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Influence of the internal anatomy on the leakage of root canals filled with thermoplastic technique

Al-Jadaa, Anas ; Attin, Thomas ; Peltomäki, Timo ; Heumann, C ; Schmidlin, Patrick R ; Paquè, F

Abstract: OBJECTIVES: The aim of this paper is to evaluate the influence of the internal anatomy on the leakage of root canals filled with the thermoplastic technique. MATERIALS AND METHODS: The upper central incisors (UCI) and mesial roots of the lower molars (MRLM) (n = 12 each) were tested regarding leakage using the gas-enhanced permeation test (GEPT) after root filling. The quality of the root fillings was assessed using micro-computed tomography (CT) by superimposing scans before and after treatment to calculate unfilled volume. The calculated void volume was compared between the groups and correlated to the measured leakage values. Data were analyzed using t test and Pearson's correlation tests ($p < 0.05$). RESULTS: The mean void volume did not differ between UCI and MRLM (13.7 ± 6.2% vs. 14.2 ± 6.8%, respectively). However, significantly more leakage was evident in the MRLM ($p < 0.001$). While the leakage correlated highly to the void volume in the MRLM group ($R = 0.981$, $p < 0.001$), no correlation was found in UCI ($R = 0.467$, $p = 0.126$). CONCLUSION: MRLM showed higher leakage values, which correlated to the void volume in the root canal fillings. CLINICAL RELEVANCE: Care should always be taken while doing root canal treatments, but attention to teeth with known/expected complex root canal anatomy should be considered.

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Thermoplastic root canal filling quality in different anatomies as a determinant factor in leakage

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Abstract

Aim: To study the effect of thermoplastic root canal filling quality on its influence on leakage behavior in different root canal anatomies.

Methodology: Teeth with simple and complex root canal anatomies (n=12 each) were tested with regard to leakage using the gas-enhanced permeation test (GEPT) after root filling. Quality of the root fillings was assessed using micro-computed tomography (µCT) by superimposing scans before and after treatment to calculate unfilled volume as a percentage of the whole root canal space. The calculated void volume was compared between the groups and correlated to the measured leakage value of each individual sample. Data were analyzed using Kolmogorov-Smirnov, t- and Pearson's correlation tests ($p<0.05$).

Results: Significantly more leakage was evident in the complex root canal anatomy as determined with GEPT ($P < 0.001$). However, the mean void volume did not differ between simple and complex root canal anatomy (13.7 ± 6.2% versus 14.2 ± 6.8%). Whilst there was a high correlation between the void volume and GEPT in complex root canal anatomy (R^2 = 0.981, $P < 0.001$), no correlation was found in the simple root canal anatomy (R^2 = 0.467, P= 0.126). This was explained by the observation that complex anatomies showed through-and-through voids, which were not observed in simple anatomies.

Conclusion: Complex root canal anatomy showed higher leakage values, which correlated to the root canal quality of the root canal fillings.

Introduction

The controversy about the importance of leakage testing in root-filled teeth has been initiated two decades ago and has remained a continuous source of academic dispute ever since. So far, this debate did not openly discuss all problematic aspects and how to solve them but instead was blocked by some endodontic communities. Some scientific journals even abandoned any publishing regarding this potentially important topic (JOE Editorial Board, 2007). This discussion dates back to 1993, when the efficacy of endodontic leakage testing was questioned for the first time (Wu and Wesselink. 1993). In their original investigation, a fluid infiltration method was used, and they found that the rate of leakage decreased over time. They concluded that substrate infiltration might be influenced by the entrapment of the substrate used throughout the path of leakage, resulting in a potential blockage of this path. In addition, it was reported that the temperature may increase, facilitate or even enhance the leakage values. Another important observation or – more exactly – claim was that gas bubble entrapment could retard the leakage testing process. This late observation led to the suggestion to apply vacuum on the counter part to overcome such problems.

Another method to test leakage of filled root canals was the bacterial leakage set-up using two-chambers. This model was first adapted in the field of endodontics in 1980 (Goldman *et al.* 1980) and ever since, many studies were established based on this original model. Leakage was indicated to happen within weeks (Torabinejad *et al*. 1990). These findings were not corroborated by histological studies, which indicated that the bacterial presence in the apical canal portion to be negligible or not present at all, even if the root obturation were exposed for a long time period, once the root canal filling was properly performed (Ricucci and Bergenholz 2003, Ricucci *et al*. 2009). A systematic testing of this method (Rechenberg *et al*, 2011), indicated a possible bias, resulted in false positive leakage phenomena, which were detected and influenced by unwanted routes, such as the sample-embedding interface, which may result in an unwanted gaps and pathways leading to measurement and interpretation bias.

Micro-computed tomography (µCT) is capable to assess root canals in vitro, at different stages throughout the course of the treatment. This technique can assess three-dimensionally the volume, which is either removed or added to root canal spaces (Paqué *et al*. 2012). It allows therefore for a quality assessment of root canal fillings by means of their capability of occupying the space within the root canal system (Rechenberg and Paqué 2012). However, the method *per se* cannot express the leakage status of the sample, but it can indicate the radiographic quality of the root canal filling and serve as a valuable surrogate parameter as such for the internal seal and therefore indirectly for leakage, as one could hypothesize.

In summary, there is no doubt that current leakage testing methods as described above are still lacking validated set-ups, and have never been conceivably shown to exclude possible leakage routes nor assess the initial status of tested samples. In addition, leakage testing in vitro must correspond to in situ findings of properly filled root canals. A recently published editorial in the International Endodontic Journal has therefore sent an open invitation to investigators and encouraged them to establish new experimental models to assess root canal filling quality in terms of techniques and materials using more reliable and reproducible methods (De-Deus 2012).

Based on different shortcomings on leakage evaluation techniques as described above, the current investigation aimed to compare the leakage after root canal filling of teeth with simple root canal anatomy to corresponding values in counterparts with complex canal anatomy. In addition, the leakage values were correlated to the corresponding root canal filling quality as assessed by µCT evaluation. It was hypothesized that root canals with complex anatomy should have more voids when root-filled, which result in increased leakage.

Material and methods

Teeth

To study the influence of root canal anatomy on the root filling quality and sealability, teeth with two different root canal morphologies were selected based on µCT pre-scans. The group with a simple root anatomy consisted of upper central incisors (UCI) with a single root canal (n=12). The group with the complex root anatomy included mesial roots of lower molars (MRLM) selected roots had to exhibit two canals and an isthmus in between (n=12). None of the teeth should have had previous root canal treatment, visible signs of caries or cracks in their roots. All roots had a fully formed apex and were extracted for reasons, which were not related to the study. All teeth were stored in 0,2% thymol at 5°C for no longer than 1 year and were pre-scanned with a µCT device (µCT 40:Scanco Medical, Brüttisellen, Switzerland) with an isotopic resolution of 72 µm at 70 kV and 114 µA, to confirm their suitability to the study purpose as mentioned above.

Root canal preparation

All treatments were done by a graduate dentist who was not aware of the study aims. She was taught the technique of rotary root canal preparation (ProTaper universal System) and the continuous wave of condensation technique for root canal filling.

Tooth length was adjusted to 18 mm, by reducing the crown from its occlusal side with a slow speed diamond saw (0.4 mm, Struers GmbH, Birmensdorf, Switzerland) under water-cooling (Fig 1, B). Molar teeth were sectioned at the furcation area in order to obtain the single mesial roots with a diamond disc (Super-Flex 911HH, Busch & CO., Engelskirchen, Germany).

Root canal preparation was then accomplished under the magnification of a stereomicroscope (Stemi 1000, Zeiss, Oberkochen, Germany) and an access cavity was prepared through the crown with a diamond bur has a grit-size of 80 µm (Bur 837 KR, 8614, Intensive SA, Grancia, Switzerland) and attached to a high-speed handpiece (Sirius, Micro Mega, Besancon, France) with. Canal orifices were identified and the initial working length was determined by the first appearance of a headstrom file size 10 (Dentsply-Maillefer, Ballaegues, Switzerland) observed at the apical foramen. The final working length was further confirmed by a standard X-ray technique (Heliodent Plus, Sirona, Germany) with digital X-rays (Digora Optime, Soredex, Münchenstein, Switzerland). The canals were then prepared with a chemo-mechanical preparation approach according to the manufacturer's recommendations with the ProTaper rotary system (ProTaper universal, Dentsply-Maillefer, Ballaegues, Switzerland), which ran on a rotary motor (Endo-Mate TC2, NSK, Tochigi, Japan). Irrigation was performed using a side vented needle of a diameter 0.30 mm (Max-i-Probe; Hawe-Neos, Dentsply, Gioggio, Switzerland) inserted to 1mm shorter than the working length, with repetitive rinsing of 1 ml 1% hypochlorite solution after each file size preparation. Canals were prepared to file size F3. A final irrigation with 5 ml 17% EDTA took place, canals were then dried with paper points and the build-up was established as described in details below.

Sample embedding

A cylindrical coronal build-up of 11 mm diameter and 10 mm height was casted in a custom-made Teflon mold, which covered the coronal 7 mm of the tooth (Fig. 1, C). Before proceeding with the build-up and embedding process, the pulp chamber was secured with a cotton pellet and a temporary filling (Cavit, 3M ESPE, Seefeld, Germany), which was placed on top. The coronal part was then conditioned with a bonding system (Clearfil SE Protect, Kuraray America Inc., New York, USA), light cured for 20 seconds in a light cure chamber (Spectramat, Ivoclar-Vivadent, Schaan, Liechtenstein). The build-up was made using Luxacore build-up material (Luxa Core Automix, DMG, Hamburg, Germany). Samples were then light cured again for 5 minutes.

All teeth samples were then embedded in custom-made PVC rings, which had an outer diameter of 15 mm and an inner counterpart of 10 mm with a thickness of 3 mm. These rings were conditioned on their inner surface by sandblasting with 50-µm aluminum oxide (Benzer-Dental AG, Zurich, Switzerland).

Teeth with their build-ups were embedded with a light-cured nail build-up material kit (Sina, Shenzhen Cyber Technology Ltd, Mainland, China). The nail build-up gel material consisted of a primer, gel and glaze. The teeth as well as the rings were primed on their inner surface and subsequently light-cured for 4 minutes in a light-cure chamber (Spectramat, Ivoclar-Vivadent, Schaan, Liechtenstein). The parts were then held together in position in a custom-made rubber carrier, which was made of a putty material (Optosil, Heraeus Kulzer GmbH, Hanau, Germany). The gel was applied in one increment on the topside to fill the space between the ring and sample and was then light-cured for 4 minutes. The sample was turned upside down, the gel was optimized and extended on the root surface leaving the last three mm of the embedding free. It was then light cured again for another 4 minutes. Care was taken not to allow excess material formation on the upper and lower ring surfaces. Finally, a glaze layer was applied to both, the upper and lower gel surfaces to strengthen and eliminate any imperfections in the embedding. Everything was finally light cured for another extra 4 minutes.

Root canal filling

The root canal filling was performed after the build-up was fabricated and the samples were scanned for the first time with the µCT and the baseline measurement with Gas-enhanced Permeation Test device (GEPT) determined. The access cavity was regained, the canals were recapitulated to the full working length. Patency of the canal was insured for by inserting a headstrom file size 15 (Dentsply-Maillefer, Ballaegues, Switzerland) to 1mm beyond the apex subsequently followed by irrigation with 5ml EDTA 17 %. The root canal filling was made by implementing the continuous wave of condensation technique as follows: Master point gutta-percha was fitted to the full working length (F3 GP, Dentsply-Maillefer, Ballaegues, Switzerland) and the fitting of the master point was confirmed with a standard X-ray. The canals were then dried with paper points (ISO size 30, 0.04 taper, Dentsply-Maillefer, Ballaegues, Switzerland) and an epoxy resin root canal sealer (MM Seal, Micro Mega, Besancon Cedex, France) was then mixed on a glass slap. The master point was soaked into the sealer and presented to the full working length in the canal. With the aid of vertical thermal plugger (Xtra Fine, 0.04 taper, System B, Sybron Endo, California, USA) the master point was cut down to 3-4 mm coronal to the apex. A gutta-percha back fill was finally achieved (Obtura III, Sybron Endo, California, USA).

The access cavity was secured with a cotton pellet and the on top was sealed with a temporary filling (Cavit, 3M ESPE, Seefeld, Germany) and left to dry in a humid box at 35°C for 24 hours. In between tests, the samples, were kept in a humid box and at a temperature of 35 °C (Heraeus UT6420, Thermo Fisher Scientific, Dreieich, Germany)

Gas enhanced permeation test (GEPT)

The GEPT, the technical aspects and the validation, has already been described in a previous publication (Al-Jadaa *et al.* 2014). In short, the test took place in a temperature-controlled chamber (Temperature 35°C). The sample was mounted in a split-chamber with an O-ring lubricated with silicon grease (Molykote 111 compound, DOW Corning GMBH, Germany) and the compartments were differently pressurized (Fig. 2). In addition, the upper compartment of the chamber was filled with 2.5 ml 0.9% NaCl before the split chamber was tightly mounted. Overall, a net pressure difference of 1030 hPa was created by pressurizing the upper compartment with 860 hPa using N^2 and under-pressurizing the lower compartment by vacuum application to -170 hPa. The pressure difference was stabilized and secured by closing the valves leading to the positive and negative pressure sources. A pressure difference measuring device (Testo 526, Testo AG, Lenzkirch, Germany) was connected to the system on both sides in order to measure the pressure changes over time. This device was connected to a computer with an installed program (V 4.2 SP2, Testo AG, Germany) to monitor the pressure range change at a rate of 1 measurement/sec over a testing period of 40 minutes. Giving the hypothesis that the sample is creating a tight seal, no change in the pressure difference between the two chambers over time will occur. If the sample would be leaking, the pressure difference between the two chambers would start to drop, which then would result in a curve, which shows the rate of sample leakage over time. The rate of change represented by a slope value of the curve in hPa/min. To confirm the measured leakage to be true, the penetrating fluid volume was calculated and correlated to the measured pressure difference change slope value.

The test was carried at the following time points:

- After the samples embedding in the PVC rings and before access cavity reopening, to determine the baseline of the sample and to ensure the embedding tightness.
- After root canal filling with the access cavity opened to measure the leakage through the root canal filling.

- After the root apex sealed from its outer surface to ensure no leakage happened through unwanted routes within the embedding components. This took place at the end of testing, the last free 2 mm of the apex were sealed with composite (Filtek Supreme, 3M ESPE, Seefeld, Germany) after a standardized conditioning and bonding (Syntac Classic, Ivoclar, Vivadent, Schaan, Liechtenstein). Subsequently, the GEPT was assessed again for a last time to ensure the baseline value could be regained.

Data extraction for GEPT

The pressure difference change over time was expressed for each test as the slope between two fixed time points (1200 sec and 2400 sec) utilizing the following equation:

Slope = $\frac{P1 - P2}{t2 - t1}$ hPa/min

The baseline value was then subtracted from the value after treatment to calculate the absolute leakage of each sample.

The collected water volume in the Eppendorf tube was also weighted and converted into volume (ml).

µCT analysis of root canal treatments

The root canals fillings were tested with the GEPT method before being evaluated for the 3D root canal filling quality. It was hypothesized that a filling compromised 100% of the root canal volume can provide a perfect tight seal which prevents leakage. To allow multiple measurements, individual custom-made carrier, made of heavy-body rubber impression material (3M ESPE Pentamix 2, 3M Deutschland GmbH, Seefeld, Germany) was established for each sample (Fig. 1, E). The rubber carriers were glued to scanning electron microscopy stubs (014001-T, Bal Tec AG, Balzers, Liechtenstein). This set-up allowed for the ease of sample removal and repositioning in the same position at each test stage. Each sample was scanned after embedding (after root canal preparation) utilizing a high-resolution µCT scanner (µCT 40: Scanco Medical, Brüttisellen, Switzerland) at an isotopic resolution of 20 µm at 70 kV and 114 µA. The lower 11 mm of the root below the mounting ring presented the area of interest. The scan was repeated at 70 kV and 114 µA with an isotropic resolution of 10 µm after the root canal filling was done. Before and after scans were superimposed with a specialized software (IPL Register 1.01, Scanco Medical, Brütisellen, Switzerland). And also the volumes of root canals before and after root canal filling were calculated (IPL V5.15, Scanco Medical, Brütisellen, Switzerland). The root canal filling, i.e. the gutta-percha and sealer, was identified and subsequently the volume was calculated as follows: The voxels defined before root canal filling, which consisted of soft tissues, fluids or air presented the total canal volume. The voxels, which were filled with a radio-opaque material after filling were considered as being filled in the common sense with the respective material. Counting these voxels allowed for volume calculation by multiplying in one voxel volume. The root canal filling volume was presented as a percentage to the total canal volume, out of which the remaining unfilled root canal volume or root canal filling defect or voids could be calculated as well.

Statistical analysis

To compare groups for the resulted filled canal volume as well for their performance under GEPT, t-test was applied ($P \leq 0.05$). To test for correlation between the GEPT measurements to the root canal filling defective volume, as well the GEPT measurements to the permeated fluid volume in milliliters, the Pearson correlation coefficient was applied for both situations with the probability of type I error set at ($\alpha=0.05$).

Results

Root canal filling defects as well as the GEPT performance were presented as mean values and standard deviation for both groups separately (table 1). One sample from the MRLM group had to be excluded after detecting a crack in the outer root wall. Therefore, only eleven samples could finally be exuded in the final analysis.

The void volumes were presented as a percentage of the whole root canal volume. Results showed a normal distribution for both groups (Table 2). No statistical significant difference with regard to void volume between the two groups was detectable. However, significantly more leakage was observed in the MRLM group ($P < 0.001$). When correlating the two factors, a high correlation between the void volume and the corresponding measured GEPT values was detected in the MRLM group ($R^2 = 0.981$, $P < 0.001$), whereas in the UCI group the respective correlation coefficient was low ($R^2 = 0.467$, $P= 0.126$; Fig. 4).

The correlation between the measured pressure difference slope and the infiltrated water volume was high ($R^2 = 989$, $P < 0.001$). All the samples exhibited leakage values close to their baseline values once the apices were sealed again, with the differences almost not detectable and ranged between 0.00-0.01 hPa/min.

Discussion

The present study aimed to elaborate the cause and effect between root canal filling quality and leakage. The detected root canal filling defect measured as void volume, in both simple and complex root canal anatomies, was relatively high accounting for 13.7% and 14.2, respectively. This finding was corroborated by a previous study where the root canal filling defect was also assessed (Rechenberg *et al.* 2013). The high defect volume can be referred mainly to the limited experience of the operator who did the root canal filling in the present study. On the other hand, this fact has resulted in an equal and normal distribution of the detected voids in both groups with no differences.

Another observation was the significantly higher leakage in MRLM group. The leakage in this group was highly correlating to the detected void volume, which was in contrast to the UCI group, where no correlation was found. This indicates that voids do not necessarily have to correlate with leakage, unless they are through and through. In teeth with complex anatomy as can be found in mesial roots of lower molars for example, the voids are most likely located at areas, which are hard-to-reach by the root canal filling, and hence, they maintain their continuity along the whole root length (Fig. 3). Whereas in a simple root canal anatomy like in the UCI group, void may exist, but a seal can still be established at any level within the root canal, i.e. the void can be entrapped within the root filling itself (Fig. 3).

There was a slight deviation from zero in the correlation between the measured slope values with regard to the collected fluid volume. This may be explained by the loss of some of the infiltrated fluid within the sample or by some evaporation of the infiltrated fluid under pressurized conditions in the lower chamber. However the correlation was still high (R^2 = 989, $P < 0.001$).

As previously mentioned, leakage has always been a controversial issue in endodontics. Clinical studies suggested different opinions weighing the importance of the coronal seal, the root filling tightness or both against each other. A recent study highlighted the importance of the root canal treatment quality as the determinant factor for success (Ricucci *et al.* 2000). Another study (Kirkevang *et al*. 2000) observed better success rates once a coronal seal was achieved regardless of the quality of the root filling. Recently, retrospective clinical studies emphasized the importance of both seals to be established to ensure the best outcome of the root canal treatment (Song *et al*. 2014, Archana *et al*. 2015). However, all dentists agree that microleakage is one of the most risky factors, which may contribute for the development of an periapical periodontitis (Muliyar *et al*. 2014).

In summary, within the limitations and under the conditions of the current investigation, mesial roots of lower molars showed higher leakage values, which highly correlated to the root canal filling quality (i.e. loads of voids). This corroborates with clinical findings where the apical periodontitis was significantly more often detected in molars presented with root canal filling short of the radiographic root apex (Zhao and Xu 2014). Di Filippo *et al.* (2014) found a high correlation between poor root canal filling quality and the presence of periapical periodontitis. Thus emphasizes again the importance of establishing a high root canal filling quality, to ensure a higher success rate of the root canal treatment and the importance of adequate laboratory models to measure these conditions.

The tested samples acquired their initial leakage status (before the establishment of the access cavity through the build-up) once their apices properly sealed again. This indicates the detected leakage can only be explained by one pathway, namely through the canal itself.

Conclusion

Under the current conditions of this investigation, the hypothesis was accepted in regard to the leakage performance in root canals with complex anatomies but not for the quality of root canal fillings. The mesial roots of lower molars presenting a complex anatomy showed the highest leakage values which correlated to the root canal filling quality. The combination of the current leakage set-up and the quantitative μCT-analysis seems to present a promising way to improve the understanding and to confirm the pathways through which leakage occurs under different anatomical variations and simulated clinical conditions. Therefore, it merits further investigation and the field is open for scrutiny.

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Tables:

Table 1

Group	Root canal filling defect (%)	Effective GEPT (hPa/min)	Effective fluid infiltration (ml)
UCI	13.74 ± 6.23 A	0.102 ± 0.072 A	0.049 ± 0.036 A
MRLM	14.17 ± 6.83 A	0.321 ± 0.154 B	0.144 ± 0.073 B

Results presented as mean values ± SD . Capital letters indicates significance (read vertical).

Table 2

Upper Central Incisor (UCI)				Mesial Roots Lower Molar (MRLM)			
Sample No.	Defect Volume (%)	Effective GEPT (hPa/min)	Effective Fluid infiltration (ml)	Sample No.	Defect Volume (%)	Effective GEPT (hPa/min)	Effective Fluid infiltration (ml)
UCI 1	17.43	0.22	0.13	MRLM 1	25.95	0.61	0.286
UCI 2	13.03	0.19	0.08	MRLM 2	10.49	0.23	0.102
UCI 3	6.13	0.04	0.025	MRLM 3	8.73	0.2	0.097
UCI 4	10.65	0.182	0.083	MRLM 4	7.76	0.19	0.076
UCI 5	18.99	0.18	0.072	MRLM 5	12.73	0.32	0.132
UCI 6	11.99	0.04	0.015	MRLM 6	22.43	0.48	0.235
UCI 7	24.22	0.11	0.053	MRLM 7	4.45	0.13	0.051
UCI 8	13.18	0.05	0.033	MRLM 8	12.66	0.28	0.121
UCI 9	5.14	0.02	0.007	MRLM 9	11.03	0.21	0.11
UCI 10	20.96	0.09	0.041	MRLM 10	18.71	0.37	0.157
UCI 11	17.43	0.072	0.027	MRLM 11	Excluded	Excluded	Excluded
UCI 12	17.43	0.03	0.02	MRLM 12	20.94	0.37	0.215

Test results presented in terms of the root filling defect volume, GEPT leakage and the infiltrated fluid volume for each sample separately

Figures:

Figure 1

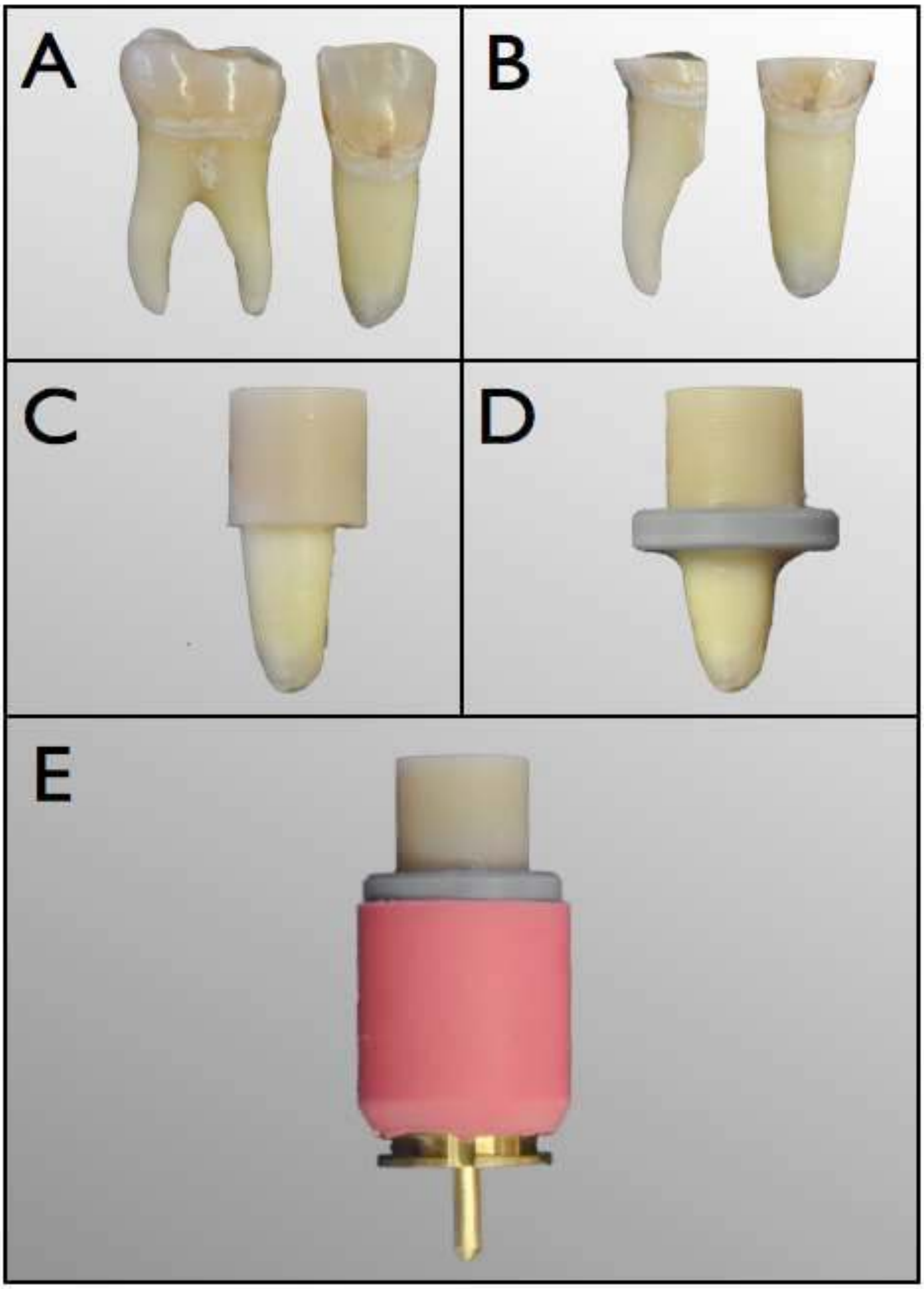

Fig. 1: Samples preparation for the root filling quality assessment.

A. Samples selection, B. Standerdization of the length to 18mm as well sectioning of roots with the anatomy of interest, C. Core build up, D. Embedding in the PVC rings, E. Mounted in the rubber carriers to carry out the μCT scans.

Figure 2

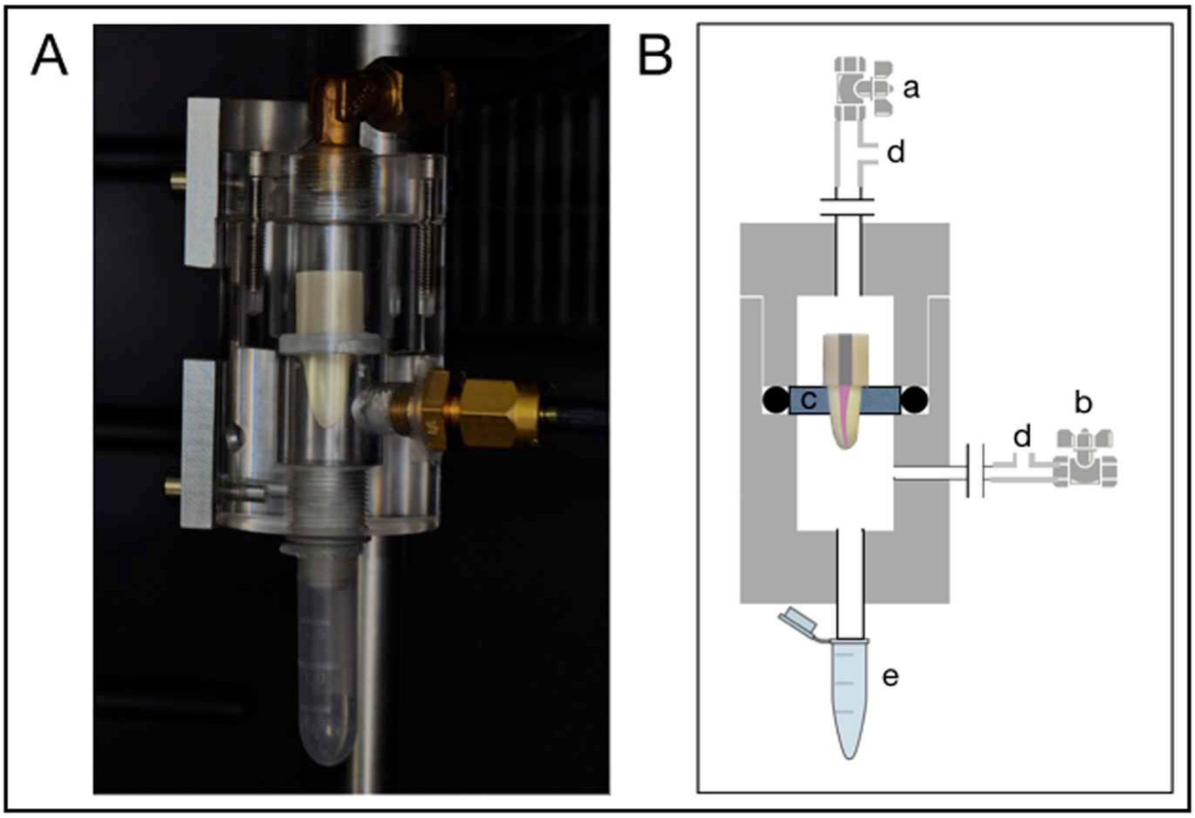

Fig. 2: The pressure difference split chamber

a. The positive pressure securing valve, b. Negative pressure securing valve, c. Sample mounted in position, d. Inlets to which the pressure measuring device is connected, e. Eppendorf tube to collect the infiltrated fluid.

Figure 3

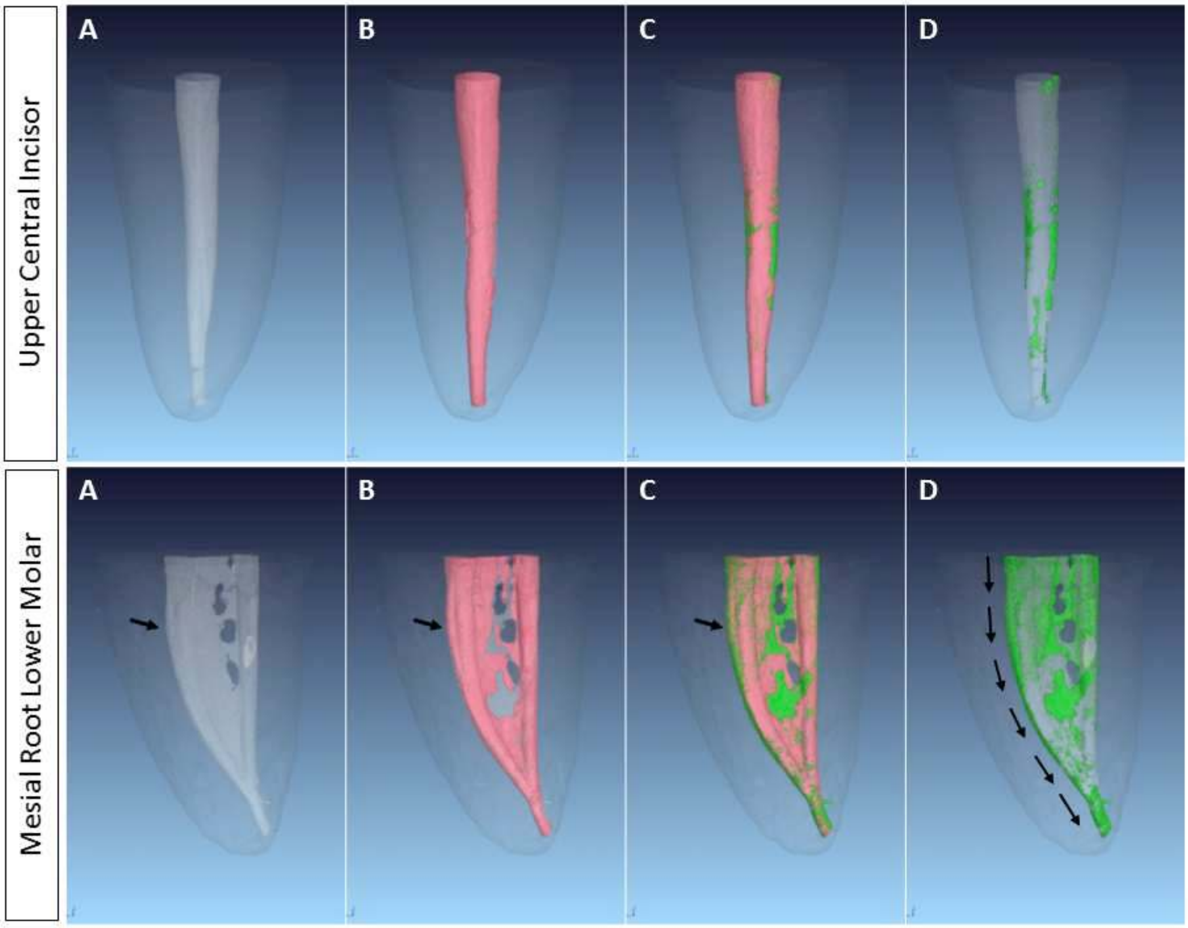

Fig. 3: Steps for 3D root canal treatment analysis.

A) Root canal scan after preparation, B) Root canal scan after filling, C) Superimposition of both scans and D) Calculation of unfilled space in the root canal system by subtracting the filled volume from the total canal space.

Figure 4

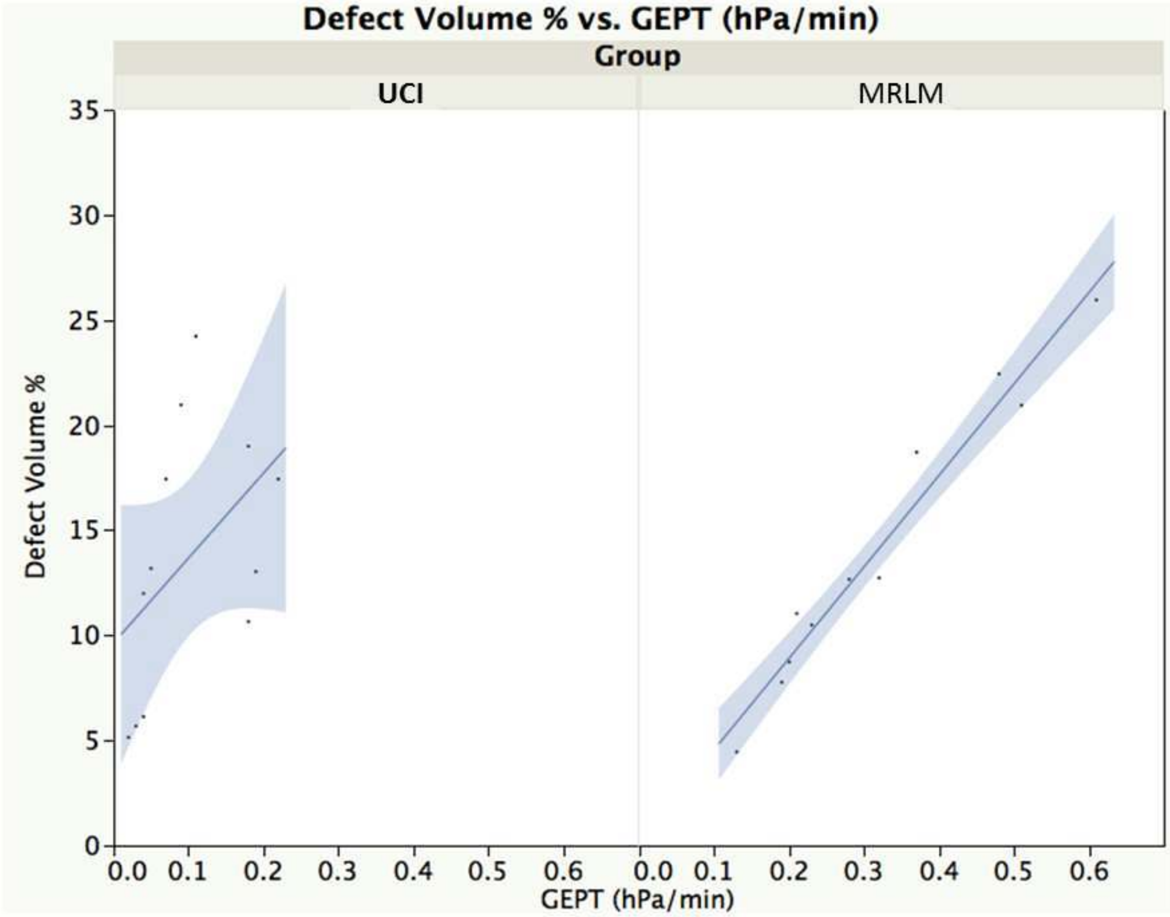

Fig. 4: Correlation of the root filling defect to the measured leakage (hPa/min).

The blue area represents the distribution of 90% of samples. The MRLM shows a higher correlation ($R^2 = 0.981$, $P < 0.001$). In contrast the UCI group, showed a lower correlation ($R^2 = 0.467$, P= 0.126).