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Original research article

The effect of thermocycling on the sealing ability of White Mineral Trioxide Aggregate: an *in vitro* study

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Abstract

Objective: The purpose of this study was to evaluate the effect of thermocycling on the sealing ability of White Mineral Trioxide Aggregate (WMTA) after application for management of furcation perforation.

Materials and Method: Thirty two human permanent mandibular molar teeth were chosen and after root amputation, the coronal parts were further trimmed and conventional access cavities were prepared. Furcation perforations were made with diamond bur and Peeso drills. Samples were divided into 3 experimental groups (n=10) and two control groups (n=1). The perforations were filled with WMTA in the experimental groups; in the control groups samples remained unfilled. Samples in the first group remained without further treatment, while in the second and third groups, teeth were thermocycled 500 and 800 times between 5 to 55 °C prior to leakage testing. Microleakage testing was done by using bovine serum albumin for 90 days. The number of days for color change was used as an indicator of protein leakage. Data were analyzed by using one-way analysis of variance and a post hoc Tukey test at a significance level of p<0.05.

Results: Non-thermocycled teeth showed significantly the longest time necessary for protein leakage to occur in comparison with the other two thermocycled groups (p<0.0001). The samples after 800 cycles showed the lowest resistance to protein leakage, while samples after 500 cycles indicated significantly more resistance against leakage (p<0.0001).

Conclusion: Thermocycling can remarkably influence the microleakage property of WMTA. Thermal changes occurring inside the oral cavity might jeopardize the sealing property of the applied cement, which can lead to microleakage and possible failure of treatment in a clinical scenario.

Keywords: Dental leakage; mineral trioxide aggregate; perforation

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Introduction

The unique sealing characteristic of Mineral Trioxide Aggregate (MTA) is well-known in the field of dentistry,[1] which makes it a suitable material for many treatments such as pulp capping,[2] pulpotomy, root canal obturation, root-end filling surgeries, and management of furcation and canal perforations.[3] This ability of MTA has been evaluated by different methods including dye leakage, fluid filtration, bacterial leakage and protein leakage in comparison with other similar material such as Super-EBA or dental amalgam.[4] In many of these investigations, authors have indicated that MTA showed a superior sealing ability than other materials.[4,5] This advantage of MTA is due to its chemical bond to the dentinal structure that is additionally enhanced by the hydration phase during its setting time.[6,7]

Establishing a good sealing ability is important to prevent or reduce the microleakage of microorganisms or their by-products, which can lead to endodontic treatment failure.[6,8] It is also of great concern to maintain this property in the dynamic oral cavity environment.[9] Many studies have focused on elements such as the thickness of

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applied MTA, or the pH value of environment, which might jeopardize the retention ability of the set cement.[5,10] In addition, there are other influential factors such as the temperature changes, which can also ne-gatively affect the applied cement's properties.[11,12] Due to temperature changes, the thermal stresses are imposed to the microstructure of Portland cements which eventually can drastically change the physical properties of the set cement.[11,12] Some investigators have studied the effect of thermal changes on other dental materials such as adhesive systems, luting agents, nano-composites, and zirconia ceramics.[13,14] In these studies the thermocycling process was used to indicate that inside the oral cavity these cycles can affect the bond strength of materials to dentin, resulting in higher microleakage after application of these mate-rials.[13,14]

Thermocycling is a reliable artificial aging method which is used in laboratory circumstances to test the effect of thermal stresses on materials.[15,16] The application regimen for biomaterials and their effect is important; previous publications confirmed that the storage temperature influences the properties of biomaterials.[8] Normally in dentistry, the tested material is subjected to a minimum of 500 cycles in water between 5 ℃ and 55 ℃, which simulate the stresses that is tolerated in a dynamic situation such as the oral cavity.[13,14,17]

The negative impact of these thermal stresses is due to the difference between the coefficient of thermal expansion of tested materials and the tooth structure.[13,14,17] This issue was also discussed by other investigators which mentioned the mismatch of the coefficient of thermal expansion of the tooth and the restorative material can result in microleakage and wear problems in applied restorative composite materials.[18] In another study, in a 1-week observation, it was reported that after 500 temperature cycles, the applied amalgam restorations showed an increase in microleakage in comparison with two types of silicate cement, restorative resins and amalgam restorations which were inserted into varnish coated cavities.[19] A similar result was reported by others, which acclaimed lower microleakage for MTA cement due to the proximity of its linear coefficient of thermal expansion to the dentinal structure.[20]

There is scarce data regarding the effect of thermocycling process on the sealing ability of MTA cement exposed to temperature changes that occur in oral cavity. Therefore, the present study was carried out to evaluate the effect of 500 and 800 cycles of thermocycling on the microleakage of white MTA (WMTA) cement after application to manage furcation perforation. The hypothesis tested was that the thermal cycles could increase the microleakage of bovine serum albumin within 90 days of observations.

Materials and Method

Sample preparation

Thirty two extracted human permanent mandibular molars with no fused roots were selected for this study. The teeth were examined under a stereomicroscope to exclude those with any fracture or crack. Sample preparation was done according to the method used by Hashem *et al.*[21] and Saghiri *et al.*[5] Briefly, samples were resected 3 mm apical to furcation and 3 mm coronal to cemento-enamel junction at a direction perpendicular to the long axis of teeth by using a water-cooled low-speed Isomet diamond saw (Buehler, Lake Bluff, NY, USA). The conventional access cavity was prepared through the reduced occlusal surface by using a tapered diamond bur. The furcation perforation was made by the same tapered diamond bur between the canal orifices. The perforated area was enlarged with #1 through #4 Peeso drills (Mani, Tochigi, Japan) in order to establish a standard 1.3 mm wide perforation in the furcation area (Figure 1A). Prepared samples were immersed in 17% EDTA and then in 1% sodium hypochlorite for 1 minute, washed in distilled water and after drying were randomly divided into three experimental groups (n=10), while two samples served as positive and negative control group.

Sachets of WMTA (tooth-colored formula, ProRoot MTA, Dentsply Tulsa Dental Specialities, Tulsa, OK, USA) were mixed according to the manufacturer's instruction and transferred to the perforated sites and the canal orifices by a manual MTA carrier (Dentsply Tulsa Dental Specialties) and packed with a hand condenser (Hu-Friedy, Chicago, IL, USA) (Figure 1B). After this stage, cotton pellets were soaked in synthetic tissue fluid (STF) which was prepared from 1.7 g of KH_2PO_4, 11.8 g of Na_2HPO_4 , 80.0 g of NaCl, and 2.0 g of KCl in 10 L of H_2O (pH 7.4) and placed in contact with the applied MTA material at the orifice of root canals and perforation site and samples were incubated in 37 °C, 98% humidity for 3 days. The cotton pellets were refreshed daily and after incubation, complete setting and solidity of the cement was verified by probing with an explorer.

Following incubation, specimens in the first group remained without further treatment. Samples in the second group were thermocycled for 500 cycles, and the third group for 800 cycles between 5 and 55 °C with 20 seconds dwell time and 5 seconds transfer time between two baths. After thermocycling regimen, whole external and internal surfaces of all samples including the pulp chamber walls and the MTA covering the canal orifices were sealed with two layers of nail varnish (Figure 1C). The only surface which remained without any varnish was the WMTA which was used in the perforation site (Figure 1C). All surfaces of the negative control group samples were coated with nail varnish, while none

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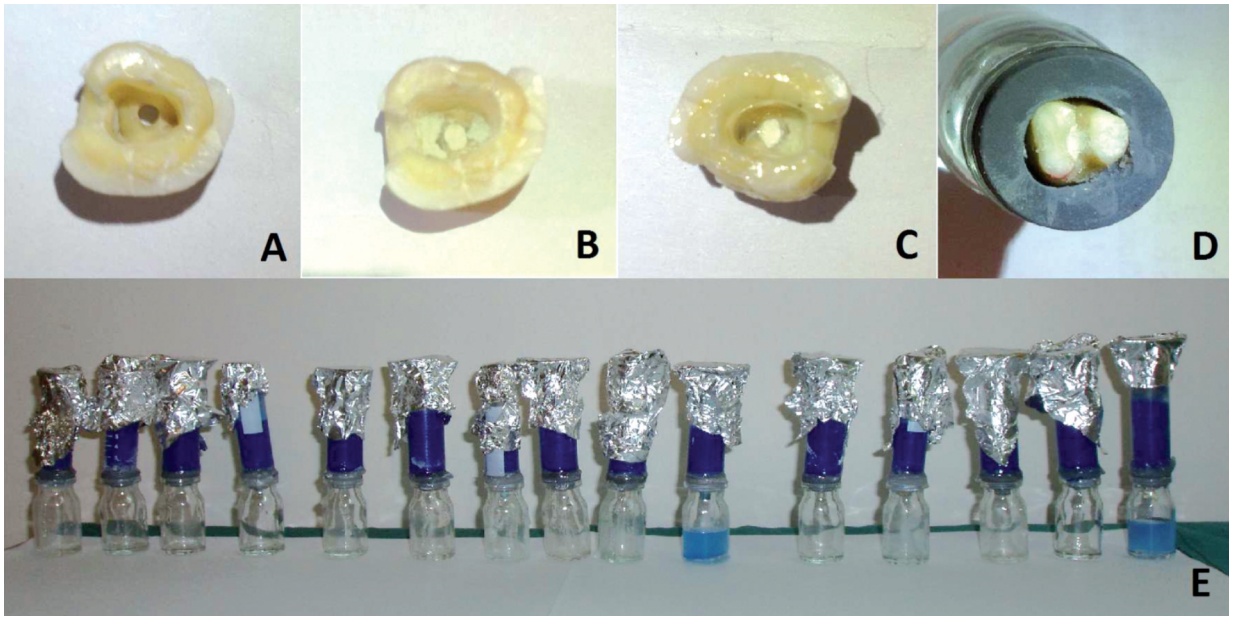

Figure 1. *Sample preparation steps;* ***A:*** *perforation made in the furcation area;* ***B:*** *WMTA placement in the perforation area and in the mesial and distal orifices (with no contact with each other);* ***C:*** *varnish coating of outer and inner surfaces except the WMTA covering the furcation perforation;* ***D:*** *placement of prepared samples inside the punched opening of a vial in a downward manner; E: leakage testing vials which present the color change.*

of the surfaces were coated with varnish in the positive control group sample. All specimens were subjected to a protein leakage analysis.

Preparation of the protein reagent

The protein reagent was prepared according to a method described by Valois & Costa.[10] Briefly, Coomassie Brilliant Blue G (B5133; 100 mg; Sigma Chemical Co, St Louis, MO, USA) was dissolved in 50 mL of 95% ethanol followed by addition of 100 mL 85% phosphoric acid. The solution was diluted with distilled water to a volume of 1 L. After filtering the diluted solution, it was stored at 4 °C until the leakage analysis.

Protein leakage analysis

Protein microleakage testing was performed according to method described by Saghiri *et al.*[5] Briefly, 10 mL glass vials were selected and after punching the rubber stopper, the samples were placed inside the punched opening with the coronal part of the teeth facing downward (Figure 1D). A plastic cylinder was then fixed on the rubber stop surrounding each sample and sealed with nail varnish. Then, 9.5 mL distilled water was dispensed into each glass vial, while the plastic cylinders were filled with 1 mL of 22% bovine serum albumin (BSA, Sigma Chemical Co) solution. All samples were incubated at 37 °C and 98% humidity for 90 days. Every day, the distilled water inside the vials was changed and the BSA solutions were refreshed during the 90 days of observation. Leakage of BSA was analyzed by using the prepared protein reagent, 0.01% (wt/vol) Coomassie Brilliant Blue G, 4.7% (wt/vol) ethanol, and 8.5% (wt/vol) phosphoric acid according to Valois & Costa.[10] The number of days taken for color to change to blue was recorded as an indicator of the protein leakage (Figure 1E). Data were analyzed by using one-way analysis of variance and a post-hoc Tukey's test at a level of significance of $p<0.05$.

Results

The color change of the distilled water in the vial of the positive control group sample was noticed at the very first day; while in the negative control group, the distilled water remained without any change in color for the entire 90 days of observation. In the experimental groups, the means ± standard deviations of the number of days before the day that the color change was noticed were: 77±3.57, 45.20±2.36 and 14.30±1.49, respectively for groups 1, 2 and 3. Statistical analysis showed significant differences between the non-thermocycled and the thermocycled groups ($p<0.0001$; Figure 2). The longest time necessary for leakage was observed in the first group where samples were not thermocycled ($p<0.0001$), while the earliest time was noticed in the third group after 800 cycles of thermocycling in comparison with other experimental groups ($p<0.0001$). Also, the second group showed significantly more resistance to protein leakage than the third group ($p<0.0001$; Figure 2).

Discussion

Establishment and maintenance of a good sealing property is of high importance as the most crucial reason for failure of endodontic treatments is the leakage of mic-

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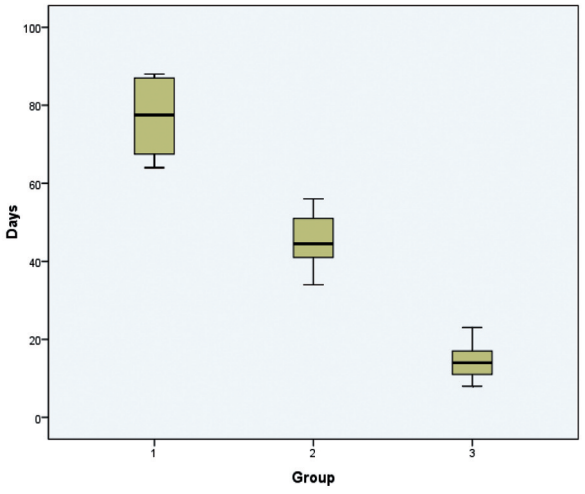

Figure 2. *The box plot graph of experimental groups showing the maximum value, 75th percentile (upper quartile), median value, 25th percentile (lower quartile) and the minimum value. Group 1: non-thermocycled, Group 2: thermocycled for 500 cycles, Group 3: thermocycled for 800 cycles. Group 1 with 77±3.57 days showed the lowest microleakage value, while Group 2 with 45.20±2.36 days showed greater microleakage and Group 3 with 14.30±1.49 days showed the greatest microleakage value.*

roorganisms and their by-products from the tooth surrounding tissues.[22,23] MTA cement has accomplished this goal through its chemical bond to dentin surface and the enhancing hydration phase which improve the bond strength remarkably.[7] However, the environmental variables such as temperature changes might have negative effects on the retention characteristic of Portland based cements. Lothenbach *et al.*[11,12] suggested that due to temperature increasing beyond 50 °C, the structure of Portland cement had more porosity, which can influence the cement properties such as compressive strength.

Artificial aging is a laboratory process which uses thermocycling cycles between 5 °C to 55 °C in order to simulate these temperature changes in a dynamic situation which can predispose a material to many thermal loads.[24] These undesirable thermal stresses might eventually result in irreversible alterations in the core structure of the subjected material and negatively affect the physical properties of the applied cement after the setting time.[11,12] In the present study, thermocycling was used to evaluate the effects of temperature changes on the microleakage of the MTA cement. According to a previous review article, thermocycling regimens used for dental materials showed a wide range;[25] and the number of cycles that can be experienced *in vivo* is only suggested for approximately 10,000 cycles per year based on a provisional estimate.[25] The number of these cycles in the present investigation was set according to ISO TR 11450 standard which mentioned a minimum of 500 cycles between 5 °C to 55 °C for evaluations of the effect of temperature changes on a material.[24]

The results of the present study indicated that 500 and 800 cycles of thermocycling can significantly increase the microleakage of MTA samples in comparison with non-thermocycled samples. This finding is consistent with other studies reporting the negative consequences of thermocycling on the physical properties of Portland cement.[11,12] Some authors have attributed the negative effect of thermocycling process to the difference of the coefficient of thermal expansion of the tooth and the restorative materials.[22] The mismatch of the coefficient of thermal expansion can explain the results of the present study to some extent; however, Santos *et al.*[20] reported that the linear coefficient of thermal expansion (CTE) was 8.86±0.28 μstrain/°C for MTA-Angelus that is close to the dentin CTE mentioned by Xu *et al.* which is $10.59\pm2.38 \times 10^{-6}$/°C.[26] The other explanation for this issue might be the structural changes occurring inside the set material due to the fluctuation of temperature which can lead to more porosity and eventually increase the leakage value.[11,12,27] Lothenbach *et al.*[11] considered that these changes are likely to happen in temperatures higher than 50 °C in Portland cements, especially in those cements for which the ratio of $Al_2O_3/SO_3<1.3$.

In a literature review performed by Gale & Darvell, the authors demonstrated that regardless of the various regimens used throughout history, the tested dental material has shown an increased leakage pattern due to thermal stresses.[25] This finding is consistent with the results of the present investigation while the samples after 800 cycles showed earlier time for leakage in comparison with samples thermocycled 500 cycles and non-thermocycled samples. This difference can also be justified by the mismatch of coefficient of thermal expansion between MTA/dentin and the amount porosity in the set material.[26] Obviously, 800 cycles of thermocycling can produce more thermal loads on the tested material which brings more changes in the integrity of cement particles and increased the porosity[11,27] that can affect the protein leakage of the applied cement.

Conclusion

Thermocycling can drastically affect the sealing properties of WMTA cement applied in the furcation area. The sealing ability of WMTA can be affected by the structural changes of the cement produced by thermocycling. As the thermocycling cycles increased, the amount of leakage value also increased. The 800 thermocycles can remarkably amplify the microleakage of WMTA cement more than the 500 cycles. This issue causes earlier time for protein leakage because of greater amount of porosity which is the main result of thermal stresses resulting from thermocycling.

Conflict of interest disclosure: The authors declare no conflict of interest related to this study.

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Termosiklusun beyaz Mineral Trioxide Aggregate tıkama etkinliği üzerine etkisi: *in vitro* çalışma

Özet

Amaç: Bu çalışmanın amacı, termosiklusun furkasyon perforasyonu tamirinde kullanılan beyaz Mineral Trioxide Aggregate'nin (WMTA) tıkama etkinliği üzerine etkisini araştırmaktır.

Gereç ve Yöntem: Çekilmiş 32 adet insan daimi mandibular molar dişi seçildi, kökleri kesildi, kalan koronal parçanın yüzeyi düzeltildi ve geleneksel giriş kavitesi açıldı. Elmas frez ve Peeso frezleri yardımıyla furkasyon perforasyonu gerçekleştirildi. Örnekler 3 deney grubuna (n=10) ve 2 kontrol grubuna dağıtıldı (n=1). Perforasyonlar, deney gruplarında WMTA ile dolduruldu; kontrol grupları doldurulmadı. Birinci grup dişlerine başka hiçbir işlem yapılmazken, ikinci ve üçüncü grup dişlerine, 5 ile 55 °C arasında sırasıyla 500 ve 800 kez termosiklus uygulandı, ve ardından tüm dişler sızıntı testine tabi tutuldu. Mikrosızıntı testi 90 gün süresince sığır serum albumini kullanılarak yapıldı. Proteinin sızıntısına bağlı olarak meydana gelen renk değişikliğinin kaçıncı günde meydana geldiği kaydedildi. Veri, tek-yönlü varyans analizi ve post hoc Tukey testi kullanılarak p<0.05anlamlılık düzeyinde analiz edildi.

Bulgular: Termosiklus uygulanmayan dişlerde protein sızıntısı, termosiklus uygulanan dişlere göre anlamlı olarak daha uzun sürede gerçekleşti (p<0.0001). Protein sızdırma süresi en hızlı 800 siklus grubunda görülürken, 500 siklus grubu sızdırmaya karşı anlamlı olarak daha dirençli bulundu (p<0.0001).

Sonuç: Termosiklus işlemi WMTA'nın mikrosızıntısını belirgin şekilde etkileyebilmektedir. Ağız boşluğu içerisinde meydana gelen ısı değişiklikleri, uygulanan doldurucunun tıkama özelliğini etkileyerek mikrosızıntıya yol açabilir ve klinik şartlarda tedavinin başarısızlığında rol oynayabilir.

Anahtar Kelimeler: Dental sızıntı; mineral trioksid agregat; perforasyon

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