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Comparing apical bacterial leakage using two different obturation techniques in human upper incisors: An in vitro pilot study

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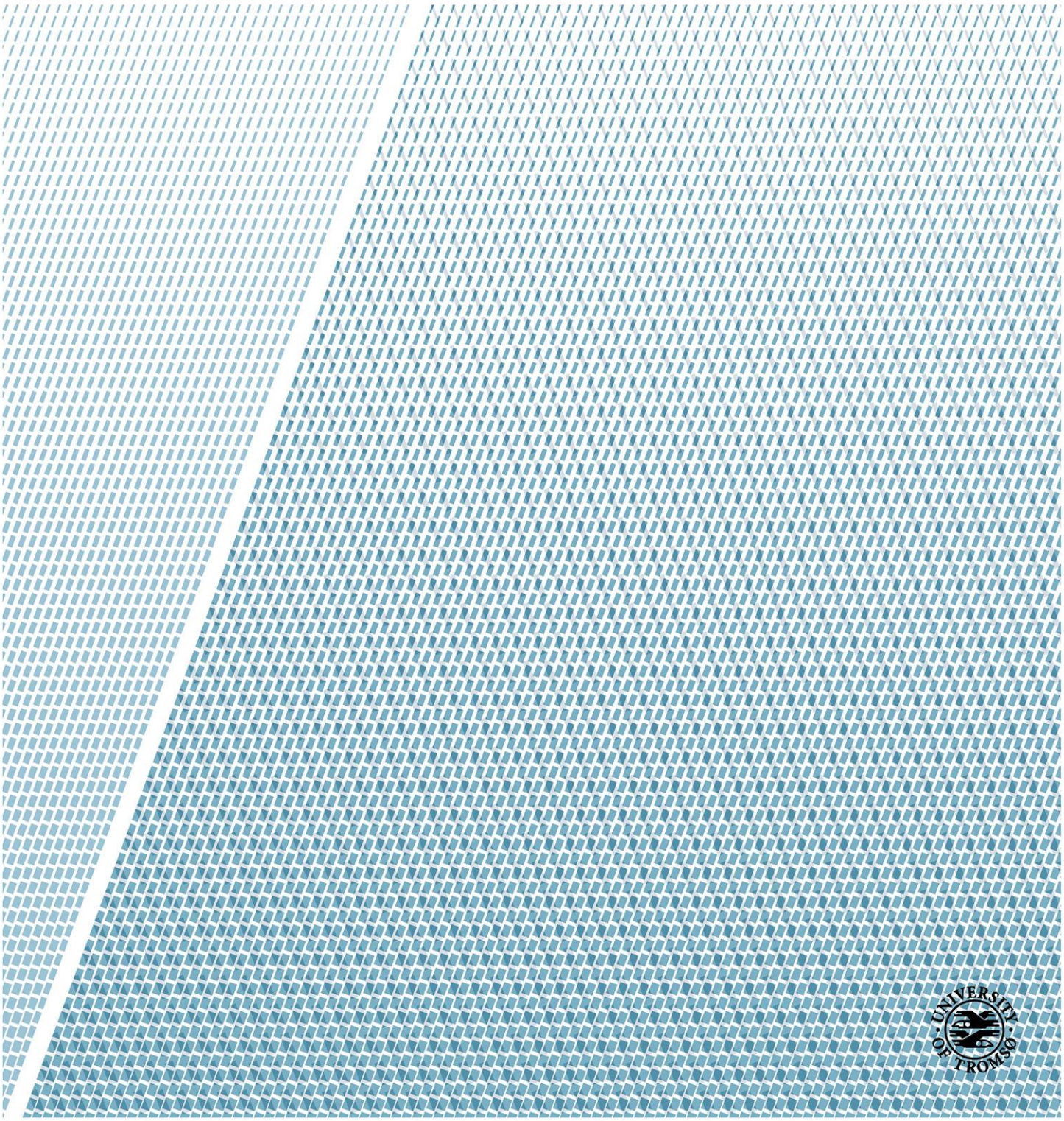

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Acknowledgement

Working on this master thesis has been demanding and educational, and we are very proud to have completed our own pilot study. We both started to read through relevant articles for the theme of the master thesis and, with guidance from our supervisor and co-supervisor, made suggestions on how to do the laboratory experiment. We conducted the experiment together so that both gained experiences within this. For the writing we distributed tasks and research.

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Abstract

Background – The materials and techniques for root canal obturation is critical for treatment success and prevention of secondary infections. Obturation techniques can be divided into two main groups: cold and requiring heat. Cold lateral condensation is a widely used obturation technique. A core carrier system requires heat and has been developed to overcome disadvantages with previous obturation techniques. GuttaCore is a relatively new and unstudied core carrier technique in vitro. Though several microleakage tests are available to compare obturation materials and techniques, the bacterial leakage test has been regarded as the most clinically relevant. However, comparing obturation techniques with regard to bacterial leakage has been questioned. No studies could be found comparing GuttaCore to lateral condensation using bacterial leakage models. Therefore, the aim of this in vitro study was to investigate the feasibility to compare cold lateral condensation and GuttaCore root filling techniques regarding bacterial leakage using a two-chamber system.

Material and method – 25 human upper incisors were radiologically examined, cleaned and autoclaved before selected into 2 experimental and 2 control groups. All teeth were mechanically cleaned and shaped using ProTaper Next rotary files (Dentsply Sirona, North Carolina, USA) and appropriate finishing hand files before obturation. The experimental groups were obturated by cold lateral condensation and GuttaCore techniques. A two-chamber system was set up to compare bacterial leakage, with *Enterococcus faecalis* as the bacterial mediator. Environmental contamination was investigated. The experiment lasted for 42 days.

Results –three positive control and two GuttaCore specimens showed bacterial contamination. No specimens in lateral condensation group showed signs of leakage. One specimen showed environmental contamination.

Conclusion – Within the limitations of this pilot study, a two-chamber system appears to be a feasible method to compare GuttaCore with other methods regarding bacterial leakage. To confirm our results, it needs future studies using a larger sample size.

Keywords: Bacterial leakage, two-chamber system, obturation techniques, GuttaCore, Lateral condensation.

1. Introduction

The biological aim of endodontic treatment is either to prevent or cure apical periodontitis (AP) (1). When the pulp tissue gets exposed to the bacteria in the oral cavity, it gradually breaks down, become necrotic and infected by bacteria and AP develops as an immune response to primary infection (2, 3). Persistence of infection in the root canal system may result from inadequate treatment procedure, but the secondary infection is caused by bacteria not present in the root canal system before treatment but introduced during the treatment or failure in coronal seal (4). Microbiology in failed root-filled teeth is mainly composed of Gram-positive facultative anaerobic bacteria, but enteric bacteria have also been found and the most common species in this group is *Enterococcus faecalis* (2).

Clinically, endodontics is perceived as chemical-mechanical cleaning and shaping of the root canal system, and the obturation (4, 5). The main objective of obturation is to create a tight seal (4). Root canal filling quality, the materials and techniques used to obturate a root canal may affect the amount and severity of the bacterial leakage.

The standard filling materials are a combination of core material, gutta-percha (GP), and sealer (5). GP is a trans-isomer of polyisoprene, resembling natural rubber, and is regarded as the gold standard material for root canal obturation (6). The α- and β-form of GP are used for endodontic purposes, where it is combined with other materials, such as zinc oxide (up to 75%) and metal salts that provide contrast for x-ray detection (2, 7).

Sealers are fluent materials used in just the amount that is necessary to complement the gaps and irregularities between the GP cones and the walls of the root canal.

Obturation techniques can be divided into two groups: cold and techniques requiring heat. Cold obturation techniques rely on compaction of cold GP into the canal(s), whereas the warm techniques use an external heat source to soften the GP and thus it is expected to adapt to the root canal anatomy.

The cold lateral compaction technique is one of the most common techniques for root canal obturation, partly because the operator has sufficient control throughout the treatment, and over- or under filling seldom occurs (2). Together with sealer, a master cone is placed and accessory cones are added to the canal – tightly compacted with a spreader, aiming at

maximizing the density of GP in the canal(s) (1, 2). However, the technique is operator dependant and time consuming. Another popular cold obturation method is the single-cone technique. Here, a single GP cone is matched to the prepared root canal(s), relying on the sealer to fill the potential gaps between the GP and interradicular dentine (2, 8). The disadvantage is therefore the reliance of distribution of sealer into the irregularities of the canal(s) which is unpredictable and may be imperfect (2).

In order to overcome some of the disadvantages of the cold obturation techniques, several techniques requiring heat has been introduced over the past years. Among them are warm lateral compaction, warm vertical compaction, injectable GP, and Carrier devices. The general concept in carrier devices is coating of a core - manufactured from plastic or steel, with solidified thermoplastic GP that is heated before placement in the root canal(s) (2). Thermafil (Dentsply Sirona, USA) is a solid core carrier device coated with thermoplasticized GP that has become popular over the years because of its simplicity and accuracy (1). Revision of teeth obturated with solid core carriers such as Thermafil has however shown to be challenging (2).

To overcome the difficulties of revision treatment with early carrier systems, GuttaCore (Dentsply Sirona, USA) obturation system was introduced to marked, where the thermoplastic GP is coated around a carrier consisting of cross linked GP (2, 5). The cross-linked thermoset gutta-percha does not melt by the heat used in an obturator oven, and is insoluble in common organic solvents employed for root canal retreatment. Removal of this modified obturator during retreatment may be achieved by mechanical trephination through the carrier (9).

Several in vitro methods exist to study bacterial leakage through root filled teeth. Amongst these are the dye study using dye molecules to investigate apical leakage; electrochemical studies to inspect flaws around fillings; fluid filtration studies to measure a material's sealing capacity, and bacterial studies to measure apical leakage. One of the disadvantages of the dye studies is the deficiency of clinical relevance because of the differences in attributes between the dye molecules and the bacterial virulence factors, which risks both overestimation and underestimation of the material's leakage (10). When it comes to electrochemical and fluid filtration studies, a disadvantage is their lack of standardization of the methodology (11). Different use of materials (electrolyte, electrode type, distance between the two electrodes etc) in the electrochemical studies leads to different electrical potential which affect the leakage measurements (10, 12). The pressure used in fluid filtration experiments varies

between different studies. In addition, some experiments used much higher pressure than the physiologic environment which gives unreasonable conclusions (10).

The bacterial study has been considered more clinically relevant than the dye studies, and the most popular design is the two-chamber method developed by Goldman et al. in 1980 (10). The design comprises two chambers with a root filled tooth suspended in between. The upper chamber is filled with bacteria that will act as the medium through which leakage is measured. In the lower chamber, the apex of the tooth is suspended into a liquid, often a sterile broth. Given that bacterial leakage from the upper chamber through the root canal occurs, the bacteria will reach the broth in the lower chamber and multiply. Depending on the composition, bacterial contamination may show as turbidity or colour change of the liquid in the lower chamber (13, 14).

Using this method, many root canal materials and methods have been tested and compared to each other over the years, where lateral compaction usually has been compared to other techniques. Yücel et al. (2006) compared bacterial penetration trough 5 different obturation techniques after 30 and 60 days using the two-chamber system (15). In their study, cold lateral condensation (Lc), Thermafil (T), System B (Sb), single cone ProTaper GP (P) and lateral condensation with ProTaper GP(PLc) techniques were compared (15). They found that after 30 days, the PLc-and Sb-groups showed lowest ratio of penetration, but after 60 days no statistical difference was observed between any of the groups (15). Results supporting no significant difference in bacterial leakage between obturation techniques was also found by Nabeshima et al. (2013) (16). Their study compared lateral condensation, modified single cone and continuous wave technique using the two-chamber system, and after 30 days there was no significant difference in bacterial leakage between the three groups (16).

A study by Karagenç et al. (2006) compared lateral condensation and Thermafil obturation techniques by using 4 different leakage methods (17). Results in regard to bacterial leakage in their study varied between the different leakage methods where lateral condensation techniques showed less leakage in the fluid test, but Thermafil showed less leakage using bacterial leakage test (17). Using the electrochemical leakage study and vacuum dye leakage test, there was no significant difference in leakage between the two techniques (17).

Only one in Vitro study could be found where GuttaCore was compared to other obturation methods and materials, though lateral condensation technique was not included. Hwang et al.

(2015) compared GuttaCore to GuttaFlow and EndoSeal MTA using confocal laser-scanning microscope (CLSM) and found that GuttaCore showed less bacterial leakage than GuttaFlow (18). No microleakage studies comparing GuttaCore and lateral condensation was found.

Comparing obturation techniques with regard to bacterial leakage has been questioned, mostly due to the discrepancies of methodology (14, 19, 20). Therefore, the aim of this in vitro study was to investigate the feasibility to compare cold lateral condensation and GuttaCore root filling techniques regarding bacterial leakage using a two-chamber system.

2. Material and Methods

2.1 Ethics

Since human teeth were used in this study, an application was sent to The Regional Ethical Committee (REK) for medical and health research ethics. REK concluded that this study was not a subject of health research legislation (reference 2019/933/REK nord).

2.2 Collection and examination of teeth

Twenty-five human central upper incisors were collected from storage at The University dental simulation clinic at UiT. These teeth were collected from different external dental clinics. Teeth were extracted due to common oral or dental conditions and cannot be traced back to original source.

To ensure similarity in root anatomy and eliminate other pathways of bacterial leakage, 9 exclusion criteria were set.

Exclusion criteria:

1. Previously endodontically treated
2. Large restorations close to the enamel-cementum junction (ECJ)

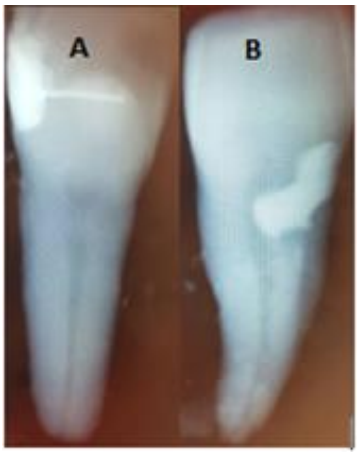
3. Restorations on the root surface
4. Caries on the root surface
5. Cracks that extended beyond the ECJ
6. More than one canal
7. Obliterated canal
8. Obliterated cavum extending to coronal third of root canal
9. Curved canal

All teeth were radiologically and clinically examined. Five teeth were discarded by the exclusion criteria. Twenty teeth were selected for the pilot study to be distributed into four groups: Lateral compaction (Lc-group), GuttaCore (Gc-group), positive control (Pc-group) and negative control (Nc-group). All teeth were kept in 75% ethanol.

Ten of the twenty teeth were considered most uniform in shape and size and were selected to be in the Lc-group and Gc-group, while the remaining 10 were selected for the Pc-group and Nc-group. This was to ensure that the teeth obturated by the compared techniques were most uniform.

The teeth were put into an envelope by operator 1 (SJ) in random order, and blindly selected into the respective groups by operator 2 (SM). This was conducted two times, one for the Lc and Gc groups and later for the Nc and Pc groups.

The teeth were mechanically cleaned carefully using a periodontal curette (LM curette 201-202 SXI Gracey, Loser & Co. GMBH) to remove any calculus and debris before being steam autoclaved at 121˚ Celsius.



Picture 1. A) included due to no caries or fillings below the ECJ, single, straight canal with no obliteration. B) excluded due to large filling and caries on the root, curved canal.

2.3 Preparation and obturation

Access cavity preparation on the teeth was done using a high-speed diamond burr. The working lengths (less than 2 mm from apex) were determined by inserting a K-file #15 into the canal until it was just visible at the apical foramen, then withdrawn 1 mm back and verified with radiographs. Glide path was made using K-files up to #20. Thereafter all the teeth were cleaned and shaped using ProTaper Next rotary files (Dentsply Sirona, North Carolina, USA) X1 (17/0.04), X2 (25/0.06) and X3 (30/0.07) at 350 rpm and torque of 4 Ncm (MotorX-smart; Dentsply Sirona, North Carolina, USA). For each tooth in the Pc-group, Nc-group and Lc-group an apical box was prepared using NiTi #40 hand files by rotating the file 2X contra clockwise at working length before removal. The size of the root canal in the Gc-group was confirmed with a GuttaCore Size Verifier NiTi X3 (Dentsply Sirona, North Carolina, USA). During the root canal preparation recapitulation was done between each file in every group. Sufficient irrigation with sodium hypochlorite (NaOCl) 3% was done during the preparations. After instrumentation the canals were dried with sterile paper points, irrigated with EDTA 17% for 2 minutes to remove the smear layer, and then dried again before obturation.

In the Pc-group, one single GP master cone (40/0.02) was placed in the canal to the working length without sealer or any accessory GP points. A hot plugger was used to remove excess GP coronally. The roots were coated with nail polish leaving 2 mm of the apical part clean.

In the Nc-group and the Lc-group, the teeth were obturated using the cold lateral compaction technique. AH Plus sealer (Dentsply Sirona, North Carolina, USA) was introduced to the canal along with the GP master cone (40/0.02) to the working length. Lateral compaction was achieved using accessory cones and a finger spreader. Excess GP was removed coronally with a hot plugger followed by cold condensation. The roots of the Nc-group were coated with nail polish only on the apical 2 mm whilst the teeth in the Lc-group were coated with nail polish leaving 2 mm of the apical part clean.

The teeth in the Gc-group were obturated using GuttaCore obturation system (Dentsply Sirona, North Carolina, USA). A thin layer of AH Plus sealer was coated into the walls of the canal using a paper point. The GuttaCore obturator X3 (Dentsply Sirona, North Carolina, USA) was softened with heat in the ThermaPrep Heater Obturator Oven (Dentsply Sirona, North Carolina, USA) and inserted to the working length. The carrier was twisted off and the excess GP was removed using a low speed hard metal rose head burr. The roots were coated with nail polish except for 2 mm at the apex.

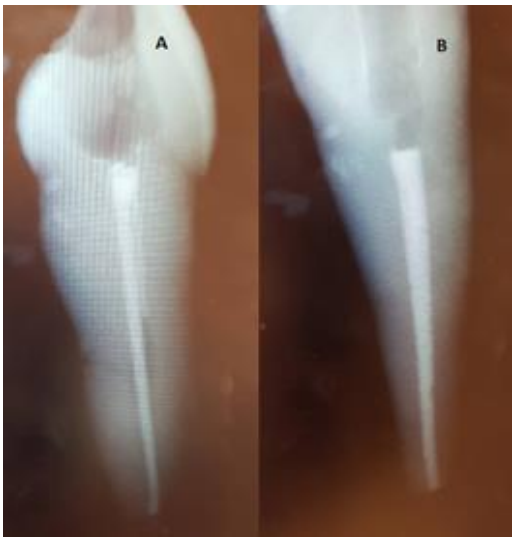
Both operators (SJ and SM) performed obturation on 2 teeth in each group. It was not noted which sample was obturated by which operator.

During obturation one tooth in Lc-, Nc- and Pc-groups cracked at the root. From the Gc-group all teeth cracked during obturation. Seven new human upper central incisors were collected from the same supply as previous teeth and prepared in the exact same way as described above.

After obturation all teeth were radiologically examined to determine if the obturation was acceptable. Exclusion criteria:

- Visible bubbles or imperfections in root canal filling
- Obturation more than 0-2 mm from the apex
- GP not removed from the cavum
- Visible cracks or deformation of the tooth

The crowns of all the teeth were cut horizontally at the ECJ using high speed diamond burrs.



Picture 2. Radiograph after obturation, before removal of crown. A) positive control with no sealer or condensation of the GP B) Lateral condensation.

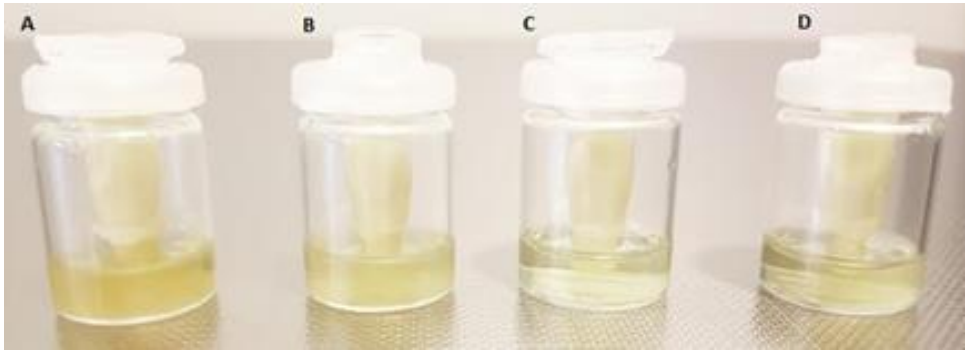
2.4 Two-chamber set-up

The experiment was done in the laboratory at UiT - The Arctic University of Norway, Faculty of Health Sciences, Institute of Clinical Dentistry (IKO). To get permission to work in the laboratory both operators had to complete a bio-safety course in addition to read local laboratory rules. The course was approved by the laboratory manager.

The bacterial leakage model was set up as introduced by Yanpiseth et al. (2018) (21). The upper chamber was made using 2 ml Eppendorf vials (Eppendorf AG, Hamburg, Germany). The lower chamber was made from glass-vials with air-tight lids.

For sealing between the chambers, a test-study was executed to determine what material would provide best seal ability between the upper and lower chamber and between the upper chamber and the teeth. The test-study was done using plastic, non-hollow teeth that were rinsed with Ethanol 70% so to ensure no leakage through the teeth. Three materials were selected for sealing between the segments of the set-up: Sticky Wax (KaVo Kerr, Orange California, USA), SDR bulk fill flowable composite (Dentsply Sirona, North Carolina, USA) and cyanoacrylate cement (CO super glue liquid, nr. 1907/2006 - ISO 11014-1, Clas Ohlson AB) and a combination of the latter with SDR between the tooth and the upper chamber and cyanoacrylate cement between the upper and lower chamber. The upper chamber was filled

with sterile LB broth Miller (VWR International, Radnor, Pennsylvania, USA) to cover the entire coronal part of the plastic teeth, while sterile LB broth in the lower chamber was to cover 2-3 mm of the apical part. The chamber set-up was done under a sterile cabinet and was left in an incubator at 37° Celsius for one week. The Eppendorf and lids of the lower chamber were prepared as described below. Environmental contamination would show as clouding and change of colour of the liquid in the lower chamber. The test showed that cyanoacrylate cement gave best seal ability with no contamination at the end of the test, and Sticky Wax showed least sealability with contamination after only 1 day.



Picture 3: test-setup with different sealing materials after 1 week. Contamination can be seen by change of colour in the lower chamber, with C as reference (no contamination). A) sticky wax B) SDR resin C) cyanoacrylate cement D) SDR composite and cyanoacrylate cement.

For the pilot study, the Eppendorf vials were cut at the tip using rotary high-speed discs so the root could be penetrated through the opening, approximately exposing 4-5 mm of the root apex. The lids of the lower chamber were cut using high-speed diamond burrs so the Eppendorf vials could be suspended through.

Cyanoacrylate cement was used to seal between the tooth and the Eppendorf and between the Eppendorf and the lid of the lower chamber. The seal was checked for cracks and bubbles after 24 hours and adjusted as needed.

The Eppendorf-lid-tooth complex was suspended in 3% sodium hypochlorite (NaOCl) for 10 minutes and air-dried in a sterile cabinet before attached to the lower chamber. The lower chamber was autoclaved and filled with sterile LB broth so approximately 2 mm of the apex of each tooth was suspended in the liquid. The complex was then attached to the lower chamber.

For the upper chamber, a solution of clinically isolated *Enterococcus faecalis* was made. The bacteria were grown overnight in an incubator at 37° Celsius and added to a sterile test-tube containing 10 ml of sterile LB broth. The absorbance was measured by Multiscan Go at 600 nm wavelength to 0,5 McFarland standard. 200 µl from the bacterial solution were added to each upper chamber, covering the entire canal-opening. All chambers were then set in an incubator at 37° Celsius.

After three days 300 µl of the sterile LB broth were added to the upper chambers. After that 300 µl from the upper chamber was taken out and 300 µl of LB broth was added at 3-4-day intervals. The LB broth was stored in the incubator at 37° Celsius and checked for contamination before use.

To ensure viability of bacteria in the upper chamber, a new bacterial solution was made at day 14 to 0,5 McFarland standard using the same method as described above. 300µl of the LB-bacteria-solution in the upper chambers were removed, and 300 µl from the new bacterial solution was added.

For all removal and adding of solutions, 1 µl-pipettes were used, and the procedure carried out under the sterile cabinet.

2.5 Contamination-procedure

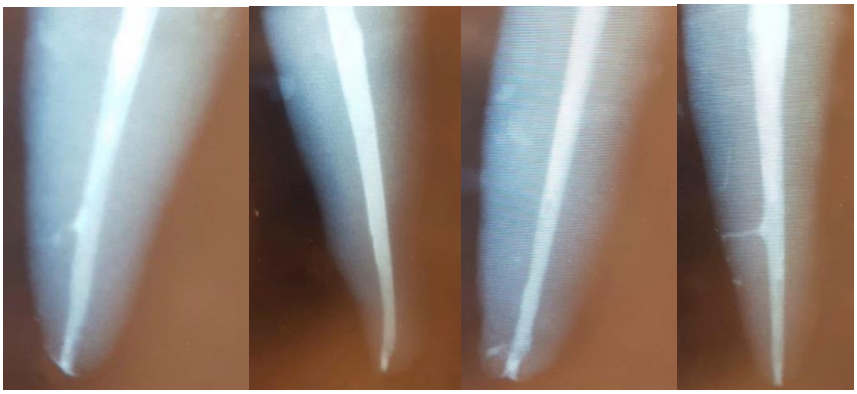
To check for bacterial leakage, each setup was checked every day by SJ and SM the first three weeks, and by an Engineer (MM) the remaining weeks of the experimental time (42 days). The lower chamber was held up to a bright lamp in order to see any disturbance in the LB broth. If the lower chamber had been contaminated with bacteria, it would show as opaque or murky flakes in the clear broth.

When contamination occurred the day was noted, and the lower chamber shaken to distribute the bacteria evenly. Two Eppendorf vials were filled with 999 µl of LB broth. Then 1µl was taken from the lower chamber and put into one of the vials, which was then shaken. 1µl from this solution was put into the other vial containing only LB broth. The dilution in two vials contained respectively 1×10^{-3} and 1×10^{-6} bacteria. One drop from each vial was put to a plate of sterile LB agar Miller (VWR International, Radnor, Pennsylvania, USA) and put into the incubator at 37° Celsius for 48 hours before checking for bacterial growth. The bacterial

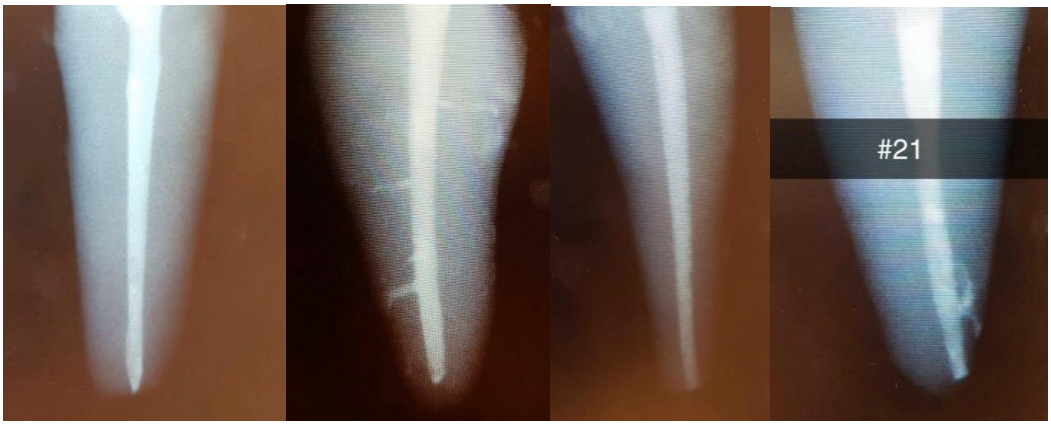
growth on the agar plate was compared to samples of *E. faecalis* and for purity (no presence of other cultures) (Picture 4).

After the experiment had ended, 15 µl from the upper chamber in specimens showing no leakage was placed on LB agar plates and kept in an incubator at 37° Celsius to ensure bacterial growth and to check for environmental contamination.

3. Results



Picture 4: Radiograph after obturation of teeth in the **Gc-group** showing dense root canal filling, ending at the radiographic apex of the root.



Picture 5: Radiograph after obturation of teeth in the **Lc-group** showing dense root canal filling, ending at the radiographic apex of the root.

Five of the sixteen samples were contaminated during the 42 days of the experiment. Two samples became infected in the Gc-group on days 14 and 34, but none in the Lc-group. All

but one sample in the Pc-group showed leakage on days 11 and 12 (Table 1). In this Pc sample (Pc4) environmental contamination was found in the upper chamber. The Nc-group did not show contamination during the experiment.

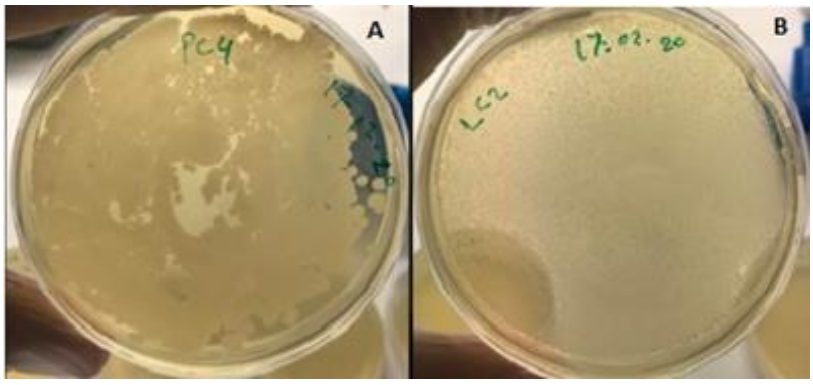
Positive *E. faecalis* growth on agar plates was found in the two Gc samples and three Pc samples that showed bacterial contamination in the lower chamber, no environmental contamination could be detected.

After the experiment was concluded, all chambers that showed no leakage were tested for bacterial viability in the upper chamber. Viability was found in all specimens.

Group/number	contamination	Day of contamination	Agar-result*	Purity of lower chamber**	environmental contamination upper chamber
PC1	+	11	positive	+	
PC2	+	12	positive	+	
PC3	+	12	positive	+	
PC4	-				yes
NG1	-				no
NG2	-				no
NG3	-				no
NG4	-				no
LC1	-				no
LC2	-				no
LC3	-				no
LC4	-				no
GC1	+	14	positive	+	
GC2	-				no
GC3	+	34	positive	+	
GC4	-				no

*bacteral growth
***E. faecalis only bacteria found*

table 1: results of bacterial leakage study.



Picture 6: Environmental contamination of upper chamber (A), *E. faecalis* in the upper chamber, no environmental contamination detected (B).

4. Discussion

The experiment lasted 42 days. Because of time and resource constraints we had to stop the experiment. This does not appear to affect our results and is the same as in several other previous bacterial leakage studies, where the studies were reported to last 42 days or fewer (14).

Our results show that the Gc-group had twice as many leaks as the control Lc-group, and leakage occurred on days 14 and 34. Time constraints appear to be an important factor in considering differences in bacterial penetration, and it has been shown that a significant difference occurs at 30 days, but not at 60 days (15).

In this experimental study, we used the maxillary central anterior teeth, and this is another important factor; because the anatomy is very different in certain groups of teeth and can affect the quality of root preparation and obturation. It has been suggested that the roots and teeth used in bacterial leakage tests may impact the results (22). Radiographic findings showed dense fillings in both experimental groups as well as filling of lateral canals along the main canal (Picture 4 &5). This indicates that both techniques have an ability to fill lateral canals in upper central incisors. It has been claimed that core-carrier obturation techniques will fill lateral canals and irregularities better and produce a more homogenous filling than cold lateral condensation (2, 23). A study comparing the percentage of GP filled area (PGFA) between Thermafil, System B and lateral condensation found that Thermafil showed significantly higher PGFA than the other two groups (23). Conversely, the X-rays do not distinguish between GP and sealer, so whether lateral canals are filled with sealer or GP is unknown.

In our study, none of the 4 Lc samples leaked. Gilbert et. al (2001) (24) found that single rooted teeth exposed to bacteria and obturated by lateral compaction leaked more rapidly than teeth obturated with Thermafil. The limitation of their study was a lack of report on which single-rooted teeth they used. The anatomy of the single-rooted teeth in anterior and lower incisors, and canines and the premolars differ significantly (25). The investigators suggested that searing off the ends of the GP cones after lateral condensation without vertical compaction might lead to a non-homogenous mass that creates voids between the GP cones.

In this experimental study, we performed vertical compaction after removing excess GP from the cavity. This could have resulted in a more homogeneous GP mass, resulting in less bacterial penetration.

The positive control group in this pilot study showed leakage at a median of 12 days, and one specimen showed no leakage during the test period. There was found environmental contamination of the upper chamber in this specimen. In order to ensure viability of *E. faecalis*, replenishing of lb-broth and bacteria was essential. To accomplish this, the lid of the Eppendorf had to be opened, exposing the bacteria and cervical part of the tooth in the upper chamber. Even though the procedure was conducted carefully in a sterile cabinet, contamination in the upper chamber of the Pc4 specimen was found. However, as only one of the specimens showed any contamination of the upper chamber, it is not regarded by the operators as an essential flaw of the two-chamber system.

The positive control teeth were set up to simulate a poor root filling with only one GP cone and no sealer. The most common set up for positive controls in other studies have been to leave the canals completely open and bacterial leakage is commonly found within a few days (15-17, 24, 26). Torabinejad et al. (1990) (27) also prepared a positive control to model poor root filling. Though the positive controls in their study showed leakage after 1-4 days, one of the specimens did not show sign of contamination after 90 days. This indicate that positive controls simulating a poor root fillings may withstand bacterial leakage for a long time, and teeth in Pc-group should be left without obturation material to accurately determine positive bacterial leakage.

The negative controls in this study showed no signs of leakage throughout the study period. Viability of *E. faecalis* was controlled and no environmental contamination was found in the upper chamber. This suggests that the two-chamber set up was not penetrated by environmental bacteria, also shown in the material test study. All the teeth in the negative control group were sealed at the apical 2mm to control that bacterial penetration in the experimental groups happened through the apex.

The bacteria *E. faecalis* was chosen for this study because of its relation to failed root fillings and ability to survive in dentine tubules even after instrumentation, intracanal medication and obturation (28). *E. faecalis* has commonly been used in bacterial leakage studies, but other

species such as *Staphylococcus epidermis, Proteus mirabilis, Streptococcus mutans, Streptococcus mitis* and *Fusobacterium nucleatum* have also been used (11, 14). The use of so many different strains of bacteria in different bacterial leakage studies may be causative for the discrepancies in results, as the method depends on the bacteria (11). Both Confirming and contradicting results were found both when compared to studies using *E. faecalis, S. epidermis* and *Proteus vulgaris* (15, 17, 24, 26), and so the discrepancies in results between this pilot study and other studies cannot be based entirely on the bacterial strains used.

Detection of bacterial leakage was in this study performed by visual inspection of disturbance in the LB-broth of the lower chamber. Though the changes were both controlled by the operators and the laboratory assistant, the change in the clear liquid might be hard to see and could, if in small quantities or if bacterial contamination had just begun, be missed. Bacterial contamination of the lower chamber in specimens where no visual disturbance could be detected was not controlled for. During the experiment, focus was to detect viability of the bacteria in the upper chamber rather than confirming or disproving trace of bacteria in the lower chambers without visual confirmation of turbidity. It can therefore not be excluded that bacterial penetration might have occurred also in these specimens. This is regarded as a weakness of this pilot study, and further investigations should aim to detect both viable bacteria in upper and lower chamber of all specimens. Even so, the evidence of more rapid bacterial penetration of the Gc group remains, as Gc1 specimen showed clear disturbance of the lower chamber broth after only 14 days compared to no visual disturbance of Lc specimens after 42 days.

Bacterial contamination found in the lower chambers were confirmed by growth on LB-agar in 1×10^{-3} and 1×10^{-6} dissolutions and compared to known growth pattern and visual hallmarks of *E. faecalis.* No presence of other bacterial cultures (environmental contamination) from the lower chambers of these specimens was found. The bacterial contamination of the positive control groups was initially tried confirmed by Polymer Chain Reaction (PCR), but no contamination could be detected. The reason for this is unknown. It was decided to control the results on LB-agar plates, where bacterial growth was confirmed. The growth and hallmark pattern found in the agar plates were deemed acceptable evidence of viability and presence of *E. faecalis* without contamination of other bacterial species. Several studies comparing bacterial leakage have used agar plating to verify the purity of bacteria

either in the lower or the upper chamber (15, 16, 26, 29, 30), and so the decision of using agar instead of PCR is not considered to be a limitation in this pilot study.

No histological or microscopically examination was conducted to determine bacterial penetration of the root canal in this pilot study. Brosco et al (2010) (31) demonstrated that even with no bacterial leakage shown by the two-chamber bacterial leakage test, microscopic examination shows bacterial infiltration of dentine tubules in the root canal system(s). Results from this pilot study cannot conclude that no bacterial leakage occurred in groups with no visual turbidity in the lower chamber. Even if bacterial penetration might occur in other parts of the root, results indicate that bacterial leakage penetrated further and faster when teeth are obturated by GuttaCore compared to lateral condensation. However, in order to accurately determine bacterial leakage, bacterial penetration into all parts of the root must be investigated (19). Therefore, we consider it a weakness that it was not assessed in this pilot study.

The most common sealing materials used for the two-chamber system in bacterial leakage studies are cyanoacrylate cement, Sticky wax or a combination of the two (14). Sealing is important to ensure that the contamination of the lower chamber results from leakage through an obturated root canal and not from environmental source. To select a suitable material, the sealability of adhesive wax, SDR composite material and cyanoacrylic cement material were compared in a pre-pilot experiment. The experiment showed that cyanoacrylate cement gave no environmental contamination after one week, and Sticky Wax showed environmental contamination of the lower chamber after only one day. The bacteria found in the lower chamber of specimens with visual bacterial leakage was tested and shown to be pure (no environmental contamination). Results from the pre-pilot study indicate that materials used for sealing between chambers and teeth are important to avoid environmental contamination. These results and no contamination of the negative control groups indicate that sealing materials used in this pilot study were suitable to prevent environmental contamination.

Instrumentation and obturation of all teeth in experimental and control groups were divided equally between the two operators in this pilot study. Obturation was performed together, at the same time, and quality controlled by both operators. It was not recorded which specimen in each group was obturated by which operator. Based on X-rays, clinical performance between the two operators was similar. Though possible explanations for the faster ratio of bacterial leakage when using GuttaCore have been discussed, it cannot be excluded that

clinical technique and experience are important factors in this experimental study. Both operators were better trained in Lc compared to Gc technique.

Little to no evidence is found of the bacterial penetration on GuttaCore compared to other obturation techniques. Comparing results from this study to other core-carrier obturation materials is suboptimal. Although previous versions of these materials are based on the same principles, GuttaCore is a newer and relatively untested material in vitro.
The need for further investigation and standardization of methodology has been recommended by other researchers and publications (14, 19, 20). The discrepancies in results from other studies also emphasize the need for investigation of the study design. The experiment allowed us to test the study approach with a few test samples and to test important parameters that could affect the study design. This in vitro pilot study highlights the need for time constraints, the study of bacterial leakage pathways and the importance of teeth included in the experiment. It has also shown that material for sealing between the teeth and chambers is important to exclude environmental contamination. These parameters seam important in order to investigate whether one material or obturation technique can be compared to another. It has also shown that the two-chamber system is a useful method for investigating bacterial leakage, though the method is technically sensitive. Further studies are needed on the clinical relevance of this micro-leakage test.

5.0 Conclusion

Within the limitations of this pilot study, a two-chamber system appears to be a feasible method to compare GuttaCore with other methods regarding bacterial leakage. To confirm our results, it needs future studies using a larger sample size.

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