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Do pre-existing microcracks play a role in the fracture resistance of roots in a laboratory setting?

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Abstract

Aim: The purpose of the current study was to investigate a potential cause-effect relationship between dentinal microcracks and fracture resistance of lower incisors that had not been endodontically treated. The null hypothesis tested was that the amount of dentinal microcracks in their roots does not turn these human teeth more prone to fracture.
Methods: A sample of 60 mandibular incisors with circular-shaped canal was selected based on micro-CT pre-scans to create a homogeneous sample. Two pre-calibrated examiners screened the cross-section images of the specimens to identify and quantify the presence of dentinal microcracks. Teeth were embedded in polystyrene resin and subjected to axial compressive loading using a universal testing machine. After they fractured, roots were re-scanned and fractography analysis was performed by inspection of 3D models to verify crack propagation. Spearman's rank correlation was used to assess the correlation between the number of microcracks and force required to fracture
Results: Dentinal microcracks were detected in 79% of the specimens (n = 44). The incidence of microcracks varied between teeth from 6% to 42% of the total slices per sample, with an average of 14 ± 17%. The number of microcraks per sample varied from 0 to 1605, with an average of 412 ± 484 (median = 221 and IQR 25% = 15 / 75% = 658). The load at failure values varied from 227N to 924N, with an average of 560.3 ± 168.1N (median 561 and IQR 25% = 458 / 75% = 694). The Spearman correlation coefficient (rho) equaled 0.065.
Conclusions: It can be concluded that there is no cause-effect relationship between the amount of dentinal microcracks and fracture resistance of nonendodontically treated lower incisors in the sense that the presence and quantity of microcracks did not turn these roots more prone to fracture.

Do pre-existing microcracks play a role in the fracture resistance of roots in a laboratory setting?

Introduction

Vertical root fractures (VRF) are defined as longitudinal fractures along the vertical axis of the root. VRFs can occur in both root filled and non-root filled teeth (Kishen & Asundi 2002, Cohen *et al.* 2006, Rivera & Walton 2007), often with catastrophic consequences as they most often result in tooth extraction. Unfavorable occlusal loads, steep cuspal inclines, deep enamel fissures within the crown, over-enlarged canals, supra-osseous post and dowel placement have been reported as aetiological factors associated with VRFs. It has been suggested that dentinal microcracks identified in cross-sectional images of roots from extracted teeth may be a triggering point or "starter defects" for VRFs (Kim *et al.* 2010, Tsesis *et al.* 2010).

When analysing the body of evidence on dentinal microcracks there are approx. seventy peer-reviewed studies embracing the main assumption, that is, dentinal microcracks observable in cross-sections and micro-CT images of roots play a role in the origin and propagation of VRFs. In other words, the bulk of the scientific literature on dentinal microcracks has accepted the role of such dentinal defects on the dynamics involved in the initiation of VFRs, even though there is no experimental evidence to support a cause-effect relationship. Interestingly, other than the work by Abou *et al*. (2014), no experimental studies have been designed specifically to investigate this potential relationship. Abou *et al*. (2014) investigated the fracture resistance of oval-shaped canals and also observed the effect of various kinematics and canal shaping systems on dentinal walls. However, at that time, attention focused on canal instrumentation-induced dentinal defects, and roots were sectioned to assess the presence of microcracks under direct optical microscopy, a laboratory experimental method that has been reported to lead to misguided results and false conclusions (Bueno *et al*. 2017).

The findings from both root-sectioning and micro-CT studies that used extracted teeth following storage have reported a high prevalence of dentinal microcracks (Versiani *et al.* 2015). However, the clinical prevalence of VRFs ranges from 2% to 5%, which is a surprisingly low figure when compared to the high prevalence of potential starter dentinal defects identified in laboratory studies. Of special note is that almost all dentinal defects observed in either micro-CT or destructive root-sectioning studies have been

classified as incomplete microcracks running inwards from the external root surface towards the canal lumen (De-Deus *et al.* 2014, de Oliveira *et al.* 2017, PradeepKumar *et al.* 2017). This finding is not aligned with the current understanding of VRFs in root filled teeth, which suggest that VRFs originate internally on the canal wall (Simon 2013). Taken together, the discrepancy between the findings of most laboratory studies and clinical reality, suggests there is a strong chance that dentinal microcracks as viewed in extracted teeth are not involved in triggering VRFs *in vivo*. In other words, VRFs may be a phenomenon that is unrelated to the root dentinal microcracks described in laboratory bench-top studies using stored extracted teeth. Aligned to this rationale, De-Deus *et al* (2020) suggested that root dentinal microcracks observed in cross-sectional images of extracted roots should be referred to as *experimental microcracks* since this type of dentinal defect has never been observed in the clinical setting.

Considering the lack of clarity on the aetiology of root dentinal microstructural defects as well as the lack of specific knowledge of the role of this phenomenon on the origin, development and progression of the VRFs, the main purpose of the current study was to investigate a potential cause-effect relationship between the number of pre-existing dentinal microcracks observable on micro-CT images of extracted roots of non-root filled mandibular incisors and the fracture resistance of these roots. The prevalence, location, and pattern of pre-existing microcracks were also assessed. The working hypothesis assumed that the number of root dentinal microcracks did not predispose the roots of extracted teeth to fracture.

Material and Methods

Sample selection

After approval by the local ethics committee (protocol 931.732), a sample of 180 mandibular incisors was selected from a tooth bank. These teeth had been stored at 8º C for variable periods of time up to one year. Each specimen in the sample was pre-scanned in a micro-CT device (SkyScan 1173; Bruker microCT, Kontich, Belgium) operated at 70 kV and 114 mA, with an isotropic resolution of 14.25 µm, 180° rotation around the vertical axis, rotation step of 0.7°, camera exposure time of 250 milliseconds and frame average of 5 and reconstructed using the NRecon v 1.7.1.6 software (Bruker microCT), each under individualized parameters of beam hardening correction; ranging from 35%

to 45%, ring artefact correction from 3 to 5, and contrast limits ranging from 0 to 0.05, in order to reduce noise and to increase the quality of the images.

After reconstruction, the root canal of each specimen was segmented using ImageJ software (FIJI/ImageJ software v.1.51n; Fiji, Madison, WI, USA) and by the use of the aspect ratio tool available in the shape descriptors plugin, categorized as circular, oval and long-oval (**Supplementary file 1**). The aspect ratio tool provides the major axis/minor axis ratio value of an ellipse fitted to each cross section of the reconstructed sample, allowing the analysis of the shape along the entire root canal. Circular canals were selected from the results extracted from this analysis. Thus, root canals that had an aspect ratio close to one were considered as circular (Rechenberg *et al.* 2013).

Eventually, 92 teeth containing circular canals were chosen for further analysis: the dentine volume along the entire root and the mesiodistal width of the root were considered paramount for the selection of anatomically similar samples. Therefore, a further segmentation was performed with the region of interest focused only on the volume of dentine in the root. Using the segmented image sets, new measuring tools were used. The MinFeret measurement, available at Analyze Particles (FIJI/ImageJ software, https://imagej.net/Fiji/Downloads), was adopted to verify the thickness of the root wall on the mesial and distal aspects combined (dentine mesiodistal diameter) and its values were plotted on a graph that was later used to group teeth with similar dimensions (**Figure 1**). For measurement of canal volume, the 3D Objects Counter tool (FIJI/ImageJ software) was used. With these results, 60 teeth were finally selected with their dentine volume varying from 107 to 187 mm^3 and dentine thickness with similar graphical curves (**Figure 2**). In order to create a homogeneous sample at baseline, teeth were also decoronated 12 mm from the apex to standardize the roots that were now similar with respect to their main anatomical features, i.e. mesiodistal diameter and root length, creating a homogeneous sample at baseline.

Assessment of dentinal microcracks

After selection, two pre-calibrated examiners used a proforma with predefined criteria to analyse the cross-sectional images of the specimens to identify the position, number of dentinal microcracks and to measure with a scale (FIJI/ImageJ software), the horizontal and vertical extensions of the microcracks in each root, a process that involved around 800 slices per specimen. The horizontal and vertical extensions of the microcracks were measured using ordinary tolls from FIJI/ImageJ software. The image analysis was

performed using a 34’ high-quality computer monitor with the possibility of enlarging the micro-CT images and also reverse the colour mode, which rendered a precision of better than 1 pixel. To validate the analytical process, analyses were repeated twice at 10-days intervals to appraise the measurement reproducibility.

Twelve teeth with no pre-existing microcracks were selected as a control group.

Simulated periodontal ligament and alveolar bone

The root surfaces were covered with a thin layer of polyether impression material (Impregum F, 3M-Espe, Seefeld, Germany) to simulate the function of the periodontal ligament as described previously (Soares *et al.* 2005). In brief, the root surfaces were dipped into molten wax and then removed, so that a 0.2-0.3 mm layer of wax remained on the root surface. The wax-covered roots were then mounted individually in plastic cylinders and embedded in polystyrene resin up to 5 mm below the cementoenamel junction (CEJ). After resin polymerization, the roots were detached from the cylinders, and the wax removed from the root surfaces. The polyether impression material was mixed and placed in the space created in the resin cylinders and the roots re-inserted with any excess material being removed with a scalpel.

Fracture resistance test

The embedded roots were mounted in a metal holder and subjected to axial compressive loading with a universal testing machine (Galdabini Sun 500, Cardano al Campo, Italy) at a crosshead speed of 1 mm/min until fracture was detected. This was done using a 5 mm diameter metallic sphere (antagonistic tooth) positioned so that it contacted the flat root face on both mesial and distal sides to spread the load uniformly on the root surface. The fracture threshold was defined as the load which revealed the first fracture (a sudden load-drop during compression), resulting in a peak formation on the extension curve. A 100-kgf load cell was used and values were recorded in Newtons (N).

Fractography

After they fractured, roots were re-scanned using the parameters described above. Fractography analysis was performed by inspection of 3D models to verify crack propagation. Mode of failure was classified (Barcellos *et al.* 2013) as follows: type I – fracture at the cervical third of the root canal involving the root face and type II – vertical

root fracture. Type I was considered to be a repairable fracture, potentially allowing tooth restoration, while type II was considered a catastrophic fracture that compromised tooth integrity and its restorability. To complement the fractographic analysis, pre-existing microcracks were grouped according to concentration areas along the root thirds and compared against the position of catastrophic failures. To achieve this, the concentration areas were mapped with the position of the catastrophic failures along the Z-axis of the root using a reference coordinate system, which is based on a landmark-based registration algorithm (Analyze software, Biomedical Imaging Resource; Mayo Clinic, Rochester, MN, USA).

Statistical analysis and data presentation

The preliminary data analysis indicated that the number of microcracks did not adhere to a Gaussian distribution (D'Agostino & Pearson omnibus normality test). Thus, a nonparametric statistical analysis (Spearman's rank correlation) was used to assess the correlation between the number of microcracks and force required to fracture. The alpha-type error was set at 0.05 and Prisma 5.0 (GraphPad Software Inc, La Jolla, CA, USA) and Origin 6.0 (Microcal Software Inc., Northampton, MA, USA) were used as analytical tools. Non-parametric data are shown as median values and inter-quartile ranges (IQRs), whilst normally distributed counterparts are presented as means and standard deviations.

Results

Four specimens were lost during the embedding process. Thus, the experiment ran with a total of 56 specimens. Pre-existing dentinal microcracks were detected in 79% of the specimens (n = 44). The incidence of microcracks varied between teeth from 6% to 42% of the total number of slices per specimen, with an average of 14 ± 17%. The number of microcracks per specimen varied from 0 to 1605, with an average of 412 ± 484 (median = 221 and IQR 25% = 15 / 75% = 658). The distribution of the number of microcracks is shown in Figure 1A; however, this data did not consider the position (inner or outer wall) nor the vertical length of the dentinal defect. In other words, the correlation analysis was based on the '*number*' of microcracks.

Root dentinal microcracks originating from the outer surface of the root made-up 99.8% of the total, while no complete microcracks extending from the outer surface into the canal lumen were observed. Root dentinal microcracks originating from the canal walls accounted for only 0.2% of the total number. The vertical length (cervico-apical

direction) of the microcracks varied substantially from 0.12 to 3.21 mm; the average vertical length being 2.17 mm along the root.

The load at failure values varied from 227N to 924N, with an average of 560.3 ± 168.1N (median 561 and IQR 25% = 458 / 75% = 694). The distribution of force values is shown in Figure 1B.

The Spearman correlation test was unable to identify dependence between the number of dentinal microcracks and the force required to fracture (P=0.636), which is clearly demonstrated in the scatter plot X-Y graph in Figure 1C. The Spearman correlation coefficient (rho) equalled 0.065, indicating no correlation between these variables. This means that the '*number*' of pre-existing dentinal microcracks was able to explain only 0.65% of the root fractures.

The fracture analysis revealed that catastrophic failure (type II) was the predominant fracture type (71.4%) while cervical fracture (Type I) accounted for the remainder (28.6%). Typical fractures are presented by 3D models in Figure 2.

The quantity of unrestorable fractures (catastrophic) was low among those specimens that demanded greater forces to achieve fracture. The specimens that demanded greater loads to fracture were associated with unrestorable fractures (type II); in only 4 out of 18 specimens. Most of time, catastrophic fractures were not correlated to microcrack concentrations on the middle/apical thirds of roots. In the 3D qualitative evaluation, it was obvious that pre-existing microcracks were not associated with the propagation of fracture lines. In short, the type of fracture was not linked to the position/depth of pre-existing microcracks.

Teeth without pre-existing microcracks (control group) had similar resistance to fracture as their counterparts with significant microcrack concentrations. Among the 12 teeth with no pre-existing microcracks, only 3 required a high force to fracture.

Discussion

In the current study, the potential relationship between the number, horizontal position and vertical extension of dentinal microcracks observed on micro-CT cross-sectional images of extracted human mandibular incisor roots and their resistance to fracture was assessed. The prevalence and the number of dentinal microcracks in non-root filled mandibular incisors was thus determined by micro-CT imaging and correlated to the load required to fracture the respective roots. The results revealed that the number

of dentinal microcracks was able to explain only 0.65% of the root fractures (Spearman's rho = 0.065), which was not significant (P > 0.05). These results, for the first time, provide evidence from a laboratory setting that the number of microcracks observed in cross-sectional images does not predispose roots to fracture in a laboratory setting. This means that the dentinal microcracks identified in almost all destructive and non-destructive studies over the last decade cannot be associated, without further experimental and theoretical evidence, as trigger points for VRFs.

At first glance, the current finding may seem rather controversial and peculiar, since most laboratory studies performed since 2009 have implied that VRFs would occur as a consequence of such dentinal defects. This false hypothesis was used to justify the study of dentinal microcracks as surrogate outcomes for VRFs, e.g. their relationship with endodontic procedures such as canal preparation and filling. However, the present result can be regarded as timely considering the systematic lack of experimental studies specifically designed to evaluate the causality between microcracks and VRFs. Thus, to a degree, it is fair to say that the endodontic scientific community has been assigning an unproven clinical significance to dentinal microcracks identified in laboratory studies of extracted teeth.

VRFs originate from regions with excessive stress concentrations and propagate from that area (Wilcox *et al.* 1997). Logic would thus dictate that a VRF is likely caused by the propagation of a dentinal microcrack observed in either a sectioning or a micro-CT study. Clinical investigations have revealed that indeed, vertical root fractures appear to be extensions of dentinal microcracks that were initially incomplete and limited in extent (Tawil *et al.* 2015). VRFs, however, are macroscopic and usually appear in the middle of the root or at the root tip (von Arx & Bosshardt 2017). Indeed, it is important to highlight that science rarely disobeys seemingly logical reasoning. It is worth remembering that the endodontic scientific community has been guilty of overestimating the relationship between logic, that is deductive reasoning, and reliable scientific evidence before. For example, De-Deus *et al*. (2012, 2017b) concluded that sealer penetration of dentinal tubules, which had been used historically as an indication of the superiority of a given root-filling technique or material had no basis in fact and that there was no experimental evidence to support that claim. Interestingly, that false rationale had also been used as promotional material to launch new root filling materials. Other systematic errors in Endodontics include the purported importance of microleakage along

root fillings as a surrogate measure for their quality (Rechenberg *et al.* 2011) and, classically, the hollow tube theory by Rickert & Dixon (Torneck 1966).

In the context of VRFs, it is fair to say that the importance of microcracks identified in laboratory studies has been overestimated since the first studies appeared in 2009. Therefore, the assumption that dentinal microcracks are trigger points for VRFs has been a working hypothesis, which means it was a provisionally accepted hypothesis that demanded further scrutiny using appropriate scientific methods in order to either confirm or disprove it. Any given working hypothesis without proper experimentation, however, is unsubstantiated and speculative conjecture. This is emphasized by recent results from teeth inside bone-blocks removed from fresh cadavers where the lack of pre-existing root dentinal microcracks was demonstrated (De-Deus *et al.* 2020). The authors screened all cross-section images from 178 teeth (n = 65 530) inside bone-blocks from the cementoenamel junction to the root apex and failed to identify any dentinal defects. That result raises doubts that dentinal microcracks observed in cross-sectional images of extracted teeth or even in micro-CT screening really exist in the clinical setting (De-Deus *et al.* 2020).

Based on the purpose of the present study, a single large experimental group was used. There is no rationale to justify the use of two or three groups when the goal was limited to verify a potential cause-and-effect relationship between two variables that affect the same root. The current correlation analysis gained power by using a single large experimental group as it assumed that random factors affect only individual subjects. The teeth used were collected from a tooth bank, which means that there was only limited control regarding some of the variables able to play a role in their resistance to fracture as well as the frequency of pre-existing dentinal microcracks. These variables include the age of the patients when the teeth were extracted and storage period. Reviewing the literature on tooth-cracks related to Endodontics, no study has used age as a strict inclusion criterion for sample selection.

Moreover, a substantial sample size of 60 teeth were selected using strict micro-CT guided inclusion criteria on the anatomical features of the canal and the volume of dentine in roots, counterbalancing the uncontrolled variables mentioned above. This careful control of the specimens is confirmed by the similar average values and standard deviations that are somewhat lower than several previous studies on resistance to fracture (Akkayan & Gülmez 2002, Krishan *et al.* 2014, Santos-Filho *et al.* 2014). Additionally, the use of non-root filled teeth aids in assuring the control of potentially unknown

variables associated with mechanical shaping and chemical irrigation of root canals. The prevalence of dentinal microcracks in the current study is in line with previous micro-CT based studies using stored teeth from a tooth bank (De-Deus *et al.* 2015, 2016, Shemesh *et al.* 2018).

The application of a direct load over the root with no crown was an experimental feature that does not intend to mimic the clinical situation but rather it allows a better control of the undesirable effect of several variables related to the testing of a restored tooth. Likewise, Krishan *et al.* (2014) studied the fracture resistance of roots without coronal restoration in order to avoid confounding variables. Moreover, static loading needs to use greater strength values when compared to the physiological forces at work in the oral cavity, as they are necessary to assess fracture resistance in the laboratory setting (Türker *et al.* 2018). To understand the progression of root fractures, it is essential to consider their volumetric extension through dentine and, despite the scanning procedures which allowed better visualization of the fractured teeth, such 3D analysis was not carried out in the present study due to the limitations imposed by the software; however, such further analysis is ongoing. Future work should focus on affording theoretical, experimental and clinical models able to provide 3D information on root dentinal microcracks and thus, create a better understanding of the mechanics of root fractures in order to verify if and how these dentinal defects may or may not contribute to the development of VRFs *in vivo*.

Conclusions

There was no cause-effect relationship between the number of dentinal microcracks and fracture resistance of non-root filled mandibular incisors in the sense that the presence and quantity of microcracks did not predispose roots to fracture. Future research is awaited to provide a better understanding of the cause(s) of vertical root fractures.

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Figure 1

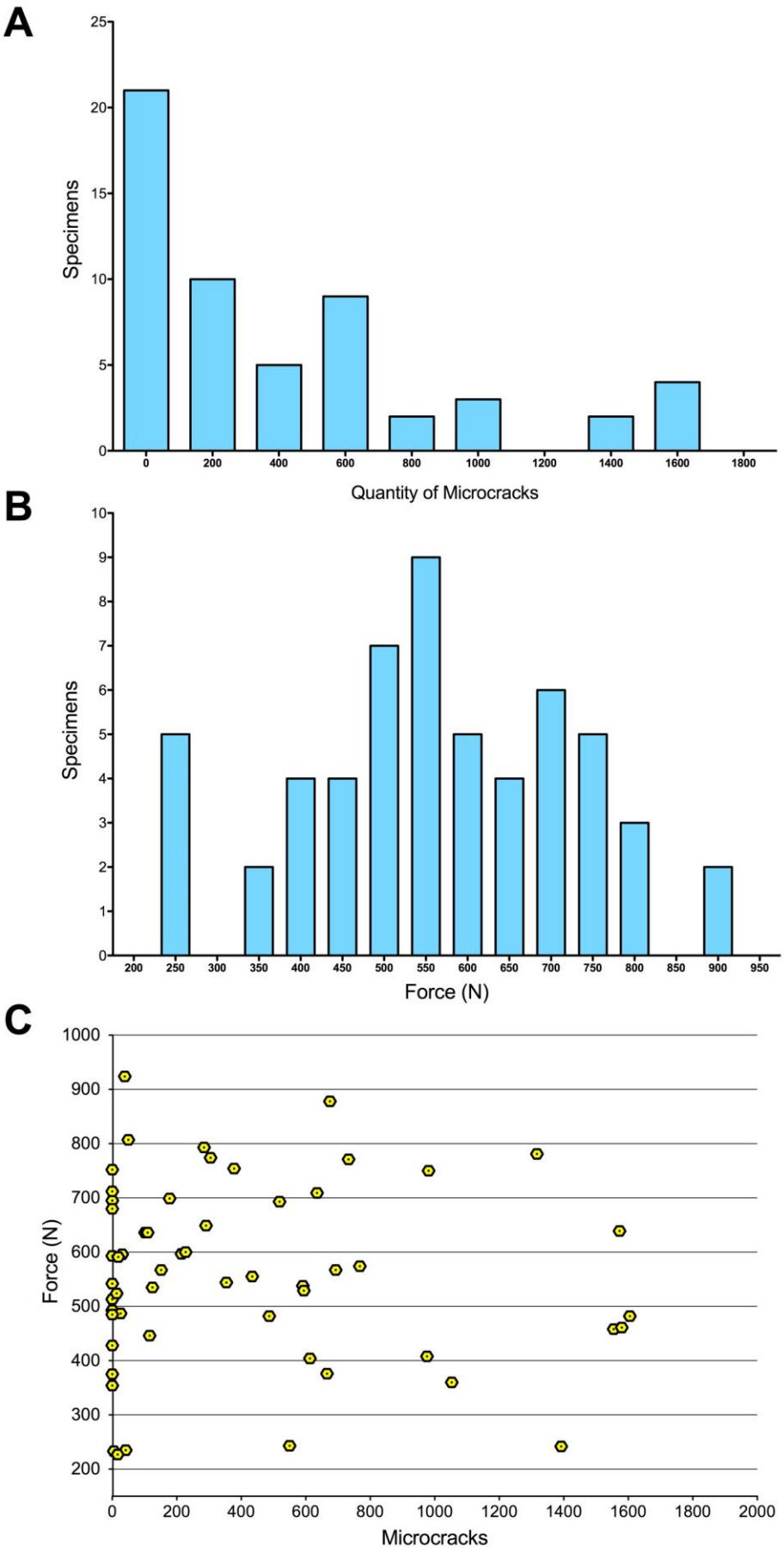
A
Specimens
Quantity of Microcracks
B
Specimens
Force (N)
C
Force (N)
Microcracks

Figure 2

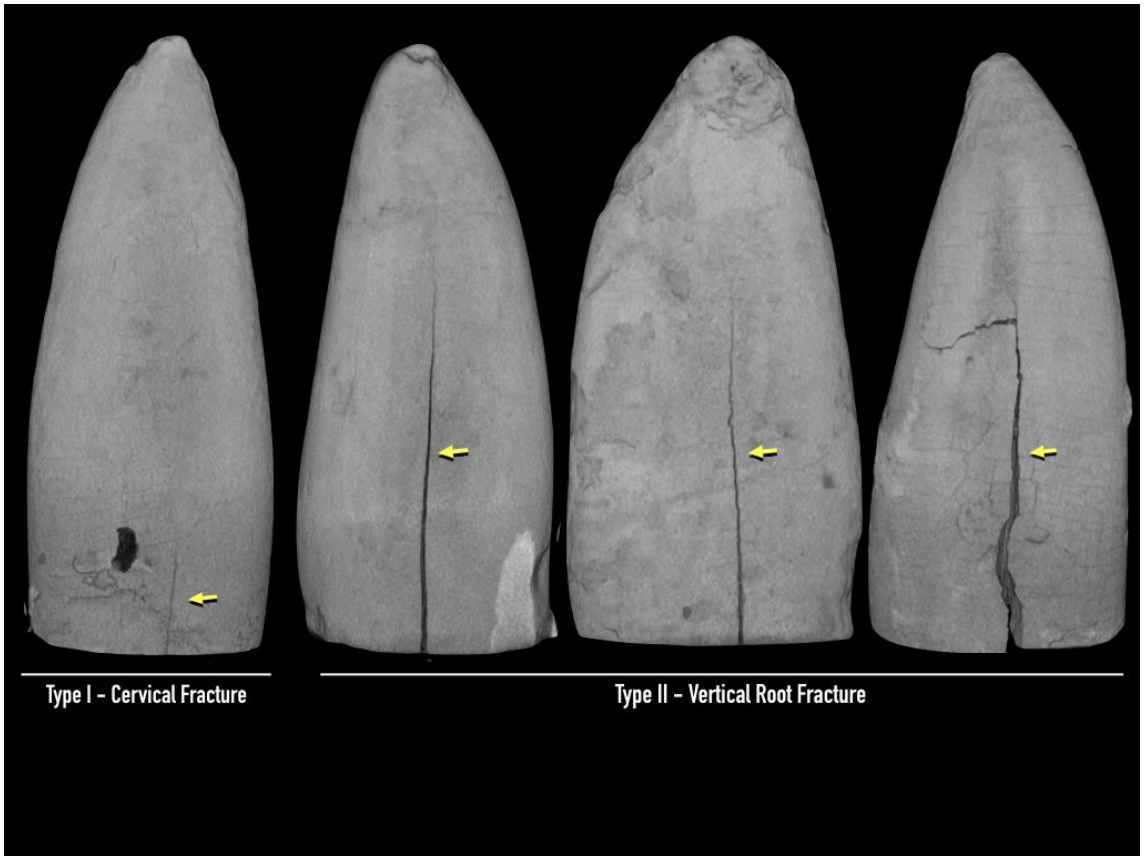

Figure 3

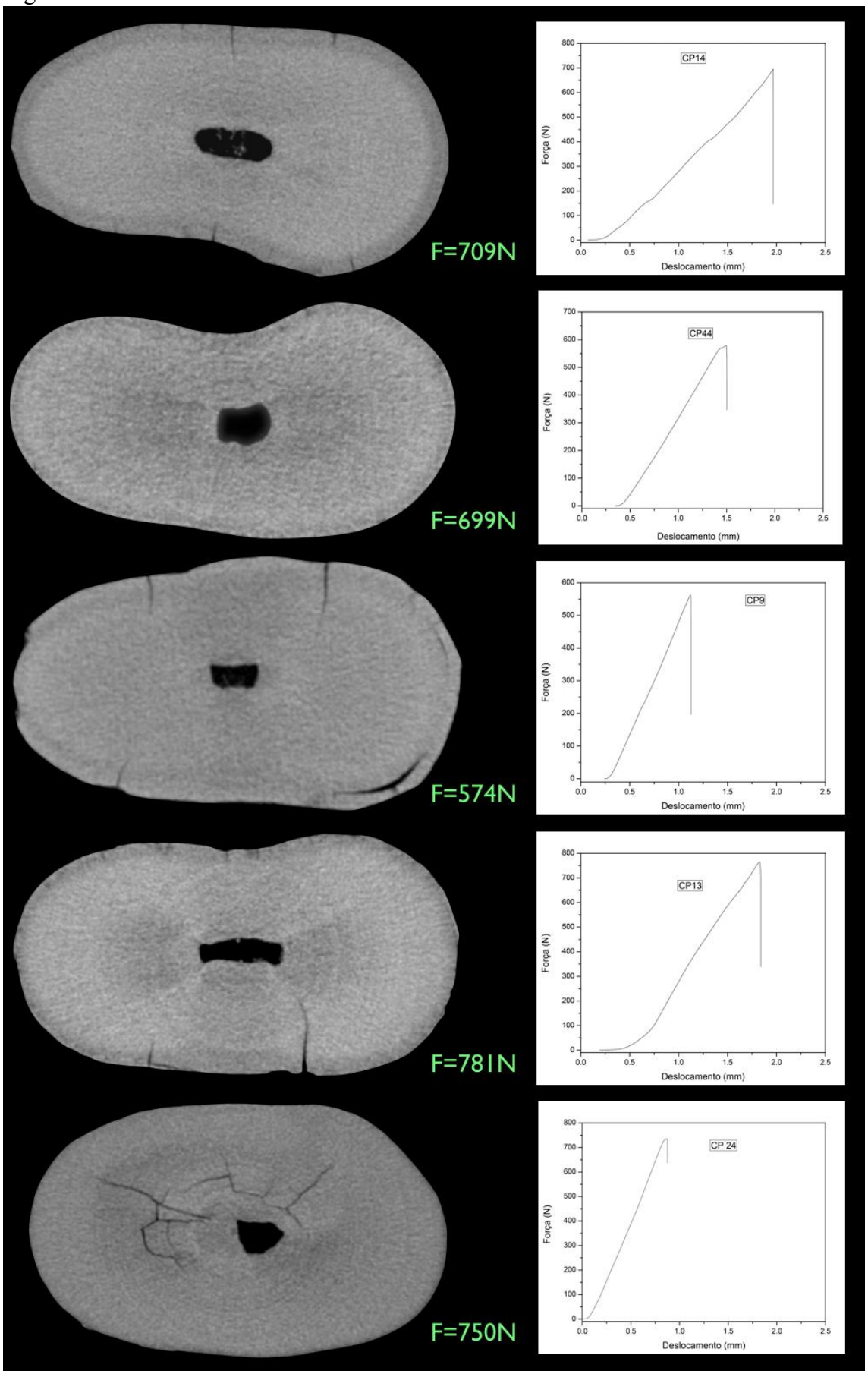
F=709N
CP14
F=699N
CP44
F=574N
CP9
F=781N
CP13
F=750N
CP 24
Força (N)
Deslocamento (mm)

Figure captions

Figure 1. 1A: Histogram illustrating microcracks data distribution; 1B: Histogram illustrating fracture load data distribution; 1C: Scatter plot X–Y graph showing the lack of correlation between two variables studied: quantity of microcracks per root and fracture resistance of the same.

Figure 2. Representative 3D models of the 2 failure modes obtained in the present study. Type I was considered as repairable fracture, allowing restoration, while type II was considered catastrophic fracture that definitely compromised tooth integrity.
2A: Type I – fracture in the cervical third of the root canal.
2B: Type II – vertical root fracture.
The arrows refer to the fracture lines.

Figure 3. Representative cross-sectional images illustrating the lack of correlation between the amounts of microcracks and force necessary to fracture the teeth

Captions to supplementary material

Figure 1. Visual representation of sample selection based on root canal anatomical configuration. (A1) Cross-sections reconstructions (A2) Root canal segmentation (A3) Aspect Ratio chart, demonstrating the bi-dimensional behaviour of the entire root canal. (B) Three-dimensional micro-CT reconstruction: 1 circular; 2 oval and 3 long oval

Figure 2. Visual representation of teeth with similar dentinal volumes. (A) Three-dimensional micro-CT reconstruction; (B) 1 – Cross-section of the reconstruction; 2 – segmentation of the cross-sections with the MinFeret diameter representation on the smaller diameter; 3 – Graph of the MinFeret values of all over the cross-sections. (C) three graphs together demonstrating numerical similarity of the mesiodistal diameter (MinFeret measure) throughout the entire root.