Porosity distribution in root canals filled with gutta percha and calcium silicate cement

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\textbf{ABSTRACT}

Objective. Gutta percha is commonly used in conjunction with a sealer to produce a fluid-tight seal within the root canal fillings. One of the most commonly used filling methods is lateral compaction of gutta percha coupled with a sealer such as calcium silicate cement. However, this technique may result in voids and worse, the filling procedures may damage the root.

Methods. We compared the volume of the voids associated with two root canal filling methods, namely lateral compaction and single cone. Micro-computed tomography was used to assess the porosity associated with each method in vitro. An automated, observer-independent analysis protocol was used to quantify the unfilled regions and the porosity located in the sealer surrounding the gutta percha.

Results. Significantly less porosity was observed in root canals filled with the single cone technique (0.445% versus 3.095%, \(p < 0.001\)). Porosity near the crown of the tooth was reduced 6 fold, whereas in the mid root region porosity was reduced to less than 10% of values found in the lateral compaction filled teeth.

Significance. Our findings suggest that changing the method used to place the endodontic biomaterials improves the quality and homogeneity of root canal fillings.

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1. Introduction

Present-day approaches to the treatment of infected root canals combine chemo-mechanical disinfection and creation of a fluid-tight seal [1,2]. Mechanical shaping of the internal root canal walls is necessary so as to make it possible to effectively clean and disinfect these internal root spaces, and to facilitate sealing by placement of designated root canal (endodontic) filling materials. Adequate cleaning as well as complete filling of the root canal spaces are known to promote healing following root canal therapy. To be successful, the filling needs to extend along the entire canal length, ending just slightly shy of the root tip, where the system of canals end and splay [3]. Classical biomaterials used in endodontic therapy are not intended to provide structural/mechanical reinforcement of the roots. Rather, root canal treatment biomaterials are typically biologically inert and have much lower elastic...
moduli than the tooth tissues that they fill [4]. Their main purpose is to prevent root canal reinfection, providing favorable conditions for post-treatment recovery processes that are expected to take place in the living tissues surrounding the root (the periodontal tissues) [1]. The material most frequently used for sealing human root canals, is a polyisoprene-based material termed gutta percha, the use of which is the standard of care [1]. Gutta percha is available in rigid semi-crystalline cone-shaped forms of various diameters, that can be easily inserted along the prepared root canal space. It can also easily be removed, in the event that root canal retreatment is necessary e.g. in cases of persistent or resistant infections. However, because of the complex shape of the root canal system, even after its preparation, prefabricated gutta percha cones often poorly fit the canal geometry. To solve this problem, slow-setting cements (commonly termed sealers) are used to seal remaining gaps between the cones and surrounding root walls. In order to improve the adaptation of endodontic fillings to the root canal geometry during treatment, mechanical compaction techniques are often used to mold the biomaterials to better fit the prepared empty root canal.

Of the techniques available clinically for root canal treatment, the lateral compaction (LC) method is often quoted as being the gold standard against which other techniques are typically assessed [5]. It is indeed the most common root canal filling technique used by general dental practitioners in the United States [6] and the main root canal filling technique taught in dental schools in Europe. The homogeneity of root canal fillings obtained by LC vary considerably, as they seem to rely on the skills of the dentist. Furthermore and unfortunately, the forces resulting from compacting adjacent gutta percha cones against the internal root walls by the LC method may even induce damage to root dentin [7]. An emerging alternative treatment approach relies on the use of a single cone (SC) technique with wider-taper gutta percha cones. This approach offers an interesting and simple treatment alternative, provided that one obtains an adequate adaptation of the filling material to the root canal geometry. The SC biomaterial-placement technique requires insertion of a properly matched cone in conjunction with a root canal sealer to completely fill the entire canal. It may thus offer a sorely-needed robust treatment alternative that may also potentially reduce the propensity of treatment to damage the dentinal walls [8]. Using both of the above mentioned root canal filling approaches (LC or SC), a sealer is used to completely fill the gaps between the cones and the dental tissues. Several types of sealers are available for this purpose. Of these, calcium-silicate-based root canal sealers are of great interest as they rely on moisture for their setting mechanism and exhibit potential bioactive swelling leading to improved sealing while forming a bond with dentin [9]. Their setting mechanism is based on the absorption of moisture from the surrounding root canal environment [10] and they typically contain zirconium oxide and various calcium-based compounds (Ca3SiO4, Ca(H2PO4)2, Ca(OH)2). Adequate wetting and full coating of cones and dental surfaces by the sealer remains a treatment challenge, since voids and air bubbles often become entrapped within the filled root canal [11]. Such voids are of great concern because they create porosity, reduce the quality of the filling, serve as hubs for microbial housing and may even link up to tunnel and transport contaminants along the filled root canal. All these lead to re-infection and treatment failure [12] with possible danger of tooth loss.

The aim of this study was to evaluate and compare the voids associated with two endodontic filling techniques, namely the lateral compaction (LC) and single cone (SC) methods. Both methods were used by combining gutta percha and a calcium silicate based sealer. The tested null hypothesis was that there is no difference in the void (%) that result following the application of the 2 filling techniques.

2. Materials and methods

2.1. Treatment specimen selection

With the approval of the ACTA dental school ethical committee, 20 maxillary and mandibular human canines, recently extracted for reasons unrelated to the present study, were selected and stored in thymol 0.1% at room temperature. A minimum of 7 teeth per group would suffice to detect a standardized effect of size r = 0.65 between the two experimental groups (determined based on the results of a previous study [13]) at 80% power and with a two-tailed probability of a-type error of 0.05. Criteria for tooth selection included the absence of root caries, lack of resorption and calcification of the root canals and a complete (fully formed and undamaged) root tip anatomy. The presence of a single straight (curvature <10°) untreated root canal was confirmed by radiographs examining the bucco-lingual and mesio-distal orientations along the root axis. The radiographs were used to estimate the root canal dimensions at 2, 5, 9 and 12 mm from the root tip in order to exclude canals with severe ovality (diameter ratio >2. Root canals presenting unusual anatomy (e.g. a diameter larger than the largest file used during instrumentation, placed to the full canal length) were excluded. The tooth crowns were removed with a diamond bur mounted on a high-speed dental handpiece, to standardize the root lengths to 14 mm.

2.2. Root canal preparation: Instrumentation and irrigation

All specimens were prepared and filled by a single operator, according to the following procedures (schematically illustrated in Fig. 1), following standard clinical practices. An ISO size-10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was placed inside the canal to determine the treatment working length, about 1 mm short of the full root canal length. All root canals were instrumented to a size 40/0.06 taper using a series of nickel–titanium files with increasing diameters (MTwo, VDW GmbH, Munich, Germany) and a torque-control motor (VDW Silver, VDW GmbH). Consequently, the internal canal diameter was enlarged along the root length, with the widest diameter found in the crown and the narrowest region, $\theta = 400 \mu m$, found near the root tip. To remove tissue remnants during instrumentation, the canals were repeatedly irrigated using 2% NaOCl (Denfect, IL Zoetermeer, the Netherlands) after each instrument using a 3G needle (NaviTip, Ultradent Products Inc, South Jordan, UT, USA) attached to a syringe (Terumo

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European, Leuven, Belgium). At the end of the preparation, 3 mL of 17% EDTA solution (Vista dental products, Inter-med Inc., Racine, WI, USA) was delivered into the root, and the solution was left in place for 3 min before flushing with 2% NaOCl. Ultrasonic activation was then performed by means of a size 25 stainless-steel ultrasonic tip (IrriSafe, Acteon, Merignac, France), inserted to 1 mm short of the working length and driven by a piezoelectric dental ultrasonic device (Satelec P5 booster, Acteon, Merignac, France) for 10 s at 35% of maximum power setting (level 7/20). Ultrasonic activation of the irrigant was repeated 3 times while flushing of the canals with NaOCl between each activation phase, followed by rinsing with distilled water to remove remnants of chemicals. The canals were briefly blotted with size 40/0.02 taper paper points.

### 2.3 Root canal filling

Specimens were allocated to two groups by simple randomization with the help of a true random number generator (www.random.org). Smartpaste Bio® (DRF Ltd., Barnack, Stamford, U.K.). A pre-mixed calcium-silicate sealer, was used. The treatment biomaterials were placed in the canals, recapitulating standard clinical practices as follows (Fig. 1e–i):

**SC group:** The sealer was injected in the root canal to about 4 mm short of the working length using the syringe and plastic needle supplied by the manufacturer; the plunger of the syringe was pressed while slowly withdrawing the tip. A single size 40/0.06 taper gutta percha cone (Mtwo, VDW GmbH, Munich, Germany) was adjusted to the length of the canal (SmartGauge, EndoTechnologies LLC, MA, USA) and then seated (Fig. 1e), reaching the full preparation length.

**LC group:** The same sealer and placement procedure was used as in the SC group. A size 40/0.02 taper gutta percha cone (Dentsply Maillefer, Ballaigues, Switzerland) was fitted to the canal working length (Fig. 1g) and used as a master cone. Lateral compaction was performed with a smooth size-B nickel–titanium finger spreader (Dentsply, Maillefer) (Fig. 1b) placed up to 1 mm short of the working length, to compact the master cone laterally and create space for the insertion of size 25/0.02 taper accessory cones (Dentsply Maillefer), dipped into the sealer prior to placement. This process was repeated incrementally until the root canal was completely filled. The filling material was trimmed at the root canal entrance using a warm instrument, and gently condensed into the root canal using an endodontic plugger instrument (Dentsply Maillefer).

Subsequently, the crown sides of all roots in both groups were cleaned from material remnants. The upper canal walls were etched for 10 s using Ultra-Etch 37% phosphoric acid (Ultradent, South Jordan, UT, USA) and rinsed with water for 5 s before being sealed by a dentin bonding system (Clearfill Photobond, Kuraray, Tokyo, Japan). The root filling was covered with a flowable dental composite resin (DC Core, Kuraray, Tokyo, Japan) in order to seal the root opening, simulating the usual clinical conditions in the mouth (Fig. 1e and i). The roots were then stored at 37°C and 100% humidity conditions for

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10 days, to allow the sealer to set completely before further analysis.

2.4. Three dimensional data acquisition

The specimens were mounted on stubs fitting the specimen stage of a μCT scanner (Scanco 40 μCT, SCANCO Medical AG, Brütisellen, Switzerland). In order to avoid any movement during scanning, the root-tip side of each specimen was embedded in self-curing acrylic resin (Vertex Self-curing, Vertex dental, Zeist, The Netherlands) taking special care to leave the root-tip canal exit uncovered and visible. Silicone tubes matching the external diameter of the acrylic stubs were fitted and filled with phosphate buffered saline, in order to prevent dehydration of the roots during the scanning procedures in the μCT. Each specimen was scanned using a 10-μm spatial resolution and acquisition times of 300 ms. The scan peak voltage was set at 70 kV (114 μA). A 0.5-mm aluminum filter and a correction algorithm of the manufacturer software were used to reduce beam-hardening artifacts. The system was calibrated following company directives using phantoms with densities of 0, 100, 200, 400 and 800 mg HA/cm³. Two scans were performed for each tooth: one performed immediately after canal preparation/instrumentation, and a second scan was performed 10 days after root canal treatment completion.

2.5. Data visualization and image processing

The reconstructed pairs of datasets were visualized using CTvox (v2.4, BrukerCT-Skyscan, Kontich Belgium) and cross-correlated with Amira (v5.3, FEI Visualization Sciences Group, Bordeaux, France) with additional image processing performed with ImageJ (v1.49 Wayne Rasband, NIH, U.S.A) and it’s free implementation Fiji. Fig. 2 shows pseudocolor renderings of typical samples (one per group) as well a schematic illustration of the image analysis steps performed. For each sample, the two 3D reconstructed datasets (one prior to and one after biomaterial placement) were mutually co-aligned along the root canal (long) axis, by cross-correlation and spatial realignment of the volumes (employing a scaled mutual information method). The datasets were cropped and mildly filtered in 3D (using a small Gaussian filter, sigma = 2.0). Each of the now 3D co-aligned datasets were segmented (binarized) as depicted in Fig. 2 panels (d) and (g) using Otsu’s algorithm, applied at every slice corresponding to different heights along the root. This resulted in binary images (Fig. 2d and g) further used in a series of morphological operations to identify and quantify voids within the 3D data. The threshold for dentin (T1) was obtained from the first of each pair of scans, and used on this data to automatically determine the empty root canal volume (VC) and the diameter in every slice along the entire root, using the multi-measure function of Fiji.

The threshold for the filling biomaterials (T2) was determined from the second scan of the same samples and used to determine the filling dimensions. A “fill holes” procedure applied to the binarized data from the first scan provided a “tooth + canal” mask (Fig. 2e). By multiplication between the segmented datasets (see Fig. 2d and g) and after removing the surrounding air (the large “void” surrounding each root) we obtained the voids within the filled root canal (Fig. 2h).

The volume of the voids (VV) was determined similarly to the empty root canal volume. The percentage of voids (void%) was calculated as:

\[
\text{void} \% = 100 \times \frac{\text{VV}}{\text{VC}}
\]

For quantitative comparative analysis of the different roots, three regions of interest (ROIs: apical (root tip), middle and coronal, see Fig. 2) were defined in each dataset, within the 9 mm of filling above the edge of the instrumented section of the canal (about 1 mm from the tooth root tip), extending coronally toward the crown. These regions in the root are of paramount concern for root canal therapy [7]. The accumulation and leakage of pathogens, particularly difficult to eradicate in these regions, typically lead to chronic infection and ultimately to tooth loss [14]. Fig. 3 shows typical plots of two analyzed canal diameters, revealing the gradual taper and reduction in the lateral dimension along the length of the root.

Beyond the working length, no treatment takes place and the canal diameter suddenly drops as it is not mechanically enlarged.

2.6. Statistical analysis

The void% were considered separately for the coronal, middle and apical ROIs in each sample and revealed a non-normal distribution, validated with the Shapiro–Wilk test. The results are therefore reported as medians with [interquartile ranges]. The median void distributions at different root heights were compared using the Friedman-Rank test. Differences between the treatment groups were analyzed with the Mann–Whitney test. Effect sizes for pairwise comparisons were reported as absolute values of Pearson’s correlation coefficient r. Statistical analysis was performed using SPSS (v 22.0 SPSS Inc., Chicago, IL, USA). Graphs were plotted using SciData (v.1.88 Free Software Foundation Inc., Boston, MA, USA) and GraphPad Prism (v 4.0 GraphPad Software, La Jolla, CA, USA). A p-value <0.05 was considered as statistically significant.

3. Results

The void distribution along all slices within the different teeth for both treatment groups are shown topographically in Fig. 4. Note that values above 25% are truncated for ease of graphical representation.

Fig. 5 plots the median void% of the different ROIs of both experimental groups. When considering the void%, pooling the 3 ROIs, the SC group exhibited significantly lower median void% than the LC group (0.445 [0.168–0.825] versus 3.095% [1.027–5.072] respectively, p = 0.001, r = 0.727). Considering each ROI separately (apical, middle, coronal), the SC group exhibited significantly less void% than the LC group in the coronal and middle ROIs (0.254% [0.052–0.648] versus 1.569% [0.915–4.643], p = 0.001, r = 0.744 and 0.167% [0.011–0.223] versus 2.057% [1.348–4.189], p <0.001, r = 0.811, respectively). In the apical (near root-tip) ROI however, the median void% in both groups were not significantly different, despite an observed trend for fewer void% appearing in the SC group (0.493% [0.297–2.213] versus 2.260% [0.379–8.536], p = 0.096, r = 0.372). Within each
group, no significant difference could be detected between the median void% of the different ROIs (p > 0.05).

4. Discussion

Our results show a systematic difference between the distribution of voids in roots treated by SC and conventional-LC root canal treatment techniques. The SC technique exhibits far less porosity. Unfilled zones in the filled root are of great concern, as such spaces may lead to regrowth of microorganisms or allow their ingress by microleakage [15]. Indeed, several studies have demonstrated an association between the presence of voids within the filled root canal and poorer clinical, long-term treatment outcomes [3]. The use of techniques capable of reducing the prevalence of empty spaces is therefore a goal for improving root canal therapy outcome.

Clinically, the presence of gaps in root canal fillings is typically determined from the radiographic homogeneity of root canal filling biomaterials [16]. The extent to which endodontic biomaterials are able to be seated and occupy the root canal volume has been investigated by a wide array of methods ex vivo that have been so far unable to evaluate the sealing ability of the root canal filling in an objective and reproducible way [1]. The use of quantitative and objective methods to evaluate the root canal treatment biomaterials and thus potentially lead to the improvement of clinical outcomes is still in sore demand. µCT is a widely-used imaging tool capable of providing high-resolution 3D views of the internal structure of many objects [17] and is well suited to visualize and evaluate the root canal filling nondestructively [11]. Recent years have witnessed an increase in its applications to endodontic research although clearly-defined and comprehensive 3D analysis protocols are still lacking.

While scan energy and other settings are easily standardized between different samples and treatments, the data processing steps and data interpretation, particularly during the quantification processes, may significantly influence the results and conclusions of such studies. In the present study, we identified the two important structures of interest (tooth material, and filling biomaterials) using different scans to maximize contrast and we used the automated Otsu method of threshold determination, which is an observer-independent objective method to obtain reproducible segmentation of both structures of interest, separately. Otsu’s algorithm finds the threshold that separates pixels into two classes in such a way

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that it minimizes the ‘intraclass variance’ [18] typically separating two different distributions of gray values. The method basically automatically finds the value separating two different densities in the images and can therefore be considered as highly standardized and observer independent. In comparison, a visual threshold assignment may be considered highly subjective [19]. Further alignment of the 3D datasets by digital image correlation made it possible to directly compare the different zones of each tooth at micrometer resolution. One particularity of the present study is the method used to accurately select and compare identical ROIs in different teeth. Selecting the outer (physically visible) root tip (apex) as a reference point for comparison would bias the results because of the variability of the anatomical location of the root canal, ending somewhere on the apex surface [20]. Furthermore, the geometric variability of the root canal anatomy at the apex complicates any attempt to reproducibly define a standard reference point among different samples and even more between different studies, when considering the high resolution measurements provided by μCT [19]. To allow comparison between teeth with different treatments, we thus selected ROIs that matched the internal specimen anatomy, based on precise canal geometries. The diameter of the instrumented root canal measured along the whole sample length facilitated discrimination of the untreated tooth region from the treated one. As is required for standard-of-care preparation procedures for root canal therapy, the working length is slightly shorter than the canal length and hence no tooth-material removal takes place beyond this length (near the root tip) such that a sharp reduction in canal diameter is expected and is indeed observed (Fig. 3, black arrows). Our study thus goes well beyond classical material distribution studies comparing slices obtained at fixed distances from the tooth apex [21], as it facilitates analysis of the exact same ROIs between different samples and guarantees unbiased comparisons of the treatment groups. Future studies using such a methodology will promote inter-study comparability. To our knowledge, the present study is the first to compare the SC and LC methods using the same sealer by means of μCT analysis. Previous studies have shown that the SC technique provides a filling result equivalent to thermoplasticized filling techniques [22,23] while the LC technique on the other hand is unable to fill the root canal as effectively [21,22].

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In the present work, a calcium silicate sealer was used with both treatment methods. Calcium silicate cements are thixotropic pseudo-plastic materials with relatively low viscosity [24]. They have therefore good flowing properties, particularly when delivered at relatively rapid rate. The placement of a tapered gutta percha cone prior to sealer setting results in hydraulic displacement of the sealer toward the confines of the root canal system. The LC method requiring the repeated insertion of a spreader instrument that compacts the endodontic biomaterials in the canal, further displaces the sealer and, according to our findings, increases the percentage of voids rather than improving the seal. Interestingly, the effects of compaction in LC can graphically be identified as periodic spreader tracks, appearing as peaks along the void% plots (Asterisks (*) marked in Fig. 3b and video b in Supplementary material). These tracks are thus produced as a result of conventional LC treatment, presumably degrading the filling quality, and merit revisiting this treatment approach, so as to improve its outcome.

A larger volume of sealer, as was used in the present study, has been advocated with the SC technique in order to fill all the interfacial spaces between the cone and the canal walls [25]. Noteworthy is the exquisite homogeneity of the filling material distribution along the root in the SC group, as

Fig. 4 – Pseudo 3D representation of the distribution of void/pores along the different roots in the (a) SC and (b) LC treatment groups. Note that the void% is plotted along the root treatment length, starting at the root tip side, as defined for the ROIs shown in Fig. 2. The coronal, middle and apical ROIs are marked by black lines sketched across all samples. The origin of the y-axis was determined by the narrowing of the instrumented canal (see Fig. 3). Significantly higher void% observed in the coronal and middle ROIs for the LC technique. Void% greater than 25% are truncated for ease of graphical representation.

Fig. 5 – Box-plot representing the void% with the SC and LC techniques at the different ROIs. X-axis: The defined ROI’s at different root canal levels (SC and LC plotted in pairs to ease comparison), Y-axis: Median void percentage (%), SC: Single cone, LC: Lateral compaction, Horizontal lines with asterisks represent pairs that are significantly different with * p<0.05 and ** p<0.01. Note that the median value of the SC group of the apical ROI coincides with the lower rim of the box.
compared to the LC group (see movies in the Supplementary online material). The 3D data provided by \( \mu \)CT is an ideal approach to observe this difference. The LC technique demonstrated significantly more void% than the SC method in the main part of the root (coronal and middle ROIs) and hints to this same trend in the apical ROI, although the void% was not statistically significantly different (\( p = 0.096 \)) between the two techniques.

Previous findings have indeed shown that a higher percentage of the root canal space can be filled by LC in round geometries as compared to oval ones [26] and that root canals tend indeed to be more circular in the apical region [27]. While a larger sample size might demonstrate a statistically significant difference between the LC and SC methods, both methods reveal voids near the tooth root apex and hence the apical region of the filling remains a shortcoming of root canal treatment, requiring further improvement (see Figs. 3 and 4 and reconstructions in Supplementary movies). The apical zone of the root canal is notorious for having an irregular anatomy which is a serious limitation of all current cleaning and filling strategies during root canal treatment. Canal branching and ramifications are frequent [28] and branching creates ‘side-canals’, (see video (b) in Supplementary material), that serve as hubs where microorganisms can grow and lead to chronic inflammation. It is therefore essential to further develop filling strategies with high sealing ability of the apical region, in order to entomb the microorganisms remaining after the shaping and disinfecting procedures. Our findings clearly demonstrate the superiority of the SC technique on LC in its ability to volumetrically fill the root canal space. Further studies need to evaluate the impact of the present findings on the long term outcome of root canal therapy.

5. Conclusions

The present study compares the void% associated to SC and LC root canal filling techniques with gutta percha and a commonly-used calcium silicate cement sealer. Our findings are based on 3D data with 10 \( \mu \)m voxel size, obtained from each sample both before and after treatment and analyzed with an observer-independent, reproducible image-analysis protocol. The void% associated with the LC technique was found to be significantly higher than with the SC technique. Within the limitations of the present study, the SC filling technique can thus be regarded as a superior alternative to the commonly used LC filling technique, suggesting that the current gold standard needs to be reconsidered by dental practitioners.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.dental.2015.06.009.

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