

# **Bacterial leakage in ex vivo teeth after apicoectomy using two tricalcium silicate-based cements as root-end filling materials**

Stud. odont. Tamar A. Tinnan, Siril Nergård & Siri B. Ellingsen

**Supervisor:**

Eero Kerosuo, Professor, IKO, Faculty of Health Sciences, University of Tromsø, Norway

**Co-supervisors:**

Laura Andriukaitiene, PhD candidate, IKO, Faculty of Health Sciences, University of Tromsø, Norway

Xiaobo Song, Associate professor, IMB, Faculty of Health Sciences, University of Tromsø, Norway

Masters thesis in Odontology, June 2014



### Acknowledgements

We would like to thank our supervisor Eero Kerosuo for providing the subject for our Masters Thesis, and for always being available when we needed him. We would also like to thank co-supervisors Laura Andriukaitiene and Xiaobo Song for the help with the practical part of the study. A great thanks to the people who helped us collect teeth, the project would not have been possible without you.

## Abstract

### **Background**

The main cause of pulpal and periradicular pathosis are microorganisms and their by-products in the root canal system. These have to be eliminated to promote healing. Because it's impossible to achieve a bacteria-free root canal an additional goal of endodontic therapy is to seal the root canal system from the outside environment. Today the standard method for filling the root canals are a combination of a core material and a root canal sealer, usually gutta-percha and epoxy resin sealer. Sometimes periapical healing is not achieved even after conventional root canal treatment, if retreatment is not an option or failed, surgical endodontic treatment is indicated. Through retrograde approach the surgery comprises elimination of pathological tissue, root resection, preparation of root-end cavity and placement of a root-end filling material. For many years amalgam was considered to be a suitable material for root-end fillings, but today MTA (Pro Root MTA, Dentsply Tulsa, Dental, Tulsa, OK, USA) is the gold standard, and has been associated with high success rates.

Recently a new dentine replacement material Biodentine (Septodont, Saint-Maur des Fossés, France) has been introduced on the market. Biodentine (BD) has shown to have similar biocompatibility and bioactivity as MTA, and because BD has shorter setting time than MTA, it should be considered an interesting alternative.

The only leakage tests done on BD are glucose leakage and dye leakage, which have shown promising results for BD, but no research has been published on the bacterial leakage of BD when used as a retrograde filling.

### **Aim**

The aim of this study was to compare the bacterial leakage in single canal roots when either BD or MTA was used as a root-end fillings material.

### **Results**

There were pre-test failures in BD, MTA, AHPlus and positive control groups, reducing the number of samples. The positive control leaked in 2 days and there was no leakage in either of the negative control samples. The proportion of leaking samples (in 60 days) were 6/6 and 4/5 for BD and MTA, respectively. The mean number of days (+/- SD) for the resistance of the leakage were 4.3 (+/-1.9) and 14.0 (+/- 22,0) for BD and MTA, respectively. The mean

OD600 values were 1.9 and 1.2, for BD and MTA, respectively. None of these differences between the BD and MTA groups were statistically significant.

### **Conclusion**

Due to the weak power of the present study, it could not show any statistically significant difference between the tested materials, but there was a clear trend showing better sealing ability for MTA compared to BD. As long as there are no studies to indicate at least as good in vitro sealability for BD as shown previously for MTA, the clinical use of BD as a root-end filling material is not warranted.

# **Bacterial leakage in ex vivo teeth after apicoectomy using two tricalcium silicate-based cements as root-end filling materials**

## Introduction

The main cause of pulpal and periradicular pathosis are microorganisms and their by-products in the root canal system (Takehashi et al 1965). Microorganisms must be eliminated from infected root canal to promote healing. Several studies have shown that it is impossible to achieve a bacteria-free root canal space in all cases, even after thorough cleaning, shaping and irrigation with disinfectants and antiseptics (Ørstavik et al 1991, Peters et al 2002). Therefore, an additional goal of endodontic therapy is to seal the root canal system from the outside environment with an obturation material and entomb any residual microorganisms, preventing periradicular tissues from the ingress of bacteria or their by-products (Sundquist & Figdor 1998).

Standard methods for filling the root canal system are combinations of core material, which usually is gutta-percha (GP), and root canal sealer. The sealer will fill voids and minor discrepancies between the GP and the root canal wall. The epoxy-based sealer is one of the most used sealers and has been used for more than 40 years. Epoxy resin sealers have good mechanical and sealing properties, no expected effect on general health, allergic reaction are rare and the antimicrobial properties are good (Schmalz & Hørsted-Bindslev 2010). Coronal leakage is a clinical phenomenon implicated in all steps of endodontic therapy and it may lead to treatment failure. To avoid treatment failure it is important that the endodontic filling materials have the qualities needed to prevent leakage. To assess and compare the materials, one can do various tests to investigate for leakage and most of them are performed in vitro and include dye penetration, fluid perfusion test and bacterial leakage model (Wu & Wesselink, 2003).

Surgical endodontic treatment is indicated as a complementary procedure when the conventional endodontic treatment failed or when retreatment with orthograde approaches are technically difficult or impractical. Through retrograde approach the surgery comprises elimination of pathological tissue, root resection, preparation of root-end cavity and placement of a root-end filling material (Velvart 2010).

The apicoectomy is performed by raising a flap, removing bone and curettage of the soft-tissue lesion to get surgical access to the apical part of the tooth. The 2-3 mm (Gutman & Harrison, 1991) tip of the root has to be cut to get a convenient access to the root canal for the apical instruments and to remove bacterial organisms in the accessory canal and delta. The cut must be done with an angle of 0-10 degrees (Kim & Kratchman, 2006), to reduce the number of exposed dentinal tubules, since the tubules can serve as pathway for bacteria. The root-end cavity is then sealed to prevent leakage of tissue fluid to the root canal space and an eventual bacterial leakage from the root canal to periapical tissue. The best outcome of apicoectomy is achieved by apical cavity preparation with a root-end filling. Studies has shown that teeth treated with a root-end filling materials had significantly better healing (96%) than teeth treated only with smoothing of the orthograde GP root filling (52%) (Christiansen et al, 2009). In a study done by Molven et al (1996) it was shown that 92% of the teeth with periapical surgery without a root-end filling, had incomplete healing.

The materials that have been used as retrograde fillings are amalgam, Intermediate Restorative Material (IRM), Super Ethoxy-Benzoic Acid (Super-EBA) and Mineral Trioxide Aggregate (MTA). For many years amalgam was considered to be a suitable material for root-end fillings, but Dorn & Gartner (1990) showed that Super EBA and IRM had a higher success rate than amalgam, and they are more tissue-tolerant (Song & Kim, 2012). Today MTA is considered to be the gold standard for root-end fillings and it has been associated with high success rates in clinical trials (Torabinejad et al 1995, Fischer et al 1998, Chong et al 2003)

Recently, a new dentine replacement material Biodentine (BD) has been introduced. It consists of a powder component and a liquid component. The powder component mainly consists of tricalcium silicate, with the addition of  $\text{CaCO}_3$  and  $\text{ZrO}_2$ . The liquid component has calcium chloride ( $\text{CaCl}_2$ ), as setting accelerator, in a water reducing agent (Laurent et al 2008).

The producer claims that the product can be used as a dentine replacer both in the crown and in the root. This includes temporary enamel restorative material, permanent dentine restorative material, restoration of deep and/or large coronal carious lesions (sandwich

technique) and deep cervical and/or radicular lesions, pulp capping and pulpotomy, in addition to repair of root perforations, furcation perforations, perforating internal and external resorption, apexification and root-end filling in endodontic surgery (retrograde filling) (Septodont, data on file 2014)

It has been shown that BD has similar biocompatibility and bioactivity as MTA (Laurent et al 2008). Because of its bioactivity BD is considered as a suitable material for direct pulp capping. In pulp cells from mice BD caused differentiation into odontoblast-like cells and stimulated biomineralization (Zanini et al 2012). Human dental pulp capped with BD or MTA, both showed complete dentinal bridge formation and absence of inflammatory pulp responses in the majority of the specimens (Laurent et al 2011, Tran et al 2012, Nowicka et al 2013). Studies done on the effects of BD on dentine surface show formation of tag-like structures just beneath the interface (Han & Okiji, 2011, Atmeh et al, 2012). Because BD also has a much shorter setting time than MTA, it should be considered an interesting alternative for MTA in both pulp capping and as a root-end material after apicoectomy.

When MTA and BD are exposed to endodontic irrigants like NaOCl, chlorhexidine and saline, they lose much of the push-out bond strength (Guneser et al 2013). The same phenomenon has been shown after the removal of the smear layer (El-Ma'aita et al 2013), which will negatively influence BD's use as a root filling material. BD has shown a high wash-out effect and low durability when tested as a posterior restoration, and consequently it is recommended to use in sandwich-restorations only (Koubi et al 2012, Grech et al 2012).

The root-end filling materials have been ranked according to their ability to resist bacterial leakage as follows: MTA > IRM > amalgam (Fischer et al, 1998). The inferiority of amalgam in this respect is clearly reflecting also the clinical outcome, as MTA and IRM have been shown to yield clearly the best clinical success, speaking for the importance of the sealing ability of the root-end filling material (Chong et al, 2009).

The in vitro micro leakage of BD has been shown to be less than that of zinc oxide eugenol by glucose leaking method (Wang et al, 2012). In cervical cavities Raskin et al (2012) found less dye leakage with bonded BD, compared to a light cured glass ionomer (GI). With the glucose

leakage method Koubi et al (2012) could not find any significant difference in leakage between BD and a light cured GI.

No research has been published on the bacterial leakage of BD when used as a retrograde filling. The aim of this study was to compare the bacterial leakage in single canal roots when either BD or MTA was used as a root-end filling material.

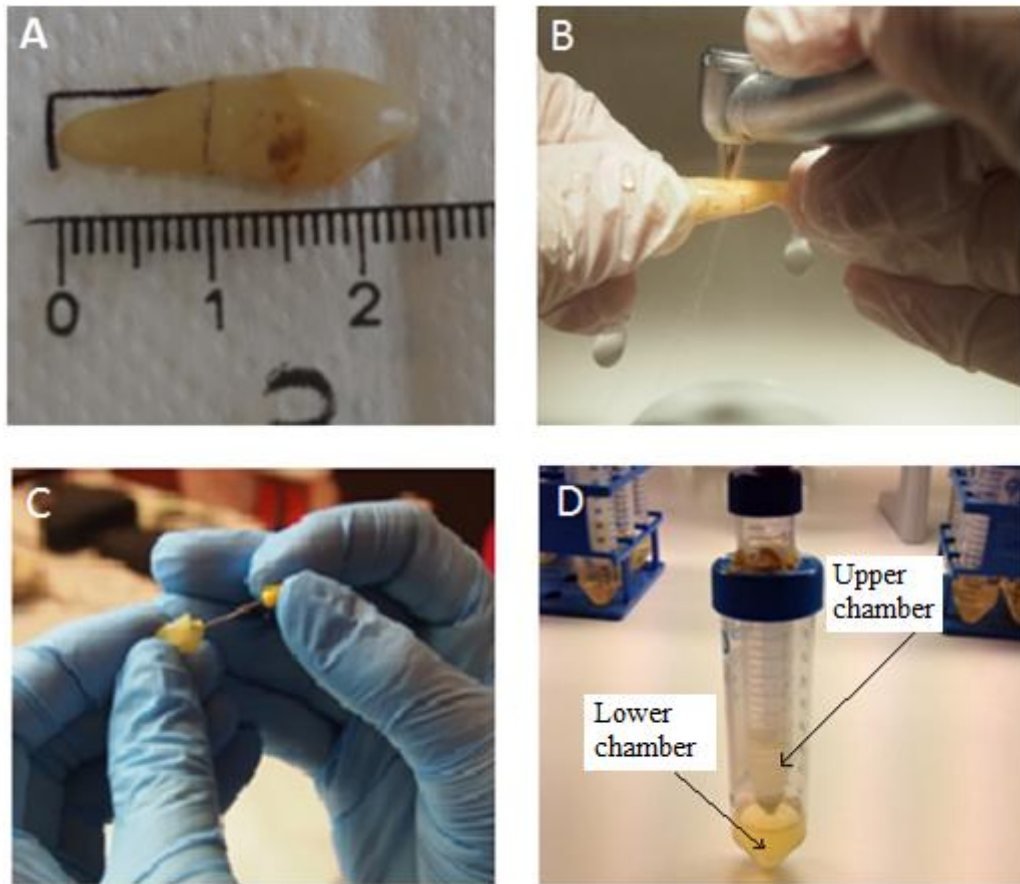
### Materials and methods

A total of 30 single-rooted human teeth, extracted for reasons not linked to this study, were selected and stored in 1% ethanol. Calculus, soft- and hard tissue remaining on them was removed with periodontal scaling instruments. The crowns were removed under water cooling using a diamond burr with a diameter of 0,8 mm (ISO 310204107002008, Komet, Rock Hill, USA) mounted on a turbine, leaving the root 10 mm in length (Fig. 1A-B).

All roots were inspected with a dental operating microscope (G6, Global, St. Louis, MO, USA) under x12.8 magnification for the presence of cracks, two or more canals, accessory canal openings coronal to the apical 2 mm, caries or other damages on the root, in which case they were discarded.

The working length was determined by introducing a small K-file through the apex, and withdrawing it 1mm. The ProTaper NiTi rotary files (Dentsply/Maillefer, Ballaigues, Switzerland) and stainless steel K-files (Dentsply/Maillefer) were used for the preparation of the canals up to size # 60 (Fig. 1C). Sodium hypochlorite (0.5% NaOCl) was used for the irrigation of the root canal after the use of each file. The roots were rinsed with 3 ml of 17% EDTA for five minutes to remove the smear layer (Saleh et al 2004). The roots were then again stored in 1% ethanol until they were autoclaved.





**Figure 1.** Laboratory procedures. A) Measuring of the apical 10 mm. B) Cutting of the apical 10 mm. C) Preparation of the canal. D) Two-chamber system.

Six roots were randomly selected to receive a standard endodontic obturation with GP using lateral condensation technique. GP cones (#60) and spreader size A, and AH Plus sealer (Dentsply/Maillefer) were used. These roots were kept for 24 hours in sterile saline at 37° C to allow the sealer to set.

In order to get a maximum bacterial challenge, but yet a physical barrier to the root-end filling, 24 roots were filled with GP cone #60 using single-cone technique, but without any sealer.

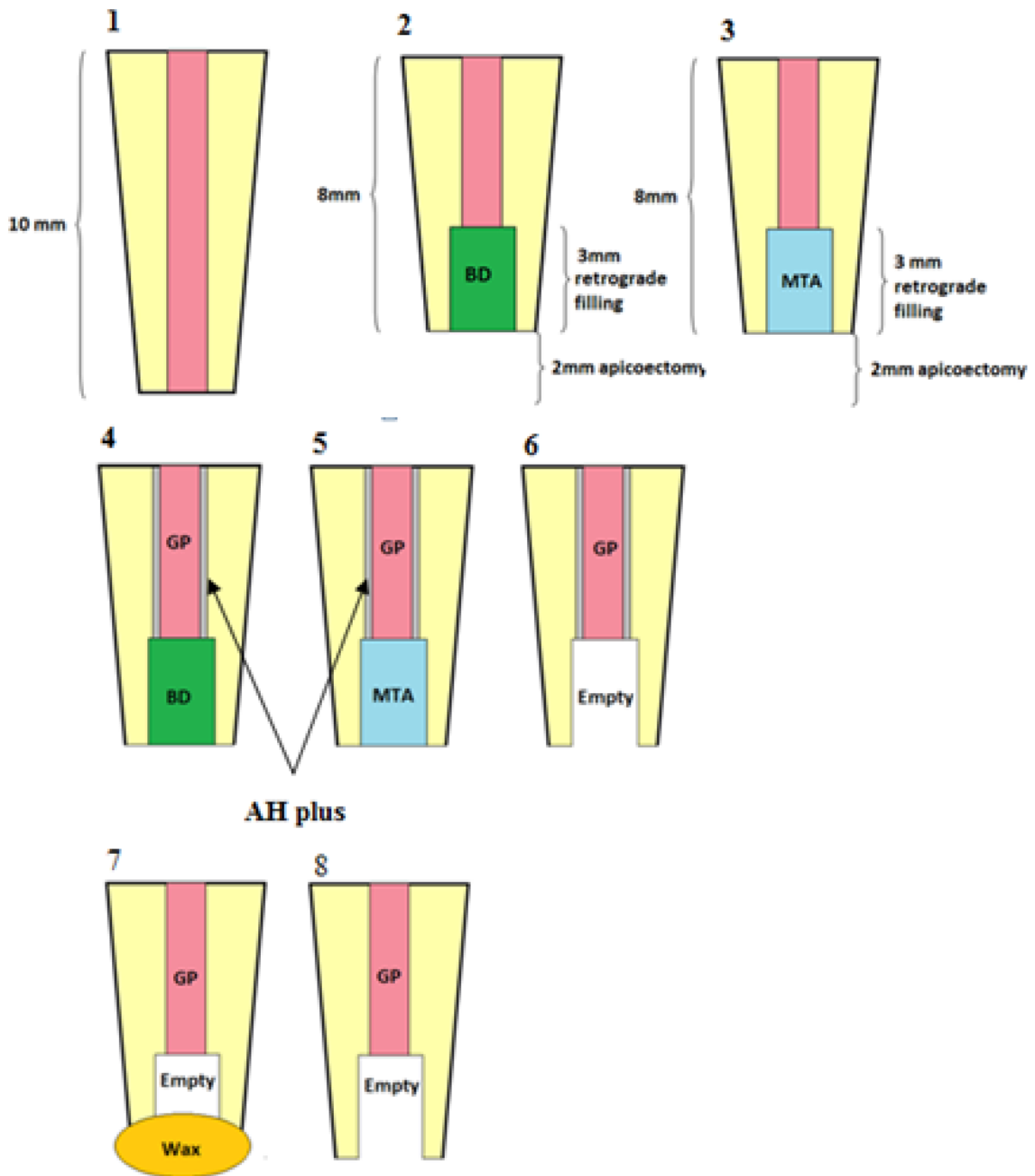
The apicoectomy was simulated by cutting the apical 2 mm of the roots (Gutmann & Harrison 1991) with a diamond fissure burr size 1.0 mm (Komet) using sterile saline for cooling. The root-end cavities were prepared to a depth of 3 mm with a tungsten carbide fissure burr with a diameter of 0.8 mm (Komet), parallel to the canal, leaving a 3 mm deep root-end cavity free of GP. Sterile saline in a syringe was used as cooling.

The 24 roots filled with the single-cone technique were randomly sub-grouped as follows: 10 roots in BD (Septodont, Saint-Maur des Fossés, France) group, 10 roots in MTA (Pro Root MTA, Dentsply Tulsa, Dental, Tulsa, OK, USA) group, 2 roots in a positive and 2 roots negative control group.

In the BD and MTA groups the root-end cavities were filled with their respective filling material using endodontic pluggers, fitting into the root-end cavity. The MTA was mixed according to the manufacturers manual. The BD was also mixed according to manufacturers instructions but the consistency of the material was too dry to work with, hence-it was added a few more drops of the liquid until the appropriate consistency was achieved.

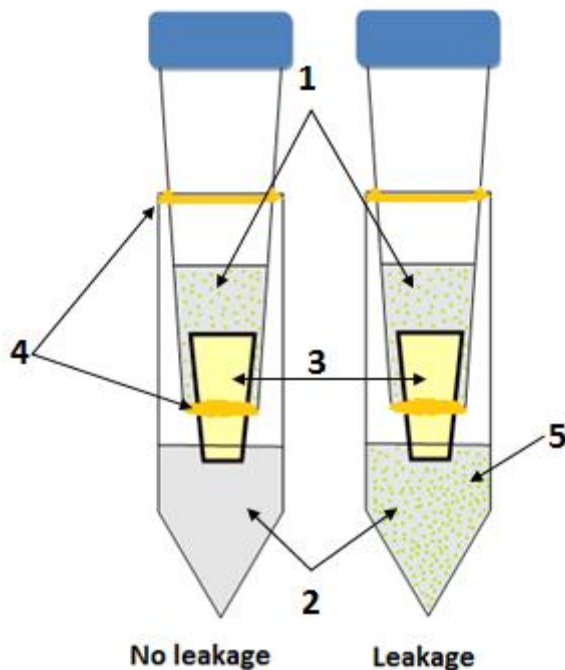
The six roots filled with lateral condensation, were randomly assigned to three groups consisting of two roots each: one was filled with MTA, the second with BD and in the third group the root-end cavities were left empty (Fig. 2).

Aseptic techniques were maintained throughout the entire procedure. All the specimens were kept in sealed tubes with sterile saline for 48 hours at 37°C to allow the root-end filling to set.



**Figure 2.** Schematic drawing of the groups in the experiment. 1) Root + GP, before apicoectomy. 2) BD group: GP + BD (N= 10). 3) MTA group: GP + MTA (N=10). 4) Control group: GP+ BD+ AH plus (N=2). 5) Control group: GP+ MTA+ AH plus (N=2). 6) Control group: GP+ AH plus (N=2). 7) Negative control (N=2). 8) Positive control (N=2)

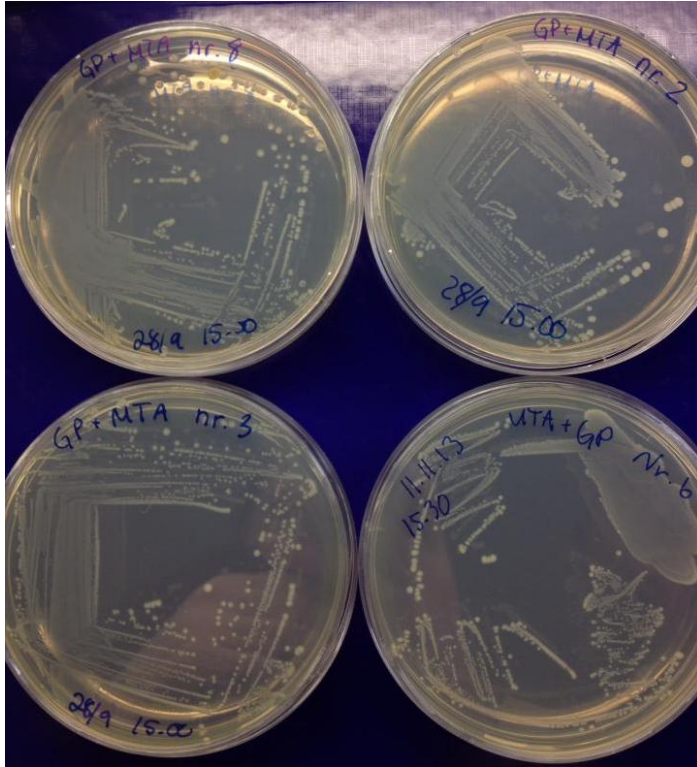
The two-chamber method described by Torabinejad et al (1990) and modified by Saleh et al 2008 was used. The tip of the 15 ml polyethylene tubes were cut off to accommodate the coronal ends of the specimens. The specimens were attached with sticky wax (Kerr, SpofaDental a.s.) to the tubes that served as a bacterial reservoir, leaving 2-3mm of the coronal part of the root uncovered. The mounts were tightly sealed with sticky wax to sterile 40 ml polyethylene tubes containing 8 ml of sterile trypticase soy broth (TSB; Oxoid Ltd, Basingstoke, UK). The apices extruding from the polyethylene tubes were soaked vertically 2 mm into the broth (Fig. 1D and Fig.3). In the negative control group the roots were completely covered with wax and in the positive control group the root-end cavities were left empty (Fig. 2).



**Figure 3.** Schematic drawing of the 2-chamber method used. 1) Upper chamber with trypticase soy broth containing *E. faecalis*. 2) Lower chamber. 3) Root. 4) Wax. 5) Turbidity

Three ml of 72 hours culture of American Type Culture Collection of *Enterococcus faecalis* (ATCC 29212) strain in TSB was added to each upper chamber. Each mount was kept at 37°C throughout 60 days or until the last leakage occurred. The bacterial suspension in the upper chamber was replaced with 3 ml fresh culture every 4<sup>th</sup> day. To discover a bacterial leakage the bottom chambers were inspected every second day for turbidity. On observation of turbidity, the day of leakage was recorded for each leaking sample and the number of leaking samples was recorded per group. Thereafter the cap of the lower chamber was removed and 1

ml of the suspension from the this chamber was used for optical density ( $OD_{600}$ ) determination (Ultraspec 2000, Pharmacia Biotech Ltd, Cambridge, UK), followed by colony identification plating. The plates were incubated at 37°C for 24 hours, and thereafter inspected for uniform appearance of the colonies to rule out any contamination (Fig. 4).



**Figure 4.** Uniform bacterial colonies

Student's t-test was used to analyse the difference in OD-values and the statistical difference in mean time to resist the leakage (survival) between the two groups. A p-value of  $<0.05$  was to be considered significant.



## Results

**Table 1.** Proportion of the samples that leaked during 60 days, and the mean OD-values of the lower chambers of the leaked samples by each group.

Group	Original No. of samples	No. of samples excluded due to pre-test failure	Final No. of samples to follow up	Proportion of the samples leaked in 60 d	Mean OD-values of the leaked samples	Mean days of survival before leakage (SD)
BD	10	4	6	6/6	1,949	4,33 (2,07)
MTA	10	5	5	4/5	1,244	14,0 (22,0)
AH Plus/BD	2	1	1	1/1	2,336	2
AH Plus/MTA	2	1	1	1/1	1,273	2
AH Plus only	2	1	1	1/1	2,287	2
Neg. ctr.	2	0	2	0/2	-	-
Pos.ctr	2	1	1	1/1	-	2

Statistical evaluation with Student's t-test

Mean survival time (days): MTA vs. BD,  $p > 0.05$

Mean OD<sub>600</sub> value: MTA vs. BD,  $p > 0.05$

Already after a few hours some of the samples showed leakage of the entire upper chamber content to the lower chamber. As this happened in all groups, these empty upper chambers were regarded as pretest failures and were excluded from the study. See details in Table 1.

All the samples that leaked showed a turbid lower chamber that was confirmed by OD-value measurements, ranging from 0,292 A to 2,385 A. There was no significant difference between OD-values from the BD group and the MTA-group (Table 1).

The MTA group resisted the bacterial leakage longer than the BD group. The last two BD filled roots leaked on the seventh day, giving a mean value of 4,3 days. The second last last MTA root leaked on the 47th, giving a mean leakage value of 14 days. The statistical difference between these two groups was not significant (Table 1).

All samples of bacterial identification plating showed a uniform colony characteristic to *E. faecalis* (Fig. 4).

The negative control group did not leak at all. The positive control group which was expected to leak due to no retrograde filling suffered one pretest failure. The remaining sample showed a leakage after 3 days. The three different groups in the AH+ control group lost one sample each to pretest failures, leaving only one sample in each group.

### Discussion

The clinical relevance of leakage tests done in vitro has been criticized (Wu 1995, AliGhamdi & Wenneberg, 1994). Dye leakage studies can give a false positive reading if their molecules are small enough, and it has been shown that air bubbles may prevent dye penetration (Goldman et al 1980). A bacterial leakage model was developed to overcome the limitations that dye leakage studies have. Bacteria perform better than dye in testing for leakage of hydrophilic materials, but it is not known if the result of bacterial leakage test in vitro may be transferred to in vivo conditions because the state of bacteria, lipopolysaccharides and immunological factors in an inflammatory process can't be reproduced in in vitro studies (Torabinejad et al, 1990, Barthel et al, 1999). The bacterial species *E. faecalis* was chosen for this study because they are a part of the normal flora in humans, and it has been reported that they are one of the major pathogens of post-treatment apical periodontitis, with a reported prevalence of 29-77% (Sundquist & Figdor, 2003).

There may be many different reasons for why the samples leaked so quickly, many of them pointing to methodological errors. The teeth used in this experiment had been stored in 70% ethanol for several months. This may have influenced the structure of the teeth thereby affecting the end-result of the experiment in unknown ways.

When cutting the apices and preparing the retrograde cavity, the cones loosened quite easily from the root canal. The cause was most probably the lack of coronal restoration holding the GP-cone in place. This may have influenced the condensation of the BD and MTA against the GP when doing the retrograde filling. The same problem occurred in the AH Plus-control group, even though these roots were obturated with sealer, accessory cones and lateral condensation.

According to the instructions from the manufacturer, one capsule with BD-powder is supposed to be mixed with 5 drops of liquid for 30 seconds in a capsule mixer. When following these instructions the BD got a dry, sand-like consistency which was difficult to use. Hence more liquid was added to get the wanted consistency, mixed by hand. This may also be a source of methodological error, as the consistency is difficult to standardize.

The manufacturer indicates that during the initial setting time of 10 minutes, BD should not be in contact with water or liquid. The roots treated with BD were put directly in saline water to avoid the roots from drying. This also simulates a clinical situation where the wound is closed as soon as possible, exposing the material to body fluids. This might have affected the setting reaction of BD.

There were three different operators, therefore individual variations in the quality of the retrograde filling could have influenced the results. The compaction of the MTA and BD may have been different, not only between roots but between operators as well.

A possible methodological error explaining the pretest failures could be that the tubes in the two chamber system were cut inappropriately, resulting in a distance too large between the upper chamber and the root for the wax to cover. The handling of the wax may also not have been satisfactory. There might have been too little wax between the upper chamber and the root, or the wax was too fixed when applied to the gaps. There were three different operators setting up the two-chamber systems, this may have lead to different quality of the systems.

The main result of this study was not statistically significant, though MTA showed longer survival time (14.0d) against the bacterial leakage, compared to BD (4.3 d). The lack of statistical significance is explained by small amount of samples, which became even smaller due to pretest failures.

In conclusion, due to the weak power of the present study, it could not show any statistically significant difference between the tested materials, but there was a clear trend showing better sealing ability for MTA compared to BD. As long as there are no studies to indicate at least as good in vitro sealability for BD as shown previously for MTA, the clinical use of BD as a root-end filling material is not warranted.

## References

AliGhamdi A, Wennberg A. Testing of sealing ability of endodontic filling materials. *Endod Dent Traumatol.* 1994 Dec;10(6):249-55.

Atmeh AR, Chong EZ, Richard G, Festy F, Watson TF. Dentin-cement interfacial interaction: calcium silicates and polyalkenoates. *J Dent Res.* 2012 May;91(5):454-9. doi: 10.1177/0022034512443068. Epub 2012 Mar 20.

Barthel CR, Moshonov J, Shuping G, Orstavik D. Bacterial leakage versus dye leakage in obturated root canals. *Int Endod J.* 1999 Sep;32(5):370-5.

Chong BS, Pitt Ford TR, Hudson MB. A prospective clinical study of Mineral Trioxide Aggregate and IRM when used as root-end filling materials in endodontic surgery. 2003. *Int Endod J.* 2009 May

Christiansen R, Kirkevang LL, Hørsted-Bindslev P, Wenzel A. Randomized clinical trial of root-end resection followed by root-end filling with mineral trioxide aggregate or smoothing of the orthograde gutta-percha root filling--1-year follow-up. *Int Endod J.* 2009 Feb; 42(2):105-14. doi: 10.1111/j.1365-2591.2008.01474.x.

Dorn SO, Gartner AH. Retrograde filling materials: a retrospective success-failure study of amalgam, EBA, and IRM. *J Endod.* 1990 Aug;16(8):391-3.

El-Ma'aita AM, Qualtrough AJ, Watts DC. The effect of smear layer on the push-out bond strength of root canal calcium silicate cements. *Dent Mater.* 2013 Jul;29(7):797-803. doi: 10.1016/j.dental.2013.04.020. Epub 2013 May 22.

Fischer EJ, Arens DE, Miller CH. Bacterial leakage of mineral trioxide aggregate as compared with zinc-free amalgam, intermediate restorative material, and Super-EBA as a root-end filling material. *J Endod.* 1998 Mar;24(3):176-9.

Goldman LB, Goldman K, Kronman JH, Letourneau JM. Adaptation and porosity of poly-HEMA in a model system using two microorganisms. *J Endodon* 1980;66:863-6.

Grech L, Mallia B, Camilleri J. Investigation of the physical properties of tricalcium silicate cement-based root-end filling materials. *Dent Mater*. 2013 Feb;29(2):e20-8. doi: 10.1016/j.dental.2012.11.007.

Guneser MB, Akbulut MB, Eldeniz AU. Effect of various endodontic irrigants on the push-out bond strength of biodentine and conventional root perforation repair materials. *J Endod*. 2013 Mar;39(3):380-4. doi: 10.1016/j.joen.2012.11.033. Epub 2013 Jan 16.

Gutmann JL, Harrison JW. *Surgical endodontics*. Boston: Blackwell Scientific Publications, 1991; 213-14

Han L, Okiji T. Uptake of calcium and silicon released from calcium silicate-based endodontic materials into root canal dentine. *Int Endod J*. 2011 Dec;44(12):1081-7. doi: 10.1111/j.1365-2591.2011.01924.x. Epub 2011 Jul 21.

Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. *Oral surg oral med oral pathol*. 1965 sep;20:340-9.

Kim S, Kratchman S. Modern endodontic surgery concepts and practice: a review. *J Endod*. 2006 Jul;32(7):601-23. Epub 2006 May 6.

Koubi S, Elmerini H, Koubi G, Tassery H, Camps J. Quantitative evaluation by glucose diffusion of microleakage in aged calcium silicate-based open-sandwich restorations. *Int J Dent*. 2012;2012:105863. doi: 10.1155/2012/105863. Epub 2011 Dec 12.

Koubi G, Colon P, Franquin JC, Hartmann A, Richard G, Faure MO, Lambert G. Clinical evaluation of the performance and safety of a new dentine substitute, Biodentine, in the restoration of posterior teeth - a prospective study. *Clin Oral Investig*. 2013 Jan;17(1):243-9. doi: 10.1007/s00784-012-0701-9. Epub 2012 Mar 14.

Laurent P, Camps J, De Méo M, Déjou J, About I. Induction of specific cell responses to a Ca(3)SiO(5)-based posterior restorative material. *Dent Mater*. 2008 Nov;24(11):1486-94



Laurent P, Camps J, About I. Biodentine(TM) induces TGF- $\beta$ 1 release from human pulp cells and early dental pulp mineralization. *Int Endod J.* 2012 May;45(5):439-48. doi: 10.1111/j.1365-2591.2011.01995.x. Epub 2011 Dec 22.

Laurent P, Camps J, About I. Biodentine(TM) induces TGF- $\beta$ 1 release from human pulp cells and early dental pulp mineralization. *Int Endod J.* 2012 May;45(5):439-48

Molven O, Halse A, Grung B. Incomplete healing (scar tissue) after periapical surgery--radiographic findings 8 to 12 years after treatment. *J Endod.* 1996 May;22(5):264-8.

Moodnik RM, Levey MH, Besen MA, Borden BG. Retrograde amalgam filling: a scanning electron microscopic study.

*J Endod.* 1975 Jan;1(1):28-31.

Nowicka A, Lipski M, Parafiniuk M, Sporniak-Tutak K, Lichota D, Kosierkiewicz A, Kaczmarek W, Buczkowska-Radlińska J. Response of human dental pulp capped with biodentine and mineral trioxide aggregate. *J Endod.* 2013 Jun;39(6):743-7. doi: 10.1016/j.joen.2013.01.005. Epub 2013 Apr 10.

Orstavik D, Kerekes K, Molven O. Effects of extensive apical reaming and calcium hydroxide dressing on bacterial infection during treatment of apical periodontitis: a pilot study. *Int Endod J.* 1991 Jan;24(1):1-7.

Peters LB, van Winkelhoff AJ, Buijs JF et al Effects of instrumentation, irrigation and dressing with calcium hydroxide on infection in pulpless teeth with periapical bone lesions. *Int Endod J* 2002; 35:13-21.

Raskin A, Eschrich G, Dejoui J, About I. In vitro microleakage of Biodentine as a dentin substitute compared to Fuji II LC in cervical lining restorations. *J Adhes Dent.* 2012 Dec;14(6):535-42.

Saleh IM, Ruyter IE, Haapasalo M, Ørstavik D. Survival of *Enterococcus faecalis* in infected dentinal tubules after root canal filling with different root canal sealers in vitro. *Int Endod J.* 2004 Mar;37(3):193-8.

Saleh IM, Ruyter IE, Haapasalo M, Ørstavik D. Bacterial penetration along different root canal filling materials in the presence or absence of smear layer. *Int Endod J*. 2008 Jan;41(1):32-40. Epub 2007 Oct 3.

Schmalz and Hørsted-Bindslev, Root canal fillings materials, page 202-207 in *Textbook of Endodontology of* Bergenholtz G, Hørsted-Bindslev P, Reit C. Wiley-Blackwell 2010.

Song M, Kim E. A prospective randomized controlled study of mineral trioxide aggregate and super ethoxy-benzoic acid as root-end filling materials in endodontic microsurgery. *J Endod*. 2012 Jul;38(7):875-9. doi: 10.1016/j.joen.2012.04.008. Epub 2012 May 16.

Septodont, data on file: <http://www.septodont.co.uk/node/1005>. Accessed 27.01.2014.

Sundquist G, Figdor D. Endodontic treatment of apical periodontitis. In Orstavik D, Pitt Ford TR, eds. *Essential Endodontology*, 1<sup>st</sup> edn. Oxford, 1998, UK: Blackwell Science Ltd, p.253.

Sundqvist G, Figdor D. Life as an endodontic pathogen. *Endodontic Topics* 2003;6:3-28

Torabinejad M, Ung B, Kettering JD. In vitro bacterial penetration of coronally unsealed endodontically treated teeth. *J Endod* 1990;16:566-9.

Torabinejad M, Rastegar AF, Kettering JD, Pitt Ford TR. Bacterial leakage of mineral trioxide aggregate as a root-end filling material. *J Endod*. 1995 Mar;21(3):109-12.

Tran XV, Gorin C, Willig C, Baroukh B, Pellat B, Decup F, Opsahl Vital S, Chaussain C, Boukpepsi T. Effect of a calcium-silicate-based restorative cement on pulp repair. *J Dent Res*. 2012 Dec;91(12):1166-71. doi: 10.1177/0022034512460833. Epub 2012 Sep 13.

Velvart. Surgical endodontics, page 348-349 in *Textbook of Endodontology of* Bergenholtz G, Hørsted-Bindslev P, Reit C. Wiley-Blackwell 2010.

Vallés M, Mercadé M, Duran-Sindreu F, Bourdelande JL, Roig M. Influence of light and oxygen on the color stability of five calcium silicate-based materials. *J Endod*. 2013 Apr;39(4):525-8. doi: 10.1016/j.joen.2012.12.021. Epub 2013 Feb 10.

Wang X, Chang J, Hu S. A study on the sealing ability and antibacterial activity of Ca<sub>3</sub>SiO<sub>5</sub>/CaCl<sub>2</sub> composite cement for dental applications. *Dent Mater J.* 2012 ;31(4):617-22.

Wu MK, Wesselink PR. Endodontic leakage studies reconsidered: Part I. Methodology, application, and relevancy. *Int. Endod. J.* 1995; 28:86-90

Zanini M, Sautier JM, Berdal A, Simon S. Biodentine induces immortalized murine pulp cell differentiation into odontoblast-like cells and stimulates biomineralization. *J Endod.* 2012 Sep;38(9):1220-6. doi: 10.1016/j.joen.2012.04.018. Epub 2012 Jul 24.