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Original Article

Evaluation of the bond strength of root-end placed mineral trioxide aggregate and Biodentine in the absence/presence of blood contamination

Huseyin Akcay[1], Hakan Arslan[2], Merve Akcay[3], Merve Mese[3], Naciye Nur Sahin[3]

[1]Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Izmir Katip Çelebi University, Izmir, Turkiye,
[2]Department of Endodontics, Faculty of Dentistry, Ataturk University, Erzurum, Turkiye,
[3]Department of Pedodontics, Faculty of Dentistry, Izmir Katip Çelebi University, Izmir, Turkiye

Correspondence: Dr. Huseyin Akcay
Email: huseyin.akcay@ikc.edu.tr

ABSTRACT

Objective: Mineral trioxide aggregate (MTA) has been accepted as an appropriate root-end filling material in endodontic microsurgery because of setting ability in the wet environment. The aim of this study was to assess the bond strength of root-end placed MTA and Biodentine (Septodont, Saint Maur des Fossés, France) in the absence/presence of blood contamination. **Materials and Methods:** Forty-eight single-rooted maxillary incisors were used. subsequent to root-end resection and apical preparation using ultrasonic retro-tips, the specimens were randomly separated into two groups according to the root-end filling materials: MTA (Cerkamed Medical Company, Stalowa, Poland) or Biodentine. The specimens were then separated into two subgroups according to storage condition (absence/presence of blood) (n = 12). After obtaining 2.0 ± 0.1 mm slices, push-out tests were performed. Each slice was examined under a stereomicroscope to evaluate the failure mode. The data were analyzed using two-way analysis of variance and Tukey's *post hoc* test for multiple comparisons. The failure modes were analyzed using the Chi-square test (P = 0.05). **Results:** The bond strength was significantly affected by the presence of blood contamination and root-end filling material type (P < 0.001). Biodentine had better bond strength than MTA (P < 0.001). The most common failure type was adhesive failure. According to the Chi-square test, there were no statistically significant differences among the groups (P = 0.394). **Conclusions:** Biodentine had better bond strength values compared to MTA, and the bond strength of both MTA and Biodentine as root-end filling materials was negatively affected by the presence of blood.

Key words: Blood, endodontics, microsurgery, mineral trioxide aggregate, tricalcium silicate

INTRODUCTION

Surgical endodontics is usually indicated for the treatment of teeth with periapical lesions when orthograde root canal treatment fails.[1] Mineral trioxide aggregate (MTA) has been accepted as an appropriate root-end filling material following ultrasonic root-end preparation because of its bioactive, hard tissue conductive-inductive, antimicrobial, and biocompatible properties, as well as its ability to set in the presence of moisture.[2-5] Recently, a new tricalcium silicate-based cement, Biodentine (Septodont, Saint Maur des Fossés,

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France), has been developed. This product is suggested to be superior to other products because of its reduced setting time, and it is also advertised as a biocompatible and bioactive material. The powder component contains tricalcium silicate, dicalcium silicate, calcium carbonate and oxide, iron oxide, and zirconium oxide. The liquid component of Biodentine differs from MTA as it contains calcium chloride as an accelerator and as a hydrosoluble polymer.[6]

Because of the setting ability of MTA in the wet environment, this material has also been used in areas with bleeding.[7] Moreover, in most applications, MTA comes into contact with excessive blood during placement. The aim of this study was to evaluate the bond strength of root-end placed MTA and Biodentine in the absence/presence of blood contamination as there are limited data on this subject. The null hypothesis was that there was no statistically significant difference in the bond strength of MTA and Biodentine with/without blood contamination.

MATERIALS AND METHODS

Specimen preparation

Forty-eight single-rooted maxillary central incisors with comparable dimensions were chosen from a pool of teeth that had been extracted for causes unconnected to this study. The noninterventional clinical studies Institutional Review Board approved the protocols for this study (no. 223). The specimens were submerged in 0.5% chloramine-T solution (Merck, Darmstadt, Germany) for 48 h for disinfection, and were then stored in 4°C distilled water until use.

Root canal preparation

Regular coronal access cavity preparation was performed, and root canal shaping procedures were performed with ProTaper rotary instruments (Dentsply Maillefer, Ballaigues, Switzerland) up to an F4 (size 40, 0.06 taper) master apical file size. The root canals were irrigated with 2 ml of 2.5% NaOCl (Werax, Izmir, Turkey) between instrument alterations. A final rinse was performed with 5 mL of 17% ethylenediaminetetraacetic acid (Werax, Izmir, Turkey) for 1 min to remove the smear layer. This was followed by rinsing with 5 mL of 2.5% NaOCl for 1 min, and then 5 mL of distilled water. The teeth were then dried with paper points. The single Gutta-percha cone (F4, Dentsply Maillefer, Balaigues, Switzerland) was then slightly coated with an epoxy resin-based sealer (AH Plus; Dentsply DeTrey, Kontanz, Germany), and placed into the root canal to the working length. Mesiodistal and buccolingual radiographs were taken to affirm complete filling. After root filling, the coronal opening was filled with a temporary filling material (Cavit; 3M ESPE, Seefeld, Germany), and the specimens were kept at 100% humidity and 37°C for 1 week to completely set.

Apical resection and root-end preparation

Under a dental operating microscope (Carl Zeiss, Oberkochen, Germany), the roots were resected from 3 mm above the root tip at an angle of 90° to the longitudinal axis of the tooth using a water-cooled diamond bur. Root-end preparation to a depth of 3 mm was performed using ultrasonic retro-tips with a 0.6 mm apical diameter and a 0.75 mm coronal diameter, adapted to an ultrasonic preparation system (VarioSurg NSK, Tochigi, Japan) under constant water spray irrigation. The specimens were randomly separated into two groups, and the cavities were filled with either MTA (Cerkamed Medical Company, Stalowa, Poland) or Biodentine, which were mixed following the manufacturer's instructions. The specimens were then separated into two subgroups depending on the storage condition (absence/presence of blood) ($n = 12$). The specimens for the blood contaminated group were kept in Eppendorf tubes (Isolab, Istanbul, Turkey) containing blood, and the nonblood contaminated group specimens were stored in Eppendorf tubes containing sterile physiological saline. The specimens were stored at 37°C and 100% humidity for 1 week.

One slice of 2.0 ± 0.1 mm thickness was obtained from each root tip with a precision saw (Isomet 1000; Buehler, Lake Bluff, IL., USA) at a low speed with water cooling. The thickness of the slices was documented after computation with a digital caliper.

Push-out test

The diameter of each cement was measured under a stereomicroscope (Zeiss Stemi 2000C) at ×32 magnification. The push-out test was completed on each specimen with a universal test machine (AGS-X; Shimadzu Corporation, Tokyo, Japan) using a 0.5-mm diameter cylindrical stainless steel plugger at a crosshead speed of 0.5 mm/min. The highest load value at failure was saved in Newtons (N) and transformed to MPa by dividing the load by A, the bonded area. The bonded area of the root canal filling was calculated using the following equation: $A = (\pi r1 + \pi r2) \times L$, where, $L = \sqrt{(r1 - r2)^2 + h^2}$ $r1$ is the minor radius, $r2$ is the greater radius of the canal width (mm), h symbolizes the thickness of each section (mm), and π is fixed at 3.14.[8]

Following the test process, the root section was evaluated with stereomicroscope at ×32 magnification to assess the failure mode. Three categories of failure were recognized: Adhesive failure (between the cement and root dentin), cohesive fracture (within the dentin or cement layer), and mixed (a mixture of, cohesive and adhesive).

Statistical analysis

Data obtained from the bond strength test, in MPa, were analyzed with Levene's test of homogeneity of variance, the Kolmogorov–Smirnov test of normality, two-way analysis of variance (ANOVA) considering two factors (presence of blood contamination and root-end filling material type), and Tukey's *post hoc* test for multiple comparisons. The failure mode data were analyzed using the Chi-square test (P = 0.05). All statistical analyses were performed using software (SigmaStat for Windows version 3.5; Systat Software, Inc., Erkrath, Germany) at a significance level of 0.05 and a confidence interval of 95%.

RESULTS

Analysis of the data confirmed a normal distribution (P = 0.302) and homogeneity of variance (P = 0.102); therefore, parametric tests were used to analyze the data.

Two-way ANOVA showed that the bond strength was significantly affected by the presence of blood contamination and the root-end filling material type (P < 0.001). However, there were no significant interactions between the presence of blood contamination and the root-end filling material type (P = 0.153) [Table 1].

Biodentine had higher bond strength than MTA (P < 0.001). Blood contamination negatively affected the bond strength of both materials (P < 0.001) [Table 2], and the most common failure type was an adhesive failure [Figure 1]. According to the Chi-square test, there were no statistically significant differences between the groups (P = 0.394).

DISCUSSION

In this study, all controllable factors except for the root-end filling material and blood contamination were standardized to the greatest extent possible. Specimens with similar dimensions were chosen, and the root canals were prepared and filled with a standard technique. Root resections were performed at the same level, and root-end cavities were prepared using standard ultrasonic retro-tips.

Table 1: Two-way ANOVA for the presence of blood contamination and root-end filling material type and the interaction according to the push-out bond strength data (P<0.05)

Source of variation	Sum of squares	df	Mean squares	F	*P* value*
Blood contamination	29.650	1	29.650	38.166	<0.001
Root-end filling material type	40.288	1	40.288	51.859	<0.001
Blood contamination × Root-end filling material type	1.639	1	1.639	2.110	0.153
Total	105.760	47	2.250		

*Statistically significant difference at P<0.05

Table 2: Mean (standard deviation) of the push-out bond strength values (MPa) of root-end filling materials to root dentin in retrograde cavity in the presence/absence of the blood contamination

Blood Contamination	Material type	
	MTA	Biodentine
+	0.60 (0.45)^{A,a}	2.07 (0.71)^{A,b}
-	1.81 (1.24)^{B,a}	4.01 (0.88)^{B,b}

Mean values represented with same lowercase letters (row) are not significantly different according to Tukey's test (P>0.05). Mean values represented with same uppercase letters (column) are not significantly different according to Tukey's test (P>0.05)

In this study, both root-end filling materials – MTA and Biodentine – were filled into the retrograde cavities in the presence or absence of blood contamination. The different filling materials were compared in terms of their bond strengths to the root dentin.

MTA is biocompatible and has frequently been used as a promising biomaterial for root-end filling.[9-11] This material contains calcium oxide and silicon, which are fine hydrophilic particles that set, even in the presence of moisture.[2] Because of these advantages, MTA was selected for the control group.

According to the outcomes of this study, Biodentine had higher bond strength values compared to MTA. Blood contamination negatively affected the bond strength of both materials used. Therefore, the null hypothesis that there was no statistically significant difference in the bond strength of MTA and Biodentine with/without blood contamination can be rejected. The high-bond strength of cement to the root dentin via micromechanical retention or frictional resistance may be beneficial in maintaining the integrity of the cement-dentin interface. The interlocking between the cement and the root dentin may improve the dislocation resistance of intra canal filling materials.[12,13] Pull-out,[14-16] push-out,[17,18] and

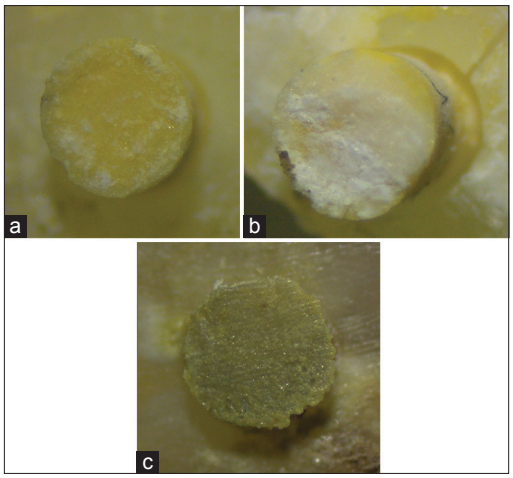

Figure 1: Stereomicroscopy photographs of the failure types at ×32 magnification. (a) Adhesive failure in the absence of blood. (b) Mixed failure in the absence of blood. (c) Adhesive failure in the presence of blood

micro-tensile tests[19] are conventional methods of assessing the bond strength of endodontic materials cemented into root canals. MTA and Biodentine are not suitable for pull-out and micro-tensile test methods. Goracci *et al*.[20] also demonstrated that the micro-tensile technique seems to have a high premature failure rate and large data variability. In this study, the push-out test method was used, which results in fewer premature failures and provides a uniform stress distribution.[20,21]

There are numerous reports on the influence of blood on MTA's properties. Charland *et al*.[22] demonstrated that the setting time for MTA samples was not significantly different in various media, including blood. Torabinejad *et al*.[23] demonstrated that the presence or absence of blood had no significant influence on the amount of dye leakage of MTA. Rahimi *et al*.[24] assessed the resistance of blood contaminated MTA in a simulated furcation perforation model and found that it resulted in an increase in bond strength. On the contrary, Vanderweele *et al*.[25] evaluated the effect of blood contamination on retention of MTA in simulated furcation perforations, and found that blood contamination negatively affected the adhesion of MTA. In this study, MTA was used as a root-end filling material, and it was found that the bond strength of MTA was negatively affected by the contamination of blood.

Nekoofar *et al*.[26] stated that the surface microstructure of MTA with blood exhibited substantial differences in crystalline formations, with a lack of acicular crystals. The outcome of this study showed that blood contamination adversely affects the bond strength of both MTA and Biodentine. The reduced bond strength in this study was associated with the prevention of complete hydration and the complete setting of cement, depending on surface differences in the blood contamination groups.

Kim *et al*.[27] showed that prolonged exposure of MTA to fetal bovine serum adversely affect the setting of MTA and causes it to remain unset up to 2 mm from the contact area. The same study also found that 10% calcium chloride helped the setting of MTA. MTA and Biodentine have similar chemical compositions; both are composed mainly of tricalcium and dicalcium silicate. However, the liquid of Biodentine differs from that of MTA, containing calcium chloride as an accelerator and hydrosoluble polymer. The presence of calcium chloride, and the concordant reduced setting time and contact time, probably provided the high-bond strength in the Biodentine group.[6,28,29]

Biodentine overcomes some disadvantages of MTA including the reduction of a prolonged setting time, which makes it, especially advantageous for apical surgery.[30] However, there are limited data about this material. In a recent study, Biodentine exhibited significantly greater push-out bond strength than MTA as a furcation repair material under blood contamination.[31] Despite of Biodentine being used in retrograde cavities in this study, this result is in agreement with our investigation. Elnaghy indicated that Biodentine showed higher bond strength to root dentin compared with MTA after exposure to different pH values.[32] In addition, other studies also stated that Biodentine provided higher push-out bond strength values compared with those of MTA[33,34]

This higher push-out bond strength values regardless of the blood presence could be attributed to the higher bio-mineralization ability of Biodentine which might have provoked the formation of tag-like structures at the cement-dentine interface and enhanced the dislodgement resistance of Biodentine as compared with MTA.[28,35] The higher content of calcium-releasing products in Biodentine than in MTA may promote to higher bio-mineralization and higher bond strength.[35-37] The differences in bond strengths among the Biodentine and MTA may also be attributed by the differences in the particle sizes, which may have an effect on the penetration of cement into dentinal tubules.[38] Compared to the MTA, smaller particle size of Biodentine may be caused to the formation

of tag-like structures and better micromechanical adhesion to dentine[36,39]

In a recent study, Biodentine was found to be bioactive since it increased cell proliferation and bio-mineralization in comparison with controls.[40] The authors suggested that Biodentine can be regarded as an appropriate material for clinical indications of dentin-pulp complex regeneration. In this study, blood contamination resulted in a statistically significant change in the bond strength of Biodentine; however, this bond strength was similar to that of MTA without blood contamination.

CONCLUSIONS

Within the limitations of this study, it can be concluded that (1) Biodentine had higher bond strength values compared to MTA, and (2) the bond strengths of MTA and Biodentine as root-end filling materials were negatively affected by the presence of blood. These findings indicate that Biodentine may be advantageous, especially for apical surgery, even the presence of excessive blood.

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Conflicts of interest
There are no conflicts of interest.

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