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Original Article

Comparison of apical microleakage of mineral trioxide aggregate, calcium-enriched mixture cement and biodentine as root end filling materials

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Abstract

Introduction: The aim of this in vitro study was to assess the apical microleakage of mineral trioxide aggregate (MTA), calcium enriched mixture (CEM) cement, and biodentine.

Materials & Methods: The study was performed on 76 single-canal human teeth. Root canals were prepared by ProTaper rotary system, then obturated with gutta-percha. Thereafter, the apical section of the teeth was cut from 3 mm above the apex, and 3 mm of gutta-percha was removed by an ultrasonic device from the apical part of the root canals. Then, 60 teeth were randomly assigned to 3 groups and filled with MTA, CEM cement, and biodentine. Control groups were also prepared. All surfaces of the samples were covered with two layers of nail polish, except for the surfaces near the apical filling. In each group, half of the samples were immersed in indian ink for 3 days and the other half for 7 days. After clearing, the samples were examined using stereo microscope with 20x magnification and dye penetration was evaluated. Data analysis was performed using Repeated measures and One-way ANOVA and $p<0.05$ was considered significant.

Results: The mean microleakage for MTA, CEM cement and biodentine on the third day were 2576.80 and 2567.60, 2370.20 and the mean on the seventh day were 2431.50, 1516.50 and 1560.70, respectively. The mean leakage was not statistically different in samples on the third and seventh days. The difference of microleakage was statistically significant among these materials.

Conclusion: It seems the biodentine compared to MTA and CEM cement have better apical sealing ability.

Keywords: Biodentine, Mineral trioxide aggregate, Dental leakage, Root canal filling materials

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مقایسه میزان ریزنشت اپیکالیMTA ، سمان غنی شده کلسیمی و بیودنتین به عنوان ماده پر کننده انتهای ریشه دندان

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چکیده

مقدمه: هدف از این مطالعه آزمایشگاهی بررسی میزان ریزنشت اپیکالی سه ماده MTA، CEM cement و بیودنتین می باشد.

مواد و روش ها: این پژوهش با ۷۶ نمونه دندان تک کانال انجام شد. آماده سازی کانال ها با فایل های روتاری پروتیپر انجام گردید و کانالها به طور کامل با گوتا پرکا پر شدند. ۳ میلی متر انتهای ریشه هر یک از نمونه ها قطع گردید و به کمک دستگاه اولتراسونیک ۳ میلی متر از گوتا پرکا از سمت اپکس خارج گردید و به طور تصادفی و در گروههای مساوی ۶۰ نمونه با MTA ، Cem cement و بیودنتین پر گردید و همچنین گروه های کنترل آماده گردید. تمامی سطوح نمونه ها به جز ناحیه مجاور پر کردگی با ۲ لایه لاک ناخن پوشیده شد. در هر گروه نیمی از نمونه ها به مدت ۳ روز و نیمی دیگر به مدت ۷ روز در جوهر هندی غوطه ور شدند. بعد از شفاف سازی ، نمونه ها با استریومیکروسکوپ با بزرگنمایی ۲۰ ارزیابی شدند و میزان نفوذ رنگ مشخص شد. آنالیز داده ها توسط آنالیز واریانس یک طرفه و آنالیز واریانس با داده های تکراری انجام شده و سطح معنی داری $p<0.05$ تلقی گشت.

یافته ها: میانگین ریزنشت MTA، CEM cement و بیودنتین در روز سوم به ترتیب؛ ۲۵۷۶/۸۰ ، ۲۵۶۷/۶۰ و ۲۳۷۰/۲۰ و نیز میانگین ریزنشت در روز هفتم به ترتیب ۲۴۳۱/۵۰، ۱۵۱۶/۵۰ و ۱۵۶۰/۷۰ بدست آمد. میانگین ریزنشت نمونه ها در روزهای سوم و هفتم تفاوتی را نشان نداد. همچنین تفاوت ریزنشت بین این سه ماده معنا دار بود.

نتیجه گیری: به نظر می رسد بیودنتین نسبت به MTA و CEM cement توانایی سیل کردن بهتری را در ناحیه انتهای ریشه دارد.

واژگان کلیدی: بیودنتین، Mineral trioxide aggregate ، نشت دندان، مواد پر کننده کانال ریشه

Introduction

Periapical surgery is usually conducted to eliminate a part of the root with unprepared canal. It is also done in cases for them non-surgical root canal treatment with coronal access is impossible. The primary indications of periapical surgery are anatomic considerations such as irretrievable material in the root canal, blocked or unidentifiable root canals and severe root curvature, intraoperative accidents, biopsy, symptomatic cases, horizontal root fracture, and corrective surgeries. [1] Apical resection is one of the main procedures in periapical surgery. The apical resection includes beveling the apical segment of the root, providing two objectives. Firstly, the unprepared apical part of the root is removed, allowing the surgeon to determine the reasons for failure. Secondly, a smooth surface is provided for appropriate apical cavity preparation and placement of suitable filling material. Apical filling material must possess features like resistance to resorption, tolerability by the periapical tissues, good sealing ability, easy placement, detectability on radiographs, not being affected by water, and providing

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the possibility of tissue regeneration. [2] The mineral trioxide aggregate (MTA) is a material developed exclusively for sealing of the apical section of teeth. Its main constituents are calcium carbonate, calcium silicate, calcium aluminate, bismuth oxide and calcium sulfate. The MTA compared to super EBA amalgam and IRM has shown less leakage when used for apical filling and management of lateral perforations. [1,2] The MTA is the first recommended material to obturate the apical part of the canal. In addition, it is used for pulp capping, pulpotomy, apexogenesis and dental perforations. [3]

The MTA has the most tissue compatibility compared to other canal obturating materials including amalgam, gutta-percha, zinc oxide eugenol, glass ionomer and composite. [4] Calcium enriched mixture (CEM) cement is another material with different characteristics compared to MTA though its tissue compatibility is as good as MTA. [5] The CEM cement's formula is different from calcium. This material possesses a low cytotoxicity. Studies have suggested that the CEM cement releases phosphate and calcium ions and then transforms into hydroxyapatite. Its clinical applications are similar to those of MTA with similar usage in pulp capping and restoration of perforations. [6,7] The antibacterial activity of CEM cement is similar to calcium hydroxide and higher than MTA. Compared to MTA, the CEM cement has higher flowability, less working time and similar sealing ability. Another feature of CEM cement is its long setting time. [8,9]

Biodentine is recently applied as an innovative material for pulp capping. Features including biocompatibility, the ability to induce differentiation of odontoblasts and mineralization of dentine and suitable sealing ability are reported for biodentine. Mechanical and handling properties of biodentine are more favorable than MTA and CEM cement. Its application as a pulp capping and restorative material has been proven, too. Its setting time is about 10-14 minutes, making it more suitable than MTA and CEM cement.

Biodentine is available as capsules with specific amounts of powder and liquid. The powder contains tricalcium silicate (main ingredient), decalcium silicate (second main ingredient), calcium carbonate (filler), zirconium oxide (radioapacifier) and ferrous oxide (dye). [10] The liquid contains calcium chloride which has the role of a water-soluble polymer as an accelerator and acts as a reducing agent similar to water. However, the manufacturer has not revealed the exact percentages of these constituents. [11] Biodentine has a pH of 12 and can release calcium and silicate ions, leading to stimulation of mineralization and formation of a mineral infiltration zone at the dentin-cementum interface, eventually causing better sealing ability. [12]

Therefore, due to different characteristics of these materials and considering the few number of studies on comparing the biodentine with two other materials, this study was conducted. The aim of this study was to compare the microleakage of these apical filling materials.

Materials & Methods

This study was evaluated after obtaining the ethical approval from Babol University of Medical Sciences (IR.Mubabol.HRI.REC.1398.112). Then, 76 extracted single-canal maxillary central incisors were used. The selected teeth had no resorption, crack or fracture below the cementoenamel junction (CEJ). Moreover, the teeth had complete and closed apex and were not endodontically treated. The teeth were cleaned immediately after extraction and the attached soft or hard tissues were removed. The samples were disinfected through soaking in 5.25% sodium hypochlorite (Golrang Co., Tehran, Iran) for 24 hours. Thereafter, they were kept in sterile 0.9% physiologic serum (Tehrandarou, Tehran, Iran) at the room temperature until the experimental phase. Then, the teeth were cut perpendicular to the long axis below the CEJ by a diamond disk (010, Tizkavan, Tehran, Iran) using copious amounts of water. Samples with an approximate length of 10 mm were prepared.

ProTaper (Dentsply, Tulsa USA) rotary files were used for canal preparation according to the manufacturer's instructions. After root canal preparation, the canals of 68 teeth were obturated with gutta-percha (Gapadent, Seoul, Korea) and AH26 sealer (Dentsply, DeTrey, Konstanz, Germany) by lateral condensation technique. Furthermore, 8 teeth were selected as positive controls and were not filled to show complete penetration. Then, the apical 3 mm of all samples was cut by the disk. In order to provide the suitable space required for filling materials, 3 mm of gutta-percha was removed by an ultrasonic device from the apical part of the obturated root canals.

Next, 60 samples were divided into two major groups (A and B) with 30 samples in each group. Each major group was then divided into 3 groups, each with

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10 samples. Samples in these 3 groups were filled with MTA (Angelus, Londria, PR, Brazil) (Fig1), CEM cement (Doustkam Co., Tehran, Iran), or biodentine (Septodont, Saint-Maur-des-Fosses Cedex, France) (Fig2), respectively. Besides, 8 negative control samples were filled with warm gutta-percha (Fig3).

After that, all samples were placed in wet cotton balls to allow complete setting of the materials. The samples were then placed in normal saline and kept in the incubator for 1 week. Thereafter, all surfaces of the samples were covered with 2 layers of nail polish (Doobina Co., Tehran, Iran), except for the area near the apical filling material. For the negative control samples, the area near the apical filling was also covered with nail polish. After that, the samples of group A along with half of the samples of the positive (Fig4) and negative control groups were immersed in indian ink (Pelikan, Tehran,Iran) for 3 days. The samples of group B and the remaining positive and negative control samples were immersed in the ink for 7 days. After these periods, the samples were removed from the ink container and rinsed with tap water for 5 minutes. Clearing was performed to visualize the amount of microleakage.

For this purpose, the nail polish was removed from the samples by acetone-soaked cotton balls. Then, all samples were placed for 72 hours in 5% nitric acid solution (Merck, Germany) at the room temperature for decalcification. The nitric acid solution was daily replaced, and manual mixing of the samples in the acid was performed 3 times a day. The end point of decalcification was determined by an explorer and radiographic examination. At the end of this stage, the roots had an elastic consistency. After complete decalcification, the roots were rinsed with tap water for 4 hours.

Finally, dehydration was performed by ethylic alcohol (Merck, Frankfurt, Germany) with different percentages. The samples were kept in 75% alcohol for 24 hours, 85% alcohol for 1 hour and 3 one-hour sessions in 96% alcohol. Then, the teeth were placed in methyl salicylate liquid (Merck, Germany). After 3-4 hours, as shown in figures, the samples become completely clear and their internal contents become visualized



Fig 1. A specimen of a MTA microleakage



Fig 2.A specimen of a biodentine microleakage



Fig3. A specimen of negative control without microleakage



Fig4. A specimen of a positive control with maximum microleakage

To measure the dye penetration, a stereomicroscope (Dewinter, Italy) with a magnification of 20 was used. The highest amount of dye penetration along the gutta percha was recorded by Dewinter Capture Pro 4.6 software for each sample (Fig5).

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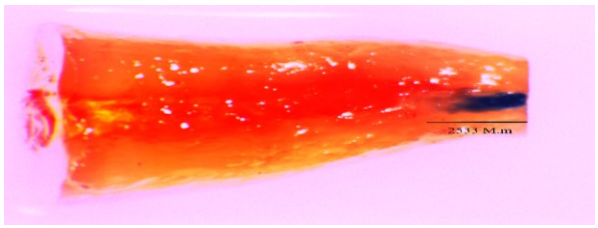


Fig5. A specimen of a measure of microleakage amount from the apical end in the horizontal dimension (2533 µm)

Measuring the microleakage in the surface with the highest dye penetration was performed by a computer and a digital camera. For all 60 samples containing apical filling materials, the amount of microleakage was represented by a number in the micrometer scale and statistically analyzed. Samples in the negative control group had no microleakage, while all positive control samples showed complete microleakage. The data related to the 60 samples in A and B groups were statistically analyzed using SPSS 22. Repeated measures and One-way ANOVA were used to compare microleakage and time. Level of significance was set at α=0.05.

Results

Data analysis in table1 reveals that the mean values and standard deviations of the apical microleakage for these 3 materials after 3 and 7 days are as follows:

Table1. Comparison of the mean microleakage on the third and seventh days by material type

Microleakage amount / Material type	Third day		Seventh day		P*
	Mean	SD	Mean	SD	
MTA	[a]2576.80	268.38	[a]2567.60	474.42	0.95
Biodentine	[b]1516.50	571.90	[b]1560.70	899.98	0.91
Cem cement	[a]2370.20	410.81	[a]2431.50	421.89	0.73
P**	<0.001		0.003		-

Similar letters indicate no significant difference at the 0.05 level

P**: One-way analysis of variance, P*: Analysis of variance with repeated data

To compare the mean microleakage on the third and seventh days based on the type of the filling material, the repeated measure analysis of variance showed that the type of material significantly was associated with microleakage (p<0.001, df=2, F=29.58). Further, the mean value of the microleakage for different materials was not significantly different between days 3 and 7 with a 9.2 µm reduction for MTA (p=0.95), a 44.2 µm increase for biodentine (p=0.91) and a 61.3 µm increase for CEM cement (p=0.73).

The mean value of microleakage in day 3 for biodentine samples was significantly less than that for MTA and CEM cement samples (p<0.001). Similarly, in day 7, this value was significantly less for biodentine compared to MTA and CEM cement (p<0.001). The interaction between the type of material and time was not significant (p=0.98, df=2, F=0.01).

Discussion

The findings of this study suggested that the apical microleakage of biodentine was significantly less than the other materials. Although the biodentine appears to have less microleakage than MTA and CEN cement, it is best to evaluate the bacterial microleakage to confirm the result of this study. In 2017, Ramezanali et al. compared the coronal microleakage in MTA, CEM cement and biodentine in the orifice region. Their findings indicated that the CEM cement and MTA had the least and highest penetration rates in the orifice region, respectively. [13] The difference between their study and the present study was the location of material placement. In the current study, the biodentine showed significantly less apical microleakage compared to MTA and CEM cement. The similarity between the ongoing study and study of Ramezanali et al. was that the CEM cement performed better than MTA in sealing ability. [13]

In another study in 2016, Agrafioti et al. evaluated the sealing ability and microstructure of MTA and biodentine in different environments. Their results demonstrated that both materials are suitable for application in acidic environments. [14] This study highlights the effective application of biodentine compared to MTA. Placing biodentine in acidic environment simulates the metabolic environment of bacteria present in periapical lesions. Therefore, evaluation of apical filling materials in this condition is also beneficial.

In 2015, Wälivaara et al. assessed the sealing ability of biodentine as an apical filling material in a case

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series. They have stated that the biodentine is an appropriate material for apical filling in apical surgeries. [15] This study is noteworthy because it has assessed the performance of biodentine from a clinical standpoint. The location of material placement in their study was similar to the present study, but no comparison was performed in their study. Nevertheless, the application of biodentine in endodontic surgeries was somehow approved.

In 2014, Vemisetty et al. compared the marginal compatibility of biodentine, MTA and glass ionomer cement. The marginal gap between these materials and dentin was measured using a microscope. Biodentine displayed the least marginal gap and best marginal compatibility compared to the other two materials. [16] Although the materials used in this study were not the same as the present study, the biodentine illustrated better performance compared to MTA, which is consistent with our findings. The marginal compatibility which is effective in sealing ability of materials was evaluated in this study.

In another study in 2014, Soleymani et al. compared the apical microleakage of MTA Fillapex and AH26 in dry and blood-containing environments. Though the apical microleakage was less in AH26, the microleakage was not statistically different between these two materials. [17] The advantage of similar studies was the use of different areas of the tooth and different biological environment to compare these materials; however, the major drawback of these studies was the lack of clinical condition with long-term follow-up and use of randomized clinical trials (RCT) to test the adequacy of these materials.

Another procedure performed in the present study was the clearing technique to determine the amounts of microleakage. This technique possesses several advantages compared to other techniques for visualizing root canals under the stereomicroscope. Various techniques are available for comparison of apical microleakage in different materials including dye penetration, dye diffusion, fluid filtration, bacterial and endotoxin infiltration, glucose, caffeine and protein infiltration, radioisotope penetration, animal studies and electrochemical microleakage tests. In the dye penetration technique, performing through soaking the teeth in various dyes such as 5% eosin, 0.5-2% methylene blue, 0.5-1% black Indian dye, rhodamine, fuchsine, and other dyes, the amount of microleakage can be assessed. The clearing technique used in the present study allowed for three-dimensional visualization of the root canal anatomy so that no component of the root canal was missed, and the leakage area became evident on the samples. This technique is simple and fast and requires no complex equipment. [18] Moreover, this technique allows for observation of lateral and accessory canals [19] as well as clearly depicts the relationship between the endodontic sealers and apical foramen.

In general, all mentioned techniques can be useful, provided that an adequate number of samples are used, appropriate control groups are selected and the selected technique can be standardized. [20] Moreover, other studies mentioned in the literature review have revealed that the biodentine is at least equal to the other evaluated material, and no study showed the worse performance of biodentine compared to other materials used in the study. Due to the novelty of biodentine and its suitable performance, further clinical studies with longer follow-up periods are recommended to evaluate the biodentine. Animal studies can also be performed for evaluation of its properties and comparing them to other endodontic materials. This in vitro study was free of pathogens and microorganisms.

Conclusion

Thus, comparing the results with actual oral environment in presence of bacteria needs further studies which assess the microleakage properties of this material in an environment similar to the oral cavity.

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Conflict of interest: We declare no conflict of interest

Authors' Contribution

The study was designed by Ali Soleymani. The study data were collected by Abbas Ghobadi. Analysis and data were performed by Hemmat Gholinia and Abbas Ghobadi. Study supervision was performed by Ali Soleymani.

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