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COMPUTED TOMOGRAPHIC DETECTION OF CRACKS AFTER LIQUID NITROGEN CRYOTHERAPY OF ROOT APEXES

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ABSTRACT

Este Cryotherapy with liquid nitrogen (N_2) has been effective to eliminate microorganisms such as *Enterococcus faecalis*, but its effects on dental tissues have not yet been well established. This *in vitro* study evaluated the formation of cracks in root apexes after cryotherapy. Forty uniradicular human teeth were endodonticall treated, resected (apicectomy), retroprepared and submitted to two 60-second freezing cycles with N_2. The number of cracks along the 5-mm apical root segment was observed by using cone beam computed tomography and data submitted to McNemar and Wilcoxon tests ($p<0.05$). A significant increase in the number of teeth with complete cracks after cryotherapy was observed ($p=0.017$). Therefore, the use of the present cryotherapy protocol is still not safe as adjuvant method for parendodontic surgeries in order to eliminate microorganisms in refractory periapical lesions.

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INTRODUCTION

The periapical lesion or periradicular periodontitis occurs as a result of an immune and inflammatory response to microorganisms that infected the root canals system. The presence of persistent infection is the main reason for endodontic treatment failure. Several factors can be related to the effectiveness of the endodontic treatment, such as the patient's systemic conditions, age, sex, complex anatomy of the root canal system due to the presence of accentuated radius of curvature (Pimentel *et al.,* 2020) and refractory periapical infections. Aerobic and facultative anaerobic microorganisms, the most commonly species found in treated canals and periapical area, are typically observed in higher amounts in refractory periapical infections (Krithikadatta; Indira; Dorothykalyani, 2007; Pavaskar *et al*., 2012). Among them, Enterococcus faecalis (E. faecalis) is the most prevalent due to its virulence, ability to compete with other microorganisms, to invade dentinal tubules and to resist to nutritional deprivation (Stuart, 2006; Zhang; Du; Peng, 2015); this specie can colonize the root canal even after chemical-mechanical preparation and intracanal medication (Baik *et al*., 2008). Paraendodontic surgery is an alternative treatment for persistent periapical lesion, in cases whereas endodontic retreatment is discarded and tooth preservation is intented. Modern paraendodontic surgery with the use of ultrasonic tips, operating microscope, microinstruments and biocompatible retrofilling materials presents an approximately 90% success rate (Chen *et al*., 2015). *In vitro* cryotherapy has been investigated as adjuvant method to eliminate E. faecalis and increase surgical success rate (BATISTA, 2006). Cryotherapy is an effective therapeutic method of controlled and non-selective tissue destruction that involves the application of cryogenic substances, such as liquid nitrogen (N_2) at extremely low temperatures (approximately -196 °C) (Prasad *et al*., 2009).In comparison to other treatment methods, the cryotherapy offers unique

advantages particularly in bone tissue since, despite of the efficient cell destruction, the inorganic bone matrix is preserved and serves as a substrate for the formation of new tissue (Benagliam; Jardim; Mendonça, 2014). There are still no clear guidelines for the safe clinical use of N_2 in root canals or surfaces with refractory apical periodontitis, due to the lack of information regarding the occurrence of cracks. The cracks can cause bone loss due to microorganism colonization of the root surface and lead to tooth extraction (Ricucci *et al*., 2015).

MATERIALS AND METHODS

After receiving approval of the study by the Ethics Committee for Human Research of the Federal University of Espírito Santo under file number 1.042.984, forty unirradicular human teeth were gathered and cleaned with manual and ultrasonic scalers (Enac, OsadaEletric, Tokyo, Japan). The endodontic access was performed with aid of tapered conical burs and ultrasonic tips (TRA24D, Dental Trinks, Sao Paulo, Brazil) ultrasound inserts; then, teeth were immersed in 1% NaOCl during 24 hours and washed in running water for 10 minutes. After determining their actual lengths, the root canals were instrumented with a rotary system up to file X2 (ProTaper Next, Dentsply-Maillefer, Konstanz, Switzerland), irrigated with 1mL of 2.5% NaOCl between each instrument change and rinsed with 3 mL at the end. Then, teeth were irrigated with 3 mL of 17% EDTA (ethylenediaminetetraacetic acid) and 5 mL of 0.9% physiological solution, dried with with absorbent paper cones and filled with a resin-based root-canal sealer (AH Plus, Dentsply-Maillefer) and tapered gutta-percha cones by using the Tagger's hybrid technique. Subsequently, apicectomy was performed perpendicularly to the long axis of each tooth: 3 mm of the root apex was resected with aid of 28-mm-length Zekrya bur, retroprepared with ultrasonic tips (TRA18D, Dental Trinks) and irrigated with physiological solution. All procedures were performed with the use of an operating microscope (DF Vasconcellos, São Paulo, Brazil). Dental crowns were fixed on wax bases and root apexes remained exposed; thus, N_2 was sprayed through a 0.57-mm-diameter-aperture bent cryoprobe (Cry-AC model #103-20, Brymill, Ellington, CT, USA) connected to a portable cryogenic device (CRY-AC-3, Brymill). The cryoprobe was positioned 1 mm from the root apex (Figure 1) and the following protocol was used: one 60-second freezing cycle, followed by a 4-minute thawing interval and another application of 60 seconds. Next, teeth were stored in 0.9% physiological solution at room temperature.

Figure 1.a) Bent spray cryoprobe Model #103-20;b) Portable cryogenic device (CRY-AC-3); c) N_2 sprayed 1 mm from the root apex

Before and after cryotherapy, teeth were fixed on wax bases in order to maintain the same position and scanned with cone beam computed tomography (CBCT). Images were acquired by using the i-CAT Cone Beam 3D Dental Imaging System (Imaging Science International, Hatfield, PA, USA) preset with 0.2 mm voxel size, 120 kVp and 5 mA. Multiplanar reconstructions of 1-mm-thick slices were obtained by using a specific software (Xoran version 3.1.62, Xoran Technologies, Ann Arbor, MI, USA) with aid of Angio Sharpen Medium filter and 280% zoom. CBCT images were examined by three evaluators (one endodontist and two radiologists) on a 20.1-inch high resolution monitor (FlexScan L887, EizoNanao, Matto-Ishikawa, Japan), up to a maximum of 15 teeth per session, with a 48-hour interval between sessions in order to avoid visual fatigue. The evaluators were blinded regarding the acquisition moment of all images (before or after cryotherapy) and registered the presence of crack (absent or present), type (complete, intradentin and incomplete) and the number of cracks along the 5-mm apical root segment (0 to 1 mm, 1.01 to 2 mm, 2.01 to 3 mm, 3.01 to 4 mm, and 4.01 to 5 mm). The presence of cracks before and after cryotherapy was compared by using the McNemar test, while the Wilcoxon test was used to evaluate the number of cracks along the 5-mm apical root segment.

RESULTS

The number teeth with complete, intradentin and incomplete root cracks are shown in Table 1. A significant difference was found between the number of complete cracks before (18 teeth or 45%) and after cryotherapy (30 teeth or 75%) (p=0.017).

Table 1. Absolute values and percentages of the number teeth with different crack types before and after the application of N_2

Crack types	Numberofteeth				
	Before cryotherapy		After cryotherapy		p-value
	n	%	n	%	
Complete	18	45.0	30	75.0	0.017*
Intradentin	8	20.0	9	22.5	1.000
Incomplete	6	15.0	10	25.0	0.388
Teeth	40	100.0	40	100.0	-

n: number; %: percentage. *Significant difference (McNemar test, p<0.05).

Table 2. Descriptive statistics of the number of cracks per type diagnosed before and after the application of N_2

Crack types	Numberof cracks				
	Before cryotherapy		After cryotherapy		p-value
	Mean	SD	Mean	SD	
Complete	0.3500	0.4336	1.0748	0.8453	0.000*
Intradentin	0.1163	0.2874	0.1665	0.3459	0.524
Incomplete	0.1080	0.3050	0.1663	0.3535	0.302
Total	0.5750	0.7472	1.4083	0.9042	0.000*

SD: standard deviation.*Significant difference (Wilcoxon test, p<0.05).

Table 3. Descriptive statistics of complete cracks distributed along the 5 mm apical root portion, before and after cryotherapy

Apical root segment (mm)	Evaluationmoment	Mean	SD	p-value
0 a 1	Before cryotherapy	0.0915	0.2264	0.118
	After cryotherapy	0.2163	0.3958	
1.01 to 2	Before cryotherapy	0.0912	0.1845	0.005*
	After cryotherapy	0.3415	0.4862	
2.01 to 3	Before cryotherapy	0.0830	0.2353	0.023*
	After cryotherapy	0.3083	0.4919	
3.01 to 4	Before cryotherapy	0.0582	0.1671	0.271
	After cryotherapy	0.0248	0.0880	
4.01 to 5	Before cryotherapy	0.0250	0.1169	0.020*
	After cryotherapy	0.1835	0.4134	
Total	Before cryotherapy	0.3500	0.4336	0.000*
	After cryotherapy	1.0748	0.8453	

SD: standard deviation.*Significant difference (Wilcoxon test, p<0.05).

It should be noted that the same tooth may account for more than one crack type. On average, a significant lower number of cracks (0.575±0.747) was detected before N_2 application in comparison to

the amount registered after cryotherapy (1.408±0.904) (p=0.000). An increase in the number of all crack types was observed after cryotherapy; however, a significant difference was only found for complete cracks (p=0.000) (Table 2). Table 3 describes the number of complete cracks per millimeter along the 5-mm apical root segment. Before and after cryotherapy, a significant difference in the number of complete cracks was found at the following ranges: "1.01 to 2mm" (p=0.005), "2.01 to 3mm" (p = 0.023), and "4.01 to 5 mm" (p=0.020). The increase in the number of both intradentin and incomplete cracks before and after the application of N_2 was not significant (p>0.05) along the 5-mm apical root segment.

DISCUSSION

In this study, a significant increase in the number of complete cracks was found after the application of N_2. Fayad, Ashkenaz and Johnson (2012) and Neves *et al*. (2014) mentioned that complete cracks are more easily visualized than incomplete cracks. Several studies suggest that endodontic treatment (both preparation with nickel-titatium files and condensation of filling material) may cause root cracks. According to Capar *et al*. (2014) and Li *et al*. (2015), cracks may occur due to root canal shaping procedures and rotary instrumentation. The authors observed that ProTaper Next system caused fewer dentinal cracks compared with the ProTaper Universal (Dentsply-Maillefer, Switzerland). Conversely, De-Deus *et al.* (2015) and Zuolo *et al*. (2017) reported that rotary instrumentation did not induce the formation of cracks in root canal dentin. For Rose and Svec (2015), the presence of periodontal structures may prevent cracks or dentinal damage in endodontically treated teeth. According to Shemesh *et al*. (2009), root canal filling also causes a significant increase in the number of cracks when compared to sound teeth. However, De-Deus *et al*. (2017) observed dentinal micro-cracks only after instrumentation, while root canal filling did not induce the development of new dentinal micro-cracks.In our study, all teeth were submitted to endodontic instrumentation with the Protaper Next rotary system (Dentsply-Maillefer, Switzerland) and CBCT images were acquired after instrumentation, obturation, apicectomy and retropreparation; thus, cracks diagnosed after cryotherapy may be related to N_2 application. Arx *et al*. (2010) showed the occurrence of cracks on the apical root surface after apicoectomy with high-speed bur, by contrast, Aydemir*et al*. (2014) did not observe crack formation. Layton *et al*. (1996) found apical cracks after ultrasonic retropreparation, while other studies did not observe an increase in the number of micro-cracks when roots were retroprepared by using stainless steel and zirconium nitride coated ultrasonic instruments (2014).

In accordance with Fayad, Ashkenaz and Johnson (2012) and Neves *et al*. (2014), intracanal gutta-percha negatively influences the diagnosis of root fractures, since hypodense lines can be misdiagnosed as root fractures because of its similar radiographic appearance. Nevertheless, the presence of gutta-percha did not significantly reduce the accuracy of vertical root fractures (VRFs) diagnosis in the study of Elsaltani, Farid and Ashmawy (2016). The authors also reported that among several CBCT systems, i-CAT (Imaging Science International, USA) showed the highest accuracy in the detection of VRFs in endodontically treated teeth.In our study, albeit all teeth were obturated with gutta-percha, the 3 mm of the apexes were retroprepared (removal of filling material) and could have facilitated cracking diagnosis due to a smaller number of artefacts on CBCT acquired with i-CAT (Imaging Science International, USA). CBCT has been considered a powerful tool for detecting VRFs with different thicknesses (Fayad; Ashkenaz; Johnson, 2012; Neves *et al*., 2014; Özer, 2010) and shows higher accuracy than digital radiography (Özer, 2010). The tomographic acquisition parameters influence the quality of images and, consequently, the diagnosis of cracks. In this line, 0.2 mm voxel size has been suggested due to reduced radiation to the patient and excellent diagnostic accuracy (Özer, 2011; Hassan *et al*., 2010). However, Uzun*et al*. (2015) reported no significant differences among different voxel resolutions of two CBCT systems. N_2 has been used as adjuvant therapy for excision of tumors with high recurrence rate; however, there is still no consensus regarding the number of freezing cycles and both two (Beltrão, 2003) and three 60-second applications have been reported (Schmidt; Pogrel, 2004). In the present study, a significant increase in the number of cracks visible to naked eyes was found after using two freezing cycles of 60 seconds and 4-minute thawing interval.It should be noted that an increase in the number of cycles may increase the risk of damage to tooth structure, soft tissue and bone (Lehugeur, 2009). In addition, the interval between the N_2 applications is an important factor to be considered since a longer time for natural thawing results in increased tissue destruction (Gage; Baust, 1998). The evaluation of two *in vitro* cryotherapy protocols (2 freezing cycles of 15 seconds and 3 cycles of 60 seconds, both with 4-minute thawing intervals) revealed some cracks visible to naked eyes, which suggests that cryotherapy may cause damages if repeated several times on the same tooth. However, tooth structure will probably not be undermined since only a single use (3 freezing cycles) would be used in clinical practice (Borges, 2007). The comparison among several methods to eliminate *E. faecalis* reveals that N_2 is not the best approach (Lehugeur, 2009). Although cryotherapy is effective in reducing bacterial biofilm, cracks are observed and its*in vivo* applicability is still questioned due to the aggression that extremely low temperatures around -35 °C may cause to periodontium and alveolar bone (Granzotto, 2009). The sudden drop in temperature caused by N_2 may influence the growth of *E. faecalis* and partially reduce the bacterial population; however, it seems not enough to attribute a "bactericidal effect" to N_2 (Batista, 2006). Temperatures reached after 15 seconds of N_2 intracanal application are able to cause cell death in tooth supporting structures around the root apex (Smidt, 2006). Recent studies on cryopreservation of teeth recommend the use of cryoprotectants such as dimethylsulfoxide or glycerol to reduce the risk of dentin cracking. When evaluating root surfaces by means of micro-CT, Kühl (2012) observed that cryopreservation with N_2 does not generate cracks, which were associated with forceps application during tooth extraction. Studies on cryotherapy of the root surface with N_2 are still at early stages; protocols are not yet well established and unclear outcomes regarding crack formation that may result in tooth loss. Future research may investigate the effect of cryoprotectants on root surfaces prior to cryotherapy.The use of N_2 at the root apex as adjuvant in paraendodontic surgeries to eliminate microorganisms in refractory periapical lesions is still not safe due to a higher occurrence of root cracks.

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