EFFECT OF LOW CONCENTRATIONS OF ANTIBIOTIC INTRACANAL MEDICAMENTS ON CROWN DISCOLORATION AND PUSH-OUT BOND STRENGTH

by

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DEDICATION

I owe thanks to God in the first place.
This thesis is dedicated to my wife Jumana Alashi,
and my son, Youssof Yaghmoor. They have been the key to my success
in my educational career. Also, I dedicate this thesis to my mother, brother, and
sisters, who have always supported me. A special dedication to my role model,
my ill father Bahjat Yaghmoor, who devoted his life, efforts, and money
to educate me well. Lastly, I dedicate this work to my wife's family, who
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TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

INTRODUCTION

Traditionally, the first choice for the clinician to treat immature traumatized teeth is apexification because of its high success rate.[1] Several drawbacks of apexification have been investigated in the literature. These drawbacks include increased risk of root fracture,[2] inability to regain the pulp vitality,[3] and the fact that it is a long-term treatment with multiple visits.[4] Hypothetically, previously mentioned drawbacks might be minimized when endodontic regeneration (ER) is used instead of apexification. ER is the most recent treatment option for immature teeth with necrotic pulps and is defined as **"**biologically based procedures designed to replace damaged structures, including dentin and root structures, as well as cells of the pulp-dentin complex." [5]

The American Association of Endodontists (AAE) has recommended the following procedure for regenerative treatment.[6] At the first appointment, the canal is irrigated with 20 mL of 1.5 percent of sodium hypochlorite (NaOCl) for 5 minutes, dried with paper points, filled with Triple (TAP) or double (DAP) antibiotic paste (0.1-1 mg/mL) or $Ca(OH)_2$, and temporized with Cavit for 1 to 4 weeks. At the second visit, the root canal is rinsed with 20 mL of 17-percent ethylenediaminetetraacetic acid (EDTA) followed by sterile saline. Bleeding is then stimulated by extending a sterile instrument from the apex into the apical tissues, and a collagen membrane is placed on the blood clot 3 mm below the cemento-enamel junction. Finally, a 3 mm to 4 mm thickness of root cement is applied and the access is restored with glass ionomer followed by composite resin.

Endodontic regeneration demands effective disinfection of the root canal,[7-9] which can be achieved through irrigation solutions and intracanal medicaments.[10-12]

The most commonly used irrigation solutions are NaOCl and chlorhexidine.[5] Various intracanal medicaments have been advocated during endodontic regeneration, including $Ca(OH)_2$,[13] TAP (equal amounts of metronidazole, ciprofloxacin, and minocycline),[14,15] and DAP (equal parts of metronidazole and ciprofloxacin).[16]

Typical clinical concentrations of TAP and DAP (1000 mg/mL) were suggested to have harmful effects on the survival of the pulp fibroblasts,[17] stem cells from apical papillae,[18] and dental pulp stem cells,[19] Furthermore, high concentrations of currently used antimicrobial medicaments were proposed to adversely affect the physical,[20,21] chemical,[22] and mechanical structure of radicular dentin.[22,23] AAE proposed to use low concentrations of DAP and TAP, ranging from 0.1 mg/mL to 1 mg/mL, instead of the currently used concentrations in an attempt to create a biocompatible biological environment within the root canal during endodontic regeneration.[6] However, these low concentrations are in a liquid form and cannot be used as an inter-appointment dressing during regenerative endodontics. Recently, a series of articles proposed a novel approach to load these low concentrations of antibiotic combinations into a water-based methylcellulose system in an attempt to create antimicrobial pastes with controlled antibiotic concentrations.[24-26] This approach claimed to maintain the antimicrobial effects of these antibiotics,[21,27] minimize the cytotoxic potential of these medications,[26] and reduce the notorious effects of these medicaments on physio-mechanical properties of radicular dentin.[21-24,25]

Little is known about potential effects of intracanal medicaments used in endodontic regeneration on the bonding of calcium-silicate-based cements to dentin, and recent studies have demonstrated conflicting findings. Recent studies reported significant negative effects of $Ca(OH)_2$,[28] as well as typical clinical concentrations of

DAP [28,29] and TAP[28] on bond strength of calcium-silicate-based cements to root dentin. On the other hand, another study showed that $Ca(OH)_2$ and TAP did not significantly compromise the bond strength of root cements to dentin.[29] Nevertheless, none of the previous studies were able to test the effect of low concentrations of DAP or TAP on bond strength of root cements as it is technically impossible to maintain the liquid form of low antibiotic concentrations within the root canal over an extended period of time. The ability of loading low concentrations of DAP and TAP into a methylcellulose system would enable us to accurately estimate the effect of these low concentrations on bond strength of various calcium-silicate cements.

Several studies demonstrated clear evidence of significant tooth discoloration after TAP application.[30-32] The reason for this discoloration was mainly attributed to the presence of minocycline, a tetracycline derivative, within the TAP composition. [30,33] Therefore, some studies suggested sealing the dentin within the pulp chamber with dental adhesive before TAP application.[32,34] Other studies recommended using minocycline-free antibiotic medicaments such as DAP [33] or replacing the minocycline present in TAP with non-tetracycline antibiotics.[31] Indeed, a recent study demonstrated that the application of DAP or $Ca(OH)_2$ did not cause any significant tooth discoloration.[33] However, other studies have shown that the $Ca(OH)_2$ and high concentration of DAP can also lead to significant tooth discoloration,[35-37] No previous study was able to explore the ability of 10 mg/mL or 1 mg/mL concentrations of DAP or TAP intracanal medicaments to induce tooth discoloration.

Objectives: The aims of this study were 1) to evaluate the effects of low concentrations of TAP and DAP (1 mg/mL) loaded into an aqueous methylcellulose system on push-out bond strength of various root cements and 2) to evaluate the

effects of mid and low concentrations of TAP and DAP (10 mg/mL and 1 mg/mL) loaded into an aqueous methylcellulose system on crown discoloration.

First Null Hypothesis: Low concentrations of TAP and DAP medicaments will not cause significant change in bond strength of calcium-silicate cements to radicular dentin.

First Alterative Hypothesis: Low concentrations of TAP and DAP medicaments will cause significant reduction in bond strength of calcium-silicate cements to dentin.

Second Null Hypothesis: Mid and low concentrations of DAP and TAP medicaments will not cause significant tooth discoloration regardless of the use of an adhesive bonding agent.

Second Alterative Hypothesis: Mid and low concentrations of DAP and TAP medicaments will cause significant tooth discoloration regardless of the use of an adhesive bonding agent.

REVIEW OF LITERATURE

ENDODONTIC REGENERATION

Of all dental traumatic injuries in children, 22 percent are in permanent teeth.[38] Most of those are immature teeth.[38] Traditionally, the first choice for the clinician to treat immature traumatized teeth is apexification because of its high success rate.[1] Apexification's technique is based on the formation of an apical barrier to prevent extrusion of a root canal filling using either calcium hydroxide or MTA.[39] This technique's concern is periapical healing with no root growth.[40] In an attempt to overcome the apexification drawbacks, ER was recently used by clinicians.[1] The idea of ER is based on Nygaard Ostby's 1961 study in which he used blood clots to form new blood vessels, which caused the formation of new tissue.[41] The engineering concept of ER is to regenerate the dentin and pulp in the root canal space. Moreover, ER has some advantages over apexification, such as short-term treatment, maintaining the tooth vitality, and increasing the root length and thickness.[39,42] In 2001 Iwaya et al. concluded in the first published case about ER that revascularization of the immature teeth with necrotic pulp can be achieved.[43] After that, Banches et al. in 2004 published another case report that showed ER can regain the vitality in the necrotic immature teeth as well as regenerate roots.[42] In the two previously mentioned studies, the clinicians used the intracanal medicaments as a disinfected material, which has a major role in a teeth revascularization.

INTRACANAL MEDICAMENTS

The intracanal medicaments are used daily in dental clinics to control dental infection and decrease inflammation, pain, and root resorption.[42,44] Providing a

suitable intracanal environment in the necrotic immature tooth depends mainly on disinfection.[45] Minimal mechanical instrumentation with NaOCl has been shown to create an insufficiently disinfected environment for ER.[46] Moreover, bacteria is not only present in the root canal space but may also exist in dentinal tubules, accessory canals, or any other regions in the root canal system.[47] For this reason, canal disinfection in ER is usually achieved by chemical means through using irrigants and medicaments to assure a maximum disinfection of the root. Irrigation agents do not provide enough sterilization because of the presence of complex bacteria in the root canal system.[48] Sato et al. have proven that the TAP combination of antibiotics can infiltrate through the dentin competently.[49] The most common intracanal medicament used clinically in ER is TAP.[14,15] This triple antibiotic was investigated to prove its efficiency on bacteria *in vitro* and *in vivo*.[49,50] TAP has bactericidal components (metronidazole and ciprofloxacin) and a bacteriostatic component (minocycline).[43] Iwaya et al. and Banches et al. used DAP and TAP respectively as intracanal medicaments.[42,43] In addition to the TAP and DAP, $Ca(OH)_2$ is considered an intracanal medicament.[51] Due to the low water solubility of $Ca(OH)_2$, it needs 3 weeks to 4 weeks to reach the maximum concentration in the peripheral dentin.[52] This is the length of time recommended in the AAE considerations.[6] The antibacterial effect of the $Ca(OH)_2$ is accomplished by increasing the pH (12.5-12.8), which makes an unfavorable environment for bacteria when they come in direct contact.[53] This highly alkaline environment is achieved by the release and diffusion of hydroxyl ions (OH).[54] However, some concerns have been raised when using intracanal medicaments, like crown discoloration,[32,35] bacterial resistance, and allergic reactions.[34]

PUSH-OUT BOND STRENGTH

The bond strength laboratory tests have been classified into 1) qualitative tests which are concerned with the type of bond failures and 2) quantitative tests that calculate the amount of force required for the bond to fail.[55] Furthermore, the bond strength tests can be done on the macro level (bonding surface area >3mm^2) or on the micro level (bonding surface area <3mm^2).[56] Some of the advantages of performing laboratory tests over clinical tests are 1) a specific parameter can be measured while all other variables are constant, 2) data can be more easily collected and analyzed than in clinical studies, and 3) different experimental groups can be tested in one study.[57] On the other hand, it is impossible to perform or design a single laboratory test that can predict the exact clinical behavior of specific materials.[58] Immature teeth treated by ER are always exposed to occlusal force that may affect the sealing and bonding of the intracanal cements to the radicular dentin. For that reason, the push-out bond strength test has been commonly used to evaluate the shear bond strength of the intracanal filling materials to the radicular dentin.[28,29,59-61] The push-out test procedure was explained in the literature as when the load or force is applied perpendicular to the testing material through a plunger that is attached to the universal machine without touching the radicular dentin.[59] Moreover, the intracanal filling materials that showed high push-out bond strength numbers have high longevity and good prognosis.[62,63] The main concern about the push-out test is the variations in the test methods that make it difficult to compare the results of different studies.[64] Recent meta-analysis review concluded that root filling materials, root filling techniques, tooth type, root cylindrical section and thickness, storage time, and load velocity all affect the push-out bond strength test.[65] Also, the effects of different intracanal medicaments on the mechanical and surface properties of the radicular dentin have

been studied.[23,24] These studies found that such effects have impact on the bond strength of intracanal cements to radicular dentin.[29,66]. Topcoglu et al.[29] and Turk et al.[28] concluded in their studies that using intracanal medicaments in ER treatment decreases the bond strength of intracanal cements to radicular dentin.

INTRACANAL CEMENTS

Calcium-silicate-based cements have been used widely in ER treatment. Moreover, calcium-silicate-based cements have favorable properties (such as biocompatibility, ability to kill bacteria, sealing ability, bioactivity, ability to set in a moist environment, and acceptable mechanical and physical properties) that make it the material of choice in ER.[67,68] Different calcium-silicate based cements are similar in their main chemical composition but have some variances (Table I). It is well known that mineral trioxide aggregate (MTA) is the gold standard material between all calcium-silicate cements.[69] MTA was first established as a root repair material by Torabinejad in 1993 at Loma Linda Uneversity.[70] Basically, the MTA cement is composed of calcium-silicate powder that reacts with water to produce calcium hydroxide and calcium-silicate through a hydration reaction.[71] The calcium ions produced from the reaction act as a stimulator for the osteoblast and odontoblast to differentiate and proliferate while the hydroxide group increases the alkalinity to create an unfavorable environment for the bacteria to grow.[72-74] Although MTA is the gold standard for the calcium-silicate-based cements, it has some drawbacks, e.g. long setting time (3 hours to 4 hours), tooth discoloration, high cost, low radiopacity, difficult manipulation, and poor handling properties.[75,76] In order to overcome these disadvantages, the manufacturers compete to produce new materials that also maintain the desirable properties of the MTA. Biodentine and Bioceramic putty are two examples of new calcium-silicate-based materials. Biodentine first started in 2009

as a dentin replacement material and has also been used in root repair material, pulp capping, and retrograde filling.[77] It is supplied as a pre-capsulated powder and liquid and triturated with an amalgamator. In addition, studies showed that Biodentine has superior physical and mechanical properties, faster setting time (12 min), higher bond strength to the radicular dentin, and better sealing ability than MTA.[78] These improved properties are due to adding reducing agent in the liquid, which results in a low powder/liquid ratio.[78] In addition, the manufacturer claimed that the decreased setting time is an effect of increasing the surface particle size, adding accelerators, and reducing the amount of liquid needed in the reaction.[79] On the other hand, Bioceramic putty was first developed in 2007 as a premixed ready-to-use material. The setting reaction and setting time (2 hours to 4 hours) of this material depend on the amount of moisture present in the dentinal tubules.[80] Bioceramic putty has good mechanical, biological, and antibacterial properties, and it is better than MTA in handling properties, sensitivity to moisture, radiopacity, and sealability.[81-84] Contact of Bioceramic putty and the physiological fluid results in hydroxyapatite-like precipitate.[85]

CROWN DISCOLORATION

Clinicians should not emphasize the biological and functional issues and ignore the esthetic one. According to Sulieman et al., esthetic discoloration problems in patients are considered a more significant issue than restoring normal tooth shape.[86] For that, clinicians need to be aware of the causes and clinical manifestations of discoloration in order to provide the best treatment.[87] Previous studies stated that some intracanal medicaments caused tooth discoloration during and after ER.[30-32, 35] Akcay et al. compared the effects of different antibiotics used in ER on crown discoloration of bovine teeth.[33] The result showed that TAP with the minocycline

group causes the highest degree of crown discoloration, exceeding the perceptibility threshold (3.7). Kim et al. did an *in-vitro* study to know which component of TAP is responsible for crown discoloration; the result showed that neither metronidazole or ciprofloxacin caused discoloration, but the minocycline did.[32] Minocycline is a semisynthetic derivative of tetracycline.[32] The mechanism of discoloration caused by minocycline is not known yet. However, Tanase et al. concluded that a possible mechanism is due to the minocycline incorporated into dentin and enamel by binding to Ca^{2+} through chelation.[88] Moreover, the possible theoretical mechanism of crown discoloration is caused by $Ca(OH)_2$ that, post dissociation into Ca^{2+} and OH^-, Ca^{2+} diffuses within dentinal tubules to react with hydroxyapatite-forming crystals that may result in white or yellow color changes in teeth.[36]

Several suggested solutions for crown discoloration have been proposed. One study stated that reducing the application time to 24 h may prevent crown discoloration, but in other studies the discoloration effect appeared after 1 h of TAP application.[32,89] The AAE recommend delivering the intracanal medicaments via a syringe and when using the TAP, keeping it below the level of the cementoenamel junction in an attempt to prevent possible crown discoloration.[6] Other ways to prevent crown discoloration were proposed by Thibodeau et al. and Trop et al. when they replaced the minocycline with cefaclor and Arestin in the TAP, respectively.[7,16] This approach reduced the crown discoloration but didn't prevent it. Furthermore, Reynolds et al. and Kim et al. used adhesive bonding agents in the pulp chamber prior to TAP application in an attempt to seal the dentinal tubules to prevent the diffusion of the antibiotics.[32, 34] The results of these case reports showed that applying adhesive bonding agent reduces the crown discoloration but does not prevent it.

Several methods have been used in previous studies to measure tooth

discoloration, e.g. detecting discoloration visually, color matching with color tabs, looking at digital photos, using colorimeters, and using spectrophotometers.[32,36,90] However, the visual methods are not consistent between the observer and are considered very subjective.[91] On the other hand, many studies done on the spectrophotometer concluded that it is a reliable, reproducible, accurate, and objective way to evaluate the color.[92-94] Because of this, the spectrophotometer is a gold standard in color science.[95] Paul et al. compared the conventional way, the human shade assessment, with the spectrophotometer to conclude that spectrophotometer was more accurate by 33 percent and a more objective match by 93.3 percent.[94] Spectrophotometers contain a source of optical radiation, and the device has a detector to measure the amount of light energy reflected from the object (1 nm to 25 nm intervals along the visible spectrum) as well as a means of converting it into quantitative data.[96] The spectrophotometer relies on the Commission International de I'Eclairage's (CIE) L*a*b* system, approved by the International Organization for Standardization.[97] This system is a three-dimensional uniform color space. The color differences can be quantified by the Euclidean distance (ΔE value) without indicating the direction of the color differences.[97]

In a previous study done by Johnstone and KAO, the main purpose was to establish the relation between the subjective color reading method and the objective color reading method.[98] They considered the United States Public Health Service (USPHS) visual criteria as a subjected method and the (CIE) L*a*b* system, using a colorimeter, as an objective numerical method.[98] The result showed that the color match by the USPHS was overlapped with the ΔE reading range from 2.2 to 4.4 in the (CIE) L*a*b* system.[98] They concluded that the 3.7 color change level is the average of the acceptable clinical color difference (perceptible threshold).[98]

MATERIALS AND METHODS

PUSH-OUT BOND STRENGTH EXPERIMENT

Sample Preparations

Intact, single, straight, conical-rooted human teeth (n = 144) were selected for this study following local Institutional Review Board (IRB) guidelines (IRB # 1408889870, 2016). The teeth were stored at 4 °C in 0.1-percent thymol solution and used no more than 6 months after extraction. Samples were horizontally decoronated 0.5 mm apical to the facial/buccal cementoenamel junction using a water-cooled low-speed diamond saw (Buehler ltd, Lake Bluff, IL). Furthermore, the apical 3 mm of each root were removed, resulting in 8 ± 1mm root sections. The internal diameter of the roots was standardized by mechanical preparation with Peeso reamers (Dentsply, Johnson City, TN) (size 1 to size 5) to a final diameter of 1.5 mm. After the use of each size of Peeso reamer, root canals were irrigated with 2 mL of 1.5% NaOCl for 1 min using a 27-gauge needle. After instrumentation was completed, each canal received a final rinse with 5 mL of 1.5% NaOCl for 2 min, 5 mL of 17% EDTA (Vista, Racine, Wisconsin) for 2 min, and 5 mL of sterile water for 2 min.

Intracanal Medicament Preparation

Preparation of intracanal medicaments was performed according to previous studies.[24,25] To prepare the commonly used clinical concentration of TAP, 1000 mg of United States Pharmacopeia grade antibiotic powders compounded of equal portions of metronidazole, ciprofloxacin, and minocycline (Champs Pharmacy, San Antonio, TX) were mixed with 1 mL of sterile water. To prepare the commonly used clinical concentration of DAP, 1000 mg of United States Pharmacopeia grade antibiotic

powders compounded of equal portions of metronidazole and ciprofloxacin (Champs Pharmacy) were mixed with 1 mL of sterile water. To prepare low concentrations of antibiotic medicaments, 100 mg TAP or DAP powders were dissolved in 100 mL of sterile water. Then, 8 g of methyl cellulose powder (Methocel 60 HG, Sigma-Aldrich, St. Louis, MO) were gradually added to the 100 mL of 1 mg/mL solution of TAP or DAP and mixed for 60 minutes using a magnetic stir bar to obtain a final homogenous paste with 1 mg/ mL concentration of TAP or DAP. The methylcellulose served as a vehicle to retain the low concentrations of TAP or DAP (1 mg/ml) in the root canal. In other words, the methylcellulose system increased the viscosity of the low concentration TAP or DAP to make its consistency clinically applicable. Commercial $Ca(OH)_2$ intracanal dressing was also used (UltraCal XS, Ultradent, South Jordan, Utah).

Treatment Procedure

The prepared roots were randomly divided into 6 treatment groups according to the type and concentration of intracanal medicament (n = 24 per group): no-treatment control group, $Ca(OH)_2$, typical clinical concentration of TAP (1000 mg/ml), low concentration TAP loaded into a methylcellulose system (1 mg/mL), typical clinical concentration DAP (1000 mg/mL), and low concentration DAP loaded into a methylcellulose system (Figure 1).

Intracanal medicaments (0.05 mL) were injected into the root canals in their respective groups using 1 mL disposable syringes (BD, Franklin Lakes, New Jersey) and intracanal capillary tips (Ultradent). After extrusion of the intracanal medicament from the apical opening, excess was removed and apical openings as well as coronal access were sealed using a light cure flowable composite (Kerr, Orange, CA). The flowable composite was bonded by a one-step self-etch adhesive bonding agent (G-

aenial, GC, Alsip, Illinois) on the external surface of the root with extreme caution to avoid touching the internal wall of the radicular dentin (Figure 2**)**. Roots were incubated in deionized water at 37 °C for 4 weeks. The four-week application period reflects the clinical guidelines of endodontic regeneration procedures recommended by the AAE.[6]

After incubation, roots treated with each intracanal medication were randomized into three subgroups based on type of root cement (8 = per subgroup): MTA cement (Proroot, Dentsply, Tulsa, OK), Biodentine cement (Septodont, Sant-Maur-des-Fosses, France), and Endosequence Bioceramic putty cement (Putty, Endosequence, Savannah, GA). The roots were re-accessed, and each root canal was irrigated with 5 mL 17-percent EDTA followed by 5 mL of sterile water to remove the intracanal dressing. Each of the three cements was mixed according to the manufacturer's instructions and applied into roots with different sizes of endodontic pluggers. The roots were then re-sealed with flowable composite and stored in a humid environment at 37 ^{o}C for 2 weeks to ensure the complete setting of the calcium-silicate cements. Conventional radiographs were taken buccolingually and mesiodistally to evaluate the compactness of the intracanal cement. After incubation, 2 cylindrical cross sections with 1.5-mm thickness were coronally obtained from each root using a water-cooled diamond saw (Figure 2). The apical and coronal sealed areas of the roots was excluded from the root cylinders to avoid any possibility of the undesirable effect of bonding agent on the intracanal medicaments and, consequently, on the intracanal cement bond to radicular dentin. Root canal diameters and the thickness of each root cylinder were measured to the nearest 0.01 mm utilizing a digital caliper (Mitutoyo, Japan). The area of adhesion between the cement and each root cylinder was estimated according to the following equation:

Adhesion surface area (mm^2) = $(D1 + D2)/2 \times \pi \times h$

where D1 and D2 are upper and lower cylinder diameters, respectively, π is the constant 3.14, and h is the thickness of the root cylinder.

Push-Out Bond Strength Test

This test was conducted utilizing a universal testing machine (Sintech Renew 1123, MTS, Eden Prairie, MN) as described in a previous study.[99] The root cylinders were stabilized, with the apical side facing upward, on the center of a metal disc that had a central hole (Figure 3 and 4). The central hole within the metal disc was larger than the root cylinder internal diameter to maintain the root cylinder in the correct position while allowing easy dislodgment of the root cement. A compressive force was applied at a crosshead speed of 0.5 mm/min using a cylindrical metal plunger (1.3 mm in diameter) connected to the loading cell (2500N). The metal plunger diameter was smaller than the internal root diameter by approximately 0.2 mm. The force of dislodgement of the dental cements was reported in newtons, and the push-out bond strength (MPa) was computed for all samples using the equation below:

Push-out bond strength (MPa) = the dislodgment force (N)/ adhesion surface area (mm^2).

Following the push-out experiment, the failure pattern of each sample was inspected using stereomicroscopy (Nikon UM-2, Tokyo, Japan) at X40 magnification and classified according to the following criteria: 1) adhesive failure (between dentin and the root cement), 2) cohesive failure (within the root cement), or 3) mixed failure.

DISCOLORATION EXPERIMENT

Sample Preparation

Intact human third molars, free of cracks, fractures, caries, abrasions, and visible discoloration (n = 160), were selected following local IRB guidelines. The teeth were stored at 4 °C in 0.1-percent thymol solution and were used no more than 6 months after extraction. Soft tissue was removed by hand scaling and polishing performed by a lab-polishing machine (Handler, NJ) with pumice (Henry Schein, Melville, NY). Teeth were horizontally sectioned 1 mm apical to the buccal cementoenamel junction using a water-cooled low-speed diamond saw to create crowns with a 1 mm extension to the roots. Access cavity preparation was not performed. Dental pulps were extirpated with a spoon excavator, and the internal axial walls of the pulp chambers were mechanically debrided through the retrograde access by spoon excavators. In addition, a surgical endo tip #3 (ProUltra, Dentsply, Johnson City, TN) was attached to an endodontic ultrasonic (ASI, Englewood, CO) hand piece, which was used to remove any remnants of pulp tissue. Then, 5 mL of 1.5-percent NaOCl followed by 5 mL of 17-percent EDTA were used to rinse each pulp chamber using 27-gauge needles. Additionally, each pulp chamber received a final irrigation with 5 mL of sterile water for one min. The main reason behind choosing the molar teeth – The retrograde access of molars is wide, facilitating the removal of pulp tissue from the pulp chamber.

Samples were randomly divided into 8 groups based on the type and concentration of intracanal medicament (n = 20 per group): no-treatment control group, $Ca(OH)_2$, typical clinical concentration TAP (1000 mg/mL), mid-concentration TAP (10 mg/mL), low-concentration TAP (1 mg/mL), typical clinical concentration DAP (1000 mg/mL), mid-concentration DAP (10 mg/mL) and low-concentration

DAP (1 mg/mL). Furthermore, every group was divided into two subgroups: with and without dental adhesive (n = 10 per subgroup). The dental adhesive agent was used to coat the internal wall of each pulp chamber before the application of the assigned intracanal medicament as an approach to minimize crown discoloration[32,34] (Figure 5).

APPLICATION OF DENTAL ADHESIVE AND INTRACANAL MEDICAMENTS

The internal walls of the pulp chambers for half of the samples in each group were coated with a one-step self-etch adhesive bonding agent (GC) according to the manufacturer's recommendation: apply the bonding agent, wait for 10 seconds and air dry for a maximum of 5 seconds. After that, an LED light curing unit (Ivoclar Vivadent Inc, Amherst, NY) was used at a distance of < 1mm (without contacting the tooth) for 10 seconds through the retrograde access opening. Intracanal medicaments were then delivered, using 1 mL disposable syringes (BD) and intracanal capillary tips (Ultradent), into the pulp chamber through the retrograde access. All internal walls of each pulp chamber were completely covered with the 0.05 mL of the corresponding intracanal medicament. After that, the retrograde access was sealed with A3 shade regular composite (Kerr) using a plastic hand instrument. The composite was applied on the external surface of the root with extreme caution to avoid pushing the composite into the pulp chamber. A one-step self-etch adhesive bonding agent was used to bond the composite. Then, each group was incubated in 100-percent humidity at 37 °C for 4 weeks.

After 4 weeks, the pulp chambers of all samples were re-accessed by removing the retrograde composite. The pulp chambers were rinsed by 5 mL of 17-percent EDTA followed by 5 mL of sterile water through the retrograde access to remove the intracanal medicaments. Then, the retrograde access was resealed with A3

shade regular composite (Kerr). All samples were then subjected to artificial aging (5000 thermal-cycles), which corresponds to approximately 6 months of *in-vivo* functioning (ISO TR 11450). For each cycle, the samples were alternated between two water baths of 5 ^{o}C and 55 ^{o}C with a dwelling time of 30 seconds at each temperature extreme (Thermocycler, SD Mechatronik, Feldkirchen-Westerham, Germany).

Color Measurement

Color measurement was performed at the following intervals for each sample: prior to intracanal medicament application (baseline), 1 day after application, 1 week after application, and 4 weeks after application, as well as after thermo-cycling, which was performed directly after the 4 weeks reading point. Crown color measurements were recorded with a Spectrophotometer Vita Easyshade (Vident, Brea, CA) under standard lab illuminations. To standardize the area of color measurement and the path of application of the spectrophotometer during repeated measurements, a close-fit custom-made cap with a circular hole 6.2 mm in diameter was prepared using clear, soft thermoplastic sheets 2-mm thick (Ultradent) (Figure 6). The hole was made by a Soft Tissue Puncher with a 6.2-mm diameter (Omnia Spa, Parma, Italy). The diameter of the hole matched the diameter of the spectrophotometer tip that was used for color measurement.

Each sample was mounted into its own custom-made cap before each color measurement. The device was calibrated at each time interval and the color measurements were reported by using the CIE L*a*b* system. The value of L* is the lightness (from 0 [black] to 100 [white]), and the values of a*and b* are the red-green axis (from +80[red] to -80[green]) and the yellow-blue axis (from +80[yellow] to -80[blue]) in the chromaticity parameter, respectively. The mean value of three

measurements of the total color change (ΔE) was calculated at each time interval by the spectrophotometer. ΔE describes the color difference between the time point before intracanal medicament placement and each subsequent time point measurement.

ΔE of each sample was calculated by the following equation:

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{½}$$

The proposed acceptance for color matching that was adopted in this study was 3.7 ΔE units (perceptibility threshold), beyond which the differences are clinically perceivable.[98]

STATISTICAL ANALYSIS

Summary statistics (mean, standard deviation, standard error, minimum, maximum) were calculated for the push-out bond strength for each treatment-cement combination at the two root cylinder locations. Bond strength was evaluated using three-way ANOVA with factors for Cements (MTA, Biodentine and Endosequence putty), Treatment (Control, $Ca(OH)_2$, 1000mg/ml TAP, 1mg/ml TAP, 1000mg/ml DAP and 1mg/ml DAP) and location on the root (coronal and middle), as well as all two-way and three-way interactions among the factors, a random effect was added to get correlation between the coronal and middle locations. All pair-wise comparisons from ANOVA were made using least significant differences to control the overall significance level at 5 percent (only the significant interactions were included). Bond strengths were summarized by cements, treatments and locations. Generalized estimating equation (GEE) methodology for cumulative logistic regression was used to evaluate the effects of cement type, treatment, and location on failure mode; two-

way and three-way interactions were included in the model. The GEE method allows for clustering of the two locations within each specimen.

Summary statistics (mean, standard deviation, standard error, minimum, maximum) were calculated for the color change for each treatment-adhesive bonding agent combination at each time point. One-sample t-tests were used to determine if the color change for each treatment-adhesive bonding agent combination for each time point was significantly different from 3.7, the perceptibility threshold. Repeated measures ANOVA was used to evaluate the effects of treatment, adhesive, and time on color change; two-way and three-way interactions were included in the model. Time was repeated within each specimen, allowing different variances at each time and non-constant correlations between times. A 5-percent significance level was used.

Sample Size Justification

Based on a previous study the standard deviation for push-out bond strength was estimated to be 2.2 MPa.[99] With a sample size of 7 roots per treatment-cement combination, the study had 80-percent power to detect a difference of 3.6 between any two subgroups, assuming two-sided tests each were conducted at a 5-percent significance level. The coefficient of variation for color change was estimated to be 0.5. With a sample size of 9 crowns per treatment-adhesive combination, the study had more than 90-percent power to detect a color change of 3.7 within each treatment-adhesive bonding agent combination for each time point and 80-percent power to detect a difference of doubling of the color change between any two subgroups, assuming two-sided tests were each conducted at a 5-percent significance level.

RESULTS

PUSH-OUT BOND STRENGTH EXPERIMENT

One-way ANOVA showed that all three variables (Treatment, Cement, and Location) had a significant effect on the bond strength (P-value < 0.0001). Moreover, the two-way interactions between previously mentioned factors showed a significant difference on bond strength with (Treatment*Cement) (P-value < 0.0001) and (Treatment*Location) (P-value = 0.0002) but no significant difference when Cement interacted with Location. However, the three-way interactions between all three variables revealed no significant difference in bond strength.

Location of Root Cylinders

Four treatment groups (control, $Ca(OH)_2$, 1000 mg/mL TAP, and 1 mg/mL TAP) had significantly higher bond strength in the coronal root cylinders than the middle root cylinders (P-value < 0.0001), as did 1 mg/mL DAP (P-value = 0.0003). On the other hand, 1000 mg/mL DAP did not have a significant difference between the coronal and middle root cylinders.

Types of Cement

The 1 mg/mL DAP group showed that the bond strength of Biodentine was significantly higher than MTA (P-value < 0.0001) and Endosequence putty (P-value = 0.0408), and the bond strength of Endosequence putty was significantly higher than MTA (P-value = 0.0044). Moreover, the $Ca(OH)_2$ and 1 mg/mL TAP groups presented no significant difference between all three types of cements. Furthermore, the 1000 mg/mL TAP and 1000 mg/mL DAP groups displayed the same trend in bond strength between the three cements as following: Endosequence putty and

Biodentine were significantly higher than MTA (P-value < 0.0001) but did not show a significance difference between each other. Finally, the control group revealed that bond strength of Endosequence putty was significantly higher than MTA (P-value < 0.0001) as well as significantly higher than Biodentine (P-value = 0.0020), but the bond strength between MTA and Biodentine did not have a significant difference (Table II).

Types of Treatment

Both coronal and middle root cylinders showed the same pattern of significant bond strength between different treatment groups within the same type of cement (Table II).

MTA Cement in Coronal Root Cylinders

Although the 1 mg/mL DAP group showed the highest bond strength, it also had no significant difference from the $Ca(OH)_2$ and 1 mg/mL TAP groups but did have significantly higher bond strength than the control, 1000 mg/mL TAP, and 1000 mg/mL DAP groups (P-value < 0.0001). The $Ca(OH)_2$ group did not have a significant difference with 1 mg/mL TAP but did show significantly higher bond strength than the control, 1000 mg/mL TAP, and 1000 mg/mL DAP groups (P-value < 0.0001). Moreover, the 1 mg/mL TAP group displayed significantly higher bond strength than the control (P-value = 0.0002), 1000 mg/mL TAP (P-value = 0.0001), and 1000 mg/mL DAP (P-value < 0.0001) groups. The bond strength of the control group did not show a significant difference with the 1000 mg/mL TAP group but was significantly higher than the 1000 mg/mL DAP group (P-value = 0.0120). Lastly, the 1000 mg/mL TAP group was significantly higher than the 1000 mg/mL DAP group (P-value = 0.0204) in bond strength (Figure 7).

Biodentine Cement in Coronal Root Cylinders

The highest bond strength in the 1 mg/mL DAP group was significantly different from all other treatment groups (P-value < 0.0001). Furthermore, bond strength of the 1000 mg/mL TAP group was not significantly different than the $Ca(OH)_2$, 1 mg/mL TAP, and 1000 mg/mL DAP groups. On the contrary, it was significantly higher than the control group (P-value < 0.0001). $Ca(OH)_2$ did not have a significant difference in bond strength with the 1 mg/mL TAP and 1000 mg/mL DAP groups but was significantly higher than the control group (P-value = 0.0008). The 1 mg/mL TAP group was not significantly different, regarding bond strength, with the 1000 mg/mL DAP group, but it was significantly higher than the control group (P-value = 0.0001). Finally, the 1000 mg/mL DAP group was significantly higher than the control group (P-value = 0.0001) in bond strength (Figure 8).

Endosequence Putty Cement in Coronal Root Cylinders

The 1 mg/mL DAP group had the highest bond strength and was not significantly different from the $Ca(OH)_2$ group. However, it was significantly higher than the 1 mg/mL TAP (P-value = 0.0264), control (P-value = 0.0086), 1000 mg/mL TAP (P-value = 0.0043), and 1000 mg/mL DAP (P-value = 0.0083) groups. The $Ca(OH)_2$, 1 mg/mL TAP, control, 1000 mg/mL TAP, and 1000 mg/mL DAP groups did not show significant difference from each other (Figure 9).

MTA Cement in Middle Root Cylinders

The highest bond strength in the $Ca(OH)_2$ group had no significant difference from 1 mg/mL TAP and 1 mg/mL DAP but was significantly higher than the 1000 mg/mL TAP, control, and 1000 mg/mL DAP groups (P-value < 0.0001). The 1 mg/mL TAP group was not significantly different from the 1mg/mL DAP group but

had significantly higher bond strength than the 1000 mg/mL TAP (P-value < 0.0001), control (P-value = 0.0002), and 1000 mg/mL DAP (P-value < 0.0001) groups. Moreover, the 1 mg/mL DAP group displayed significantly higher bond strength than the 1000 mg/mL TAP (P-value < 0.0001), control (P-value = 0.0001), and 1000 mg/mL DAP (P-value < 0.0001) groups. The bond strength of the 1000 mg/mL TAP group did not have a significant difference from the control group but did have significantly higher bond strength than the 1000 mg/mL DAP group (P-value = 0.0204). Lastly, the control group was significantly higher than the 1000 mg/mL DAP group (P-value = 0.0120) in bond strength (Figure 10).

Biodentine Cement in Middle Root Cylinders

The highest bond strength in the 1 mg/mL DAP group showed a significant difference from all other treatment groups (P-value < 0.0001). Furthermore, the bond strength of the 1000 mg/mL DAP group was not significantly different than the 1 mg/mL TAP, $Ca(OH)_2$, and 1000 mg/mL TAP groups. In contrast, it was significantly higher than the control group (P-value = 0.0001). The 1 mg/mL TAP group did not show a significant difference in bond strength from the $Ca(OH)_2$ and 1000 mg/mL TAP groups but did have significantly higher bond strength than the control group (P-value = 0.0001). The $Ca(OH)_2$ group did not have a significant difference regarding bond strength with the 1000 mg/mL TAP group, but it was significantly higher than the control group (P-value = 0.0008). Finally, the 1000 mg/mL TAP group was significantly higher than the control group (P-value = 0.0005) in bond strength (Figure 11).

Endosequence Putty Cement in Middle Root Cylinders

The 1 mg/mL DAP group had the highest bond strength but was not

significantly different from $Ca(OH)_2$ group. However, it was significantly higher than the 1000 mg/mL DAP (P-value = 0.0083), 1000 mg/mL TAP (P-value = 0.0043), 1 mg/mL TAP (P-value = 0.0264), and control (P-value = 0.0086) groups. Finally, the $Ca(OH)_2$, 1000 mg/mL DAP, 1000 mg/mL TAP, 1 mg/mL TAP, and control groups were not significantly different from each other (Figure 12).

Failure Mode

Location: The coronal location had higher proportion of cohesive failures than the middle location for Biodentine cement in the control group (P-value = 0.0137), for MTA cement in the DAP 1000 mg/mL (P-value = 0.0497) group, and for MTA cement in the TAP 1 mg/mL group (P-value = 0.0026). The coronal location had a lower proportion of cohesive failures than the middle location for MTA cement in the DAP 1 mg/mL group (P-value = 0.0003). No other statistically significant location differences were found.

Cement: MTA had a lower proportion of cohesive failures than Endosequence putty (P-value < 0.0037) and Biodentine (P-value < 0.0281) for the DAP 1 mg/mL group at the coronal location.

Treatment: The DAP 1 mg/mL group had a higher proportion of cohesive failures than the control (P-value = 0.0417) and TAP 1000 mg/mL (P-value = 0.0247) groups for Endosequence putty. The DAP 1 mg/mL group had a lower proportion of cohesive failures than the DAP 1000 mg/mL (P-value = 0.0066), TAP 1 mg/mL (P-value = 0.0279), and TAP 1000 mg/mL (P-value = 0.0123) groups for MTA at the coronal location. The control group had a lower proportion of cohesive failures than the DAP 1000 mg/mL (P-value < 0.0326) and TAP 1 mg/mL (P-value < 0.0307) groups for Endosequence putty at the middle location, as well as a lower proportion of cohesive failures than the TAP 1 mg/mL group for Biodentine at the middle location

(P-value = 0.0324).

DISCOLORATION EXPERIMENT

Color changes showed significant ($p<0.05$) increase ($\Delta E > 3.7$) only in the following groups: TAP 10 with no adhesive at week 1 and after thermocycling (Figure 13); TAP 1000 with adhesive at week 1, week 4, and after thermocycling (Figure 14); and TAP 1000 with no adhesive at all time points (Figure 13).

Adhesive Effect on Color Change

Color change was significantly lower with adhesive than without adhesive for TAP 10 mg/mL after week 1 (P-value = 0.0206) and after thermocycling (P-value = 0.0001). In addition, color change was significantly lower with adhesive than without adhesive for TAP 1000 mg/mL after day 1 (P-value < 0.0001), week 1 (P-value < 0.0001), and week 4 (P-value < 0.0001).

Time Effect on Color Change in Adhesive Groups

The $Ca(OH)_2$ group demonstrated significantly higher color change in 1 week and 4 weeks than in 1 day and after thermocycling. The no-treatment (control) group showed significantly higher color change in 1 week and 4 weeks than 1 day and after thermocycling. It is worth mentioning that there were no significant differences in between all time points regarding the DAP 1 mg/mL and DAP 10 mg/mL groups. DAP 1000 mg/mL group displayed significantly higher color change in 1 day than after 4 weeks and thermocycling; also, the 1-week time point had significantly higher color change than after thermocycling. TAP 1 mg/mL group displayed significantly higher color change at the 1-week time point than the other three time points. TAP 10 mg/mL group revealed significantly higher color change in 1 week than 1 day and after thermocycling, and the 4-week time point showed significantly higher color

change than 1 day. Finally, TAP 1000 mg/mL group showed significant color change between all time points in the following sequence, starting from the highest to the lowest: after thermocycling, 4 weeks, 1 week and 1 day (Table III).

Time Effect on Color Change in Non-Adhesive Groups

The $Ca(OH)_2$ group showed that the 4-week time point had significantly higher color change than the 1-day time point and after thermocycling; after 1 day and after 1 week showed significantly higher color change than after thermocycling. The no-treatment (control) group showed significantly higher color change in 1 week and 4 weeks than 1 day and after thermocycling. The DAP 1 mg/mL group illustrated significantly higher color change in 4 weeks and after thermocycling than after 1 week. However, the DAP 10 mg/mL group showed the opposite result where 1 week had significantly higher color change than 4 weeks and after thermocycling. Moreover, the DAP 1000 mg/mL group showed higher significant color change in 1 day and 1 week than 4 weeks and after thermocycling. TAP 1 mg/mL group showed significantly higher color change in 1 week than 1 day and 4 weeks. The 4-week and after-thermocycling time points illustrated significantly higher color change than 1 day. TAP 10 mg/mL group showed after 1 week and after thermocycling to have a significantly higher color change than after 1 day and after 4 weeks, and the 4-week time point reading had significantly higher color change than the 1-day time point. Finally, TAP 1000 mg/mL group revealed that the color change at 4 weeks was significantly higher than all other three time points, and after thermocycling had significantly higher color change than 1 day (Table III).

Treatment Effect on Color Change in Adhesive Groups

The 1-day time point (Figure 15) showed that TAP 1000 mg/mL and DAP 1000 mg/mL had significantly higher color change than $Ca(OH)_2$ and no treatment. In addition, DAP 1 mg/mL had significantly higher color change than DAP 10 mg/mL and TAP 1 mg/mL; however, DAP 1 mg/mL had significantly lower color change than DAP 1000 mg/mL and TAP 1000 mg/mL. Also, DAP 1000 mg/mL and TAP 1000 mg/mL displayed significantly higher color change than DAP 10 mg/mL. Furthermore, the DAP 1000 mg/mL demonstrated significantly higher color change than TAP 10 mg/mL and TAP 1 mg/mL. Finally, TAP 1000 mg/mL displayed significantly higher color change than TAP 10 mg/mL and TAP 1 mg/mL (Table V).

The 1-week time point (Figure 16) showed that $Ca(OH)_2$ and no treatment had significantly higher color change than DAP 1 mg/mL and DAP 10 mg/mL; however, TAP 1000 mg/mL had significantly higher color change than $Ca(OH)_2$ and no treatment. Furthermore, DAP 1000 mg/mL, TAP 1 mg/mL, TAP 10 mg/mL, and TAP 1000 mg/mL had significantly higher color change than DAP 1 mg/mL and DAP 10 mg/mL. Moreover, TAP 1000 mg/mL revealed significantly higher color change than DAP 1000 mg/mL, TAP 1 mg/mL, and TAP 10 mg/mL (Table V).

The 4-week time point (Figure 17) showed that $Ca(OH)_2$ had significantly higher color change than DAP 10 mg/mL and TAP 1 mg/mL, but it had significantly lower color change than TAP 1000 mg/mL. In addition, the no-treatment group had significantly higher color change than DAP 1 mg/mL, DAP 10 mg/mL, or TAP 1 mg/mL, but it had significantly lower color change than TAP 1000 mg/mL. Moreover, TAP 1000 mg/mL had significantly higher color change than DAP 1 mg/mL. DAP 1000 mg/mL, TAP 10 mg/mL, and TAP 1000 mg/mL had significantly higher color change than DAP 10 mg/mL. Furthermore, TAP 1000 mg/mL revealed

significantly higher color change than DAP 1000 mg/mL, TAP 1 mg/mL, and TAP 10 mg/mL (Table V).

After thermocycling (Figure 18) showed that DAP 1 mg/mL and TAP 1000 mg/mL had significantly higher color change than $Ca(OH)_2$. Moreover, TAP 1000 mg/mL had significantly higher color change than the no-treatment, DAP 10 mg/mL, DAP 1000 mg/mL, TAP 1 mg/mL, and TAP 10 mg/mL groups. In addition, DAP 1 mg/mL had significantly higher color change than DAP 10 mg/mL, but it showed significantly lower color change than TAP 1000 mg/mL (Table V).

Treatment Effect on Color Change
in Non-Adhesive Groups

The 1-day time point (Figure 19) showed that DAP 1000 mg/mL and TAP 1000 mg/mL had significantly higher color change than the $Ca(OH)_2$, no-treatment, and DAP 10 mg/mL groups; also, $Ca(OH)_2$ had significantly higher color change than TAP 1 mg/mL. Furthermore, DAP 1000 mg/mL and DAP 1000 mg/mL had significantly higher color change than DAP 1 mg/mL. On the other hand, DAP 1 mg/mL displayed significantly higher color change than TAP 1 mg/mL and TAP 10 mg/mL. Moreover, DAP 1000 mg/mL had significantly higher color change than TAP 1 mg/mL and TAP 10 mg/mL, but significantly lower color change than TAP 1000 mg/mL. In addition, TAP 1000 mg/mL has significantly higher color change than TAP 1 mg/mL (Table VI).

The 1-week time point (Figure 20) showed that DAP 1000 mg/mL, TAP 10 mg/mL, and TAP 1000 mg/mL had significantly higher color change than $Ca(OH)_2$, and the $Ca(OH)_2$ group has significantly higher color change than DAP 1 mg/mL. Moreover, the no-treatment group had a higher color change than DAP 1 mg/mL; however, no treatment illustrated significantly lower color change than TAP 10

mg/mL and TAP 1000 mg/mL. Furthermore, DAP 1000 mg/mL, TAP 1 mg/mL, TAP 10 mg/mL, and TAP 1000 mg/mL had significantly higher color change than DAP 1 mg/mL and DAP 10 mg/mL. In addition, TAP 1000 mg/mL had significantly higher color change than DAP 1000 mg/mL, TAP 1 mg/mL, and TAP 10 mg/mL. Also, TAP 10 mg/mL had significantly higher color change than TAP 1 mg/mL (Table VI).

The 4-week time point (Figure 21) showed that TAP 1000 mg/mL was significantly higher in color change than all other treatment groups. $Ca(OH)_2$ had significantly higher color change than DAP 10 mg/mL and TAP 1 mg/mL. Moreover, the no-treatment, DAP 1 mg/mL, DAP 1000 mg/mL, and TAP 10 mg/mL groups had significantly higher color change than DAP 10 mg/mL. In addition, TAP 10 mg/mL had significantly higher color change than TAP 1 mg/mL (Table VI).

After thermocycling (Figure 22) showed that TAP 1000 mg/mL was significantly higher in color change than all other treatment groups. DAP 1 mg/mL and TAP 10 mg/mL had significantly higher color change than $Ca(OH)_2$ and no treatment. Moreover, DAP 1 mg/mL had significantly higher color change than DAP 10 mg/mL but significantly lower change than TAP 10 mg/mL. In addition, DAP 1000 mg/mL, TAP 1 mg/mL, and TAP 10 mg/mL had significantly higher color change than DAP 10 mg/mL. The color change with TAP 10 mg/mL was significantly higher than with DAP 1000 mg/mL and TAP 1 mg/mL (Table VI).

TABLES AND FIGURES

TABLE I

Chemical compositions and the roles of different materials used in the study[49,69,84,100,101]

Material (Lot number)	Manufacturer	Ingredients		Role
Triple Antibiotics Paste (TAP) (Lot: 171705MS5)	Champs Pharmacy, San Antonio, TX	Metronidazole		Antibiotics
		Ciprofloxacin		
		Minocycline		
Double Antibiotics Paste (DAP) (Lot: 170305MS1)	Champs Pharmacy, San Antonio, TX	Metronidazole		Antibiotics
		Ciprofloxacin		
Calcium hydroxide (Lot: BDNLL)	UltraCal XS, Ultradent, South Jordan, Utah	Calcium hydroxide		Antibacterial
		Barium sulfate		Radiocontrast agent
		methyl cellulose		Improve handling properties
ProRoot MTA (Lot: 201404-01)	Dentsply, Tulsa, Oklahoma	Tricalcium silicate		Bioactive agent
		Dicalcium silicate		Bioactive agent
		Tricalcium aluminate		Accelerator
		Tetracalcium aluminoferrite		Increase hardening
		Bismuth oxide		Radiopacifiers
Biodentine (Lot: B123559)	Septodont, Sant-Maur-des-Fosses, France	powder	Tricalcium silicate	Bioactive agent
			Dicalcium silicate	Bioactive agent
			Calcium carbonate and Oxide filler	Improve physical properties
			Zirconium oxide	Radiopacifier
		Liquid	Calcium chloride	Accelerator

(continued)

TABLE I (cont.)

Chemical compositions and the roles of different materials used in the study[49,69,84,100,101]

Biodentine (Lot: B123559)	**Septodont, Sant-Maur-des-Fosses, France**	Liquid	Hydrosoluble polymer	Reducing agent
Endosequence putty (Lot: 1602BPP)	Endosequence, Savannah, Georgia	Calcium silicates		Bioactive agent
		Zirconium oxide		Radiopacifier
		Tantalum oxide		Radiopacifier
		Calcium phosphate monobasic		Improve physical properties

TABLE II

Mean ± (SD) of the push-out bond strength (MPa)
in coronal and middle root cylinders*

Coronal						
Treatment / Cement	No treatment (Control)	$Ca(OH)_2$	1000 mg/ml TAP	1 mg/ml TAP	1000 mg/ml DAP	1 mg/ml DAP
MTA	6.81 (2.37) B b	10.14 (3.36) A a	6.04 (2.23) B b	10.12 (2.42) A a	2.22 (0.6) B c	10.56 (2.18) C a
Biodentine	7.63 (2.51) B c	11.59 (3.54) A b	12.55 (4.49) A b	11.47 (1.39) A b	10.26 (2.37) A b	15.34 (2.71) A a
Endosequence putty	10.7 (1.25) A b	11.58 (2.22) A ab	9.73 (3.21) A b	11.48 (0.73) A b	9.51 (2.73) A b	13.32 (1.35) B a
Middle						
Treatment / Cement	No treatment (Control)	$Ca(OH)_2$	1000 mg/ml TAP	1 mg/ml TAP	1000 mg/ml DAP	1 mg/ml DAP
MTA	3.58 (0.87) B b	9.15 (3.38) A a	3.92 (1.25) B b	8.36(1.70) A a	2.74 (1.14) B c	8.18 (1.73) C a
Biodentine	4.88 (1.78) B c	8.22 (2.93) A b	7.63 (3.72) A b	9.39 (3.74) A b	10.69 (3.23) A b	13.98 (1.93) A a
Endosequence putty	8.53 (1.21) A b	10.00 (1.56) A ab	8.99 (3.13) A b	8.65 (1.95) A b	9.69 (2.04) A b	11.59 (2.41) B a

*Different uppercase letters within each treatment group represent significant differences between different types of cements. Different lowercase letters within each cement group represent significant differences between different types of treatments.

TABLE II

Discoloration experiment, P-values when comparing different time points to each other within the same treatment and adhesive group

Treatment	Adhesive	Time Comparison			p-value
Control		Day 1	vs.	Week 1	<.0001
		Day 1	vs.	Week 4	0.0001
		Day 1	vs.	Thermocycling	0.8446
		Week 1	vs.	Week 4	0.9422
		Week 1	vs.	Thermocycling	<.0001
	Adhesive	Week 4	vs.	Thermocycling	<.0001
		Day 1	vs.	Week 1	0.0008
		Day 1	vs.	Week 4	0.0065
		Day 1	vs.	Thermocycling	0.6182
		Week 1	vs.	Week 4	0.7175
		Week 1	vs.	Thermocycling	0.0059
	No adhesive	Week 4	vs.	Thermocycling	0.0016
$Ca(OH)_2$		Day 1	vs.	Week 1	0.0015
		Day 1	vs.	Week 4	0.0005
		Day 1	vs.	Thermocycling	0.4332
		Week 1	vs.	Week 4	0.4463
		Week 1	vs.	Thermocycling	0.0001
	Adhesive	Week 4	vs.	Thermocycling	<.0001
		Day 1	vs.	Week 1	0.3654
		Day 1	vs.	Week 4	0.0151
		Day 1	vs.	Thermocycling	0.2112
		Week 1	vs.	Week 4	0.0527
		Week 1	vs.	Thermocycling	0.0259
	No adhesive	Week 4	vs.	Thermocycling	<.0001
DAP_1		Day 1	vs.	Week 1	0.4504
		Day 1	vs.	Week 4	0.6823
		Day 1	vs.	Thermocycling	0.3778
		Week 1	vs.	Week 4	0.1975
		Week 1	vs.	Thermocycling	0.0913
	Adhesive	Week 4	vs.	Thermocycling	0.5033
		Day 1	vs.	Week 1	0.0868
		Day 1	vs.	Week 4	0.1552
		Day 1	vs.	Thermocycling	0.0532
		Week 1	vs.	Week 4	0.0005
		Week 1	vs.	Thermocycling	0.0002
	No adhesive	Week 4	vs.	Thermocycling	0.4643
DAP_10		Day 1	vs.	Week 1	0.4594
		Day 1	vs.	Week 4	0.5643
		Day 1	vs.	Thermocycling	0.7482
		Week 1	vs.	Week 4	0.9165
		Week 1	vs.	Thermocycling	0.7080
	Adhesive	Week 4	vs.	Thermocycling	0.7165
		Day 1	vs.	Week 1	0.0966
		Day 1	vs.	Week 4	0.4237
		Day 1	vs.	Thermocycling	0.1828
		Week 1	vs.	Week 4	0.0070
		Week 1	vs.	Thermocycling	0.0024
	No adhesive	Week 4	vs.	Thermocycling	0.4498
		Day 1	vs.	Week 1	0.4855
DAP_1000	Adhesive	Day 1	vs.	Week 4	0.0417

(continued)

TABLE III (cont.)

Discoloration experiment, P-values when comparing different time points to each other within the same treatment and adhesive group

DAP 1000	Adhesive	Day 1	vs.	Thermocycling	0.0101
		Week 1	vs.	Week 4	0.0924
		Week 1	vs.	Thermocycling	0.0334
		Week 4	vs.	Thermocycling	0.4377
	No adhesive	Day 1	vs.	Week 1	0.1366
		Day 1	vs.	Week 4	0.0009
		Day 1	vs.	Thermocycling	0.0001
		Week 1	vs.	Week 4	0.0155
		Week 1	vs.	Thermocycling	0.0062
		Week 4	vs.	Thermocycling	0.4854
TAP_1	Adhesive	Day 1	vs.	Week 1	0.0002
		Day 1	vs.	Week 4	0.0894
		Day 1	vs.	Thermocycling	0.3575
		Week 1	vs.	Week 4	0.0388
		Week 1	vs.	Thermocycling	0.0064
		Week 4	vs.	Thermocycling	0.2656
	No adhesive	Day 1	vs.	Week 1	<.0001
		Day 1	vs.	Week 4	0.0301
		Day 1	vs.	Thermocycling	0.0073
		Week 1	vs.	Week 4	0.0075
		Week 1	vs.	Thermocycling	0.0577
		Week 4	vs.	Thermocycling	0.4569
TAP_10	Adhesive	Day 1	vs.	Week 1	<.0001
		Day 1	vs.	Week 4	0.0037
		Day 1	vs.	Thermocycling	0.0824
		Week 1	vs.	Week 4	0.2965
		Week 1	vs.	Thermocycling	0.0247
		Week 4	vs.	Thermocycling	0.0896
	No adhesive	Day 1	vs.	Week 1	<.0001
		Day 1	vs.	Week 4	<.0001
		Day 1	vs.	Thermocycling	<.0001
		Week 1	vs.	Week 4	0.0191
		Week 1	vs.	Thermocycling	0.6733
		Week 4	vs.	Thermocycling	0.0009
TAP_1000	Adhesive	Day 1	vs.	Week 1	<.0001
		Day 1	vs.	Week 4	<.0001
		Day 1	vs.	Thermocycling	<.0001
		Week 1	vs.	Week 4	<.0001
		Week 1	vs.	Thermocycling	<.0001
		Week 4	vs.	Thermocycling	<.0001
	No adhesive	Day 1	vs.	Week 1	0.0877
		Day 1	vs.	Week 4	<.0001
		Day 1	vs.	Thermocycling	0.0251
		Week 1	vs.	Week 4	<.0001
		Week 1	vs.	Thermocycling	0.4380
		Week 4	vs.	Thermocycling	<.0001

TABLE IV

Discoloration experiment, P-values when comparing different treatment groups to each other within the same time point and adhesive group

Adhesive	Time	Treatment Comparison			p-value
Adhesive	Day 1	Control	vs.	$Ca(OH)_2$	0.8165
			vs.	DAP_1	0.1625
			vs.	DAP_10	0.3615
			vs.	DAP_1000	<.0001
			vs.	TAP_1	0.5315
			vs.	TAP_10	0.5984
			vs.	TAP_1000	0.0001
		$Ca(OH)_2$	vs.	DAP_1	0.2434
			vs.	DAP_10	0.2529
			vs.	DAP_1000	<.0001
			vs.	TAP_1	0.3914
			vs.	TAP_10	0.4483
			vs.	TAP_1000	0.0002
		DAP_1	vs.	DAP_10	0.0218
			vs.	DAP_1000	0.0010
			vs.	TAP_1	0.0441
			vs.	TAP_10	0.0554
			vs.	TAP_1000	0.0085
		DAP_10	vs.	DAP_1000	<.0001
			vs.	TAP_1	0.7737
			vs.	TAP_10	0.6990
			vs.	TAP_1000	<.0001
		DAP_1000	vs.	TAP_1	<.0001
			vs.	TAP_10	<.0001
			vs.	TAP_1000	0.4875
		TAP_1	vs.	TAP_10	0.9210
			vs.	TAP_1000	<.0001
		TAP_10	vs.	TAP_1000	<.0001
Adhesive	Week 1	Control	vs.	$Ca(OH)_2$	0.2527
			vs.	DAP_1	0.0002
			vs.	DAP_10	<.0001
			vs.	DAP_1000	0.5348
			vs.	TAP_1	0.1772
			vs.	TAP_10	0.3856
			vs.	TAP_1000	<.0001
		$Ca(OH)_2$	vs.	DAP_1	0.0076
			vs.	DAP_10	0.0007
			vs.	DAP_1000	0.5996
			vs.	TAP_1	0.8357
			vs.	TAP_10	0.7814
			vs.	TAP_1000	<.0001
		DAP_1	vs.	DAP_10	0.4503
			vs.	DAP_1000	0.0015
			vs.	TAP_1	0.0136
			vs.	TAP_10	0.0033
			vs.	TAP_1000	<.0001
		DAP_10	vs.	DAP_1000	0.0001
			vs.	TAP_1	0.0014
			vs.	TAP_10	0.0003
			vs.	TAP_1000	<.0001
		DAP_1000	vs.	TAP_1	0.4642

(continued)

TABLE IV (cont.)

Discoloration experiment, P-values when comparing different treatment groups to each other within the same time point and adhesive group

Adhesive	Week 1		vs.	TAP_10	0.8044
		DAP_1000	vs.	TAP_1000	<.0001
			vs.	TAP_10	0.6278
		TAP_1	vs.	TAP_1000	<.0001
		TAP_10	vs.	TAP_1000	<.0001
Adhesive	Week 4		vs.	$Ca(OH)_2$	0.7470
			vs.	DAP_1	0.0347
			vs.	DAP_10	0.0002
			vs.	DAP_1000	0.0941
			vs.	TAP_1	0.0113
			vs.	TAP_10	0.1589
		Control	vs.	TAP_1000	<.0001
			vs.	DAP_1	0.0725
			vs.	DAP_10	0.0008
			vs.	DAP_1000	0.1753
			vs.	TAP_1	0.0265
			vs.	TAP_10	0.2763
		$Ca(OH)_2$	vs.	TAP_1000	<.0001
			vs.	DAP_10	0.1047
			vs.	DAP_1000	0.6554
			vs.	TAP_1	0.6654
			vs.	TAP_10	0.4749
		DAP_1	vs.	TAP_1000	<.0001
			vs.	DAP_1000	0.0393
			vs.	TAP_1	0.2323
			vs.	TAP_10	0.0202
		DAP_10	vs.	TAP_1000	<.0001
			vs.	TAP_1	0.3800
			vs.	TAP_10	0.7882
		DAP_1000	vs.	TAP_1000	<.0001
			vs.	TAP_10	0.2522
		TAP_1	vs.	TAP_1000	<.0001
		TAP_10	vs.	TAP_1000	<.0001
Adhesive	After thermo-cycling		vs.	$Ca(OH)_2$	0.7468
			vs.	DAP_1	0.0513
			vs.	DAP_10	0.8038
			vs.	DAP_1000	0.1262
			vs.	TAP_1	0.6356
			vs.	TAP_10	0.2146
		Control	vs.	TAP_1000	<.0001
			vs.	DAP_1	0.0236
			vs.	DAP_10	0.9407
			vs.	DAP_1000	0.0647
			vs.	TAP_1	0.4260
			vs.	TAP_10	0.1186
		$Ca(OH)_2$	vs.	TAP_1000	<.0001
			vs.	DAP_10	0.0284
			vs.	DAP_1000	0.6702
			vs.	TAP_1	0.1384
			vs.	TAP_10	0.4737
		DAP_1	vs.	TAP_1000	<.0001
			vs.	DAP_1000	0.0760
			vs.	TAP_1	0.4704
			vs.	TAP_10	0.1370
		DAP_10	vs.	TAP_1000	<.0001

(continued)

TABLE IV (cont.)

Discoloration experiment, P-values when comparing different treatment groups to each other within the same time point and adhesive group

			vs.	TAP_1	0.2893
			vs.	TAP_10	0.7709
		DAP_1000	vs.	TAP_1000	<.0001
			vs.	TAP_10	0.4415
	After thermo-	TAP_1	vs.	TAP_1000	<.0001
	cycling	TAP_10	vs.	TAP_1000	<.0001
No adhesive	Day 1		vs.	$Ca(OH)_2$	0.1095
			vs.	DAP_1	0.0585
			vs.	DAP_10	0.6444
			vs.	DAP_1000	<.0001
			vs.	TAP_1	0.4359
			vs.	TAP_10	0.7465
		Control	vs.	TAP_1000	<.0001
			vs.	DAP_1	0.7675
			vs.	DAP_10	0.2529
			vs.	DAP_1000	<.0001
			vs.	TAP_1	0.0181
			vs.	TAP_10	0.0550
		$Ca(OH)_2$	vs.	TAP_1000	<.0001
			vs.	DAP_10	0.1508
			vs.	DAP_1000	<.0001
			vs.	TAP_1	0.0080
			vs.	TAP_10	0.0272
		DAP_1	vs.	TAP_1000	<.0001
			vs.	DAP_1000	<.0001
			vs.	TAP_1	0.2156
			vs.	TAP_10	0.4329
		DAP_10	vs.	TAP_1000	<.0001
			vs.	TAP_1	<.0001
			vs.	TAP_10	<.0001
		DAP_1000	vs.	TAP_1000	<.0001
			vs.	TAP_10	0.6480
		TAP_1	vs.	TAP_1000	<.0001
		TAP_10	vs.	TAP_1000	<.0001
No adhesive	Week 1		vs.	$Ca(OH)_2$	0.3816
			vs.	DAP_1	0.0022
			vs.	DAP_10	0.2203
			vs.	DAP_1000	0.1498
			vs.	TAP_1	0.4475
			vs.	TAP_10	0.0027
		Control	vs.	TAP_1000	<.0001
			vs.	DAP_1	0.0267
			vs.	DAP_10	0.7243
			vs.	DAP_1000	0.0214
			vs.	TAP_1	0.1033
			vs.	TAP_10	0.0001
		$Ca(OH)_2$	vs.	TAP_1000	<.0001
			vs.	DAP_10	0.0614
			vs.	DAP_1000	<.0001
			vs.	TAP_1	0.0002
			vs.	TAP_10	<.0001
		DAP_1	vs.	TAP_1000	<.0001
			vs.	DAP_1000	0.0082
			vs.	TAP_1	0.0482
		DAP_10	vs.	TAP_10	<.0001

(continued)

TABLE IV (cont.)

Discoloration experiment, P-values when comparing different treatment groups to each other within the same time point and adhesive group

No Adhesive	Week 1	DAP_10	vs.	TAP_1000	<.0001
		DAP_1000	vs.	TAP_1	0.4935
			vs.	TAP_10	0.1110
			vs.	TAP_1000	<.0001
		TAP_1	vs.	TAP_10	0.0235
			vs.	TAP_1000	<.0001
		TAP_10	vs.	TAP_1000	<.0001
No adhesive	Week 4	Control	vs.	$Ca(OH)_2$	0.3320
			vs.	DAP_1	0.7578
			vs.	DAP_10	0.0072
			vs.	DAP_1000	0.7312
			vs.	TAP_1	0.2759
			vs.	TAP_10	0.3114
			vs.	TAP_1000	<.0001
		$Ca(OH)_2$	vs.	DAP_1	0.5075
			vs.	DAP_10	0.0003
			vs.	DAP_1000	0.1897
			vs.	TAP_1	0.0405
			vs.	TAP_10	0.9663
			vs.	TAP_1000	<.0001
		DAP_1	vs.	DAP_10	0.0028
			vs.	DAP_1000	0.5147
			vs.	TAP_1	0.1629
			vs.	TAP_10	0.4809
			vs.	TAP_1000	<.0001
		DAP_10	vs.	DAP_1000	0.0185
			vs.	TAP_1	0.1046
			vs.	TAP_10	0.0003
			vs.	TAP_1000	<.0001
		DAP_1000	vs.	TAP_1	0.4548
			vs.	TAP_10	0.1760
			vs.	TAP_1000	<.0001
		TAP_1	vs.	TAP_10	0.0366
			vs.	TAP_1000	<.0001
		TAP 10	vs.	TAP_1000	<.0001
			vs.	DAP_1	0.0089
			vs.	DAP_10	0.2302
			vs.	DAP_1000	0.2479
			vs.	TAP_1	0.1995
			vs.	TAP_10	<.0001

(continued)

TABLE IV (cont.)

Discoloration experiment, P-values when comparing different treatment groups to each other within the same time point and adhesive group

No Adhesive	After Thermo-cycling	Control	vs.	$Ca(OH)_2$	0.7842
			vs.	DAP_1	0.0089
			vs.	DAP_10	0.2302
			vs.	DAP_1000	0.2479
			vs.	TAP_1	0.1995
			vs.	TAP_1000	<.0001
		$Ca(OH)_2$	vs.	TAP_1000	<.0001
		DAP_1	vs.	DAP_10	0.0002
			vs.	DAP_1000	0.1376
			vs.	TAP_1	0.1746
			vs.	TAP_10	0.0248
			vs.	TAP_1000	<.0001
		DAP_10	vs.	DAP_1000	0.0194
			vs.	TAP_1	0.0138
			vs.	TAP_10	<.0001
			vs.	TAP_1000	<.0001
		DAP_1000	vs.	TAP_1	0.8977
			vs.	TAP_10	0.0002
			vs.	TAP_1000	<.0001
		TAP_1	vs.	TAP_10	0.0004
			vs.	TAP_1000	<.0001
		TAP_10	vs.	TAP_1000	<.0001

TABLE V

Mean ± (SD) of color change in adhesive groups with combination of treatment and time*

Treatment	Day 1	Week 1	Week 4	After Thermocycling
$Ca(OH)_2$	1.78 (0.73)^{ab}	3.15 (0.96)^{b}	3.46 (1.06)^{cd}	1.41 (0.64)^{a}
Control	1.68 (0.97)^{ab}	3.66 (1.05)^{b}	3.63 (0.82)^{d}	1.59 (0.71)^{ab}
1 DAP	2.27 (0.79)^{b}	1.95 (0.68)^{a}	2.47 (0.34)^{abc}	2.69 (0.61)^{b}
10 DAP	1.30 (0.87)^{a}	1.61 (0.46)^{a}	1.57 (0.74)^{a}	1.45 (0.50)^{a}
1000 DAP	3.68 (1.67)^{c}	3.39 (0.89)^{b}	2.71 (0.32)^{bcd}	2.45 (0.63)^{ab}
1 TAP	1.42 (0.39)^{a}	3.06 (0.92)^{b}	2.23 (0.65)^{ab}	1.86 (0.66)^{ab}
10 TAP	1.46 (0.46)^{ab}	3.28 (0.82)^{b}	2.86 (0.78)^{bcd}	2.29 (1.08)^{ab}
1000 TAP	3.39 (1.49)^{c}	7.01 (1.95)^{c}	9.85 (1.87)^{e}	11.29 (2.42)^{c}

*Treatments with the same superscript letter were not significantly different at $p<0.05$.

TABLE III

Mean ± (SD) of color change in no adhesive group with combination of treatment and time*

Treatment	Day 1	Week 1	Week 4	After Thermocycling
$Ca(OH)_2$	2.18 (0.69)^{BC}	2.57 (0.98)^{BC}	3.35 (0.97)^{C}	1.59 (0.65)^{AB}
Control	1.51 (0.57)^{ABC}	2.96 (1.30)^{BCD}	2.82 (0.91)^{BC}	1.74 (0.92)^{AB}
1 DAP	2.31 (0.61)^{C}	1.58 (0.48)^{A}	2.98 (0.42)^{BC}	3.23 (0.73)^{C}
10 DAP	1.70 (0.74)^{ABC}	2.41 (0.86)^{AB}	1.32 (0.68)^{A}	1.07 (0.39)^{A}
1000 DAP	4.24 (1.18)^{D}	3.60 (0.40)^{DE}	2.63 (1.01)^{BC}	2.39 (1.01)^{BC}
1 TAP	1.18 (0.55)^{A}	3.30 (0.53)^{CD}	2.22 (0.88)^{AB}	2.47 (0.93)^{BC}
10 TAP	1.37 (0.41)^{AB}	4.31 (0.52)^{E}	3.37 (0.80)^{C}	4.50 (0.76)^{D}
1000 TAP	10.54 (1.51)^{E}	11.27 (1.62)^{F}	13.56 (3.46)^{D}	11.61 (3.36)^{E}

*Treatments with the same superscript letter were not significantly different at $p<0.05$.

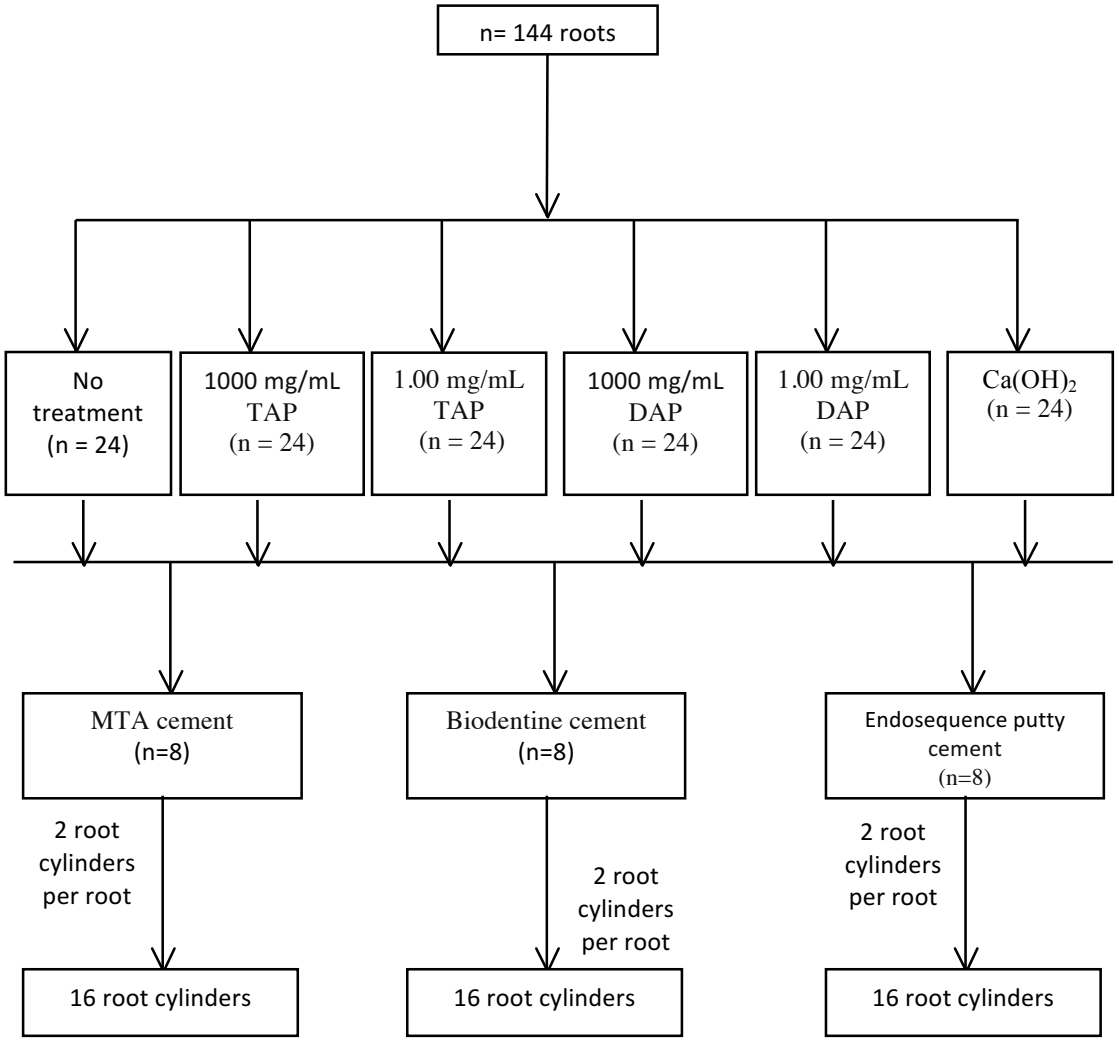

FIGURE 1. Flow chart of the study design of the push-out bond strength experiment.

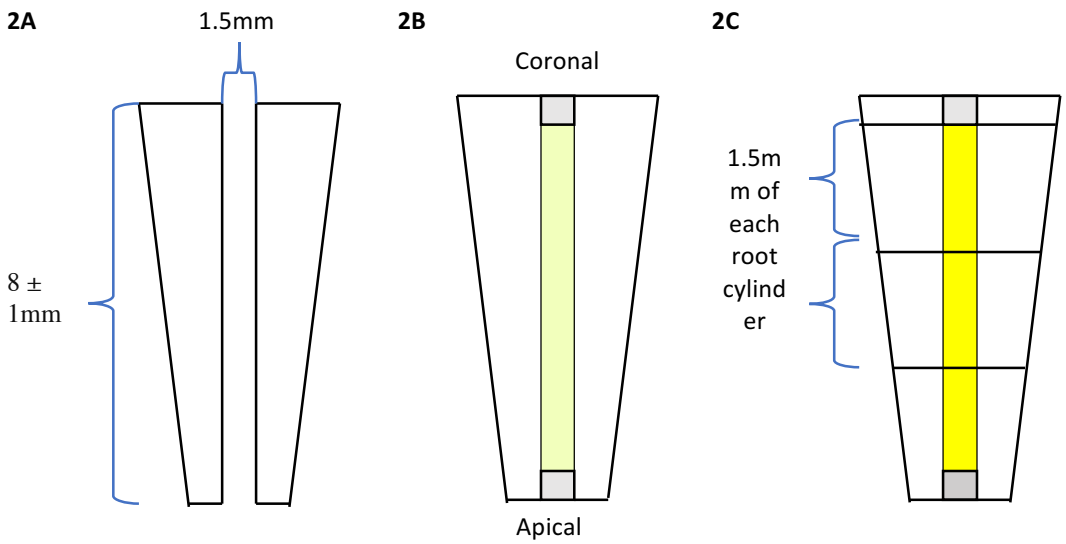

FIGURE 2. Root samples prepared (2A), filled with intracanal medicaments (2B) and sectioned after root cements application (2C).

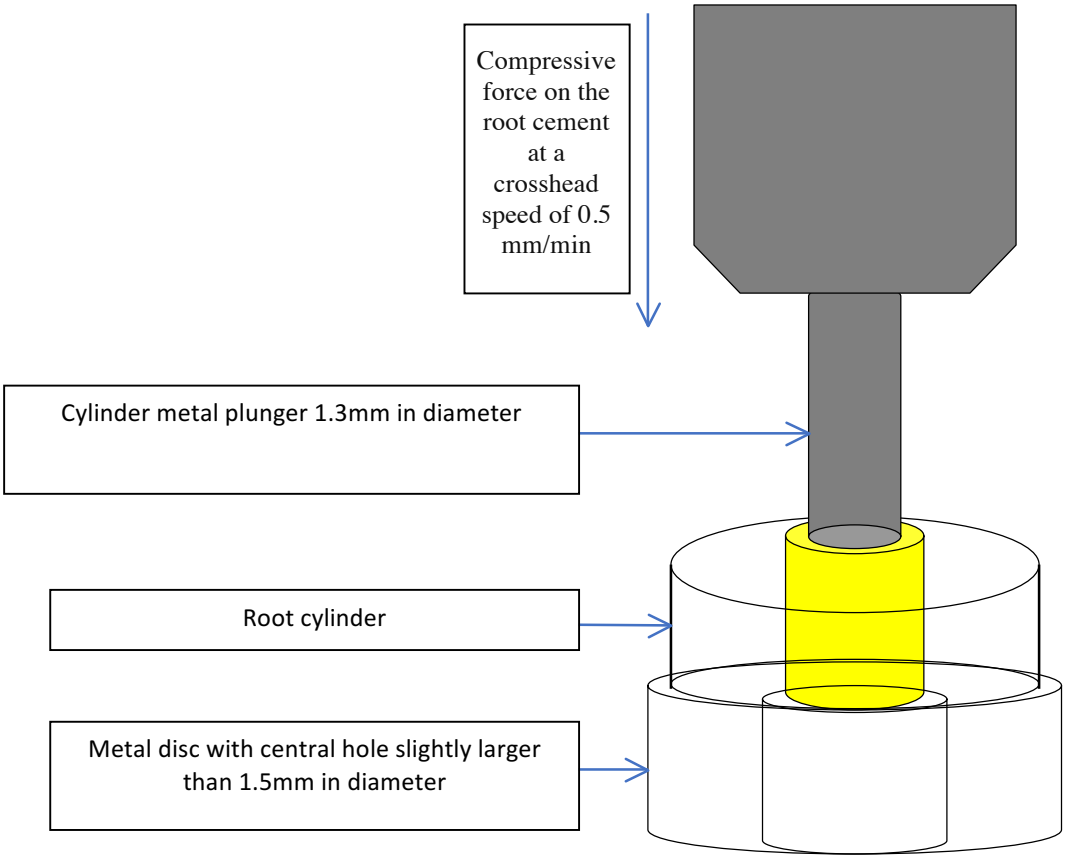

FIGURE 3. Root cylinder sample fixed on the metal disc for the push-out bond strength test.

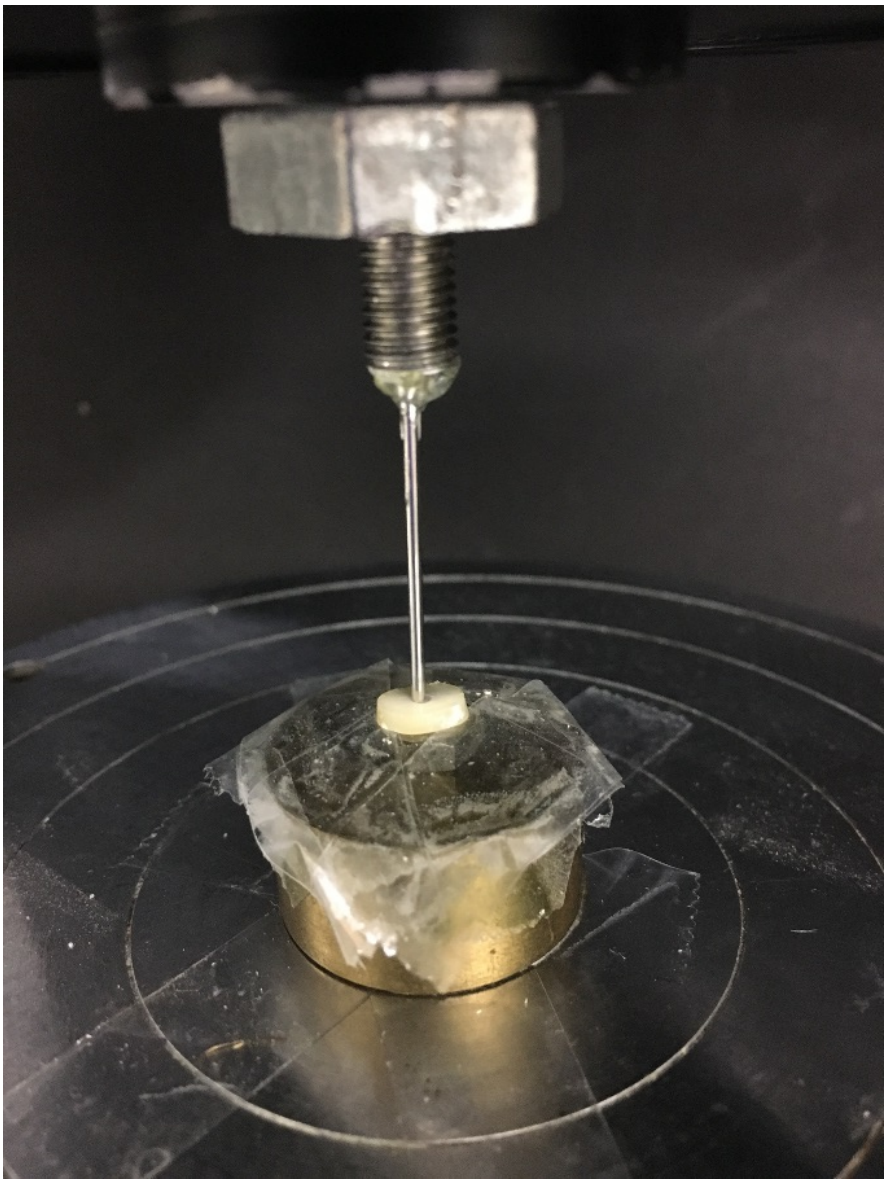

FIGURE 4. Push-out bond strength test setup.

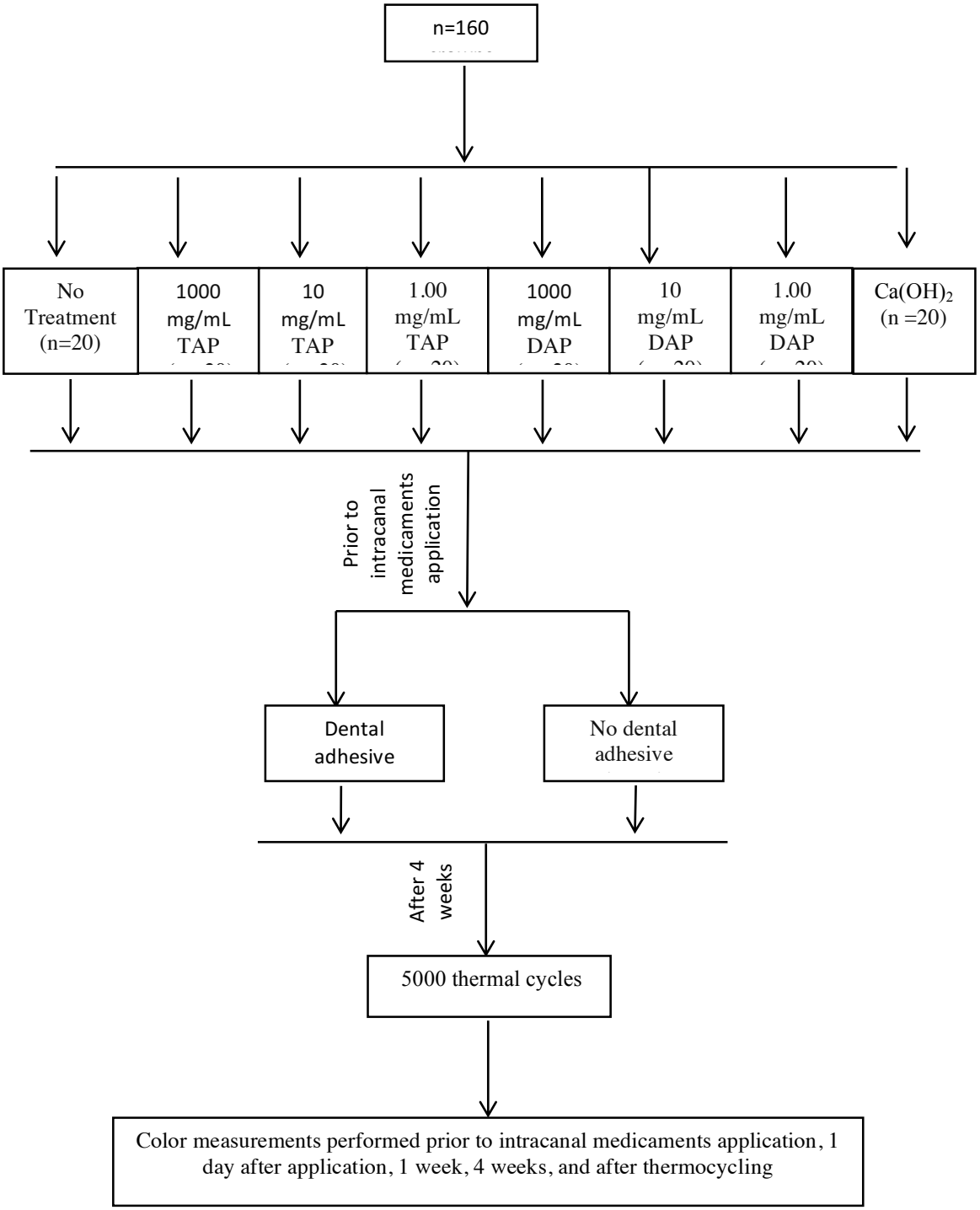

FIGURE 5. Flow chart of the study design of the discoloration experiment.

FIGURE 6. Crown sample mounted in the custom-made close-fit cap made by a clear soft thermoplastic sheet.

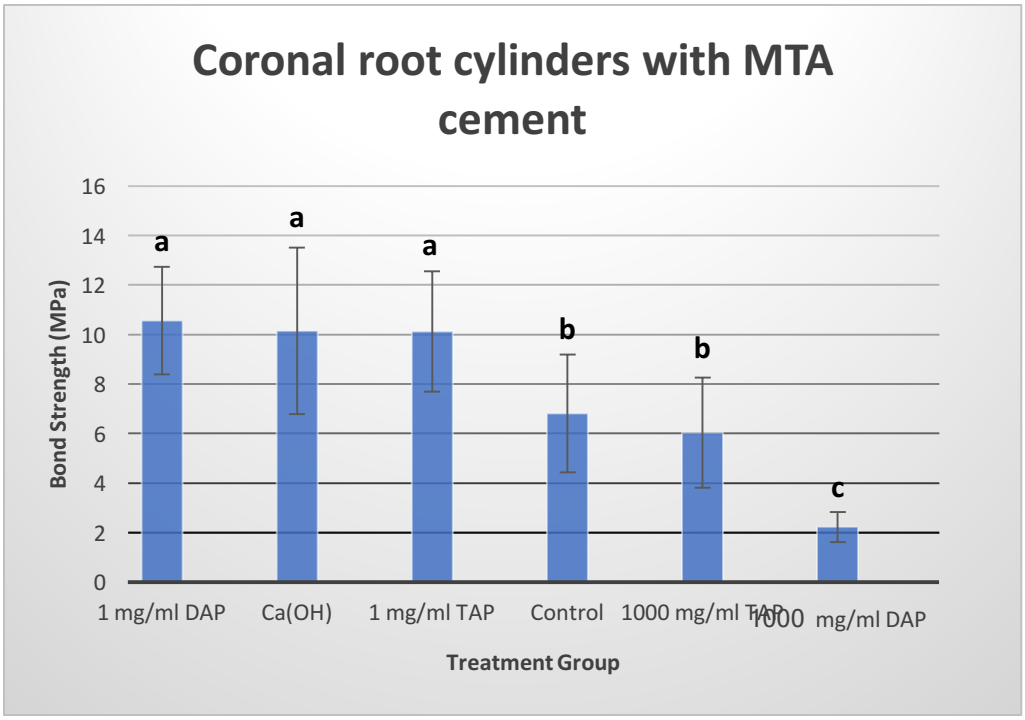

FIGURE 7. Mean ± (SD) of the push-out bond strength (MPa) of MTA cement in the coronal cylinder when using different treatments. Different lowercase letters represent significant differences between different types of treatments.

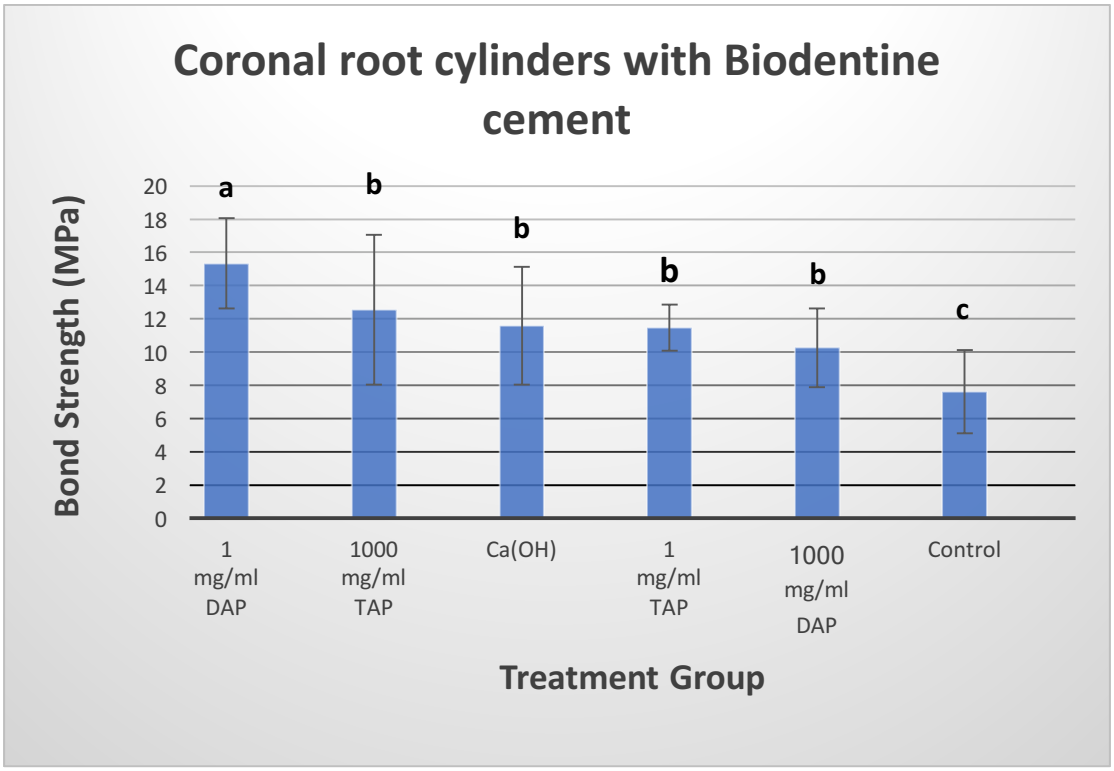

FIGURE 8. Mean ± (SD) of the push-out bond strength (MPa) of Biodentine cement in the coronal cylinder when using different treatments. Different lowercase letters represent significant differences between different types of treatments.

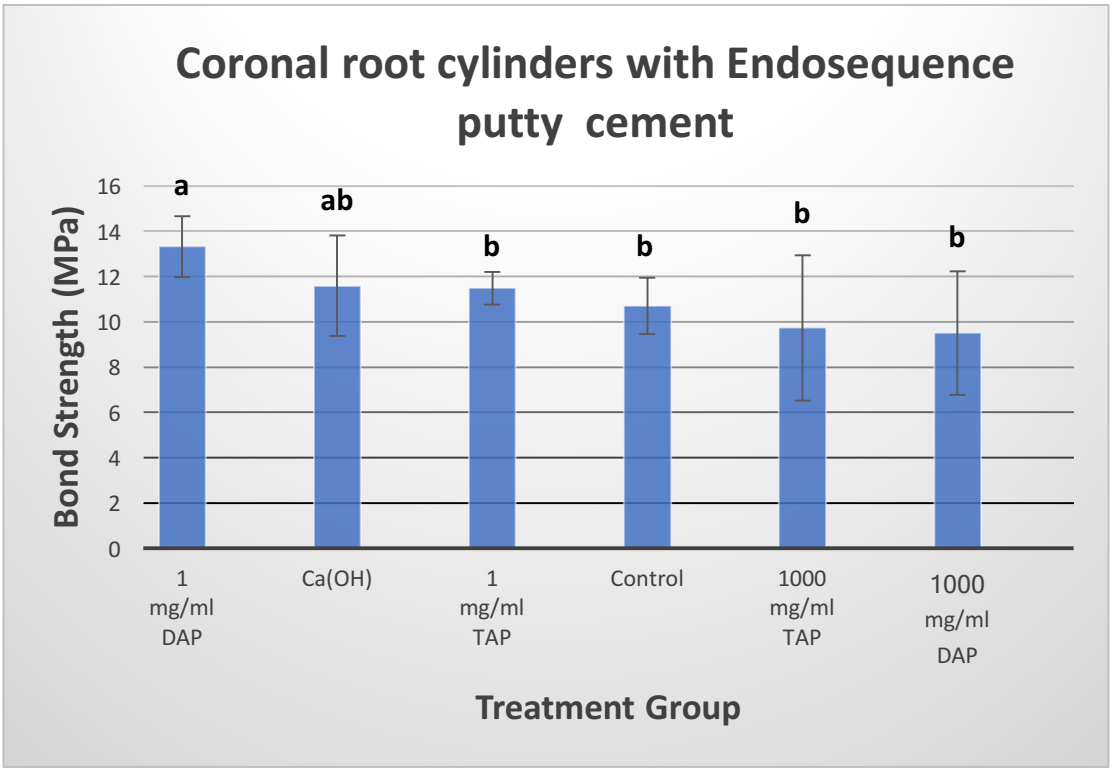

FIGURE 9. Mean ± (SD) of the push-out bond strength (MPa) of Endosequence putty cement in the coronal cylinder when using different treatments. Different lowercase letters represent significant differences between different types of treatments.

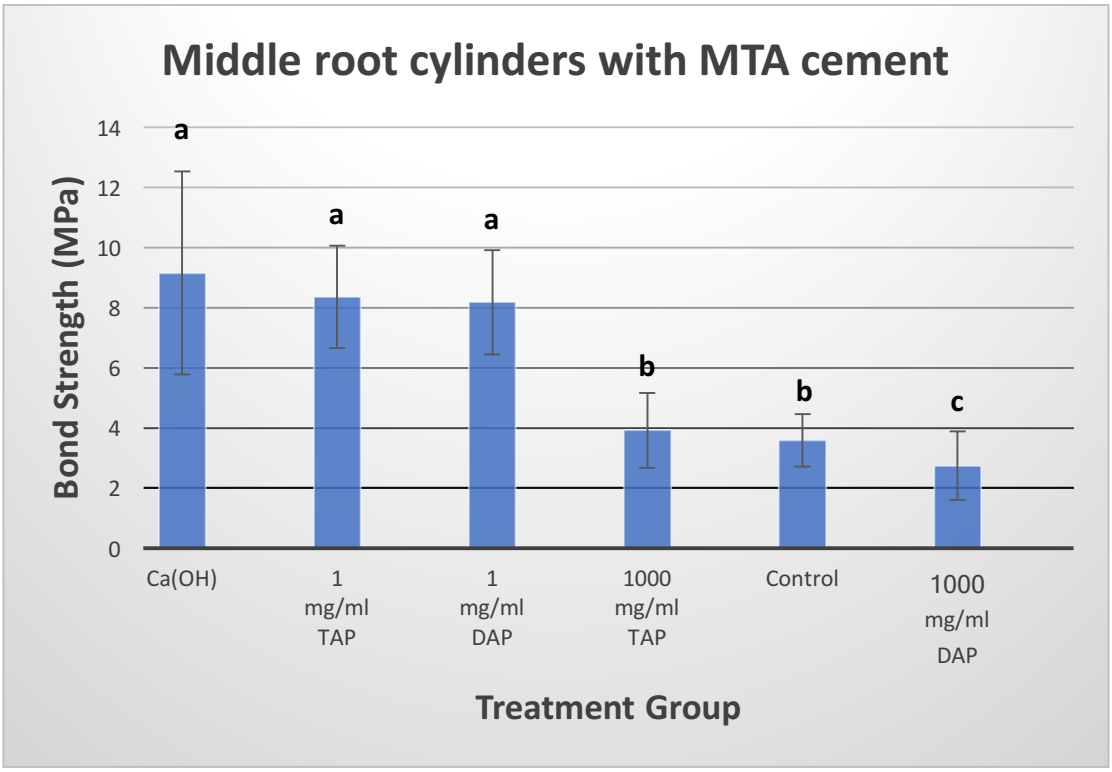

FIGURE 10. Mean ± (SD) of the push-out bond strength (MPa) of MTA cement in the middle cylinder when using different treatments. Different lowercase letters represent significant differences between different types of treatments.

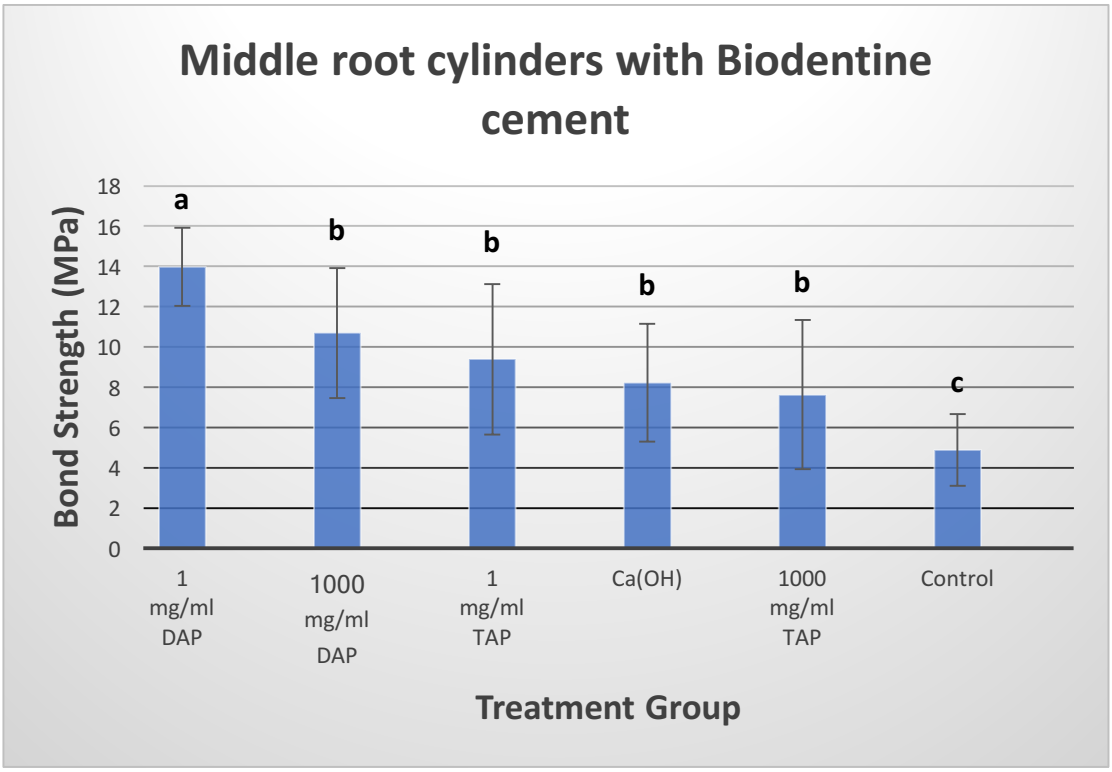

FIGURE 11. Mean ± (SD) of the push-out bond strength (MPa) of Biodentine cement in the middle cylinder when using different treatments. Different lowercase letters represent significant differences between different types of treatments.

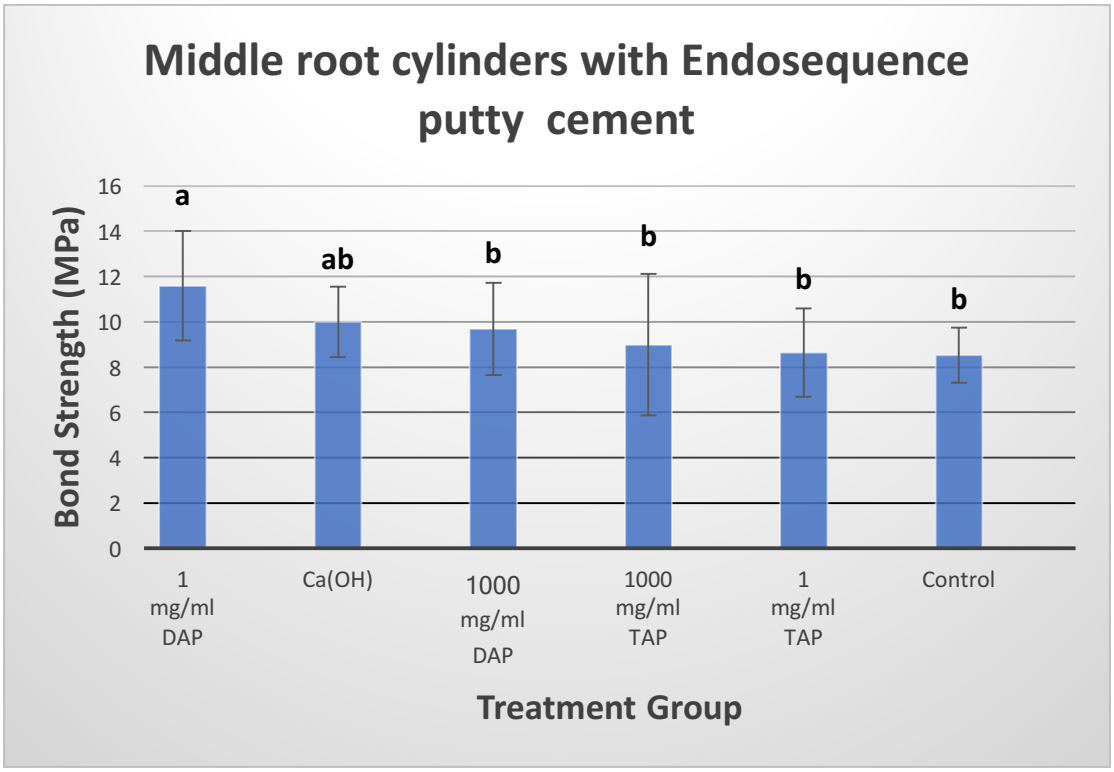

FIGURE 12. Mean ± (SD) of the push-out bond strength (MPa) of Endosequence putty cement in the middle cylinder when using different treatments. Different lowercase letters represent significant differences between different types of treatments.

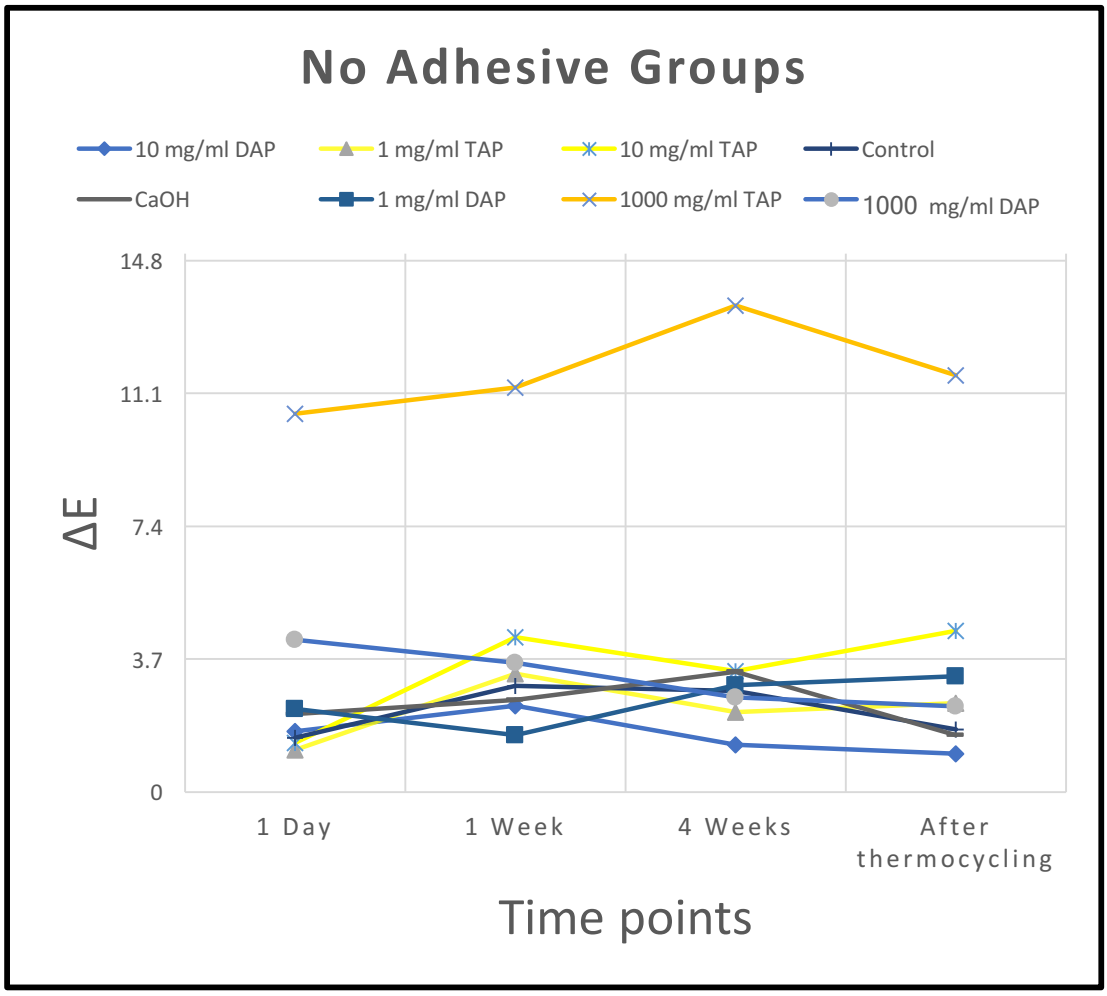

FIGURE 13. Mean ± (SD) of the push-out bond strength (MPa) of Endosequence putty cement in the middle cylinder when using different treatments. Different lowercase letters represent significant differences between different types of treatments.

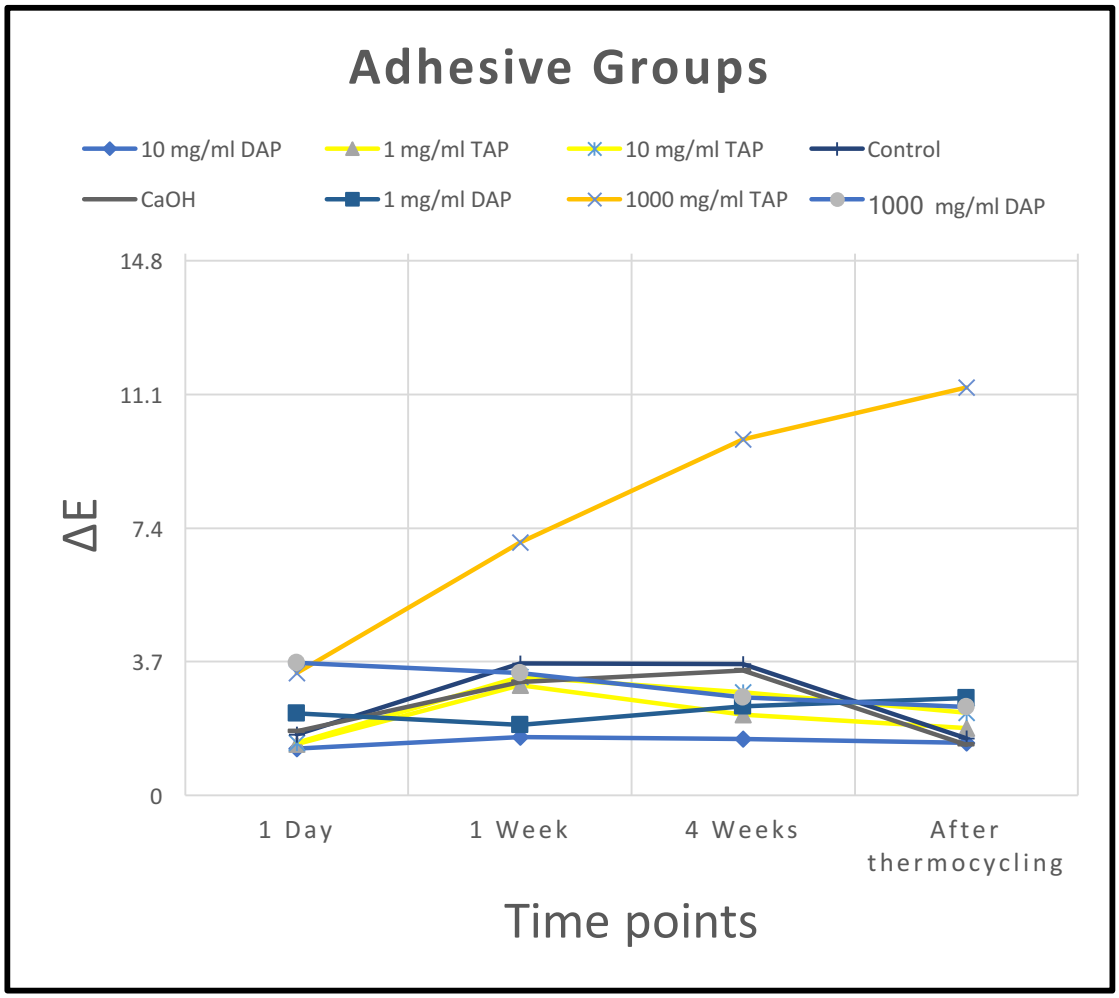

FIGURE 14. Line graph illustrates the means of color change in different treatment groups at different time points where adhesive was used.

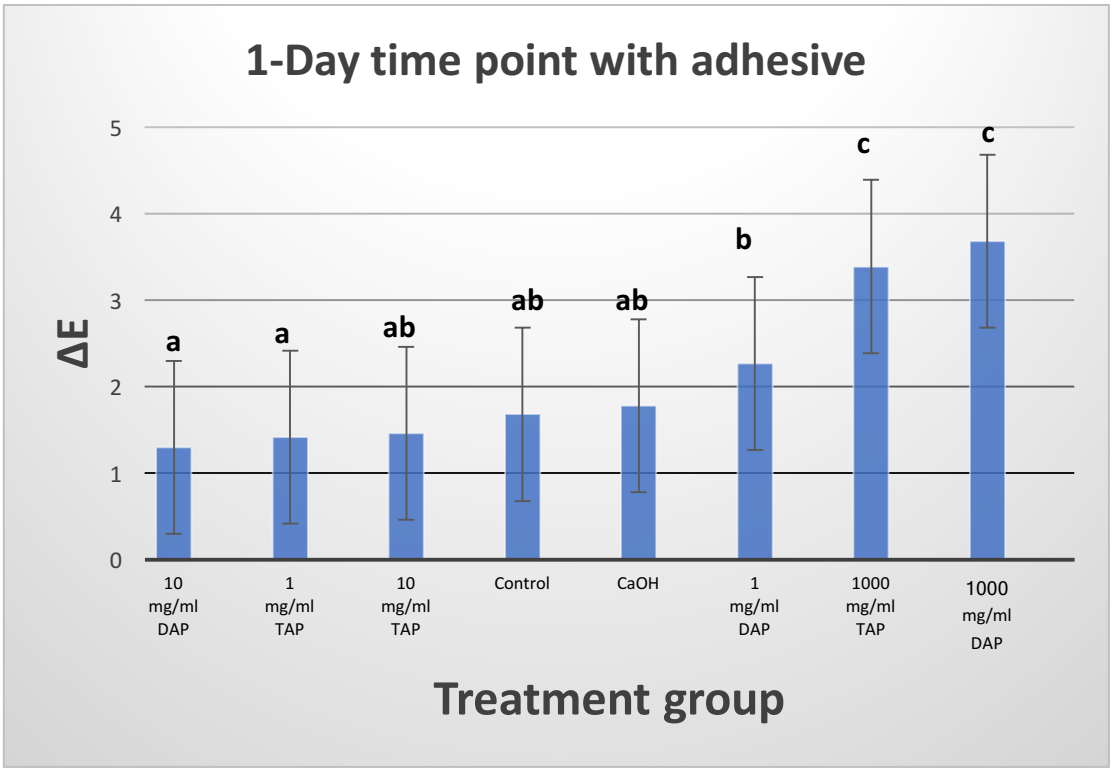

FIGURE 15. Mean ± (SD) of color change in different treatment groups at the 1-day time point where adhesive was used. Treatments with the same superscript letter were not significantly different at $p<0.05$.

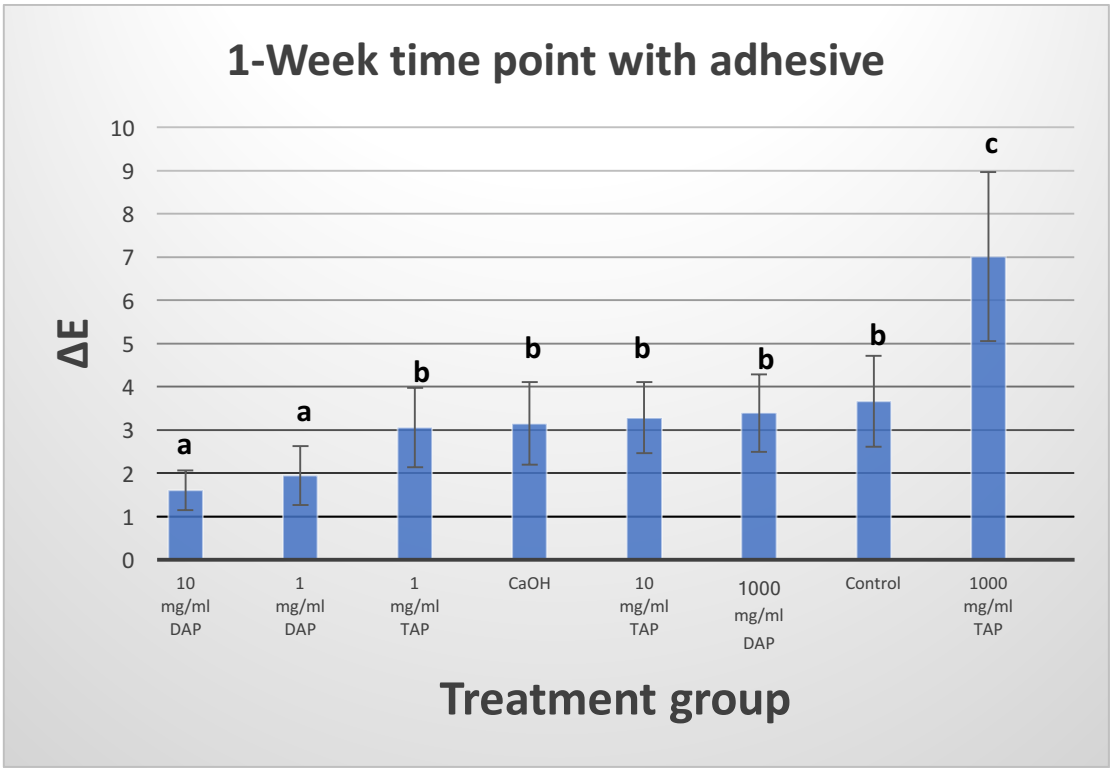

FIGURE 16. Mean ± (SD) of color change in different treatment groups at the 1-week time point where adhesive was used. Treatments with the same superscript letter were not significantly different at $p<0.05$.

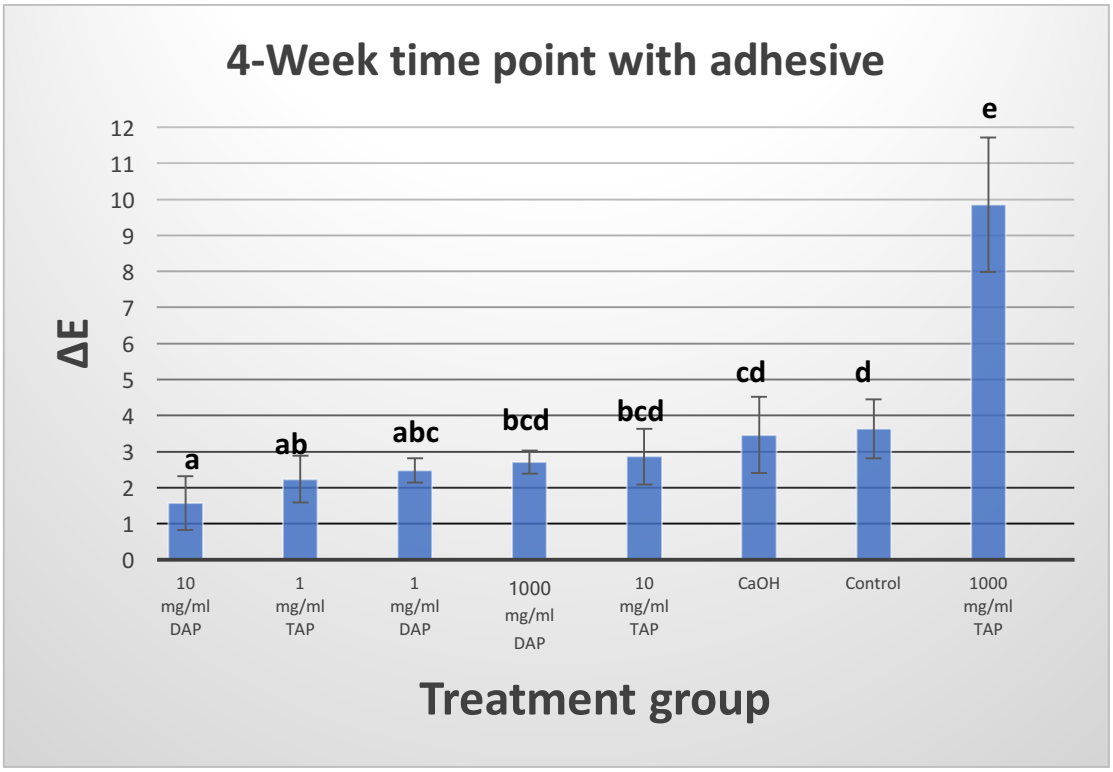

FIGURE 17. (SD) of color change in different treatment groups at the 4-week time point where adhesive was used. Treatments with the same superscript letter were not significantly different at $p<0.05$.

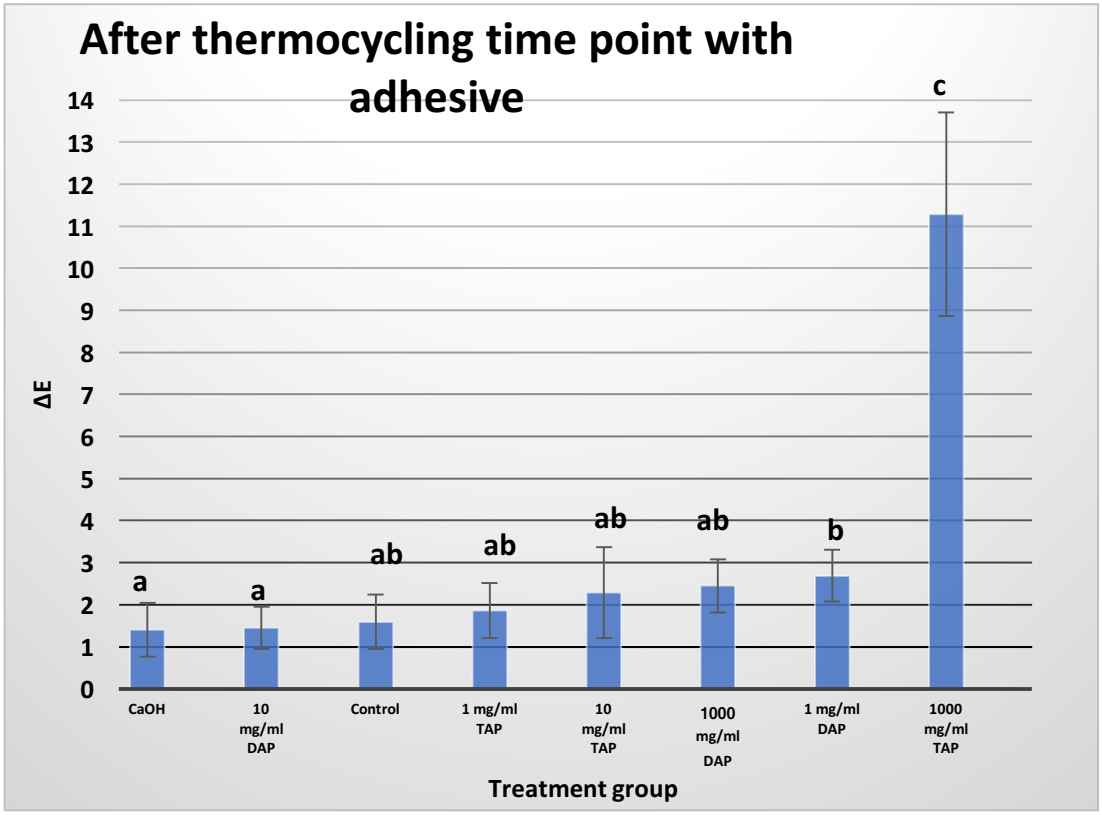

FIGURE 18. Mean ± (SD) of color change in different treatment groups at the after-thermocycling time point where adhesive was used. Treatments with the same superscript letter were not significantly different at $p<0.05$.

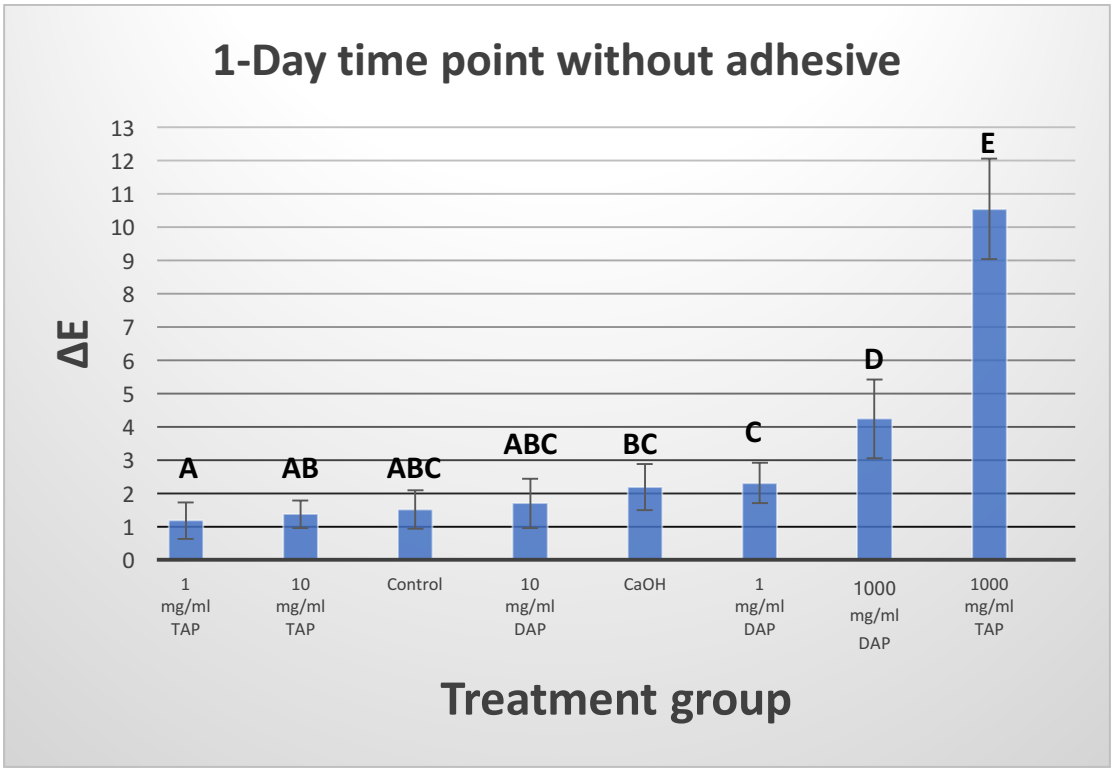

FIGURE 19. Mean ± (SD) of color change in different treatment groups at the 1-day time point where no adhesive was used. Treatments with the same superscript letter were not significantly different at $p<0.05$.

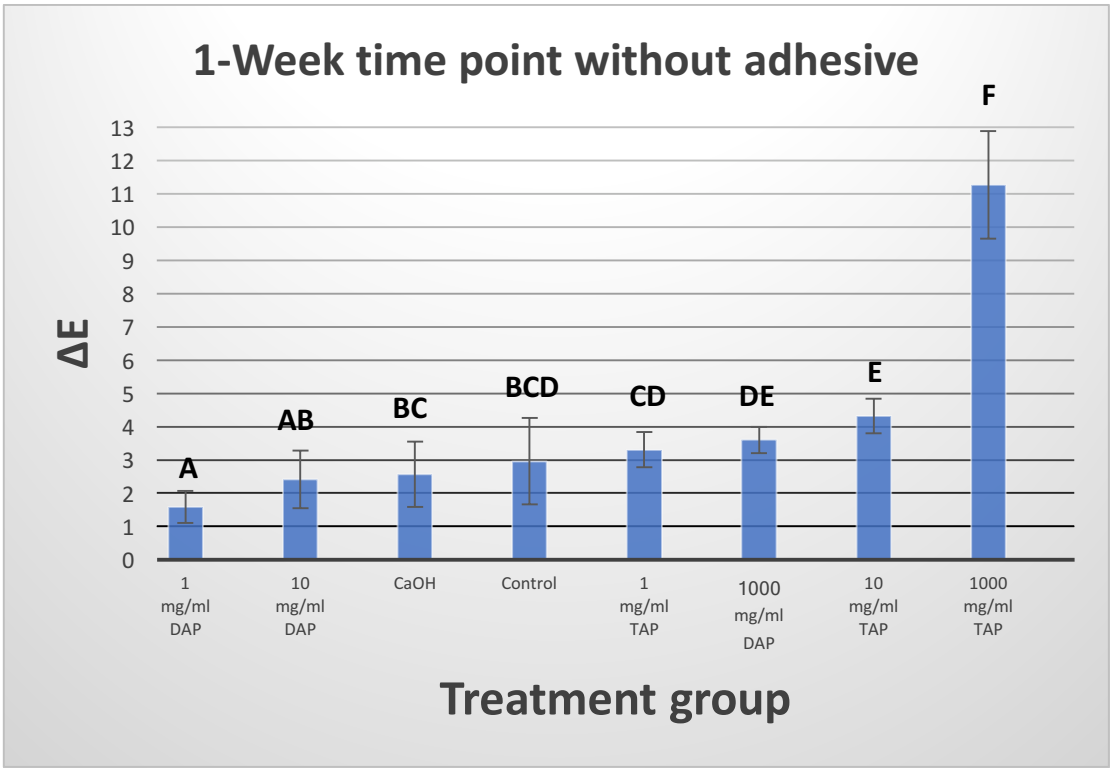

FIGURE 20. Mean ± (SD) of color change in different treatment groups at the 1-week time point where no adhesive was used. Treatments with the same superscript letter were not significantly different at $p<0.05$.

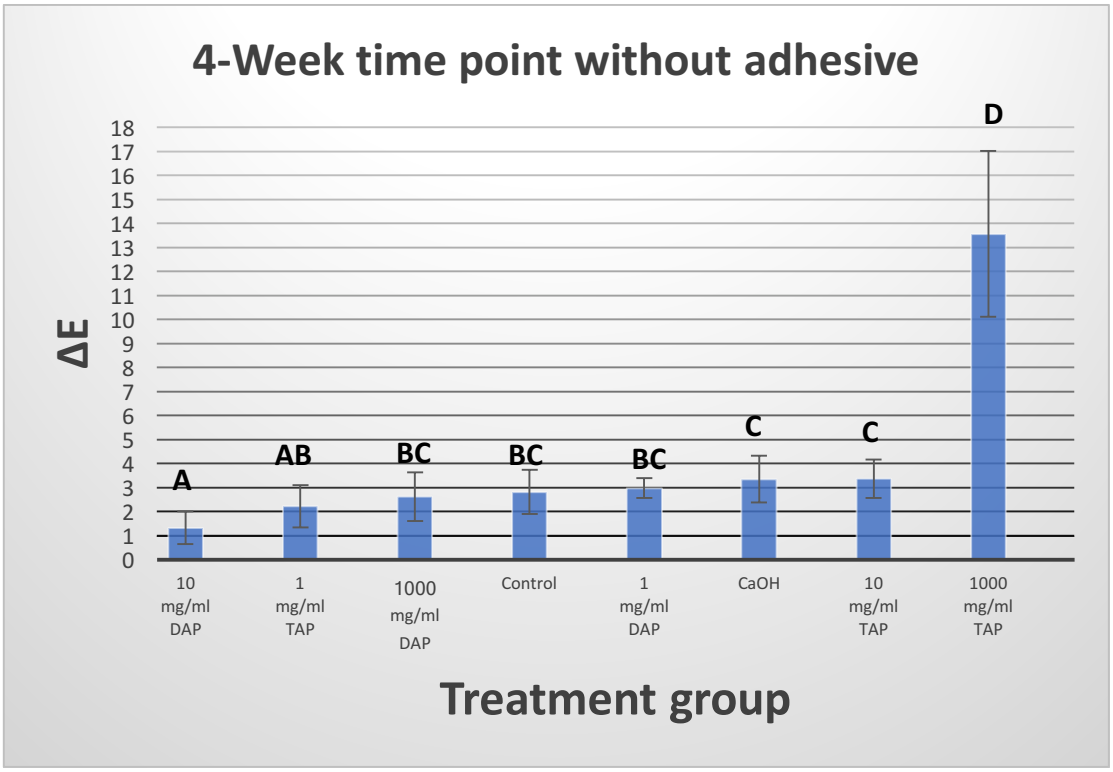

FIGURE 21. Mean ± (SD) of color change in different treatment groups at the 4-week time point where no adhesive was used. Treatments with the same superscript letter were not significantly different at $p<0.05$.

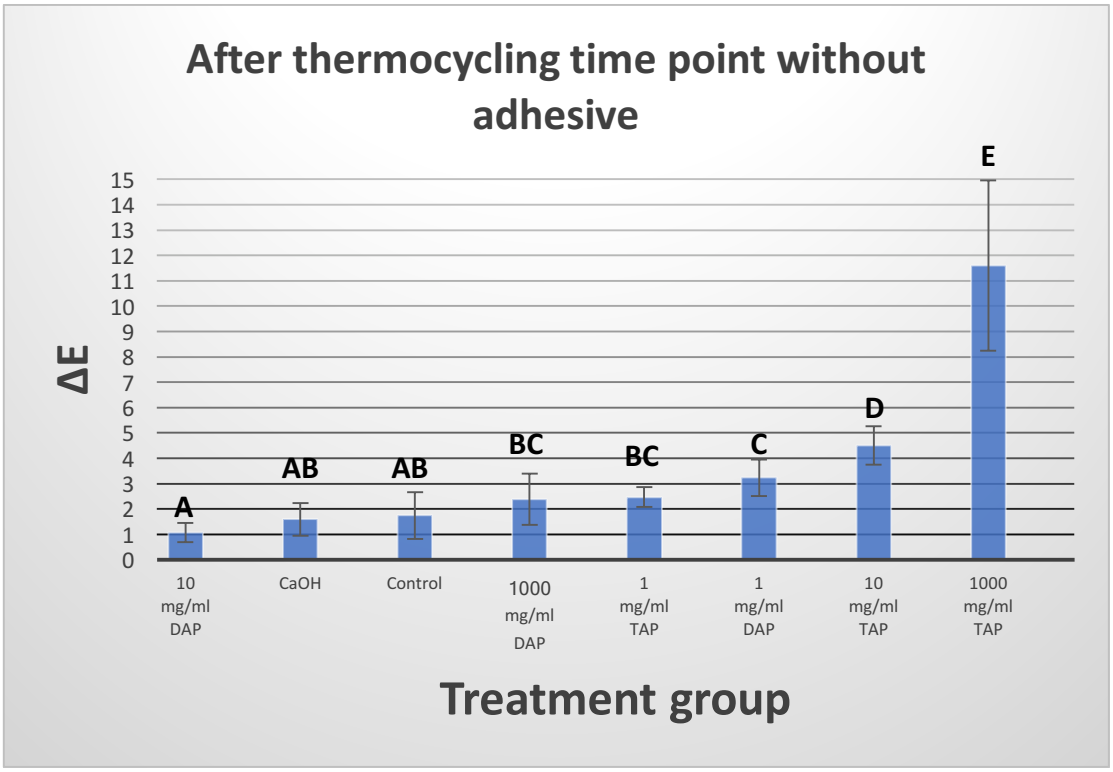

FIGURE 22. Mean ± (SD) of color change in different treatment groups at the after-thermocycling time point where no adhesive was used. Treatments with the same superscript letter were not significantly different at $p<0.05$.

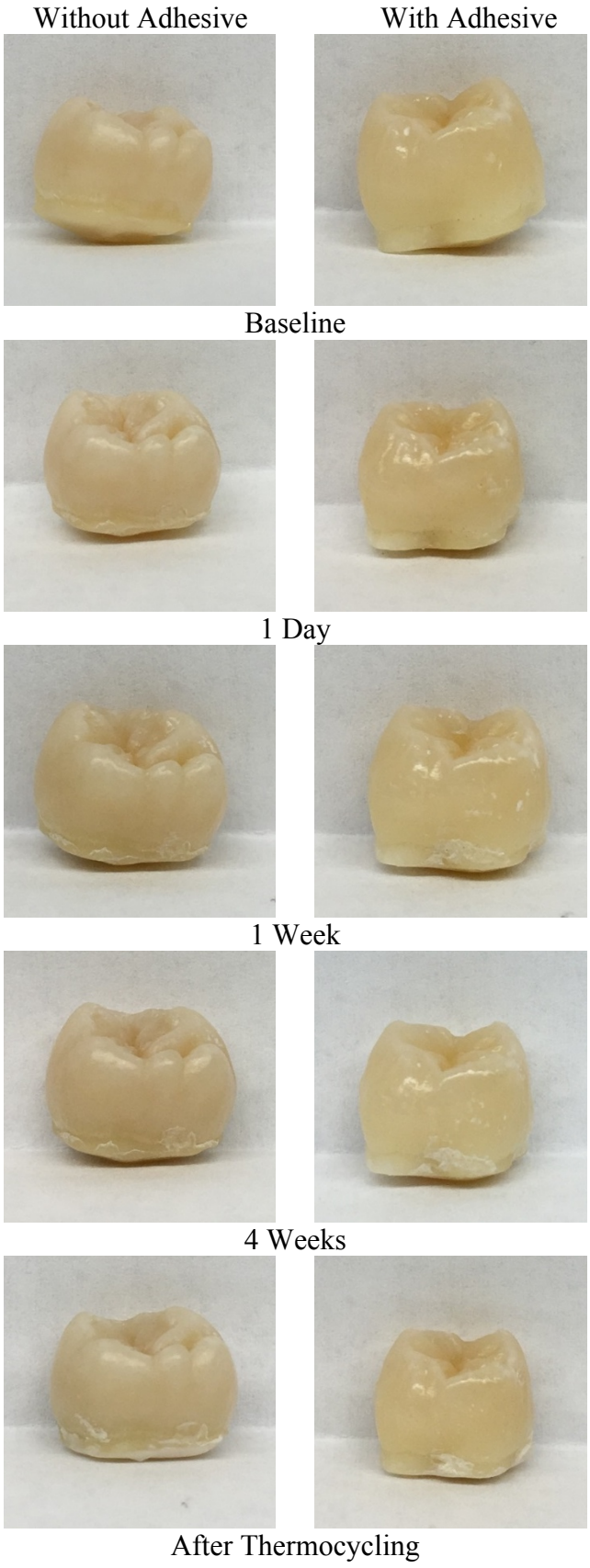

FIGURE 23. Crown samples of 10 mg/mL TAP group without adhesive and with adhesive at different time point.

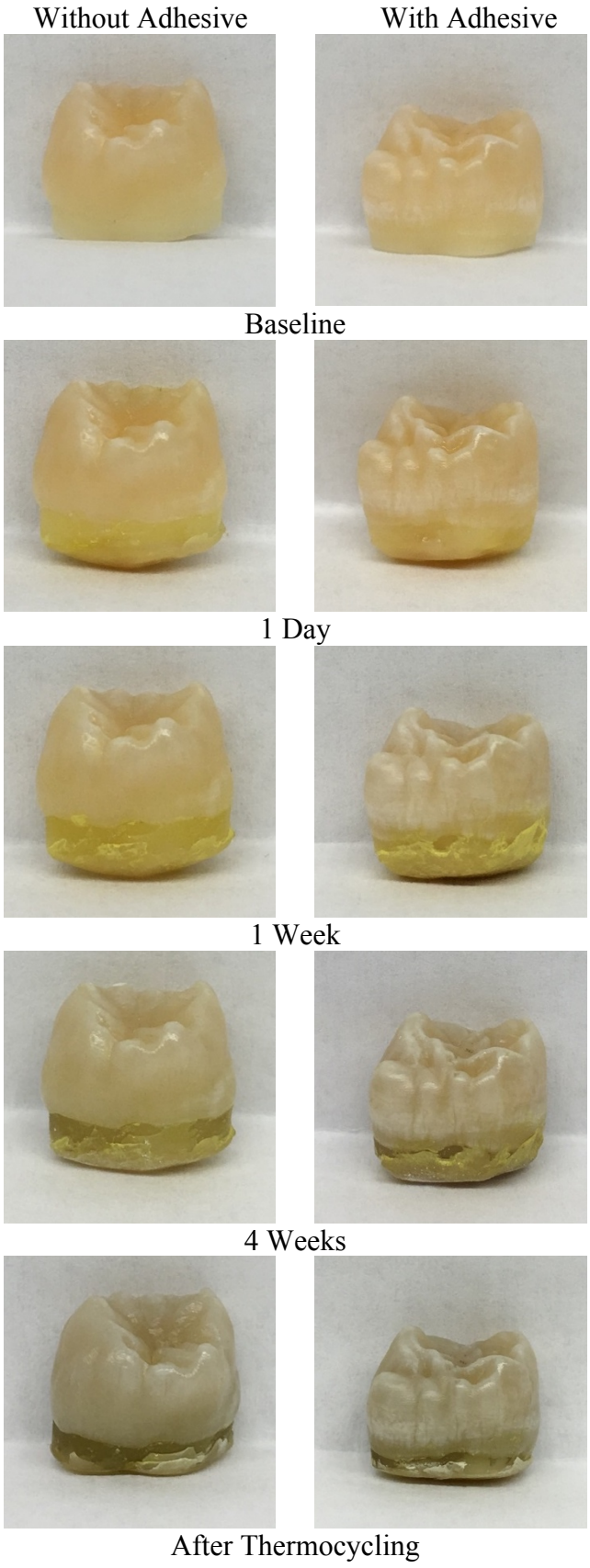

FIGURE 24. Crown samples of 1000 mg/mL TAP group without adhesive and with adhesive at different time points.

DISCUSSION

PUSH-OUT BOND STRENGTH EXPERIMENT

Push-out bond strength is an important factor for the intracanal cement in the ER procedure. High bond strength of intracanal cement is desired to ensure the better sealing ability of intracanal cements against the passage of bacteria. Also, the bond strength plays an essential role in resisting the possible dislocation force that may happen indirectly by the occlusal functional force.

This study evaluated the effects of low concentrations of TAP and DAP (1 mg/mL) loaded into an aqueous methylcellulose system on the push-out bond strength of MTA, Biodentine, or Endosequence putty cements. None of the previous studies evaluated the effects of low concentrations of TAP and DAP on push-out bond strength due to the lack of paste-like medicaments. However, in the present study the low concentrations of TAP and DAP were formed in a paste-like material.

The results of this study showed that the coronal cylindrical sections have higher bond strength than the middle cylindrical sections in all treatment groups except for the 1000 mg/mL DAP group, where the coronal and middle section have similar bond strengths regardless of the intracanal cements. In conjunction with the present study, one meta-regression analysis review concluded that apical third sections have lower bond strength than coronal ones.[65] In addition, another study found the existence of regional differences in penetration of sealer cement between apical and coronal areas, where the apical region has the least penetration, and consequently the bond strength to radicular dentin decreases.[102] Some possible explanations of the present study's results are that different areas in the root have different dentinal tubule densities, orientations, or degrees of sclerosis. In addition,

moving apically, the diameter of the dentinal tubule becomes smaller.[52, 103-105] However, this result is not compatible with some of the previous studies, which showed no difference in the bond strength of intracanal cements in different root section locations.[66,99] The difference in results between the present study and previous ones may be due to different exposure times of the medicaments to radicular dentin or different allowable setting times for the cements before the push-out bond strength test was performed.

In general, the push-out bond strength of the intracanal cements in the current study showed that Biodentine and Endosequence putty cements had comparable bond strength numbers, while MTA cement had significantly lower numbers. This result agreed with the previous studies.[29,106-108] In the present study, the push-out bond strength of the intracanal cements showed the same pattern in the coronal and middle locations within the same treatment group. The 1 mg/mL DAP group showed the highest bond strength for Biodentine, followed by Endosequence putty, and MTA had the lowest bond strength. Moreover, the $Ca(OH)_2$ and 1 mg/mL TAP groups presented the same bond strength in all three tested cements. Furthermore, the 1000 mg/mL TAP and 1000 mg/mL DAP groups displayed the same trend in bond strength between the three cements as following: Endosequence putty and Biodentine, then MTA. Finally, the control group revealed that Endosequence putty had the highest bond strength followed by the other two tested cements. The study by Gokturk et al. agreed with the present study's results where they found that Endosequence sealer had higher bond strength than MTA sealer when used with no medicament or 1000 mg/mL DAP.[109] Moreover, a previous study, showed that Biodentine cement showed significantly higher bond strength, followed by Endosequence putty and MTA cements.[110] It is worth mentioning that researchers in that study looked at the effect of

blood on the bond strength of the calcium-silicate base cements. However, three studies that compared the bond strength of Biodentine with MTA showed that Biodentine was superior.[66,78,107] Moreover, these studies evaluated the effects of three different factors on the bond strength: smear layer, irrigant solutions, and use of apical root sections.[66,78,107] In another study, they compared Endosequence putty bond strength to MTA in an acidic environment, which showed the higher bond strength of Endosequence putty cement.[111]

Nagas et al. studied the effects of different intracanal medicaments on the bond strength of MTA and Biodentine; their results showed higher bond strength of Biodentine regardless of the type of medicaments used.[106] The difference in the protocols of the Nagas et al. study and the present study may be the cause of different results; for instance, Nagas et al. stored the samples for only 1 week after application of the medicaments as well as after application of the cements. Also, in their study they applied the cements after roots had been sectioned. However, 1000 mg/ml TAP in both studies displayed higher bond strength with Biodentine than MTA. Another study, which compared the bond strength of these three types of cements, showed that MTA cement has significantly higher bond strength than Biodentine and Endosequence putty cements when NaOCl is used.[112] However, in the control groups of the same study, Biodentine had significantly higher bond strength than MTA, and Endosequence putty's bond strength was significantly lower than MTA.

There are three possible explanations of the higher bond strength with Biodentine and Endosequence putty. The first explanation is due to their smaller particle size that allowed better penetration of the dentinal tubules by the material, which increased the micromechanical retention.[113,114] The second is the high ability of Biodentine to form tag-like structures because of high calcium and silicon uptake

(Biomineralization) into dentin.[113] The third explanation was suggested by Aydin and Buldure who stated that the interaction between Biodentine and the radicular dentin causes water to move between these two surfaces to hydrate the Biodentine; consequently, the Biodentine more thoroughly penetrates the dentinal tubules.[108]

Both coronal and middle root cylinders in the present study showed the same pattern of bond strength between different treatment groups within the same type of cement. In the coronal or middle root cylinders, MTA cement showed the highest bond strength when 1 mg/mL DAP, $Ca(OH)_2$, and 1 mg/mL TAP were used, followed by the control and 1000 mg/mL TAP groups. The 1000 mg/mL DAP group had the lowest bond with MTA cement. Turk et al. had results similar to the present study; they found that DAP decreased the bond strength of MTA to radicular dentin.[28] Moreover, the present study agreed with a portion of the results reported by Topcuoglu et al. where DAP decreased the bond strength of MTA and TAP had no effects on the bond strength of MTA.[29] In contrast to the present study's results, previous studies showed that there were no effects on the bond strength of MTA when the $Ca(OH)_2$, TAP or DAP were used.[28,106,109]

In the current study, when Biodentine intracanal cement was used in the coronal or middle sections, treatment groups displayed results in the following order: the 1 mg/mL DAP group had the highest bond strength, followed by all other treatment groups and then the control group, which had the lowest bond strength. In contrast to the present study, Nagas et al. showed in their study that TAP and $Ca(OH)_2$ had no effects on the bond strength of Biodentine.[106] A recent study showed different results than the present study: the no medicament group and the $Ca(OH)_2$ group revealed the highest bond strength of the Biodentine, followed by TAP and then DAP.[108] In the previously mentioned study, differences in the protocol from the

current study might be the reason behind this disagreement. These differences include incubation time of the medicaments, allowable time for the cements to achieve complete setting, and the fact that application of the cements was done after the root cylinders were sectioned.

In the current study, when Endosequence putty cement was used, the 1mg/mL DAP group showed the highest bond strength alongside the $Ca(OH)_2$ group, both of which were then followed by all other treatment groups. A study by Gokturk et al. showed similar results to the present study where the no medicament, $Ca(OH)_2$, and DAP groups had no significant difference on the bond strength of the Endosequence sealer.[109] A recent study showed results different than the present study: the no medicament group and the $Ca(OH)_2$ group revealed the highest bond strength of the Endosequence putty followed by TAP and then DAP.[108] The differences in the protocols have already been mentioned.

It is worth mentioning that 1 mg/mL of TAP and DAP have not been used before in any published study to evaluate the bond strength. Furthermore, TAP and DAP used in all previously mentioned studies were in the typical clinical concentrations.

Studies that evaluated the effects of TAP, DAP, and $Ca(OH)_2$ on the chemical compositions of the radicular dentin concluded that the typical clinical concentrations of TAP and DAP have the highest demineralization effect, followed by $Ca(OH)_2$ and 1 mg/mL TAP.[22,24,25] 1 mg/mL antibiotic combination have a lesser demineralization effect than the typical clinical concentrations of TAP and DAP.[21,25] Consequently, in the present study, 1 mg/mL DAP showed higher bond strength than other treatment groups. Although 1 mg/mL TAP had the same demineralization effect, the presence of minocycline negatively affected the bond strength by chelating to the calcium in

the radicular dentin, which decreased the chemical bonding of calcium-silicate-based cements to the radicular dentin.[115] In addition to the lesser demineralization effect of the $Ca(OH)_2$, it has a positive effect on the bond strength of the calcium-silicate-based cements when the chemical bonding occurs between the cements and the residual $Ca(OH)_2$.[116] In addition, the results of previous studies showed that the typical clinical concentration of TAP increases surface roughness on the radicular dentin more so than 1 mg/mL TAP and $Ca(OH)_2$.[21,117] A previous study concluded that increasing the degree of demineralization and roughness of the radicular dentin negatively affects the initial mechanical adhesion of calcium-silicate-based cements, which decreases the chemical binding.[118] The explanation of this is that the initial mechanical adhesion of calcium-silicate-based cements transform into chemical bonding.[118] Previous studies have shown that 1 mg/mL antibiotic combinations should have a lesser effect on the surface roughness of the radicular dentin than other treatment groups as well as lesser level of demineralization, which may increase the bond strength of intracanal cements.[25,117] Another study indicated that bonded material caused by the biomineralization process of calcium silicate cements contained mainly calcium and phosphate.[119] Therefore, hypothetically, loss of calcium and phosphate, by a demineralization process, from the radicular dentin surface may decrease the bond strength of calcium silicate cements. Based on the results of the study, the null hypothesis was rejected because the low concentrations of TAP and DAP (1 mg/mL) significantly increased the bond strength of calcium-silicate cements to radicular dentin.

DISCOLORATION EXPERIMENT

Many factors may have a discoloration effect on the tooth's crown during or after ER.[120] Examples of these materials or factors include types and concentrations of

the intracanal medicaments, type of intracanal cement, and the presence or absence of blood inside the root canal system.[30,31,35,37,121] In the present study, the focus was solely on the types and concentrations of the intracanal medicaments in order to evaluate the pure discoloration effects of different intracanal medicaments.

This study evaluated the effects of low and mid concentrations of TAP and DAP (1 mg/mL and 10 mg/mL) loaded into an aqueous methylcellulose system on crown discoloration with or without using an adhesive bonding agent on the internal wall of the pulp chamber after 1 day, 1 week, 4 weeks, and after thermocycling.

The rationale behind exposing the samples to thermocycling after removing the intracanal medicaments is the possibility of the debonding of the adhesive agent after thermocycling, which may allow the remnants of the intracanal medicament to diffuse into the dentinal tubules to discolor the crown. According to previous studies, either the bonding interface might hydrolyze by the hot water of the thermocycling, debonding might occur due to different thermal contraction/expansion coefficients of the adhesive agent compared to the tooth tissue, or both processes could occur simultaneously.[122,123]

None of the previous studies evaluated the effects of 10 mg/mL or 1 mg/mL concentrations of TAP and DAP on crown discoloration. However, in the present study the 10 mg/mL or 1 mg/mL concentrations of TAP and DAP were formed in a paste-like material. The present study showed that when an adhesive bonding agent was used prior to the mid-concentration TAP (10 mg/mL), the crown had less discoloration than without adhesive at the 1-week and after-thermocycling time points. The same applied for the typical clinical concentration TAP (1000 mg/mL) at day 1, week 1, and week 4. This result is comparable with Kime et al.'s results, although they used the typical clinical concentration of TAP and the change in color

was only followed for 14 days.[32] However, the tetracycline group, represented by minocycline in the TAP, had the affinity to bind to the Ca^{2+} that is present in the dentin in order to form an insoluble complex.[88] Based on this idea, the sealing of the dentinal tubules by an adhesive bonding agent in order to prevent direct contact of the TAP to radicular dentin explains the reduction in the discoloration of the adhesive groups compared to the no-adhesive groups.

Regarding the time variable in the present study, $Ca(OH)_2$ and the control group showed the least color change at the 1-day time point regardless of the use of adhesive. A study by Akcay et al. showed the same pattern.[33] In addition, a recent study by Fundaoglu et al. showed that control group had the same pattern as the present study shows.[124] It can be suggested that there is a positive relationship between time and crown discoloration. Similar to the 1-day time point, $Ca(OH)_2$ and the control group showed the least color change after thermocycling regardless of the use of adhesive. A possible explanation is that the medicaments were removed from the pulp chamber of the samples before they were exposed to thermocycling. An additional cause could be the washing effect of hot and cold baths during the thermocycling process.

In the current study, when an adhesive bonding agent was used prior to mid and low concentrations DAP (10 mg/mL and 1 mg/mL), no significant color change was detected between all reading time points. This observation supports the idea of using an adhesive bonding agent before application of the intracanal medicaments. The typical clinical concentration of DAP (1000 mg/mL) groups showed an inverse relationship between time and crown discoloration. Akcay et al. showed that the typical concentration of DAP caused an unclear pattern of discoloration at different time points: the color changed more in 1 day than in 1 week but then increased again

after 2 weeks.[33]

In the current study, regardless of concentrations and presence of adhesive, TAP groups displayed the least color change at the 1-day time point when compared to all other time points. Previous studies showed the same result with the typical clinical concentration and 0.1 mg/mL concentration.[32,33,124] As exposure time of the TAP to the radicular dentin increases, the amount of minocycline that diffuses into the dentinal tubules also increases, and vice versa; this could explain why the least color change was observed at the 1-day time point.

In the current study when comparing the different treatment groups at each time point, nearly the same pattern was shown regardless of whether or not the adhesive bonding agent was used. In a recent study, although they did not used an adhesive bonding agent, the treatment groups showed the same pattern at all color reading time points.[124] Moreover, at all time points regardless of adhesive used, mid or low-concentrations DAP (10 mg/mL or 1 mg/mL) had either the least color change of all treatment groups or it is not significantly different from the group with the least color change. Alongside the present study, Fundaoglu et al., concluded that low concentration (0.1 mg/mL) of DAP had the least ΔE values at all time points.[124] Furthermore, the typical clinical concentration of TAP showed the highest ΔE value at all time points whether adhesive bonding agent was used or not. Prior studies evaluated the discoloration effect of typical clinical concentration of TAP.[30,33] The current study showed the same results as previous ones. One possible explanation of the high discoloration effect of TAP 1000 mg/mL is the diffusion of the medicament into dentin through dentinal tubules in order to bind with Ca^{2+}[88]. Moreover, a previous study showed that more than 85 percent of TAP remained in the dentin after irrigation.[125] In the same study, the TAP was found in the dentin to a depth greater

than 350μm.[125] The low or mid concentrations (1 mg/mL or 10 mg/mL) of TAP showed less discoloration than the high concentration. Also, the low or mid concentrations (1 mg/mL or 10 mg/mL) of DAP showed discoloration less than or comparable to the high concentration. A possible cause of this is that a lower amount of TAP or DAP particles leads to a lower amount of medicament diffusion into the dentin.

Although in the current study there were significant color differences between treatment groups at different time points, the only groups that displayed a significant increase from the perceptibility threshold (ΔE>3.7) were: 10 mg/mL TAP with no adhesive after week 1 and after thermocycling (Figure 23); 1000 mg/mL TAP with adhesive after week 1, week 4, and after thermocycling (Figure 24); and 1000 mg/mL TAP with no adhesive at all time points (Figure 24). Interestingly, a recent study evaluated the color change of the 0.1 mg/mL of TAP and DAP and showed that 0.1 mg/mL TAP exceeding the 3.7 level at all time points. The potential causes of the difference between the results in the previous and the current study are the difference in sample design and the method of the medicament preparation, which was not fully explained in the previous study.[124] This is a very important finding, since the change in the crown's color cannot be detected by the naked eye until this change exceeds ΔE = 3.7.[98,126,127] Therefore, it is not valuable clinically to know the pattern of the color change caused by the intracanal medicaments for ΔE values below the perceptibility threshold (3.7). However, the 3.7 is not an absolute point but it is an average of a range of numbers.[98] Johnston and Kao found that the color matches when the ΔE ranges from 2.2 to 4.4.[98] This means that if the ΔE value is between 3.7 and 4.4, the possibility of clinically undetectable discoloration is still present. On the other hand, if the ΔE number falls between 2.2 and 3.7, there is a possibility of clinically

detectable discoloration. Based on the results of the present study, the null hypothesis was rejected because the mid-concentration TAP (10 mg/mL) showed significant discoloration as well as because the use of an adhesive bonding agent with that concentration of TAP significantly decreased the color change.

Limitations of the present *in-vitro* study, for the push-out bond strength experiment, included the absence of apical tissue, blood, and a resorbable matrix, which may have other effects on the bond strength of the intracanal cement. Moreover, lack of a healing process is also considered a limitation; in some situations, leaving the intracanal medicament for longer than 4 weeks is necessary depending on the sign/symptoms of persistent infection.[6] More *in-vitro* studies are needed to investigate the effects of blood and resorbable matrices on the bond strength of intracanal cements. For the discoloration experiment, the absence of blood, resorbable matrix, and intracanal cements may have other effects on crown discoloration. The model used in this discoloration study has the advantage of using retrograde access to fill the pulp chamber instead of using coronal access in order to avoid disturbance of the intact crown. On the other hand, in this model the sample thickness was not fully standardized. Thickness of the object has an effect on the diffusion and reflection of light, which may affect the color reading.[128] In order to minimize the effect of different thicknesses in the present study, a close-fit custom-made cap was made to use for each sample before each color reading.

A well-designed study is needed in order to investigate the exact explanation of the high bond strength of the new calcium silicate based materials (Biodentine and Endosequence putty) to radicular dentin. More *in-vitro* studies are needed to know the appropriate concentration of TAP and DAP that can be used to achieve sufficient antibacterial effect, the least cytotoxicity, as well as the least negative effects on the

bond strength of the intracanal cement and on the crown discoloration. Clinical studies are required to support the use of low- or mid-level concentrations of TAP and DAP in ER to reduce the adverse effect of the currently used clinical concentration on the radicular dentin and on the crown discoloration.

SUMMARY AND CONCLUSION

PUSH-OUT BOND STRENGTH EXPERIMENT

Within the limitations and according to the results of the current study, 1 mg/mL DAP loaded into an aqueous methylcellulose system and $Ca(OH)_2$ did not have a significant negative effect on the bond strength of calcium-silicate-based cement to radicular dentin. Therefore, using 1 mg/mL DAP loaded into an aqueous methylcellulose system or $Ca(OH)_2$ instead of the typical clinical concentration of TAP and DAP is highly recommended during disinfection in ER.

DISCOLORATION EXPERIMENT

Within the limitations and according to the results of the current study, Low and mid concentrations (1 and 10 mg/mL of DAP) and $Ca(OH)_2$ had the least effect on the color change of the human tooth crown of all intracanal medicaments used in this study. Therefore, 1mg/mL to 10 mg/mL of DAP or $Ca(OH)_2$ is recommended to use during disinfection in ER.

Within the limitations and according to the results of the current study, using an adhesive bonding agent prior to typical clinical concentration or mid-concentration of TAP (1000 mg/mL or 10 mg/mL) application significantly reduces the discoloration effect. Therefore, using an adhesive bonding agent on the internal wall of the pulp chamber prior to application of medicaments is recommended in ER.

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ABSTRACT

EFFECT OF LOW CONCENTRATIONS OF ANTIBIOTIC INTRACANAL MEDICAMENTS ON CROWN DISCOLORATION AND PUSH-OUT BOND STRENGTH

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Introduction: Some intracanal medicaments used in regenerative endodontics may compromise the bond strength of root cements and lead to tooth discoloration.

Objectives: To evaluate the effects of 1) low concentrations of TAP and DAP (1 mg/mL) on push-out bond strength of various root cements, and 2) low concentrations of TAP and DAP (1 mg/mL and 10 mg/mL) on crown discoloration.

Materials and Methods: Single rooted human teeth (n = 144) were horizontally decoronated and instrumented according to standardized protocol. The samples were randomized into six experimental groups ($Ca(OH)_2$, 1000 mg/mL TAP and DAP, 1 mg/mL TAP and DAP, and no medicament control group. After four weeks, the medicaments were removed and each group was divided into three subgroups to

receive MTA cement, Biodentine cement, or Endosequence Bioceramic putty cement for two weeks. Then, two root cylinders were obtained from each root and push-out bond strength testing was performed. For the crown discoloration experiment, 160 crowns were obtained from intact human molars and randomized into experimental groups as described earlier with the addition of two groups (10 mg/mL TAP and DAP). The pulp chambers in half of the samples from each group were coated with an adhesive bonding agent before receiving the assigned intracanal medicament. Color changes (ΔE) were detected by spectrophotometer at 1 day, 1 week, and 4 weeks after application, as well as after thermocycling.

Results: In the push-out bond strength experiment, 1 mg/mL DAP generally demonstrated significantly higher bond strength of root cements compared with the other treatment groups. For the crown discoloration experiment, when an adhesive bonding agent was used prior to (10 mg/mL or 1000 mg/mL) TAP, the crowns had significantly less discoloration than those without adhesive. DAP 10 mg/mL had the least significant color change at all time points regardless of whether adhesive was used.

Conclusion: 1) 1 mg/mL DAP and $Ca(OH)_2$ did not have significant negative effect on the bond strength of calcium-silicate-based cement to radicular dentin. 2) 1 mg/mL and 10 mg/mL of DAP and $Ca(OH)_2$ had significantly less effect on the color change of the human tooth crown than all intracanal medicaments used in this study.

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