Introduction

Matching the filling material to a soundly cleaned and shaped root-canal is a central objective of root-canal treatment (Sjogren *et al.* 1990) aimed at promoting healing of the supporting tissues. Under *in-vivo* conditions, the match between the root-canal filling and the dentine is a measure of the quality of treatment that is judged mainly by clinical radiography, of well known shortcomings (Kersten *et al.* 1987). For *in-vitro* studies, additional methods are available for determining how well the filling materials (usually gutta-percha and sealer) are adapted to the prepared root-canal. Root canal treatment qualities are often determined by leakage tests and/or cross-sectional observations: the former (Wu and Wesselink 1993) are used to directly quantify root obturation outcome (Shemesh *et al.* 2006) whereas the latter provide direct, visual estimates of the relative dimensions and relations of the canal and filling materials.

Observing root-canal filling cross-sections through the microscope has the advantage of being fast and practical, allowing for inter- and intra-root comparisons of a given treatment. Indeed such cross sectional observations have frequently been used to report the outcomes of root-canal filling procedures and to grade new products and treatment approaches by light microscopy (LM) (Mannocci et al. 1998, Özok *et al.* 2008, van der Borden *et al.* 2010). Such grading often seeks to quantify the percentage of gutta-percha filled the canal (PGP); reported values span 66-99% (Jung *et al.* 2003, Wu and Wesselink 2001)

where 66% PGP indicates that only 2/3 of the canal cross-section are actually filled with gutta-percha.

Electron microscopy (EM) has also been used for assessing the quality of root canal fillings, by studying the interface between fillings and root-canal walls (Shipper *et al.* 2004, Gondim *et al.* 2003). An increase in the availability of micro-tomography (µCT) makes for an attractive non-destructive alternative to observing real cross-sectional slices by microscopy (Jung *et al.* 2005). Virtual slices can easily be created in three-dimensional (3D) reconstructed tomography scans, circumventing the need for sectioning the roots. Sectioning may result in the formation of artifacts and only produces limited numbers of samples. Furthermore, substantial differences in contrasts often produce difficulties in reliably identifying and differentiating between the root-canal walls and the filling components.

Recent phase-contrast high-resolution X-ray imaging techniques, usually obtained with synchrotron-radiation research facilities, provide extensive details due to high-resolution and high-brilliance of these X-ray machines (particularly when coupled with partial-coherence, see Cloetens *et al.* 1997). The strong signals arising at internal interfaces and material boundaries make phase-contrast enhanced micro-CT (PCE-CT) well suited for detecting micrometer-sized discontinuities and voids, typical to what is found in teeth (Zaslansky *et al.* 2010). However, with respect to root-canal fillings, it is not known how PCE-CT data relate to conventional microscopy and micro-CT measurements, previously reported in the literature. Furthermore, all evaluation methods require a human

observer to determine where one material ends and where other materials begin and consequently inter-observer discrepancies may exist. Thus, treatment outcomes determined by different methods in different studies need to be calibrated.

The purpose of this study was to investigate the type and quality of details that may be seen in treated roots by PCE-CT and to compare measurements of the areas occupied by the materials within the root-canal fillings, as 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 continously immersed in water, except during the 25 min scan times when they were placed in the high-resolution microtomography setup of the BAM*line* at BESSY-II (Berlin, Germany) (Rack *et al.* 2008). The setup was operated at 28keV. Similar to conventional tomography where multiple projections of the sample are needed (seen from different angles around the object), 900 radiographs were recorded for each root at angular rotation steps of 0.2º, with 0.2 seconds exposure times per projection. A detector with 2.5 µm effective pixel size, situated 430 mm behind the sample (Zaslansky *et al.* 2010) was used. The X-ray images all contained the radiographic apex of treatment including 2 mm of root-canal filling as well as 0.5 mm of the canal beyond the gutta-percha tip. Each series of radiographs was next normalized and reconstructed conventionally by the backprojection method (Octopus V8.1,

Zwijnaarde, Belgium, Vlassenbroeck *et al.* 2007). All reconstructed PCE-CT volumes were visualized (Amira 4.1, Visage Imaging GmbH, Germany) and virtually sliced in the computer memory (PCT-CE slices) and then five teeth were chosen for sectioning and imaging by the conventional methods mentioned above. In this manner, corresponding features in images obtained by LM and EM were matched and identified. For such imaging, three of the roots were acrylic-embedded (see next paragraph) whereas two of the roots were kept wet and prepared as follows: first the teeth were serially sectioned across the root long axis, using a water cooled slow-speed diamond wheel (Isomet Buehler LTD, Lake Bluff, IL). Slices were thus obtained at 600-1000 µm increments coronal to the gutta-percha tip. These sectioned but unpolished slices (LM-U slices) were imaged by LM (observed in a Leica DFC 480+DM-RXA2 with a 0.5 µm effective pixel size, Leica-Microsystems GmbH, Wetzler, Germany) and were later wet machine-trimmed utilizing a series of polishing papers and diamond slurries (Logitec PM5 + METADI diamond pastes, Coventry, UK) down to 1 µm. The now-polished samples were again imaged by LM (LM-P slices) and then observed at 1 µm pixel size in a low-vacuum water-vapor 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 lab-based µCT (Skyscan 1072 Kontich, Belgium) at 100 keV, 3.1 µm effective pixel size, 6 sec exposures. They were then reconstructed (Nrecon 1.6) and visualized similar to the PCE-CT data, such that virtual images of the samples and particularly of the polished surfaces were obtained (µCT slices).

The three teeth designated for acrylic-embedding were dehydrated in a series of increasing ethanol-water exchange solutions. Each sample was embedded in Poly-methyl methacrylate (PMMA, Merck, Darmstadt, Germany), sectioned and polished along and across the root axis for comparative dry-imaging by LM-P, LVEM, µCT versus the PCE-CT scans of the original intact roots. Note however that substantial dimensional distortions were revealed following this preparation, presumably due to restricted exchange of water within the root-canal filled teeth, or possibly due to stress and strain evolution during liquid exchange and polymerization shrinkage of the embedding material. These samples were therefore used for comparative identification of the main root-canal filling constituents: gutta-percha, dentine and voids, as seen by each imaging method (data not shown).

Quantification of the wet-sliced cross-sectional areas detected by each method was performed on an identical section that was imaged by all methods (Fig. 1). ImageJ (Rasband 1997-2009) was used to manually delineate a line along the margins of each material (tracing the internal edges of the canal, the margins of gutta-percha and the perimeter of the void) so as to enclose areas on the cross-section observed by each method. We thus obtained 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 3 times by 3 independent evaluators, instructed to identify and trace the aforementioned interfaces. PGP was then

calculated for each method by averaging the ratios of filling-areas less void areas, divided by canal areas (for details see Wu and Wesselink 2001).

Two-way ANOVA with Bonferroni adjustment, $p<0.05$, (Sigmaplot 11, Systat software inc) was used to analyze the results.

Results

Figure 1 (a-e) shows a typical sectional image as seen by all imaging methods (LM-U, LM-P, EM, µCT & PCE-CT). Note marked differences that exist in the appearances of gutta-percha, voids and dentin. The contrast of the sealer varies significantly from one imaging method to another, precluding reliable identification and tracing of sealer margins. Figure 2 shows the results of area measurements of all observers, grouped by (a) inner canal (b) outer gutta-percha rim and (c) outer edge of finger-spreader void. Lines below the abscissa indicate lack of significant difference of the means ($p>0.05$); inter-observer differences that are significant are indicated by starred brackets above the respective bars ($p<0.05$).

Overall it can be seen that areas measured on the unpolished slices are larger than the same areas measured by any other imaging method. The largest discrepancies found between identical structures measured by the different methods were 41% for the void, 15% for the gutta-percha and 9% for the canal. Pooled calculated 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% 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 detail observed in the PCE-CT scans, where details down to the sizes of silver particles in the AH26 are revealed.

Discussion

PCE-CT offers exciting opportunities to provide non-destructive in-vitro information about root canal fillings, which hopefully may further improve understanding of endodontic preparation and treatment. PCE-CT emphasizes the gutta-percha, dentin and void interfaces at high resolution (see example in the supplementary online video), such that relations between the natural tissue and treatment materials can be quantified at the micrometer length scale and in 3D.

The LM-U preparations were similar to those reported in other studies (e.g. Jung et al 2003 and Őzok et al 2008), and presumably they represent the typical level of detail that one may expect by this standard imaging method. All LM-U measurements revealed average greater area estimates and exhibited blurring of canal and gutta-percha edges. An explanation for the reduced visibility of the LM-U filling and canal rims (Fig. 1a) may be related to the presence of surface scratches, surface-height irregularities and a thick smear-layer on the observed surface following conventional-sectioned. Such surface-roughening features degrade the image as seen by light microscopy. Unpolished slices thus

lead to overestimates of the gutta-percha and dentine cross-sectional areas, leading to an underestimation 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 and 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 canal filling, as compared with LM-U and µCT. Note that the central finger-spreader void appears to exhibit some smearing after polishing. Empty voids such as this, may obviously trap debris during the polishing procedures and one cannot exclude the possibility that a thin smear-layer may exist, despite the fact that far less blurring is evident as compared with LM-U. The LM-P method is thus advantageous, although not flawless.

EM and lab-based µCT images (Figs. 1c,d) yielded similar lower average area estimates which is attributable to dehydration that must have occurred during the hours-long scan times. While 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. The extreme contrast differences seen by µCT and the well-known resulting image artifacts make tracing interfaces of gutta-percha and dentin difficult. This is in agreement with the findings of Huybrechts *et al.* (2009) who found that CBCT scans of root-canal fillings have significant artifacts caused by the filling material as well as the reduced resolution. Conventional µCT thus

offers less information than PCE-CT and LM-P and should thus be used with caution.

When converting all area measurements into PGP (Wu and Wesselink 2001), the LM-P and PCE-CT methods revealed intermediate values, highlighting the sub-optimal quality of this particular filling. PGP values as determined by LM-U however appear to be misleadingly high, erroneously suggesting high quality ranking of this filling which is incorrect. Thus, PCE-CT or LM-P (polished LM) might be important to avoid overestimation of the PGP in such 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 favorable PGP ratios. The reasons for lower PGP values reported by µCT are less clear and may possibly 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 where either gutta-percha or dentine end. Thus, contrast differences, blurring effects and to some extent - reduced resolution render µCT the least accurate of all methods studied here.

Small differences were seen between observers, and the relative inter-observer uncertainty is less than 3% by all methods. Thus the manual process of identifying and dliniating where root-canal interfaces are located is not the cause for the differences exhibited by different methods discussed here. This too is 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-canal

filling by different methods was high overall. There was a striking difference of 41% between EM and LM-U cross-sectional area-estimates for the finger-spreader void, which may be explained by the fact that the method of imaging plays a greater role when determining smaller cross-sectional areas. Thus, LM-P or PCE-CT should be used for studying small inclusions voids in root-canal filling materials. As no evaluation of the sealer was carried out in this study, clearly further research is still needed.

Conclusions

PCE-CT provides detailed non-destructive root-canal filling information, with area estimates similar to those seen by LM-P sections. For some measurements, there are marked differences in the cross-sectional areas of the canal, gutta-percha and void when determined by different methods and it is important to consider the effects of sample preparation on the analyzed images.

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Figure legends

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-vapor imaging electron microscopy (EM) d) conventional laboratory µCT e) Same slice, located within a volume obtained by PCE-CT. Note lack of crack artifact on lower-left rim, visible in all the later imaged slices, produced during sample sectioning. Also note the variable visibility of the sealer, mainly in the finger-spreader void area, precluding reliable delineation. The noticeable ring-artifacts are a common noise in computed tomography, but they have negligible effects on the measurement reported here.

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 micro tomography (PCE-CT), lab-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 are indicated by starred brackets above the graph columns. Error bars indicate standard deviations.

Supplementary (online only) video and 3D figure:

Video. An animation and 3D rendering of a typical phase-contrast enhanced microtomogram of a root and root-canal filling. A slice across this root corresponds to the data shown in figure 1. Exquisite details are revealed within the root and root-canal filling. Colors are arbitrary and represent intensity values.

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 thetype of data obtained and the spatial distribution of micrometer length-scale details within the root-canal filling and surroundings.