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Identification of root filling interfaces by microscopy and tomography methods

P. Zaslansky[1], P. Fratzl[1], A. Rack[2], M-K. Wu[3], P. R. Wesselink[3] & H. Shemesh[3]

[1]Department of Biomaterials, Max-Planck Institute of Colloids and Interfaces, Potsdam, Germany; [2]European Synchrotron Radiation Facility, Grenoble, France; and [3]Department of Endodontology, Academic Centre of Dentistry Amsterdam (ACTA), University of Amsterdam and VU University, Amsterdam, The Netherlands

Abstract

Zaslansky P, Fratzl P, Rack A, Wu M-K, Wesselink PR, Shemesh H. Identification of root filling interfaces by microscopy and tomography methods. *International Endodontic Journal*, **44**, 395–401, 2011.

Aim To assess differences in observed cross-sectional areas of root canals and filling materials, as imaged by three microscopy and two tomography methods.

Methodology Six roots filled with laterally compacted Gutta-percha and AH26 were scanned with phase-contrast enhanced microtomography in a synchrotron facility. Reconstructed virtual slices were compared with sections of both wet and acrylic-embedded roots, evaluated also by light and electron microscopy (EM) and laboratory-based microtomography (µCT). The different contrasts of Gutta-percha, voids, sealer and root dentine were identified and correlated. Inner canal border, outer Gutta-percha rim and the external margin of a void were manually delineated, and the enclosed areas were repeatedly measured by three observers. Interobserver and interimaging method differences were tested by 2-way ANOVA with Bonferroni adjustments ($P < 0.05$). Percentages of Gutta-percha-filled canal areas (PGP) were determined.

Results Phase-contrast enhanced microtomography revealed internal interfaces and detailed 3D volumes of accentuated voids as well as micrometre-sized particles and gaps within the treated roots. Overestimates in the cross-sectional areas were obtained by light microscopy, whereas underestimates were obtained by µCT and EM. Differences exceeded 40%; however, PGP values by all methods were within 5% for the same slice. Differences between observers were sometimes significant, but they were not method related (<3%).

Conclusions Phase-contrast enhanced microtomography is a powerful non-destructive *ex vivo* investigation method for studying the interfaces within root canals and filling materials at a micrometre resolution. The method does not require damage-prone sectioning/polishing during sample preparation procedures. Caution should be used when quantifying the extent of Gutta-percha in root fillings by measurements using µCT, light and EM.

Keywords: imaging, microCT, PGP, phase-contrast enhanced radiography, root canal filling quality, synchrotron.

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Introduction

Effectively filling the cleaned and shaped root canal is a central objective of root canal treatment, aimed at promoting healing of the supporting tissues (Sjogren *et al.* 1990). Under *in vivo* conditions, the adaptation between the root filling and dentine is judged mainly by clinical radiography with well-known shortcomings (Kersten *et al.* 1987). In the laboratory, however, various methods are available for determining how well the filling materials adapt to the prepared root canal. Root filling qualities are often determined by leakage tests and/or cross-sectional observations; the former (Wu & Wesselink 1993) are used to directly quantify the permeability of the root canal system (Shemesh *et al.* 2006), whereas the latter provide direct, visual estimates of the relative dimensions and relations of the canal and filling materials.

Correspondence: Dr Paul Zaslansky, Department of Biomaterials, Max-Planck Institute of Colloids and Interfaces, Wissenschaftspark Potsdam D-14424, Germany (Tel.: +49 331 567 9439; fax +49 331 567 9402;
e-mail: paul.zaslansky@mpikg.mpg.de).

Evaluating root treatments by observing cross-sections of fillings through the microscope has the advantage of being rapid and practical, allowing for inter- and intraroot comparisons of a given treatment. Cross-sectional observations by light microscopy (LM) have frequently been used to report the outcomes of root filling procedures and to grade new products and treatment approaches (Mannocci *et al.* 1998, Őzok *et al.* 2008, van der Borden *et al.* 2010). Such grading often seeks to quantify the percentage of Gutta-percha-filled canal (PGP). Reported PGP values span 66–99% (Wu & Wesselink 2001, Jung *et al.* 2003) where 66% PGP indicates that only 2/3 of the canal cross-section is actually filled with Gutta-percha.

Electron microscopy (EM) has also been used for assessing the quality of root fillings, by studying interfaces between fillings and canal walls (Gondim *et al.* 2003, Shipper *et al.* 2004). An increase in the availability of microtomography (μCT) makes for an attractive non-destructive alternative to the above-mentioned methods (Jung *et al.* 2005). Virtual slices can be created easily within three-dimensional (3D) reconstructions of tomography scans, circumventing the need for sectioning the roots. This is important because sectioning may result in the formation of artefacts due to mechanical damage and it is also only useful for creating a small number of slices per given root. Presently, tomography also has limitations related to artefact formation (Tofts & Gore 1980). Furthermore, even the best μCT images often contain substantial differences of contrast between the root and filling materials. These result in great difficulties in identifying and differentiating between the canal walls and the filling components.

Recent phase-contrast high-resolution X-ray imaging techniques, usually obtained in synchrotron radiation research facilities, provide extensive details and high resolutions. Some of these radiation facilities also deliver partially coherent X-ray beams, such that strong signals may arise at internal interfaces and material boundaries within the irradiated objects (Cloetens *et al.* 1997). Radiographs obtained in this manner can be used to create phase-contrast enhanced micro-CT (PCE-CT) reconstructions. These are well suited for detecting micrometre-sized discontinuities and voids that are typically found in teeth (Zaslansky *et al.* 2010). With respect to root fillings however, it is not known how PCE-CT data relate to conventional microscopy or μCT scans. Furthermore, because current image analysis methods require a human observer to determine where one material ends and where other materials begin, interobserver discrepancies may exist. Thus, it is important to establish how reliably each of the materials in root fillings is identified, traced and measured when imaged by different methods.

The purpose of this study therefore was to investigate the type and quality of details that are observed in treated root canals scanned by PCE-CT and to compare measurements of cross-sectional areas occupied by the materials within root fillings, when imaged by PCE-CT versus LM, EM and μCT.

Materials and methods

Six roots filled by lateral compaction of Gutta-percha and AH26, randomly chosen from a pool of 60 treated teeth (Shemesh *et al.* 2006), were scanned with PCE-CT. Teeth were continuously immersed in water, except during the 25-min scan times when they were placed in the high-resolution microtomography set-up of the BAM*line* at BESSY-II (Berlin, Germany) (Rack *et al.* 2008, Zaslansky *et al.* 2010). The set-up was operated at 28 keV and, similar to conventional tomography, multiple projections of the sample were obtained from different angles around the rotation axis. In this manner, 900 radiographs were recorded for each root at angular rotation steps of 0.2°, requiring 0.2 s exposure times per projection. A detector with 2.5-μm effective pixel size was used, positioned 430 mm behind the sample (Zaslansky *et al.* 2010). All X-ray images contained the radiographic apex of every treated tooth, including 2 mm of root filling as well as 0.5 mm of the canal beyond the Gutta-percha tip. Each series of radiographs was concomitantly normalized and reconstructed by the backprojection method (Octopus V8.1, Zwijnaarde, Belgium) (Vlassenbroeck *et al.* 2007). The reconstructed PCE-CT volumes were visualized (Amira 4.1; Visage Imaging GmbH, Berlin, Germany) and virtually sliced within the computer memory (PCT-CT slices) where five teeth were chosen for actual sectioning and imaging by conventional methods. Accordingly, three of the roots were embedded in acrylic, whereas two of the roots were kept wet and prepared as follows: first, the teeth were serially sectioned across the root orthogonal to the crown-root axis, using a water cooled slow-speed diamond wheel (Isomet Buehler LTD, Lake Bluff, IL, USA). Slices were thus obtained at 600- to 1000-μm increments coronal to the Gutta-percha tip. These still unpolished slices were imaged by LM (LM-U slices), equipped with a camera (Leica DFC 480 + DM-RXA2; Leica-Microsystems GmbH, Wetzler, Germany) with a 0.5-μm effective pixel size. Each slice was then wet machine-polished utilizing a series of grinding papers and diamond slurries (Logitec PM5 diamond paste; Logitec Ltd. Old Kilpatrick, UK, and METADI diamond pastes, Coventry, UK) down to 1 μm. The now-polished slices were again imaged by LM (LM-P slices) and then observed in a low-vacuum water-vapour EM (EM slices; obtained in an FEI Quanta 600, Eindhoven, the Netherlands). Three of these now-polished slices were eventually X-ray scanned in a laboratory-based μCT (Skyscan 1072, Kontich, Belgium) at 100 keV, 3.1-μm effective pixel size, 6 s exposures. They were then reconstructed (Nrecon 1.6, Skyscan, Kontich, Belgium) and visualized in a manner similar to the PCE-CT data, such that virtual images of the samples and particularly of the polished surfaces were obtained (μCT slices). Corresponding features in images obtained by LM, EM and μCT were matched and identified in the PCE-CT slices.

The three teeth designated for acrylic-embedding were dehydrated in a series of increasing ethanol–water exchange solutions and embedded in Poly-methyl methacrylate (PMMA; Merck, Darmstadt, Germany). These samples were then sectioned and polished along and across the root axis for comparative dry-imaging by LM-P, EM and μCT and compared with the PCE-CT scans of the original intact roots. Substantial dimensional distortions were revealed following this standard laboratory preparation approach; therefore, these samples were only used for comparative identification of the main root filling constituents, namely Gutta-percha, dentine and voids, as seen by each imaging method (data not shown).

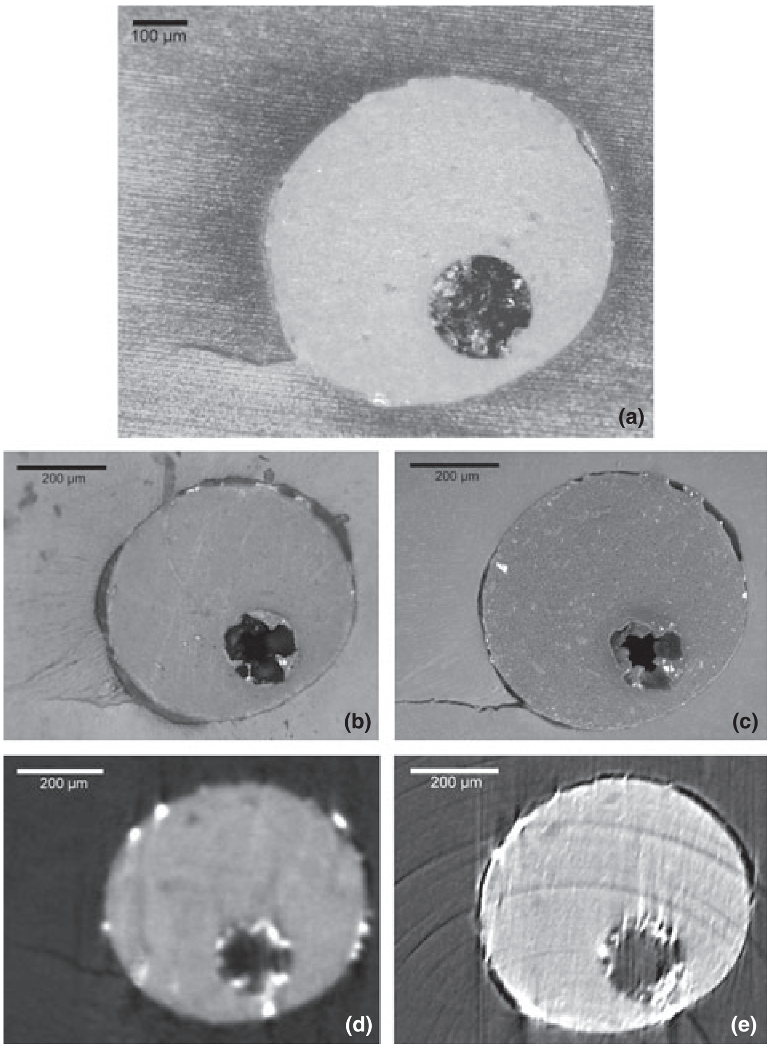

Figure 1 A typical root section imaged by different methods: (a) Light microscopy unpolished (LM-U) (b) Light microscope following polishing (LM-P) (c) Low-vacuum water-vapour imaging electron microscopy (EM) (d) laboratory microtomography (μCT). (e) Same slice located within a volume obtained by phase-contrast enhanced microtomography (PCE-CT). The noticeable ring-artefacts are a common noise in high-resolution tomography, but they have negligible effects on the measurements reported here. Note absence of crack on lower-left rim of the sample only in this image. The crack must have developed during sectioning and is seen in panels a–d. The variable visibility of the sealer by the different imaging methods, specifically within the finger-spreader void area, precluded reliable delineation.

Quantification of the cross-sectional areas was performed on an identical section that was imaged by all methods (Fig. 1). ImageJ (Rasband 1997–2009) was used to manually trace the internal edges of the canal, the margins of Gutta-percha and the perimeter of a void, so as to enclose areas on the surface of the section that was imaged by each method. The data obtained included cross-sectional areas of the inner canal wall, the outer rim of Gutta-percha and the margin of a finger-spreader void (identified by chance within Gutta-percha in this particular slice). Each measurement was repeated thrice by three independent evaluators, instructed to identify and trace the aforementioned interfaces. PGP was then calculated by averaging the ratios of filling-areas less void areas, divided by canal areas (Wu & Wesselink 2001). Two-way anova with Bonferroni adjustment, $P < 0.05$, (Sigmaplot 11; Systat Software Inc., Chicago, IL, USA) was used to analyse the results.

Results

Figure 1(a–e) shows a typical section as seen by all imaging methods (LM-U, LM-P, EM, μCT & PCE-CT). Marked differences in contrast are evident in the appearances of Gutta-percha, voids and dentine. The contrast of the sealer varies immensely from one imaging method to another, effectively precluding reliable identification and comparison.

Figure 2 graphically depicts the area measurements of all observers for all methods. Overall, it can be seen that areas measured on the unpolished slice are larger than the same areas measured by the other imaging methods. The largest discrepancies found between identical structures were 41% for the void, 15% for the Gutta-percha and 9% for the canal. Pooled PGP means and standard deviations revealed that EM and LM-U provided the highest PGP values (90.8 ± 0.8% and 89.6 ± 1.6%, respectively) followed by 87.2 ± 1.0% for PCE-CT, 86.1 ± 4.2% for μCT and 85.6 ± 1.3% for LM-P. The greatest differences between observers: 2.8% for the canal and 2.4% for the Gutta-percha areas were statistically significant. No significant differences were found between observer measurements of the void area. The supplementary online 3D reconstruction movie and additional stereo-image (red/cyan anaglyph, requiring use of red/cyan 3D viewing glasses) demonstrate the exquisite details observed in the PCE-CT scans, where features down to the sizes of silver particles in the AH26 are revealed.

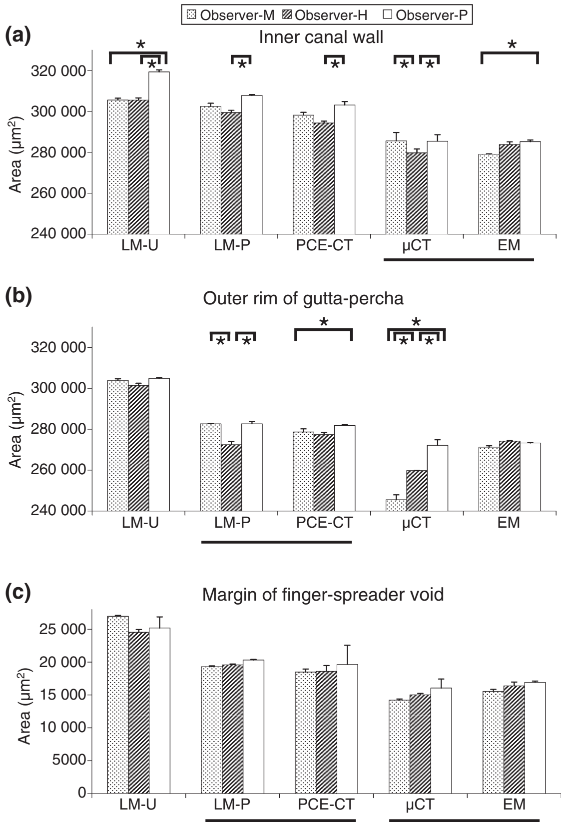

Figure 2 Areas [μm^2] determined by three observers for (a) dental canal, (b) Gutta-percha rim and (c) void within Gutta-percha. Data grouped by method: unpolished light microscopy (LM-U), polished light microscopy (LM-P) phase-contrast enhanced microtomography (PCE-CT), laboratory-based micro-CT (μCT) and electron microscopy (EM). Lines beneath the abscissa indicate non-significant differences between the means ($P > 0.05$). Measurements found to be significantly different between observers ($P < 0.05$) are indicated by starred brackets above the graph columns. Error bars indicate standard deviations.

Discussion

Phase-contrast enhanced microtomography offers exciting opportunities to provide non-destructive information about root fillings in the laboratory, which may further improve understanding of root canal preparation procedures and treatment outcomes. The PCE-CT method emphasizes the Gutta-percha, dentine and void interfaces at high resolution (Video S1), such that relations between the natural tissue and treatment materials can be quantified at the micrometre length scale and in 3D. PCE-CT also does not suffer from the extreme contrast differences seen with μCT, although edges may appear overly enhanced resulting in streak artifacts (Fig 1e).

The LM-U results were similar to those reported in other studies (Jung *et al.* 2003, Őzok *et al.* 2008), and presumably they represent the typical level of detail that one may expect to obtain by this standard imaging method. All LM-U measurements revealed average greater area estimates and exhibited blurring of canal and Gutta-percha edges, when compared with the other imaging methods. An explanation for the reduced visibility of root filling details by LM-U following conventional sectioning (Fig. 1a) relates to the state of the imaged cross-section surface: scratches, height irregularities and a thick smear-layer make focusing difficult whilst concealing the dentine-filling interfaces, and consequently degraded images were seen by LM-U. Unpolished slices thus lead to overestimates of the Gutta-percha and dentine cross-sectional areas, and they result in an underestimate of the degree of mismatch between these materials.

The LM-P measurements revealed area estimates similar to those obtained by PCE-CT, and clear views of edges with micrometre-sized details were obtained (Fig. 1b). Both the LM-P and PCE-CT methods allowed unequivocal identification and delineation of dentine and Gutta-percha edges, providing superior information about the root filling when compared with LM-U and μCT. Note that the central finger-spreader void appears to exhibit some smearing after polishing (Fig. 1b,c). Empty voids such as this may inadvertently trap debris during the polishing procedures, and one cannot exclude the possibility that a thin smear-layer remains even though minimal blurring is seen, when compared with LM-U. The LM-P method is thus advantageous, but not flawless.

EM and laboratory-based μCT images (Fig. 1c,d) yielded similarly lower average area estimates, which is attributable to dehydration that accompanied the hours-long scan times. Whilst a shrinkage exceeding 10% appears in the current study, different sample dimensions and dehydration conditions result in variable loss of water and consequent unpredictable (although moderate) dimensional changes. Even when humidity fluctuations are controlled in the μCT, extreme contrast differences emerge coupled with well-known reconstruction image artefacts (Tofts & Gore 1980) resulting in difficulties of tracing interfaces of Gutta-percha and dentine by this method. This is in agreement with the findings of Huybrechts *et al.* (2009) who reported that cone-beam CT scans of root fillings have significant artefacts caused by the filling materials as well as the limited resolution. Laboratory investigative μCT thus offers less information than PCE-CT, LM-P and EM and should be used with caution for studying interfaces in root fillings.

When converting all area measurements into PGP (Wu & Wesselink 2001), the LM-P and PCE-CT methods revealed intermediate values, highlighting the suboptimal quality of the particular filling shown in Fig. 1. PGP values as determined by LM-U, however, appear to be misleadingly high, erroneously suggesting a good quality of this filling. Thus, PCE-CT or LM-P might be important to avoid overestimation of the PGP in root fillings. The high PGP estimates found by EM were surprising, given the reduced Gutta-percha and canal areas that were observed. Presumably the shrinkage of dentine in the low-vacuum EM chamber exceeded that of Gutta-percha, resulting in favourable PGP ratios. The reasons for lower PGP values reported by μCT are less clear. They may be related to the greater standard deviation values seen in the pooled μCT PGP estimates, attesting to the difficulty of different observers to clearly determine the interfaces of Gutta-percha and dentine.

Small differences were seen between observers, and the relative interobserver uncertainty is <3% by all methods. Thus, the manual process of identifying and delineating root canal interfaces is not the reason for the differences exhibited by the imaging methods discussed here. This is also in agreement with Huybrechts *et al.* (2009) who concluded that the correlation between the ability of different observers to detect a void within the root filling by different methods was high overall.

The striking difference of 41% between EM and LM-U cross-sectional area measurements for the finger-spreader void suggests that only the high-precision imaging methods (LM-P and PCE-CT) should be used for studying small inclusion voids in root filling materials.

Conclusions

Phase-contrast enhanced microtomography provides substantial information, revealing cross-sectional area estimates in root fillings that are similar to those obtained by LM-P sections. For endodontic research, PCE-CT has the advantage of providing information non-destructively and in three dimensions. Marked differences exist in the cross-sectional areas of the canal, Gutta-percha and void when determined by 2D and 3D imaging methods. Sample preparation sectioning and imaging conditions may inadvertently change the root canal and treatment dimensions: non-destructive imaging methods such as PCE-CT should thus be used, at least as control measurements.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. A stereo-image (red/cyan anaglyph), showing in 3D a reconstruction of a phase-contrast enhanced (PCE-CT) root tip, providing a qualitative estimate of the type of data obtained and revealing the spatial distribution of micrometre length-scale details within the root canal filling and surroundings.

Video S1. An animation and 3D rendering of data in a typical phase-contrast enhanced microtomogram (PCE-

CT) of a root and root canal filling. A slice across this root corresponds to the data shown in Fig. 1. Exquisite details are revealed within the whole, wet sample. Colours however are arbitrary and correspond to intensity values. For estimates of dimensions see Fig. 1.

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