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ORIGINAL ARTICLE

Enlargement of the apical foramen of mature teeth by instrumentation and apicoectomy. A study of effectiveness and the formation of dentinal cracks

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ABSTRACT

Objective: In the last few years there have been attempts to revascularize mature necrotic teeth instead of performing a standard root canal treatment. Apical foramen enlargement (AFE) would be necessary for regenerative treatments of mature teeth. In the literature, AFE has been made through apicoectomy and instrumentation. However, no standardized methods have been described yet, which may affect the success of the therapy. Our aim was to describe the effectiveness and damage to dental structures of five methods for AFE.

Methods: Two hundred and ten human teeth were assigned to one control group ($n = 10$) and four treatment groups ($n = 50$ each): instrumentation was up to file #80 0.5 mm coronal to the apex (I), at apex level (II), 0.5 mm beyond the apex (III) and apicoectomy at 2 and 4 mm from the apex (IV). The apical foramen diameter was measured before and after treatment. The formation of clinically visible fractures (CVF) and microcracks was analysed clinically and with ESEM, respectively. Thirty-two *in situ* sheep's teeth were also instrumented, to compare damage in *in situ* and *ex vivo* teeth.

Results: The foramen diameter was augmented by 0.15, 0.47, 0.54 0.06 and 0.32 mm in human teeth of groups I, II, III, apicoectomy at 2 and 4 mm, respectively. CVF were more frequent as the working length was augmented. No statistical differences were found for microcrack formation. *In situ* teeth showed significantly less damage.

Conclusions: Instrumentation at apex level seems to be the most effective and least harmful technique for AFE, while apicoectomy is not a useful method.

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Introduction

Traditionally it has been considered that a closed apex is a requirement for a successful root canal treatment. Recently however, a more conservative and biologically-based approach for the treatment of necrotic teeth has been proposed: regeneration or revitalization of the pulp tissue [1], which would benefit from a wide apical foramen. The absence of an apical constriction, and therefore a wide apical foramen, would allow the ingrowth of blood vessels and nerve tissue, as well as cell migration into the root canal [2]. Previous studies have indicated that the size of the apical foramen is not the all-decisive factor for successful revascularization and ingrowth of new tissue [3]. However, a recent study showed that apical diameter does influence the success of regenerative procedures, since root thickness, length and apical narrowing are greater in teeth with an apical diameter greater than 1 mm [4]. This is according to a study in autotransplanted and replanted teeth, which indicated that revascularization is unpredictable when the apical foramen diameter was smaller than 1 mm [5]. Thus, enlargement of the apical foramen in order to allow revascularization of mature teeth would be necessary in three different clinical procedures: autotransplantation, replantation and regenerative endodontic therapy [3].

According to the literature, enlargement of the apical foramen for the treatment of mature teeth is performed by instrumentation [6–8] or apicoectomy [3]. However, there is no detailed description of the clinical protocol for performing enlargement of the foramen, which may be influencing the success of the therapy. It is likewise unclear whether instrumentation and apicoectomy are effective methods for foramen enlargement, and what effects might be caused to dental structures by instrumentation at apex level with wide files.

Studies on foramen enlargement do not report the length at which the instrumentation or apicoectomy is done [3,8,9]. In cases of foramen enlargement by instrumentation, since the position of the foramen varies depending on several factors [10], it is clinically relevant to establish a working length to effectively enlarge the foramen. Sub-instrumentation (with respect to the foramen) would not enlarge the foramen, and over-instrumentation would affect the periapical tissues, leading to symptoms in the patient [11]. For apicoectomy, the distance at which the root is resected directly affects the final diameter of the enlarged foramen. The radicular canal has a

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conical anatomy in which its diameter diminishes apically. Additionally, it is important to know how many millimetres the enlarged apical foramen will measure after the apicoectomy, and therefore whether this method is effective. An *in vivo* study performed apicoectomy on mature dog's teeth, in which the final diameter of the foramina varied from 0.24 to 1.09 mm [3]. However, the size of the foramen before apicoectomy was not measured and therefore it is not possible to evaluate whether the apicoectomy was effective.

The object of our study was to assess the effectiveness and damage to the dental structures of five methods (instrumentation until file K #80 at three different working lengths, and apicoectomy 2 and 4 mm from the apex) used to enlarge the apical foramen of mature human extracted teeth. Additionally, 32 sheep's teeth, which were set in the alveolar bone were also instrumented in order to compare damage in *in situ* and *ex vivo* teeth.

Materials and methods

Human teeth

The project was approved by the Bioethics Commission of the Universitat de Barcelona (IRB 00003099).

The sample size was 210 extracted human teeth, obtained from the dental hospital of Universitat de Barcelona. Only mature, single rooted (incisor and canine), mandibular and maxillary teeth were included. Teeth with external or internal resorption, with filling materials, posts or root fractures and/or dilacerated teeth were excluded.

Teeth that complied with the inclusion criteria were washed in 0.12% chlorhexidine and cleaned manually with a universal curette (Hu-Friedy, Chicago, IL) to remove calculus and remaining periodontal tissue. They were then preserved in saline for 1–3 months, until instrumentation/apicoectomy.

The teeth were randomly assigned to one of the five groups:

Group I ($n = 50$): working length 0.5 mm coronal to the tooth apex (sub-instrumentation);

Group II ($n = 50$): working length at the level of the tooth apex;

Group III ($n = 50$): working length 0.5 mm beyond the tooth apex (over-instrumentation);

Group IV ($n = 50$): apicoectomy at 2 and 4 mm from the tooth apex;

Group V ($n = 10$): control teeth, without any treatment.

The crowns of the teeth in groups I, II and III were sectioned with a carborundum disc at the level of the cementoenamel junction. Once the root canal had been located, instrumentation was done as follows: Teeth were manually instrumented with 25 mm K-Flexofile #15 progressing up to file #80 (Dentsply Maillefer, Ballaigues, Switzerland), using the balanced force technique under copious irrigation with 2.5% sodium hypochlorite. Files were renewed after instrumentation of 10 teeth. Teeth were kept moist with saline during the instrumentation. The length of the tooth (from the cementoenamel junction to the apex) was measured with a file # 0.8 and the working length for the different groups were establish according to this measure. The working length was 0.5 mm coronal to the tooth apex for group I, at the level of the tooth apex for group II and 0.5 mm beyond the apex for group III.

The teeth of group IV underwent apical resection with a 22 mm carborundum cutting disc (Renfert Dynex, Hilzingen, Germany). The first resection was performed 2 mm from the root apex and the second apicoectomy was performed 4 mm from the root apex.

The maximum diameter of each apical foramen was measured pre-treatment and post-treatment by a single operator, previously trained and calibrated. The calibration process consisted of theoretical training and measurement of the apical foramen of 20 teeth, repeated 10 days later. The intra-observer agreement was calculated using the intra-class correlation coefficient with SPSS software (V. 20.0, Chicago, IL). The measurements were taken under a 10× binocular stereo microscope (Premiere, Manassas, VA) with a crown-gauge-thickness instrument (Patterson Iwanson Spring Caliper, Saint Paul, MN) (accuracy of a tenth of a millimetre), and entered in an Excel document. Any adverse event such as root/apex fracture was recorded.

Fifteen teeth of each treatment group and 10 control teeth (not instrumented/apicectomized) were randomly selected, and were examined and photographed by environmental scanning electron microscope (ESEM) (Quanta 200, FEI Co., Hillsboro, OR). Teeth were coated with silver and gold, and analysed individually under 80× magnification at high vacuum conditions. One observer blindly analysed each image. The examiner was previously calibrated. The calibration process consisted of theoretical training and measurement of the apical foramen of 20 teeth, repeated 10 days later. The intra-observer agreement was calculated using the Cohen's kappa coefficient with SPPS 20.0 software. To be considered a complete dentinal crack, the fracture had to compromise both interior and exterior walls of the root (Figure 1(A–D)) and its extension on the exterior wall had to attain 0.5 mm or more. The presence/absence of complete dentinal cracks was evaluated and the results were dichotomized (presence/absence of dentinal cracks).

Sheep's mandibles

Sheep's teeth set in the alveolar bone were instrumented up to file #80 at the same working lengths as human extracted teeth, in order to evaluate damage to dental structures of instrumentation in non-extracted teeth.

Four heads of 4-year-old sheep were used. The heads were donated by another study, unrelated to the oral cavity. The heads were frozen for approximately 7 days. Teeth were treated in the mandibles. Crown access was created and the radicular canal was located. Pulp tissue was extracted with a K-Flexofile #15 (Dentsply Maillefer, Ballaigues, Switzerland), the teeth were radiographed with a portable digital radiograph system (Dexco ADX6000, Pasadena, CA) and tooth lengths were measured. The incisors ($n = 32$) were randomly assigned to the experimental groups.

Group a ($n = 10$): working length 0.5 mm coronal to the tooth apex (sub-instrumentation);

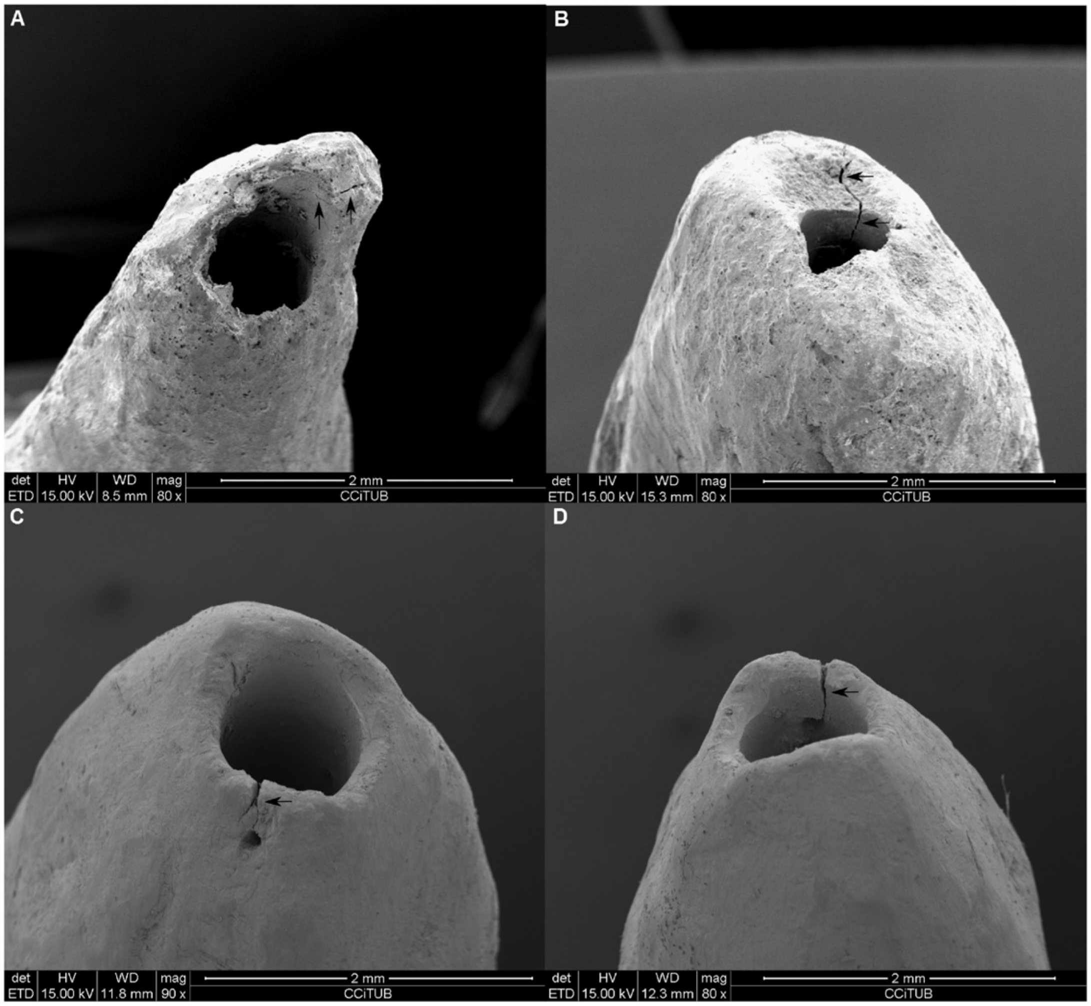

Figure 1. (A) Dentinal crack in a sub-instrumented tooth. (B) Dentinal crack in a tooth instrumented at apex level. (C) External wall view of the dentinal crack in an over-instrumented tooth. (D) Internal wall view of the dentinal crack in an over-instrumented tooth. Black arrows: dentinal cracks.

Group b ($n = 11$): working length at the level of the tooth apex;

Group c ($n = 11$): working length 0.5 mm beyond the tooth apex (over-instrumentation).

The root canals were manually instrumented up to file #80 (Dentsply Maillefer, Ballaigues, Switzerland) using the balanced force technique and passive irrigation with 2.5% sodium hypochlorite. Teeth were carefully extracted with forceps and washed with saline. No visible damage to the dental structures was observed after the extraction. Additional cleaning was carried out with a universal curette to gently remove remaining periodontal tissue. Foramen diameters were measured with the same protocol used for human teeth, and any adverse event observed clinically, such as root/apex fracture, was recorded. Subsequently, five teeth of each group, chosen at random, were analysed under ESEM as previously described.

Statistical analysis

Human and sheep's teeth were analysed separately. The collected data were entered into an Excel file and statistical analysis was performed with SPSS software (V. 20.0, Chicago, IL). Descriptive statistics were calculated, including mean and standard deviation. The homogeneity of variance was determined through Kolmogorov–Smirnov test. Pearson Chi-square, Fisher Exact Test and Wilcoxon Signed Rank test were used to evaluate statistical differences. In all statistical analyses a significance level of .05 was used.

Results

The intra-class correlation coefficient that determines intra-observer agreement for measurements was 0.96 (almost perfect) and the Cohen's kappa coefficient was 0.9.

Human teeth

The mean pre-treatment diameter of all teeth was 0.41 mm (±0.14), ranging between 0.0 (obliterated) and 0.9 mm. The four groups presented similar pre-treatment diameters of the apical foramen ($p > .05$). Table 1 shows the frequency of the pre-treatment diameters for each group.

Group I: sub-instrumentation

The mean pre-treatment apical foramen diameter was 0.42 mm (±0.15). After instrumentation up to K file #80, only

Table 1. Number of human teeth with pre-treatment diameter in each category, aleatorized in the treatment groups.

Group	Foramen diameter (mm) 0.0–0.2	0.3–0.4	0.5–0.6	0.7 or more	Total
Sub-instrumented teeth	1	27	20	2	50
Working length at tooth apex level	4	29	14	3	50
Over-instrumented teeth	3	30	14	3	50
Apicoectomy	9	20	19	2	50

Chi^2 test showed no statistical differences between the groups ($p < .001$).

Table 2. Number of teeth with increased, decreased and unvarying apical diameter after treatments.

Group	*N* teeth with increased diameter from original	*N* teeth with same diameter as original	*N* teeth with decreased diameter from original	*N* teeth with increased diameter after 2 mm apicoectomy	*N* teeth with same diameter after 2 mm apicoectomy	*N* teeth with diameter >/1 mm
Sub-instrumented teeth	25	25	0	NA	NA	5
Working length at tooth apex level	49	1	0	NA	NA	16
Over-instrumented teeth	50	0	0	NA	NA	41
2 mm apicoectomy	23	17	10	NA	NA	1
4 mm apicoectomy	43	6	1	42	8	8

Chi^2 test showed statistical differences between the groups with respect to the increased, equal or decreased diameters ($p < .001$).

Table 3. Prevalence of microcracks and clinically visible fractures in human and sheep teeth.

Group	*N* of teeth with dentinal cracks (%)	Mean of dentinal cracks, of the teeth with microcracks (SD)	*N* of clinically visible apex fractures (%)
Human sub-instrumented teeth	7 (46.7)	1.71 (0.88)	4 (8%)
Human teeth, working length at tooth apex level	6 (40%)	1.33 (0.47)	6 (12%)
Human over-instrumented teeth	9 (60%)	1.44 (0.68)	10 (20%)
Apicected Human teeth (4 mm)	3 (20%)	1.33 (0.47)	0 (0%)
Sheep sub-instrumented teeth	0 (0%)	0 (NA)	0 (0%)
Sheep teeth, working length at tooth apex level	1 (20%)	2 (NA)	0 (0%)
Sheep over-instrumented teeth	0 (0%)	0 (NA)	0 (0%)

Microcracks were analysed by ESEM, with a sample size of 15 human teeth per group and five sheep teeth per groups. Clinically visible fractures were analysed in all teeth (200 human teeth and 32 sheep teeth). NA, not applicable.

25 teeth (50%) showed an enlarged apical foramen; the foramen size of the remaining teeth was unchanged (Table 2). Augmentation of the foramen size was only 0.16 mm. The final foramina diameter for this group was 0.57 mm (±0.23). According to the Wilcoxon Signed Rank test, pre-treatment and post-treatment foramen diameters were statistically different. 46.7% of the teeth presented dentinal cracks and 8% presented clinically visible fractures (CVF) (Table 3).

Group II: working length at apex level

The mean pre-treatment apical foramen diameter was 0.40 mm (±0.17). After instrumentation up to K file #80, 49 teeth (98%) showed an enlarged apical foramen (Table 2). Augmentation of the foramen size was 0.47 mm. The final foramina diameter for this group was 0.87 mm (±0.17). According to the Wilcoxon Signed Rank test, pre-treatment and post-treatment foramen diameters were statistically different. Forty percent of the teeth presented dentinal cracks and 12% presented CVF (Table 3).

Group III: over-instrumentation

The mean pre-treatment apical foramen diameter was 0.43 mm (±0.11). After instrumentation up to K file #80, all teeth showed an enlarged apical foramen (Table 2). Augmentation of the foramen size of teeth with enlarged foramina was 0.54 mm. The final foramina diameter for this group was 0.97 mm (±0.02). According to the Wilcoxon Signed Rank test, pre-treatment and post-treatment foramen diameters were statistically different. Sixty percent of the teeth presented dentinal cracks and 20% presented CVF (Table 3).

Group IV: apicoectomy

The mean pre-treatment diameter was 0.4 mm (±0.14). After the apicoectomy 2 mm from the apex the mean apical diameter increased by only 0.07mm, to a final apical foramen diameter of 0.47 mm (±0.13) According to the Wilcoxon Signed Rank test, pre-treatment and post-treatment foramen diameters were not statistically different. Moreover, it is important to note that only 23 teeth presented increased foramen diameter, and only one tooth achieved an apical diameter of 1 mm (Table 2).

After the 4 mm apicoectomy, the mean apical diameter increased by 0.32 mm from the original apical size, to a final apical foramen diameter of 0.72 mm (±0.21). According to the Wilcoxon Signed Rank test, pre-treatment and post-treatment foramen diameters were statistically different.

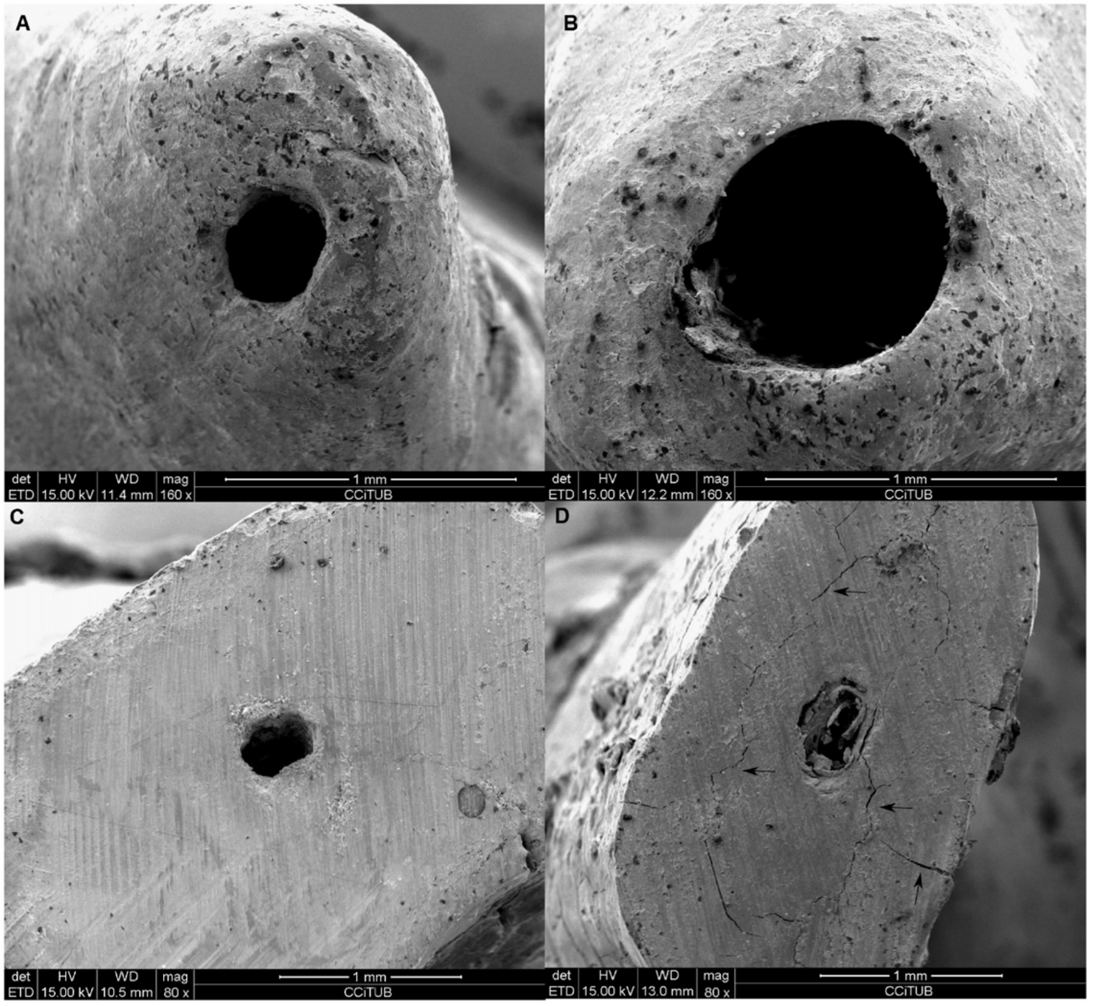

Figure 2. (A) Control tooth, foramen intact and without the presence of microcracks or clinically visible fractures. (B) Over-instrumented tooth, the foramen has an almost perfect round form and without the presence of dentinal cracks. (C) 4 mm apicectomized tooth, no dentinal crack is observed. (D) 4 mm apicectomized tooth, with microcracks. Black arrows: dentinal cracks.

Forty-three teeth presented an increased apical diameter and eight achieved an apical diameter of 1 mm (Table 2). Twenty percent of the teeth presented dentinal cracks and no teeth presented CVF (Table 3).

The form of the foramina of apicoectomized teeth was irregular (Figure 2(C,D)), with a maximum and minimum diameter; the over-instrumented teeth by contrast presented a round foramen (Figure 2(B)).

Control teeth had no CVF, and only two presented dentinal cracks (Figure 2(A)). The differences in dentinal cracks and CVF between control and treated teeth were statistically significant ($p < .05$).

Intergroup comparison (human teeth)

Table 4 shows the frequency of the post-treatment diameters for each group. The differences between the post-treatment apical foramen sizes were statistically significant between treatment groups ($p < .05$). Teeth over-instrumented and instrumented at level apex showed greater foramen diameters. The frequency of teeth with augmented, equal and diminished apical diameter was statistically different between the groups. Teeth over-instrumented, instrumented at apex level and with 4 mm apicoectomy had significantly more teeth with augmented foramen diameter (Table 2).

All groups of instrumented teeth (groups I, II and III) presented CVF. The frequency of CVF was statistically greater in over-instrumented and teeth instrumented at level apex in comparison to sub-instrumented and apicectomized teeth ($p < .05$) (Table 3).

All groups contained teeth with dentinal cracks (Figures 1(A–D) and (D), Table 3). Despite the fact that the presence and number of dentinal cracks was higher in over-instrumented teeth, there were no statistically significant differences between groups (Table 3).

Sheep's teeth

The mean post-treatment apical foramen diameters were 0.67 mm (±0.61), 0.82 mm (±0.21) and 0.88 mm (±0.18) for sub-instrumented, instrumented at apex level and over-instrumented teeth, respectively. These differences were not statistically significant.

No teeth presented CVF after instrumentation. The presence of microcracks, evaluated by ESEM, was observed in only one tooth, instrumented at apex level (Figure 3(A)).

Table 4. Number of human teeth with post-treatment diameter in each category.

Group	0.2–0.3 mm (*n*)	0.4–0.5 mm (*n*)	0.6–0.7 mm (*n*)	0.8–0.9 mm (*n*)	1 or more mm (*n*)	Total (*n*)
Sub-instrumented teeth	8	16	15	6	5	50
Working length at tooth apex level	1	1	8	24	16	50
Over-instrumented teeth	0	0	0	9	41	50
Apicoectomy at 2 mm from the tooth apex	8	31	10	0	1	50
Apicoectomy 4 mm from the tooth apex	3	9	13	16	9	50

Chi^2 test showed statistical association between the type of treatment and the obtained apical foramen diameter ($p < .001$).

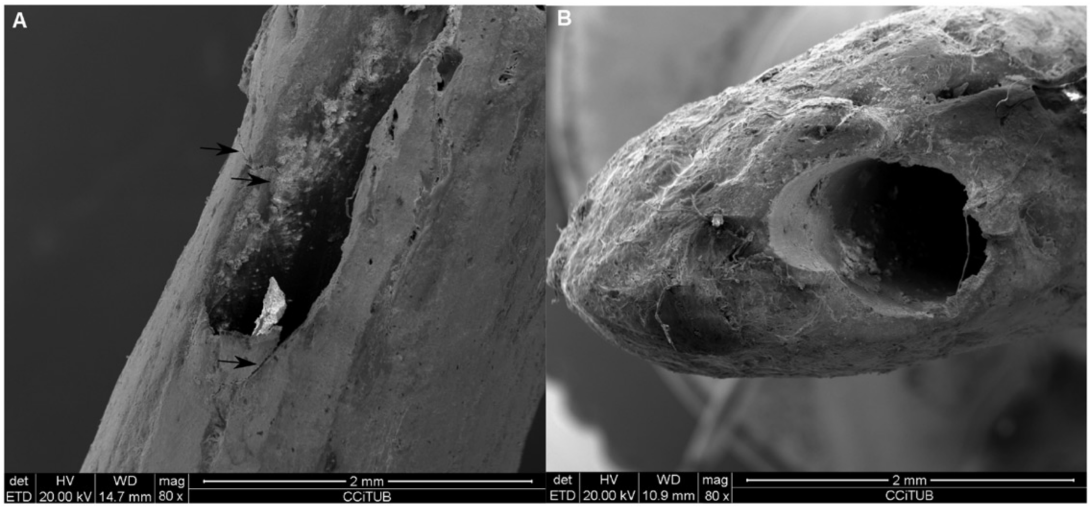

Figure 3. (A) Sheep's tooth, instrumented at apex level; microcracks are observed (black arrows). (B) Sheep's tooth, instrumented at apex level; no dentinal cracks are observed.

According to the Fisher Exact test these differences were not statistically significant. The rest of the teeth had no microcracks (Figure 3(B))

Human ex vivo *teeth* versus in situ *sheep teeth*

No statistically significant differences were found for the prevalence of CVF between human and sheep teeth. The prevalence of dentinal cracks was greater in human teeth ($p = .01$).

Discussion

The current paradigm shift in endodontics from obturation to pulp regeneration implies that the new therapy would benefit from a wide apical foramen. In 1990, Andreasen and co-workers showed that the revascularization of autotransplanted and replanted teeth was unpredictable if the apical foramen measured less than 1 mm [5]. This has been confirmed by recent research in regenerative endodontics, which showed that teeth with narrower apical diameters had significantly lower hard tissue formation compared with teeth with apical diameter greater than 1 mm, indicating that apical diameter is a factor influencing the progress of revascularization [4]. As has been shown in this study, the mean pre-treatment apical foramen of these selected anterior mature teeth is just 0.41 mm, so it would be necessary to perform apical foramen enlargement when a regenerative treatment is planned for mature teeth.

The location of the apical foramen varies in response to several factors [10], and the exact location must be known for foramen enlargement to be achieved. An imaging study showed that the mean distance between the apical foramen and the apex was 0.9 mm, but that in 68% of teeth the distance was 0.5 mm shorter or longer than the mean [10], which is a large variance in clinical terms. Cone Beam Computerized Tomography (CBCT) and electronic apex locators have been used to determine the location of the apical foramen. However, the literature shows that several factors affect the precision of electronic apex locators in determining working length [12], and that CBCTs are less reliable that electronic apex locators [13]. Thus, it is important to establish a standardized clinical protocol to achieve widening of the apical foramen, since use of inaccurate methods could fail to achieve widening, which would compromise the regenerative procedure. Unnecessary over-instrumentation, on the other hand, could lead to patient discomfort and/or apex fracture.

While foramen enlargement has been applied in studies of attempts to regenerate pulp in mature teeth, no details are reported of the length at which instrumentation or apex resection has been performed [3,8,9]. As seen in our study, variations of only 0.5 mm in working length produced very different clinical outcomes (98% of foramina were enlarged with working length at apex level, and only 25% in sub-instrumented teeth). Likewise, the distance from the apex at which the resection is performed is a critical factor in the size of the widened foramen. Several studies have used apicoectomy as a method for foramen enlargement of mature teeth [3,9]. However, according to our results resection at 2 mm from the apex causes almost no enlargement in foramen size, and in 20% of the cases causes a decrease in the foramen diameter, meaning that it is not a useful method for foramen enlargement.

The most effective methods for foramen enlargement are over-instrumentation, instrumentation at apex level and resection of 4 mm of the root; these methods enlarged the foramen by 0.54, 0.45 and 0.32 mm, respectively. However, it should be considered that over-instrumentation and 4 mm resection are invasive methods (20% of the over-instrumented roots presented CVF and 4 mm root resection causes an important loss of dental tissue). Moreover, the fact that over-instrumentation provokes patient pain and discomfort must be considered [14].

An ideal method for foramen enlargement should be both effective and less invasive. Thus, according to the results of our study instrumentation at apex level would be the most suitable method for foramen enlargement. Instrumentation at apex level produces a mean apical diameter size of 0.87 mm. In future investigations would be necessary to establish if files with 0.04 or 0.06 taper achieve better results in terms of greater diameter and fewer fractures and microcracks, or if it is mandatory to use files up to #100 in order to obtain a 1 mm diameter. Instrumentation at apex level causes CVF in 12% of the teeth, however this prevalence probably would be minor if instrumentation is performed in the mouth as it is shown in the *in situ* sheep teeth, where no CVF were observed.

The working lengths in this study were established with respect to the apex, and considering the fact that the apical foramen could be located 3 mm or more coronal to the apex [15], that the instrumentation is generally asymmetrical [16], and that some apices were fractured (which leads to greater diameters) a 1 mm final foramen diameter was achieved with #80 files in some teeth (Table 2).

The prevalence of complete dentinal cracks in this study was higher than in the results of other investigations [17,18]. This could be explained by differences in methodology, since some studies analysed dentinal cracks in the apical, middle and coronal thirds of the tooth, and not at apex level [17,18]. The apex is a thin structure compared with the apical, medium or coronal thirds of the root, and for this reason is more prone to fracture. Thus, the higher the root third analysed, the lower the prevalence of cracks [17]. Ceyhanli et al. [19] analysed microcracks by micro-CT in the apical 10 mm of roots; the prevalence of microcracks was similar to our findings. Another important reason that could explain the high prevalence of cracks in our study is the use of large files (#80) to widen the foramen. Studies that instrumented teeth only up to file #25 reported considerably fewer dentinal cracks [20].

Finally, a limitation of this study is that the extracted teeth used are more prone to fracture, due to the lack of surrounding tissue support. This could explain why the sheep's teeth, which were instrumented in their mandibles, presented a significantly lower prevalence of dentinal cracks and no CVF. However, it is important to note that the anatomy and dimension of sheep's teeth are different from human teeth. As we observed in this study, dentine walls at the apex level of sheep's teeth had greater dimensions compared to human teeth, which decreases the probability of formation of fractures or microcracks. Studies in non-extracted human teeth (such as cadaveric material) would elucidate whether fracture and microcrack prevalence is lower when teeth are instrumented *in situ*.

According to the results of this study instrumentation at apex level would be the most suitable method for foramen enlargement. Apicoectomy and 0.5 mm sub-instrumentation with respect to the apex are not effective methods. Over-instrumentation by 0.5 mm is an effective but harmful technique.

Disclosure statement

The authors report no conflict of interest

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