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Original Article

Dentistry Section

Comparison of Sealing Efficiency of Mineral Trioxide Aggregate and Biodentine used in Perforation Repair in Acidic and Neutral Environments- An In-vitro Study

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ABSTRACT

Introduction: Furcation perforation is one of the complications that occur during endodontic treatment due to anatomical differences or iatrogenic causes. Repairing the perforation with a biocompatible material eliminates the connection with the gingival sulcus and positively affects the prognosis. The sealing ability of the repair materials is very important and is affected by the conditions of the environment in which they are applied.

Aim: To compare the sealing efficiency of Mineral Trioxide Aggregate (MTA) (Angelus, Londrina, PR, Brazil) and Biodentine (Septodont, Saint Maur des Fosses, France) materials used in the repair of furcation perforations in acidic and neutral environments.

Materials and Methods: This in-vitro experimental study was carried out in the Department of Endodontics at Dicle University Faculty of Dentistry, Diyarbakır, Türkiye and Dicle University Science and Technology Application and Research Center from 10th June 2020 to 22nd July 2020. A total of 96 mandibular molars were used in the study. The specimens were randomly divided into two equal groups (n=48). When the perforations were repaired with MTA and Biodentine materials, each group was divided into four subgroups (n=12). These subgroups were kept in Phosphate Buffered Saline (PBS) and Acetic Acid (AA) solutions for different periods of time from 4 or 34 days, and all groups were immersed in methylene blue solution. The dye penetration level of the divided sections was examined under a stereomicroscope (Leica, Wetzlar, Germany). Microleakage data of all groups were statistically analysed using Kolmogorov Smirnov, Shapiro Wilk's and Mann-Whitney U Test. Statistical significance was set at 0.05, it was stated that there was a significant difference when p-value <0.05.

Results: The short-term (4 days) microleakage level of biodentine material in the AA environment was significantly lower than that of MTA material (p-value=0.005). There was no statistically significant difference between the materials in terms of long-term (34 days) microleakage levels in the AA environment (p-value >0.05). The long-term microleakage level in PBS environment of biodentine material was significantly lower than that of the AA environment (p-value=0.008).

Conclusion: Lower microleakage levels were observed in the neutral environment than the acid environment with both MTA and biodentine during both holding periods.

Keywords: Acetic acid, Dye penetration, Phosphate buffered saline, Stereomicroscope

INTRODUCTION

Furcation perforation can be expressed as the enlargement of the pulp chamber of the tooth from the pulp floor to the Periodontal Ligament (PDL) space. Inflammation, gingival enlargement, or resorption of the bone structure may occur in that area due to perforation. There are surgical or non-surgical treatment options for perforation. If it is repaired with a biocompatible material with sealing property without any delay in the intervention, the prognosis of the tooth is usually excellent [1].

MTA is one of the materials preferred most by researchers due to its excellent biocompatibility, sealing ability, and antimicrobial properties [2]. Although it was a material originally used to repair perforations, today it is used in vital pulp therapy as root end filling material, in devital teeth with open apex as an apical plug, in the treatment of dentine hypersensitivity and in regenerative endodontic treatment [3,4]. However, it has some disadvantages including long setting time, the difficulty of manipulation, causing discolouration of the tooth structure and high cost [5].

Biodentine (Septodont, Saint Maur des Fosses, France), a new calcium silicate-based restorative cement, has recently been released. It is a fast-setting restorative material that can be used in the same practices as MTA [6]. This calcium silicate-based product was first released in 2009 and was originally designed as a "dentin replacement" material. Biodentine has wide areas of use in endodontic treatments as retrograde filling material in root perforations, apexification, resorptive lesions, and endodontic surgery and in restorative dentistry as dentin replacement material. The material was actually formulated with the improvements in MTA based cement technology and some of the properties of these types of cement, such as physical nature and manipulation ability [7].

The environment in which a material exists may affect its marginal adaptation to dentin, microleakage, and the microstructure and surface morphology of the material [8]. It was reported that pH changes in the surrounding tissues due to pre-existing inflammation during the use of the material might affect the physical and chemical properties of the materials [9].

Many researchers have investigated the properties of MTA and biodentine, such as compressive strength, micro crack formation, marginal adaptation to dentine and impermeability, they compared the advantages of these materials over each other [2,8-10]. However, the effect of pH level on the physical and chemical properties of the materials in the areas they contact during an application is still controversial. In clinical practice, furcation perforations that occur during treatment or pre-exist may be accompanied by an inflammation in the adjacent periradicular tissue. This can alter the pH levels, and therefore, the properties of the material applied.

The aim of this study was to compare the sealing properties of biodentine and MTA using a dye penetration model for quantitative analysis of microleakage following exposure to acidic and neutral environments.

MATERIALS AND METHODS

This in-vitro experimental study, approved by Dicle University Faculty of Dentistry Ethics Board (Protocol no: 2019/31), was conducted in Department of Endodontics at (Dicle University Faculty of Dentistry, Diyarbakır) Türkiye and Dicle University Science and Technology Application and Research Center from 10th June 2020 to 22nd July 2020.

Inclusion criteria: A total of 96 double-rooted mandibular molars that were extracted due to periodontal reasons were used.

Exclusion criteria: Teeth with a large decay extending into the furcation area, whose roots were not separated from each other or whose furcation levels were not the same as the other teeth were excluded.

Sample size calculation: In this study, 96 specimens were included, which were divided into four subgroups with 12 specimens in each group, considering a margin of error of 5% for microleakage, with a confidence level of 95% and a theoretical study power of 99% [11].

Procedure

The selected teeth were cleaned with soft brush and distilled water to remove debris and blood stains and were preserved in saline solution until study procedure.

After the crown lengths of the teeth were shortened to 5 mm coronal to the cementoenamel border by using a high speed bur, endodontic access cavities were prepared. After access opening, perforations were created in the furcation areas of the teeth under water cooling with round burs of 1.4 mm diameter.

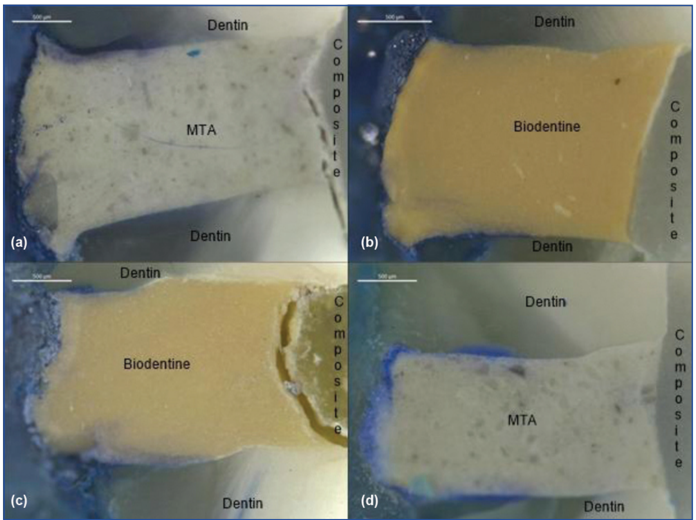
The specimens were randomly divided into two groups based on repair materials: Group A with MTA (n=48) and Group B with biodentine (n=48). The specimens in both the groups were also randomly divided into four subgroups based on storage environment and holding period (n=12). After the perforation areas were covered with the relevant materials, the endodontic access cavities were restored with composite filling material. Group A1 and Group B1 were exposed for four days to PBS solution that mimics the neutral environment (pH 7.4). Group A2 and Group B2 were exposed for four days to an AA solution buffered to pH 4.5. Group A3 and Group B3 were exposed to PBS for 34 days, while Group A4 and Group B4 were kept in PBS solution, which mimics the neutral environment, for 30 days following a period of four days in the AA solution [11].

Cotton pellets were soaked in the solutions (AA or PBS) previously used between the roots of the teeth before the repair in the groups, and the pellets were placed under the perforation area of the furcation sites to reproduce the inside of the mouth. The specimens were then placed in styrofoam, and the perforation areas were covered with the repair materials. It was removed from the styrofoam after it hardened according to the manufacturer's instructions. Gauze pads soaked in the relevant solutions based on their groups were placed between the roots in a way to come into contact with the furcation areas of the teeth taken out from the styrofoam. Afterwards, the specimens in all groups were placed in 15 mL falcon tubes with cap to ensure the continuity of the humid environment. The relevant groups (Group A3 and Group B3) of the specimens to be followed for a long period were kept in the PBS solution for 34 days for that purpose, and the solutions were replaced at 5-day intervals [11]. The groups (Group A4 and Group B4) to be exposed to the AA solution were kept in the solution for four days, and the pads which were soaked in the AA solution were removed. The same specimens were kept in the PBS solution by replacing the solution at 5-day intervals for 30 days. The specimens to be followed for a short period (Group A1, B1, A2 and B2) were kept in the PBS/AA solution for four days. All specimens were kept stable at 37°C in the incubator (Nüve Incubator EN 055, Ankara, Turkey) during the holding period.

All study groups kept in the incubator for the specified periods were removed out of the tubes, washed with distilled water, and cleaned up from the solutions. Dried specimens were dyed with two-coat nail polish covering all surfaces, except the surrounding area of 2 mm to the perforation area to prevent possible leakage from the apical foramen or the microcracks on the surface. All specimens were immersed in 50 mL of 1% methylene blue solution together with their own groups, and they were kept in the solution for 24 hours [12].

The specimens were rinsed with distilled water for 15 minutes to remove excess dye. The molars were fixed to 2×4 cm wooden blocks, and they were then divided into two in the buccolingual direction using the Isomet (Iso-Met® 1000 Precision Saw, Buehler Ltd., Illinois, EUA) device in a way to include the furcation area.

The images of the sections obtained from the specimens were recorded under a stereomicroscope (Leica, Wetzlar, Germany) at X20 magnification [Table/Fig-1]. The level of dye penetration in the recorded images was measured using the Motic Images Plus version 3.0 (Motic Inc., Ltd., Hong Kong, China) software. During the measurement, the level of dye penetration in the area from the bottom of the perforation area to the coronal of the tooth was identified in mm on both walls, and the mean value of these measurements was provided.



[Table/Fig-1]: a) MTA kept in PBS environment for short-term; b) Biodentine kept in Acetic Acid (AA) environment for short-term; c) Biodentine kept in PBS environment for short-term; d) MTA kept in Acetic Acid (AA) environment for a long time.

STATISTICAL ANALYSIS

The study data were analysed using the Statistical Package for the Social Sciences (SPSS) (SPSS, Chicago, IL, USA) version 21.0 software. The Kolmogorov Smirnov Test or the Shapiro-Wilk Test was used to analyse the results of the normal distribution of the variables. The Mann-Whitney U test was used in case the variables were not obtained from the normal distribution while evaluating the differences between the groups. As the variables were not obtained from the normal distribution, the Wilcoxon Test was used while evaluating the difference between two dependent variables. A p-value of 0.05 was considered significant.

RESULTS

Comparison of the Materials According to the Storage Environments and the Periods

The short-term microleakage level of biodentine material in the AA environment (Group B2) was significantly lower than that of MTA material (Group A2) (p-value=0.005). There was no statistically significant difference between the materials in terms of short-term microleakage level in the PBS environment (Group A1 and B1) (p-value >0.05).

There was no statistically significant difference between the materials in terms of long-term microleakage levels in the (Group A3, B3, A4 and B4) PBS and AA environments (p-value >0.05) [Table/Fig-2].

	Short-term (4 Days)		Long-term (34 Days)	
	PBS	AA	PBS	AA
Materials	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)
Biodentine	0.36 (0.3-0.48)	0.33 (0.19-0.5)	0.34 (0.22-0.4)	0.39 (0.32-0.55)
MTA	0.33 (0.18-0.44)	0.48 (0.32-0.58)	0.31 (0.06-0.5)	0.44 (0.37-0.48)
p-value	0.14	0.005*	0.37	0.58

[Table/Fig-2]: Comparison of the materials according to the storage environments and the periods (mm).
The Mann-Whitney U Test *: statistically significant

Comparison of the Environments According to the Repair Materials and the Periods

The short-term (Group A1) and long-term (Group A3) microleakage levels of MTA material in the PBS environment were significantly lower than those of the AA environment (Group A2 and A4) (p-value <0.05).

There was no statistically significant difference between the environments in terms of the short-term microleakage levels of biodentine material (p-value >0.05). However, the long-term microleakage level in the PBS environment (Group A3 and B3) was significantly lower than that of the AA environment (group A4 and B4) (p-value <0.05) [Table/Fig-3].

	Short-term (4 Days)		Long-term (34 Days)	
	MTA	Biodentine	MTA	Biodentine
Environment	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)
PBS	0.33 (0.18-0.44)	0.36 (0.3-0.48)	0.31 (0.06-0.5)	0.34 (0.22-0.4)
AA	0.48 (0.32-0.58)	0.33 (0.19-0.5)	0.44 (0.37-0.48)	0.39 (0.32-0.55)
p-value	0.001*	0.34	0.002*	0.008*

[Table/Fig-3]: Comparison of the environments according to the repair materials and the periods (mm).
The Mann-Whitney U Test; *: statistically significant

Comparisons of the Periods According to the Repair Materials and the Storage Environments

There was no statistically significant difference was observed between short-term (Group A1, A2) and long-term (Group A3, A4) of the microleakage levels of MTA material in the PBS and AA environments (p-value >0.05).

There was no statistically significant difference between the periods in terms of the microleakage levels of biodentine material in the PBS environment (p-value >0.05). On the other hand, the short-term (Group B2) microleakage level of biodentine material in the AA environment was significantly lower than the long-term (Group B4) microleakage level (p-value=0.045) [Table/Fig-4].

	MTA		Biodentine	
	PBS	AA	PBS	AA
Periods	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	Median Min-Max)
Short-term	0.33 (0.18-0.44)	0.48 (0.32-0.58)	0.36 (0.3-0.48)	0.33 (0.19-0.5)
Long-term	0.31 (0.06-0.5)	0.44 (0.37-0.48)	0.34 (0.22-0.4)	0.39 (0.32-0.55)
p-value	0.407	0.054	0.145	0.045*

[Table/Fig-4]: Comparisons of the periods according to the repair materials and the storage environments (mm).
Wilcoxon Test was statistically significant

DISCUSSION

Perforation is a pathological connection between the root canal system and the surrounding periodontal tissues. It may develop during pathological processes, such as caries and internal/external resorption, or may occur during endodontic treatment and intracanal post implementations [13]. Perforation is one of the reasons for endodontic treatment failure. This pathological connection causes an inflammatory reaction in the tissue with which it comes into contact, and it significantly affects the long-term prognosis of teeth [14,15]. Therefore, perforation areas should be repaired with a suitable material. Today, furcation repair materials preferred most are bioactive substances such as MTA and biodentine. Many in-vitro and in vivo studies have shown that the sealing ability and biocompatibility of MTA is superior to conventional materials [1,16]. MTA has high pH and radiopacity levels [17]. However, MTA has some disadvantages, such as the length of the initial setting time (3-4 hours), difficulty in using (depending on water/powder ratio), and high cost [5]. On the other hand, biodentine, which is a calcium silicate-based restorative cement, is a fast setting restorative material that can be used in the same practices as MTA [18].

An important factor affecting the success in the repair of perforations is the sealing property of the materials used [19]. An ideal material should provide complete sealing. The sealing ability of MTA has been investigated in different in-vitro studies using dye penetration method or fluid filtration technique [20,21]. In the present study, biodentine was used by considering this situation, and the sealing ability of biodentine was compared with MTA which suggested better sealing results compared to other traditional materials in the literature review.

In many studies comparing biodentine and MTA, it was indicated that there was no statistically significant difference between the two materials in microleakage level [22-24]. However, in a study, it was reported that biodentine provided better results compared to MTA, although the results were not statistically significant in repairing furcation perforations [25]. On the other hand, some studies suggested that MTA was superior to biodentine in prevention against microleakage when used to repair perforations [26,27]. The results of the present study suggested that there was no significant difference in microleakage levels between MTA and biodentine used to cover the perforation areas within the neutral pH range.

The environment where the material is applied may change marginal adaptation to dentine, microleakage, and the microstructure and surface morphology of the material [28]. Changes in the pH of the relevant tissues due to a pre-existing inflammation during the implementation may affect the physical and chemical properties of these materials [29]. The surface of the unset repair material applied to the area to repair furcation perforations may be exposed to a lower pH [30]. Torabinejad M and Chivian N, stated that MTA could remain unset when applied to a highly inflamed perforation area and that the inflammation area surrounding a tooth may have an acidic pH level of as low as 5.5-5.6 [31]. Moreover, Nilforoushan MR and Sharp JH, reported that lithium salts in the environment, including sodium chloride, had a significant effect on the chemical reactions of calcium silicate-based materials [32]. Therefore, it can be assumed that environmental factors such as acidic pH or the types of ion in the environment may affect the setting reactions of MTA. It was also reported that the surface hardness of MTA deteriorated in an acidic environment [33]. Elnaghy AM observed that a significant change occurred in the microstructure of biodentine and MTA exposed to different pH levels and stated that the surface microhardness, compressive strength, and the binding affinity of the repair material and dentine changed. As suggested by the results of the study by Elnaghy AM, MTA may be more sensitive than biodentine to environments with acidic pH [34]. The results of the studies in which the changes in the physical and chemical properties of the materials exposed to different pH levels were reported showed that biodentine seemed a more suitable option in an acidic environment compared to MTA [33,35]. However, there are still limited data in the literature regarding the effect of low pH on biodentine. In the present study, repair materials with changing physical and chemical properties in the acid environment were evaluated about microleakage in light of this information. The results of the present study were similar to other studies in the literature, and biodentine had a better sealing property in environments with low pH levels than MTA [33,35].

After the study groups were exposed for three days to environments with pHs of 4.4, 5.4, 6.4, and 7.4, Saghiri MA et al., filled retrograde cavities with MTA and evaluated microleakage using bovine serum albumin. The highest microleakage level was observed at pH 4.4, followed by pH 5.4, 6.4, and 7.4, respectively. They stated that a significantly longer period of time was required for leakage to occur in the samples stored at higher pH levels [36]. In the present study, MTA coming into contact with pH 7.4 PBS solution mimicking the neutral environment showed more sealing ability than MTA samples coming into contact with pH 4.5 AA solution. Our study results were similar to the results of the study by Saghiri MA et al., Roy CO et al., who compared the sealing ability of retrograde filling materials, and reported that the contact of MTA with an acid environment did not affect its sealing ability and that when the Calcium Phosphate Cement (CPC) matrix was used, the sealing property of MTA in the acidic environment increased [36,37]. The use of a CPC matrix, acid with a different type and not buffered, and the short exposure time (24 hours) of MTA to acidic solutions may be the reason for the inconsistency with the results of our study.

In a study comparing the solubility of MTA and biodentine materials in environments with different pH levels, Pushpa S et al., reported that acidic pH (pH 4.4) increased the solubility of both materials. They directly correlated the sealing potential and dimensional changes of an endodontic repair material with its solubility that might leave gaps supporting bacterial colonisation and its penetration into periapical tissues [38]. Accordingly, we can suggest that acidic pH negatively affects the sealing properties of MTA and biodentine. The results of our study were in line with this inference.

Agrafioti A et al., evaluated the sealing ability and morphological microstructure of biodentine compared to MTA in dentine discs after storage in an acidic environment. In the 3rd month measurements, there was statistically significantly superior sealing property with MTA kept in PBS solution than biodentine that was kept under the same conditions. However, when the materials were stored in an acidic environment, no statistically significant difference was observed in the 3rd month measurements. Moreover, they reported that the exposure of biodentine to an acid environment increased its sealing ability over time [39]. In the present study, the long-term storage condition was determined to be 34 days, and it was observed that coming into contact with the acid environment during this period negatively affected the sealing property of both materials. There was no significant difference between the sealing properties of the two materials kept for a long time in both environments. The differences between studies may result from different types of acid used and different levels of pH, the fact that the prepared solutions were not buffered, varying periods of contact with the solutions, and different techniques used for microleakage examination. Studies evaluating the microleakage of biodentine and MTA have been compared in [Table/Fig-5].

Elnaghy AM, examined the microstructure of MTA in environments with different pH levels using a scanning electron microscope, and in the images obtained, they showed that the microstructure changed towards acidic pH levels and a shift from amorphous weakly crystalline superficial gel structure containing spherical particles and microchannels to pores with laminated cross-stratified structures occurred [34]. Moreover, in another study, they found compatible results of the compressive strength of biodentine and MTA following exposure to different pH levels with the findings of microhardness values [40]. The compressive strength of the tested materials decreased significantly following exposure to low pH levels, and biodentine, as in micro-hardness, had higher compressive strength and showed more resistance to acidic environment compared to MTA. It is suggested that the development of a hybrid layer on dentine interface space using calcium silicate based materials in an acidic environment may be impaired [33]. The results of our

Author	Place of study	Method of evaluation	Results
Nikoloudaki GE et al., (2014) [27]	Greece	Dye penetration	Statistical analysis revealed that perforations restored with MTA exhibited less microleakage than biodentine.
El Khodary MH et al., (2015) [23]	Saudi Arabia	Fluid filtration	Mineral Trioxide Aggregate and biodentine showed similar capabilities in sealing the furcal perforations of the primary molars.
Ramazani N and Sadeghi P (2016) [24]	Iran	Bacterial leakage	Biodentine had no notable difference compared to MTA in terms of in-vitro bacterial leakage.
Agrafioti A et al., (2016) [39]	Greece	Fluid transport	When the materials were stored in an acidic environment, no statistical significant differences were found regarding their ability to prevent fluid movement between the 24 hours and the three months measurements.
Pathak S and Sharma D, (2018) [22]	India	Dye extraction	Not significant difference was found as-No significant difference between biodentine and MTA Angelus. They both are same for microleakage.
Övsay E et al., (2018) [26]	Turkey	Bacterial leakage	Pro Root MTA was determined as the most successful in terms of preventing microleakage when compared with biodentine.
Mohan T M et al., (2019) [25]	India	Dye penetration	The results showed that furcation perforation repaired with biodentine showed decrease in microleakage compared with that of ProRoot MTA. However, there was no statistically significant difference between the ProRoot MTA and biodentine.
Yuzer GM and Kaya SD, (2021) [present study]	Turkey	Dye penetration	The results of the present study suggested that there was no significant difference in microleakage levels between MTA and biodentine used to cover the perforation areas within the neutral pH range.

[Table/Fig-5]: Studies evaluating the microleakage of biodentine and MTA.

present study suggested that the low sealing property of the repair materials kept in an acidic environment compared to the neutral environment may result from defects in the development of this hybrid layer caused by the acidic environment. It may be proposed that the sealing properties of the materials change together with the changes in the microstructure.

Limitation(s)

The main limitation of the study was that the microleakage was examined with a stereomicroscope. This method, which allows for two dimensional inspection, may interfere with the exact calculation of the microleakage volume.

CONCLUSION(S)

Both long-term and short-term contact of MTA with PBS showed better sealing properties compared to that with AA. In biodentine, on the other hand, there was no difference when came into contact with short-term storage environments. The molars coming into contact with PBS during the long-term presented better sealing properties. Low pH levels adversely affected the sealing properties of both materials; however, in the presence of inflammation, biodentine may be preferred to repair an area of perforation as it provides better results in terms of microleakage. The current study evaluates the microleakage volume in two dimensions and we think that the use of micro CT for future research can prevent this limitation in volume evaluations.

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