Hard-tissue debris accumulation analysis by high-resolution computed tomography scans

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Abstract

INTRODUCTION: Hard-tissue debris accumulation is a potential side effect of root canal instrumentation that has not been systematically investigated. In the current communication, a method to assess this debris using high-resolution microcomputed tomography (microCT) is presented.

METHODS: Based on prescans, mandibular molars with joining mesial root canals and isthmuses between these were selected (n = 6). The mean volume filled with apparent hard-tissue debris after instrumentation without irrigation was calculated over 2 mm of the mesial canal system by multiplying the voxel volume with the number of voxels representing acquired radiopaque material. Backscattered electron imaging was used to compare the calcium-phosphorus content of this material with that of the root dentin in the same specimen.

RESULTS: Backscatter scans showed that the accumulated debris viewed in the microCT scans was consistent with root dentin. In the selected canal segments, 29.2% +/- 14.5% of the original canal volume was filled with accumulated debris, which represented a significant change from the preoperative scan (p < 0.01, one-sample t test against zero). Three-dimensional reconstructions of the microCT images visualized the accumulated hard-tissue debris in the whole canal system.

CONCLUSIONS: The current method appears suitable to quantitatively compare different instrumenting/irrigating regimens on dentin debris accumulation.
Hard Tissue Debris Accumulation Analysis by High-Resolution Computed Tomography

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Abstract

Introduction Hard tissue debris accumulation is a potential side effect of root canal instrumentation that has not been systematically investigated. In the current communication, a method to assess this debris using high-resolution micro-computed tomography (µCT) is presented.

Methods Based on pre-scans, mandibular molars with joining mesial root canals and isthmuses between these were selected (n = 6). The mean volume filled with apparent hard tissue debris after instrumentation without irrigation was calculated over 2 mm of the mesial canal system by multiplying the voxel volume with the number of voxels representing acquired radiopaque material. Backscattered electron imaging was used to compare the calcium-phosphorus content of this material with that of the root dentin in the same specimen.

Results Backscatter scans showed that the accumulated debris viewed in the µCT scans was consistent with root dentin. In the selected canal segments, 29.2 ± 14.5% of the original canal volume was filled with accumulated debris, which represented a significant change from the pre-operative scan (p<0.01, one-sample t-test against zero). Three-dimensional reconstructions of the µCT images visualized the accumulated hard tissue debris in the whole canal system.

Conclusions The current method appears suitable to quantitatively compare different instrumenting/irrigating regimens on dentin debris accumulation.
Current concepts in root canal debridement, in absence of good alternatives (1), still largely rely on mechanical instrumentation of the canal system. However, canal preparation has its side effects. Some of these, such as alteration of the original canal anatomy, instrument separation, and the production of a smear layer on instrumented surfaces, have been investigated in detail (2, 3). Other untoward effects, on the other hand, have not gained much attention. Among these, accumulation of debris in canal fins, isthmuses, and ramifications could be clinically important, yet this outcome has not been assessed systematically.

Recent advances in scanning and computer technology made it possible to study tooth anatomy using high-resolution micro-computed tomography or short $\mu$CT (4, 5). This technology bears the advantage over the conventional anatomical methods that it is non-destructive. Teeth can thus be screened before and after treatment. This feature enabled researchers to measure previously unknown outcomes such as alteration of canal volumes by different instrumentation protocols and the amount and distribution of canal wall areas that have not been altered by an instrument (6). Thus far, $\mu$CT assessments have been related to the amount of hard tissue that was removed. However, the current technology theoretically also permits the evaluation of hard tissue debris that is not removed but rather transported into canal recesses.

The aim of the present study was to develop a method to view and analyze accumulated hard tissue debris in the isthmus area of mandibular molars that is created during rotary root canal instrumentation by $\mu$CT.

**Materials and Methods**

**Experimental teeth**

20 human mandibular first and second molars with fully developed roots were selected from the department’s collection of extracted teeth. The current research protocol was according
to the Guideline for Good Clinical Practice (ICH, Geneva, Switzerland) and did not alter the
treatment plan of any of the involved patients, who gave informed consent that their extracted
teeth could be used for study purposes. The institutional ethics committee approved the
procedures. Teeth had been stored in a 0.1% thymol solution for less than one year at 5°C.

**Preliminary tomography for selection of teeth**

Specimens were pre-scanned using a high-resolution micro-computed tomography system
(μCT 40, Scanco Medical, Brüttisellen, Switzerland) with an isotropic resolution of 72 μm at 70
kV and 114 μA. After three-dimensional reconstruction only teeth with joining mesial root canals
were included in further investigations. Isthmuses between mesiolingual and mesiobuccal root
canals were calculated in vertical dimensions. Thus, six mandibular molars containing mesial
isthmuses with a length of at least 4 millimeter could be selected for the current study. They were
mounted on SEM carriers (014001-T, Bal-Tec AG, Balzers, Liechtenstein) to allow exact
repositioning in the scanning system.

**Preparation of teeth**

Root canals of the molars were accessed using a diamond-coated bur and mesial canals
were instrumented using ProTaper instruments (Dentsply Maillefer, Ballaigues, Switzerland).
ProTaper SX, S1 and S2 were used to flare the orifice, coronal and middle third of the root canals
to get straight-line access. Care was taken to use SX only in the coronal part and S1 and S2 in the
coronal and middle part of the root canals. Subsequently canals were negotiated with K-files ISO-
size 10 (Dentsply Maillefer) until the tip was just visible at the main portal of exit. Working
length was determined by subtracting 1 mm from that length and a glide path was maintained by
using K-files size 15 and 20 to working length. Shaping of the canals was continued using S1, S2,
F1, F2 and F3 to working length. No irrigation solution was used during or after the instrumentation.

**Micro-CT measurements and evaluations**

Specimens were scanned before and after preparation using a commercially available micro-computed tomography system (µCT 40, Scanco Medical). Teeth were scanned at 70kV and 114 µA with an isotropic resolution of 20 µm resulting in 600 to 800 slices for each root. The volume of interest was determined for 100 slices in the main isthmus area of each mesial root resulting in a vertical length of 2 mm for comparative quantitative evaluation. A fixed threshold was applied to separate dentin from root canals and binary images of the root canals were produced. Although the mounting on SEM carriers ensured almost exact repositioning of the specimens for both scanning procedures, superimposition was calculated subsequently with newly developed software (IPL Register 1.01beta, Scanco Medical). Thus, the outer root contour was automatically registered of the post- versus pre-treatment scan. The two 3D scans were co-registered with each other with rigid 3D rotation and translation, determined by maximizing the cross-correlation of the two overlaid 3D data sets of the outer hull of the tooth which is unchanged by the root canal treatment. This co-registration was performed with an accuracy better than 1 voxel as determined on two test scans of an untreated tooth, where the subtraction image of the co-registered scans showed discrepancies less than 1 voxel (i.e. only a few isolated noise points remained).

Volumes of matched root canals before and after preparation were calculated using specially developed software (IPL V5.06B, Scanco Medical). Hard tissue debris was identified and calculated as follows: voxels that were identified as soft-tissue, liquid or air (canal volume) in the pre-operative scan but then were filled with radiopaque material in the post-operative scan
were assumed to be filled with hard tissue debris. Counting those voxels, multiplied with the volume of one voxel thus resulted in volume of apparent hard tissue debris filling the original root canal space. Voxels representing canal wall areas that were removed during instrumentation but then filled with debris could not be included in this calculation, because it is not possible to unambiguously discern between accumulated hard tissue debris and dentin on the scans.

For visualization of the complete mesial canal system before and after root canal preparation the volume of interest for each tooth was selected extending from the furcation region to the apex of the roots. Subsequently, the voxels that turned from radiolucent to radiopaque reflecting the accumulated hard tissue debris were superimposed on the original canal anatomy. Accumulated hard tissue debris could thus be visualized in the complete mesial canal system (Fig. 1).

**Electron microscopy**

After the µCT scans, teeth were infiltrated and finally embedded in ascending concentrations of an isobornyl methacrylate resin (Technovit 7200 VLC, EXAKT, Norderstedt, Germany). Resin blocks polymerized with white and blue light were cut to expose root trans-sections in the isthmus area using a diamond-coated saw (Isomet, Bühler, Switzerland) under water-cooling. Root sections were mounted on individual stubs and raw-polished using 1200 and then 2400 grit silicon paper. Thereafter, specimens were polished using diamond pastes of particle sizes down to 0.5 µm. The exposed surfaces were carbon-coated in an electron-beam evaporator (BAL-TEC MED 020, Bal-Tec AG). The specimens were examined in a scanning electron microscope (Supra 50 VP, Zeiss, Oberkochen, Germany) equipped with an annular mono-crystal scintillation type (YAG) backscatter detector in backscatter mode at an accelerating voltage of 10 kV and 9 to 12 mm working distance. Digital images were taken at magnifications
of 150 to 250 x. Subsequently, mapping with EDX (energy-dispersive x-ray analysis) was performed by integrating 102 frames for calcium and phosphorus.

**Data generation and analysis**

The total volume of apparent accumulated hard tissue debris was calculated in \( \text{mm}^3 \) per 2 mm of the selected root section containing the isthmus. This value was related to the original canal volume (main canals plus isthmus) in that section. To test the hypothesis that instrumentation caused a significant accumulation of hard tissue debris, the mean debris-filled volume in % of the original canal area was tested against zero (one-sample t-test against zero). The alpha-type error was set at .01.

**Results**

In the evaluated root sections, instrumentation of the mesial roots of lower human molars using the ProTaper system without irrigation left a mean of 29.2±14.5% of the original canal system filled with apparent hard tissue debris. This debris accumulation was statistically significant (one-sample t-test against zero, \( p<0.01 \)). Qualitative observation of the three-dimensional reconstructions of the \( \mu \text{CT} \) scans showed that the whole isthmus areas were almost completely filled with apparent hard tissue debris (Fig. 1). In addition, parts of the canal walls that had not been shaped by the instruments were filled with accumulated debris also, as were the apical portals of exit.

EDX analysis revealed that the accumulated debris depicted on the \( \mu \text{CT} \) scans contained as much calcium and phosphorus as the root dentin (Fig. 2).
Discussion

The current research resulted in a model that offers new possibilities in the assessment of root canal debridement. To the best of our knowledge, this would be the first time that hard tissue accumulation during instrumentation was monitored both qualitatively and quantitatively in human teeth. This first communication reports a proof of principle or methodological study. No irrigation was used during instrumentation. Future studies will show whether the issue is of clinical importance. This could be the case especially in canal systems with isthmuses that are in contact with the apical delta, as are commonly encountered in the mesial roots of mandibular and mesiobuccal roots of maxillary molars (7). As has been shown, accumulated debris certainly has a negative impact on the sealability of root canals (8), but it also may hamper disinfection in cases with apical periodontitis (9, 10). This, however, remains to be shown in future investigations.

The limitation of the current study is the fact that extracted teeth were used. The high radiation required for μCT scans makes it impossible to assess debris accumulation in situ. Furthermore, the necessity to perform pre-scans to select study teeth with similar anatomy would also, apart from ethical concerns, exclude the possibility to treat teeth in patients, extract them, and then assess debris accumulation. A further limitation is the fact that only hard tissue debris can be viewed, the remaining soft tissue is invisible. This is because μCTs are based on radiographic images. As indicated in the Materials and Methods section, the volume of accumulated hard tissue debris had to be related to the original canal volume and not to the canal volume after instrumentation for quantitative analysis.

In the current investigation, no irrigation was used during or after instrumentation to obtain the maximum possible amount of accumulated debris. Future studies could investigate the
effect of irrigation between instruments with an inert irrigant, as well as the impact of chelating agents and/or ultrasonic irrigant activation. One advantage of the µCT imaging employed here over other assessment methods is that it is non-destructive. Consequently, the same specimens can be scanned several times to view and compare hard tissue debris after different sequential treatment steps.

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References


Captions

**Figure 1.** Three-dimensional reconstructions of µCT scans of the mesial root canal systems of the six mandibular molars under investigation. (A) Pre-operative situations with the complex isthmus areas. (B) Corresponding three-dimensional µCT reconstructions after instrumentation. Note that a major part of the isthmus and apical delta volume has disappeared, because it was filled with radiopaque material. (C) Superimposition of the apparent accumulated hard tissue debris (grey areas) over the original canal anatomy (row A).

**Figure 2.** (A) µCT scan through in the apical third of a representative specimen. (B) The corresponding backscattered electron image of the polished root slice infiltrated with isobornyl methacrylate resin. Note the high similarity of radiopaque areas in the µCT scan (panel A) with the correlation of backscattered electrons registered by the backscatter detector in the scanning electron microscope (panel B). (C) Sampling for calcium (green); (D) sampling for phosphorus (red). Note that there was no apparent difference regarding those elements between the dentinal wall and the accumulated debris.