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Root dentinal microcracks: a post-extraction experimental phenomenon?

Introduction

During the investigation of vertical root fractures (VRFs), the microstructural integrity of root dentine and cementum has been evaluated using destructive (tooth-sectioning) (Hin *et al.* 2013, Liu *et al.* 2013, Arias *et al.* 2014, Ashwinkumar *et al.* 2014, Karataş *et al.* 2015, Saber & Schäfer 2016, Bahrami *et al.* 2017, Kfir *et al.* 2017) and non-destructive (micro-computed tomography [micro-CT]) experimental models (De-Deus *et al.* 2014, 2015, 2016, 2017a, 2017b, Bayram *et al.* 2017, PradeepKumar *et al.* 2017, Zuolo *et al.* 2017). Most of these studies used either teeth that had been stored for varying periods of time (Hin *et al.* 2013, Liu *et al.* 2013, De-Deus *et al.* 2014, 2015, 2016, 2017a, Karataş *et al.* 2015, Bayram *et al.* 2017, Zuolo *et al.* 2017) or were freshly extracted (Ashwinkumar *et al.* 2014, Saber & Schäfer 2016, Kfir *et al.* 2017, PradeepKumar *et al.* 2017) with only a few studies being conducted using cadaveric models (Arias *et al.* 2014, Bahrami *et al.* 2017, De-Deus *et al.* 2017b).

The use of a non-destructive high-resolution imaging technology, i.e. micro-CT, has made it possible to gain a more reliable insight into the phenomenon of dentinal microcrack formation. Micro-CT allows the internal structure of opaque objects (e.g. teeth) to be observed by screening hundreds of slices per specimen, where the full extent of crack lines can be mapped (De-Deus *et al.* 2014, 2015, 2016, 2017a, 2017b, Bayram *et al.* 2017, PradeepKumar *et al.* 2017, Zuolo *et al.* 2017). The use of micro-CT thus allows root dentine and cementum to be observed in their original state, i.e. after extraction, and then examined again after root canal procedures. Based on this method, two main conclusions have been drawn: (i) the lack of relationship between dentinal microcrack formation and mechanical preparation of root canals with nickel-titanium (NiTi) instruments *per se* (De-Deus *et al.* 2014, 2015, 2016, 2017a, 2017b, Bayram *et al.* 2017, Zuolo *et al.* 2017) and (ii) the recognition of preexisting microcracks as a phenomenon in untreated teeth (De-Deus *et al.* 2014, 2015, 2016, 2017a, 2017b, Bayram *et al.* 2017, PradeepKumar *et al.* 2017, Zuolo *et al.* 2017). Preexisting microcracks

are microstructural defects in roots of non-endodontically treated teeth with their aetiology being credited to factors such as age, parafunctional stresses (Yang *et al.* 1995, Chan *et al.* 1998), or restorative procedures (Kishen 2006, Shemesh *et al.* 2009).

Although rarely reported in destructive sectioning studies, preexisting microcracks have been observed in non-endodontically treated specimens even in the initial studies that focused mostly on the relationship between the development of dentinal defects and root canal preparation techniques (Arias *et al.* 2014, Karataş *et al.* 2015, Bahrami *et al.* 2017, Kfir *et al.* 2017). Interestingly, the use of micro-CT technology in studies using stored teeth revealed a high incidence (ranging from 12.31% to 41.44%) of preexisting microcracks in the baseline images acquired from untreated teeth (De-Deus *et al.* 2014, 2015, 2016, 2017a, Bayram *et al.* 2017, Zuolo *et al.* 2017). Actually, information provided by untreated control groups have been significant and controversial. Most often, no microcracks can be observed when stored sound teeth are sectioned horizontally (Shemesh *et al.* 2009, Karataş *et al.* 2015, Kfir *et al.* 2017), while in some studies using cadaveric models microcracks have been reported in the untreated control groups (Arias *et al.* 2014, Bahrami *et al.* 2017). Conversely, a low prevalence of preexisting microcracks was reported when evaluating freshly extracted teeth (7.1%) (PradeepKumar *et al.* 2017) or in a cadaveric model (2.46%) (De-Deus *et al.* 2017b) when using micro-CT technology. This means that the phenomenon of preexisting microcracks must be reconsidered as a consequence of the new evidence provided by the micro-CT imaging method and the use of either freshly-extracted teeth (De-Deus *et al.* 2017b, PradeepKumar *et al.* 2017) or teeth within a cadaver model (De-Deus *et al.* 2017b).

In summary, the existence of preexisting microcracks has been controversial. The somewhat puzzling occurrence of preexisting microcracks has created interest in potential aetiological factors as well as in determining whether VRFs are preceded by such microstructural defects. Considering its as-yet-unknown aetiology as well as the lack of knowledge on this phenomenon, the current study aimed to investigate the prevalence, location, and pattern of preexisting microcracks in non-endodontically treated teeth from fresh cadavers. Micro-CT technology was used as the analytical

tool enabling full screening of the root dentine with the teeth retained in their original alveolar socket. The core hypothesis being tested was that preexisting microcracks occur at a high frequency in non-endodontically treated teeth.

Materials and methods

Sample selection

Forty-two dentoalveolar maxillary and mandibular bone-blocks, each containing 3-5 adjacent teeth (a total of 178 teeth), were collected postmortem during the autopsy of several adult donors. The family members gave their informed consent which was obtained under a research protocol approved by the local Forensic Department and the National Committee on Health Research Ethics (protocol no. 931.732). The age of the donors ranged from 19 to 44 years (mean age, 31 years). Inclusion criteria were the presence of non-carious maxillary or mandibular first and second premolars and molars surrounded by alveolar bone and periodontal ligament. Bone-blocks with teeth were stored at -20 ºC and submitted to the experimental procedures within 40 days from their collection.

Before the scanning procedures, frozen bone-blocks were removed from the freezer and placed into a refrigerator at a constant temperature of 8 ºC for a slow defrost. After 3-4 hours, each bone-block was scanned in a micro-CT device (SkyScan 1173; Bruker-microCT, Kontich, Belgium) using an isotropic resolution of 13.18 µm at 90 kV and 88 mA through 360º rotation around the vertical axis, with a rotation step of 0.5º, camera exposure time of 1000 milliseconds, and frame averaging of 5. The x-rays were filtered with a 1-mm-thick aluminum filter. The acquired images were reconstructed into cross-sectional slices with NRecon v.1.6.10 software (Bruker-microCT) using standardized parameters for beam hardening (15%), ring artifact correction (5), and contrast limits (0.0095-0.03), resulting in the acquisition of 1300-1600 transverse cross-sections per bone-block.

Pilot study - validation method

Validation of the present method was based on 4 high-resolution micro-CT scans of a single bone-block containing 3 teeth (one premolar, one first molar and one second molar) following the same parameters previously described. The sequence of micro-CT scans was: (i) entire bone-block, (ii) extracted tooth, (iii) dehydrated extracted tooth, and (iv) entire bone-block after reinsertion of the extracted tooth into its alveolar socket (**Fig. 1**). The integrity of the dentine (presence of dentinal microcracks) was evaluated by screening the cross-sectional images obtained in the reconstruction step, from the cementoenamel junction to the root apex, by 3 blinded calibrated examiners. The calibration process was based on viewing sessions using cross-sectioning images with previously identified microcracks. The image analysis was repeated twice at 2-week intervals to validate the process of microcracks identification.

In the first scan, no microcracks were observed (**Fig. 2 [a] and [b], and Fig. 3 [a] and [b]**). Then, the maxillary second molar tooth was atraumatically removed from the bone-block by avoiding touching or damaging the surrounding tissues (**Fig. 1 [c], [d] and [e]**). This technique involved a careful detachment of 2/3 of the roots with periotomes until luxation occurred and, to minimize potential tooth damage, extraction forces were used only for tooth withdrawal, and not to loosen it. The extracted molar was immediately scanned and the cross-sectional images were screened as described above. No microcracks were observed in the second scan (**Fig. 2 [c] and Fig. 3 [c]**).

Aiming to induce the development of dentinal defects, the second molar tooth was submitted to a dehydration process using a standard graded series of alcohols (50%, 60%, 70%, 80%, 90% and 100% ethanol). Then, the tooth was placed into an auto-desiccator cabinet (Bel-Art automatic desiccator clear 2.0, Wayne, NJ, USA) and scanned on a weekly-basis to verify the presence of microcracks. After a period of 3 months, the scan (third scan) clearly revealed the presence of dentinal microcracks (**Fig. 2 [d] and Fig. 3 [d]**). Subsequently, the specimen was carefully reinserted in its original alveolar socket and the entire bone-block was rescanned (fourth scan). The image analysis of the cross-sections revealed that the microcracks observed when the tooth was outside the bone-

block remained detectable when the entire bone-block was scanned (**Fig. 2 [e] and [f], and Fig. 3 [e] and [f]**).

Image analysis

Visualization and qualitative analysis of the reconstructed image stacks of the 42 bone-blocks were assessed using CTVol v.2.3 software (Bruker-microCT). All cross-sectional images of the 178 teeth (n = 65,530) were screened from the cementoenamel junction to the apex to identify the presence of dentinal defects. Three previously calibrated examiners, blinded to the experimental design, screened all images at 2-week intervals. In case of divergence, images were evaluated together until full agreement was reached (De-Deus *et al.* 2016).

Results

In the pilot study, microcracks observed when the tooth was outside the bone-block remained detectable when the entire maxillary segment was scanned, which validated the method of assessing dentinal microcracks in a fresh cadaver model through micro-CT technology (**Figs. 1 to 3**).

From a total of 178 teeth in the bone-blocks removed from cadavers, 65,530 cross-section images were analyzed and no dentinal microcracks were detected. **Figs. 4 and 5** show representative images from coronal, middle, and apical thirds of a selection of teeth evaluated in the study.

Discussion

In the current study, the incidence of dentinal microcracks in non-endodontically treated teeth was assessed *in situ* through micro-CT images of 178 teeth in maxillary and mandibular bone-blocks obtained from 42 fresh cadavers. No preexisting dentinal microcrack were observed, refuting the core hypothesis. The absence of such dentinal microcracks in a methodology that is under close to *in vivo* conditions – a human cadaver model - suggests that microcracks may occur due to post-extraction manipulation or the storage conditions of the experimental teeth. This finding means that such

dentinal microcracks - observable in cross-sectional images of the roots - may not exist in the clinical setting; in fact, thus far this type of dentinal defect has been observable only under post-extraction experimental conditions (De-Deus *et al.* 2014, 2015, 2016, 2017a, Bayram *et al.* 2017, PradeepKumar *et al.* 2017, Zuolo *et al.* 2017).

The present result contrasts with the accumulated knowledge regarding dentinal microcrack formation that was been published since 2009 (De-Deus *et al.* 2014, 2015, 2016, 2017a, Bayram *et al.* 2017, PradeepKumar *et al.* 2017, Zuolo *et al.* 2017). Actually, the concept that dentinal microcracks are a post-extraction experimental phenomenon are partially supported by recent insights on this topic. Shemesh *et al.* (2018) reported the impact of environmental conditions on dentinal tissue and demonstrated that loss of water produces stresses that are sufficient to induce spontaneous dentinal defects, demonstrating experimentally that the biomechanical response of root dentine is highly influenced by its degree of hydration. This is in accordance with previous findings that showed that residual microstrain concentrations in hydrated roots was a controlled phenomenon and also that dehydrated dentine had lower toughness (Jameson *et al.* 1993, Kahler *et al.* 2003. Kruzic *et al.* 2003) and was more brittle (Huang *et al.* 1992). Thus, results reported in the study of Adorno *et al.* (2013) may be thought of as a consequence of tooth dehydration, since microcrack propagation continued in root slices even after 1 month of storage, even though no further stress was applied to the dentine. In this sense, the dehydration process that teeth endure outside of the oral environment may explain the high prevalence (12.31% to 41.44%) of dentinal microcracks in the baseline images of non-endodontically treated stored teeth evaluated through micro-CT technology (De-Deus *et al.* 2014, 2015, 2016, 2017a, Bayram *et al.* 2017, Zuolo *et al.* 2017), considering that the extracted teeth were obtained from tooth banks that used a variety of storage conditions.

Another important study used micro-CT to evaluate the prevalence, location, and pattern of preexisting dentinal microcracks in 633 freshly-extracted non-endodontically treated teeth and found dentinal defects in 45 teeth (7.1% of the sample) (PradeepKumar *et al.* 2017). Similarly, De-Deus *et al.* (2017b) using a cadaveric model also reported 2.46% of dentinal defects in the baseline images

from non-endodontically treated teeth. In accordance with the present results, these important outcomes establish a more realistic prevalence of preexisting dentinal microcracks in extracted teeth, in contrast with the substantial number of defects reported in previous micro-CT studies using stored teeth (De-Deus *et al.* 2014, 2015, 2016, 2017a, Bayram *et al.* 2017, Zuolo *et al.* 2017). These findings emphasise the low prevalence of preexisting microcracks and raise serious doubts about the validity of most studies on dentinal cracks using extracted teeth, since cracks are likely to be a consequence of the post-extraction experimental conditions. Based on this scientific evidence, it may be inferred that dehydration of dentinal tissue is the main cause of microcracks in non-endodontically treated teeth reported over the last decade. The prevalence of this phenomenon is thus a function of the interplay between the origin of the specimen - *storage versus freshly-extracted teeth/cadaveric model*, and the analytical method used - *sectioning versus non-destructive micro-CT*.

In a broader sense, the development of VRFs in endodontically-treated teeth is usually attributed to factors such as age, the root and root canal anatomy, masticatory function and/or the presence of excursive interferences or parafunctions that teeth could be subjected to during a patients' life (Arias *et al.* 2014). However, it is possible that VRFs develop as a consequence of cracked or split teeth initiated originally from the crown. Hence, it is important to consider the possible implications of the conditions under which teeth are stored when analysing the results of laboratory studies since unintentional dehydration will introduce systematic experimental flaws, irrespective of care taken during the rest of the experiment. This strongly suggests that *in situ* approaches such as models using fresh cadavers should be considered as a gold standard to assess the behaviour of dentinal tissue in terms of crack initiation and propagation.

The methodology used in the current study appears to be the close to an ideal experimental model to study the phenomenon of microcracks and the overall status of the dentine. The use of an *in situ* fresh cadaveric model, in which the bone and periodontal ligament remained preserved and also, the viscoelastic properties of the attachment apparatus, together with a highly accurate and non-destructive imaging method (micro-CT) for the assessment of the integrity of dentinal tissue has clear

advantages over other methodologies used previously in the study of dentinal defects, that is, sectioning and micro-CT analysis of stored teeth. Furthermore, the cadaveric model avoids the impact of tooth extractions and thus the use of periotomes, luxators or forceps, which usually are suggested as generators of dentinal defects. However, it is necessary to emphasise that the sampling used in the current study has one limitation, the age range of the cadavers which was between 19 and 44 years old. Thefore, future work should focus on assessing the presence of dentinal defects in older cadavers.

As stated in the first study on dentinal microcracks using a cadaveric model and micro-CT (De-Deus *et al*. 2017b), there is no international agreement, general regulations, or standards of tissue banking on a specific storage temperature for teeth inside bone-blocks. A statement from the American Association of Tissue Banks (2008) recommended a storage temperature of -20 °C for up to 6 months and -40 °C for longer periods of deep frozen preservation. However, the influence of storage time and freezing temperatures on the biomechanical properties of teeth are not entirely understood and are still to be determined. In the present study, the storage temperature of the cadaveric bone-blocks followed that used by De-Deus *et al.* (2017b) and did not affect the structure of the bone or teeth, which was -20 ºC, as recommended, with a period of slow defrost before scanning and further experimental procedures.

Concerns regarding whether the scanning resolution of micro-CT images may or may not be sufficient to detect smaller microcracks have also been raised (Pop *et al.* 205, De-Deus *et al.* 2016, PradeepKumar *et al.* 2017). Nevertheless, the validation of the micro-CT imaging method for the observation of the dentinal defects in extracted teeth has already been reported (De-Deus *et al.* 2016); it was demonstrated that defects visualized by direct observation of dentine with reflected light microscopy (using the sectioning method) are also visualized in the reconstructed cross-sectional images obtained by high-resolution micro-CT scans. However, the same is not necessarily valid when the teeth are scanned within cadaveric bone-blocks. Therefore, due to the innovative character of the observation of the dentinal defects in the images scanned from cadaveric bone-blocks, the validation of the method was necessary to eliminate any possibility of false-negative results. The results revealed

that the screening process was able to demonstrate the presence of the same microcracks in both experimental setups (tooth outside and inside the maxillary bone-block), validating the method to assess dentinal microcracks in a fresh cadaver model through micro-CT technology.

The result of this study suggests that future works should focus on the existence of dentinal root dentinal microcracks in non-endodontically treated teeth. In the meantime, until proven otherwise, it shoud be assumed that dentinal microcracks observed in stored extracted teeth subjected to root canal procedures are in fact a result of the extraction process and/or the post-extraction storage conditions. As a consequence, the presence of such dentinal microcracks in stored extracted teeth - observevable in cross-sectional images of the roots under experimental conditions - should be refered to as *experimental dentinal microcracks*.

Conclusions

This *in situ* cadaveric model revealed the absence of preexisting dentinal microcracks in non-endodontically treated teeth. This means that the prevalence of dentinal microcracks observed in previous cross-sectional images of stored extracted teeth is flawed. It also questions whether microcracks - observable in cross-sectional images of roots in extracted teeth really occur in non-endodontically treated teeth in the clinical setting.

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Figure legends

Fig. 1 Sequence illustrating the atraumaticaly extraction of a molar tooth used in the validation process. (a, b) 3D model and image of a maxillary bone-block of a fresh cadaver. (c, d) The atraumatic technique involved the careful detachment of 2/3 of the roots with periotomes until luxation occurred. (e) extracted second molar.

Fig. 2 (a) Typical cross-sectional image of a maxillary bone-block of a fresh cadaver. The molar tooth is visualized inside the alveolar bone socket. No microcracks are observed. (b) Detail of the distal-buccal (DB) root of the second molar underlining the integrity of the root dentine, where no microcracks are observed. (c) Cross-sectional image of the second molar tooth immediately after tooth removal. No microcracks are observed. (d) Cross-sectional image of the extracted second molar tooth scanned 3 months after the controlled dehydration process - a microcrack was induced and clearly observable in the DB root of the second molar tooth (arrow). (e) Cross-sectional image of the second molar tooth after reinsertion in its original place into the alveolar bone socket. The induced microcrack is clearly observable in the DB root of the second molar tooth (arrow). (f) Detail of the DB root of second molar underlining the presence of the microcrack (arrow) that was not present in images (a) and (b).

Fig. 3 Typical cross-sectional image of a maxillary bone-block of a fresh cadaver. The molar tooth is visualized inside the alveolar bone socket. No microcracks are observed. (b) Detail of the distal-buccal (DB) root of the second molar underlining the integrity of the root dentine, where no microcracks are observed. (c) Cross-sectional image of the second molar tooth immediately after tooth removal. No microcracks are observed. (d) Cross-sectional image of the extracted second molar tooth scanned 3 months after the controlled dehydration process - a microcrack was induced and clearly observable in the DB root of the second molar tooth (arrow). (e) Cross-sectional image of the second molar tooth after reinsertion in its original place into the alveolar bone socket. The induced

microcrack is clearly observable in the DB root of the second molar tooth (arrow). (f) Detail of the DB root of second molar underlining the presence of the microcrack (arrow) that was not present in images (a) and (b).

Fig. 4 Cross-sectional images of roots from a maxillary bone-block containing pre-molars and molars teeth where it is possible to observe the status of the dentinal tissue without any dentinal defect.

Fig. 5 Cross-sectional images of roots from a maxillary bone-block containing pre-molars and molars teeth where it is possible to observe the status of the dentinal tissue without any dentinal defect.