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Original Article

Effect of varying durations of intracanal medicament application used in regenerative endodontic treatment on the push-out bond strength of a novel cement: NeoMTA Plus

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Abstract

Aim: The aim of the present study was to evaluate the effect of varying durations of intracanal medicament application used in regenerative endodontic treatment on the push out the bond strength of a novel cement-NeoMTA Plus.

Materials and Methods: A total of 60 extracted single-rooted maxillary anterior teeth were decoronated. Roots were instrumented uniformly until Peeso Reamer size #5 to simulate open apices. A total of 60 roots were then divided into four groups according to intracanal medicaments used ($n = 15$): Group 1: Triple antibiotic paste (TAP); Group 2: Double antibiotic paste (DAP); Group 3: Calcium hydroxide paste (CH); and Group 4 (control): No medicament. Samples were kept in saline solution for 2, 4, and 12 weeks, after which time five roots were selected randomly from each group, representing the samples of each time point. After removal of the medicaments, NeoMTA Plus was placed into 8 mm of the coronal third of the roots and samples were incubated. Roots were sectioned to obtain 2 discs per root ($n = 10$). A push-out test was used to measure the sealing efficacy of NeoMTA Plus. Data were analyzed using a one-way ANOVA followed by Tukey's pairwise comparisons.

Results: CH, DAP, and TAP application resulted in significantly lower values of the push-out bond strength of NeoMTA Plus after 12 weeks compared to 2 weeks ($P < 0.05$).

Control versus calcium hydroxide	P=0.0002
Control versus double antibiotic paste	P=0.0312
Control versus triple antibiotic paste	P=0.0002

No significant differences were found between the time points in the control group ($P > 0.05$).

Changes from 2-12 weeks	Mean	SD
Control	0.60	0.81

SD: Standard deviation

DAP showed lowest push-out bond strength.

Group	2 weeks		4 weeks		12 weeks		Changes from 2 weeks to			
							4 weeks		12 weeks	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Double antibiotic paste	2.82	0.35	1.89	0.41	1.46	0.40	0.93	0.55	1.36	0.38

SD: Standard deviation

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Conclusion: CH, DAP, and TAP cause an alteration in dentinal surface properties leading to negative effect on bond strength of NeoMTA Plus. The effect is more evident in DAP and as treatment time is prolonged. The type and duration of medicament application have to be monitored to achieve a maximum therapeutic value as well as to avoid compromise on the coronal seal.

Keywords: Antibiotic pastes; coronal seal; intracanal medicaments; NeoMTA Plus; regenerative endodontic treatment

INTRODUCTION

Ever since its inception, regenerative endodontic treatment (RET) has been generating tremendous interest. From starting out as just an idea, it is quickly receiving sufficient attention from clinicians and patients alike to be recognized as a specialty in itself. RET is based on the concept of recreating an ideal microenvironment within the tooth and surrounding structures to recapitulate the steps taking place during embryogenesis and organogenesis. This is shown to reconstitute the original biological structures replicating their form and function.[1] While endodontic treatment in mature teeth has shown predictable results with a consistently high-success rate over the years, the scenario has not been as favorable with young, immature teeth. Needless to say, this has been the major driving force for continued aggressive research in the field of regeneration. The main reasons for failed attempts at RET have been residual infection due to inadequate cleaning and shaping of weak root canal walls susceptible to fracture, and reinfection due to inability to hermetically seal wide open apices by conventional techniques.[2] Thus, it is clear that the one factor which cannot be compromised on for pulp tissue regeneration is the sterility of the root canal space. To avoid damage to the already fragile canal walls, minimal instrumentation is recommended. As an adjunct, disinfection is accomplished with irrigation solutions and intracanal medicaments. Of the myriad of medicaments tested, the ones most commonly used today include antibiotic pastes (metronidazole and ciprofloxacin with or without minocycline) and calcium hydroxide (CH).[3] While some studies have indicated that a 2–4 week application of antibiotic pastes and CH is sufficient to obtain maximum remedial effects, others have shown a wide range of preferences from 1 week to several months.[4] However, their long-term use has generated increasing reports of demineralization and deterioration of surface and mechanical properties of radicular dentin, eventually leading to propagation of microcracks and vertical root fractures. After the suppression of symptoms, the coronal seal is generally obtained using MTA.[4] Its use in pulpotomies brings it close to the visible coronal tooth structure. Since it was initially only available as a gray variant, discoloration of marginal gingiva was reported. In 2002, in an attempt to curb it is unesthetic properties, a white MTA was introduced. However, this also failed to meet the standard and showed evidence of crown discoloration. The cause of discoloration is debatable, but most fingers would point to the presence of radio-opacifiers like bismuth oxide and its reaction with dental tissues and irrigants. Recently, a new age MTA has been formulated with alternative nonstaining opacifiers-NeoMTA Plus (Avalon Biomed Inc, Brandenton, FL). This has been particularly marketed for pulpotomies in anterior teeth keeping in mind the esthetic concerns of young patients.[5] NeoMTA Plus retains the osteoconductive and inductive properties of its predecessors, as well as their chemical bond to dentin.[5] Since pretreatment of dentin with intracanal medicaments has shown to negatively affect it is microstructure and properties, it may also lead to compromised bonding.[6] Bond strength, also measurable as dislocation resistance, is directly comparable to sealing efficacy of restorative materials to root canal walls. This is a critical factor in RET since teeth are exposed to occlusal and procedural forces in the oral cavity that may cause dislodgement of MTA. This, in turn, will give rise to leakage of saliva and bacterial contaminants into the canal thus rendering our efforts at cleaning and shaping void. Recently studies have reported that the time-dependent application of medicaments used in RET affects the dislocation resistance of MTA.[6] However, the coronal sealing ability of the novel cement NeoMTA Plus has not yet been evaluated. Therefore, the aim of the present study is to evaluate the effect of varying durations of intracanal medicament application used in regenerative endodontic treatment on the push out the bond strength of NeoMTA Plus.

MATERIALS AND METHODS

The study protocol was approved by the Institutional Review Board and Ethical Committee of the Institution (IRB No – 2017/P/CONS/52).

A pilot study was conducted, and the sample size was obtained.

$n = Z_{(1-\alpha/2)}{}^{2}\, SD^2/d^2$

$Z_{(1-\alpha/2)} = 1.96$

SD = Standard deviation of variable = 0.133 (as determined in study)

d = precision = 0.05

$n \approx 28 \approx 30$

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Sixty intact maxillary anterior teeth extracted for periodontal reasons were collected for this study from the Department of Oral and Maxillofacial Surgery. The specimens were immersed in Chloramine T solution (Merck, Darmstadt, Germany) for 48 h for disinfection. They were then stored in distilled water until used. The presence of a single straight canal was radiographically confirmed. The exclusion criteria included the presence of multiple canals, calcifications, curved canals, previous root canal treatment, resorption, cracks, caries, and developmental defects. Buccolingual and mesiodistal dimensions at the cementoenamel junction were measured using a digital caliper (Mituyoto, Kawasaki, Japan) to provide standardization and teeth deviating more than 20% from average values were excluded from the study.

Sample preparation

Teeth were sectioned apically 12 mm below and coronally 2 mm above the cementoenamel junction using a diamond disk. Remnant pulpal soft tissue was removed using a size 30 Hedström file (Dentsply Maillefer, Ballaigues, Switzerland) [Figure 1]. Working length was determined radiographically using size 20 K-files (Dentsply Maillefer, Ballaigues, Switzerland) and was kept 2 mm more than normal to over-instrument the canals simulating immature open apices. Rotary instrumentation was carried out using ProTaper rotary instruments (Dentsply Maillefer, Ballaigues, Switzerland) to standardize to master apical size 30 corresponding to F-3. Next Peeso reamers (Dentsply Maillefer, Ballaigues, Switzerland) were used sequentially from #1 to #5 to obtain larger canal space and foramen. The canals were irrigated with 2 mL 2.5% sodium hypochlorite (NaOCl) (Sigma-Aldrich, St. Louis, MO) between the instrument changes. A final flush was applied using 5 mL 17% ethylenediaminetetraacetic acid (EDTA) (Sigma-Aldrich, St. Louis, MO) for 1 min and 5 mL 2.5% NaOCl for 1 min and then dried using paper points (Dentsply Maillefer, Ballaigues, Switzerland)

Preparation and placement of intracanal medicaments

The specimens were randomly divided into a control group (without intracanal dressing) and three experimental groups that received an intracanal dressing with either CH, double antibiotic paste (DAP), or triple antibiotic paste (TAP) ($n = 15$).

- Group 1: TAP paste: 1:1:1 mixture of ciprofloxacin (Bayer, Leverkusen, Germany), metronidazole (Sanofi Aventis, Frankfurt, Germany), and minocycline (Ratiopharm, Ulm, Germany) prepared with sterile distilled water (w/v 3:1), as described in a study by Yassen *et al*.,[7] was delivered into the canals using a lentulo spiral (Dentsply Maillefer, Ballaigues, Switzerland)
- Group 2: DAP paste: 1:1 mixture of ciprofloxacin (Bayer, Leverkusen, Germany) and metronidazole (Sanofi-Aventis, Frankfurt, Germany) prepared with sterile distilled water (w/v 2.5:1) was delivered into the canals as described previously
- Group 3: CH paste prepared by mixing CH powder (Kalsin; Spot Dis Deposu AS, Izmir, Turkey) with sterile distilled water (w/v 2:1) was placed into the root canals as described previously
- Group 4: Unfilled control group.

The apical and coronal root orifices were sealed using modeling wax (Hindustan Modelling Wax, Hindustan Dental Products, Hyderabad). All specimens were stored in a saline solution that was refreshed every 7 days until they were required for evaluation. After 2, 4, and 12 weeks, 5 teeth were selected randomly from each group, representing the samples of each time point. At the end of the incubation period, modeling wax was peeled off followed by the removal of TAP, DAP, and CH using 2 mL of 2.5% NaOCl and 17% EDTA. The canals were then dried using absorbent paper points.

NeoMTA Plus (Avalon Biomed Inc., Brandenton, FL, USA) was prepared according to manufacturer's instructions. With the help of an amalgam carrier, it was placed 8 mm into the coronal third of the root canal under radiographic control. They were allowed to set in the atmosphere for 3 h and were then stored for a week at 37°C at 100% humidity in a humidity chamber (Biotechnics, India) to allow the setting of the MTA completely.

Push out test

After storage period was complete, each specimen was sectioned perpendicular to its long axis using a precision saw (IsoMet 1000; Buehler, Lake Bluff, IL, USA) at a slow speed under water cooling. Two slices were obtained from each tooth ($n = 10$ for each group) at depths of 2 and 5 mm from cementoenamel junction and approximately 1 ± 0.1 mm thickness. Size of slice and diameter of the filled canal were measured with a digital caliper. After standardization was accomplished, a test tip of 1.2-mm diameter was positioned over the filled portion so as it touched only the NeoMTA Plus and did not stress the canal walls. A push-out test was performed on each specimen with a universal testing machine (AGS-X; Shimadzu Corp, Tokyo, Japan) at a crosshead speed of 1 mm/min until failure occurred.

The force required to dislocate NeoMTA Plus was recorded in Newtons. The maximum load had applied to the filling material before failure was recorded in Newtons and converted to megapascals (MPa) according to the following formula:

Push-out bond strength (MPa) = maximum load (N)/ adhesion area of NeoMTA Plus (A) (mm^2)

$2 \pi r \times h$

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where π is the constant 3.14, *r* is the root canal radius, and h is the thickness of the root slice in millimeters.

Failure mode analysis

After bond strength measurement was completed, samples were viewed under 20x magnification to determine the failure mode as shown in Figure 2. Failure type was classified into three categories as done in a study by Shokouhinejad *et al*.[8]

1. adhesive failure between NeoMTA Plus and dentin
2. cohesive failure within NeoMTA Plus or
3. mixed failure which includes NeoMTA Plus and dentin together.

Statistical analysis

The effects of the type of medicament used and the duration of the medicament treatment on the coronal sealing efficacy of NeoMTA Plus were examined using a one-way ANOVA followed by *post hoc* adjustment with Tukey's test for pairwise comparisons. Statistical significance was set at $P < 0.05$ with a 95% confidence interval. All statistical analyses were performed with IBM SPSS Statistics 20 software (IBM Corporation Software Group, Somers, NY, USA).

Figure 1: NeoMTA Plus manipulation

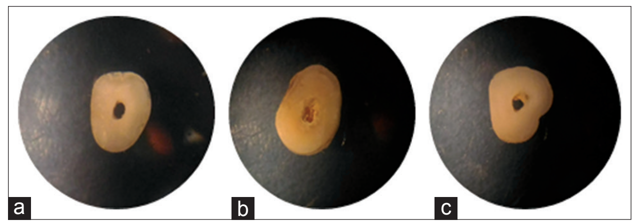

Figure 2: Modes of failure of NeoMTA Plus at dentinal interface. (a) Adhesive failure, (b) Cohesive failure, (c) Mixed failure

RESULTS

Mean push-out bond strength values at 2, 4, and 12 weeks are shown in Figure 3. By one-way ANOVA, no significant difference was found between the control group at all time-points ($P > 0.05$).

The time factor displayed a definite effect on the push-out bond strengths of all the experimental groups; prolonged treatment time caused a decrease in values. The 12 weeks samples of TAP, DAP, and CH showed significantly lower values as compared to the control group ($P > 0.05$). Within the experimental groups, push-out bond strength values were the highest for CH and the lowest for DAP. Modes of failure are listed in Table 1. Cohesive failure was the most frequent failure type in NeoMTA Plus, with no predominant mode of failure affected by the duration of the placement of CH, DAP, and TAP.

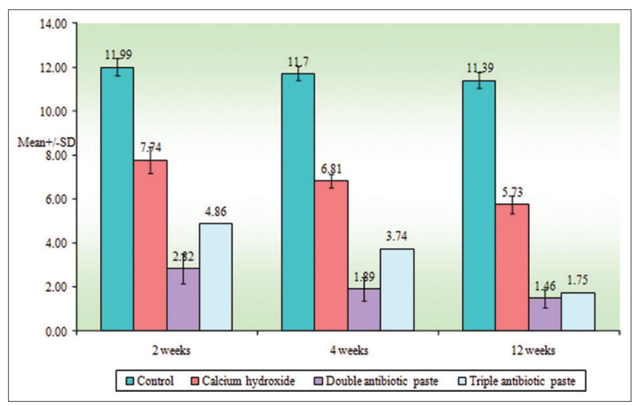

Figure 3: Mean value and standard deviation of push-out bond strength values of NeoMTA Plus

Table 1: Type of failure numbers for each group as evaluated by 20 x magnification

	Adhesive	Cohesive	Mix
Control	3	6	1
CH	2	5	3
DAP	4	4	2
TAP	2	6	2

CH: Calcium hydroxide paste, TAP: Triple antibiotic paste, DAP: Double antibiotic paste

DISCUSSION

Conventionally, CH had been used for pulp therapy of immature permanent teeth. It was gradually taken over by MTA due to its comparable results and shortened treatment time. MTA can be used as a pulp capping agent, in the repair of perforations, apexification, and lately in RET. However both, its gray and white variants, possessed a discoloration potential which is unacceptable, especially in today's age of high-esthetic demand. Thus, there was a need for an alternative filling material which could provide both, a stain-proof restoration, as well as retain the biological and mechanical benefits of MTA. This was fulfilled by the introduction of a novel cement, NeoMTA Plus.[5] The formulation varies from traditional MTA by altered particle size and replacement of bismuth oxide as

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a radio-opacifier by the stain-free tantalum oxide,[5] truly commencing an era of esthetic endodontics.

Along with its cosmetic appeal and regenerative potential, another important feature of NeoMTA Plus as a restorative should be its bond strength. Recently, there has been a paradigm shift in the endodontic treatment philosophy stating that coronal seal, by preventing recontamination, has a greater impact on success than root canal preparation itself.[9] NeoMTA Plus is subjected to various dislodgement forces in the oral cavity due to restorative procedures and mastication. The dislocation resistance, which is directly comparable to bond strength of a restorative, should be our prime concern. This can be measured as adhesion of a cement to the canal walls by tensile, shear, and push-out tests. Of these, push-out tests have been shown to be efficient and reliable to measure the sealing efficacy and has been utilized in many studies.[6] The push-out bond strength may be affected by humidity, thickness of cement, pH, and physical properties of dentinal walls.[10]

Since it is the sine qua non in cases of RET for canals to be chemically disinfected by intracanal medicaments, NeoMTA Plus will rarely be in contact with untreated dentin. This leads to altered chemical and mechanical properties affecting its adhesion and sealing ability. Previous studies have evaluated the influence of intracanal medicaments on the dislocation resistance of MTA.[6] It was concluded that dislocation resistance was significantly affected when TAP application was longer than 2 weeks and the CH was longer than 4 weeks. According to results of the present study, the bond strength of NeoMTA Plus to pretreated dentin was also influenced by both, the type of medicament and treatment time. DAP negatively affected the coronal seal showing the lowest push-out bond strength gradually decreasing over time, followed closely by TAP. Both antibiotic pastes, as well as CH, showed a reduction in seal starting at week 2 itself, significantly decreasing over the 12 week evaluation period. This can be explained by a study conducted by Yassen *et al*.[7] who stated that antibiotic pastes show definite demineralization of dentin due to their acidic nature and this effect increases in a time-dependent fashion. CH application was also shown to cause collagen degradation of dentin.[7] Furthermore, it was put forth that intracanal medicament application caused alteration of dentinal Ca and *P* and in turn increased the surface roughness and led to substandard micromechanical properties. A combination of these factors may be responsible for the poor seal of NeoMTA Plus to pretreated dentin.

Although this explanation suffices for the mechanical mode of adhesion between NeoMTA Plus and dentin, Sarkar *et al*.[11] have shown that the secondary adhesion is more of chemical binding. This is mediated by a diffusion controlled reaction between the apatite layer of MTA and dentin. An adhesive interfacial layer is formed by leaching of Ca^{2+} ions by MTA, which on further examination was revealed to be a structure similar in composition to hydroxyapatite and composed primarily of calcium, phosphorus, and oxygen. Triggering the formation of this adhesive layer may enhance the bond between MTA and dentin. This is a possible explanation of why the NeoMTA

Table 2: Comparison of four groups at 2 weeks, 4 weeks, and 12 weeks values by one way ANOVA

Groups	2 weeks		4 weeks		12 weeks		Changes from 2 weeks to 4 weeks		Changes from 2 weeks to 12 weeks	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	11.99	0.39	11.70	0.60	11.39	0.68	0.29	0.84	0.60	0.81
Calcium hydroxide	7.74	0.32	6.81	0.31	5.73	0.54	0.93	0.51	2.02	0.49
Double antibiotic paste	2.82	0.35	1.89	0.41	1.46	0.40	0.93	0.55	1.36	0.38
Triple antibiotic paste	4.86	0.40	3.74	0.37	1.75	0.31	1.12	0.37	3.11	0.58
F	1155.2600		960.4330		850.2850		3.7520		33.0420	
P	0.0001*		0.0001*		0.0001*		0.0192*		0.0001*	

**P*<0.05. SD: Standard deviation

Table 3: Pair-wise comparison of four groups at 2 weeks, 4 weeks, and 12 weeks values by Tukeys multiple *post hoc* procedures

Groups	*P* 2 weeks	4 weeks	12 weeks	Changes from 2 weeks to 4 weeks	Changes from 2 weeks to 12 weeks
Control versus calcium hydroxide	0.0002*	0.0002*	0.0002*	0.0955	0.0002*
Control versus double antibiotic paste	0.0002*	0.0002*	0.0002*	0.0918	0.0312*
Control versus triple antibiotic paste	0.0002*	0.0002*	0.0002*	0.0171*	0.0002*
Calcium hydroxide versus double antibiotic paste	0.0002*	0.0002*	0.0002*	0.9990	0.0741
Calcium hydroxide versus triple antibiotic paste	0.0002*	0.0002*	0.0002*	0.8807	0.0011*
Double antibiotic paste versus triple antibiotic paste	0.0002*	0.0002*	0.5750	0.8884	0.0002*

**P*<0.05

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Plus bond to CH in the present study was higher than that of antibiotic pastes. Use of nonsetting CH has been advocated before MTA placement to neutralize the pH in an acidic environment and improve the sealing ability of MTA. This is due to the formation of calcium carbonate or reaction of MTA with residual CH, both supplying Ca^{2+} necessary for the production of the adhesive interfacial layer as aforementioned. Among antibiotic pastes used in the present study, sealing ability of NeoMTA Plus in teeth treated with TAP was higher than in those treated with DAP. Studies have shown that TAP cannot be effectively removed once placed and about 80% is retained in the root canal regardless of the irrigant used. Minocycline in the TAP binds to calcium via chelation-forming insoluble complexes in tooth matrix. The presence of minocycline in TAP over DAP and its reaction with dentin may be responsible for better adhesion.[7]

The present study shows that the predominant mode of failure of NeoMTA Plus was of the cohesive type. This was not in agreement with Shokouhinejad *et al*.[8] who stated that failure was usually at MTA-dentin interface or of adhesive type. Studies have shown that the particle size affects the adhesion of cements to dentin.[9] Since NeoMTA Plus has a smaller particle size as compared to conventional MTA variants, it may be the reason for enhanced penetration into dentinal tubules, in turn increasing the bond strength.

In the present study, CH, DAP, and TAP all caused a significant decrease in bond strength and consequently sealing efficacy of NeoMTA Plus to dentin at successive time intervals from 2–12 weeks [Tables 2 and 3]. This coincides with previous studies[6] done to test the dislocation resistance of MTA under the influence of intracanal medicaments showing that NeoMTA Plus has similar adhesive properties as conventional MTA. Yassen *et al*. in a study[7] evaluating the effect of CH, DAP, and TAP on microhardness and fracture resistance of roots stated that antibiotic pastes negatively affected the microhardness after 1–3 months application, whereas CH increased the microhardness at all time periods. However, all medicaments have shown to decrease the fracture resistance of roots at a 3 months period as compared to 1 week.

CONCLUSION

Within limitations of this *in vitro* study, CH, DAP, and TAP have all shown to decrease the sealing efficacy of NeoMTA Plus over a period from 2–12 weeks of application. The bond strength values of intracanal medicaments deteriorated as treatment time was prolonged. The values were observed to be the lowest for teeth treated with DAP. On examination of failure mode between NeoMTA Plus and dentin, cohesive failure was found to be the most predominant, regardless of the medicament used or duration of application. Therefore, NeoMTA Plus can be used as a suitable esthetic alternative to previously used CH and conventional MTA in cases of RET in young, immature anterior teeth. The one thing however that must be kept in mind is the appropriate selection of medicament as well as the duration of treatment to obtain maximum remedial effects without compromising on the coronal seal of the endodontically treated tooth.

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Conflicts of interest

There are no conflicts of interest.

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