



MASTEROPPGAVE

Biodentine™ as a root-end filling

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Abstract

Aim and hypothesis

The purpose of this study is to evaluate Biodentine™ as a root-end filling.

The working hypothesis is that Biodentine™ provides apical bacterial seal as MTA when applied in root-end filling therapy.

Materials and methods

For background literature, an electronic search was conducted in PubMed. Key words related to apicoectomy, root-end filling materials and micro leakage were used. Apart from PubMed, the web pages of the manufactures were used to include the information that only has been published in the form abstract or being just "data on file" without any other forum of publication.

Simultaneously dye micro leakage lab experiments were conducted, testing Biodentine™, IRM® and ProRoot MTA™ as root-end fillings. The experiments were performed using extracted human teeth and fuchsin-colored dye solution. The experimental protocol was compiled using well-known principles with references. A total of three experiments were performed; a pilot experiment (Experiment 1), Experiment 2 and Experiment 3. The pilot experiment (Experiment 1) aimed to evaluate the feasibility of the dye leakage experiment for ex vivo teeth. The results of the pilot experiment were used as a guideline for the dye leakage experiment in Experiment 2. In Experiment 3 ProRoot MTA™ was used as root-end fillings as a standard to investigate dye leakage after different immersion times.

Results

In Experiment 1, two out of five canals filled with Biodentine™ could resist dye penetration. None of the canals filled with ProRoot MTA™ or IRM® resisted dye penetration.

In Experiment 2 only one canal filled with ProRoot MTA resisted dye penetration. Biodentine™ and IRM® displayed total leakage through the root-end filling material. However, the specimens with Biodentine™ had less leakage through the gutta-percha compared to IRM®.

In Experiment 3 total dye leakage was not seen in specimens immersed in a 1- to 3-hour time frame. Total leakage of the root-end filling was first seen after 6 hours. After 12 hours, dye had penetrated both the root-end filling and the gutta-percha.

Conclusion

In Experiment 2, ProRoot MTA™ was the only material to resist apical leakage. The proper dye immersion time was established in Experiment 3, using MTA samples only. The experiment should now be repeated in order to establish the ability of Biodentine™ to resist apical leakage compared to ProRoot MTA™.

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Introduction

Apicoectomy

Upon failure of endodontic therapy, one can choose either to re-treat the tooth non-surgically, with an orthograde root filling, or surgically, with apicoectomy and a retrograde root filling. The procedure can be performed by either simply cutting off the apical portion of the root and then planing the cut surface, or include the preparation of an apical class I cavity as well. The apical cavity is then filled with a material suitable for root-end filling.

The rationale for removing the most apical three millimetres of the root is that (all) accessory canals apically should be removed to achieve apical bone healing. Removal of 3mm also removes 98% of apical ramifications, and 93% of lateral canals (Kim & Kratchman 2006).

Bevel angles

When preparing the apically resected root surface using the traditional technique, it is necessary, in order to gain access to the root canal, to create a bevelled surface. In traditional apicoectomy procedures, this angle will be between 45 and 60 degrees. In the modern microsurgery approach, however, an angle of 0 to 10 degrees is standard (Kim & Kratchman, 2006). Increasing the bevel angle will among other consequences result in an increase of the surface area of the cut apical end, and thus increasing the risk of apical leakage through lateral dentinal tubules. (Bergenholtz et al., 2010)

Root-end filling

Studies have shown that the best outcome of surgical retreatment is achieved by apical cavity preparation and placing an apical filling material (Christiansen et al., 2008).

The function of the root-end filling is to provide an ideally hermetic seal of the root canal, to inhibit coronal leakage of pathogens and their products into the periradicular tissues, and thus promote healing and formation of cementum on the cut dentinal surface (Chong & Pitt Ford, 2005).

Sealing ability

Sealing ability refers to the materials ability to resist micro leakage through the entire thickness of the material. Inadequate apical seal is a major cause of surgical endodontic failure. It is common to examine leakage using following methods: Fluid filtration, dye leakage, protein leakage or bacterial leakage.

Choice of root-end filling materials

Amalgam, IRM, resins, MTA and many other dental materials have been tried and tested as root-end endodontic filling materials. At the present, IRM and MTA are widely used materials for this procedure, and both have been associated with high success rates in clinical trials (Chong et al., 2003).

Bench testing of filling materials

Several methods may be employed in order to evaluate dental materials: Micro leakage of materials has

been evaluated with passive penetration of dyes, fluid perfusion tests, bacterial penetration models and capillary flow porometry (Tobón-Arroyave et al., 2007). Micro leakage is defined as the passage of bacteria, fluids and chemical substances between the root structure and filling material, and in order to assess micro leakage, passive penetration of fuchsin, as well as several other dye agents including Indian ink, rhodamine and methylene blue, have been employed by numerous micro leakage studies (Tobón-Arroyave et al., 2007). Advantages with dye penetration testing include that they are easy to perform; the equipment needed is cheap and easily available. Additionally it is possible to make identical setups to compare the samples tested (Roberecht et al. 2011).

However, dye penetration experiments give questionable results. There are several factors affecting the dye leakage; the thickness of the dentinal walls, the dye pH, the type of dye used, the tooth storage environment and if the material tested is set before put in the dye solution (Parirokh & Torabinejad, 2010b). When dye leakage samples are ready to be examined, the teeth are usually cut longitudinally. This random cut does not necessarily go through where the leakage of the dye is at its deepest, potentially giving a wrong impression of the materials resistance to penetration (Camps & Pashley, 2003). However, Orosco et al. (2010) suggested that a gap between the material and dentinal wall did not represent the entire condition of the apical plug. This suggests that the apical plug as a whole could resist total leakage of dye, regardless of how deep dye would have penetrated the material.

There have been discussions whether dye leakage experiments have clinical relevance since the dye molecule is much smaller in size compared to e.g. bacteria. In theory one could imagine that the dye molecule would penetrate in between the material tested and the dentinal walls much easier than e.g. bacteria. Barthel et al. (1999) did a bacterial vs. dye leakage study in obturated root canals. Ninety-six single-rooted teeth were divided in to three groups using different sealers for each group. The roots were first exposed to soy broth containing *Staphylococcus epidermis* and thereafter 48 hours to basic fuchsin. In the bacterial experiment they found no significant difference between the three groups. In the dye leakage experiment they found significantly greater leakage in one group compared to the other, concluding there is no correlation between the results of the two tests and that molecular size perhaps is not the relevant parameter when testing sealability of root-canal fillings.

Requirements of an ideal root-end filling material

Chong & Pitt Ford (2005) summarized the requirements of an ideal root-end filling material as follows: The material should adhere or bond to tooth tissue and "seal" the root-end three-dimensionally; not promote, and preferably inhibit, the growth of pathogenic microorganisms; be dimensionally stable and unaffected by moisture in either the set or unset state; be well tolerated by periradicular tissues with no inflammatory reactions; stimulate the regeneration of normal periodontium; be nontoxic both locally and systemically; not corrode or be electrochemically active; not stain the tooth or the periradicular tissues; be easily distinguishable on radiographs; have a long shelf life, be easy to handle.

Literature review on materials used for root-end filling

Search methodology

For background literature, an electronic search was conducted in PubMed. Key words related to apicoectomy, root-end filling materials and micro leakage was used. We focused on studies published in the journals with the highest impact factor in the field of endodontics and materials science; International Endodontic Journal, Journal of Endodontics and Journal of Dental Materials. Apart from PubMed, the web pages of the manufactures were used to include the information that only has been published in the form abstract or being just "data on file" without any other forum of publication.

Amalgam

For many years, amalgam was in many ways regarded as a high-grade filling material when used for root-end fillings. However, Scanning electron microscopy (SEM) revealed gaps between amalgam fillings and the root canal walls, as first demonstrated by Moodnik et al. (1975). Cytotoxicity studies have further opened up for discussions surrounding amalgam use in the clinical setting (Bruce GR et al., 1993). Such science has since contributed to amalgam's popularity decline in relation to root-end fillings, because of questionable sealing ability, handling difficulties during the procedure, corrosion (causing discoloration and tattooing of soft tissues) and mercury content. Clinical follow-up studies have shown poor outcomes related to retrograde filling with amalgams (Bergenholtz et al., 2010). In addition, certain countries have now placed universal bans on amalgam treatment, including Norway, due to the material's mercury content and the possible adverse biological and environmental effects, thus eliminating amalgams from the root-end filling discussion completely.

Chemical and physical properties

Hohenfeldt et al. (1985) showed that electrochemical erosion of amalgam caused failure in apical amalgam fillings. Badr et al. (2010) showed that MTA has better marginal adaptation to resin root-segment replicas than amalgam.

Biocompatibility and cytotoxicity

Badr et al. (2010) showed that amalgam is more cytotoxic than MTA, using culture of human PDL fibroblasts.

Clinical outcomes

The work published by Dorn & Gartner (1990), showed that when comparing the results for two individual and geographically distinct dental practices, when all healing cases were combined, the success rate for amalgam in both practices was 75% when rounded to the nearest percentage point. The success rates of SuperEBA and IRM were 95% and 91%, respectively (Dorn & Gartner, 1990). This comparison demonstrated the inferiority of amalgam when compared to other available filling materials, in a retrospective study.

Biodentine™

Biodentine™ with Active Biosilicate Technology™ was announced by dental materials manufacturer Septodont in September of 2010, and made available in January of 2011. According to the research and development department of said manufacturer, "a new class of dental material which could conciliate high mechanical properties with excellent biocompatibility, as well as bioactive behaviour" (Septodont Biodentine™ scientific file, 2010) had been produced. According to the manufacturer, the material can be used as a "dentine replacement material whenever original dentine is damaged."

Biodentine™ is a calcium silicate based material used for crown and root dentin repair treatment, repair of perforations or resorptions, apexification and root-end fillings. The material can also be used in class II fillings as a temporary enamel substitute and as permanent dentine substitute in large carious lesions (Septodont Biodentine™ scientific file, 2010). The manufacturer points out the biocompatibility and the bioactivity of the material, which is important since the use of the material involves indirect and direct pulp capping and pulpotomy. According to the manufacturer, Biodentine™ preserves pulp vitality and promotes its healing process. (Septodont Biodentine™ scientific file, 2010).

Biocompatibility and cytotoxicity

Laurent et al. (2008) tested a new Ca_3SiO_5 -based material to evaluate its genotoxicity, cytotoxicity and effects on the target cells specific functions. The study concluded that the new material is biocompatible. The material was not found to affect the specific functions of target cells and thus could safely be used in the clinic.

About et al. (2010) investigated Biodentine™ bioactivity by studying its effects on pulp progenitor cells activation, differentiation and dentine regeneration in human tooth cultures. The study concluded that Biodentine™ is stimulating dentine regeneration by inducing odontoblast differentiation from pulp progenitor cells.

Laurent et al. (2012) did further a study to investigate the capacity of Biodentine™ to affect TGF- β 1 secretion from pulp cells and to induce reparative dentine synthesis. Biodentine™ was applied directly onto the dental pulp in a human tooth culture model, resulting in a significant increase of TGF- β 1 secretion from pulp cells and thus inducing an early form of dental pulp mineralization shortly after its application.

Han & Okiji (2011) compared calcium and silicon uptake by adjacent root canal dentine in the presence of phosphate buffered saline using Biodentine™ and ProRoot® MTA. The results showed that both materials formed a tag-like structure composed of the material itself or calcium- or phosphate rich crystalline deposits. The thickness of the Ca- and Si-rich layers increased over time, and the thickness of the Ca- and Si-rich layer was significantly larger in Biodentine™ compared to MTA after 30 and 90 days, concluding that the dentine element uptake was greater for Biodentine™ than for MTA.

Biodentine™ as a root-end filling

In this literature review we have concentrated on Biodentine™ as a root-end filling. For this use there is no published studies, whereas the only documentation available is from the manufacturer. The material has

indications similar to calcium silicate containing materials e.g. MTA. Septodont claims that Biodentine™ has features as an endodontic repair material that are superior to MTA: Biodentine™ has better consistency, better handling and safety, and faster setting time which creates no need for a two step obturation.

Clinical outcomes

Clinical outcomes for Biodentine™ used in root-end filling are currently lacking.

IRM®

Background information

Dentsply IRM® (Intermediate Restorative Material) is a polymer reinforced zinc oxide eugenol cement, first described as a root-end filling material in 1990 (Dorn & Gartner, 1990). Zinc oxide eugenol cements were introduced as a dental material in the 1890s and have been used as temporary restorations, as cement in temporary placement of fixed bridges and as impression paste, among other applications and uses. Today IRM® is used for temporary restorations; restoration of deciduous teeth; restorative emergencies; and as base material under non-resin permanent restorations (Dentsply Caulk, IRM® product information sheet). IRM® is in addition widely used as a retrograde root-end filling material (Kim & Kratchman, 2005). It is cost-effective, easy to mix and easy to handle (Tawil et al., 2009)

Chemical and physical properties

Cavity preparation must provide mechanical retention for the filling material (Dentsply Caulk, IRM® product information sheet). The setting time of IRM® is 6 minutes +/- 30 seconds (Torabinejad et al. 1995). IRM® has a radiopacity greater than recognizable dentin and is less radiopaque than gutta-percha, making it distinguishable on a radiograph when used as a root-end filling material (Torabinejad et al., 1995). Zinc-oxide eugenol cements exhibit a pH of approximately 7 at the time of placement, potentially making them the least irritating of all dental materials (Chiayi, 2003).

Biocompatibility and cytotoxicity

IRM® contains eugenol, which has raised questions about harmful effects on the periapical tissues. Studies has shown that IRM® does not enhance new tissue formation when used as a root-end filling material (Economides et al., 2003), and that adhesion of human osteoblasts is poor (Zhu et al., 2000). In one *in vivo* study, the outcomes showed that IRM® is suitable as retrograde root-end filling material, providing appropriate healing response from periapical tissues (Tawil et al., 2009).

Clinical outcomes

IRM® used as a root-end filling material has shown success rates of 76% after 12 months and 87% after 24 months (Chong et al., 2003), and in 91% of the cases in another study (Dorn & Gartner, 1990) IRM® has been shown to have antibacterial effects, causing complete inhibition of *P.aeruginosa*, as well as delayed or limited growth of *E.faecalis* (Eldeniz et al., 2006).

Mineral Trioxide Aggregate

Background information

Over the years, several different retrograde root-end filling materials have been used, such as silver amalgam, IRM®, glass ionomer and composite resins. However, these mentioned materials have not met the requirements for an ideal root-end filling material (see p.8 *Requirements of an ideal root-end filling material*). Mineral trioxide aggregate (MTA) was developed by Dr. Mahmoud Torabinejad and Dean J. White in the early 1990s as a potential root-end filling material or as a repair material for lateral root perforations (Torabinejad et al., 1993; Lee et al., 1993; Torabinejad & White, 1995). Mineral trioxide aggregate was later approved by the U.S. Federal Drug Administration and became commercially available in 1998 under the product name ProRoot® MTA (Tulsa Dental Products, Tulsa, OK, USA) (Camilleri, 2008).

Recently Shahi et al. (2011) published a study, comparing the sealing ability of MTA and Portland cement (PC) when used as root-end filling materials in a dye leakage experiment. This study showed that there was no significant difference in sealing ability between the two materials. Due to the similar sealing ability and the low cost of PC, the authors suggest that PC may be an alternative to MTA (Shahi et al. 2010).

At first MTA was introduced with the colour gray (GMTA). However, the GMTA proved to have some discoloration potential, as it would discolour overlying tissues. White MTA (WMTA) was therefore developed and introduced by Dentsply Tulsa Dental in 2002 as an aesthetic alternative to GMTA. A study by Matt et al. (2003) were performed to compare leakage of WMTA versus GMTA when used as apical barriers in simulated open root apices. The study concluded that GMTA had significantly less leakage than WMTA (Matt et al., 2004). In late 2003 Dentsply Tulsa Dental improved the WMTA formula. Due to this, newer studies have concluded that WMTA is more or less equivalent to GMTA with respect to leakage (Ferris et al., 2004; Shahi et al., 2009).

Clinical applications of ProRoot® MTA include the following: Repair of furcal and lateral root perforations, repair of internal root resorption, vital pulp therapy (direct pulp capping, partial pulpotomy and pulpotomy), as an apical plug during apical surgery and as an apical plug in non-vital teeth with open apex (Material Data Safety Sheet, 2002; Vosoughosseini et al., 2008).

Relative to the previously mentioned root-end filling materials (silver amalgam, IRM, glass ionomer and composite resins), ProRoot® MTA has shown favourable results. The advantages of ProRoot® MTA as a root-end filling material, in relation to the other mentioned alternatives, include greater sealing ability and better marginal adaptation (Torabinejad et al., 1993; Lee et al., 1993; Torabinejad et al., 1995). ProRoot MTA™ also has the favourable ability to set under moist conditions (Torabinejad & White, 1995) and studies show that MTA has an antibacterial and antifungal effect (Parirokh & Torabinejad, 2010b).

However, ProRoot® MTA has its disadvantages: It contains toxic arsenic, the material is expensive, it is difficult to handle and it has a long setting time. No solvent exists, and hence removal of MTA is difficult. GMTA has a discoloration potential when used as a pulpotomy material (Parirokh & Torabinejad, 2010c).

Chemical and Physical properties

ProRoot® MTA is a fine powder of particles with hydrophilic properties that set in the presence of water. According to the ProRoot® MTA Material Data Safety Sheet, MTA contains approximately 75% Portland cement, 20% bismuth oxide and 5% gypsum by weight. The main compounds are tricalcium silicate, bismuth oxide, dicalcium silicate, tricalcium aluminate and calcium sulphate dihydrate/gypsum (Material Data Safety Sheet, 2002). The bismuth oxide gives MTA its radiopaque appearance on radiographs (Torabinejad & White, 1995).

The chemical difference between WMTA and GMTA were investigated by Asgary et al. (2005) and Camilleri et al. (2005), and the studies concluded that WMTA contains significantly lesser amounts of aluminium and iron compared with GMTA (Asgary et al. 2005; Camilleri et al. 2005). The gray colour in GMTA is caused by tetracalcium aluminoferrite, which lacks in WMTA (Storm et al. 2008; Camilleri et al. 2005; Camilleri et al. 2006). The main compounds of GMTA are tricalcium silicate, dicalcium silicate and bismuth oxide. WMTA primarily contains tricalcium silicate and bismuth oxide (Camilleri et al. 2005; Parirokh et al. 2010a).

The presence of toxic arsenic in MTA has raised some concern regarding potential toxicity to the organism, though studies have concluded that the amount of arsenic leakage to surrounding tissues is very low: The low amount of leakage is due to the insolubility of MTA, the small amounts used, and the fact that ferric oxide in MTA has a stabilizing effect on arsenic (Parirokh et al. 2010a).

Preparation of MTA is done by mixing MTA powder and sterile water in a 3:1 ratio. The fact that MTA hardens with water as its reactant allows MTA to set in the moist conditions within the human body. This gives MTA an important advantage over other, commonly used root-end filling materials (Torabinejad & White, 1995).

When hydrated, the fine powder forms a colloidal gel that solidifies to form a strong impermeable barrier (Material Data Safety Sheet, 2002).

Mineral trioxide aggregate requires a working time of about 5 minutes. The mean setting time is approximately 2 hours and 45 minutes, but setting time may vary depending on several factors: The type of MTA used (WMTA/GMTA); the place of deposition related to the degree of moisture on site and air entrapped during mixing. In dry conditions, setting time is prolonged and bacterial leakage is adversely affected (Parirokh et al., 2010; Chogle et al., 2007). This barrier of solidified MTA cures over a four-week period (Material Data Safety Sheet, 2002). The long setting time may be considered one of the major drawbacks of ProRoot® MTA.

In 2001, MTA-Angelus (Angelus Soluções Odontológicas, Londrina, PR, Brazil) was introduced on the Brazilian dental market as an alternative to ProRoot® MTA (Orosco et al., 2010).

The chemical difference between ProRoot® MTA and MTA-Angelus is that MTA-Angelus lacks calcium sulphate dihydrate as one of its main compounds (Material Data Safety Sheet 2005). This gives MTA-Angelus

a reduced setting time relative to ProRoot® MTA (2 h and 45 minutes for ProRoot® MTA and 10 minutes for MTA-Angelus) (Hashem & Hassanien 2008). An *in vitro* study simulating root-end filling using ProRoot® MTA and MTA-Angelus has shown that there is no statistically significant difference in sealing ability between the two materials (Lolayekar et al., 2009).

In 2004 yet another material similar to MTA appeared on the dental market – this time in Argentina. The new material got the brand name CPM™ (Egeo S.R.L., Buenos Aires, Argentina) and has a setting time of approximately 1 hour (Orosco et al. 2010).

Marginal adaptation and sealing ability

The marginal adaptation describes the degree of proximity and interlocking the filling material create to the cavity wall of a tooth. When analyzing the marginal adaptation, it is common to use Scanning Electron Microscopy (SEM) analysis.

Several studies have shown that MTA has better marginal adaptation than glass ionomer cements, SuperEBA™, IRM® and amalgam (Parirokh et al. 2010b). A recent study by Munhoz et al. (2011) also evaluated the marginal adaptation of MTA compared to the sealer AH26 when used as a root-end filling. The marginal adaptation was measured using a 3D profilometry and SEM. The study concluded that MTA had better marginal adaptation than Sealer 26.

One might think that marginal adaptation and sealing ability has a strong correlation. However, studies done by Orosco et al. (2010) and Xavier et al. (2005) have shown that this is not always the case. In the study by Orosco et al. (2010), CPM™ had the best marginal adaptation, but represented the worst results regarding micro leakage. Thus, the following was proposed: A gap may not represent what really occurred in the entire apical plug (Orosco et al. 2010).

A literature review by Torabinejad & Parirokh (2010b) systematically went through articles from 1993 to 2009 regarding the sealing ability and biocompatibility of ProRoot® MTA. The review divided the sealing ability of MTA in relation to the leakage method used.

In fluid filtration tests, the literature review by Torabinejad & Parirokh (2010b) concluded that ProRoot® MTA is superior compared to IRM®, SuperEBA™ and amalgam. A study by De Bruyne et al. (2005) showed that MTA leaked more than Fuji IX. However, re-doing the same experiment with only slight adjustments of the protocol, using bovine teeth instead of human teeth, gave conflicting results (Parirokh & Torabinejad, 2010b).

Reviewing dye leakage experiments, Torabinejad & Parirokh concluded the following: MTA is one of the most resistant root-end filling materials to dye penetration. It is however emphasized that there is several factors affecting the dye leakage of MTA. These factors include the thickness of dentinal walls, the dye pH,

the type of dye used, the tooth storage environment and if the MTA has set before being put in dye solution (Parirokh & Torabinejad 2010b).

Few studies of protein leakage in MTA have been performed, but Valois & Costa (2004) found that an apical plug of 4mm was significantly more effective than an apical plug of 2-3mm. A study by Saqhiri et al. (2008) suggested that there was less leakage in samples stored at higher pH values, rather than in an acidic environment. Another study by Shahi et al. (2009) investigated the sealing ability of MTA and PC, concluding that PC has better sealing ability than MTA when used for furcal perforation repair (Shahi et al. 2008).

Biocompatibility

As an endodontic root-end filling material will come in close contact with human tissue, the biocompatibility properties of the material are of concern. The literature review by Parirokh & Torabinejad (2010b) states that several studies have shown MTA to be a more biocompatible material compared to SuperEBA™, IRM® and amalgam. Additionally MTA is believed to be a bioactive material with several favourable properties, such as stimulating cementum- and hard tissue formation, and having an antibacterial effect due to its alkaline pH (Parirokh & Torabinejad, 2010b).

Clinical outcomes

Kim & Kratchman (2006) did a review article on modern endodontic surgery concepts and practice, and found MTA to be the most biocompatible root-end filling material at that time. Chong et al. (2003) compared IRM® and MTA as root-end filling materials to assess the success rate of MTA in a prospective clinical study. The success rate for IRM® was 76% after 12 months and 87% after 24 months. For MTA the success rate was 84% after 12 months and 92% after 24 months. However, no statistically significant differences between the two filling materials were found.

Saunders (2008) did a prospective clinical study using MTA as a root-end filling material. Out of a total of 276 teeth examined, 163 showed complete radiographic healing with no symptoms; 82 teeth had no symptoms but showed incomplete healing; 31 teeth showed no healing. The success rate including all teeth with no symptoms was 88.8%

Another prospective clinical study done by Lindeboom et al. (2005) evaluated MTA and IRM® as retrograde sealers in surgical endodontics, examining a total of one hundred single-rooted teeth. 50% of the teeth treated with IRM® and 64% of the teeth treated with MTA showed complete healing. Incomplete healing was seen in 36% of the IRM® treated teeth, and in 28% of the MTA treated teeth. Unsatisfactory healing was seen in 14% of the IRM® treated teeth, and 6% of the MTA treated teeth. No statistically significant differences between the two filling materials were found.

Resins

Background information

Resin composites have existed as dental restorative materials for more than 50 years (Margeas, 2012; Rueggeberg, 2002). Application has included endodontic root-end filling, and the method has been documented in clinical studies on such materials as Retroplast™, used in combination with GLUMA® Desensitizer as a dentine bonding agent (von Arx et al., 2010).

Technique

Retroplast™ differs from other materials in that it is not placed in an apical class I cavity, but on an apical surface that has been made slightly concave (Retroplast™ Instructions for use). The technique of sealing the resected surface was first proposed by Rud et al. (1991), in an attempt to combat apical leakage through exposed dentinal tubules.

In practical considerations, according to Otani et al. (2011), sealing with resins may be an option whenever root-end cavities cannot be prepared, because of morphology, or previous prosthodontic treatment. Since resins are widely used by dentists for many other purposes than root-end filling, sealing with resins may represent a clinically familiar alternative to other materials.

Biocompatibility

Otani et al. (2011) reported after animal studies on root-end sealing with Super Bond C&B® that although no cementum-like tissue formation had occurred, few inflammatory cells were present at the resin-interface; cementum-like tissue formation was only observed when using MTA. The authors concluded that root-end sealing with the resin was significantly better than using no retrograde filling material. (Otani et al., 2011)

Clinical outcomes

A prospective clinical study by von Arx et al. (2010) concluded that Retroplast™ should be used with caution for root-end sealing in apical surgery of mandibular premolars and molars. The authors stated that published success rates of Retroplast™ when compared with other root-end filling techniques in clinical studies have always been lower than those of MTA, but because the MTA and Retroplast™ techniques use two different methods of root-end preparation, differences in treatment outcome cannot solely be attributed to the filling material itself (von Arx et al. 2010)

Dye leakage in root-end endodontic filling materials

Background

Apicoectomy is a surgical approach to endodontic treatment, indicated in a number of situations e.g. failed re-treatment cases or complex root canal anatomy. Another example is cases with acceptable endodontics and a new prosthetic restoration, e.g. post and crown or bridge, but a continued or progressive periapical lesion. Disassembling the construction and orthograde retreatment may be more time consuming and more expensive than apicoectomy (Kim & Kratchman, 2006). The success of such endodontic surgery relies on removal of all necrotic tissue and the complete sealing of the entire root canal system. For a previously endodontically treated tooth, the most important cause of a periapical lesion is a leaking apical seal. In order to treat such a case, a tight apical seal has to be produced, either by non-surgical re-treatment or by apicoectomy and placement of a root-end endodontic filling (Kim & Kratchman, 2006).

A number of different dental materials have been tried and tested as root-end endodontic fillings. Previously amalgam was widely used, but at the present ZOE containing materials, such as IRM® and SuperEBA™, are more popular and show better sealing ability and biocompatibility than amalgam. Today MTA is considered to be the ideal root-end endodontic filling material because of its superior healing induction potential and biocompatibility than any other root-end filling material available (Kim & Kratchman 2006).

Biodentine™ with Active Biosilicate Technology™ was announced by dental materials manufacturer Septodont in September of 2010, and made available in January of 2011. Biodentine™ is a calcium silicate based material used for crown and root dentin repair treatment, repair of perforations or resorptions, apexification and root-end fillings. The material has indications similar to calcium silicate based materials e.g. MTA. Septodont claims that Biodentine™ has features as an endodontic repair material that are superior to MTA, that it has been shown that Biodentine™ is not mutagenic (Harmand, 2003) and that it can resist micro leakage (Tran et al, 2008)(Septodont Biodentine™ scientific file).

Purpose

The purpose of the planned series of experiments is to evaluate Biodentine™ for its ability to resist micro leakage when used as a root-end endodontic filling material.

The working hypothesis is that Biodentine™ provides apical seal as the prevailing materials MTA and IRM®, in an *ex vivo* experiment simulating apicoectomy.

Materials and methods

Experiment 1 (pilot)

This pilot study aimed to evaluate the feasibility of the dye leakage experiment for *ex vivo* teeth.

Root-end filling materials tested

For the apical leakage test a new silicate cement product, Biodentine™ (Septodont, Saint Maur des Fosses, France), was tested against mineral tri-aggregate cement, ProRoot® MTA (Dentsply Maillefer, Ballaigues, Switzerland) and a temporary filling material, IRM® (Dentsply Maillefer).

Preparation and filling of root canals

Three parallel samples were used for each material. One tooth without any root-end filling was tested as a positive control, and another tooth, entirely covered with sticky wax, was used as a negative control.

Thirteen human teeth, stored in 0.01% NaOCl since extraction, are prepared. Crowns were removed using a diamond burr mounted on a turbine, under water cooling, leaving 13mm of the root. A #10 K-file (Dentsply Maillefer) was first extended through the apex, then retracted to 1mm from the apex, and the root canal was then prepared up to #20 K-file, under irrigation with 1% NaOCl. The root canal was further prepared using ProTaper Universal Rotary files up to a final size of F5 (REF A 0411-F5, Dentsply Maillefer). The root canal was dried with sterile paper points (size F5 A022W00090200, Dentsply Maillefer) and obturated with a size F5 gutta-percha master cone (A022X0090200, Dentsply Maillefer), and the appropriate number of size B gutta-percha accessory cones (A022F, Dentsply Maillefer) simulating the clinical obturation procedure. No sealer was used, as the evaluation concerns the apical filling only. The apical three millimetres of the root were cut off using a diamond burr, producing a 10mm long root segment. The apical end of the root canal is prepared to a depth of 4mm using a size 008 fissure burr (ISO 310204107002008, Komet, Rock Hill, USA) held parallel to the root, and leaving a cylindrical root-end cavity with a 0.8mm diameter free of gutta-percha. The root-end cavity was then filled using either Biodentine™, ProRoot® MTA or IRM®.

Dye leakage test

Kerr Sticky Wax (Kerr Corporation, Orange, CA, USA) was melted in a cauldron and the root segments were dipped in it to achieve a noticeable wax-covering of the apical and lateral portions of the root, but avoiding wax coverage on the coronal dentine surface. During the wax covering procedure, the root segments were held using an endodontic spreader (see: Fig. 1). The specimen were then transferred into test tubes containing a diluted dye solution consisting of two parts fuchsin-colored Caries Detector (US043, Kuraray Medical Inc., Okayama, Japan) and three parts sterile saline solution, then soaked for 48 hours at 37 °C. After the soaking period, the specimen were rinsed and fixed on a metal board using TempBond NE (Kerr Corporation, Orange, CA, USA), and then split in half longitudinally using a diamond burr mounted on a turbine. The results were photographed using a Canon 450D SLR camera with a Canon EF 100mm f/2.8 USM macro lens.



Fig. 1. The illustration shows how the specimens were covered in wax in preparation for dye immersion.

Control groups

The control groups were comprised of one positive control specimen and one negative control specimen. The controls were prepared as described for the experimental groups, with the following modifications: In the positive control, the root-end cavity was left empty. In the negative control, the specimen was completely covered in sticky wax before immersion.

Experiment 2

The results of the pilot experiment were used as a guideline for the dye leakage experiment.

Root-end filling materials tested

For the apical leakage test a new silicate cement product, Biodentine™ (Septodont), was tested against mineral tri-aggregate cement, ProRoot® MTA (Dentsply Maillefer) and a temporary filling material, IRM® (Dentsply Maillefer).

Preparation and filling of root canals

Eleven human teeth, stored in 0.01% NaOCl since extraction, were used. Three parallel samples were used for each material. The root segments were prepared and filled in the same manner as in experiment 1.

Mixing of the materials were standardized, an they were mixed in the following ways:

From each Biodentine™ capsule, the same amount of powder was removed using the tip of a spatula as a measuring tool. The remaining powder in each capsule was mixed with 1 bottle of Biodentine™ liquid. Biodentine™ capsules were mixed with 3M™ Espe CapMix™ Mixing Unit for 30 seconds, at 4300 oscillations per minute. The application of Biodentine™ was performed using ProRoot® MTA carrier tubes. To each standardized capsule of IRM® powder and liquid, 1 scoop of IRM® powder was added. The capsule contents were then mixed with 3M™ Espe CapMix™ Mixing Unit for 10 seconds, at 4300 oscillations per minute. The application of IRM® was performed using hand instruments only.

MTA liquid was gradually mixed with more and more ProRoot® MTA powder until a creamy consistency was achieved. MTA was applied using ProRoot® MTA carrier tubes.

The root segments were then incubated in sterile saline solution for 7 days at 37 °C to allow the materials to set.

Dye leakage test

The specimen were covered in Kerr Sticky Wax (Kerr Corporation) like in experiment 1, and soaked for 48 hours at 37 °C in the same diluted dye solution. This procedure differed at the wax covering procedure; the root segments were held using a bent endodontic spreader instead of a straight one (see: Fig. 2 and 3), and the wax were covering the coronal and lateral portions of the root, but avoiding wax coverage on the apical dentine surface. The results were photographed using a Canon 450D SLR camera with a Canon EF 100mm f/2.8 USM macro lens.

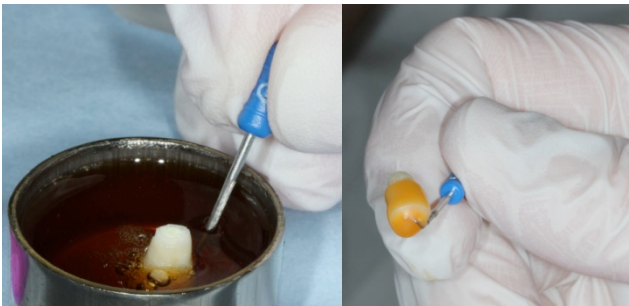


Fig. 2. The illustration shows how the specimen were held and covered in wax in preparation for dye immersion.

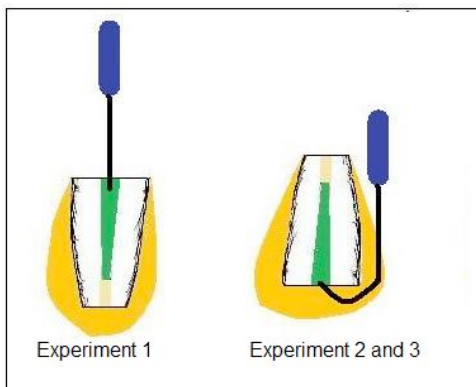


Fig.3. The illustration shows how the root segments were held using a bent endodontic spreader during the wax covering procedure, and how this method of holding differed between experiment 1 and experiment 2 and 3. (Wax = yellow colour, gutta-percha = green colour, root-end filling = beige colour.

Criteria for success

A definite criterion for either success or not success of the root-end filling was the ability to resist dye penetration beyond the most coronal part of the filling material. The results was classified as no-, partial- and total leakage through the root-end filling material in the sample; and as no-, partial- and total leakage through the gutta-percha when the root-end filling material has total leakage (see: Fig.4). The assessment of leakage was done on high-resolution digital photographs.

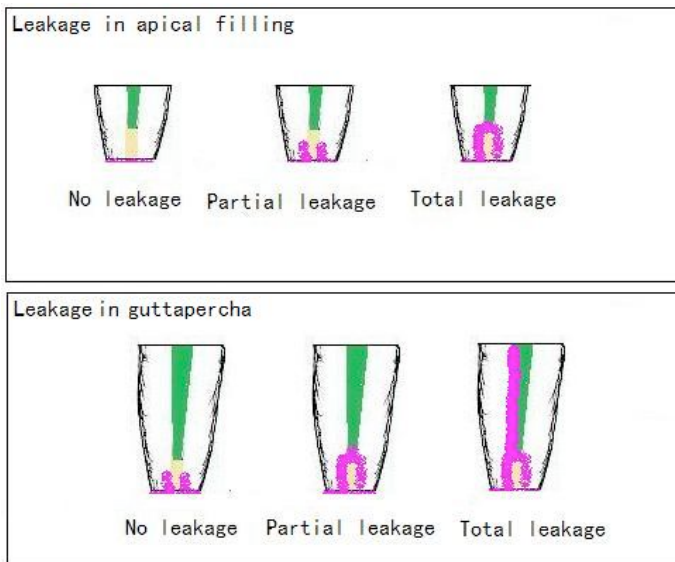


Fig.4. The illustration shows how leakage was measured, depicting the criteria both for the apical filling and the guttapercha.

Experiment 3

Four single rooted teeth were prepared as earlier described, with the only difference that all four of the root-end cavities were filled with ProRoot® MTA. To ensure good quality of the root-end fillings, the specimens were radiographed before incubation (Fig. 5). After the incubation they were covered in Kerr Sticky Wax and divided in four groups given from when they were taken out of the solution: 1 hour, 3 hours, 6 hours and 12 hours. The results were photographed using a Canon 450D SLR camera with a Canon EF 100mm f/2.8 USM macro lens, through a Global microscope with the magnification 19.2 and a field of 10 mm.

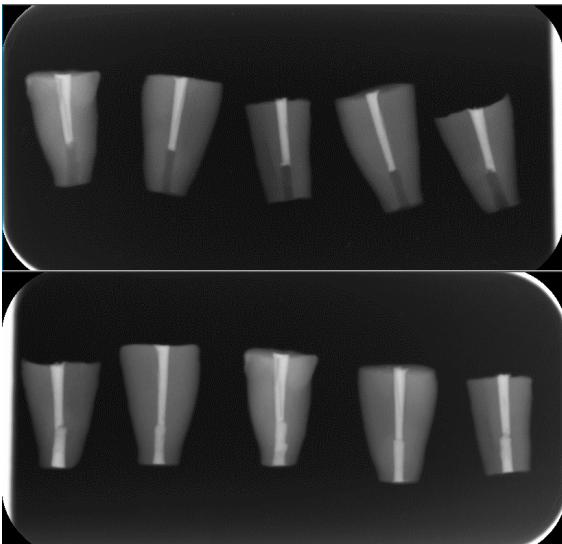


Fig.5. Radiograph of specimen before and after placing root-end fillings.

Results

Experiment 1

Two out of five canals filled with Biodentine™ could resist the dye penetration, while none of the canals filled with MTA and IRM® could. Kerr Sticky Wax was a suitable material for sealing the specimens, the

concentration of the dye solution seems suitable, as does the immersion duration. The canal size and the length of the root-end preparations varied among the specimen.

Experiment 2

After 48 hours immersion in the dye, only one specimen in the MTA group could at least partly resist the dye leakage through the root-end filling. Both Biodentine™ and IRM® showed total leakage in the root-end material, but Biodentine™ showed less leakage along the gutta-percha (Fig. 5A-C, table 1). The positive control showed through-out leakage, whereas the negative control gave a perfect seal.

Table 1. The distribution of dye leakage in the three tested materials expressed separately to affect the root-end filling only (apical filling) and the rest of the canal (gutta-percha). 48 hour immersion.

	Material					
	MTA	BIODENTINE	IRM	POS. CONTROL	NEG. CONTROL	TOTAL
Teeth tested(n)	3	3	3	1	1	11
Leakage in apical filling						
Non					1	1
Partial	1					1
Total leakage	2	3	3	1		9
Leakage in gutta-percha						
Non	2				1	3
Partial		3				3
Total leakage	1		3	1		5

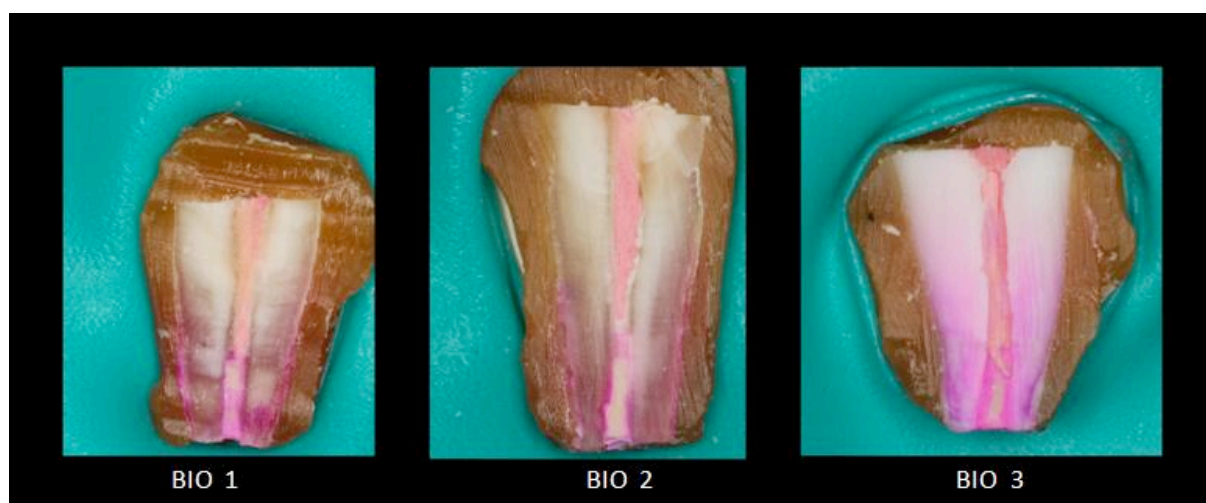


Fig.6A. Results in the Biodentine™ group.

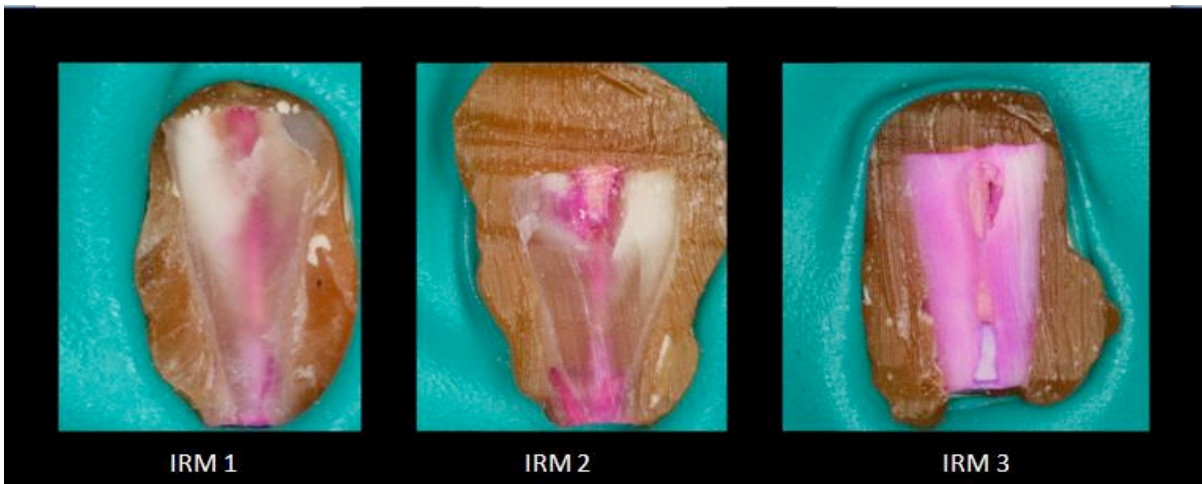


Fig.6B. Results in the IRM® group.

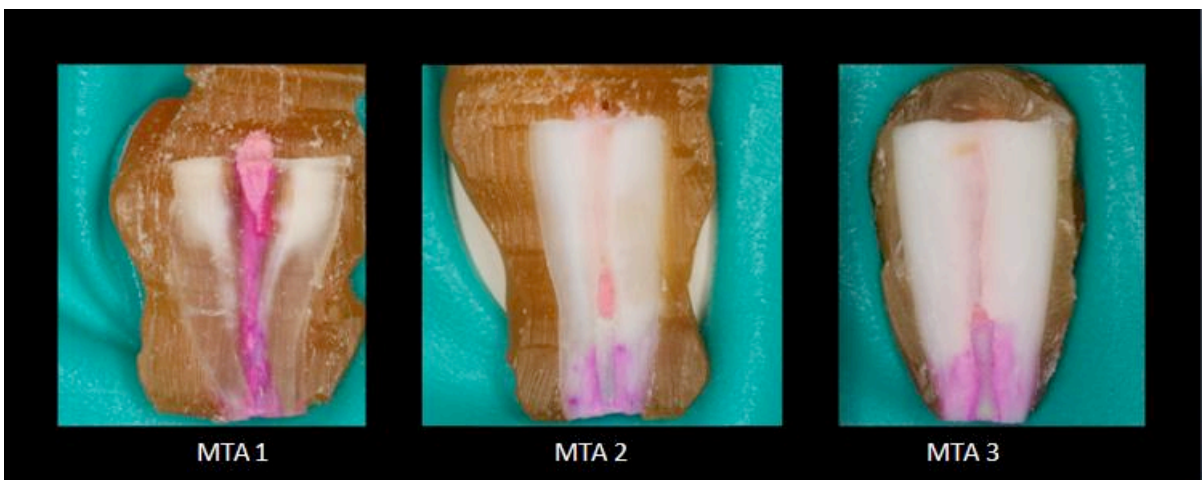


Fig.6C. Results in the MTA group.

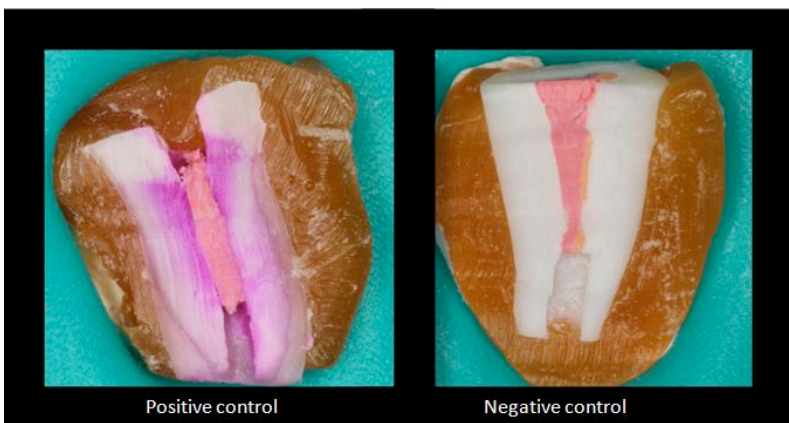


Fig.6D Results in the control groups.

Experiment 3

Figures 7A-D show dye leakage in samples with MTA root-end fillings after different immersion times. No leakage was seen after 1 or 3 hours (Figs. 7A and 7B), but after 6 hours the dye had penetrated entirely the root-end filling and to some extent along the gutta-percha (Fig. 7C). After 12 hours there was a total leakage (Fig. 7D)



Fig.7A. No leakage seen after 1 hour



Fig.7B. No leakage after 3 hours

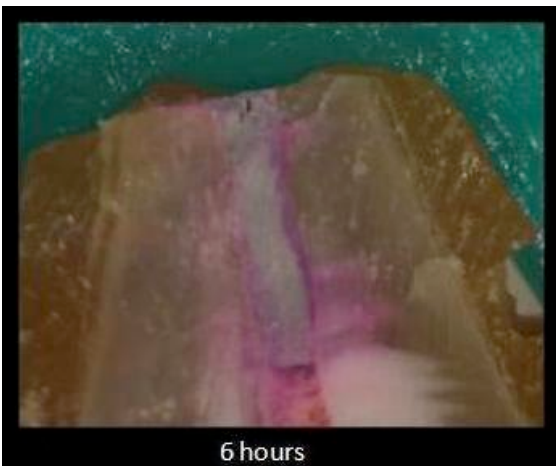


Fig.7C. The dye has penetrated entirely the root end filling and to some extent along the gutta-percha.

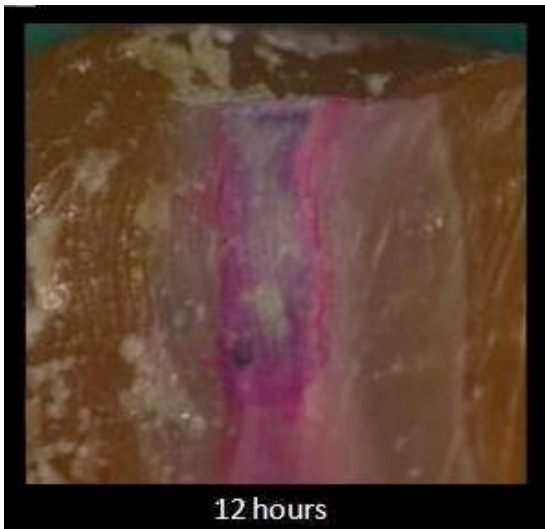


Fig.7D. After 12 hours total leakage is seen along the root end filling and the gutta-percha.

Discussion

Negative and positive controls

The negative control group showed no dye penetration, and there was no fuchsin colour contamination of the sample. In the positive control group, all of the dentinal tooth substance had taken on the fuchsin colour from the dye solution. The size of the dye molecules is small enough to penetrate between the canal wall and the gutta-percha, but not through the sticky wax that gives a total seal.

Pilot experiment results vs. Experimental results

Several parameters have been changed from the experiment 1 to the actual dye leakage experiments, and thus the results should not be compared. However, in the pilot experiment two out of five canals filled with Biodentine™ showed favourable results (no leakage), whereas in experiment 2 all three Biodentine™-filled canals leaked. We cannot be sure whether the results vary due to changes in the method. Canal size and the length of the root-end filling varied among the specimen in the first experiment, which may have an influence on the results. This was standardized in experiment 2, by using only one operator. The application method for MTA and Biodentine™ was standardized after experiment 1, by using suitable carrier tubes. Mixing techniques varied between the two first experiments; some modifications were done to the manufacturers' mixing procedures for each of the tested materials. When using ProRoot® MTA™ carrier tubes, it is desirable to achieve a more creamy consistency of both Biodentine™ and MTA. As a result a higher liquid/powder ratio was necessary. Carrier tubes were not used for experiment 1, but were used both in the case of Biodentine™ and MTA in experiment 2. The change in liquid/powder ratio may have caused the result differences between the experiments, but the root-end fillings look more homogenous and precise in experiment 2, and no voids may be seen between the filling and gutta-percha.

Measuring apical- instead of coronal leakage to evaluate a root-end filling

In experiment 1 *coronal* leakage was evaluated. Reversing the penetration direction was motivated by the aim to evaluate the root-end filling, and not the root canal filling. This modification of the protocol presented

a practical challenge when covering the specimen in sticky wax: The specimen became more difficult to hold while performing the waxing procedure (See: Fig.3)

Radiographic control of the quality of the root-end fillings

Operator inexperience may have had effects on the quality of the apical fillings. The quality of the fillings was not checked using radiographs in the two first experiments. In experiment 3 radiographic pictures were taken, showing that some of the root-end-fillings were not good enough. The fillings were then changed, so that every root-end-filling was at good quality. This might have been the situation in experiment 2 as well, that some of the fillings were at bad quality, affecting the results.

Storage of root-end filling materials

ProRoot® MTA had not been kept in unopened, sealed packets, but had been portioned into autoclaving pouches, and then autoclaved and kept at room temperature. This method of MTA™ storage is practiced in all cases at the Student clinic.

Biodentine™ capsules were close to their expiration date, but not past it. Both of these facts may have affected the results.

Immersion time

Immersing the specimens in dye solution for 48 hours may have been too long. This may have caused false positive results, or unnecessarily poor stratification of the results. After the first experiment it was concluded that the immersion time was suitable for the planned experiment 2. Even though, the second experiment showed total leakage in almost every specimen. The negative control group showed no leakage at all, and it was concluded that the error was according to immersion time. A separate experiment was done to establish the proper dye immersion time, showing that total leakage occurs somewhere between three and six hours.

Conclusion

MTA was the only material to at least partly resist apical leakage in experiment 2. In order to differentiate between the samples of the different materials, the proper dye immersion time had to be established in a separate third experiment, using MTA samples only. Experiment 3 showed that total leakage occurs between three and six hours. The experiment should now be repeated in order to establish the ability of Biodentine™ to resist apical leakage compared to MTA.

Currently we do not have the substance to draw conclusions on neither the experimental outcomes nor the clinical outcomes of Biodentine™. There are no papers reviewing the outcomes of Biodentine™ at this time.

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