Original paper

Comparison of the methods of disinfection/sterilization of extracted human roots for research purposes

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Conflict of interest
None declared

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Abstract

Background. Extracted human teeth are used to simulate dental procedures and are essential for practical education and research studies.

Objectives. The aim of this study was to evaluate the efficacy of different sterilization methods for extracted human roots and to assess the effects of these methods on dentin microhardness.

Material and methods. The crowns of 40 mandibular incisors were removed. The roots were sectioned at 10 mm and divided into 4 groups ($n = 10$ per group): G1 – no sterilization (control); G2 – microwave radiation (650 W, 5 min); G3 – ethylene oxide (288°C, 3 h); and G4 – autoclave (121°C, 15 min). The roots were immersed in brain heart infusion (BHI) and incubated at 37°C in variable oxygen atmospheres. After 14 days, the samples were assessed for turbidity. Three slices were obtained from each root, and indentations were made at 30, 60 and 120 µm from the root canal lumen. The microbiological data was analyzed with the Kruskal–Wallis test and Dunn's post-hoc test. Microhardness was evaluated by means of the two-way analysis of variance (ANOVA) and Tukey's test ($p < 0.05$).

Results. The roots submitted to autoclaving were 100% sterile, which differed from the other methods ($p < 0.05$); the control specimens had 0% sterility. For microhardness, significant differences were found between the methods, particularly for the apical third (68.06 ±12.50) ($p < 0.05$).

Conclusions. Although all the evaluated techniques reduced dentin microhardness, autoclaving should be used as the most reliable method of sterilization of extracted dental roots.

Keywords: hardness test, autoclave, ethylene oxide, microwave oven, tooth root

Introduction

Extracted human teeth are used to simulate dental procedures and are essential to practical education and research studies.[1–3] In vitro and in situ studies provide scientific support for developing new materials, and allow the control and simulation of real situations in a laboratory environment.[4,5]

The American Dental Association (ADA) and the Centers for Disease Control and Prevention (CDC) recommend a thorough removal of microorganisms capable of transmitting diseases from non-disposable materials used in patient care.[1,6,7] The effective elimination of microorganisms is necessary to perform in vitro and in situ research in an attempt to maintain the substrate structure unaltered.[7–10] This objective is hard to achieve due to the abundance of microorganisms present in the oral cavity, biofilm and saliva.[2,7] Therefore, there is a high risk of infection and cross-contamination when handling extracted teeth.[2,7–9]

Disinfection refers to an action that reduces the microbial load present on the surface of an object, whereas sterilization refers to a process that removes any detectable microbial load.[11,12] Various chemical sterilants have been tested for the disinfection of extracted teeth, including formalin, glutaraldehyde, iodopovidone, alcohol, vinegar, thymol, and chloramine.[3,12] The literature also reports the use of physical agents, autoclaving, gamma radiation, microwave radiation,[2,8,13] and gases, such as ethylene oxide and formaldehyde,[11] to sterilize extracted teeth.

The effectiveness of any method of sterilization depends upon time, contact, temperature, pressure, and how the specimens are stored, washed and decontaminated.[9–12] Autoclaving consists in using pressurized steam to achieve the hydrolysis and coagulation of cellular proteins; it kills bacteria, viruses, spores, and all other microbial forms.[3,8] It is recommended to follow the manufacturer's protocol and never interrupt the sterilization cycle. After the process, the sterilizer must be depressurized and the packs must remain inside for drying.[11]

The process of sterilization in ethylene oxide steam alternates between ethylene oxide gas cycles and ventilation periods.[14,15] Microwave radiation is initially effective; however, depending on the time of exposure, cellular disintegration may occur due to an increase in temperature.[13,16] There have been concerns about the effects of infection control on extracted teeth, namely on dentin permeability and the bond strength of restorative materials.[1,17] It is unknown whether autoclaving affects the chemical and micromechanical relationship between dentin and dental materials.[9] Studies have shown changes in the chemical properties of dentin and a reduction in its microhardness.[2,18,19]

Another important consideration is that studies that evaluated the microbiological effectiveness of sterilization methods used small sectioned dental slabs,[2] which fails to simulate the real conditions of in situ studies. The standardization of the experimental design and of the teeth are required to produce valid results in laboratory research.[20–22] Previous investigations did not evaluate the growth of microorganisms under different oxygen incubation conditions (e.g., aerobic and anaerobic).[6,7,12] Conversely, this study used different oxygen environments in an attempt to eliminate every type of microorganism present.

Considering the aforementioned facts, it is important to investigate the effectiveness of sterilization methods for large dental slabs, such as roots, before using them for educational or research purposes. This study aimed to evaluate the efficacy of different sterilization methods that are commonly used for extracted human dental roots as well as their effects on dentin microhardness. The null hypotheses were: 1. There is no difference in sterilization effectiveness between the methods tested; and 2. There is no difference in dentin microhardness after using the different sterilization methods.

Material and methods

Sample preparation

Forty freshly extracted human incisors were stored in 0.1% thymol solution at 4°C. They were cleaned and examined to verify that there were no structural anomalies and that they had complete root formation. A radiographic examination was conducted to confirm that there was a single root canal in each tooth. The 40 teeth had their crowns removed (IsoMet™ 1000; Buehler, Lake Bluff, USA) and the roots were trimmed coronally to a standardized length of 10 mm. The final dimensions were checked with a digital caliper (Mitutoyo, Suzano, Brazil).

Dental root sterilization

The roots were divided into 4 groups (n = 10 teeth per group):

– G1 (control): no sterilization method was used;
– G2 (microwave): the specimens were placed in glass containers, individually immersed in 100 mL of sterile distilled water and exposed to microwave radiation in a domestic oven (Electrolux, Pinhais, Brazil) at 650 W for 5 min;
– G3 (ethylene oxide): the specimens were submitted to 50–80% relative humidity at 288°C for 3 h (Wimac Kliniekdiensten, Rotterdam, the Netherlands); and
– G4 (autoclave): the specimens were stored in a glass vessel containing distilled water and autoclaved at 121°C with 15 psi for 15 min in an industrial autoclave (model AG 523; Ortosintese Indústria e Comércio, Jaragua, Brazil).

All roots were immersed in sterile distilled water before and after sterilization to equally corrode the dental substrates.

Broth turbidity analysis

The roots from each group were individually immersed and transferred to test tubes containing 10 mL of sterile brain heart infusion (BHI). Each tube was agitated for 1 min in an agitator (Vortex, São Paulo, Brazil). For each experimental group, 3 tubes with the BHI broth and the roots (n = 12) were incubated under anaerobic conditions. Another 3 tubes for each group (n = 12) were incubated in a microaerobic atmosphere. Finally, 4 tubes for each group (n = 16) were incubated in an aerobic atmosphere at 37°C. In this way, it was possible to evaluate the growth of every possible type of microorganisms – aerobic, facultative anaerobic and anaerobic.

After a 14-day incubation period, the presence or absence of turbidity was assessed. Turbidity in the sample was regarded as the evidence of microbial growth in the broth; no visible growth in the broth was considered effective disinfection. The broth turbidity analysis is shown in Fig. 1.

Microhardness test

The specimens were mounted on a low-speed diamond machine (IsoMet 1000). Two slices of 2-millimeter thickness were obtained from each third of each root. The 1st slice of each third was used to determine microhardness.

The specimens were ground wet with 600- and 1,200-grit silicon carbide papers, polished with felt disks, and embedded in aluminum oxide paste at a low speed. Then, they were washed under running water, dried with gauze and examined at ×40 magnification to confirm smoothness. Dentin microhardness was measured with a Knoop indenter (HMV-2; Shimadzu, Barueri, Brazil) under a load of 25 g at a dwell time of 10 s. For each slice, 12 indentations were made (3 at each quadrant) at each depth – 30, 60 and 120 µm from the root canal lumen. The means for the quadrants were calculated.

Statistical analysis

The microbiological data was evaluated with the Kruskal–Wallis test and Dunn's post-hoc test. The microhardness data was evaluated according to normal and homogenous distribution (the Shapiro–Wilk test and Levene's test, respectively), followed by the two-way analysis of variance (ANOVA) (with regard to the sterilization methods and the root thirds) and Tukey's test. The analyses were performed using the IBM SPSS Statistics for Windows software, v. 25.0 (IBM Corp., Armonk, USA), at a 5% significance level.

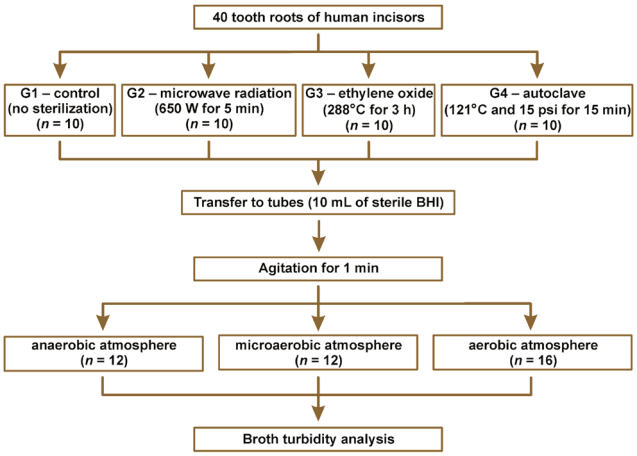

Fig. 1. Diagram of broth turbidity analysis
BHI – brain heart infusion.

Results

Broth turbidity analysis

The 1st null hypothesis of this study was rejected. The statistical analysis revealed significant differences between the sterilization methods. The roots submitted to autoclaving were 100% sterile (100% of samples were non-turbid), which was different from the other methods ($p < 0.05$). Ethylene oxide had 50% sterility. The control group (no sterilization) had 0% sterility; it was statistically similar to microwave radiation, which produced only 10% sterility ($p > 0.05$).

The patterns of turbidity, sterility, and the mean values for each group are presented in Table 1 and illustrated in Fig. 2. Figure 3 shows the samples after the experiment.

Table 1. Broth turbidity after 14 days per visual analysis and the percentage of root sterility for each experimental group (n = 10)

Groups	Broth turbidity†	Sterility [%]	*Me*	*Z*-value	*M*
Microwave radiation	(9/10)	10	2.0	1.87	1.9[a]
Ethylene oxide	(5/10)	50	1.5	–0.62	1.5[b]
Autoclave	(0/10)	100	1.0	–3.75	1.0[c]
Control	(10/10)	0	2.0	2.50	2.0[a]

Me – median; *M* – mean. † Broth turbidity indicates the presence of microorganisms. Different letters in superscript indicate statistically significant differences between the groups (Kruskall–Wallis test ($p = 0.001$) and Dunn's post-hoc test ($p < 0.05$)).

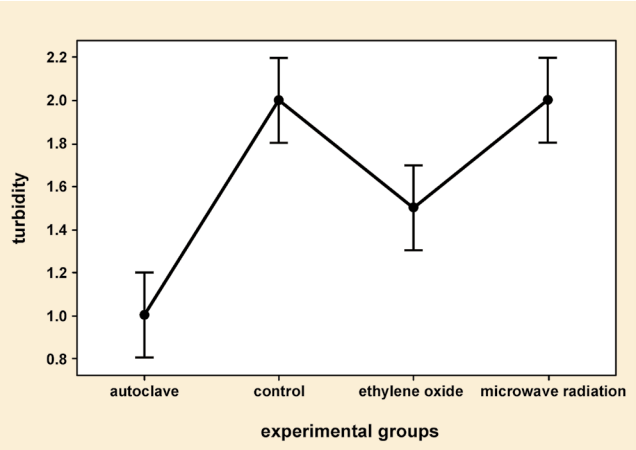

Fig. 2. Microbiological data for each experimental group
Data presented as median (*Me*) and 95% confidence interval (*CI*).

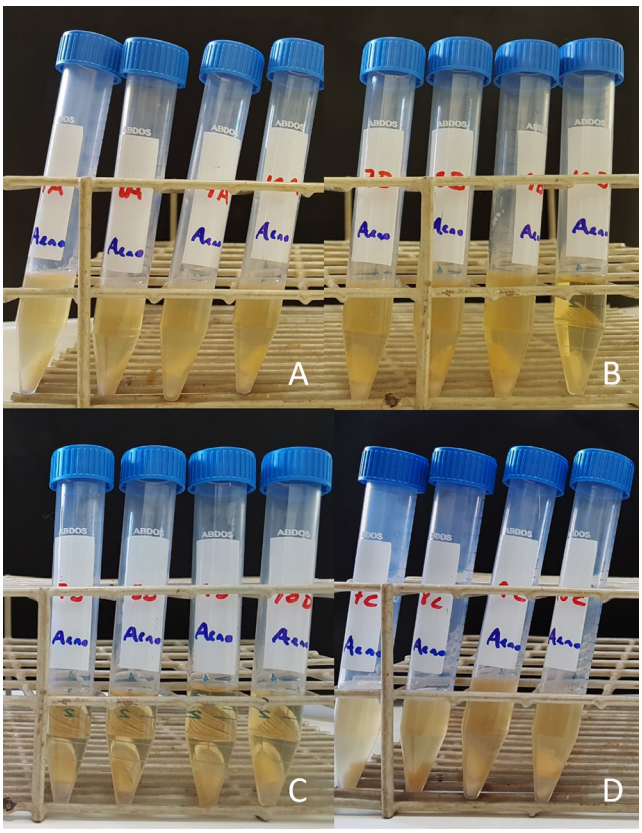

Fig. 3. Roots individually stored in tubes containing brain heart infusion (BHI), showing clear and turbid samples
A – microwave radiation; B – ethylene oxide; C – autoclave; D – control (no sterilization).

Microhardness test

The 2nd null hypothesis of this study was rejected, as some of the tested protocols significantly reduced dentin microhardness (expressed as Knopp hardness number (KHN)) in human root canal dentin. The two-way ANOVA revealed significant differences in the microhardness values between the sterilization methods ($p < 0.0001$), the root thirds ($p = 0.0041$), and with regard to the interaction of the 2 factors ($p = 0.0265$). The control group showed the highest mean value (91.09 ±4.08[a]), which was statistically significantly different from those for the microwave radiation (66.69 ±9.43[b]), ethylene oxide (71.42 ±9.61[b]) and autoclave (73.53 ±15.02[b]) groups. The highest microhardness values were found in the cervical (77.73 ±10.48[a]) and middle (81.25 ±15.26[a]) thirds ($p > 0.05$). The lowest values were found in the apical third (68.06 ±12.50[b]). The microhardness mean and standard deviation (*M* ±*SD*) values for each experimental group are detailed in Table 2.

Concerning the interaction of factors, the ethylene oxide, autoclave and control groups showed the highest microhardness values in the cervical and middle thirds ($p < 0.05$), whereas the microwave radiation group showed the lowest values in the middle third.

Discussion

In the current coronavirus disease 2019 (COVID-19) context, worldwide concern has arisen due to the risk of cross-contamination. Dental practice, education and research are some of the most highly impacted fields.[23] The oral cavity linked with the respiratory tract is a natural habitat for many microorganisms, including several bacterial species, several potentially pathogenic fungi, and viruses, such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[24,25]

To reduce the risk of transmitting blood pathogens, tooth roots must be biologically safe before they are used. At the same time, they should have their normal mechanical properties maintained.[5,7,26] However, taking into account bacterial colonization, the tubular invasion of bacteria[27] and the complex microstructure of the teeth, tooth samples are difficult to sterilize, as sterilization measures can modify the tooth properties, and even a minor change can affect the results of the analysis.[3]

Table 2. Microhardness [KHN] according to the different methods of sterilization and root thirds

Root third	Microwave radiation	Ethylene oxide	Autoclave	Control
Cervical	77.57 ±19.74^{Ba}	75.25 ±29.40^{Ba}	66.42 ±22.49^{Ba}	91.70 ±4.65^{Aa}
Middle	60.85 ±18.75^{Ba}	78.52 ±20.96^{Ba}	90.79 ±19.29^{Ba}	94.83 ±7.94^{Aa}
Apical	61.65 ±8.71^{Bb}	60.49 ±23.10^{Bb}	63.38 ±9.68^{Bb}	86.73 ±10.13^{Ab}

Data presented as mean ± standard deviation (*M* ±*SD*).
Different uppercase letters in superscript indicate statistically significant differences between the sterilization methods ($p < 0.05$). Different lowercase letters in superscript indicate statistically significant differences between the root thirds ($p < 0.05$).

Previous authors have already tested the sterilization methods assessed in this study, and these protocols are commonly used in health services and research.[8,12,15]

Broth turbidity is a widely used test that is simple to perform. When correctly conducted, the turbidity measurements guarantee the quality and accuracy of the results.[6,28] Contrary to other studies,[6,7,12] we evaluated the growth of all types of microorganisms (aerobic, facultative anaerobic and anaerobic) to better simulate clinical conditions.

Mandibular incisors were used in this study due to their smaller size and thickness as well as lower anatomical variability as compared to other dental groups.[5] Furthermore, to the best of our knowledge, this is the first investigation to use 10-millimeter-long contaminated roots and not only small dental slabs.[2] This study aimed to support in situ research using acrylic palatal devices with human roots to allow biofilm accumulation.[5]

The 1st null hypothesis of this study was rejected. The outcomes revealed differences among the sterilization methods with respect to the presence of viable microorganisms. The roots submitted to autoclaving were 100% sterile; thus, this was the only method that was able to completely eliminate microorganisms from the extracted roots. The autoclaving process is the method recommended by ADA and by previous studies for materials exposed to body fluids.[1,6,7] Its main advantages are its rapidness and efficiency, excellent reliability, and cost-effectiveness. The limitation is that this method cannot be used with sensitive equipment and materials.[11] Autoclave sterilization is recommended for preventing cross-contamination during laboratory bonding tests with human teeth.[4] Talge Carvalho et al. stated that the autoclave sterilization of teeth slabs produced no significant changes in dentinal tubule morphology or dentin chemical composition.[28] Western and Dicksit considered autoclaving to be 100% effective and reliable.[29] However, extracted teeth with amalgam restorations should not be autoclaved because of mercury release.[30]

Concerning ethylene oxide sterilization, the literature reports that it provides powerful gas penetration and is the gentlest for delicate materials.[31] However, it is expensive, toxic, explosive, and requires long time cycles and a specialized aeration chamber.[11] For these reasons, it is better for hospitals and not practical for dental clinics. Ethylene oxide destroys microorganisms by chemically reacting with nucleic acid, and interfering with cellular metabolism and reproductive processes, which renders the affected microbes nonviable.[32] The results of the current study showed lower sterility than expected (50% of the roots were sterile), which is probably due to the parameters used. Other authors have also warned about the inability of ethylene oxide to sterilize human teeth.[4,15] Thomas et al.[14] found a significant interaction between the depth of caries lesions in enamel and ethylene oxide sterilization in vitro.[14] Ethylene oxide is only 20–36% effective against *Bacillus subtilis* in extracted teeth.[15] The literature does not provide any evaluation of the ethylene oxide sterilization of human teeth with the consideration of different equipment parameters.

Microwave radiation has been indicated for the disinfection/sterilization of laboratory materials.[31] However, few studies have assessed its ability to reduce the microbial load on dental substrates.[13,33] In the current study, microwave radiation was not a reliable method for sterilizing human roots. The mechanism by which microwave radiation kills microorganisms is not entirely understood. Microwaves promote decontamination by both thermal and nonthermal processes, since the electromagnetic field induced by microwaves causes biological and chemical alterations in cells.[13,33] Therefore, microwave radiation is generally used as a disinfectant, but it is ineffective for use with extracted teeth in laboratory research.

Microhardness measurement is one of the simplest methods of non-destructive mechanical characterization.[2] Dentin and enamel microhardness depend on the amount of mineral structure content, and provide indirect evidence of mineral loss or gain in hard dental tissues.[2] The mineral content of dentin decreases with age, which leads to dental fragility.[17]

The 2nd hypothesis of this study was rejected, since dentin microhardness was reduced after conducting the different sterilization processes. These results are consistent with those of previous studies, in which the sterilization methods also decreased microhardness.[2,8,9,18] This effect has been associated with calcium loss, which results in dentin demineralization and softening. It is hypothesized that the high temperature and pressure during sterilization disrupt collagen and denature the organic component in dentin, which affects microhardness.[1,2,29]

Reduced microhardness values may influence the results of studies that assess mechanical properties, such as bond strength, of resin-based materials.[1,8,17–19] Moreover, the sterilization process might increase the risk of tooth fracture, which is unwanted in preclinical endodontic training.[17] Other authors measured the surface microhardness, roughness and bond strength of denture teeth and acrylic resin denture bases, and verified the decrease in material microhardness; this outcome was considered a common and standard consequence of all sterilization methods.[27,34]

Finally, it is important to emphasize that infection control is not limited to the disinfection/sterilization of extracted teeth. Students and researchers should wear gloves, masks and eye protection when handling extracted teeth.

In this study, the best sterilization method for eliminating microorganisms was autoclaving, even though it reduced microhardness. The choice of method depends on particular situation, analysis and the study objective. For in situ and in vitro research with human teeth, we recommend autoclave sterilization before the laboratory steps. Further studies with additional methods should be conducted to determine sterilization methods and protocols that cause the least alteration of tooth mechanical properties.

Conclusions

Based on the experimental methods and results of this study, it can be concluded that autoclaving is the most effective sterilization method to eliminate microorganisms from 10-millimeter-long human roots. Regardless of the method tested, the roots submitted to disinfection/sterilization had their dentin microhardness reduced.

Ethics approval and consent to participate

This study was approved by the institutional Ethics Committee at the Ribeirão Preto Dental School, University of São Paulo, Brazil (No. of approval: 37032114.1.0000.5419).

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

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