

Knoop microhardness test in polymers used for surgical guide manufacturing after the disinfection process

Teste de microdureza knoop em polímeros utilizados na confecção de guias cirúrgicos após o processo de desinfecção

Gustavo Macedo PEREIRA¹ , Beatriz Cristina DE PAULO¹ , Carolina Gonzales FRANCA¹ , Saulo Pereira CONCEIÇÃO¹ , Tatiana Cursino PEREIRA¹ , Guilherme de Siqueira Ferreira Anzaloni SAAVEDRA¹ 

1 - Universidade Estadual Paulista, Instituto de Ciência e Tecnologia, Departamento de Materiais Odontológicos e Prótese. São José dos Campos, SP, Brazil.

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ABSTRACT

Objective: To analyze the Knoop microhardness of samples made from Dima® Print Guide polymer (Kulzer – Brazil) after different disinfection methods. **Material and Methods:** A total of 15 samples of the Dima® Print Guide polymer (Kulzer – Brazil) were fabricated and divided into five groups with three samples in each group as follows: 1 - control group (C), in which the samples did not undergo any disinfection method; 2 - disinfected with 2% chlorhexidine (CHX); 3 - disinfected with peracetic acid (PA); 4 - disinfected with 70% alcohol (AL); and 5 - sterilized in an autoclave (AS). Three microindentations were made on the surface of each specimen using a 300 g load for a dwell time of 30 seconds, measured with a microhardness tester model HMV-G21DT (Shimadzu Corporation, Kyoto, Japan). **Results:** The microhardness results were submitted to one-way ANOVA, which showed statistically significant differences ($p < 0.001$), followed by Tukey's test. The mean and standard deviation of the samples by group were: C ($33.26 \pm 1.49a$); CHX ($29.71 \pm 1.69a$); AP ($32.23 \pm 0.33a$); AL ($30.81 \pm 2.10a$); and AS ($25.46 \pm 1.18b$) ($p < 0.05$). **Conclusion:** The samples sterilized in an autoclave showed a decrease in microhardness, while the other disinfection methods did not significantly alter the microhardness of the surgical guides.

KEYWORDS

Computer-assisted surgery; Disinfection; Hardness test; Sterilization; Three-dimensional printing.

RESUMO

Objetivo: Analisar a microdureza knoop de amostras do polímero dima® Print Guide (Kulzer – Brasil) após diferentes métodos de desinfecção. **Material e Métodos:** Foram confeccionadas 15 amostras do polímero dima® Print Guide (Kulzer – Brasil) divididas em 5 grupos com 3 amostras cada grupo sendo eles: 1 - grupo controle (C), o qual as amostras não passaram por nenhum método de desinfecção; 2 - desinfetado com clorexidina 2% (CLX); 3 - desinfetado com ácido peracético (AP), 4 - desinfetado com álcool 70% (AL) e 5 - esterilizado em autoclave (EA). Foram realizadas três microindentações na superfície de cada espécime com uma carga de 300 g (c) por um tempo de permanência de 30 s, as quais foram medidas utilizando um durômetro modelo HMV-G21DT (Shimadzu Corporation, Kyoto, Japão). **Resultados:** Os resultados da microdureza foram submetidos ao teste ANOVA de um fator, o qual mostrou diferenças estatísticas ($p < 0,001$) e ao teste de Tukey. Os valores médios e desvio padrão das amostras por grupo foi de: C ($33,26 \pm 1,49a$); CLX ($29,71 \pm 1,69a$); AP ($32,23 \pm 0,33a$); AL ($30,81 \pm 2,10a$); EA ($25,46 \pm 1,18b$); ($p < 0,05$). **Conclusão:** As amostras esterilizadas em autoclave apresentaram diminuição da microdureza e os demais métodos de desinfecção não alteraram significativamente a microdureza das guias cirúrgicas.

PALAVRAS-CHAVE

Cirurgia assistida por computador; Desinfecção; Teste de dureza; Esterilização; Impressão tridimensional.

INTRODUCTION

Prosthetic planning in implant dentistry is of utmost importance for treatment success. The ideal positioning of the implant considers the available bone volume, proximity to adjacent structures, and the final crown position over the implant. This encompasses morphology, angulation, as well as occlusal and proximal contacts [1].

Digital devices have played a significant role in implant dentistry by assisting in diagnosis, planning, and treatment execution. Digital planning for guided surgeries is facilitated by devices such as cone-beam computed tomography (CBCT) and intraoral scanners. CBCT captures and reproduces three-dimensional images of the patient's anatomical structures [2]. On the other hand, intraoral scanning is a tool used in dentistry to virtually record the position of the patient's teeth and mucosa. When combined with CBCT, it enables reconstructing and visualizing both hard and soft tissues in software, allowing for optimal implant positioning planning and fabrication of a surgical guide [3-5].

Surgical guides for implant placement function as templates to correctly position the drills, prevent incorrect angulations during drilling, precisely follow the preoperative plan, and eliminate the need for flap opening during surgery [5,6]. 3D guides are printed using resin-based materials, and most 3D printers employ either stereolithography (SLA) or digital light processing (DLP) technologies [7]. The SLA method uses an ultraviolet or diode laser to draw cross-sections layer by layer to construct the object, while the DLP method utilizes a digital projection screen to transfer each layer's image during object formation. Both techniques enable producing complex objects with high accuracy [8].

When compared to conventional flap surgery, guided surgery offers greater precision and safety. Because it is preoperatively planned using software, it minimizes the risk of deviation during drilling and consequently reduces intraoperative complications [7,9,10]. Patients experience less edema, bleeding, and swelling with the use of surgical guides, resulting in a more controlled inflammatory response. This has been associated with higher success rates in implant rehabilitations performed with guided surgery compared to the conventional

method [11,12]. In addition to the technical advantages, the digital method is less invasive and faster, thus providing a more comfortable and less painful intraoperative and postoperative experience for the patient [13].

Connections between dental offices and prosthetic laboratories can present a high risk of cross-contamination if disinfection protocols are not properly followed. The surgical guide comes into direct contact with the surgical site during implant placement, which allows for indirect cross-infection via surfaces, equipment, and aerosols. Therefore, proper disinfection becomes essential [14]. Contamination of the guide by pathogenic microorganisms can pose a risk to the patient's health and compromise treatment success, as inflammation may impair osseointegration. Thus, disinfection by physical or chemical methods is recommended to avoid such risks, with sterilization being the most effective for decontamination [15-17].

The most commonly used disinfection materials by dental surgeons among those available on the market include 70% alcohol and 2% chlorhexidine, particularly due to their easy accessibility in clinics and low risk of damaging the materials used [15]. However, despite their widespread use, these agents are considered intermediate-level disinfectants [18,19]. Therefore, it is necessary to evaluate alternative methods which offer greater effectiveness, such as peracetic acid, which has shown a high disinfection capacity when tested [20]. Nonetheless, the literature clearly indicates that autoclave sterilization remains the most effective method for ensuring safety and complete microorganism elimination from materials. It is a well-established technique widely used by dental professionals [21,22].

However, different disinfection and sterilization processes may affect the mechanical properties of surgical guides, leading to deformations and thus compromising their clinical performance [23,24]. This risk is directly related to the moment of intraoral positioning and implant placement when the surgical guide is subjected to multidirectional forces. Fractures or deformations at this stage compromise the precision of the previously planned implant placement and jeopardize all the benefits of guided surgery [23].

Therefore, the aim of this study was to analyze the Knoop microhardness of samples made from Dima® Print Surgical Guide & Tray polymer (Kulzer GmbH, Hanau, Germany) after application of different disinfection methods, both chemical and physical. The null hypothesis established was that the different disinfection and sterilization protocols would not significantly affect the mechanical properties or dimensional stability of the polymer used in the fabrication of surgical guides.

MATERIAL AND METHODS

Sample preparation

The sample size calculation was based on data from the study by Török et al. [25], considering a large effect size ($f = 0.4$), a significance level of 5% ($\alpha = 0.05$), and a statistical power of 80% ($1 - \beta = 0.80$) for one-way analysis of variance (ANOVA). The minimum estimated number was eight specimens per group. However, three specimens per group were analyzed due to practical limitations in time and resources. A strategy of performing three microindentations on each sample was adopted to enhance the data reliability, increasing the total number of measurements and allowing for greater precision in determining the results.

The samples ($N=15$) were designed using Rhinoceros 6.0SR8 software (McNeel North America, Seattle, USA), in bar format with dimensions of 25 mm × 10 mm × 2 mm, oriented diagonally at a 45-degree angle relative to the main axis. The model was exported in STL format to the slicing software of the printer and programmed for printing with a layer thickness of 100 μm .

The printing was performed using Dima® Print Surgical Guide & Tray polymer (Kulzer GmbH, Hanau, Germany) on a cara® Print 4.0 pro 3D printer (Kulzer) employing Digital Light Processing (DLP) technology. After printing, the samples were subjected to the manufacturer-recommended cleaning protocol, which involved immersion in isopropyl alcohol for 4 minutes (cara Print Clean pro, Kulzer, Hanau, Germany), followed by drying with compressed air. The samples subsequently underwent the post-curing process using a cara® Print LEDcure photopolymerizer (Kulzer, Hanau, Germany) with a wavelength of 365 nm for a period of 10

minutes to ensure complete polymerization and achieve the ideal mechanical properties of the resin.

Following post-curing, the samples were manually polished on both surfaces using water sandpapers of grit sizes 600, 1200, and 2000 (3M, Saint Paul, Minnesota, USA), on an Ecomet 250 polisher (Buehler), under continuous water cooling until the appropriate translucency standard was achieved. The specimens were then stored in a dry environment, protected from light, at room temperature for a period of 24 hours, in accordance with the experimental protocol.

Materials used in the disinfection process

The samples were divided into five groups, following previously established disinfection protocols. CLX Group ($n=3$) – *Chlorhexidine Digluconate* (Rioquímica S/A, São José do Rio Preto, SP): the samples were fully immersed in a container for 10 minutes, then removed, dried, and washed in an ultrasonic bath with distilled water for 8 minutes [26]. AP Group – Peracetic Acid 0.2% (*Perax Rio 0.2%*, Rioquímica S/A, São José do Rio Preto, SP): the samples were fully immersed in a container for 30 minutes, then removed, dried, and washed in an ultrasonic bath with distilled water for 8 minutes [27]. AL Group – 70% Alcohol (*Rialcool 1L*, Rioquímica S/A, São José do Rio Preto, SP): the samples were fully immersed in a metal container for 15 minutes, then removed, dried, and washed in an ultrasonic bath with distilled water for 8 minutes [27]. EA Group – Autoclave (*Vitale Class 12L Autoclave*, Cristófoli, Paraná, Brazil): the samples were packaged in surgical-grade pouches of appropriate dimensions and autoclaved for 10 minutes at 134°C. After sterilization, a 20-minute drying period was observed to ensure complete material drying [24]. C Group – Control: the samples were washed in an ultrasonic bath with distilled water for 8 minutes.

Knoop microhardness

For the Knoop microhardness test (Shimadzu Microhardness Tester HMV-G21DT – Shimadzu Corporation, Japão), three microindentations were performed on the surface of each sample using a 300 g load (c) and a dwell time of 30 seconds [27].

After the microindentations, the surface microhardness was measured using an HMV-

G21DT microhardness tester (Shimadzu Corporation, Kyoto, Japan) (Figure 1A). The Knoop Hardness Number (KHN) was obtained by measuring the longest diagonal (d) of the diamond-shaped indentation left by the pyramidal diamond indenter (Figures 1B, 1C, 1D, 1E, and 1F).

The Knoop hardness was calculated by measuring the longest diagonal (mm), according to the equation based on ASTM C 1327-03:

$$KH = 14.2 F / d^2 \quad (1)$$

In which: F = load in kgf; d = longest diagonal, in mm; KH = Knoop hardness.

The average of the three values obtained for each sample was subsequently calculated, and the results were subjected to statistical analysis.

Statistical analysis

The data obtained were subjected to descriptive analysis and the Shapiro–Wilk normality test. Given the parametric distribution of the data, one-way analysis of variance (ANOVA) was applied, followed by Tukey's multiple

comparisons test, adopting a 5% significance level. Statistical analyses were performed using the Jamovi software, version 1.6 [28].

RESULTS

According to the descriptive statistics presented in Table I, normality ($p > 0.05$) was observed among the groups when microhardness was analyzed.

A one-way ANOVA test was applied following the statistical analysis. As shown in Table II, the results demonstrated statistical significance with $p < 0.05$. Based on this outcome, Tukey's Post Hoc test was performed to allow for multiple comparisons analysis, as presented in Table III. This test revealed statistically significant differences in the following group comparisons: 2% Chlorhexidine vs. Autoclave ($p < 0.05$); Peracetic Acid vs. Autoclave ($p < 0.05$); Alcohol vs. Autoclave ($p < 0.05$); and Alcohol vs. Autoclave ($p < 0.05$).

Based on the statistical analysis and the obtained means, it was observed that the control

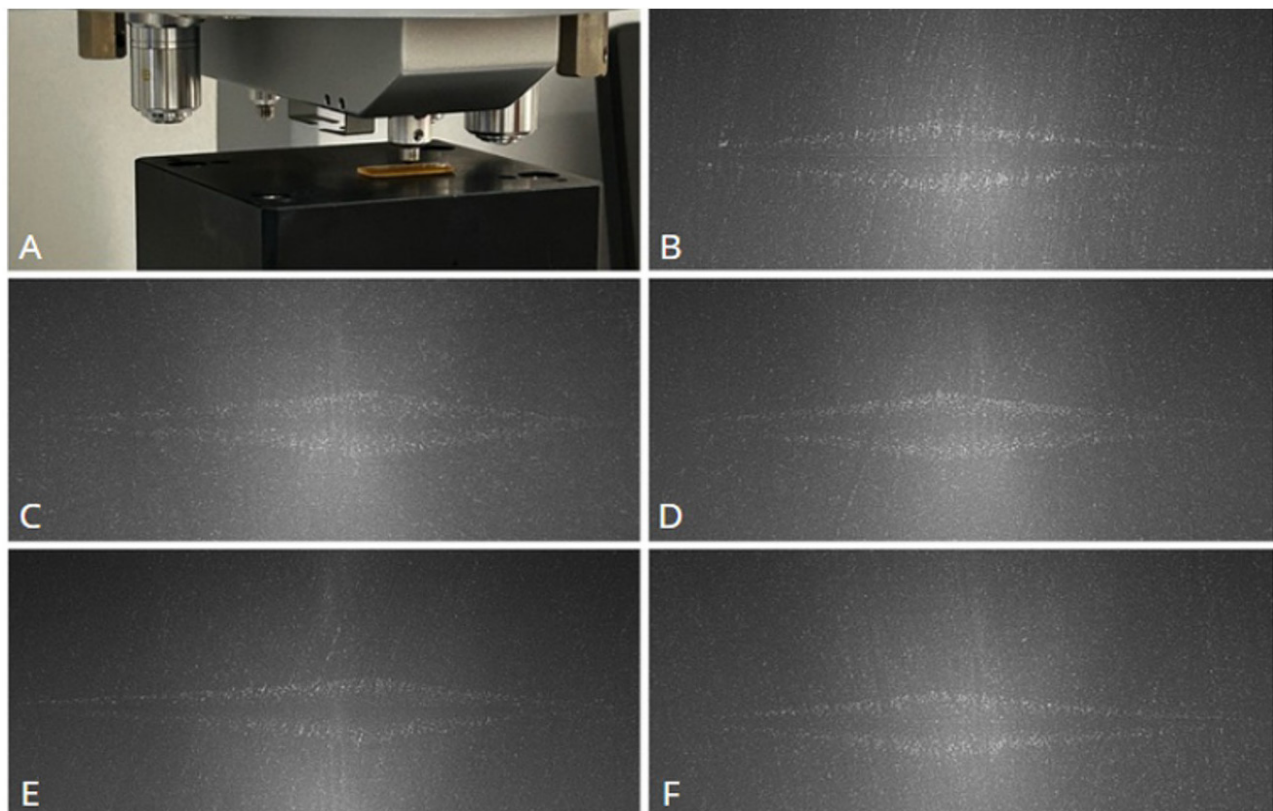


Figure 1 - Knoop Microhardness Test. (A) Sample positioned in the microhardness tester; (B, C, D, E, and F) Readings of the microindentations performed on samples disinfected with 2% chlorhexidine (CLX), 0.2% peracetic acid (PA), 70% alcohol (AL), sterilized by autoclaving (AS), and control (C), respectively.

Source: Prepared by the authors.

Table I - Descriptive statistics

Group	N	Mean	Median	Standard Deviation	Minimum	Maximum	W	p
Hk CLX	3	29.7	30.0	1.620	28.0	31.2	0.975	0.699
AP	3	32.2	32.1	0.332	32.0	32.6	0.932	0.495
AL	3	30.8	29.8	2.105	29.4	33.2	0.815	0.150
EA	3	25.5	25.6	1.189	24.2	26.6	0.985	0.768
C	3	33.3	33.9	1.491	31.6	34.3	0.863	0.276

Notes: Hk = Knoop microhardness; CLX = chlorhexidine; AP = peracetic acid; AL = alcohol; EA = autoclave; C = control group; W = Shapiro–Wilk normality test statistic; p-value = probability value.

Source: Prepared by the author.

Table II - Statistical analysis – One-way ANOVA (Welch's test)

	F	Gl1	Gl2	P
Hk	16.8	4	4.31	0.007

Notes: F = test statistic; df_1 = numerator degrees of freedom; df_2 = denominator degrees of freedom; Hk = Knoop microhardness; p-value = probability value.

Source: Prepared by the author.

Table III - Statistical analysis – Tukey's post hoc test

Tukey's HSD post hoc test						
		CLX	AP	AL	EA	C
CLX	Difference	–	-2.52	-1.10	4.24*	-3.56
	Mean					
	p-value	–	0.290	0.884	0.034	0.083
AP	Difference		–	1.42	6.77**	-1.03
	Mean					
	p-value		–	0.759	0.002	0.905
AL	Difference			–	5.34**	-2.46
	Mean					
	p-value			–	0.008	0.312
EA	Difference				–	-7.80***
	Mean					
	p-value				–	<0.001
C	Difference					–
	Mean					
	p-value					–

*p < .05. **p < .01. ***p < .001. CLX = chlorhexidine; AP = peracetic acid; AL = alcohol; EA = autoclave; C = control group.

Source: Prepared by the author.

group demonstrated the highest surface penetration resistance compared to the groups subjected to different disinfection protocols. The highest surface penetration resistance among the disinfected groups was observed in the group treated with peracetic acid, while the lowest value was identified in the group subjected to the autoclave process.

Considering that microhardness is inversely proportional to the size of the indentation diagonal,

the smaller the diagonal length, the greater the material's hardness; the materials which exhibited lower values indicate greater resistance to deformation and consequently greater surface hardness. In this context, the group subjected to autoclave sterilization stood out (Figure 1D), showing the smallest diagonal length of the diamond-shaped indentation compared to the other disinfection methods, which is corroborated

by the lower surface resistance value. This result suggests an increase in polymer hardness after autoclave treatment, and the reduction in indentation size indicates that the sterilization process caused structural changes in the material, making it more resistant to surface deformations.

DISCUSSION

With the aid of tools such as computed tomography and digital planning software, guided surgery ensures the precise positioning of implants, preserving anatomical structures and promoting both aesthetic and functional outcomes. The use of customized surgical guides makes the procedure accurate, avoiding frequent adjustments during surgery [29,30]. The minimally invasive technique reduces tissue trauma, allowing for faster and more comfortable recovery for the patient compared to conventional flap surgery [31].

Due to the direct contact of the surgical guide with the patient's blood and saliva, it is essential to perform prior disinfection or sterilization to eliminate pathogenic microorganisms. Inadequate disinfection may pose serious risks of infection to the patient, compromising implant osseointegration and thereby contradicting the initial purpose of using surgical guides [9,14].

The literature highlights the effectiveness of sterilization, mainly because it is a method which can be regularly monitored through biological indicators [32]. Furthermore, its efficiency in eliminating bacteria and viruses makes it the most recommended approach for surgical items that come into direct contact with tissues [33,34]. However, when evaluated [24,25,35,36] this method showed that thermal sterilization altered the mechanical properties of the materials used in manufacturing the guides, leading to deformation and consequently reduced accuracy, as confirmed by the present study in the results presented in Table II. Only the group sterilized in an autoclave showed a reduction in microhardness compared to the control group, which underwent no treatment (Mean EA 25.5 Hk; C 33.3 Hk).

Therefore, chemical disinfection methods based on existing literature regarding distortion are commonly used in clinical settings as a preventive measure, especially considering that surgical guides are classified as critical instruments by the Centers for Disease Control and Prevention (CDC) and the American Dental

Association (ADA). These guidelines state that heat-sensitive critical materials must undergo sterilization by immersion in an FDA-approved chemical sterilant for 6 to 10 hours, followed by rinsing with sterile water [37]. However, studies have shown that shorter exposure periods may also be effective in eliminating microorganisms. Sennhenn-Kirchner et al. [15] and Smith et al. [16] recommend immersing surgical guides in 70% alcohol for 15 minutes. Supporting these findings, the present study verified that this method does not alter the microhardness of the printed material (Mean; C 33.3 Hk; AL 38.8 Hk).

Nevertheless, although 70% alcohol is the most accessible chemical method, it is not the only one available. Other methods, such as chlorhexidine and peracetic acid, are also recognized and widely used in clinical practice [15,38]. However, the literature reveals a lack of mechanical testing associated with different chemical disinfection methods, despite their established use. Although chemical methods are generally considered less aggressive, it is essential to consider their potential impact on the accuracy and mechanical properties of the material. Therefore, this study aimed to evaluate three other commonly used chemical disinfection methods in addition to sterilization (a widely discussed method). The results showed that only autoclave sterilization ($p < 0.05$) statistically caused changes in microhardness values, while the other methods maintained similar values to the control group (C 33.3 Hk), confirming their lower interference with material properties and thus their safety in clinical use. This finding aligns with the study by Török et al. who tested the microhardness of surgical guides sterilized via autoclave, Gigasept® Instru AF (Schülke & Mayer GmbH, Norderstedt, Germany), and plasma. They concluded that autoclave sterilization at 134°C was the only method which affected material microhardness, while other methods (e.g., chemical disinfection, autoclave sterilization at 121°C, and plasma sterilization) did not result in significant changes [25].

In turn, this study demonstrated that chemical disinfection is the most suitable approach when evaluating the mechanical properties of polymers, thereby rejecting the proposed null hypothesis. Despite limited resources, it was observed that samples subjected to physical disinfection showed a reduction in microhardness, which, according to the test, indicates an increase in material hardness, as microhardness is inversely proportional to overall hardness. This increase raises the risk of material fracture during application and potential

distortion in software-based planning. In contrast, chemical disinfection methods did not cause significant changes in this parameter.

Based on the results observed in this study, we highlight the need for direct evaluation of the dimensional stability and mechanical strength of the material. Finally, further studies are warranted to investigate the mechanical consequences of chemical disinfection methods.

CONCLUSION

Although sterilization is considered the most effective disinfection method, it was observed to reduce the microhardness of the polymer used in the printing of these devices. In contrast, established chemical disinfection methods did not show a significant impact on the microhardness of the samples, with alcohol standing out as the most viable alternative for clinical use.

Author's Contributions

GSFAS: Conceptualization. BCP, CGF, SPC: Data Curation. GMP, TCP: Formal Analysis. GSFAS: Funding Acquisition. BCP, CGF, SPC: Investigation. BCP, CGF, SPC: Methodology. GSFAS: Project Administration. GMP, TCP, GSFAS: Supervision. BCP, CGF, SPC, GSFAS: Visualization. BCP, CGF, SPC: Writing – Original Draft Preparation. GMP, TCP, GSFAS: Writing – Review & Editing.

Conflict of Interest

No conflicts of interest declared concerning the publication of this article.

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Regulatory Statement

This in vitro study did not involve human or animal subjects and did not require ethical approval.

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Guilherme de Siqueira Ferreira Anzaloni Saavedra
(Corresponding address)

Universidade Estadual Paulista, Instituto de Ciência e Tecnologia, Departamento de Materiais Odontológicos e Prótese, São José dos Campos, SP, Brazil.
Email: guilherme.saavedra@unesp.br

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