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Effect of Chitosan and EDTA Solutions on Bond Strength of Two Different Calcium Silicate Based Materials

Kitosan ve EDTA Solüsyonlarının İki Farklı Kalsiyum Silikat Esaslı Malzemenin Bağlanma Dayanımına Etkisi

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Abstract

Objective: The aim of the present study was to evaluate the potential of using chitosan-based chelating agents to improve the bond strength of mineral trioxide aggregate (MTA) Angelus (Londrina, PR, Brazil) or MTA Repair high plasticity (Londrina, PR, Brazil).

Materials and Methods: A total of 60 dentine discs were obtained from 15 freshly extracted human maxillary central incisors. Two canal-like holes were drilled and the disks were divided into four groups, as following; group 1: chitosan solution with acetic acid; group 2: chitosan solution with lactic acid; group 3: 17% ethylenediaminetetraacetic acid; and group 4: distilled water. Discs were subjected to the tested chelating solution for smear layer removal for 3 min. The two holes of the same dentine discs were each randomly filled with one of the tested materials. The push-out test was performed and data were analyzed using 2-way analysis of variance test with a 5% significance level.

Results: No significant differences were observed for the type of tested material (p=0.153) and the interaction between tested material and solution (p=0.922); however, there was a significant difference among chelating agents (p=0.001).

Conclusion: Both materials showed similar bond strength regardless of the a chelating agent was used or not. All chelating agents significantly decreased the push-out strength of both materials, except for the chitosan solution prepared with acetic acid.

Keywords: Chelating agents, ethylenediaminetetraacetic acid, chitosan, mineral trioxide aggregate, dental bonding

Öz

Amaç: Bu çalışmanın amacı, mineral trioksit aggregat (MTA) Angelus (Londrina, PR, Brezilya) veya MTA Repair high plasticity (Londrina, PR, Brezilya) materyalinin dentine olan bağlanma dayanımını artırmak için kitosan bazlı şelatlama ajanlarının kullanım potansiyelini değerlendirmektir.

Gereç ve Yöntemler: On beş adet yeni çekilmiş maksiller santral kesici dişinden toplam 60 adet dentin diski elde edildi, dentin disklerinde iki adet kanala benzer delik açıldı ve dört gruba ayrıldı; grup 1: asetik asitle hazırlanan kitosan solüsyonu; grup 2: laktik asitle hazırlanan kitosan solüsyonu; grup 3: %17 etilen diamin tetra asetik asit; ve 4. grup: distile su. Diskler 3 dakika süreyle smear tabakasının uzaklaştırılması için test edilen şelatlama solüsyonuna tabi tutuldu. Aynı dentin diskinin iki deliğinden her biri, test edilen malzemelerden biri ile rastgele dolduruldu. Push-out testi gerçekleştirildi ve veriler %5 anlamlılık düzeyinde 2 yönlü varyans analizi testi kullanılarak analiz edildi.

Bulgular: Test edilen materyalin tipi (p=0,153) ve test edilen materyal ile çözelti arasındaki etkileşim (p=0,922) açısından önemli bir farklılık gözlenmedi; ancak şelatlama solüsyonları arasında anlamlı farklılık vardı (p=0,001).

Sonuç: Her iki materyal şelatlama solüsyonu kullanılıp kullanılmadığına bakılmaksızın benzer bağlanma dayanımı gösterdi. Asetik asit ile hazırlanan kitosan solüsyonu haricinde tüm şelatlama solüsyonları her iki materyalin dentine olan bağlanma dayanımını önemli ölçüde azaltmıştır.

Anahtar Kelimeler: Şelatör ajanlar, etilendiamintetraasetik asit, kitosan, mineral trioksid agregat, diş yapıştırma

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Introduction

During the root canal instrumentation, a smear layer containing organic and inorganic materials is formed. Removal of this layer may positively affect the root canal disinfection and the adaptation of obturation materials (1). Various chemicals have been investigated to remove the smear layer. The most common chemical Ethylenediaminetetraacetic acid (EDTA) chelate calcium ions in dentine and forms soluble Ca-EDTA complex. However, EDTA has cytotoxic potential and is considered as an environmental pollutant (2).

Chitosan is a natural aminopolysaccaride. Many organic acids have been recently suggested as a solvent to form chitosan conjugates. One of those acids, lactic acid has been suggested as a chelating agent in endodontics and chitosan dispersion prepared with lactic acid has been associated with low cytotoxicity, high biocompatibility, hydrophilicity and great antibacterial properties (3).

Calcium silicate based cements can be considered as the gold standard material for several clinical procedures in endodontics. One of these cements, mineral trioxide aggregate (MTA) Angelus (MTA-Ang; Londrina, Brazil) is composed of 80% Portland cement and 20% bismuth oxide. MTA Repair high plasticity (MTA-HP; Londrina, Brazil) has been introduced with high-plasticity and improved physical properties compared to MTA Angelus (4).

Bond strength has been defined as a measure of frictional sliding that can be conventionally tested using the push-out test method and influenced by root canal chelating agents leading to changes in the content of dentine and the physicochemical properties of calcium silicate-based materials (5,6). Hereby, it is recommended for flushing the area with copious amounts of distilled water to remove chelating agents completely (6). However, contact of calcium silicate based materials with chelating agents may be unavoidable for some endodontic treatments such as regenerative procedures in which chelating agents are used as final irritant to release growth factors from dentine (6). In several studies, the adhesive properties of calcium silicate-based materials have been assessed after irrigation with various solutions (7,8). Up to now, only one study has been previously investigate the effect of chitosan based chelating agents on bond strength of calcium silicate based materials (9). We conducted this study to test two null hypotheses: (i) there is no differences in the bond strength between MTA-Ang and MTA-HP, and (ii) the chelating agents do not affect the push-out performance of the MTA-Ang and MTA-HP.

Materials and Methods

Sample Selection and Preparation

The study was realized in accordance with Declaration of Helsinki and approval was obtained through the Local Ethics Committee at Ankara University (decision no: 36290600/02, date: 17.01.2020). Based on a previous study with effect size of 0.74 (10), a total of 48 disc samples were found to be necessary. The study involved 15 freshly extracted human maxillary mature central incisors without visible sign of root fracture, caries, or resorption. Teeth were embedded in cold cure acrylic (Meliodent, UK). The coronal and apical segments of each tooth were removed to leave 10 mm long middle third of root. Four horizontal dentine discs (1±0.1 mm thick) in the middle third were created using a low-speed saw. A total of 60 dentine discs (n=15 per group) were randomly divided into four groups according to the type of irrigation solution, as following; group 1: chitosan solution with acetic acid; group 2: chitosan solution with lactic acid; group 3: 17% EDTA; and, group 4 (control group): distilled water. Two canal like holes perpendicularly to the surface of each dentine disc were prepared with a 0.8 mm cylindrical carbide bur, maintaining a minimum distance of 0.5-1 mm between the holes, external root surface and inner root canal wall. Dentine discs were immersed in a 2.5% sodium hypochlorite (NaOCl) for 15 minutes and then, each dentine disc from the same tooth was subjected to the tested chelating solution for 3 minutes. For preparation of the chitosan dispersion with acetic acid (pH=2.39), 0.2 g of chitosan was diluted in 100 mL of 1% acetic acid, and stirred using a magnetic stirrer for 2 h. For preparation of the chitosan dispersion with lactic acid (pH=2,186), 0.2 g of chitosan was diluted in 100 mL of 1% lactic acid, and stirred using a magnetic stirrer for 2 h. All dentine discs were flushed with distilled water as the last irrigant for 1 minute and then dried with absorbent paper. The two holes of the same dentine disc were each randomly filled with one of the tested calcium silicate based materials: MTA-Ang or MTA-HP. All tested materials were mixed according to the manufacturers' instructions, placed into the holes using a carrier and condensed with hand pluggers. The dentine discs contacted with gauze moistened (pH 7.2) were kept at 37 °C for 7 days.

The push-out test was performed by an operator who is blinded to the experimental groups using a universal testing machine (Lloyd LRX; Lloyd Instruments Ltd., UK). A plunger tip of 0.6 mm diameter was used in a coronal-apical direction at a crosshead speed of 0.5 mm/min until dislocation. The adhesion surface area was determined through the following formula: $2\pi r \times h$. (r: radius of canal-like hole; h: height of the disc). Push-out bond strength (MPa) = maximum force (N)/adhesion surface area (mm^2).

Fractographic Analysis

All dentine discs were examined under a stereomicroscope at 40x magnification to assess mode of bond failure which were classified as follows: (i) adhesive failure occurred at the material-dentine interface, (ii) cohesive failure within the tested material and (iii) mixed failure.

Statistical Analysis

The data were evaluated with two-way analysis of variance and Tukey's post hoc test by using SPSS 20.0 software (IBM Corp, NY) to determine the effect of chelating solution, type of tested material and their interaction on push-out strength. The level of statistical significance was set at $p<0.05$.

Results

Two-way analysis revealed a significant difference for the type of solution ($p=0.001$); however, no significant differences were observed for the type of tested material ($p=0.153$) and the interaction between tested material and solution ($p=0.922$). MTA-HP group had slightly higher push-out strength values when compared to MTA-Ang group ($p>0.05$). Regardless of the type of the material used, significantly higher push-out values were obtained from group 4 (discs irrigated with distilled water; no chelating agent), compared with group 2 (discs irrigated with chitosan solution prepared with lactic acid) (Tukey, $p=0.000$) and group 3 (discs irrigated with EDTA) (Tukey, $p=0.012$). There was no statistically significant difference between group 1 and group 2 ($p=0.21$), group 1 and group 3 ($p=0.77$), group 1 and group 4 ($p=0.145$), group 2 and group 3 ($p=0.754$). Group 2 showed the least push-out bond strength. The percentages of the failure modes of the samples were presented in Table 1. Mixed failures were the most observed (Figure 1).

Discussion

This study was designed to assess whether the use of different chelating agents affect the bond strength of two different calcium silicate based materials. According to the results, first null hypothesis was accepted. All chelating agents were associated with a significant decrease in bond strength of tested materials except for chitosan solution prepared with acetic acid, necessitating the partially accepted of the second null hypothesis.

The present study utilized "intra-tooth model" for sample preparation. This model involves providing a horizontal or longitudinal dentine slice from a tooth and creating artificial canal-like holes in the same dentine slice in order to minimize the experimental confounding factors such as differences in root canal anatomy, tooth age, storage time, distribution of sclerotic dentine, and micro-hardness (10,11). Thus, a more reliable sample baseline could be established to make fair comparisons among materials and/or irrigation solutions (11).

The differences between push-out bond strength values of MTA-Ang and MTA-HP for all irrigation regimens were not statistically significant. This result can be attributed to the similarity of their chemical compositions. The only differences of MTA-HP from MTA-Ang are the addition of an organic plasticizer to its liquid and containing calcium tungstate as a radiopacifier. Addition of calcium tungstate instead of bismuth oxide as radiopacifier to calcium silicate based cements has no significant effect on physicochemical properties such as calcium ion release, solubility and pH; however, it associated with less discoloration potential on dental structures (12,13). Unlike the present results, Silva et al. (14) found significantly higher push-out bond strength values for MTA-HP when compared to MTA-Ang. Despite using push-out model similar to those utilized in the present study, there was variability in the irrigation sequence. The authors immersed dentine discs into the NaOCl after irrigation with EDTA. However, in our study, dentine discs were subjected to only distilled water after using chelating agents to eliminate possible effects of NaOCl on hydration process of MTA and organic structure of dentine. Because,

Table 1. Mean push-out bond strength values with standard deviations and failure modes of each material for each group

Groups (Solution)	N	Material	Mean (MPa)	Std. Deviation	Failure modes, % (A/C/M)
Group 1 (Chitosan dispersion mixed with asetic acid)	15	MTA Angelus	4.257	1.677	27/13/60
	15	MTA-HP Repair	4.722	1.953	27/7/67
	30	Total	4.49	1.59900	27/10/63
Group 2 (Chitosan dispersion mixed with lactic acid)	15	MTA Angelus	3.482	1.561	60/0/40
	15	MTA-HP Repair	3.635	1.953	47/0/53
	30	Total	3.5592	1.73897	53/0/47
Group 3 (EDTA)	15	MTA Angelus	3.625	1.766	27/7/67
	15	MTA-HP Repair	4.437	2.228	40/7/53
	30	Total	4.0313	2.01847	33/7/60
Group 4 (Distilled water)	15	MTA Angelus	5.259	1.578	27/13/60
	15	MTA-HP Repair	5.7613	2.258	40/7/53
	30	Total	5.5103	1.93152	33/10/57

EDTA: Ethylenediaminetetraacetic acid, MTA: Mineral trioxide aggregate, MPa: Mycophenolic acid, Std.: Standard

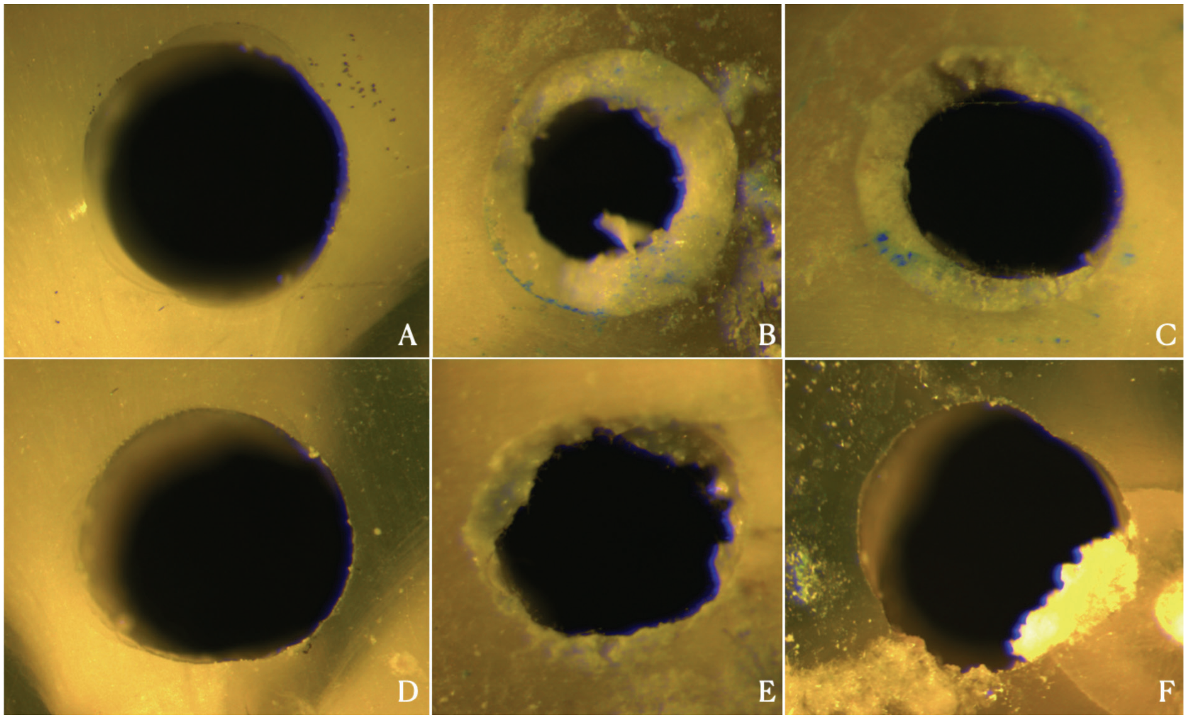

Figure 1. Representative stereomicroscopic images of the mode of bond failures. (A) Adhesive, (B) cohesive, and (C) mixed within MTA Angelus. (D) Adhesive, (E) cohesive, and (F) mixed within MTA Repair HP

MTA: Mineral trioxide aggregate, HP: High plasticity

NaOCl can alter the physical properties of MTA decreasing the portlandite peaks during the hydration and interact with bismuth oxide which forms a part of MTA (8,15). NaOCl can also lead to degrade the collagen fibrils before deposition of hydroxyapatite or carbonated apatite, which are the main components of biomineralization of MTA (16,17).

Fractographic analysis revealed that bond failures for MTA-Ang and MTA-HP were predominantly mixed type of failure followed by adhesive type of failure. The adhesive type of failure could be more observed as the bond strength decreased and this was corroborative the present push-out results. This mixed type of failure indicated that both materials generally presented cohesive and adhesive type of failures at the same time. Several factors such as storage time and particle size of the material were suggested as detrimental factors to determine the mode of bond failure during fractographic investigation. A smaller particle size and longer storage time have been associated with higher bond strength (9,16). However, the mean particle size of the MTA-HP and MTA-Ang was reported to be 11.20 μm and 15.48 μm, respectively, which are greater than the mean tubule diameter of root dentine (2.65-2.90 μm) (18,19). For this reason, the biomineralization capacity of both materials can be considered as a superior factor instead of their infiltration properties via their small particles in terms of attachment to dentin.

All chelating agents used in the present study showed similar effect on bond strength of MTA-Ang and MTA-HP. Similar to a previous study in which use of different chelating agents resulted in no alteration on bond strength of MTA, but lower push-out values were obtained compared to control group (8). Although, distilled water was used as a final rinse after all irrigation protocol to remove chelating solutions from canal-like holes, bond strength of the tested materials was varied according to the type of irrigation solution used. This result can be attributed to presence of trace amount of chelating agent on the dentinal walls or within the dentinal tubules. Additionally, particles of chitosan can deposit on the surface and predominantly within the dentinal tubules, presumably as a result of its highly charged molecular structure (9). Ozlek et al.'s (9) study has suggested that residue of chitosan particles may affect push-out bond strength of mineral trioxide aggregate-resin hybrid root canal sealer.

The hydration mechanism of MTA involves two stages: dissolution of MTA powder with releasing calcium ions into the environment and then, precipitation of the hydrated products. The use of acids during this process could disturb precipitation and crystallization of the hydrated compounds while chelating calcium ions released from MTA (6). Acidic pH could decrease its microhardness and bond strength to root dentine since the acids such as EDTA chelates calcium ions released from MTA (14). All chelating agents used in the present study had a pH level ranged from acidic to neutral and therefore both materials showed higher bond strength in dentine discs irrigated with distilled water solely.

Previous studies have been showed that the capacity of chitosan dispersions to remove calcium ions and smear layer from root canals as efficiently as EDTA is attributed to properties of chitosan and not the contribution of solvent (20). However, use of different solvents may have an effect on physicochemical properties of chitosan dispersions. In a study conducted by Soares et al. (21), chitosan dispersions were prepared with different acids such as lactic acid and acetic acid. Similar to our findings, chitosan dispersion prepared with lactic acid had lower pH level than those prepared with acetic acid (21). This minor change in pH levels of dispersions could be related to the lower push out values obtained from group 2 (chitosan mixed with lactic acid) in comparison with group 1 (chitosan mixed with acetic acid), despite that was not statistically significant. Thus, it could be suggested that the use of different acids in chitosan dispersions would not cause any change in terms of push-out values of tested materials. Additionally, acetic acid and lactic acid were not tested solely, because these solutions are not much preferred clinically.

From the clinical point of view, effect of the chelating agents on the long-term prognosis of endodontic treatments is still unknown, because the factors related to success cannot be isolated and various etiological factors play essential roles in development of post-operative apical pathology. Moreover, from the mechanical point of view, an endodontic material should have good retention properties under static conditions and during masticatory function. However, there are conflicting results regarding whether the smear layer should be removed or not for better mechanical retention (1). Especially for calcium silicate based cements material's adhesion to root dentine occurs as result of biomineralization through formation of tag-like structures into dentinal tubules and this process can be affected by environmental condition such as pH (6). In use of chelating agents, its possible effects on treatment outcome, physicochemical properties of material and material-dentine interactions should be considered. Rather than limiting the use of chelating agents, more studies need to be conducted seeking for alternative ways to buffer their relatively negative effects.

Conclusions

Both materials showed similar bond strength whether chelating agent was used or not. All chelating agents significantly decreased the push-out strength of both materials except for chitosan solution with acetic acid. The effect of chitosan dispersion prepared with lactic acid on push-out bond strength of both materials tested was similar to that of chitosan dispersion prepared with acetic acid. However, other materials can be effected differently by the irrigation sequence and type of irrigation solution and thus, further studies should assess the interaction of other materials with dentine irrigated with chitosan dispersions and characterize their effects on the tested materials.

Ethics

Ethics Committee Approval: The study was realized in accordance with Declaration of Helsinki and approval was obtained through the Local Ethics Committee at Ankara University (decision no: 36290600/02, date: 17.01.2020).

Informed Consent: This study does not require informed consent.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: C.K., K.G., N.D.K., Design: C.K., K.G., N.D.K., Data Collection or Processing: C.K., K.G., N.D.K., Analysis or Interpretation: C.K., K.G., Literature Search: C.K., Writing: C.K., K.G.

Conflict of Interest: No conflict of interest was declared by the authors.

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