

Influence of a new method of sterilization on the morphology and physical properties of extracted human teeth

Influência de um novo método de esterilização na morfologia e propriedades físicas de dentes humanos extraídos

Daylana Pacheco da SILVA^{a*}, Urias Silva VASCONCELOS^b, Valdimar da Silva VALENTE^b,
Gregório Antônio Soares MARTINS^b, Carmem Dolores Vilarinho Soares de MOURA^b

^aUNICAMP – Universidade Estadual de Campinas, Faculdade de Odontologia de Piracicaba,
Piracicaba, SP, Brasil

^bUFPI – Universidade Federal do Piauí, Faculdade de Odontologia, Departamento de Odontologia Restauradora,
Teresina, PI, Brasil

Resumo

Introdução: Métodos de esterilização adotados em Banco de Dentes Humanos podem promover mudanças estruturais no esmalte e na dentina. Assim, o método ideal deve combinar a eficácia antimicrobiana e a preservação das propriedades biomecânicas do substrato. **Objetivo:** Avaliar a morfologia e propriedades físicas do esmalte e dentina dos dentes humanos extraídos, após serem submetidos a diferentes métodos de esterilização. **Método:** Dezesseis terceiros molares extraídos foram seccionados nas seguintes regiões dentárias: mesio-distal, vestibulo-lingual e na junção amelocementária. Quarenta espécimes foram selecionados de acordo com valores de microdureza e rugosidade iniciais, e distribuídos em quatro grupos experimentais (n = 10 / grupo): Grupo 1 = Autoclave 121 °C (30 minutos); Grupo 2 = 2,5% de hipoclorito de sódio (07 dias); Grupo 3 = 5,25% Hipoclorito de sódio (07 dias); Grupo 4 = ácido acético a 30% (7 dias). Após a esterilização, foi avaliada a microdureza, rugosidade e morfologia do esmalte e dentina. Os dados foram analisados pelo teste t pareado, Wilcoxon, Kruskal-Wallis e Análise de Variância. Um nível de significância de 5% foi adotado. **Resultado:** Todos os métodos de esterilização alteraram a microdureza e a rugosidade da dentina (p <0,05). Em relação ao esmalte, a microdureza foi afetada apenas pela Autoclave e pelo tratamento com 2,5% de hipoclorito de sódio (p <0,05). Foi verificada nas micrografias, uma grande abertura dos poros do esmalte e dos túbulos dentinários. **Conclusão:** os métodos de esterilização adotados alteraram a morfologia e / ou propriedades físicas do esmalte e da dentina.

Descritores: Esmalte dentário; dureza; esterilização; dentina.

Abstract

Introduction: Sterilization methods adopted in Human Teeth Bank can promote structural changes in enamel and dentin. Thus, the ideal method should combine the antimicrobial efficacy and preservation of the substrate biomechanical properties. **Aim:** Evaluated the morphology and physical properties of enamel and dentin of extracted human teeth, after being submitted to different sterilization methods. **Method:** Sixteen extracted third molars were sectioned in the following tooth regions: mesio-distal, vestibular-lingual and at the cement enamel junction. Forty specimens were selected according to values of microhardness and roughness initials, and distributed in four experimental groups (n = 10/group): Group 1 = Autoclave 121 °C (30 minutes); Group 2 = 2.5% Sodium hypochlorite (07 days); Group 3 = 5.25% Sodium hypochlorite (07 days); Group 4= 30% Acetic Acid (7 days). After sterilization, the microhardness, roughness and morphology of enamel and dentin were evaluated. Data were analyzed by paired t-test, Wilcoxon, Kruskal-Wallis and Analysis of Variance. A significance level of 5% was adopted. **Result:** All sterilization methods altered the microhardness and roughness of the dentine (p <0.05). Regarding to enamel, the microhardness was affected only by Autoclave and 2.5% Sodium hypochlorite treatment (p <0.05). A large aperture of the enamel pores and dentinal tubules was verified in the micrographs. **Conclusion:** The sterilization methods adopted altered the morphology and/or physical properties of enamel and dentine.

Descriptors: Dental enamel; hardness; sterilization; dentin.



INTRODUCTION

Human Teeth Bank (HTB) is a nonprofit institution that stores, maintains and donates extracted human teeth, respecting ethical, legal and biosafety standards¹. Teeth manipulation can provide a risk of contamination for dentists, academics and researchers due to the presence of blood pathogens from pulp and periodontal tissues².

Previous studies highlight some methods of human teeth disinfection, such as immersion in formalina, sodium hypochlorite, glutaraldehyde or autoclave³. Related to teeth sterilization, other studies point out ethylene oxide, and gamma radiation use⁴. However, their efficacy can be altered and depends on the time of exposure, temperature, pressure, number of microorganisms and type of organic material that surrounds the teeth⁵.

Sterilization methods adopted in HTB can promote structural changes in enamel and dentin⁵. Thus, the ideal method should combine the antimicrobial efficacy and preservation of the substrate biomechanical properties, such as surface microhardness, coloration, mineral composition, microleakage, bond strength and dentin permeability⁶. Therefore, the choice of method should be careful in order to provide less variability in the studies and greater reliability of the results.

The Center for Disease Control and Prevention⁷ considers that extracted teeth used for scientific and didactic purposes should be autoclaved or disinfected with sodium hypochlorite or other germicidal solutions. However, these methods modify or remove the enamel protein matrix⁸. Considering these criteria, the most recommended method would be gamma radiation⁴, despite of its complex and expensive process⁹.

Additionally, since an ideal method for tooth sterilization has not been well established, acetic acid solution has been investigated as an available, simple, low toxicity and cheaper antimicrobial agent¹⁰. However, there are few studies evaluating the effects of this substance on the enamel and dentin properties.

Therefore, this *in vitro* study evaluated the morphology and physical properties of enamel and dentin, after being submitted to sterilization methods. The hypothesis tested was that sterilization methods would not influence the morphology and physical properties of extracted human teeth.

MATERIAL AND METHOD

This *in vitro* study was approved by the Research Ethics Committee of the Federal University of Piauí (UFPI) – nº 1.872.436, in accordance with the World Medical Association Declaration of Helsinki.

Specimens Preparation

Sixteen third molars, newly extracted from patients aged 18-25 years, were selected. Teeth were acquired from the UFPI's HTB, and stored in distilled water at 4 °C. After cleaning, the absence of defects in the enamel development, carious lesions and pre-existing fractures were verified.

The coronary portion was sectioned mesio-distal, vestibular-lingual/palatine, and at cement enamel junction (CEJ). A double-face diamond steel disc (KG Sorensen, Barueri, SP, Brazil) was used and 64 specimens containing enamel and dentin were obtained. The inner surface was polished with silicon carbide sandpaper (#400, #600 and #1200) (*Buehler, Lake Bluff, Illinois, USA*). After each sandpaper changing, the blocks were placed on ultrasound (Branson 1210 – Odontobrás, Ribeirão Preto, SP) containing deionized water for 5 minutes. This procedure was performed to prevent interference of sandpaper grains in the dental surface smoothness. Ultra-polishing was performed with SUPRA felt disc (Arotec S/A Ind. e Com., Cotia, SP) and aqueous diamond suspension (Arotec S/A Ind. e Com., Cotia, SP) with 1µm abrasive particles.

Microhardness and Surface Roughness Analyses

After finishing and polishing the blocks, values of initial microhardness (KHN_1) of enamel and dentin were determined. It was used a Knoop hardness tester (Future-Tech FM hardness tester, FM-ARS 900 software) at 50 g static load for enamel and 5 g for dentin, both for 5 seconds. Enamel indentations were performed at 150 µm below the surface, 150 µm above the dentin and between these two distances. To perform microhardness in dentin, the specimens were left at room temperature for 30 minutes in order to minimize the interference of dehydration during measurement. Indentations were done 150 µm after dentin beginning, 150 µm above its terminus and between these two distances. KHN_1 in enamel and dentin were obtained from the average of the three indentations in each dental tissue.

Roughness analysis (R_a) was determined using a Surfscorder SE 1700* rugosimeter (Kosaka Laboratory Ltd, Kosaka, Japan) with cut-off adjusted to 2.5 mm. After three readings in each specimen, the mean and initial total roughness (R_{a1}) was established.

General averages were calculated for microhardness and surface roughness against the individual values. Therefore, specimens that presented values outside the range ($\pm 20\%$ of the overall mean) were excluded of the study. The selected specimens were randomized and distributed into the treatment groups ($n = 10/\text{group}$): G1 = Autoclave 121 °C (30 min); G2 = 2.5% Sodium hypochlorite (07 days); G3 = 5.25% Sodium hypochlorite (07 days); G4 = 30% Acetic acid (7 days). After the sterilization process, new tests of microhardness (KHN_2) and roughness (R_{a2}) were performed.

Scanning Electron Microscopy Analysis (SEM)

A morphological analysis was performed on enamel and dentin treated with different methods of sterilization ($n = 4$), and without treatment ($n = 1$). The specimens were washed with distilled water, gradually dehydrated with ethanol in the following concentrations: 25% (20 min), 50% (20 min), 75% (20 min), 95% (30 min), 98.93% (60 min), 100% (60 min). Then, specimens were fixed with hexamethyldisilazane (10 min). After 24 hours at room temperature, the specimens were metallized with gold (Bal-Tec SDC 050 Sputtercoater, Balzers, Liechtenstein), and visualized at 7,500x of magnification (JSM 5600LV, Jeol, Tokyo, Japan).

Statistical Analysis

Data were analyzed using the statistical program SPSS (Statistical Package for Social Sciences) version 20.0, specific for Windows. The assumptions of equality of variances and normal distribution of errors were checked by Kolmogorov-Smirnov test. Paired t-test was used to compare normal data from the same group, otherwise the Wilcoxon test was adopted. For analyses among groups with normal values, it was used Analysis of Variance (ANOVA) followed by post hoc Tukey test. Kruskal-Wallis test was used for the others. The significance level was set at 5%.

RESULT

KHN₁ and KHN₂ analyses showed that the Autoclave and 2.5% Sodium hypochlorite methods significantly decreased enamel microhardness (p < 0.05). Regarding dentin, all treatments significantly altered its surface (p < 0.05). Additionally, it was not possible to calculate the microhardness values of the specimens treated with acetic acid, due to the high demineralization caused in enamel and dentin (Table 1). Related to the Ra₁ and Ra₂ means, a significant difference (p < 0.05) was observed in all treated groups (Table 2).

Table 1. Mean microhardness of enamel and dentin, along with the standard deviations, before and after treatment

Dental tissues	Experimental groups	KHN ₁ ± σ	KHN ₂ ± σ	P
Enamel	G1	292.28 ± 19.58	269.22 ± 20.84	0.005**
	G2	292.57 ± 13.23	266.74 ± 16.83	0.005**
	G3	289.74 ± 19.96	266.55 ± 18.00	0.985*
	G4	-	-	-
Dentin	G1	48.61 ± 3.43	45.42 ± 3.36	0.009*
	G2	47.65 ± 4.45	19.42 ± 2.03	0.000*
	G3	46.12 ± 4.67	20.5 ± 2.32	0.000*
	G4	-	-	-

p < 0.05 is statistically significant; * Value of p (paired t-test); ** p-value (Wilcoxon's test); σ = standard deviation; KHN₁ = superficial Knoop microhardness before treatment; KHN₂ = superficial Knoop microhardness after treatment; G1 = Autoclave; G2 = 2.5% Sodium hypochlorite; G3 = 5% Sodium Hypochlorite; G4 = 30% Acetic Acid.

Table 2. Means of total roughness of the specimen, accompanied by standard deviations, before and after treatment

Groups	Ra ₁ ± σ	Ra ₂ ± σ	p
G1	0.095 ± 0.125	0.143 ± 0.035	0.000
G2	0.097 ± 0.12	0.174 ± 0.059	0.002
G3	0.095 ± 0.14	0.167 ± 0.042	0.000
G4	0.095 ± 0.13	0.463 ± 0.121	0.000

p < 0.05 is statistically significant for the paired t-test; σ = standard deviation; Ra₁ = total roughness of the specimen before treatment; Ra₂ = total roughness of the specimen after treatment; G1 = Autoclave; G2 = 2.5% Sodium hypochlorite; G3 = 5% Sodium Hypochlorite; G4 = 30% Acetic Acid.

After enamel SEM analysis, it was observed that the morphology of the untreated specimen (Figure 1a) was characterized by a homogeneous, dense and smooth surface. Micrographs of the Autoclave and Sodium hypochlorite treated groups showed a porous surface (Figures 1c, e, g). In addition, it was noted that the surface exposed to acetic acid (Figure 1i) showed distinct morphological characteristics from the other treatments, presenting a porous, irregular, and with high roughness surface.

In dentin specimens, the untreated surface presented opened dentinal tubules associated to peritubular dentin (Figure 1b). Based on these characteristics, the micrographs of the surfaces treated with Autoclave and Sodium hypochlorite (Figures 1d, f, h) showed increase in the dentinal tubules opening. Also, it was observed collagen fibers alteration as the hypochlorite concentration increased. In addition, acetic acid treatment caused greater increase of the dentinal tubules and removal of collagen fibers (Figure 1j).

Therefore, it was observed that all the sterilization methods used in this study increased the porosity and widened the aperture of the dentinal tubules, demonstrating greater dissolution in the peritubular dentin region, and increasing the permeability of this tissue.

DISCUSSION

The sterilization methods adopted in this experiment altered the physical and morphological properties of the enamel and dentin, thus the null hypothesis of this study was rejected.

For Humel et al.¹¹, sterilization methods should eliminate all pathogens present in extracted human teeth to avoid the risk of contamination. The sterilization methods used in this research demonstrated efficacy in previous studies^{2,12}. Besides microbiological factor, the maintenance of the morphology and structural integrity of the enamel and dentin should be considered when sterilization methods are chosen¹³. However, there is no consensus in the literature about the ideal method that preserves physical and structural properties of dental tissues¹⁴.

In order to observe higher alterations in the substrate, roughness and microhardness data were obtained from the specimens, since these values determine mineral loss or gain¹⁵. To reduce possible bias, both analyzes were performed in the same specimen and in standardized moments. The results of this study indicated that Autoclave and 2.5% Sodium hypochlorite decreased KHN of enamel, while all treatments affected KHN in dentin. The effect caused by Autoclave was observed in previous studies^{13,16}. Parsell et al.¹⁷ explain this effect by the presence of under pressure heat, which is able to break ionic bonds between collagen and hydroxyapatite, leading to denaturation of the organic matrix. However, there is still no agreement among the authors about the effect of this method on dental substrate^{14,18}. Moreover, Patil, Uppin¹⁹ showed that microhardness values changed similarly when 2.5% and 5% hypochlorite concentrations were used.

Another study pointed out that this solution was able to weaken the dental substrate, besides changing the microhardness according to its concentration and time of action²⁰. Regarding Sodium hypochlorite concentration and its effect on the substrate,

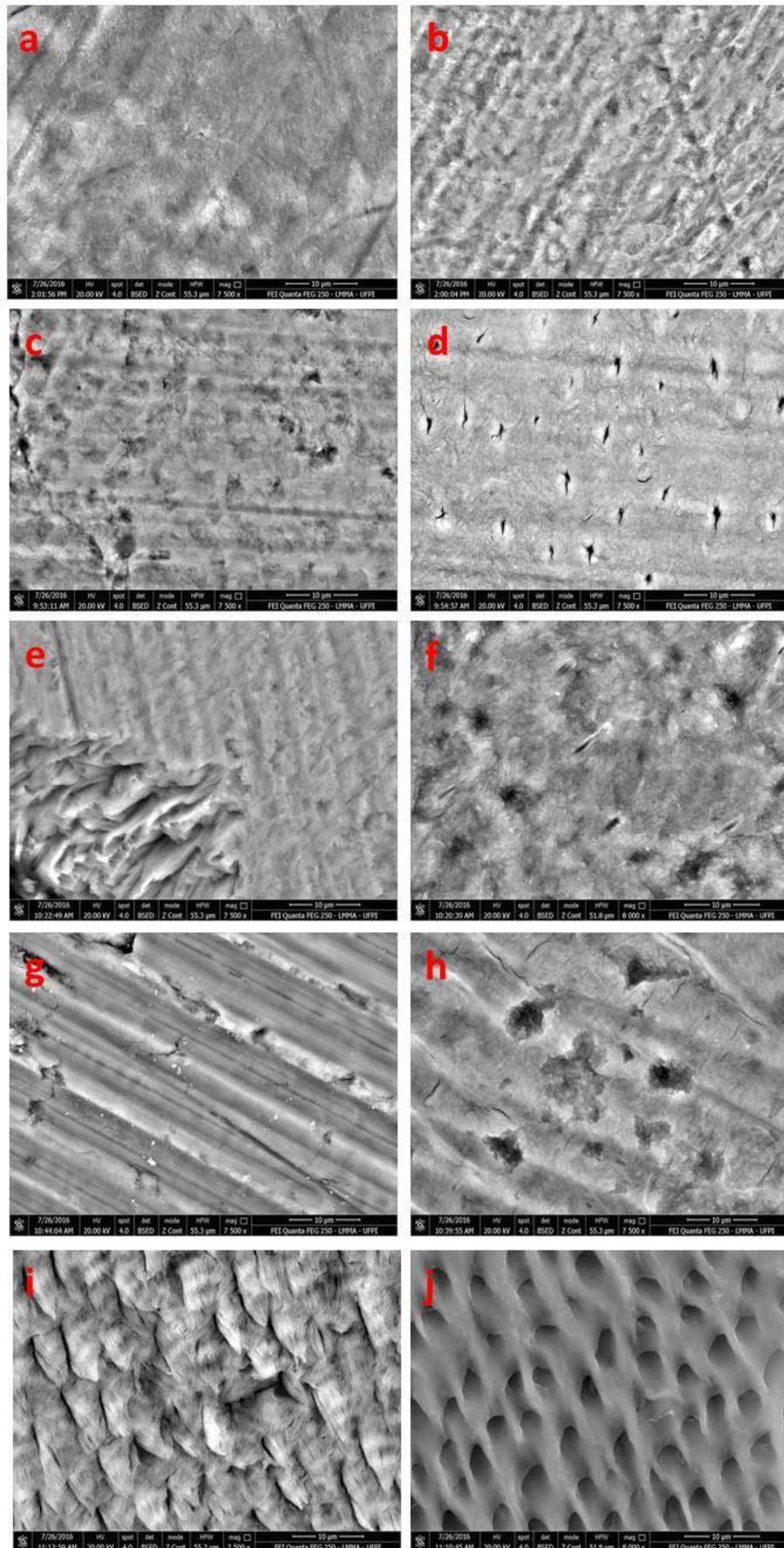


Figure 1. Scanning Electron Microscopy of enamel and human dentin submitted to different methods of sterilization and control group. (a) Untreated dental enamel (Control); (b) Untreated dental dentin (Control); (c) Enamel treated with autoclave; (d) Dentin treated with autoclave; (e) Enamel treated with Sodium Hypochlorite 2.5%; (f) Dentin treated with 2.5% Sodium Hypochlorite; (g) Enamel treated with 5% Sodium Hypochlorite; (h) Dentin treated with 5% Sodium Hypochlorite; (i) Enamel treated with 30% Acetic Acid; (j) Dentin treated with 30% Acetic Acid. Increase of 7.500 X.

it was observed that only 2.5% Sodium hypochlorite caused KHN change. This fact might be explained by the natural instability of this solution, and interference of time, light, exposure to air and high temperatures, changing the solution of the solution²¹.

Previous studies also have used acetic acid for disinfection of extracted human teeth, due to discussions about the toxicity of chlorine and other disinfectants^{22,23}. This substance is not commonly used in dentistry, but is considered an alternative disinfectant for teeth, acrylic resins and toothbrushes^{10,24}. In addition, the inclusion of this substance in this study was due to its availability, low cost and low toxicity²³.

In the evaluation of the roughness, all the methods used were able to alter teeth properties, as previously showed in the literature²⁵. Compared to other methods, acetic acid produced the most significant increase due to its acidic character and demineralizing effect on the substrate.

SEM analysis showed that all sterilization methods increased enamel pores and dentinal tubules, indicating a greater permeability of these tissues. However, these results were different from those found by Pashley et al.¹⁸, besides did not observe changes in the permeability and adhesive strength of dentin.

Therefore, it is suggested that all sterilization methods evaluated in this study provided changes in the physical and morphological properties of enamel and dentin. Furthermore, it is recommended that extracted human teeth used in research should be stored in purified water until selection of the sterilization method. Thus, the researcher must choose the method that best fits its research objectives, in order to achieve reliable results and eliminate study bias.

CONCLUSION

Despite the limitations of this in vitro study, our findings suggest that sterilization methods can alter the morphology and/or physical properties of enamel and dentin.

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CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

*CORRESPONDING AUTHOR

Daylana Pacheco da Silva, Departamento de Odontologia Restauradora, Faculdade de Odontologia de Piracicaba, UNICAMP – Universidade Estadual de Campinas, Av. Limeira, 901, Areião, 13414-903 Piracicaba - SP, Brasil, e-mail: daylanapachecos@gmail.com

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