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Morphological and chemical changes of dentin after applying different sterilization methods

Avaliação das alterações morfológicas e químicas da dentina após a aplicação de diferentes métodos de esterilização

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Resumo

Objetivo: O presente estudo teve como objetivo avaliar as alterações morfológicas e químicas na dentina geradas por diferentes métodos de esterilização, através de microscopia eletrônica de varredura (MEV) e da análise por espectrometria de energia dispersiva de raios-X. **Material e método**: Seccionaram-se 5 dentes humanos em 4 amostras, as quais foram divididas em 3 espécimes cada, totalizando 60 espécimes. Os espécimes foram separados conforme os grupos de esterilização: calor úmido sob pressão; radiação gama cobalto 60; controle (sem esterilização). Após a esterilização, os 60 espécimes foram analisados por MEV, usando 3 aumentos: 1500X, 5000X, 10000X. As imagens foram analisadas por 3 examinadores previamente calibrados, que distribuíram escores referentes às alterações observadas nos túbulos dentinários: 0 = sem alteração morfológica, 1 = baixa obliteração dos túbulos dentinários, 2 = moderada obliteração, 3 = completa obliteração. A composição química da dentina foi avaliada por meio do processo EDS, com 15 kV de incidência e penetração de 1 µm. Os dados obtidos foram submetidos ao teste estatístico de Kruskall-Wallis com nível de significância de 5%. **Resultado**: De acordo com os resultados obtidos, verificou-se que a esterilização tanto por autoclave quanto por radiação gama cobalto 60 não provocou alterações significativas na morfologia dos túbulos dentinários e na constituição química da dentina. **Conclusão**: Concluiu-se que ambos os métodos podem ser utilizados para a esterilização de dentes em pesquisa in vitro.

Descritores: Radiação; microscopia; análise química; dentina.

Abstract

Aim: The present study evaluated the morphological and chemical changes of dentin produced by different sterilization methods, using scanning electron microscopy (SEM) and energy-dispersive X-ray spectrometry (EDS) analysis. **Material and method**: Five human teeth were sectioned into 4 samples, each divided into 3 specimens. The specimens were separated into sterilization groups, as follows: wet heat under pressure; cobalt 60 gamma radiation; and control (without sterilization). After sterilization, the 60 specimens were analyzed by SEM under 3 magnifications: 1500X, 5000X, and 10000X. The images were analyzed by 3 calibrated examiners, who assigned scores according to the changes observed in the dentinal tubules: 0 = no morphological change; 1, 2 and 3 = slight, medium and complete obliteration of the dentinal tubules. The chemical composition of dentin was assessed by EDS, with 15 kV incidence and 1 µm penetration. **Result**: The data obtained were submitted to the statistical tests of Kruskall-Wallis and ANOVA. It was observed that both sterilization methods – with autoclave and with cobalt 60 gamma radiation – produced no significant changes to the morphology of the dentinal tubules or to the chemical composition of dentin. **Conclusion**: Both methods may thus be used to sterilize teeth for research conducted in vitro.

Descriptors: Radiation; microscopy, electron; chemical analysis; dentin.

INTRODUCTION

Several in vitro studies utilize extracted human teeth, which must be submitted to sterilization in order to ensure greater safety to the researcher and obviate cross contamination. Additionally, tooth sterilization is essential to allow controlled inoculation of microorganisms in the canal, in research testing for antimicrobial effectiveness. Hence, in order to obtain results closer to the clinical reality in in vitro research, no structural alteration of the tooth should occur during sterilization.

Several authors have used different methods of tooth sterilization: wet heat under pressure (autoclave)[1,2], cobalt 60 gamma radiation[3-5], ethylene oxide[6,] and chemical solutions[7]. However, it is essential to assess whether these methods produce any chemical or morphological alterations, and whether there are significant differences among them.

Pashley et al.[8] and Brown et al.[9] evaluated the use of the autoclave, and concluded that it does not interfere with dentinal permeability. However, other studies have demonstrated that dentin microhardness is affected when this kind of sterilization is used on teeth, insofar as this method could indeed interfere with the ionic bond between collagen and hydroxyapatite[2], possibly producing biomechanical changes that may have gone undetected when the Pashley and Brown assessments were performed.

Sterilization through gamma radiation has been shown to reduce microbial contamination in the food industry, preserving and extending the expiration date of foodstuffs without affecting their overall quality[10]. This radiation does not change the nutritional quality of foodstuffs because it sterilizes without having to resort to high temperatures, high pressure, or any chemical substance. In hospitals, this radiation is used on medical supplies, including surgical instruments[11]. The advantage of this sterilization method is the elimination of toxins, rendering the materials apyrogenic, a characteristic that lacks when sterilization is performed by autoclave.

Several endodontic studies have evaluated the action of irrigating agents on toxins found in root canals (endotoxins), present on the external membrane of gram-negative bacteria and released during cell duplication or death[4,5]. The teeth used in these studies should thus be submitted to a sterilization process capable of eliminating microorganisms and toxins, such as gamma radiation[12].

A study conducted by Rodrigues et al.[13] showed that there is no significant difference between the initial microhardness of enamel and that observed after sterilization with gamma radiation. However, research investigating the changes that may be produced by gamma radiation on dental structures, mainly on dentinal tubules, is still scarce in the related literature, indicating the importance of further developing this research area. Thus, the aim of the present study was to assess the morphological and chemical changes in crown and root dentin after sterilization with cobalt 60 gamma radiation or with autoclave, by means of SEM and chemical composition analysis.

MATERIAL AND METHOD

Experimental Procedures

This study was approved by the institutional research ethics committee (protocol No. 283.027). Five single-rooted human teeth, recently extracted for various reasons, were used with previous authorization by the patients through free and informed consent. The selection of teeth was performed based on the dimensions and similarities of root morphology. Teeth root surfaces were cleaned with periodontal curettes (Golgran Ltda, São Caetano do Sul, SP, Brazil) and were stored in closed containers with physiological solution, and then frozen at –18°C in a freezer, until such time as they were used.

The teeth were sectioned perpendicularly to their long axis with carborundum discs (Labordental Ltda, São Paulo, SP, Brazil) under abundant water irrigation to obtain 4 samples of each tooth: middle third of the crown, and cervical, middle and apical thirds of the root. Each sample was sectioned into 3 specimens, each of which was sterilized by a different method, as follows (n = 20): GA) wet heat under pressure (autoclave) (Cristófoli Equipamentos de Biossegurança, PR, Brasil) at 121°C for 20 min; GG) cobalt 60 gamma radiation (20 Kgy) (EMBRARAD - Empresa Brasileira de Radiação, Cotia, SP, Brazil); GC) control (not submitted to any sterilization process).

SEM Analysis

After sterilization, the specimens were fixed on *stubs*, coated with gold using a sputter coater, and then analyzed by scanning electron microscopy (SEM) at the Sensor Analysis Laboratory of the National Institute of Space Research (LAS/INPE). The analysis was performed under 3 magnifications: 1500X, 5000X and 10000X, thus producing 36 images of each tooth, for a total of 180 images, all of which were of the dentinal surface of the specimens.

The images were analyzed by 3 previously calibrated examiners to assess any morphological changes in the dentinal tubules, compared to the control group. The examiners were blinded to the groups. Thus, 3 images of each sample were displayed simultaneously on the computer screen for a simultaneous evaluation of the effects of the different methods on the same sample. Scores were assigned according to the changes observed in the dentinal tubules: 0 = no morphological change, 1 = slight obliteration of the dentinal tubules, 2 = medium obliteration of the dentinal tubules, 3 = complete obliteration of the dentinal tubules.

EDS Analysis

In addition to the morphological analysis, a qualitative analysis was performed of the changes in chemical composition of dentin. This analysis was conducted by EDS (Energy dispersive X-ray spectrometry), in the same laboratory cited above. The incident energy used for this analysis was 15 kV, for a 1-µm-deep penetration into the specimen. The area assessed was 1 mm^2 (100X magnification) in the center of the sample. The assessment was performed on 6 specimens (control, cobalt 60 gamma radiation and autoclave): 3 of the middle third of the root and 3 of the cervical third of the root.

Statistical Analysis

The scores obtained for each fragment were submitted to the non-parametric Kruskall-Wallis statistical test at a 5% level of significance (BioEstat 5.3; Instituto de Desenvolvimento Sustentável Mamirauá, Tefé, AM, Brazil).

RESULT

After applying the non-parametric Kruskall-Wallis test (5% significance) no significant statistical difference was found among the 3 groups studied (p = 0.1389; F = 2.04 and degrees of

freedom = 2). The scores obtained in the SEM analysis are shown in Figure 1, and the images obtained in the same test are shown in Figures 2, 3 and 4.

The results obtained in the EDS analysis are shown in Table 1. The chemical elements (oxygen, magnesium, phosphorus and calcium) are displayed according to their mass percentage in each specimen.

According to the results of this qualitative analysis, the percentages of oxygen, magnesium, phosphorus and calcium in the experimental specimens are similar, and no abrupt variation was observed compared to the control.

DISCUSSION

The sterilization of teeth used for in vitro research in laboratories of different dental specialties, such as Endodontics or Restorative Dentistry, should be effective in fighting the microorganisms present, without harming the mineralized tissues, to avoid inducing false results or interfering in the learning process of students. For this purpose, several sterilization means have been used and studied.

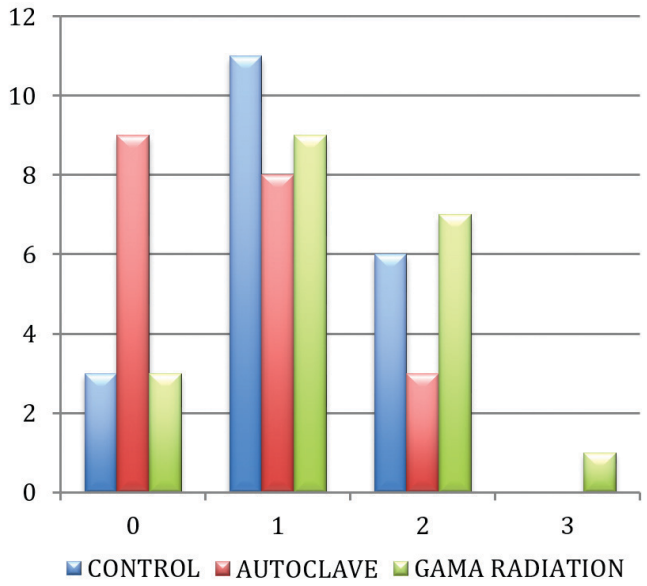

Figure 1. Scores obtained in the SEM analysis according to the study groups.

Scanning electron microscopy (SEM) scores were used to evaluate the characteristics of dentin after sterilization. This type of evaluation was chosen because most of the research conducted with SEM is based on a score system, which quantitatively assesses the surface analyzed[14].One limitation of this system is that it does not allow a reliable comparison between studies and depends on a subjective assessment by observers.

In the present study, no significant morphological changes were observed in the specimens sterilized by wet heat under pressure (autoclave) compared to those not submitted to this type of sterilization. The dentinal tubules of most specimens showed little or no obliteration, compared to those of the control group, which were not autoclaved. These results corroborate the findings of McGuckin, Pashley[15], who observed no significant difference in dentin bond strength of autoclaved and non-autoclaved teeth. These authors suggested that the effects of different sterilization methods should be further investigated to assess possible physical, morphological or chemical changes in dental tissues. Another study conducted by Pashley et al.[8] found that autoclave sterilization of extracted teeth did not produce changes in intrinsic dentin permeability or bond strength, demonstrating that dentinal tubules were unaffected by the sterilization process used. Thus, tooth sterilization by autoclave can be indicated, insofar as, according to this and other studies, it does not produce significant changes in the morphology and chemical composition of dentin. In addition to being effective in eliminating microorganisms, this method is also simple and quick, thus making it easily accessible to students and researchers.

Today, cobalt 60 gamma radiation is being employed as a method for sterilizing and depyrogenating teeth used for in vitro research. In addition to destroying microorganisms, this method neutralizes the endotoxins (LPS) released by Gram-negative bacteria after cell death or duplication[12]. Thus, research involving

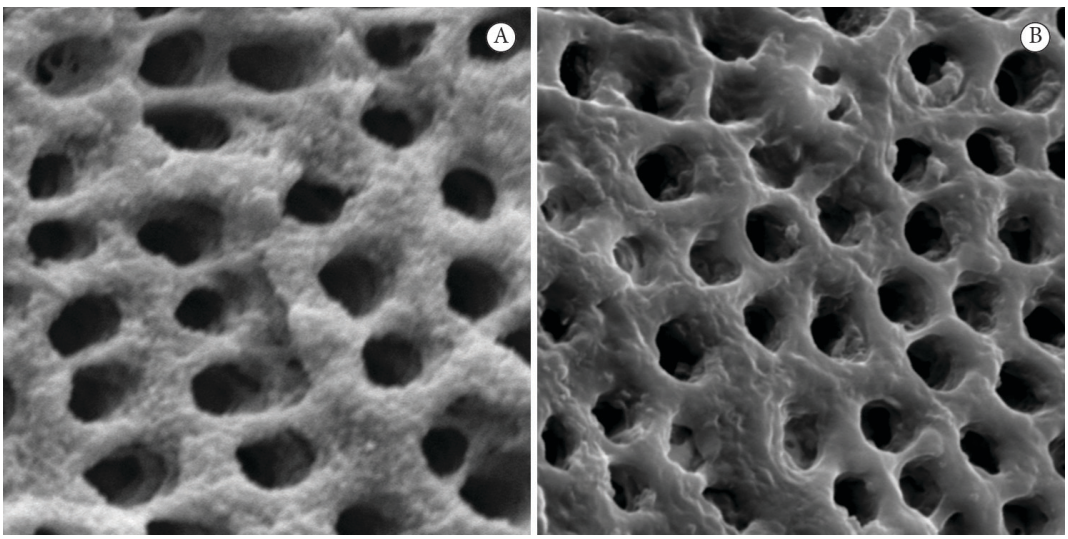

Figure 2. Specimens from GA: A) apical third of the root; B) middle third of the root (original magnification 5000X).

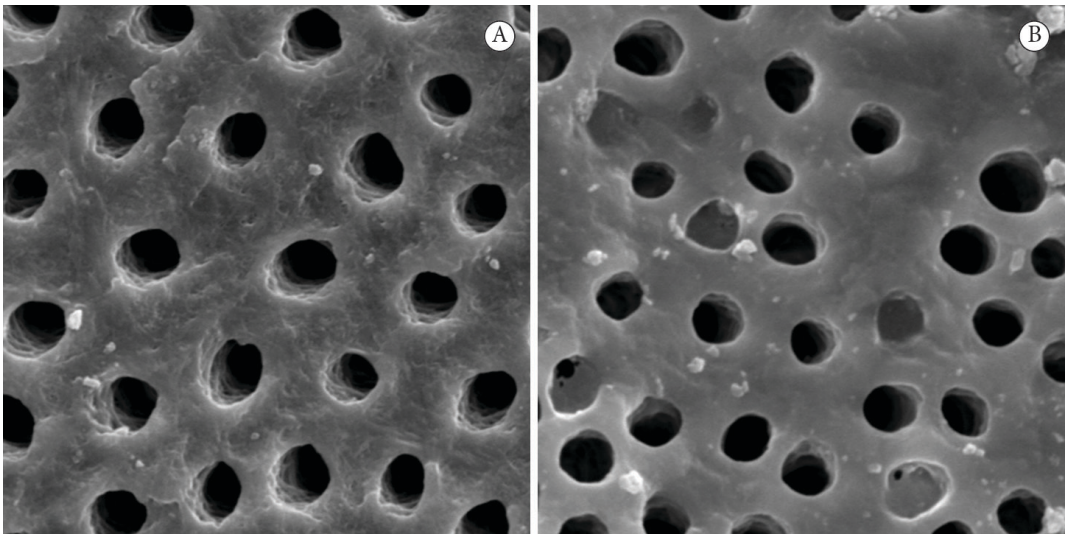

Figure 3. Specimens from GG: A) apical third of the root; B) middle third of the root (original magnification 5000X).

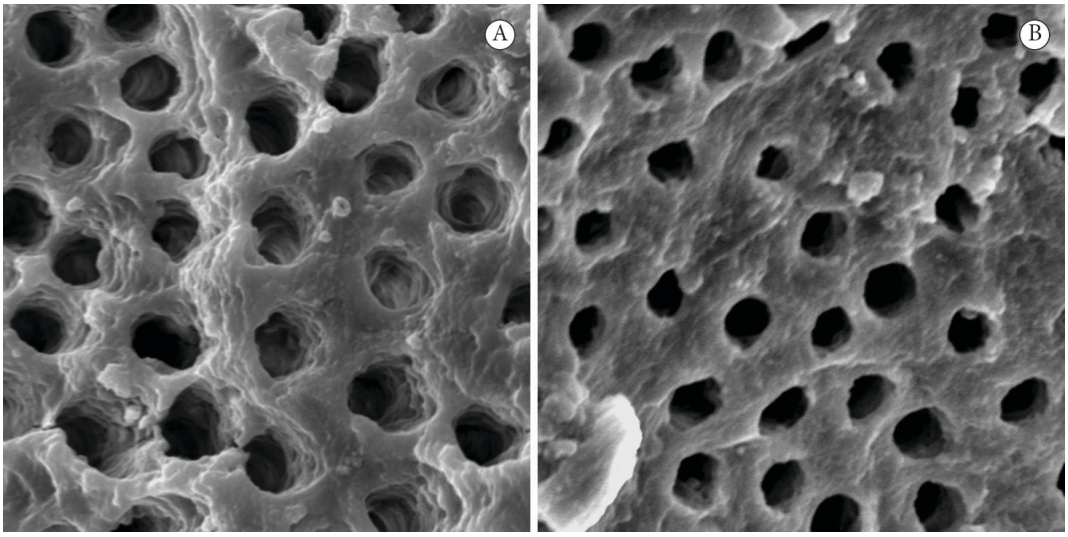

Figure 4. Specimens from GC: A) apical third of the root; B) middle third of the root (original magnification 5000X).

Table 1. Mass percentage of chemical elements present in the specimens studied

	Oxygen	Magnesium	Phosphorus	Calcium
GA – Middle root	49.06%	0.62%	16.59%	35.75%
GC – Middle root	45.39%	0.73%	17.83%	36.05%
GG – Middle root	42.44%	0.66%	18.02%	40.97%
GA – Cervical root	50.22%	0.74%	15.99%	33.05%
GC – Cervical root	49.34%	0.68%	18.69%	36.49%
GG – Cervical root	52.45%	0.72%	16.08%	30.75%

GA: wet heat under pressure; GG: cobalt 60 gamma radiation; GC: control.

LPS, particularly in the endodontic area, commonly uses cobalt 60 gamma radiation (20 Kgy) as a means of sterilization.

In the present study, cobalt 60 gamma radiation did not induce significant changes in the morphology and chemical composition of dentin, since most of the samples displayed dentinal tubules with little or no obliteration, similarly to the control and autoclave groups ($p>0.05$). These results are in agreement with those obtained by White et al.[16], who found that gamma radiation did not affect the permeability of several segments of crown dentin, similarly to the results obtained by Goodis et al.[17]. White et al.[16] further observed the effects of different sterilization methods on dentin discs, based on optical properties (infrared UV, VIS, NIR, and FTIR spectra) before and after sterilization by autoclave, ethylene oxide, gamma radiation and dry heat. According to their results, no changes

produced by gamma radiation were detected while other methods produced detectable spectrum changes. Based on these results, the authors suggested that the common methods of sterilization alter the structure of dentin, whereas gamma radiation is a promising method because it is effective against microorganisms and toxins, and does not induce detectable structural changes.

In 2001, Sperandio et al.[18] evaluated the effects of gamma radiation on the bond strength of dentin and on dentinal surface morphology, and observed that it did not affect these variables, as observed under scanning electron microscopy. In 2004, Rodrigues et al.[13] evaluated the effects of the same radiation on the mechanical properties of enamel and on demineralization, and concluded that it did not affect enamel microhardness or enamel resistance to demineralization. Although leading to similar conclusions, none of these previous studies had evaluated dentin changes, as in the present study.

Hence, cobalt 60 gamma radiation has produced quite satisfactory results both for sterilization of specimens and in terms of the integrity of dentinal morphology and mineral structure, recommending its use as a sterilization method for teeth used for in vitro research or in learning laboratories.

CONCLUSION

Based on the results obtained in this study, we concluded that both sterilization methods analyzed – with autoclave and with cobalt 60 gamma radiation – did not produce significant changes in the morphology of dentinal tubules or in the chemical composition of dentin, indicating that they can both be used safely and effectively to sterilize teeth for in vitro research.

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CONFLICTS OF INTERESTS

The authors declare no conflicts of interest related to this manuscript.

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