An analytical Micro CT methodology for quantifying inorganic dentine debris following internal tooth preparation

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ABSTRACT

Objectives: MicroCT allows the complex canal network of teeth to be mapped but does not readily distinguish between structural tissue (dentine) and the debris generated during cleaning. The aim was to introduce a validated approach for identifying debris following routine instrumentation and disinfection.

Methods: The mesial canals of 12 mandibular molars were instrumented, and irrigated with EDTA and NaOCl. MicroCT images before and after instrumentation and images were assessed qualitatively and quantitatively.

Results: Debris in the canal space was identified through morphological image analysis and superimposition of the images before and after instrumentation. This revealed that the removal of debris is prohibited by protrusions and micro-canals within the tooth creating areas which are inaccessible to the irrigant. Although the results arising from the analytical methodology did provide measurements of debris produced, biological differences in the canals resulted in variances. Both irrigants reduced debris compared to the control which decreased with EDTA and further with NaOCl. However, anatomical variation did not allow definitive conclusions on which irrigant was best to use although both reduced debris build up.

Conclusions: This work presents a new approach for distinguishing between debris and structural inorganic tissue in root canals of teeth. The application may prove useful in other calcified tissue shape determination.

Clinical significance: Remaining debris may contain bacteria and obstruct the flow of irrigating solutions into lateral canal anatomy. This new approach for detecting the amount of remaining debris in canal systems following instrumentation provides a clearer methodology of the identification of such debris.

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1. Introduction

Root canal therapy aims to eliminate infection from a diseased tooth and prevent future ingress of bacteria through filling the inside of the cleaned teeth.[1] Canals are shaped with files and irrigated with chemicals, removing dentine which may harbour bacteria.[1,2] Observations have shown biofilms to be embedded on inorganic tissue and its removal is therefore

important to successful treatment[2]. Irrigation of the inside of a tooth with a suitable disinfectant is an important part of the process. Various irrigation regimes exist with sodium hypochlorite (NaOCl) being clinically recommended because of its antimicrobial nature and ability to break down tissue.[3] Ethylene-Diamine-Tetra-Acetic acid (EDTA) is another commonly used irrigant which is capable of demineralising tissue.[3]

The removal of debris has been investigated on natural teeth[4–6] and with engineered tooth models.[7,8] Research has utilised qualitative scoring by clinicians[4,6,7] and most investigations are commonly performed on 2-dimensional (2D) cross-sections of teeth[4,5,9] which give a limited view of where debris is accumulating. The process of cross sectioning may alter the location of debris and this problem is further compounded by the complex systems present in mandibular molars.[10,11] Such issues may provide reasons why comparative studies of techniques have shown no statistical significance.[5,9,12]

Micro Computed Tomography (MicroCT) is a non-destructive imaging technique which uses X-rays to create high-resolution ($\sim$10 μm) cross-sections images through a specimen which can later be reconstructed as a 3D model. It is being used in various dental research applications. The geometry of root canals has been studied,[13] white spot lesions in enamel have been characterised[14] and demineralisation of enamel with treatment evaluated.[15] MicroCT is a valuable tool in endodontic research. A recent study has shown the successful image acquisition of isthmuses[10] while another has reported the ability to identify inorganic debris within a root canal.[16] Previous investigators have indicated the difficulty using this approach in distinguishing between structural and debris dentine as both have the same radio-opacity. Previous methodology relied upon relating the volume of debris to the original canal volume pre-instrumentation.[17] Subsequently, this method cannot quantify debris accumulation in the canal space created via instrumentation. As more effective instrumentation and irrigation methods are developed, analysis of debris in these areas will become increasingly clinically relevant.

The aim of this study was to extend current imaging approaches to develop a more robust analytical methodology for the quantification of debris without the need for qualitative clinical scoring. In addition we validated our method both systematically and computationally. The methodology was demonstrated by investigating the role of EDTA and NaOCl in canal debridement. The null hypothesis was that EDTA and NaOCl are as equally effective at facilitating the removal of debris.

2. Materials and methods

2.1. Main experiment

Twelve mandibular permanent molar teeth were obtained prior to the Human Tissue Act (UK). Teeth were divided into 3 groups for processing with: (i) no irrigation, (ii) irrigation with 17% aqueous EDTA (Vista Dental, Racine, US) and (iii) irrigation with 6% aqueous NaOCl (Vista Dental, Racine, US). The group containing teeth that were not irrigated was used as a positive control, as this was predicted to lead to the highest accumulation of debris. Canals were prepared by a Clinical specialist (Endodontist) using instruments from Dentsply Maillefer (Dentsply, Addlestone, UK). The canal length was measured with a ruler, mesial canals identified and negotiated to length to size 15 with stainless steel flexofiles. Canals were shaped to length with shaper S1, S2 and S3 files. Shaping was continued with finishers F1, F2 and F3 to ½ mm, 1 mm and 2 mm short of the working length. All files were Nickel Titanium ProTaper rotary files used at a speed of 300 rpm. Irrigant of 1 ml was applied immediately after initial canal negotiation and reshaping with each file. Irrigation was carried out with a 27G monoject needle (TycoHealthcare, Gosport, UK). Following canal preparation, teeth were analysed by the MicroCT system.

2.2. MicroCT imaging

Analysis of the anatomy of the teeth was undertaken using a MicroCT system (Skyscan 1172, e2v technologies plc, Chelmsford, UK). The lengths of the teeth were scanned at 80 kV, 124 μA, at an isotropic pixel size of 7–13 μm resulting in the acquisition of 1100–1200 transverse cross-sections per tooth. A camera exposure time of 620 ms, a rotation step of 0.4°, frame averaging of 9 and medium filtering of the data was applied. X-rays were filtered with 500 μm aluminium and a 38 μm thick copper filter. A flat field correction was taken on the day, prior to scanning to correct for variations in the pixel sensitivity of the camera. Images were reconstructed using NRecon 1.6.2 (SkyScan, e2v technologies plc, Chelmsford, UK) with a bream hardening correction of 25%, a ring correction of 20 and an attenuation co-efficient range of −0.005–0.05. To enable the teeth to be fixed onto the MicroCT stage, the crown of the tooth was cut using a diamond impregnated cutting wheel (TAAB, Aldermaster, UK), leaving a flat surface for positioning.

2.3. Quantitative image analysis

To reduce noise, all images were processed using an edge preserving smoothing algorithm as described by Gonzalez and Woods[18] and written as an Image J Plugin (University of Jyväskylä, Finland). A window of 3 and standard deviation of 15 were used.

Original canal space occupied by dentine after instrumentation was identified using a registration (superimposition) approach (see below).[17] Debris in newly created canal space was identified by its shape through mathematical morphology[19] as it had deposited in the form of small dentine chips following instrumentation.

The steps involved in quantifying debris are outlined in Fig. 1. Image of the canal before and after instrumentation were co-registered (aligned/overlayed) using Mattes Mutal Information[20] with 3D Slicer 3.6 (available from http://www.slicer.org/). This enabled dentine that had been packed into original canal space to be identified. Pixels that were occupied by air and then became dentine must be debris. Following registration, root canal space was identified through the selection of a range of MicroCT slices. The first slice in the sequence was below the pulp chamber when two roots separate to form two distinct channels. The last slice was at
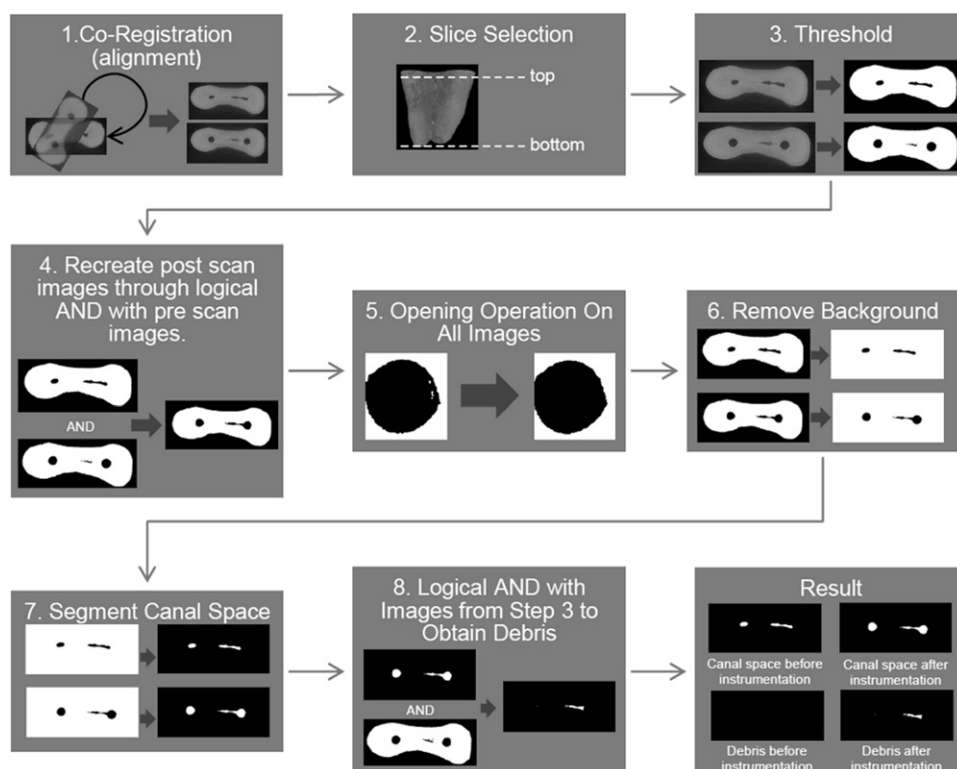
Fig. 1 – Flow chart illustrating how data was quantified.

the apex of the tooth. A global threshold was applied to construct an image which contained dentine alone. An intensity range of 90–150 was a suitable threshold for identifying dentine. Image pre-instrumentation were combined with post-instrumented canal image using a logical AND operation. The operation essentially combines the features from both data sets. This erased debris in unmodified canal space. Debris in modified canal space was removed using a morphological opening operation.[19] An opening operation removes objects which are smaller than a defined shape. Background was removed using a morphological closing operation and a subsequent region growing of the background. This prevented the background from being identified as canal space e.g. in the case where a canal opened at the surface of the tooth, into the background. A closing operation fills openings which are smaller than a defined shape, closing off any canals that exited the tooth prior to the apical foramen. Canal space was segmented using the region growing algorithm. Steps 3–8 were performed in Matlab 7.8.0.347 (The MathWorks, Cambridge, UK).

The result of the quantification process was a set of images containing the root canal space and images containing debris. Canal space and debris volumes were determined through voxel summation. Percentage of debris remaining was calculated:

$$t = \frac{d'''}{d' + d''} \times 100$$

where t is the % of total debris accumulated after instrumentation and irrigation, d' is the debris before preparation, d'' is the debris created during preparation and d''' is the debris

present after preparation. Debris before and after preparation are debris volumes acquired from the processed data. Debris created during preparation is proportional and was acquired from change in canal space volume.

The debris in the three groups was quantified in this manner. Proprietary SkyScan software CTAn was used to generate models which were then analysed using SkyScan software CTVol.

2.4. Validation of method

Five, grade 200 aluminium ball bearings of diameters 0.5, 1, 2.5, 5 and 10 mm (CCR Products, West Hartford, US) were placed in polystyrene blocks. Images were acquired by the MicroCT system using identical settings as used for teeth scanning. $V = (4/3)\,\pi \cdot r^3$.

Known spherical volume was calculated using the formula. Measured volumes as determined by MicroCT were calculated using the same method as used for canal space analysis. Analysis of the known and measured volume values enabled the calculation of percentage error.

Polystyrene blocks, containing the ball bearings were scanned 4 times, each time being removed and reinserted on the positioning stage. The computational processes were applied and quantitatively compared using tanimoto similarity coefficient.[21]

2.5. Statistical analysis

SPSS (Statistical Package for the Social Sciences) 19.0 was used for statistical analysis. The existence of normal distributions

in each data set was computed using Shapiro–Wilk analyses. Normally distributed data set was further tested for homogeneity using Levene. Since data was not homogenous, non-parametric Kruskal Wallis test and Mann–Whitney U test were applied. Power calculations were calculated using nomogram.[22]

3. Results

3.1. Method validation

Known and measured volumes of the ball bearings were similar (Fig. 2). The error for the 4 larger ball bearings was between 0.4 and 1.3% of the known volume but there was 10% for the smallest.

Tanimoto similarity coefficient was between 0.68–0.71 for debris and 0.9–30.94 for canal space. An image with the 4 repeats overlaid showed that differences were at the edges of debris build up. Calculating the percentage of debris left for each repeat of the same tooth showed a difference of 2% between the two most different image sets.

3.2. Efficacies of EDTA and NaOCl to remove debris

Location of debris was visually mapped using models (Fig. 3). Canal morphology prior to instrumentation is shaded light grey, new canal space dark grey and debris white. The dotted lines on the representative images of these models illustrate the approximate location of the corresponding cross-section. Visual inspection of the models demonstrated that there were large variations in both tooth morphology and location of debris accumulation (Fig. 3). The majority of debris accumulated in uninstrumented regions such as isthmuses (Fig. 3, A1), fins (Fig. 3, B3), and projections off the main canals (Fig. 3C2). Narrow isthmuses that were less than 68 μm were not visualised in the model (Fig. 3, tooth 4A).

Although a reduction in the mean amount of debris accumulated with no Irrigant, EDTA and NaOCl (Fig. 4), this was not statistically significant. The difference in variance, which reduced with EDTA and further with NaOCl was statistically significant.

4. Discussion

MicroCT is a useful approach for measuring debris in vitro as the canal is quantified in 3D and the tooth is not mechanically cross-sectioned. The localisation of debris can be understood through 3D maps and quantified by voxel summation. This novel study is a major advance for quantifying debris,[17] enabling the detection of debris in newly created canal space through its shape. It accounts for different amounts of debris created due to biological variation.

The ball bearings of known size were used to measure the systematic accuracy of the technique. The largest ball bearing was chosen to represent the size of a tooth and the smallest, the size of an internal micro-canal. The accuracy was unexpected for the smallest and movement artefact suggested it might be due to its lighter weight. The computational error (as measured by Tanimoto similarity coefficient) was high for canal space but lower for debris. Further analysis of the images showed that debris had moved within the canal. Debris is quantified as the total sum in the 3D canal and the results are therefore location independent. However, any debris movement during the scan would introduce a systematic error in the results. Calculating percentage debris left showed that the largest error resulted in a variance of 2%. This is small compared with the variance in a single irrigant, which was as great as 10% due to biological variability.

Despite an improved methodology for detecting debris, the study was unable to demonstrate statistical difference because of this biological variability in mandibular molars. Representative images of the 3D debris maps visualise how isthmus shape, size, protrusions and fins cause debris to accumulate in varying locations which will have differing accessibilities to the irrigant and instruments (Fig. 3). It was expected that the use of no irrigant would have produced a
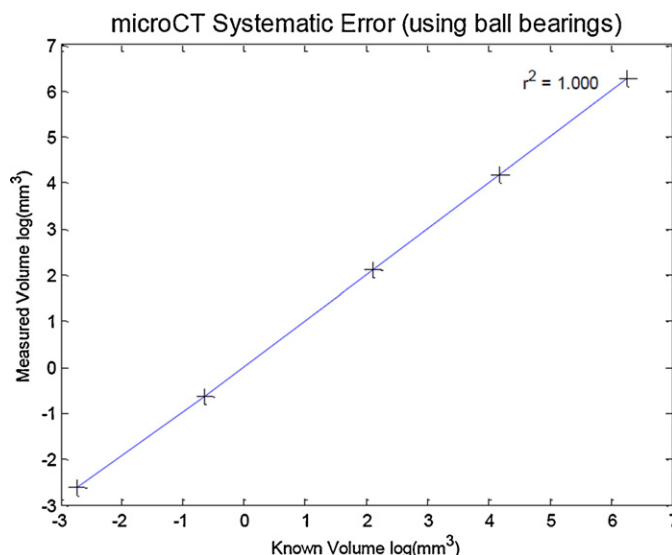

Fig. 2 – Comparison of known and volume (using ball bearings) as measured by MicroCT.
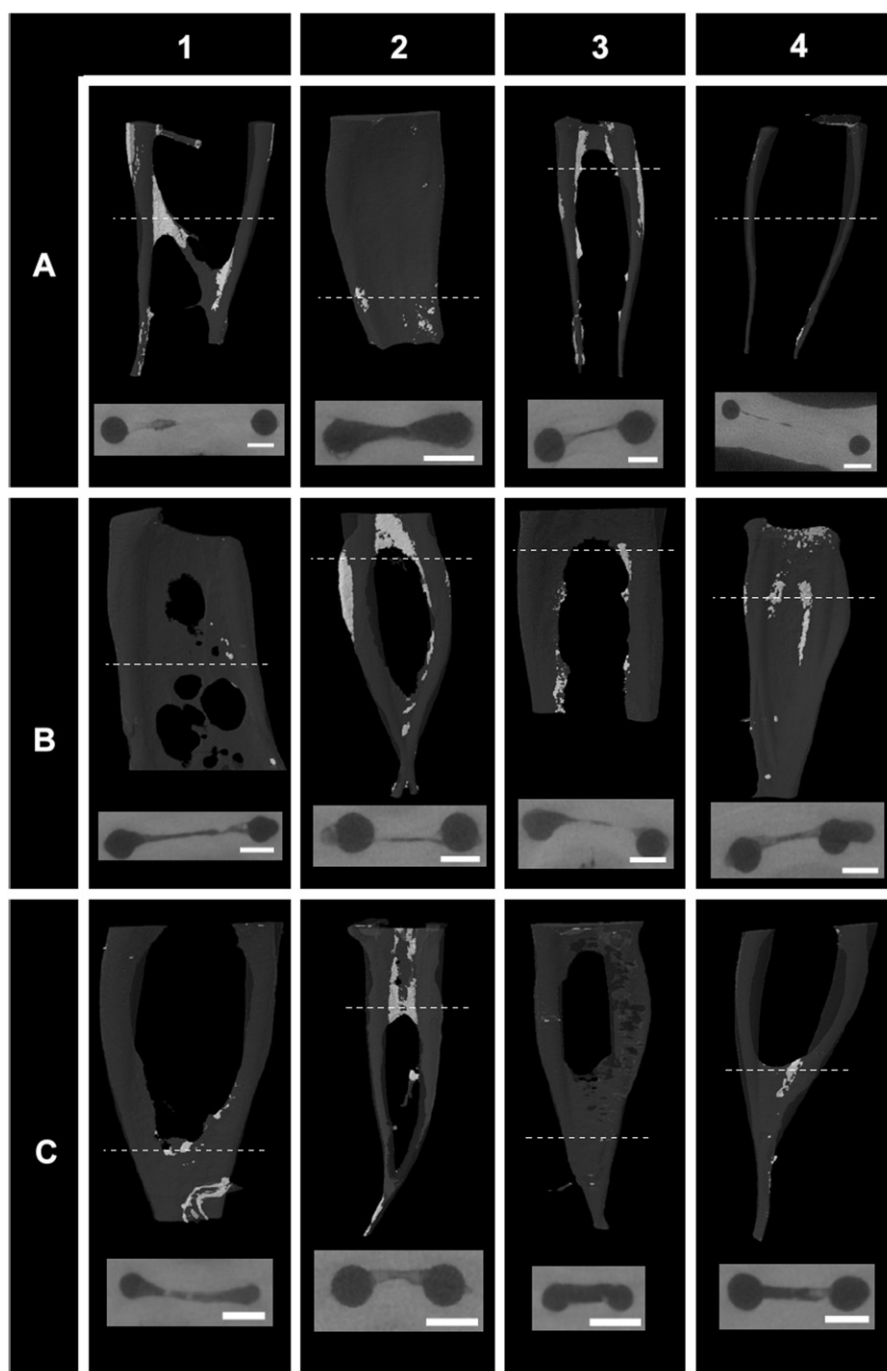
Fig. 3 – 3D representative images and cross-sectional slices of teeth from MicroCT data instrumented with no irrigant (row A), EDTA (row B) and NaOCl (row C). All scale bars represent 1000 μm.

statistically significant accumulation when compared with the use of an irrigant but there were no statistical differences (Fig. 3, compare 1A with 4A). Variation, which was statistically different, decreased with no irrigant, EDTA and NaOCl. Variation may decrease as the irrigant plays a greater role in debridement than morphology. Dentine debris consists of both organic and inorganic components. EDTA dissolves inorganic material such as hydroxyapatite, whereas NaOCl dissolves organic material such as pulpal remnants and collagen.[3] NaOCL may dissolve such organic material,

breaking down the debris into inorganic fragments, potentially enabling it to be flushed more effectively out of the canal. However, a calculation indicated that for a power of 80%, a total of 50 teeth per group were required to show differences in mean. The time for scanning each tooth together with reconstruction (~1000 h) did not make this a feasible way forward.

The particular complexity of this type of tooth has been realised in other studies[10,11,23] and the variation shown in this study is consistent with other researchers.[5,9,16,24–27] Despite
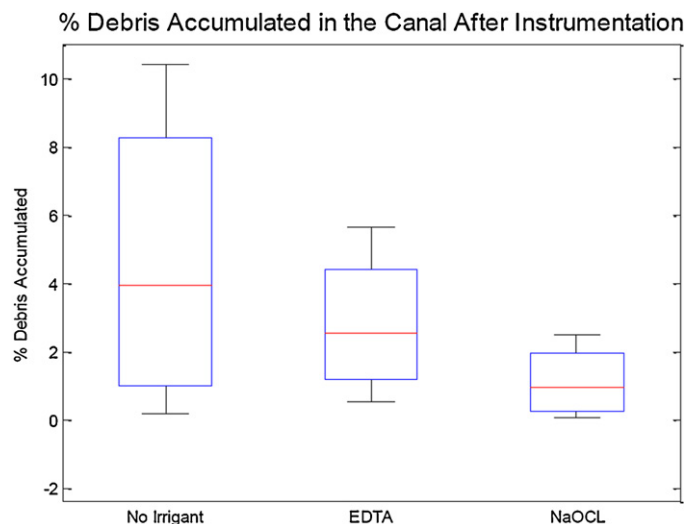
Fig. 4 – Box plot comparing the three irrigation regimes.

acknowledged variation, natural teeth are studied without prior consideration of their anatomy[5,9,16,24–27] and it is assumed that large sample sizes of 10–25 teeth per group are sufficient to show statically significance. Studies comparing different cleaning regimes have shown no statistical difference and it has been concluded that this was due to instrumentation.[5,9,16,24–27] However, the results might be because of the variation in this type of tooth rather than the lack of difference between instrumentation.

Instead of increasing sample size, it might be more feasible to carry out experiments on natural mandibular molars with similar anatomy, engineered models[7,28] or more recently computational fluid models.[29–31] A greater understanding of the effect that anatomy has on technique may enable improved methods to be developed enabling debris to be more effectively removed.

In conclusion, an improved method was presented and validated but it was not possible to discard the null hypothesis because of biological variation in mandibular molars. Isthmuses, protrusions and fins create complexities, which should be taken into account in comparative studies. This work presents a validated improved approach for distinguishing between debris and structural dentine in the full canal and offers a potential approach to overcome anatomical variability. Such a methodology may have applications, e.g. the determination of calcified tissues volumes before and after treatment.

Acknowledgements

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