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Volume and/or Time of NaOCl Influences the Fracture Strength of Endodontically Treated Bovine Teeth

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The aim of this study was to evaluate whether amplifying the volume and/or time of contact of NaOCl affects the fracture strength of endodontically treated bovine teeth. Four bovine incisors from 10 animals were allocated into 4 groups using a split-mouth design. Root canals were instrumented using a sequence of 4 manual stainless steel files and irrigated with a 5.25% alkalized NaOCl solution. The total volume and time of irrigation, per sample, varied among the groups as following: standard volume and time of contact - 15 mL/11.5 min; volume raise - 30 mL/11.5 min; time of contact raise - 15 mL/19 min; and volume and time of contact raise - 30 mL/19 min. Samples were subjected to a fracture resistance assay. At p=0.05, two-way ANOVA statistically scrutinized the results. Effect size of NaOCl time of contact and volume were also calculated (η^2). The variation in time (p=0.000), volume of irrigation (p=0.000) and the combination of both (p=0.038) negatively influenced the fracture resistance. Standard volume and time of irrigation showed the highest fracture strength while isolated increase in volume or time reduced in 25% and 37%, respectively, the fracture resistance; the simultaneous increase in volume and time of irrigation promoted a reduction of 47%. Effect size of NaOCl time of contact was superior (0.746) than the volume (0.564). Raising the volume and/or time of a 5.25% alkalized NaOCl solution reduces the fracture resistance of endodontically treated bovine teeth.

Key Words: volume and time of NaOCl, bovine teeth, endodontically treated teeth, fracture resistance, sodium hypochlorite.

Introduction

Chlorine solutions (NaOCl) are undeniably the most widely accepted irrigants to flush root canals in Endodontics (1). They are highly recommended on either vital or necrotic cases due to their unique ability to dissolve organic tissue and broad-spectrum anti-bactericidal effect (2-4), which are essential to achieve optimal cleaning and disinfection before the root canal can be considered ready for filling.

These essential features of NaOCl solutions are directly related to the amount of available chlorine (5,6). Because the amount of available chlorine rapidly declines in the root canal as it is consumed during the interaction with tissues and microorganisms (1,5,7), to achieve an optimal cleaning effectiveness, substantial volumes of NaOCl should be delivered into the root canals. This provides a constant renewing of the available chlorine (5,6) and, consequently, an efficient debridement by flushing both organic tissues and dentinal debris generated by instrumentation (1). Similarly, the overall time of contact of the solution with the root canal walls is also directly related to the number of bacteria and biofilm destroyed (1). Moreover, the overall complexity of the root canal system and the limited fluid dynamics achieved by syringe irrigation (8), give additional rationale to support the idea of improving volume and time of contact of NaOCl to root dentin, enhancing the chances of optimal root canal disinfection.

Although fundamental, tissue dissolution by NaOCl solutions negatively impacts over the dentinal tissue, leading to the depletion of its components of organic nature (9), which irreversibly changes the dentin scaffold, causing several unwanted physical and mechanical changes, such as micro-crack formation and the reduction in flexural strength, microhardness, and modulus of elasticity (10-18), as well as a dry weight reduction by 14% (19). It has been recently speculated that the proteolytic effect by different NaOCl solutions used for an average time of contact of 26 min induced a mean root weakening of about 30% (20). Following a cause-effect rationale, if volume and time of contact are raised, so it might be the overall dentin proteolysis, intensifying the side effect on the organic scaffold of dentin, ultimately increasing root proneness to fracture.

Because tooth survival may be significantly reduced in weakened roots, the present study was designed to investigate the effect of increasing the volume and/or time of irrigation with a 5.25% NaOCl solution on the fracture strength of endodontically treated bovine teeth. The null hypothesis tested was that neither volume nor time of irrigation was relevant factors to modify the root strength of bovine teeth.

Materials and Methods

Sample Size Calculation

Based on the study by Souza et al. (20), an effect size of 1,05 was identified and input together with an alpha-type error = 0.05 and power b = 0.95 to an a priori analysis of variance (ANOVA) fixed-effects model (F family, G*Power 3.1.1 for Windows, Heinrich-Heine-Universität Düsseldorf, Germany). A minimum total sample size of 21 teeth (5.25 teeth per group) and a critical F of 3.19 were required for observing significant differences.

Sample Selection and Preparation

The 4 anterior incisors from 10 cattle with similar ages were extracted from the mandibular jaws, which were donated to this study after animals slaughtering for feeding purposes. To obtain anatomic matching among the groups, each selected incisor was randomly assigned to 1 of 4 groups, resulting in 10 teeth per group and a total sample size of 40 teeth. Root diameters at the level 8 mm from the apex were measured to be within 8-10 mm. Diameters lower or higher than the established parameters resulted in the exclusion of the cattle. After extraction, the specimens were stored in saline until use.

After cross-sectioning the roots at levels 5 mm coronally and 10 mm apically to the cement-enamel junction by means of a low speed saw (VC-50 Precision Diamond Saw; Leco, Miami, FL, USA) under copious water-cooling, the samples remained with 15 mm in length. Hedströen files (Dentsply Maillefer, Ballaigues, Switzerland) removed the pulp tissue.

Preparation and Characterization of Irrigating Solutions

To obtain freshly prepared technical-grade NaOH-stabilized alkaline 5.25% NaOCl solution, a 2-mol/L NaOH solution was mixed with a standard 10% NaOCl solution (Special Farma; São Luis, MA, Brazil). Immediately before and after the experiments (20 days later), the available chlorine of the solutions was verified using a standard iodine/thiosulfate titration method. The pH of NaOH-stabilized NaOCl was also determined after 40 min of contact with a bovine root canal to confirm that the stabilization was effective using a calibrated pH electrode (Model 6.0210.100; Metrohm, Herisau, Switzerland). The root canal preparations were performed in a room with controlled temperature.

Root Canal Instrumentation and Irrigation

The root canals were instrumented using a sequence of 4 hand K-files (Dentsply Maillefer), which were selected after determining the first instrument to bind at 1 mm from the apical opening. All files remained instrumenting the fully NaOCl-filled root canal for 1 min maximum. The root canals were irrigated following different protocols according to the volume and time of contact of the NaOCl solution, as described below:

Group standard volume and time of contact (control group) - 3 mL irrigation per 1.5 min per file, resulting in a total ratio of 15 mL/11.5 min;

Group volume raise - 6 mL irrigation per 1.5 min per file, resulting in a total ratio of 30 mL/11.5 min;

Group time of contact raise - 3 mL irrigation per 3 min per file, resulting in a total ratio of 15 mL/19 min and;

Group volume and time of contact raise - 6 mL irrigation per 3 min per file, resulting in a total ratio of 30 mL/19 min.

The rate of irrigation per each group was controlled using a VATEA peristaltic pump (ReDent Nova, Ra'anana, Israel) and irrigation solution was delivered into the root canal using a 10 mm luer-lock syringe and a 27-G needle (NaviTip; Ultradent Products, South Jordan, UT) reaching 3 mm from the apex. An aspiration tip was used to suck out the extruded solution adjacently to the coronal opening to make sure that any solution was drawn off the external root surface. To prevent the extrusion of irrigating solution throughout the large apical opening created after root sectioning, the apexes of each tooth was sealed off with wax. All teeth received a final irrigation with 5 mL of distilled water for 2 min, removing any solution remnants from the root canals. Then, all root canals were stored at 37 °C with 100% humidity until the strength tests were performed.

Simulation of the Periodontal Support Apparatus

A 0.2- to 0.3-mm-thick wax layer was created over the root up to 2 mm below the cementoenamel junction by immerging the teeth in melted wax (Horus; Herpo Produtos Dentários, Petrópolis, RJ, Brazil). Further, the roots were embedded in a polystyrene resin (Cristal, Piracicaba, SP, Brazil) dispensed in 21-mm diameter and 25-mm high polyvinyl chloride cylinders. After the set of the resin, the teeth were withdrawn from the polystyrene resin, the wax removed from root surface and resin cylinder "sockets", and further reinserted into the respective cylinder socket filled with a polyether impression material (Impregum Soft; 3 M/ESPE, Seefeld, Germany). The excesses of impression material were removed, ultimately resulting in a 0.2-0.3 mm simulated periodontal ligament.

Fracture Strength Test

A compressive load driven at a crosshead speed of 0.5 mm/min was simulated by means of a servo-hydraulic universal testing machine (EMIC DL2000; EMIC Equipamentos e Sistemas de Ensaio Ltda, São José dos Pinhais, PR, Brazil) until fracture of the specimens. An apparatus that allowed a 45° angle formation with the EMIC loading tip was used to simulate a traumatic shock on the middle third of the crowns from a buccal-lingual direction. The final load demanded to fracture the samples was recorded in newton.

Statistical Analysis

Assumptions of Gaussian distribution and homogeneity of the variance were confirmed (Shapiro-Wilk and Levene tests, $p>0.05$), leading to a two-way ANOVA analysis to study the effects of the independent variables (1) volume and (2) the time of contact of the solution, as well as, (3) the combination of both independent variables in the fracture strength of bovine teeth (dependent variable). The effect size of variables volume and time of contact over fracture strength was also calculated using the squared Eta (η^2). A 5% cut-off level was set for determining significance using the SPSS v.21 for Macintosh (SPSS Inc., Chicago, IL).

Results

The variation in time of contact ($p=0.000$), volume ($p=0.000$) and the combination of both time and volume ($p=0.038$) negatively influenced the maximum load required to fracture the samples. Teeth irrigated with standard volume and time (control group) presented the highest fracture strength (1496.76 ±234.40), while the sole increase in either volume (1133.15 ±50.17) or time of contact (933.32 ±78.95) significantly reduced the fracture resistance by 25 and 37%, respectively. The simultaneous increase of both volume and time of contact (804.81 ±39.34) significantly weakened the roots by about 47%. Squared Eta revealed a superior effect size for time of contact (0.746) compared to the volume of the solution (0.564), indicating a superior negative influence of time of contact over the fracture strength of endodontically treated bovine teeth. Figure 1 represents the major findings of this study.

Discussion

The proteolytic capacity against organic tissue and non-specific antimicrobial effectiveness are quoted as the most relevant features of the chlorine-based solutions (2,3). To achieve these goals and raise the chances of optimal cleanness within the root canal, NaOCl solutions are volume and time-dependent (1). The present study demonstrated that the increase in volume and/or time of contact of a 5.25% alkalized-NaOCl has reduced the fracture strength of bovine teeth; thus, the null hypothesis was rejected. Although several previous studies demonstrated that the increase in the NaOCl concentration reduces the fracture resistance of the tooth (12,13,17,18,21), this is the first study assessing the impact of volume and/or time of contact of NaOCl on the fracture resistance of endodontically treated teeth.

The current results might be interpreted as the combination of several factors such as increased modifications in dentinal microhardness, elastic modulus and flexural strength induced by available chlorine with higher volume and time of contact NaOCl to dentin. Changes in microhardness reveal alterations in both organic and inorganic parts of root dentin, whereas a decrease in elastic modulus and flexural strength may potentially turn dentin more brittle (18). The deleterious effects of NaOCl solutions on collagen and proteoglycan matrix might result in dentin contraction, increasing stress concentration and crack propagation, and ultimately contributing to a meaningful reduction in the fracture strength (9). Usually the higher the concentration of NaOCl, the stronger are the deleterious effects over the dentine (12,13,17,18,21) that ultimately can turn teeth more brittle. These known effects of chlorine-based solutions on root dentin, especially in high concentrations, are certainly reasons for the reduction in root toughness, observed in the groups herein.

Interestingly, the increase in time of contact without the raise in volume was also able to lead to a negative reduction in root toughness by about 37%. This might indicate that the amount of available chlorine does not reduce as rapid as expected (1,5,7) maintaining, even for a longer time frame, the deleterious effect of chlorine

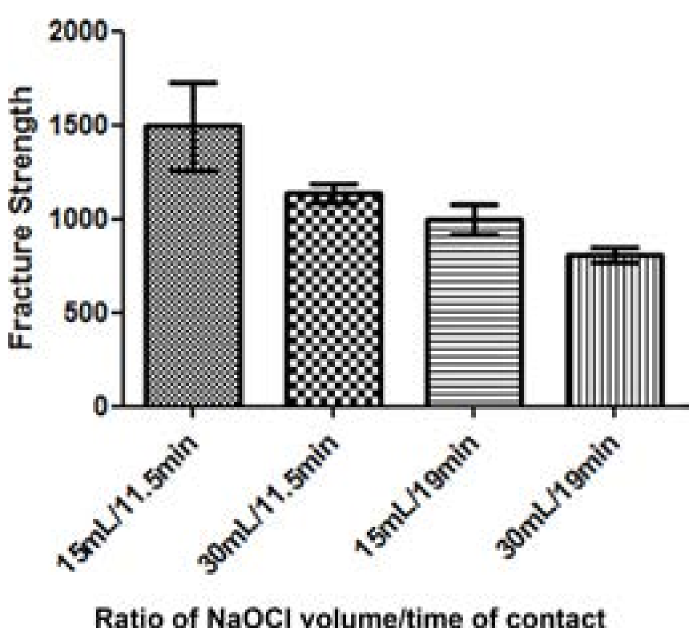

Figure 1. Fracture strength of bovine teeth per group according to the variation in the ratio of volume and time of contact with dentin by a 5.25% alkalized NaOCl solution. Increase in volume, time of contact and the combination of volume and time of contact significantly reduced the fracture strength of endodontically treated bovine teeth at a p=5%.

over the dentin scaffold. In fact, according to the effect size calculation (η^2), time of contact appears to strongly adversely affect the fracture strength compared to the raise in NaOCl volume. This is an important output to understand the role of time of contact and volume of NaOCl solutions over the dentin scaffold. Obviously, the simultaneous raise of volume exchange and time of contact of NaOCl certainly amplify both the amount of available chlorine and the time frame of contact, resulting in almost 50% reduction in root toughness as observed in the present study.

The specimens from human teeth are preferred for *ex vivo* studies because they allow testing of the study hypothesis in a more clinically relevant substrate. In this way, the use of bovine teeth in the present study could be regarded as a limitation. However, the increasing challenge of gathering viable human teeth for dental research and ethical issues has led to the use of alternatives animal samples. Among these alternatives, bovine teeth appears as a good substitute for human teeth, as they have similar modulus of elasticity, tensile strength, chemical composition and number and distribution of dentinal tubules when compared to human teeth (22-25). Moreover, bovine teeth are easier to obtain in large quantities and enable the possibility to easily standardize groups according to age. In addition, using a split-mouth design represents an experimental improvement. These approaches reduced the bias related to anatomy, which is usually difficult to exclude when using human teeth. Not less important is the simulation of the periodontal apparatus that has been found to approach the *in vitro* setup to clinical reality by distributing the fracture pattern to radicular areas (26).

From a clinical stand point, it would be sensible to use NaOCl in a suitable volume and/or time to have minimal effects on the mechanical properties of the tooth while achieving the desired debridement effect. However, this optimum protocol has not been determined yet. In addition, root canal debridement with NaOCl should be considered with caution. Although the softening effect exerted by this agent on the dentinal walls could be of clinical benefit to allow rapid root canal preparation, the alteration to root dentin may affect adhesion, as well as the sealing ability of sealers to the treated dentin surfaces, and may predispose teeth to fracture.

Under the conditions of the current study, it can be concluded that raising the volume and/or time of a 5.25% alkalized NaOCl solution negatively influence the fracture resistance of endodontically treated bovine teeth.

Resumo

O objetivo desse estudo foi o de avaliar se o aumento de volume e/ou tempo de contato do NaOCl afeta a resistência à fratura de dentes bovinos tratados endodonticamente. Quatro incisivos bovinos de 10 animais foram alocados em 4 grupos usando um desenho experimental de boca dividida. Os canais radiculares foram instrumentados usando uma sequência de 4 limas manuais de aço inoxidável e irrigados com uma solução de NaOCl alcalinizada a 5,25%. O volume total e o tempo de irrigação, por amostra, variaram entre os grupos da seguinte forma: volume e tempo de contato padrão (grupo controle) - 15 mL/11,5 min; aumento de volume - 30 mL/11,5 min; aumento no tempo de contato - 15 mL/19 min; e aumento no volume e no tempo de contato - 30 mL/19 min. As amostras foram submetidas a um ensaio de resistência à fratura. Com p=0.05, o teste two-way ANOVA analisou estatisticamente os resultados. O tamanho do efeito do tempo de contato e volume de NaOCl também foi calculado (η2). A variação no tempo (p=0,000), no volume de irrigação (p=0,000) e a interação entre ambos (p=0.038) influenciaram negativamente a resistência à fratura. O volume e o tempo padrão de irrigação apresentaram a maior resistência à fratura, enquanto o aumento isolado no volume ou no tempo de contato reduziram 25% e 37%, respectivamente, a resistência à fratura; o aumento simultâneo do volume e tempo de irrigação promoveu uma redução de 47%. O tamanho do efeito do tempo de contato com o NaOCl foi superior (0,746) ao volume (0,564). Aumentando o volume e/ou o tempo de uma solução de NaOCl alcalinizada a 5,25% reduz a resistência à fratura de dentes bovinos tratados endodonticamente.

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