Contemporary root canal filling strategies

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Chapter 6

Synchrotron-based phase contrast-enhanced microCT reveals delaminations and material tearing in water-expandable root canal fillings ex vivo

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Abstract

Introduction This study evaluated the integrity of fillings made with hygro-expandable cones (HEC) known commercially as ‘Cpoint’ or ‘Smartpoint’, and calcium silicate sealer, used as root canal filling materials.

Methods Fourteen human canines with a single straight root canal were prepared according to a standardized, conventional endodontic treatment protocol and filled with the HEC/calcium silicate sealer. 3D imaging was performed with a laboratory microCT (µCT) at its highest resolution and was compared with synchrotron phase contrast-enhanced µCT (PCE-CT) scans of the treatment extending 1 to 7 mm from the apex. A conventional ‘destructive’ method using optical microscopy validated observations in virtual slices in the tomographic data.

Results Conventional laboratory µCT at 10 µm resolution did not reveal the existing voids and defects within the root canal fillings. PCE-CT revealed elongated interfacial delamination localized mainly at the HEC-sealer interface forming extended through-and-through gaps along the root canal filling.

Conclusions Endodontic studies using conventional laboratory µCT may underestimate thin defects and delamination within root canal fillings made with HEC due to limited resolution and contrast of the lab-based broad-spectrum with low intensity X-ray sources. These limitations favor use of high-brilliance, monochromatic synchrotron based PCE-CT to reveal the important micrometer details within large (mm sized) samples. PCE-CT revealed the existence of a range of significant structural defects in the recently placed HEC fillings, which was confirmed by optical microscopy following physical sectioning. Substantial delamination spanning 20-40% of
the circumferential interface as well as other structural defects were identified within root canal fillings made of HEC and calcium silicate sealer.

**Introduction**

There is concern about some root canal filling compaction techniques, that may lead to mechanical stress (1) resulting in irreversible damage to root dentin, e.g. by root fracture (2). Filling materials and techniques that do not require significant compaction forces are therefore desirable and are the focus of a new class of materials introduced recently into clinical endodontic use. An example is the Propoint system (DRFP Ltd., Barnack, Stamford, UK), also commercialized as the Cpoint system (Endo Technologies, LLC, Shrewsbury, MA, USA) and which consists of hydro-expandable cones (HEC) made of a nylon carrier coated by a hydrogel of copolymers of acrylonitrile and vinyl pyrrolidone (3). Allyl methacrylate is used for crosslinking the copolymers, reducing the dissolution of the hydrogel and stiffening it while limiting water absorption. The hydrophilic nature of the cross-linked hydrogel promotes water absorption with the result that after placement, the filling undergoes gradual swelling. The Propoints are currently recommended for endodontic use with a calcium silicate-based root canal sealer such as EndoSequence® BC Sealer™ (Brasseler USA, Savannah, GA, USA) or Smartpaste Bio (DRFP Ltd., Barnack, Stamford, UK). Calcium silicate sealers are hydraulic cements that require water for setting (4). Both the HEC and the calcium silicate sealer may thus benefit from the moisture that is permanently present within dentin in teeth in the oral cavity. The use of a swelling polymer in conjunction with a hydraulic cement for root canal treatment is thought to produce a
functional filling, capable of adapting to the internal root morphology attaining a good seal. The dynamics of HEC expansion has been investigated \textit{in vitro} revealing significant swelling within the first 20 minutes after immersion in water (3), considered to represent the maximal expansion capacity of HEC. Adaptation to the internal canal structures as well as closure of internal gaps is thus one huge potential advantage of these materials. Whether adaptation and shape-change of the fillings is reversible or not, is still unknown. Previous research has pointed to antagonism between materials with affinity for water, when such materials were juxtaposed (5).

Laboratory source microcomputed tomography (\(\mu\)CT) is capable of providing high-resolution three-dimensional non-destructive images of the internal structures of objects (6). Recent years have witnessed an increase in the utilization of \(\mu\)CT in endodontic research (7-9), with applications such as visualization and quantification of the distribution of porosity in conventional root canal fillings (9). However, a previous study has questioned the ability of laboratory-based \(\mu\)CT to clearly reveal the voids related to specific root filling materials (10). Phase contrast enhanced microCT (PCE-CT) is a form of \(\mu\)CT that utilizes radiographs produced by laser-like (partially coherent) X-rays, typically found in synchrotron radiation facilities. Interference patterns therein enhance edges (11), revealing voids and gaps in internal structures such as root canal fillings that are often undetectable by conventional \(\mu\)CT (10).

The aim of the present study was twofold: 1. To study the structural integrity of HEC fillings several weeks after placement, specifically examining the interface between the material and the sealer. 2. To compare
the 3D data generated by a widely-used laboratory-µCT system, with PCE-CT and validated by destructive, 2D optical microscopy.

Materials and Methods

Specimen selection
Fourteen maxillary and mandibular human canines were stored in thymol 0.1% at room temperature. Criteria for tooth selection included the absence of visible root caries, lack of signs of resorption/root fracture/calcifications of the root canals as well as a complete (fully formed and undamaged) root tip anatomy. The presence of a single straight root canal was confirmed by bucco-lingual and mesio-distal radiographs. Canals with pronounced ovality (diameter ratio > 2) were excluded. The tooth crowns were removed to standardize the root lengths to 14 mm.

Root canal preparation: instrumentation and irrigation
An ISO 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) placed inside each canal was used to determine the working length, about 1 mm short of the full root length. The canals were instrumented to a size 40, taper 0.06 using Mtwo nickel-titanium files (VDW GmbH, Munich, Germany). The canals were repeatedly irrigated with 2% NaOCl (Denteck, IL Zoetermeer, the Netherlands) during instrumentation. At the end of the preparation, 3 mL of 17% EDTA solution (Vista dental products, Inter-med Inc., Racine, WI) was delivered and left in place for 3 min before flushing with 2% NaOCl. A size 25 stainless-steel tip was used for ultrasonic activation (IrriSafe, Acteon, Merignac, France) for $3 \times 10$ s while flushing the canals with NaOCl between each activation phase (12). A final rinse with distilled water was followed by brief blotting with paper points.
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Root canal filling
Smartpaste Bio was injected in the root canal to about 4 mm short of the working length. The plunger of the syringe was pressed while slowly withdrawing the tip from the canal. A single size 40, 0.06 taper Propoint, previously adjusted to the length of the canal (SmartGauge, EndoTechnologies LLC, MA, USA), was fitted along the full preparation length. The coronal access was sealed by a bonded composite (Clearfil and DC Core, Kuraray, Tokyo, Japan). The roots were then stored at 37°C and 100% humidity for 10 days. All teeth were then moved to sealed vials containing water at room temperature. Imaging was performed at least 3 weeks after treatment, assuming complete setting of the sealer.

Laboratory µCT imaging
10 specimens were mounted on custom-made stubs fitting a standard phantom-calibrated laboratory µCT (Scanco 40 µCT, SCANCO Medical AG, Brüttisellen, Switzerland). The specimens were immersed in phosphate buffered saline during scanning. Each specimen was imaged using a 10 µm resolution with 300 ms acquisition time, an energy of 70 kV (114 µA) and a 0.5 mm aluminium filter. Standard reconstruction was used employing routine beam hardening correction provided by the manufacturer (SCANCO Medical AG, Brüttisellen, Switzerland).

Phase-contrast enhanced microtomography (PCE-CT)
Several weeks after treatment, four other specimens were scanned by PCE-CT using the Helmholtz-Zentrum Geesthacht operated, partial-coherence, P05 imaging beamline of the Deutsches Elektronen-Synchrotron (DESY), Hamburg, Germany. An energy of 35 keV was used, and 1000 radiographs
Phase contrast-enhanced µCT evaluation of water-expandable root canal fillings

were obtained by rotating each sample 180° on its axis. An imaging system with an effective pixel size of 2.41 μm was used situated 50 mm behind the sample, for phase contrast-enhancement. Due to the limited field of view, imaging was restricted to the root regions extending 1~7 mm from the apex. All data were normalized and reconstructed conventionally into slices along the root axis (NRecon, Bruker CT, Kontich, Belgium).

**HEC-sealer interface delamination quantification**

Virtual slices were selected every 100 µm along the four PCE-CT scans, each representing ~2.4 μm thick sections across the root. Wherever a clear gap was observed in multiple consecutive slices, the length of delamination was measured and normalized to the total perimeter of the HEC-sealer interface. Measurements were performed along the filling axes by analyzing stacks of reconstruction slices using Fiji V2.0.0 (13) and delamination was expressed as “interfacial gap percentage”. In total, 62 slices were analyzed manually by a single observer (ATM). The data were pooled to produce histograms of “interfacial gap percentage” that show the prevalence of different degrees of delamination along the filing. Descriptive statistics are reported as median with interquartile range. Statistical analysis was performed with SPSS V22.0 (SPSS Inc., Chicago, IL).

**Validation of gap presence**

Two specimens initially scanned by PCE-CT and exhibiting delamination (interfacial gap), were scanned by laboratory µCT (Skyscan 1172, Bruker-CT, Kontich, Belgium), to aid in sample preparation for light microscopy. The physical existence of gaps observed in the HEC fillings was identified in several slices cut in root segments embedded in quick-set poly-methyl-
methacrylate (Bosworth Trim, Bosworth, Skokie, IL). Root slices were
obtained using a slow speed water-cooled diamond wheel (Isomet Buehler
LTD, Lake Bluff, IL) followed by gentle grinding and polishing, as
previously described (10). Optical images were obtained using a Leica
DVM2500 multi-focus light microscope (Leica Microsystems, Wetzlar,
Germany).

Results
Filling architecture
PCE-CT reconstructions revealed exquisite details within and around the
fillings, as shown in renderings of 3 different roots in Fig 6.1.
The virtual slices along the filling axis further revealed the nylon core
(marked C) the hydrogel layer (H), the sealer (S) and dentin (D). Many
regions exhibited a clear delamination line within the filling, all along the
H-S interface. The filling layers can also be identified in cross-sectional
reconstructions at a resolution of 10 µm (see Supplementary information Fig
6.S1) appearing to completely seal the treated canal space. Thus the
conventional lab µCT reveals the overall geometry and adaptation of the
fillings, but not subtle defects.
Figure 6.1: Renderings of 3 different HEC filled roots scanned with PCE-CT. The lower right image is a 2D slice along one of the teeth demonstrating the continuity of delamination between the sealer and polymer sheath of the filling (arrow). The different layers of the filling obtained with the HEC and calcium silicate sealer are clearly distinguishable (lower right panel: C: nylon core, H: hydrogel, S: calcium silicate sealer, D:dentin). The yellow arrow points to a delamination running corono-apically.
Supplementary Figure 6.1: Typical (10 µm) lab µCT reconstruction slices exhibiting limited contrast in zones of the fillings. Dark shadows provide hints to the existence of damage in the fillings (arrows).

Defect observations

PCE-CT-based reconstructions reveal, in addition to the materials comprising the filling, a host of different radiolucent geometries corresponding to defects within the filled root canals. We found four different types of defects/discontinuities within the HEC fillings. These are shown in Fig 6.2 and include large-scale delaminations, internal gaps/voids, tears in the hydrogel and voids/bubbles in the sealer layer.
**Figure 6.2:** HEC filling observations by PCE-CT in cross sections (a) Filling zones and delamination (arrow) observed between the HEC and the calcium silicate sealer. The different layers of the filling materials (C, H, S) are clearly distinguishable, D corresponding to dentin. Other types of defects were also observed. These include: (b) gap/rupture within the hydrogel layer (arrow), (c) tearing within the copolymer layer (arrows) associated with a non-uniform misalignment of the nylon core perimeter (arrowheads). (d) voids within the calcium silicate sealer (arrow).

Gaps at the H-S interface (Fig 6.1 inset, Fig 6.2 a) appear as continuous delamination extending tens to hundreds of μm in many regions of the fillings. A typical example of this delamination is shown in Fig 6.2a and a schematic representation is given in Fig 6.3a. The results of “interfacial gap percentage” were converted into distributions of gaps found within the apical 5 mm regions of the 4 PCE-CT imaged teeth. These are plotted in Fig 6.3b. The median interfacial gap percentage is 22.5% [with an interquartile range of 0-32%]. Curiously, one of the treated teeth did not demonstrate any
interfacial gap. However, close inspection revealed a myriad of other types of defects including hydrogel tearing and internal structural disruptions (Fig 6.2. b, c, d).

Figure 6.3: (a) Schematic representation of the different layers of HEC filled roots and a typical delamination. (b) Histograms representing the frequency of interfacial delamination percentages in HEC root fillings by PCE-CT. Root 3 did not exhibit any interfacial delamination but did contain significant signs of damage by other types of defects (see Fig 6.2 a, b).

Validation of HEC voids revealed by PCE-CT
Gaps revealed by PCE-CT were also observed by microscopy and by direct comparison with observations by careful examinations of laboratory μCT rescans (Supplementary Fig 6.S2). The renderings presented in Fig 6.S2 demonstrate limitations of laboratory μCT in providing a reliable
morphological characterization of the different layers and interfaces present in roots filled with the HEC.

Discussion
The results of this study raise concerns regarding integrity of HEC root canal fillings. HEC filling materials were developed because of their ability to expand and change shape when in contact with water. The extent of hygroscopic expansion of this material was demonstrated by Didato et al. (3) using HEC cones that were fully immersed in water. Whether the environment provided by coronally sealed root canals in vivo allows such expansion is not clear. In the present study, delamination between the cone and the sealer, as well as other structural defects such as tearing of the hydrogel were clearly observed in 3D in most although not all regions of the fillings. Hydrogels usually contain a network of crosslinked polymer chains (14) and it is known that uncontrolled expansion may ultimately lead to the rupturing of such hydrogels (15, 16) resulting in defects similar to those observed in one of the HEC specimens in the present study. A previous study evaluating the behaviour of a calcium silicate material placed against glass ionomer cement suggested that water was drawn away from the calcium silicate towards the glass ionomer (5).
Supplementary Figure 6.2: Cross section of HEC root canal fillings with different imaging modalities. a) a cross-section of a root canal filled with the HEC and calcium silicate sealer visualized under optical microscopy. The different layers comprising the filling can barely be discerned visually because of low colour contrast: (C) Central core of nylon, (H) hydrogel, (S) calcium silicate sealer, (D) dentin. A gap is observed between the hydrogel and the calcium silicate sealer (yellow arrow). b, c) Comparison of an example of identical (b) optical and (c) X-ray tomography (at 10 µm resolution) cross-sectional images of a HEC and calcium silicate sealer root canal filling. d,e) Same specimen location, scanned by lab CT at (d) 10 µm effective pixel size (Skyscan 1172 microCT scanner, similar settings as used in the SCANCO scans) (e) PCE-CT (P05, Petra III, DESY) at 2.41 µm effective pixel size, demonstrating increased contrast and detail due to some phase contrast-enhancement. An interfacial gap (white arrow) that is barely detectable by laboratory µCT can clearly be delimited by PCE-CT is clearly identifiable Gaps and voids are far easily identified and quantified.
In the case of HEC fillings, we cannot rule-out the possibility that water present within the hydrogel may be extracted by the calcium silicate sealer causing hydrogel shrinkage and delamination (Fig 6.2a, d). Excessive water uptake by the hydrogel might, in other cases, provoke tearing defects similar to those observed in one of the specimens (Fig 6.2b, c). Either way, further work is needed to understand the causes for delamination and tearing that we observed in multiple HEC fillings.

Laboratory µCT examinations allow imaging of root canal fillings non-destructively however contrast differences between the root canal filling materials and dentin (10) remain a challenge. Enhanced contrast and higher resolution are needed to characterize small voids and porosity/damage in root canal fillings with low-density (e.g. polymer-based) materials such as HEC. We observed that the same root scanned with laboratory µCT at 10 µm and using PCE-CT at 2.41 µm revealed different detail due to the higher spatial and density resolution. PCE-CT could thus clearly demonstrate gaps that were almost invisible using 10 µm settings. This illustrates the limitation of conventional laboratory µCT for the analyses of specific types of root canal fillings. Defects, which were identified in 3D tomograms, were also confirmed by optical microscopy following physical sectioning of representative samples. However a limitation of such destructive methods is that sample preparation may be causing the observed damage. Similar to other tomographic methods, PCE-CT does not require sample slicing or polishing, an advantage for studying root filling structures.

It is important to note that PCE-CT imaging has limitations, and in particular phase contrast-enhancement may result in strong artefacts. It is also difficult to gain access to the needed synchrotron X-ray sources and such imaging experiments require extensive and non-trivial processing and
computations. Still it appears essential to use such methods to elucidate the small density differences and poor contrast that exist between the HEC hydrogel and voids.

In conclusion, this work reveals the existence of significant defects, observed in root canal fillings made with HEC (Cpoint/Prosmart) and a calcium silicate sealer. Such defects are not easily detectable by conventional laboratory µCT. A better understanding of the behaviour of these materials in the root canal environment is necessary in order to improve their clinical and long-term performance.
References


