothesis testing, we evaluated whether the data presented normal distribution and homoscedasticity, using the Shapiro-Wilk test and Levene's test, respectively. Therefore, it was decided to use the one- and two-factor analysis of variance test, with Welch's robust modification for independent groups. For intergroup multiple comparison, Sheffe's post hoc was used. In addition, MANOVA based on Pillai's trace was applied. All analyses were performed considering a significance level of 5% ($P \leq 0.05$).

Results

The gutta-percha cones immersed in 2.5% NaOCl for 10 minutes presented higher tensile strength (10.79 MPa, 95% CI: 9.87–11.70 MPa), while those that were immersed in 5.25% NaOCl, presented higher tensile strength at 5 minutes (10.57 MPa, 95% CI: 9.93–11.21 MPa). On the other hand, gutta-percha cones that

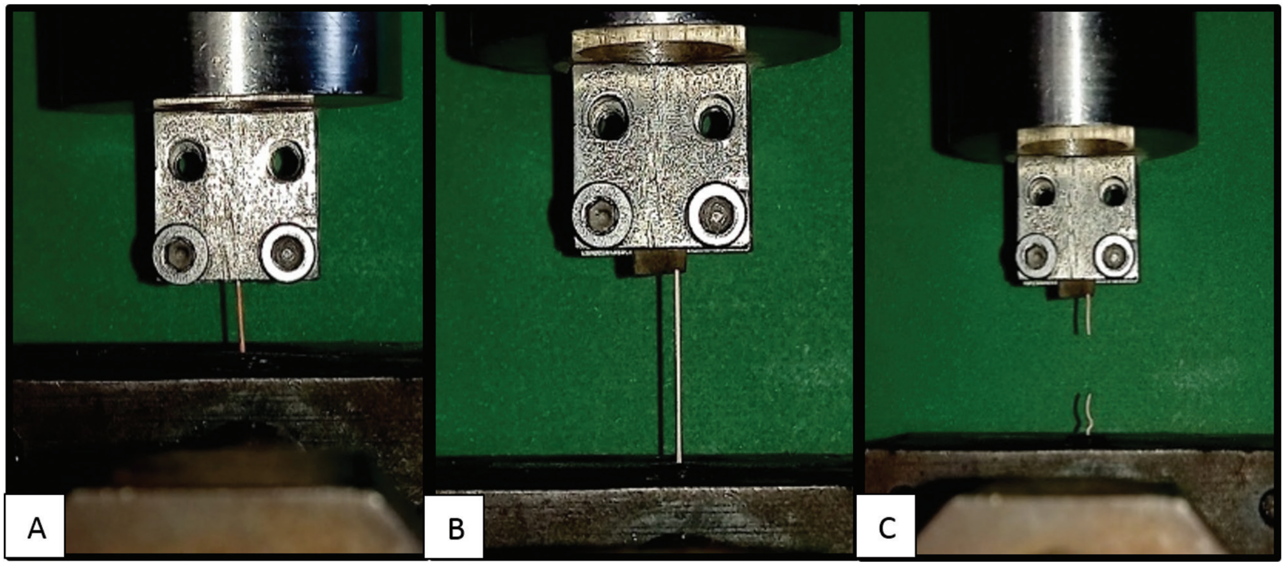

Figure 2: (A) Insertion of gutta-percha cone ends. (B) Deformation of gutta-percha cone. (C) Rupture of gutta-percha cone

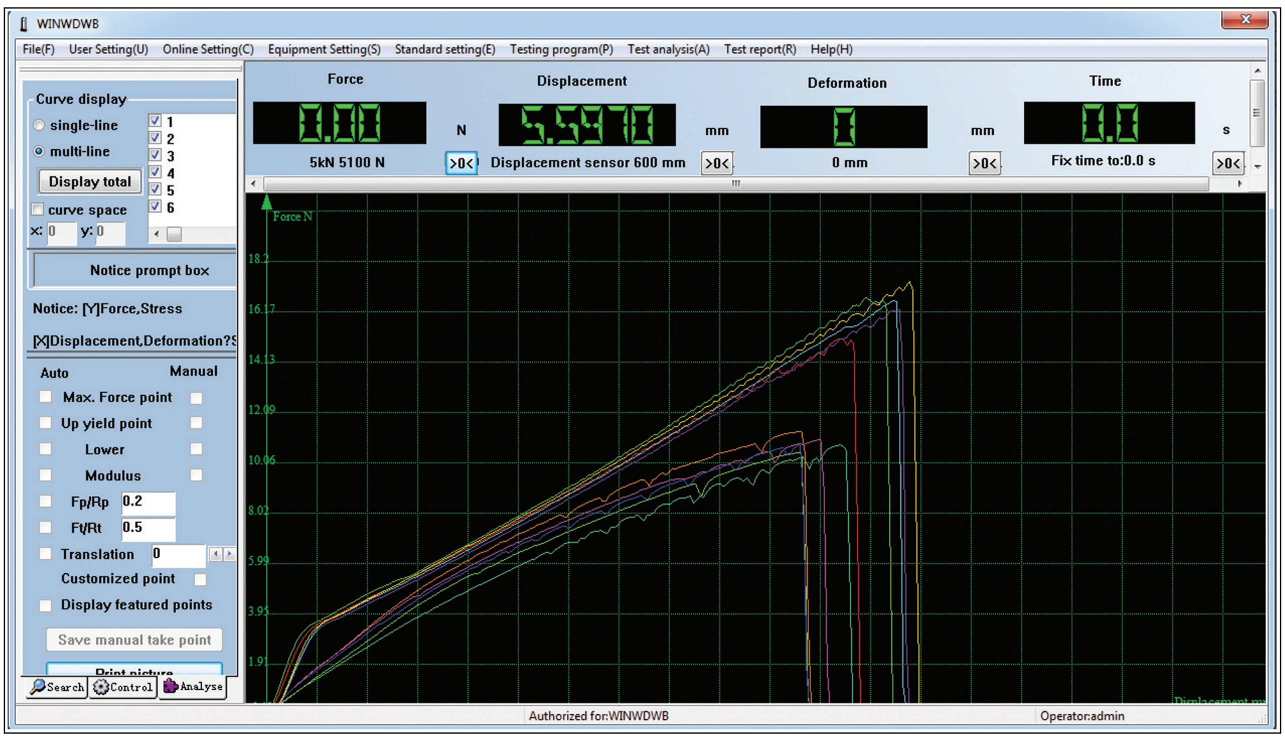

Figure 3: Computerized stress/strain diagram of gutta-percha cone samples

were immersed in distilled water (control) for 1 minute presented the highest tensile strength (10.74 MPa, 95% CI: 10.30–11.18 Mpa) [Table 1].

The gutta-percha cones immersed in 2.5% and 5.25% NaOCl, both for 5 minutes, presented higher elastic modulus values with 248.02 MPa (95% CI: 221.79–274.25 MPa) and 148.02 MPa (95% CI: 140.09–155.94 MPa), respectively. On the other hand, gutta-percha cones immersed in distilled water (control) for 1 minute presented higher elastic modulus (248.02 MPa, 95% CI: 221.79–274.25 MPa) [Table 2].

When comparing the tensile strength of gutta-percha cones, no significant differences were observed after immersion at 1, 5, and 10 minutes in 2.5% NaOCl (P = 0.715) and 5.25% NaOCl (P = 0.585), respectively. The same occurred in the control group (P = 0.772). Furthermore, when multiple comparisons were made according to concentration and immersion time, no

Table 1: Tensile strength descriptive values of gutta-percha cones immersed in different NaOCl concentrations, according time

NaOCl concentration	Time (min)	*n*	Mean (MPa)	SD	SE	95% CI		Minimum	Maximum
						LL	UL		
Control (–)	1	5	10.74	0.36	0.16	10.30	11.18	10.26	11.09
	5	5	10.48	0.53	0.24	9.82	11.14	9.61	11.05
	10	5	10.59	0.76	0.34	9.64	11.54	9.73	11.47
2.5% NaOCl	1	5	10.42	0.59	0.26	9.69	11.16	9.95	11.43
	5	5	10.50	0.83	0.37	9.47	11.54	9.61	11.39
	10	5	10.79	0.74	0.33	9.87	11.70	10.18	11.92
5.25% NaOCl	1	5	10.11	0.96	0.43	8.92	11.31	9.15	11.20
	5	5	10.57	0.51	0.23	9.93	11.21	9.99	11.13
	10	5	10.18	0.66	0.29	9.36	10.99	9.35	10.94

n = sample; SD = standard deviation, SE = standard error of mean, 95% CI = 95% confidence interval, LL = lower limit, UL = upper limit, MPa = megapascals

Table 2: Elastic modulus descriptive values of gutta-percha cones immersed in different NaOCl concentrations, according time

NaOCl concentration	Time (min)	*n*	Mean (MPa)	SD	SE	95% CI		Minimum	Maximum
						LL	UL		
Control (–)	1	5	248.02	21.13	9.45	221.79	274.25	225.56	271.25
	5	5	245.20	29.15	13.04	209.01	281.39	215.30	277.74
	10	5	236.02	23.75	10.62	206.53	265.51	196.06	253.14
2.5% NaOCl	1	5	169.07	11.15	4.99	155.23	182.92	154.10	181.97
	5	5	184.27	27.77	12.42	149.78	218.76	153.97	211.15
	10	5	157.51	6.02	2.69	150.04	164.99	149.21	163.32
5.25% NaOCl	1	5	146.25	12.70	5.68	130.48	162.02	135.71	165.07
	5	5	148.02	6.38	2.85	140.09	155.94	139.52	156.60
	10	5	139.92	4.24	1.90	134.65	145.18	134.57	146.27

n = sample, SD = standard deviation, SE = standard error of mean, 95% CI = 95% confidence interval, LL = lower limit, UL = upper limit, MPa = megapascals

significant differences were observed ($P = 0.811$) [Table 3].

When comparing elastic modulus of gutta-percha cones, no significant differences were observed after immersion at 1, 5, and 10 minutes in 2.5% NaOCl ($P = 0.090$) and 5.25% NaOCl ($P = 0.208$), respectively. The same occurred in the control group ($P = 0.185$). On the other hand, according to the concentration and immersion time, significant differences ($P \leq 0.05$) could be observed in gutta-percha cones immersed in distilled water, compared to those immersed in 2.5% and 5.25% NaOCl at 1, 5, and 10 minutes [Tables 4 and 5].

When performing the multivariate analysis according to concentration and time factors, it was observed that increase in NaOCl concentration significantly reduced the elastic modulus of gutta-percha cones, regardless of immersion time ($P < 0.001$). However, this increase in NaOCl concentration did not significantly affect tensile strength ($P = 0.338$). On the other hand, the time factor and its interaction with NaOCl concentration did not significantly affect tensile strength ($P > 0.05$) or elastic modulus ($P > 0.05$) of gutta-percha cones [Table 6 and Figure 4].

Discussion

The purpose of this in vitro study was to compare the tensile strength and elastic modulus of gutta-percha cones disinfected in 2.5% and 5.25% NaOCl, immersed for 1, 5, and 10 minutes. Therefore, when performing the multivariate analysis according to the two factors concentration and time, it could be observed that the increase in NaOCl concentration significantly reduced the elastic modulus of gutta-percha cones, regardless of the immersion time. However, this increase in NaOCl concentration did not significantly affect the tensile strength.

In the present study, the average tensile strength of gutta-percha cones immersed for one minute in distilled water (control) presented one of the highest averages (10.74 MPa), while after immersion with 5.25% NaOCl at the same time, the average decreased to 10.11 MPa. However, it should be clarified that these differences were not significant, being this in agreement with what

Table 3: Tensile strength comparison of gutta-percha cones immersed in different NaOCl concentrations, according time

NaOCl concentration	Time (min)	*n*	Mean	**P*	F	***P*	*F*	***P*
Control (–)	1	5	10.74	0.464	0.264	0.772		
	5	5	10.48	0.413				
	10	5	10.59	0.347				
2.5% NaOCl	1	5	10.42	0.083	0.345	0.715	0.549	0.811
	5	5	10.50	0.268				
	10	5	10.79	0.252				
5.25% NaOCl	1	5	10.11	0.253	0.561	0.585		
	5	5	10.57	0.410				
	10	5	10.18	0.500				

n = sample

*Based on Shapiro Wilk normality test ($P > 0.05$, normal distribution); *F*, Intergroup one-factor analysis of variance statistic

**$P \leq 0.05$ (significant differences)

Table 4: Elastic modulus comparison of gutta-percha cones immersed at different NaOCl concentrations, according time

NaOCl concentration	Time (min)	*n*	Mean	[a]*P*-value	*F*	[b]*P*-value	*F*	[b]*P*-value
Control (–)	1	5	248.02	0.305	2.282	0.185		
	5	5	245.20	0.259				
	10	5	236.02	0.071				
2.5% NaOCl	1	5	169.07	0.691	3.547	0.090	26.853	<0.001
	5	5	184.27	0.156				
	10	5	157.51	0.522				
5.25% NaOCl	1	5	146.25	0.183	1.964	0.208		
	5	5	148.02	0.977				
	10	5	139.92	0.838				

n = sample

[a]Based on Shapiro-Wilk normality test ($P > 0.05$, normal distribution), F = analysis of variance statistic with Welch's robust one-factor intergroup modification

[b]$P \leq 0.05$ (significant differences).

Table 5: Post hoc multiple comparison of elastic modulus of gutta-percha cones immersed in different NaOCl concentrations, according time

Concentration	Time (min)	Control (–)			2.5% NaOCl			5.25% NaOCl	
		1 min	5 min	10 min	1 min	5 min	10 min	1 min	5 min
Control (–)	5	1.000							
	10	0.997	1.000						
2.5% NaOCl	1	<0.001*	<0.001*	0.001*					
	5	0.003*	0.005*	0.029*	0.986				
	10	<0.001*	<0.001*	<0.001*	0.998	0.715			
5.25% NaOCl	1	<0.001*	<0.001*	<0.001*	0.858	0.251	0.998		
	5	<0.001*	<0.001*	<0.001*	0.905	0.312	0.999	1.000	
	10	<0.001*	<0.001*	<0.001*	0.612	0.102	0.965	1.000	1.000

*Based on Sheffe's Test ($P \leq 0.05$, significant differences)

Table 6: Multivariate analysis according to interaction of NaOCl concentration and time

Factors	Effect	**F*	**P*	***F*	***P*
Concentration	Tensile strength	0.971	0.388	14.400	<0.001**
	Elastic modulus	116.721	<0.001*		
Time	Tensile strength	0.089	0.915	1.252	0.297
	Elastic modulus	2.517	0.095		
Concentration* Tiempo	Tensile strength	0.569	0.687	0.562	0.806
	Elastic modulus	0.516	0.724		

*Based on MANOVA test ($P \leq 0.05$, significant effect)

**Based on Pillai trace ($P < 0.05$, significant effect)

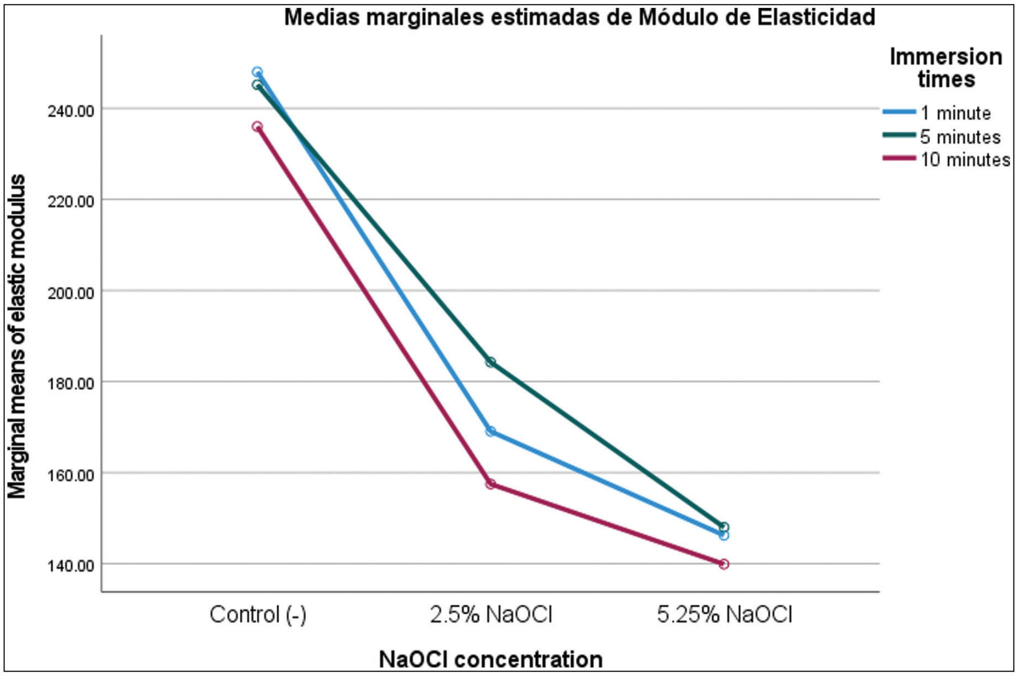

Figure 4: Elastic modulus analysis of gutta-percha cones according to NaOCl concentration and immersion time

was obtained by Naved *et al.*,[28] since they reported that gutta-percha cones immersed for one minute in 5.25% NaOCl decreased their tensile strength, although in a nonsignificant way. This is probably because the waxes/resins, as a component of the gutta-percha cone, could keep the organic content constant, and thus the tensile strength remained unchanged, as the waxes could chemically bond with the gutta-percha molecules, causing a new arrangement and movements in the gutta-percha polymeric chains, which could confer some tensile equilibrium when exposed to different NaOCl concentrations.[30] In addition, it should be noted that the wax/resin ratio and the gutta-percha itself, present in the cones, may vary in concentration according to the commercial brand, being the gutta-percha the main responsible for maintaining tensile strength in the cone. Maniglia *et al.*,[31] reported that the wax/resin chemical composition of the DENTSPLY FM brand (Brazil) has a high percentage (10.4% ± 0.11) and a gutta-percha content of 16.3%, while DENTSPLY TP contains 4% ± 0.36 of wax/resin and 21.6% of gutta-percha, while the wax/resin and gutta-percha composition used in the present study was less than 10% and 21%, respectively.[32] This could explain the contradictory results obtained by other investigations when comparing tensile strength of gutta-percha cones submerged in different NaOCl concentrations, since an increase or decrease of this property has been reported.[24,25,27,33]

On the other hand, after analyzing the results obtained, it was found that when comparing the elastic modulus mean values of gutta-percha cones during disinfection, a significant decrease was observed in those that were immersed in 2.5% and 5.25% NaOCl at 1, 5, and 10 minutes. These results are discrepant with those obtained by Ismail *et al.*, who reported an increase in the elastic modulus and a decreased elongation percentage when gutta-percha cones were immersed in 1%, 2.5%, and 5.25% NaOCl for 10, 15, and 20 minutes.[25] The reason for these differences is probably due to the fact that NaOCl coming into contact with the zinc oxide in the gutta-percha cones causes a drop in its concentration and consequently the stiffness or modulus of elasticity in the cone decreases, since this property is attributed to the presence of zinc oxide.[22,34] Likewise, this argument has been supported by Pang *et al.*,[24] as they showed that the percentage deformation of the gutta-percha cone increased when immersed in 5.25% NaOCl for 5 minutes and consequently the modulus of elasticity decreased, as it is known to be inversely proportional.[22,24,30] However, it is necessary to clarify that in the present study the modulus of elasticity of the gutta-percha cones decreased from the first minute immersed in NaOCl 5.25%. This difference may have been due to the use of gutta-percha cones of the German brand VDW® with a zinc oxide concentration of 69%,[32] while in the Pang *et al.*,[24] study, gutta-percha cones of Korean brand Meta Biomed® were used, whose zinc oxide concentration varied between 55% and 65%, being this a factor that could affect its elastic modulus and rigidity in less time.[35,36] Similarly, Carvalho *et al*.[16] demonstrated with energy-dispersive spectroscopy that zinc levels are significantly reduced when the gutta-percha cone is immersed in 1% NaOCl for disinfection during times longer than 1 hour. This suggests that, if the NaOCl concentration increases, zinc levels probably decrease in a shorter time and, therefore, the cone stiffness and its elastic modulus decrease since it has been reported that zinc is one of the main components related to the elastic modulus, together with oxygen.[22] In addition to this argument, Canalda *et al.*[34] reported that zinc oxide levels are inversely proportional to the viscoelasticity of gutta-percha cone and directly proportional to the elastic modulus or stiffness of the cone.

The importance of the present study lies in the increase of scientific evidence about mechanical alterations of gutta-percha cones due to the use of a disinfectant commonly used by dentists, and in this way, it will help to adequately choose other disinfectant solutions that may not alter the cone properties and at the same time fulfill their antimicrobial role. In addition, the study design under a robust statistical analysis, such as MANOVA, allowed us to evaluate the interaction time and concentration as possible influencing factors on the tensile strength and elastic modulus of gutta-percha cones, keeping the α-error to a minimum and identifying small differences between response variables, a situation that would not have been possible to detect if analysis of variance was used separately for each effect.[37]

Among limitations of the present study, we can mention the lack of previous studies that have used gutta-percha cones with similar chemical composition to the cones used and thus be able to discuss the results according to the composition offered by the commercial brand VDW. On the other hand, it was not possible to compare the influence of 2.5% and 5.25% NaOCl on gutta-percha cones with different diameters. Therefore, we recommend including this variable in future studies, since it has been reported that it can be an influential factor in tensile strength and elastic modulus.[35] It is also suggested to perform studies with other disinfectant solutions and compare mechanical properties of gutta-percha cone with a multivariate analysis of variance that considers time and concentration as interaction factors.

Conclusion

In summary, with limitations of the present in vitro study, it can be concluded that the increase in NaOCl concentration significantly reduced the elastic modulus of gutta-percha cones, regardless of the immersion time. However, this increase in concentration did not affect tensile strength. On the other hand, the interaction of time and NaOCl concentration did not significantly affect the elastic modulus and tensile strength.

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Conflicts of interest

None to declare.

Authors contributions

Conceived the research idea (RBG), elaborated the manuscript (RBG, ACPV, MLC, LCG), collected and tabulated the information (RBG, ACP, CLG), carried out the bibliographic search (RBG, CLG, MLC, AAM), interpreted the statistical results (CCR), helped in the development of the discussion (RBG, MLC, CLG, CCR), and performed the critical review of the manuscript (CCR, AAM, LCG, CLG, MLC). All authors approved the final version of the manuscript.

Ethical policy and institutional review board statement

This research was exempt from review by an ethics committee of the Faculty of Dentistry of the Universidad Nacional Federico Villarreal, and its execution was approved by letter No. 041-2021-COVID-FO-UNFV. In addition, the preparation of this article was based on a degree thesis supported by Rolando Bellido-Guzmán, available at https://hdl.handle.net/20.500.13084/6153

Patient declaration of consent

Not applicable.

Data availability statement

The data that support the study results are available from the author (Mr. Rolando Bellido-Guzmán, e-mail: emmabeg10@outlook.com) on request.

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