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Research / Araştırma Makalesi

The Impact of Various Irrigation Solutions on the Colour Stabilities of Five Calcium Silicate Cement: An *in-Vitro* Study

Çeşitli İrrigasyon Solüsyonlarının Beş Kalsiyum Silikat Simanının Renk Stabilitelerine Etkisi: *İn-vitro* Çalışma

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ABSTRACT

Background: This in-vitro examination aimed to determine the color changes of five calcium silicate cement [wMTA Angelus, Biodentine (BD), NeoMTA Plus, RetroMTA, OrthoMTA] in various irrigation solutions.

Methods: A total of 100 samples (n=20) were homogenously mixed and placed in cylindrical specimens (diameter of 10 mm and height of 2 mm). After setting time, the color of the samples was analyzed by using a spectrophotometer (VITA Easyshade V, Vita Zahnfabrik, Bad Sackingen, Germany). Later, each specimen was immersed in 5.25% Sodium hypochlorite (NaOCl), 2% Chlorhexidine gluconate (CHX), 17% Etilendiamintetraacetic asit (EDTA), and Saline for 24 hours, and 20 samples were left dry. Color changes were calculated. Compliance with normal distribution was assessed using the Shapiro-Wilk test. The comparison of color values, which were not normally distributed, according to material and solution, was analyzed using a two-way robust ANOVA, and multiple comparisons were examined with the Bonferroni test. Statistical significance was $p < 0.05$.

Results: The color change of wMTA Angelus was higher than that of the other materials when immersed with NaOCl. NeoMTA Plus revealed the highest color change when immersed in EDTA. The maximum discoloration of BD was observed when immersed in CHX.

Conclusion: RetroMTA may be a better choice because of less discoloration in the clinic routine.

Keywords: Calcium silicate cement; Color change; İrrigant

ÖZ

Amaç: Bu in-vitro çalışma, beş kalsiyum silikat simanının [wMTA Angelus, Biodentine (BD), NeoMTA Plus, RetroMTA, OrthoMTA] çeşitli irrigasyon solüsyonlarındaki renk değişimlerini değerlendirmeyi amaçladı.

Gereç ve Yöntemler: Toplam 100 numune (n=20) homojen bir şekilde karıştırıldı ve silindirik numunelere (10 mm çapında ve 2 mm yüksekliğinde) yerleştirildi. Sertleşme süresinden sonra numunelerin rengi bir spektrofotometre (VITA Easyshade V, Vita Zahnfabrik, Bad Sackingen, Germany) kullanılarak analiz edildi. Sonra her örnek %5,25 Sodyum hipoklorit (NaOCl), %2 Klorheksidin glukonat (CHX), %17 Etilendiamintetraasetik asit (EDTA) ve Salin içerisinde 24 saat bekletildi ve 20 örnek kurumaya bırakıldı. Renk değişimleri hesaplandı. Normal dağılıma uygunluk Shapiro-Wilk testi kullanılarak değerlendirildi. Normal dağılım göstermeyen renk değişimlerinin karşılaştırılması ANOVA ile çoklu karşılaştırmalar ise Bonferroni testi ile incelendi. İstatistiksel anlamlılık düzeyi $p < 0{,}05$'tir.

Bulgular: wMTA Angelus'un renk değişimi, NaOCl'ye daldırıldığında diğer materyallerden daha fazlaydı. NeoMTA Plus en fazla renk değişimini EDTA içine daldırıldığında ortaya çıkardı. BD en fazla renk değişimini CHX ile teması ardından görüldü.

Sonuç: Klinik rutinde daha az renk değişikliği nedeniyle RetroMTA daha iyi bir seçim olabilir.

Anahtar Kelimeler: Kalsiyum silikat siman, Renk değişimi, İrrigan

Introduction

Each year, over 24 million endodontic treatments are applied globally, with 5.5% of these including apexification, perforation repair, and endodontic apical surgery.[1] Root canal treatment may be an option in cases of failure of regenerative endodontic treatment (RET) and vital pulp treatment (VPT).[2,3] Various materials are used for this purpose, including Resin-Modified Glass Ionomer Cement (RMGIC), calcium hydroxide, tricalcium and tetracalcium phosphate, Intermediate Restorative Material (IRM), and Mineral Trioxide Aggregate (MTA).[4] Tooth discoloration after endodontic treatment is primarily caused by the penetration of endodontic material into the dental hard tissues through the dentinal tubules.[5,6] For this reason, various calcium silicate cements (CSCs) have been developed to overcome the tooth discoloration caused by MTA.[7]

CSCs are hydraulic cements that contain MTA. They are generally utilized in endodontic procedures to promote pulp regeneration and hard tissue repair. Procedures that use MTA include apexogenesis, perforation repair, root-end filling, apexification, pulp capping, and pulpotomy.[8,9] MTA is the current gold standard in the CSCs[10] and is widely used in endodontic procedures due to its biocompatible, bioactive, radiopaque, and good sealing abilities.[11] However, MTA has some disadvantages, including a long hardening time, difficulty of application, and the potential for discoloration.[12] Numerous studies have found multiple drugs and materials used during root canals to cause discoloration of the coronal tooth.[5,13,14] To eliminate the coronal tooth discoloration caused by the first MTA produced, which was gray,[15,16] white MTA (wMTA) was developed.[17] The addition of bismuth oxide causes wMTA to undergo a color change by providing radiopacity to the MTA content of the material.[18] It has been hypothesized that MTA components bind to phosphate ions or plasma proteins in the dentinal fluid, and the by-products of this binding oxidize and are converted into pigmented by-products.[19]

As a result of advancing technology, various CSCs have been developed, including Biodentine (BD) (Septodont, Saint-Maur-desFosses Cedex, France), ProRoot MTA (Dentsply-Tulsa Dental Specialties, Johnson City, TN, USA), MTA Plus Bradenton, FL, USA OrthoMTA (BioMTA, Seoul, Korea), RetroMTA (BioMTA, Seoul, Korea), and MTA Repair HP (Angelus, Londrina, PR, Brazil).[20]

BD is a calcium-silicate-based material used in endodontic treatments, such as MTA. BD is composed of powder and liquid. The powder includes tricalcium silicate, calcium carbonate, and zirconium oxide, which provides radiopacity. The liquid includes calcium chloride, which initiates the effect.[21] NeoMTA Plus (Avalon Biomed Inc, Bradenton, FL) is a dental material that contains a new, finer powder comprised of tricalcium silicate and tantalum oxide (Ta205) as a radiopacifying agent. The powder-to-gel mixing ratio in NeoMTA Plus is adjustable to aid in ease of application: it can be used as an orthograde filling at a thin consistency or as a root tip filling at a thick consistency.[22] RetroMTA (BioMTA, Seoul, Korea) is another fast-hardening CSC. According to the manufacturer, it has an initial hardening time of 180 seconds. This short

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This short hardening time may be the result of the zirconium, which accelerates the hydration of Portland cement.[23,24] Recently, a different kind of MTA, OrthoMTA (BioMTA, Seul, Korea) has been recommended for utilization as a root canal-filling substance.[25,26] OrthoMTA is composed of tricalcium silicate and has lower levels of heavy metals compared to the original ProRoot MTA.[25]

Root canal disinfection is a crucial stage in endodontic treatment. It involves using an appropriate irrigant to eliminate bacteria and other microorganisms from the root canal system.[27] An effective irrigating agent should possess antimicrobial properties and be able to dissolve organic tissue without irritating it. Additionally, it should be stable and easily stored. The agent must also be capable of removing the smear layer.[20] The commonly used irrigation regimen in conventional endodontics involves the use of organic tissue solvent and antimicrobial sodium hypochlorite (NaOCl) at concentrations ranging from 1-8%, 2% chlorhexidine (CHX) as an antimicrobial agent, and ethylenediaminetetraacetic acid (EDTA) as a demineralizing agent.[27] The American Association of Endodontists Clinical Considerations for Regenerative Procedures suggests that regenerative procedures should be prepared with canal disinfection with 1.5% -3 NaOCL and followed by EDTA in pulp regeneration treatment.[28] Distilled water and saline solution are used between the two solutions to prevent chemical reactions, such as NaOCl and CHX. However, they should not be used as main irrigants due to their lack of tissue-dissolving and antimicrobial activity.[29]

The goal of this in vitro examination is to measure the color stability of five CSCs [wMTA Angelus (Angelus Solucoes Odontologicas, Londrina, Brasil), Biodentine, RetroMTA, OrthoMTA and NeoMTA Plus] in contact with distinct irrigation solutions (EDTA, NaOCl, CHX, Normal Saline) (**Table 1**). According to the null hypothesis of this examination, there is no color change in contact of the study groups with distinct irrigation solutions.

Materials and Methods

Sample Design

The study comprised five groups, each containing 20 samples: Biodentine, wMTA Angelus, RetroMTA, OrthoMTA, and NeoMTA Plus (**Table 1**). The specimens were homogeneously mixed according to the manufacturer's recommendations and placed in cylindrical molds (10 mm in diameter and 2 mm in height). The specimens were kept at 37°C and 100% humidity during curing to achieve optimal mechanical properties. After 24 hours, each specimen was removed from the mold and the color of each sample was analyzed utilizing a spectrophotometer (VITA Easyshade V, Vita Zahnfabrik, Bad Sackingen, Germany).[30] Following color measurement, the samples were immersed in four irrigation solutions (n=4) for 24 hours at 37˚C: 5.25% NaOCl (Cerkamed, Stalowa Wola, Poland), 2% CHX (Microvem, Sakarya, Turkey), 17% EDTA (Imıcryl, Konya, Turkey) and Saline (Polifarma, Tekirdağ, Turkey), and 20 specimens were left dry. After 24 hours, the samples were removed from the irrigation solutions and dried (**Figure 1**).

Table 1. Description of experimental groups

Experimental Groups (N=100)					
	Saline	5.25 %NaOCl	2% CHX	17% EDTA	Dry
wMTA Angelus(n=20)	n=4	n=4	n=4	n=4	n=4
NeoMTA Plus(n=20)	n=4	n=4	n=4	n=4	n=4
Biodentine (n=20)	n=4	n=4	n=4	n=4	n=4
Ortho MTA(n=20)	n=4	n=4	n=4	n=4	n=4
Retro MTA(n=20)	n=4	n=4	n=4	n=4	n=4

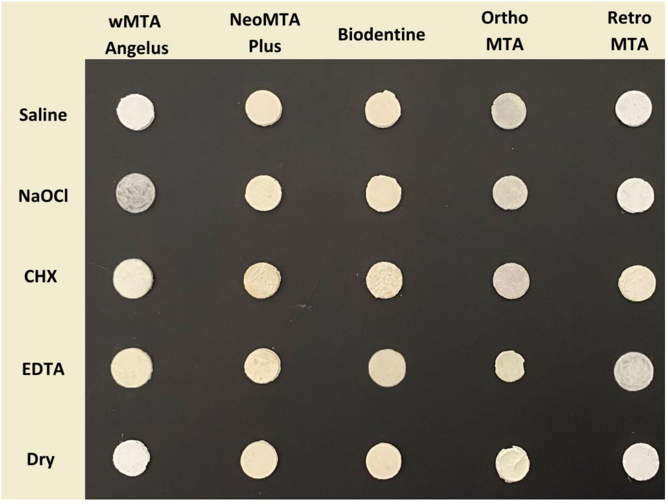

Figure 1. Images of CSCs after setting and immersion in distinct solution

Spectrophotometric Investigation

The same person obtained measurements utilizing the digital spectrophotometer before and 24 hours after immersion. The instrument was calibrated before each evaluation, and the specimens were dried. Three measurements were taken for each sample and then averaged. Color values were recorded for each sample before and after immersion. The color change was calculated using the Commission Internationale de l'Eclairage (CIE) system. The brightness (L) value and the chromatic component coordinates (a and b) were measured.

The study evaluated material color differences by measuring values in the CIELAB color space. The ΔE values, which indicate the differences between the final and initial values, were calculated using the following equation: $\Delta E^* = [(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2]^{1/2}$

The data were analyzed using the Jamovi V2.3.21 program. Compliance with normal distribution was assessed using the Shapiro-Wilk test. The comparison of color values, which were not normally distributed, according material and solution was analyzed using a two-way robust ANOVA with the Walrus package, and multiple comparisons were examined with the Bonferroni test. The analysis results are shown as median (minimum-maximum). A p-value of less than 0.05 was considered statistically significant.

Results

Table 2 shows that the median values for the Saline, NaOCl, CHX, EDTA solutions, and dry condition were 7.53, 7.79, 8.92, 10.31, and 4.17, respectively. The median color values varied depending on the material-solution interaction ($p<0.001$). A significant difference was found in the interactions between the dry condition of wMTA Angelus and CHX solution of BD, between CHX solution of BD and dry condition of OrthoMTA, between dry condition of BD and EDTA solution of BD, and between CHX solution of BD and dry condition of RetroMTA. Although the NaOCl solution in the wMTA Angelus had the highest median value of 19.90 and the dry condition in the OrthoMTA had the lowest median value of 3.28, there was no significant difference between these two interaction groups due to their distribution. All tested materials showed a statistically significant color change when immersed in all solutions compared to the dry group ($p < 0.05$).

Table 2. Descriptive statistics and multiple comparison results of color values according to material and solution

Solution	Material					Total
	Wmta Angelus	NeoMTA Plus	Biodentine	Ortho MTA	Retro MTA	
Saline	6.57 (3.02-11.92) ABCD	9.42 (0.91-18.35) ABCD	7.53 (5.02-12.00) ABCD	11.23 (2.09-12.53) ABCD	5.40 (3.17-13.64) ABCD	7.53 (0.91-18.35) b
NaOCl	19.90 (4.01-28.88) ABCD	8.23 (6.23-13.87) ABCD	5.26 (4.70-14.95) ABCD	8.56 (3.51-11.66) ABCD	4.55 (3.02-9.17) ABCD	7.79 (3.02-28.88) b
CHX	6.84 (6.33-9.98) ABCD	8.38 (4.35-10.11) ABCD	11.93 (11.32-14.12) CD	11.58 (5.34-24.23) ABCD	6.06 (2.47-19.88) ABCD	8.92 (2.47-24.23) b
EDTA	11.54 (7.77-12.60) ABCD	13.74 (9.55-24.74) ABCD	9.62 (9.35-10.60) AC	11.50 (6.89-12.85) ABCD	8.68 (4.76-18.76) ABCD	10.31 (4.76-24.74) b
Dry	4.23 (3.40-6.36) AB	5.60 (2.16-8.58) ABCD	4.72 (4.28-5.04) BD	3.28 (1.60-4.52) AB	4.00 (1.30-4.60) AB	4.17 (1.30-8.58) a
Total	7.32 (3.02-28.88)	8.68 (0.91-24.74)	8.75 (4.28-14.95)	8.56 (1.60-24.23)	4.68 (1.30-19.88)	7.72 (0.91-28.88)

a-b: There is no difference between solutions with the same letter, A-D: No difference between interaction groups with the same letter

Discussion

Based on this study's findings, the null hypothesis should be rejected. The goal of this examination was to measure the color changes of five distinct CSCs in various irrigation solutions. When selecting endodontic materials for root canal treatment, clinicians should consider aesthetic, biological, and functional criteria.[31] Visual and special instruments can be used to measure color changes in dental materials. The CIE system conforms to international standards for color detection and is accepted by the International Commission on Illumination.[32] Visual spectrophotometry is the gold standard for this purpose.[33] Spectrophotometric measurement is a widely used, reproducible, and objective method in dentistry that may detect even the slightest color changes.[34] Spectrophotometric analysis was preferred for the measurements in this study due to its sensitivity, objectivity, and reproducibility.[35]

Irrigation is a crucial step in disinfection during root canals. NaOCl is a commonly used irrigating agent for disinfection procedures, although other irrigants such as CHX, EDTA, water, and saline are also preferred.[36] In our examination, we used NaOCl, CHX, EDTA, and saline solutions for the intended purpose. It is important to note that NaOCl, due to its crystallized nature, cannot be entirely eliminated from the root canal regime, as it occludes the dentinal tubules.[37] It was observed that sodium and chlorine in NaOCl migrated into SEM examination.[38] During regenerative endodontic procedures, resorption cavity, and perforation repair, residual NaOCl may come into contact with CHX or CSCs. To compare this prolonged contact time between the irrigant and CSCs in the root canal regime, these materials can be immersed in irrigation solutions for 24 hours.[39]

The literature reports that immersion of wMTA in NaOCl results in a dark brown discoloration, which is believed to be reasoned by the reaction of bismuth oxide in MTA and NaOCl.[31] In our study, similar to the results of Keskin et al.[40] and Shah and Banga[41], wMTA Angelus changed color mostly in NaOCl solution. However, another study presented in the literature showed that wMTA Angelus was most colored in CHX solution.[42] The change in color of wMTA was interpreted as resulting from a combination of exposure to light and an oxygen-free atmosphere in the clinical setting.[34] Bismuth oxide decomposes into dark metallic bismuth and oxygen crystals when exposed to light in an oxygen-free atmosphere, causing MTA to change color. Nanocrystallite bismuth oxide is activated by both visible and ultraviolet (UV) light, with a UV-visible diffuse spectrum that extends from 300-500 nm to shorter wavelengths, peaking at 400 nm, which accelerates the discoloration of wMTA.[43]

In this study, same to the research conducted by Yılmaz et al.[44] and Keskin et al.[40], it was found that BD exhibited less discoloration after NaOCl contact was checked with wMTA Angelus. This result may be attributed to the fact that BD contains zirconium oxide instead of bismuth oxide.[21] Since BD does not contain bismuth oxide, no dark discoloration of these materials was observed after contact with NaOCl.[45]

In this study, it was found that the color of BD after contact with CHX was higher than that of other irrigants, which is consistent with previous studies in the literature.[20,39-41] CHX has been proposed as another irrigant to NaOCl owing to its antimicrobial impact.[46] On the other hand, CHX does not have tissue-dissolving characteristics and instead conditions an orange-colored toxic precipitate as it comes into contact with NaOCl.[47] CHX has been reported to cause external staining of dental tissues[48] by affecting the pellicle or plaque in filling materials containing calcium silicate at various concentrations.[49] CHX is also characterized by prolonged release with dental materials.[50]

EDTA is an important and commonly used irrigant to remove the mineralized portion of the smear layer[51,52], as it effectively removes the inorganic component of dentine and exhibits antimicrobial activity in root canals.[53] However, it can't remove the smear layer alone; a proteolytic agent, such as NaOCl, must also be used. EDTA solution is neutral or slightly alkaline and precipitates at an acidic pH. Typically, EDTA is used as a 15% or 17% solution.[46] Sobhnamayan et al.[42] found that BD demonstrated color stability when in contact with EDTA, whereas wMTA Angelus showed a greater degree of color change when compared to normal saline and dry conditions. It is important to note that BD material samples were eroded while in contact with EDTA and were therefore excluded from the study; further investigation to determine the effect of EDTA on BD microstructure is required.[42] In our study, BD showed less discoloration in the EDTA solution than the wMTA Angelus. Metlerska et al.[20] observed a statistically significant difference in all materials after contact with the EDTA solution. In our study, RetroMTA showed less coloration in NaOCl, CHX, and EDTA solutions compared to other CSCs. It is important to note that all evaluations presented here are objective and based on empirical data. However, Shokouhinejad et al.[54] reported that RetroMTA was mostly colored in NaOCl solution. In contrast, our study found that RetroMTA was mostly colored in EDTA solution.

According to the manufacturer, OrthoMTA prohibits microleakage by forming a hydroxyapatite layer between itself and the canal wall. It is also bioactive, releasing calcium ions from the apical foramen and neutralizing the apical part of the root, thereby forming an interfacial hydroxyapatite layer.[25] The released calcium ions aid in the regeneration of the apical periodontium.[55] More studies to examine the clinical operation of OrthoMTA are necessary, as its properties and mechanisms are unclear. To prevent CSC-induced discoloration, applying a dentine bonding agent in two layers between the material and the dentine bonding agent in the entrance cavity is recommended.[56]

This study's limitations include the fact that although the materials were stored at 37ºC and 100% humidity, which may not accurately reflect the physical conditions of the dentine properties and characteristics and oral environment. Additionally, CSCs were immersed in irrigation solutions for 24 hours, with all surfaces of the specimens in contact with the solutions. In clinical settings, the amount and concentration of residual irrigation in contact with the surface may vary. Therefore, it is possible that longer contact times may result in greater discoloration. It is important to note that this study measured the discoloration of the materials themselves, rather than tooth discoloration. Another limitation of this study is that it did not measure the surface structures of the materials. To better replicate clinical conditions related to tooth discoloration, additional studies with longer follow-up periods are required.

Conclusion

The study demonstrated significant differences in the ability of CSCs to change color in the presence of irrigation agents commonly used in clinical practice. Materials containing bismuth oxide exhibited color change after contact with NaOCl and CHX. Therefore, bismuth oxide-free materials, like RetroMTA, NeoMTA Plus, and BD, may be considered alternatives for use in aesthetically important areas.

Değerlendirme / Peer-Review

İki Dış Hakem / Çift Taraflı Körleme

Etik Beyan / Ethical statement

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It is declared that during the preparation process of this study, scientific and ethical principles were followed and all the studies benefited are stated in the bibliography.

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Etik Bildirim / Ethical statement

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Yazarlar çıkar çatışması bildirmemiştir. | The authors have no conflict of interest to declare.

Yazar Katkıları / Author Contributions

Çalışmanın Tasarlanması | Design of Study: ASÖ (%60), OK (%30), EUH (%10)
Veri Toplanması | Data Acquisition: ASÖ (%50), EUH (%25), OK (%25)
Veri Analizi | Data Analysis: ASÖ (%50), OK (%25), EUH (%25)
Makalenin Yazımı | Writing up: ASÖ (%60), OK (%20), EUH (%20)
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