Sealing ability of two root-end filling materials at different retro-preparation lengths

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Abstract

Purpose: The aim of the present study was to evaluate the sealing performance, expressed as microleakage (ML), of two root-end filling materials when used at different retro-preparation lengths.

Methods: Fifty single-rooted human teeth were collected for the study. The teeth were cut at the cement-enamel junction and endodontic treatment was performed. Each root was cut at 3 mm from the apex and then stored in wet condition. The teeth were divided into three groups according to the retro-preparation length: control group (no retro-preparation); group 1 (retro-preparation of 3 mm); group 2 (retro-preparation of 9 mm). The teeth were equally allocated to either Biodentine or Super EBA treatment group. The teeth were immersed in 3% methylene blue dye solution for 24 h. The samples were split longitudinally and the depth of dye penetration was examined through light microscopy.

Results: No significant statistical differences were found at different retro-preparation lengths (P > 0.05). Differences were found between materials (P < 0.05).

Conclusion: Biodentine showed significantly lower ML when compared to Super-EBA and no statistical significative differences were observed when samples were retro-prepared at 3 mm or 9 mm.

Keywords: endodontic surgery, retro-preparation length, root-end filling materials

Introduction

The objective of conventional root canal treatment is to hermetically seal any communications between the pulp and the periradicular tissue. Causes of re-contamination of endodontically treated teeth have been attributed to the leakage of either coronal restoration or apical seal that leads to the seepage of bacteria and their by-products to the surrounding periodontal tissues. In the case of failure of traditional endodontic therapy, endodontic surgery is the alternative treatment when all pre-requisites are strictly followed [1].

Endodontic surgery aims at the detension, shaping, and tridimensional obturation of the apical portion of the root canal that cannot be treated through the access cavity, but flap surgery has to be performed and the apical lesion is exposed through a local osteotomy [2]. Apicectomy is then performed as the apical root-end portion of the tooth has been thought to be the main region where persistent bacteria, such as Streptococcus species, Actinobacteria phylum and Pseudomonabacter acetoxyticus may be located [3]. Radicular resection is commonly done 3 mm at the apex, as it represents the region where the higher presence of lateral canals and apical ramifications is encountered. A retrograde preparation is then prepared of about 3 mm-long, that is usually considered an effective distance to hermatically close the lateral canals. However, little information exists on whether longer retrograde preparation depth (i.e., 9 mm) influences the seal of the root restoration. Some cases could require a deeper retrograde preparation, for example, when an orthograde approach is not practicable (presence of proper fixed dental prosthesis) and a post has been placed without correct root canal treatment. In this case, it is possible to use progressively longer ultrasonic tips (i.e., 3 mm, 6 mm, and 9 mm) (Endo Success Apical Surgery tips, Satelec SAS, Acteon Group, Merignac, France) to remove pulp remnants, debris and bacteria, related to the length of the root canal and the post. After accurate disinfection of the root canal, a retrograde filling material is compacted so as to fill the retro-prepared pulpal cavity and seal communications between coronal and apical dental portions against microorganism percolation.

The type of back-up filling material plays a major role when dealing with endodontic surgery treatment. Ideally, the material should be biocompatible and non-irritant against the host and surrounding tissues, un-resorbable, with anti-bacterial properties released over time, radiopaque, dimensionally stable, non-corrosive, resistant to humid environments, and possess regenerative ability of the periodontal ligament. Beside these properties, root-end filling materials have to provide a hermetic seal against toxins and microbes so as not to invalidate the results of the therapy.

An ideal material does not exist, and several products have been proposed over the years to be used in endodontic surgery.

Biodentine (Septodont S.A.S., Saint-Maur-des-Fosses, France) is a calcium-silicate-based cement introduced as a dentine substitute. The material is mainly composed of highly pure tricalcium silicate (which regulates setting reaction), calcium carbonate, zirconium dioxide (as radiopacifier), calcium chloride, water reducing agent, and water. The material has shown immediate and delayed sealing performances comparable to that of other commonly used root-end filling materials, together with good resistance to erosion when submitted to acidic environments, such those usually encountered during inflammation processes [4]. Biodentine has been used in vivo for the re-treatment of previously endodontically-treated teeth with incomplete healing, achieving complete infection resolution after 2-years of treatment [5]. The main benefits of Biodentine are its handling properties, as it is furnished with a low vibrating device and a disposable dispensing needle, and its setting time, which has been estimated to be inferior to Mineral Trioxide Aggregate (MTA) (12 min vs 40 min) and, conversely, more clinically acceptable [6].

Super EBA (Super EBA, Harry J Bosworth Co., Skokie, IL, USA) is a silicon-reinforced zinc oxide eugenol-based cement composed of 32% eugenol and 68% ethoxy benzoic acid (EBA). It has been introduced as a contender for MTA for root-end-filling in endodontic surgery [7]. Good marginal adaptation, biocompatibility and low cytotoxicity represent its main advantages. In particular, materials containing ZOE have been thought to possess antibacterial effects [8] and successful clinical results have been observed when the material has been used for root-end filling restoration [9].

Little information is present in the literature regarding the sealing performance of Biodentine and Super EBA at different retro-preparation depths (3 mm and 9 mm). Therefore, the objective of the study was to evaluate the root-end sealing capacity of Biodentine and Super EBA when used in retro-preparation cavities with different depths. The null hypothesis tested was that no differences would be present between the materials and that the length of retro-preparation would not influence their sealing performances.
Materials and Methods

Selection of the samples

After the approval of ethics committee (Prot. n. 17489, University of Siena, 15-06-2020), fifty teeth, incisors and premolars, extracted for periodontal reasons were collected for this study. Teeth had to be single-rooted, with one straight canal and with fully developed apices so as to be included in the study. Teeth were analyzed with an operative microscope (OPMI pico, Carl Zeiss S.p.A., Milan, Italy) and radiographically, and those presenting previous canal treatments, conservative restorations or caries, signs of fractures or resorption were excluded. Remaining residues of tartar were manually removed with a scaler. The selected teeth were stored in Chloramine T solution at 4°C until use (no more than one month before sample preparation).

Specimen preparation

The samples were prepared by a single operator. The crowns were cut with a cylindrical diamond bur (Komet Italia srl, Milan, Italy) at the cement-enamel junction. The working length was determined by viewing a #10 K-file at the apical foramina. The teeth were subjected to root canal treatment using #08 - #10 - #15 K-files in sequence to establish a glide path. The shaping of the root canals was completed with rotary Ni-Ti instruments: #10.04 - #15.05 - #20.6 - #25.06 (Mtwo; Sweden & Martina, Padua, Italy) mounted on an endodontic handpiece (VDW Gold; VDW GmbH, Munich, Germany) and set according to the manufacturer’s instructions. A 5% solution of sodium hypochlorite (Niclor 5 dentale; Ogna Lab srl, Murigli, Italy) was used as the irrigant solution during the phases of instrumentation and then the canals were dried with absorbent #25 paper points (Mynol absorbent paper points; Ada Products Company, New Franken, WI, USA). Once the fit of the gutta-percha cone (Mynol Gutta Percha Points; Ada Products Co.) was verified, a veil of sealer (Pulp canal sealer; Kerr Corporation, Bioggio, Switzerland) was placed on the walls of the root canal (white zone). The samples were longitudinally sectioned into two halves by means of a diamond blade of a cutting machine under constant water irrigation (MT micro remet; Micerium Spa, Genoa, Italy). X-rays were performed to confirm the quality of the root canal filling and to evaluate the seal of the coronal access cavity. The samples were stored in saline solution for one week until the excesses were removed using a cutting instrument.

Root-end preparation and filling

Retrograde preparation was performed using 3 mm- and 9 mm- long ultrasonic tips (Satelec Endo Success - AS 3D and AS 9D) mounted on an ultrasonic handpiece and powered according to the manufacturer’s specifications. Sterile saline solution was used to cool the tip during the retro-preparation.

Group 1 and 2 were ulteriorly divided into two subgroups according to the material used for the back filling:

- Group 1A (n = 10): no retro-preparation or back-filling (control group)
- Group 1B (n = 10): 3 mm retro-preparation + Super EBA
- Group 2A (n = 10): 9 mm retro-preparation + Biodentine
- Group 2B (n = 10): 9 mm retro-preparation + Super EBA

Biodentine is a bioactive cement and it was used according to manufacturer’s instructions: five drops of liquid were placed inside one capsule with powder, then the capsule was placed in a mixer for 30 s. The material was placed inside the root canal using a carrier (MAP One; Produits Dentaires SA, Vevey, Switzerland) and gently compacted with a retro-filling plugger (Hu Friedy, Chicago, IL, USA). All the excesses were removed with a cutting instrument.

Two measuring spoons of powder of Super EBA were mixed with a drop of liquid on a glass plate using a spatula. The material was quickly placed within a groove of a plastic model in order to obtain a cylindrical shape, and then it was placed inside the root canal with a Kayhat apical filling instrument (Hu Friedy), compacted with a retro-filling plugger and the excesses were removed using a cutting instrument.

The samples were stored at 37°C and 100% relative humidity for 48 h. A double layer of nail varnish was then applied to the whole surface of each root except the last 1 mm from the apex. After complete exsiccation of the varnish, the samples were immersed in a 3% methylene blue dye solution for 24 h.

Subsequently, teeth were carefully washed under abundant running water for no less than 15 min in order to completely remove any remnants of the dye solution. The varnish was removed with a cutter. Each tooth was longitudinally sectioned into two halves by means of a diamond blade of a cutting machine under constant water irrigation (MT micro remet; Casalecchio di Reno, Bologna, Italy). Each half of the tooth was fixed with cyanoacrylate (Locite Super Attak, Henkel Italy Srl, Milano, Italy) on a laboratory plexiglass slide.

Each section was analyzed with light microscopy (Nikon E800; Nikon, Tokyo, Japan). Images were acquired at 4× and 10× magnifications and observed by a single operator.

The presence of dye coloration along the filling material/root interface was evaluated. Greater amounts of dye along the interface, correspond to a greater amount of microleakage. Light-microscopy images showed penetration of the blue dye solution at different levels in the experimental groups (Figs. 1, 2).
Table 1 Distribution of the score variable between Groups (median, IR, IC95%)

|                  | Positive control (n = 10) | Group 1 (n = 20) | Group 2 (n = 20) | p    |
|------------------|---------------------------|------------------|------------------|------|
| Score            | 2.50                      | 2.00             | 2.50             | 0.63 |
|                  | (1.75-3.00)               | (0.25-3.00)      | (0.25-3.00)      |      |
|                  | (1.50-3.00)               | (1.00-3.00)      | (1.00-3.00)      |      |

Table 2 Distribution of the score variable between Groups (median, IR, IC95%)

|                  | Positive control (n = 10) | Biodentine (n = 20) | Super EBA (n = 20) | p    |
|------------------|---------------------------|---------------------|---------------------|------|
| Score            | 2.50                      | 1.50                | 3.00                | 0.043|
|                  | (1.75-3.00)               | (0.00-2.75)         | (1.25-3.00)         |      |
|                  | (1.50-3.00)               | (0.00-2.00)         | (2.00-3.00)         |      |

Table 3 Distribution of the score variable in the Group with 3-mm retro preparation

|                  | Positive control (n = 10) | Biodentine (n = 10) | Super EBA (n = 10) | p    |
|------------------|---------------------------|---------------------|---------------------|------|
| Score            | 2.50                      | 1.00                | 3.00                | 0.18 |
|                  | (1.75-3.00)               | (0.00-2.25)         | (0.75-3.00)         |      |
|                  | (1.50-3.00)               | (0.00-2.50)         | (1.00-3.00)         |      |

Table 4 Distribution of the score variable in the Group with 9-mm retro preparation

|                  | Positive control (n = 10) | Biodentine (n = 10) | Super EBA (n = 10) | p    |
|------------------|---------------------------|---------------------|---------------------|------|
| Score            | 2.50                      | 2.00                | 3.00                | 0.19 |
|                  | (1.75-3.00)               | (0.00-3.00)         | (1.75-3.00)         |      |
|                  | (1.50-3.00)               | (0.00-3.00)         | (1.50-3.00)         |      |

Results

The observed variable was a score measured on an ordinal scale, so it was expressed as a median (interquartile range, IR). The Kruskal-Wallis non-parametric test was used to test the significant difference between more than two independent groups and, in the case of significance, Dunn’s multiple comparison test was used. All analyses were performed by means of the Bootstrap method on 10,000 resamplings, calculating the estimates using the BCa (bias-corrected and accelerated) method to increase the power of the test. All analyses were performed by means of SPSS software (v.20) and the results associated with a P < 0.05 were considered as significant.

Sample characteristics

The final analysis sample consisted of 50 teeth, divided into three groups: Positive Control (samples were not subjected to retro-preparation or retro-filling), Group 1 (samples received 3 mm retro-preparation) and Group 2 (samples received 9 mm retro-preparation). Groups 1 and 2 were divided into two further subgroups. In Group 1 the canals were filled with Biodentine, and in Group 2 with zinc oxide eugenol cement reinforced with the addition of benzoic acid and alumina (Super EBA). The degree of infiltration was assessed through a score as follows:

0: dye not visible in any of the areas of interest;
1: penetration of the dye up to the apical area of the preparation;
2: penetration of the dye on the corner of the preparation;
3: penetration of the dye on the internal walls of the canal, along the preparation.

Comparison between Positive Control, Group 1, and Group 2

Due to the nature of the data (scores on an ordinal scale) the test used to compare the medians between the groups was the non-parametric Kruskal-Wallis test with exact method. The values in the table (Table 1) refer to the median (IR). The confidence intervals were also calculated with Bootstrap BCa method at 95%.

The analysis conducted to evaluate any significant difference between the materials used (Biodentine and Super EBA), regardless of the Group they belonged to (3 mm or 9 mm) (Table 2), showed a statistically significant difference between the groups: the Group of teeth treated with Biodentine tended to have significantly lower values than those treated with Super EBA, (P = 0.02), regardless of the type of retro-preparation performed (Fig. 4).

The analysis conducted to evaluate the possible significant difference between the materials used separately for the group treated with Biodentine and the one treated with Super EBA (Tables 3, 4), showed no statistically significant difference between the groups. Despite this, it was observed that both for the group with 3 mm retro-preparation (Group 1) and for the group treated with 9 mm retro-preparation (Group 2), the group of teeth treated with Biodentine tended to have lower values on average than those treated with Super EBA (Figs. 5, 6).

From the analysis carried out it is possible to conclude that there are no statistically significant differences for the score considered between the samples that received a retro-preparation of 3 mm, 9 mm and the sample that did not receive any retro-preparation. Despite this, it was observed on average lower values for the Group with 3 mm retro-preparation.

From the comparison between the materials used, regardless of the type of retro-preparation, the Group with Biodentine showed a significantly lower score than the Group treated with Super EBA.

Finally, comparing the score between the materials used, separately by type of retro-preparation, no statistically significant differences were observed. Despite this, the Group treated with Biodentine, once again showed average score values lower than the Group treated with Super EBA, both for the teeth treated with 3 mm and 9 mm retro-preparation.
length of retro-preparation was not a factor influencing the sealing ability of the materials. However, from a clinical point of view, a retro-preparation deeper than 3 mm is not advisable due to higher technical difficulties, such as the necessity to use longer tips in a confined space and the need to employ an ultrasonic device for a longer period of time, and this could cause increased risk of dentinal crack formation [15].

In this study a 3 mm root resection from the apex, perpendicular to the long axis of the tooth, was performed, in order to eliminate the root portion where it is more likely to find apical delta and lateral canals [16]. The retro-preparation was performed using diamond ultrasonic inserts powered at half the maximum power [17]. The introduction of ultrasonic devices has, clinically, provided many advantages in comparison to conventional burs: deeper root-end preparation along with better visual perception, preservation of the original path of the root canal, reduced osteotomy with consequent less post-operative trauma and faster healing, inferior risk of lingual injury, less smear layer production, no increase of dentine temperature [18-20].

Diamond inserts were chosen in this study due to their advantageous characteristics as they permit a faster root canal preparation when compared to the steel or Zr inserts, and they do not increase dentinal crack formations or alteration of the seal quality obtained after the canal filling [21].

Some authors have proposed the use of a laser to perform retro-preparation, but it does not seem to offer any advantages over an ultrasonic device [22]. The most important factor, during the retro-preparation phase, would seem to be the ultrasonic power which should be set at half of the maximum allowed by the device.

The ultrasonic inserts at maximum power produce a higher formation of dentinal cracks, resulting in greater cavity margin formation which may affect the sealing ability of the root-end filling material or favor bacteria growth [16].

Furthermore, Tawil et al. in their study encourage clinicians to incorporate the use of transillumination with a microscopic light emitting diode light to systematically determine the integrity of root apices during periapical microsurgery. [23].

The second result obtained in the study was that differences exist among the tested materials, as Biodentine demonstrated higher sealing quality compared to the Control Group and Super EBA. A direct comparison of microleakage expression between Super EBA and Biodentine has never been carried out. For a long time, the gold standard material for retrograde filling or root perforation repair was the MTA; this material has been mainly compared in the literature with other materials proposed for the same purposes, including Super EBA and Biodentine used in this study.

Biodentine is a new bioactive material that was introduced in 2011. The powder mainly contains tri- and dicalcium silicate (the main components of Portland cement) and calcium carbonate. The liquid is an aqueous solution of calcium chloride, to which a poly-carboxylate is added. During the setting of the cement, calcium hydroxide is formed. In the mixed state, Biodentine can be handled for about 12 min and has a consistency reminiscent of phosphate cement. According to About et al., Biodentine stimulates dentine regeneration by inducing odontoblast differentiation from pulp progenitor cells. Biodentine overcomes some disadvantages of MTA, in particular reduced setting time which makes it especially advantageous for apical surgery. [5]. Biodentine exhibited significantly greater push-out bond strength than MTA as a furcation repair material under blood contamination [24]. It has been also hypothesized that the higher amount of calcium-releasing products in Biodentine than in MTA may promote higher bio-mineralization and higher bond strength [25,26]. As found in a recent literature review by Torabinejad et al., little information is available regarding the follow-up of cases that received root-end fillings with new bioactive materials [27]. Accordingly, extended follow-up is required so as to validate real treatment outcomes.

Super EBA consists of a powder (62.5% ortho-ethoxy benzoic acid, 37.5% Eugenol) and liquid (60% zinc-oxide, 34% alumina, 6% natural resin) that have to be mixed immediately before use. Previous studies comparing MTA and Super EBA revealed that MTA determines a newly formed cementum coverage [28], better marginal adaptation [29], increased osteoblast activity [30] and is less cytotoxic than Super EBA [31]. Although a prospective randomized controlled study has shown no significant difference in the clinical outcomes of endodontic microsurgery when Super EBA
and MTA were used as root-end filling materials [32], the main advantages shown by Super EBA appear to be the handling properties and setting time. Future studies are currently ongoing to increase the number of samples employed in the study. In vivo investigations are also necessary to confirm the obtained results.

The evaluation of the sealing ability, expressed as microleakage, is a reliable method to test the use of a filling material in endodontic treatment and endodontic surgery [33]. Different methodologies have been proposed as an in-vitro test of the sealing capacity of a root-end-filling material such as: degrees of dye infiltration [6, 33], bacterial penetration [3], electro-chemical leakage [34] and fluid filtration technique [4]. Aqarabawi et al., reported that if root-end-filling materials are able to prevent dye penetration of small particles, they are able to also resist bacteria penetration and their sub-products [33], which supports the use of dye solution penetration as a valid method for microleakage evaluation.

Conflict of interest
The authors have no conflicts of interest to declare.

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