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Effect of Sodium Hypochlorite on Push-out Bond Strength of Four Calcium Silicate-based Endodontic Materials when used for repairing Perforations on Human Dentin: An *in vitro* Evaluation

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ABSTRACT

Aim: This study aimed to evaluate the push-out bond strength of NeoMTA Plus (NMTA), EndoSequence root repair material fast set putty (ERRMF), biodentine (BD), and ProRoot white mineral trioxide aggregate (PMTA) when used as perforation repair materials after exposure to 2.5% sodium hypochlorite (NaOCl) during the early setting phase.

Materials and methods: Horizontal midroot sections were prepared from single-rooted human teeth. Sections (n = 144) were randomly divided into four groups: PMTA, BD, NMTA, and ERRMF. Materials were condensed and allowed to set for 10 minutes. The groups were further divided into two subgroups. The NaOCl group included specimens that were immersed in 2.5% NaOCl for 30 minutes, and the control group included specimens on which a wet cotton pellet was placed over the test material. After 48 hours, the highest force applied to the materials at the time of dislodgement was recorded. Slices were then examined under a digital microscope to evaluate the nature of the bond failure. The surfaces of two specimens from each subgroup were observed by scanning electron microscopy. Data were statistically analyzed with two-way and one-way analysis of variances, independent t-tests, and chi-square tests. The statistical significance was set at 0.05.

Results: In NaOCl-treated groups, PMTA showed a significantly higher push-out bond strength than the other three materials ($p = 0.00$). In the control groups, the bond strength of BD was significantly higher than that of PMTA, ERRMF, and NMTA ($p < 0.05$). Compared with the control group, NaOCl treatment significantly increased the push-out bond strength of PMTA ($p = 0.00$) and ERRMF ($p = 0.00$) and significantly reduced the bond strength of BD ($p = 0.00$) and NMTA ($p = 0.03$). None of the specimens showed an adhesive type of failure. The majority of the samples exhibited a cohesive failure type. Morphological observations revealed that the surfaces exhibited cubic crystals. In ERRMF, the crystals were few in number. Sodium hypochlorite enhanced the crystallization of NMTA.

Conclusion: The push-out bond strengths of PMTA and ERRMF were significantly increased after exposure to 2.5% NaOCl in the early setting phase, and those of BD and NMTA were significantly decreased.

Clinical Significance: The results of the present study suggest that early exposure of NaOCl increase the push-out bond strength of PMTA and ERRMF. PMTA had the highest push-out values. Therefore, it would be a potentially useful perforation repair material for single visit endodontic treatment.

Keywords: Biodentine, Bond strength, EndoSequence root repair material fast set putty, Mineral trioxide aggregate, NeoMTA Plus, Perforation repair, Scanning electron microscopy, Sodium hypochlorite, Type of bond failure.

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INTRODUCTION

Root perforation is an iatrogenic or pathologic communication between the root canal system and the supporting tissues of the teeth. It has been reported that 47% of perforations occurred during endodontic treatment.[1] Ideally, a perforation should be repaired with a biocompatible, nonabsorbable, and radiopaque material that should provide a tight seal at the perforation site. Numerous dental materials have been proposed for endodontic perforation repair, including amalgam, phosphate cement, gutta-percha, zinc oxide eugenol, calcium hydroxide, glass-ionomer

cement, and resin-ionomer.[2] However, none have been able to satisfy all the requirements of an ideal endodontic repair material.

Mineral trioxide aggregate (MTA), which was first described in the scientific literature in 1993,[3] has become the preferred perforation repair material. According to the material safety data sheet, ProRoot MTA (PMTA) (ProRoot white MTA, Dentsply, Tulsa, OK) is composed of tricalcium silicate, dicalcium silicate, tricalcium aluminate, bismuth oxide, and gypsum. ProRoot MTA has many favorable properties, including biocompatibility, excellent sealing ability, bioactivity, and antibacterial properties.[4,5] The overall success rate of perforated teeth repaired with MTA was 86%.[6] However, MTA has some reported drawbacks, including difficulty in handling,[7] potential tooth discoloration,[8] and high cost. Another major disadvantage of MTA is the long setting time of 140 minutes.[9] Therefore, it is recommended to place a moist cotton pellet over MTA to allow for setting and to complete the treatment in another visit.

Many newer calcium silicate-based filling materials have been available in the market, such as biodentine (BD; Septodont, Saint Maur des Fossés, France), NeoMTA Plus (NMTA; Avalon Biomed Inc., Bradenton, FL), and EndoSequence root repair material fast set putty (ERRMF; Brassler USA, Savannah, GA). These materials have the same endodontic clinical applications as MTA, including perforation repair.

Biodentine is calcium silicate-based cement that can be used as a dentin substitute on crowns and roots. Biodentine consists of a powder and liquid. According to the company scientific file, the powder mainly contains tricalcium and dicalcium silicate, and the liquid contains calcium chloride. The powder is mixed with the liquid in a capsule in the triturator. The final setting time of BD is approximately 10 to 12 minutes. This short setting time is attributed to the addition of calcium chloride to the mixing liquid. Biodentine has the advantages of biocompatibility,[10] good sealing ability,[11] bioactivity,[12] and high compressive strength.[13]

NeoMTA is tricalcium silicate-based cement that consists of fine powder and gel. The gel is mixed into the powder until a putty-like consistency is obtained to improve the handling characteristics. According to the manufacturer, the gel imparts washout resistance. The setting time of NMTA is 15 minutes. The quality of marginal adaptation of NMTA, when used for orthograde obturation of teeth with open apices, was found to be similar to that of PMTA.[14]

Also, ERRM is a premixed cement that has been introduced as another alternative to PMTA. It is available as a paste, a condensable putty, and more recently a syringable fast set putty (ERRMF) that sets in 20 minutes. According to the manufacturer, ERRM is composed of calcium silicates, zirconium oxide, tantalum oxide, and calcium phosphate. The setting reaction begins in the presence of moisture. Previous studies have demonstrated that ERRM is a biocompatible[15] and bioactive material[16] that has sealing ability,[17] antibacterial activity,[18] and compressive strength,[19] similar to PMTA.

After perforation repair, the clinician should proceed with nonsurgical endodontic therapy that causes unavoidable contact of the irrigant with the repair material. Guneser et al[20] reported that sodium hypochlorite (NaOCl) increased the compressive strength of PMTA and BD. However, the differences in push-out bond values were not statistically significant. To our knowledge, there are no published studies that evaluate the effect of endodontic treatment on the bond strength of the above-mentioned new, fast set calcium silicate cements (CSCs) when used for perforation repair. The aim of the present *in vitro* study was to evaluate the push-out bond strength of NMTA, ERRMF, BD, and PMTA when used as perforation repair materials after exposure to 2.5% NaOCl in the early setting phase.

MATERIALS AND METHODS

Freshly extracted single-rooted human teeth were used in this study. The coronal parts of the teeth were removed. Roots were embedded vertically in a rubber mold containing epoxy resin (Vertex Orthoplast; Vertex-Dental, Zeist, The Netherlands). A mounting device was used to ensure orientation in the long axis of the tooth. The midroot was sectioned horizontally into slices with a thickness of 1.5 ± 0.05 mm using an isomet low-speed saw (Isomet; Buehler Ltd, Lake Bluff, NY, USA) with continuous water irrigation. The canal space of each slice was instrumented with a complete pass of Gates Glidden burs (Dentsply Maillefer, Ballaigues, Switzerland), sizes 2, 3, 4, 5, and 6, to achieve a diameter of 1.5 mm. All samples were rinsed with saline to remove all debris produced. Paper points were used to absorb the liquid present in the canal walls. The slices (n = 144) were randomly divided into four groups according to the test material used: PMTA, BD, NMTA, and ERRMF.

According to manufacturers' instructions, PMTA, BD, and NMTA were mixed; ERRMF is a premixed cement. Materials were placed inside the lumen of the slices and were condensed using an endodontic plugger. The excess was removed from each specimen with a scalpel. Specimens were wrapped in a piece of wet gauze, sealed in a container, and placed in an incubator at 37°C and 100% relative humidity for 10 minutes. The four groups were further subdivided into two subgroups (NaOCl or control group) of 18 samples each.

Specimens in the NaOCl groups were immersed in 2.5% NaOCl for 30 minutes. Specimens were then

removed, rinsed with distilled water, and placed in an incubator for 48 hours. For control groups, a wet cotton pellet was placed over the perforation repair material for 48 hours in an incubator.

Push-out Test

The push-out test was carried out using a universal testing machine (Instron testing machine; Model 5965, ITW, MA, USA). The samples were placed on a metal slap containing a central hole to allow for the free motion of the plunger with a 1.2 mm diameter, at a constant vertical downward pressure at a speed of 1 mm/min. The plunger tip was positioned to contact the test material only. The test was carried out until total bond failure. The highest force applied to materials at the time of dislodgement was recorded in megapascal. The operator, who made the measurements, was blinded to which sample was matched to which material.

Evaluation of Failure Patterns

To evaluate the nature of the bond failure, slices were examined under a digital microscope at 50× magnification (KH-7700; Hirox Co, Tokyo, Japan). Each sample was categorized into one of three failure modes: An adhesive failure that occurred at the dentin–material interface, cohesive failure that occurred within the material, or mixed failure, which was a combination of the two failure modes. The operator who examined the slices was blinded to which sample was matched to which material.

Scanning Electron Microscopic Examination

Two specimens from each subgroup were randomly chosen for scanning electron microscopic (SEM) examination. After air-drying overnight at room temperature, specimens were mounted and sputter-coated with gold using the fine coat ion sputter JFC-1100 (JEOL Ltd., Tokyo, Japan). We observed the specimens by SEM (JSM-6360LV; JEOL Ltd.) and acquired digital images at 5,000× for each sample. The surface characteristics of the samples were recorded with the digital imaging system.

Statistical Analysis

The results of the Shapiro–Wilk normality test revealed that the data were normally distributed. Two-way analysis of variance (ANOVA) was used to analyze the interactions between different materials and irrigation groups. A significant interaction was found (p = 0.00); hence, one-way ANOVA was used to compare the push-out bond strength for the different materials within each irrigation group. *Post hoc* testing was then accomplished using the Tukey test for multiple comparisons. An independent t-test was used to compare the means of the push-out bond strengths of the irrigation and control groups for each material. Chi-square test was used to determine if there is a significant association between the type of failure and the irrigation group for each material. Statistical analysis was performed using Statistical Package for the Social Sciences statistical software (version 21.0, SPSS IBM, Armonk, NY). The statistical significance was set at 0.05.

RESULTS

Push-out Test

The mean push-out strength values and standard deviations in each group are presented in Table 1. In NaOCl-treated groups, PMTA showed a significantly higher push-out bond strength than the other three materials (p = 0.00). In the control groups, the bond strength of BD was significantly higher than PMTA (p = 0.00), ERRMF (p = 0.00), and NMTA (p = 0.01). The ERRMF showed significantly lower bond strength than PMTA and NMTA (p = 0.00). There was no significant difference between PMTA and NMTA (p = 0.32).

Exposure to NaOCl affected the resistance to displacement of CSCs. Compared with the control treatment, NaOCl significantly increased the push-out bond strengths of PMTA (p = 0.00) and ERRMF (p = 0.00) and significantly reduced the bond strengths of BD (p = 0.00) and NMTA (p = 0.03).

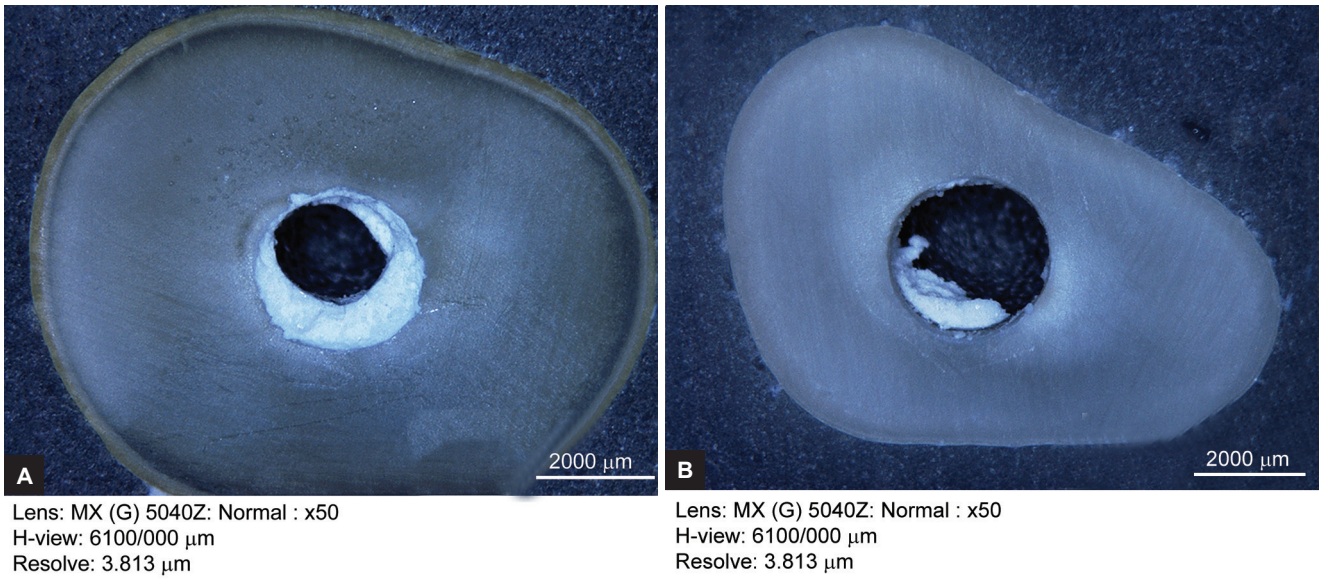
Bond Failure Patterns

Representative microscopic images of specimens with a cohesive and a mixed type of bond failure are shown in Figures 1A and B. None of the specimens in our study showed an adhesive type of failure. The majority of the samples exhibited a cohesive failure type. The NMTA specimens in the NaOCl group had equal numbers of cohesive and mixed types of failure (Graph 1). At a significance level of 0.05, chi-square tests revealed no significant association between different materials and types of failure within each irrigation group.

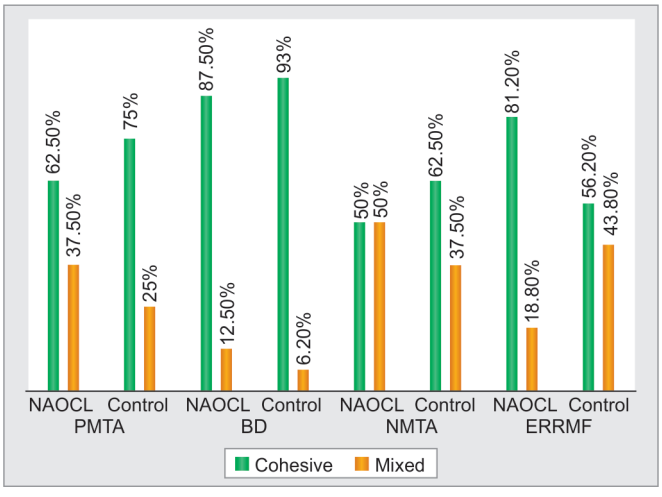
Table 1: Push-out strength values (MPa) for each group

Test material	*Group*	*Push-out (mean ± SD)*
PMTA	NaOCl	63.4 ± 5.1
	Control	48.1 ± 4.5
BD	NaOCl	45.3 ± 6.9
	Control	56.7 ± 8
NMTA	NaOCl	49 ± 3.2
	Control	51 ± 1.6
ERRMF	NaOCl	44.6 ± 3.4
	Control	31.5 ± 2.8

SD: Standard deviation



Figs 1A and B: Microscopic images of ERRMF representative samples showing: (A) Cohesive; and (B) mixed failure



Graph 1: The percentage of bond failure in each subgroup

SEM Examination

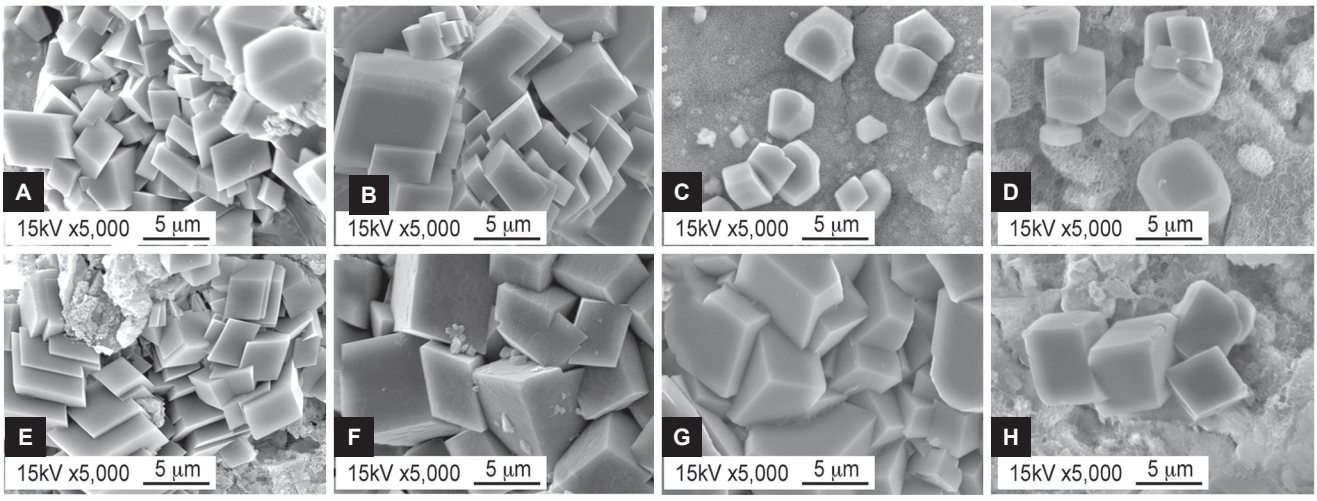
Morphological observations revealed that the microstructure of PMTA in both groups possessed irregular cubic crystals, as shown in Figures 2A and E. The BD specimens also exhibited a crystallized structure (Fig. 2B). However, when it was contacted with NaOCl, the surfaces of the cubic crystals appeared uneven (Fig. 2F). Figure 2G shows the crystallized microstructure of NMTA when immersed in NaOCl. In the NMTA control group, the cubic crystals were sparsely distributed (Fig. 2C). In ERRMF specimens, the cubic crystals were few in number in both the control and the NaOCl-treated specimens (Figs 2D and H).

DISCUSSION

When used for perforation repair, the adhesion of hydraulic cements to the surrounding dentin is essential. Compressive strength is one of the indicators of the adhesive properties of a perforation repair material to the dentinal walls. Although no clinically relevant minimum value has been universally proposed, higher push-out bond strength is more desirable.[21] This study evaluated the effect of NaOCl, the most widely accepted endodontic irrigating solution, on the push-out bond strength of new, fast set CSCs in the early setting phase. In our study, PMTA was chosen as a gold standard material because it is widely used for perforation repair in endodontics.

In the control group, the push-out bond strength of BD was significantly higher than that of the other CSCs. This finding is in agreement with previous studies[20,22] and can be explained by the small particle size of BD that improves the cement penetration into dentinal tubules.[20] Furthermore, the calcium and silicon ion uptake into dentin in BD is significantly higher than PMTA, which may increase the bond strength.[23]

The PMTA and ERRMF immersed in NaOCl showed significantly higher push-out values than the control groups. This means that NaOCl enhanced the setting reaction of both materials. Kogan et al[24] found that NaOCl gel decreased the setting time of PMTA, which might be responsible for the improvement in strength and might explain the significantly higher push-out bond strength of PMTA than the other three cements when exposed to NaOCl. Furthermore, previous studies reported a higher compressive strength of PMTA when immersed in solution.[20,25] This might be related to the hydration of the remaining unreacted mineral oxides that solidify after immersion.[20] The ERRMF is a premixed cement that sets in the presence of moisture. To the best of our knowledge, this is the first study that showed that NaOCl improved the physical property of ERRMF. A possible explanation



Figs 2A to H: Scanning electron micrographs showing the surface topography of (A and E) ProRoot MTA, (B and F) biodentine, (C and G) NeoMTA Plus, and (D and H) EndoSequence root repair material fast set putty in control (A to D) and sodium hypochlorite (E-H) groups. Scale bars = 5 µm

for this result is that an increase in the amount of moisture during the setting of ERRMF will increase the compressive strength. The results of SEM observations showed that NaOCl did not interfere with the crystallization of the cubic crystals of PMTA or ERRMF. However, the amount of crystals in ERRMF was lower than in PMTA in the NaOCl and control groups, which might be responsible for the impaired compressive strength of this material.

In our study, we found that NaOCl significantly reduced the bond strength of BD and NMTA compared with the control group. NaOCl treatment was associated with a small but statistically distinguishable difference in compressive strength in the NMTA group compared with the control group. According to our SEM examinations, NaOCl did not alter the surface morphology of BD but it enhanced the crystalline surfaces of NMTA. Crystal formation is a multifactorial process.[26] Therefore, the exact explanation for the morphologic differences in NMTA shown in this study is not known. The morphological differences may be due to the increase in the amount of moisture, which influences the dynamics of NMTA crystallization. However, the increase in the cubic microstructure in NMTA did not enhance the bond strength. Subsequent studies should be conducted to determine if NaOCl alters the chemical composition of the cements, which would weaken the bond strength of BD–dentin and the NMTA–dentin interface.

In this study, the bond failures observed in all experimental groups generally occurred within the CSC. However, differences among the bond failure groups for each material were not significant. The PMTA and BD form tag-like structures with the dentinal tubules, forming a micromechanical anchor[25,27] that might cause a cohesive bond failure. This finding is in accordance with previous studies that found that BD–dentin bond failures were predominantly cohesive.[13,20] However, others showed that PMTA–dentin bond failures were usually adhesive.[28,29] The discrepancy might be attributed to the differences in study design including the environment evaluated. To our knowledge, the mode of bond failure of NMTA and ERRMF has not been investigated in other studies. The cohesive bond failure can be explained by tubule penetration,[30] and the putty form of ERRMF can improve the adherence to dentinal walls.

Compressive strength is one of the main physical characteristics of CSCs. To our knowledge, this is the first report that compared the effect of NaOCl on the performance of PMTA and other new CSCs. The results of this study indicated that NaOCl had an adverse effect on BD and NMTA, while it improved the push-out bond strength of PMTA and ERRMF. The ERRMF is a premixed, fast-set, ready-to-use CSC that eliminates the potential of heterogeneous consistency during on-site mixing. However, when NaOCl is used, PMTA had the highest push-out values. Therefore, if a single-visit procedure is planned, the clinician can consider PMTA as a perforation repair material.

This *in vitro* method for the evaluation of the effect of NaOCl on CSCs cannot duplicate the environment that exists *in vivo* when using endodontic irrigants. In our clinical practice, there is a possibility of washout by the injection pressure of the irrigants. However, the results from this study might provide information that can aid the clinician in the selection of the best repair material for single-visit endodontics. Further studies are indicated to evaluate the effect of NaOCl on other properties of new CSCs, including sealing ability, before clinical use.

CONCLUSION

Within the limitations of this experimental *in vitro* study, it could be concluded that the push-out bond

strengths of PMTA and ERRMF were significantly increased after exposure to 2.5% NaOCl in the early setting phase, and those of BD and NMTA were significantly decreased.

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