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Effects of Chitosan on Micro-Shear Bond Strength of Self-Adhesive Cements

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Abstract

Background: The long-term success of bonding of self-adhesive cement to the tooth is questionable. Different conditioning methods were suggested to improve the bonding durability of these cements. This study aimed to investigate the effect of 1% chitosan solution on micro-shear bond strength of self-adhesive resin cements to dentin.

Methods: 28 human sound premolar teeth were divided into 4 groups. The occlusal surfaces of the teeth were ground to expose the dentine surface. In control groups, BF (Bifix) and EB (Embrace) dual-cure self-adhesive cements were applied to the dentin surface using a microtube and were light cured. In the EB+Ch and BF+Ch test groups, dentin surfaces were conditioned with 1% chitosan solution before using resin cements. The specimens were thermocycled for 2000 times between 5-55*°C* and their bond strength was measured by a mechanical testing machine at strain rate of 0.5 *mm*. Failure modes were determined using a stereo-microscope at 40X magnification. Data were analyzed using Two-Way ANOVA and Tukey's tests ($\alpha=0.05$).

Results: The results have revealed maximum bond strength in the EB+Ch test group (13.73±0.42 Mpa) and the minimum strength in the BF control group (8.01±0.42 Mpa). Data analysis showed the maximum bond strength in groups where dentin had been pretreated with chitosan solution ($p<0.001$). The failure mode was mainly adhesive type in all groups.

Conclusion: Dentin surface treated with 1% chitosan solution prior to application of self-adhesive cements increases the cement-dentin bond strength.

Keywords: Adhesive cement, Chitosan, Dentin, Resin cements

Introduction

Today, resin cements are widely used in bonding inlays and onlays, veneers, root posts, and full crown coverings (1). Recently, to simplify the cementation processes, self-adhesive resin cement has been marketed. These cements have monomers that are capable of etching and bonding to the tooth surface, without the need for separate use of an adhesive. The use of self-adhesive cements makes it easier to bond indirect restorations to the tooth surface, shorter chairside time and reduces the thickness of the cement layer between restoration and tooth (2,3). The disadvantages of self-adhesive cements can be tooth sensitivity after treatment, low bond strength with dental structure and microleakage, and reducing longevity of the restoration (4-6).

Self-adhesive resin cements contain acidic group monomers that can remove or modify the smear layer and penetrate to dentin under the smear layer and obtain a micro-mechanical interlocking with dentin surfaces (7,8).

Self-adhesive resin cements were bonded to the tooth structure by changing the smear layer and penetrating the underlying dentin and forming a hybrid layer. The smear layer can act as a barrier to the penetration of the resin into the dentin, resulting in poor physical bonding between the dental substrate and the resin (9). Self-adhesive cements demineralize the area to a certain extent depending on the thickness of the surface smear layer and penetrate the dentin, but the amount of acid monomers varies in different compositions, and ultimately, weak bond strength is observed (10).

Different conditioning methods have been proposed to increase the bond strength of self-adhesive cements to dentin (11,12). Mechanical, chemical and physical surface treatment including air abrasion, treatment with pumice, EDTA, CHX, chitosan, and various lasers are used to treat dentin (12–16). It has been reported that the use of chitosan as a chemical chelating agent with smear layer removal can increase bond strength (17,18).

It has been suggested that the use of chitosan solution prior to self-adhesive cement will increase adhesion strength to dentin. However, some previous studies have reported that the use of chitosan with self-adhesive bonding systems has no effect on bond strength (19–22).

One of the advantages of this biocompatible conditioner material is its non-toxic and antibacterial properties compared to other chelators (9,23–25). However, there are not many studies available regarding the use of chitosan solution to increase the bond strength of self-adhesive cements to dentin, and a specific concentration of chitosan solution to achieve optimal bond strength has not yet been determined.

This study aimed to evaluate the effect of 1% chitosan solution on bond strength, prior to the application of self-adhesive cements to dentin. According to the null hypothesis using of chitosan solution for 60 *s* does not influence the bond strength of cement to dentin.

Materials and Methods

This *in vitro* experimental study was conducted on 28 premolar human teeth that had been extracted due to orthodontics problems to three months ago. Teeth with cracks, caries, and restorations were excluded and replaced with sound teeth. Then, they were cleaned with a prophylactic brush and a mixture of water and pumice paste and immersed in 1% chloramine solution. A week before the test, the teeth were placed in water at room temperature. 5 to 6 mm^2 of coronal dentin was exposed by horizontally trimming the occlusal surface of each tooth crown with an orthodontic trimmer under running water without involving the pulp horns. After trimming, the resulting surfaces were flattened and finished using 600-grit abrasive papers (softflex A, Matador wet sandpaper, Germany). All the specimens were randomly divided into four groups (two pretreatment groups and two no-pretreatment groups (control) with 7 teeth in each group. On the dentin surface of each tooth, 2-3 cylindrical specimens of resin cements of 0.75 *mm* diameter and 1 *mm* length were bonded in the manner described below. Thus, each group contained 18 specimens (N=18) of cylindrical resin cement on dentin.

Control group BF: Bifix cement (Voco; Cuxhaven, Germany) was mixed by using an auto mixing tip according to the manufacturer's instructions, and then a plastic microtube (Tygon tube) with a diameter of 0.75 *mm* and a height of 1 *mm* was placed on the dentin surface and filled with resin cement. The resin composite was polymerized for 40 *s* by using a light curing unit (Demi Plus, Kerr Corp, Orange, CA, USA) at an intensity of 800 mW/cm^2. The intensity of the device was measured with a radiometer.

The control group EB: Embrace cement (Pulpdent; Watertown, MA, USA) was placed on the tooth surface and cured according to the manufacturer's instructions and the BF group method.

In the specimens of EB+Ch and BF+Ch test groups, 1% chitosan solution was applied to pretreat the dentin surface. For preparation of 1% chitosan solution, 1 *g* of chitosan powder (Sigma Aldrich, Saint Louis, MO, USA) was dissolved in 100 *ml* of acetic acid 1% using a magnetic stirrer at room temperature. According to previous studies, 1% acetic acid was used to activate chitosan in solution (9,26). To homogenize the solution, it was agitated with a magnetic stirrer for two *hr*. A 1% Chitosan solution was applied to the dentin surface for one *min* with a microbrush and the excessive solution was removed from the dentin surface using an air-water spray. Then, the dentin surface was dried with gentle air spray for 10 *s*. The other steps for the preparation of the specimens in test groups were similar to control groups.

The specimens were thermocycled for 2000 cycles and stored in water at 37*°C* for 72 *hr*. Each thermal cycle included immersion in cold and hot baths at 5-55*°C* for 20 *s* and at room temperature for 10 *s*. After completion of the thermocycling, a micro-tensile tester machine (SMT-20, SANTAM, Tehran, Iran) was used to measure the micro-shear bond strength of resin cement to dentin. The specimens were fixed to the jaw of the testing machine. A fine brass wire with 0.2 *mm* diameter was looped around each cement and cylindrical metal. The specimens were subjected to shear stress at a crosshead speed of 0.5 *mm/min* until fracture. The micro-shear bond strength of each specimen was recorded in MPa.

The normal distribution of the data was tested using the Shapiro-Wilk test, and the data were analyzed using a two-way ANOVA and Turkey's multiple comparisons at the 95% level of confidence by using SPSS software (Version 26.0, IBM Corp, Armonk, NY, USA). The significance level was set at p=0.05.

The fractured surfaces were evaluated under a stereomicroscope (ILLB100-Olympus Optical Co. Ltd., Tokyo, Japan) at ×40 magnification to determine the mode of failure (adhesive, cohesive, and mixed) by one operator. The mode of fracture was reported.

Results

The results from the study of micro-shear bond strength of self-adhesive cements before and after the application of chitosan are shown in table 1. The highest bond strength was found in the EB+Ch group (13.73±0.42 Mpa) and the lowest bond strength was in the BF control group (8.01±0.42 Mpa). The normality of the obtained data was tested using the Shapiro-Wilk test. The results indicated that the data followed a normal distribution (p>0.05).

Comparison of the groups using two-way ANOVA showed that applying chitosan solution before the application of adhesive resin cement (p<0.001) as well as the brand of used adhesive resin cement (p<0.001) had a significant effect on the bond strength of self-adhesive resin cement to dentin. As shown in figure 1, two-way ANOVA revealed that the interaction between two factors of chitosan solution and cement

Table 1. The comparison of micro-shear bond strength and mode of failure of self-adhesive resin cements to dentin in the studied groups (Mpa)

Cement	Chitosan	Mean±SD	Lower band	Upper band	p-value *	Failure mode A/M/CR/CD
Embrace	No b	8.87±1.7	8.019	9.735	p<0.001	12/5/1/0
	Yes	12.87±2.0	12.014	13.730		10/7/1/0
Bifix	No	6.51±1.4	5.624	7.404	p<0.001	11/5/2/0
	Yes b	10.91±0.9	10.055	11.771		10/6/2/0

SD: Standard Deviation; * two-way ANOVA; Groups specified with lowercase letters indicate no significant difference based on Tukey's test (p>0.05). A: Adhesive; M: Mixed; CR: Cohesive in Resin composite; CD: Cohesive in Dentin.

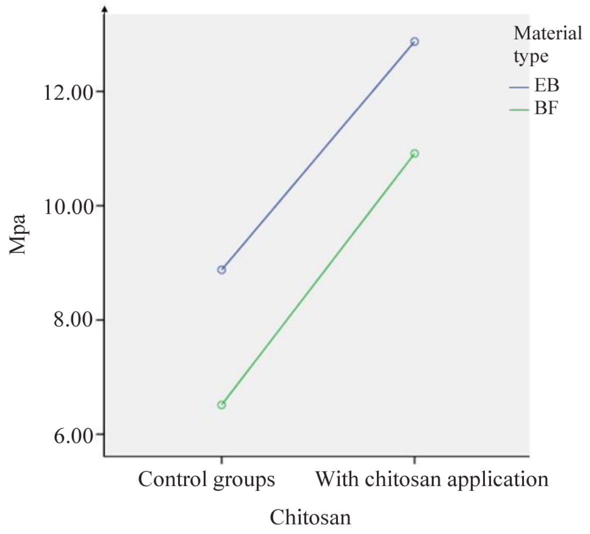

Figure 1. Comparison of the micro-shear bond strength of self-adhesive resin cements to dentin in the studied groups.

brand had no significant effect (p=0.642). The results of the comparison of two groups with complementary Tukey's HSD test are shown in table 1.

Failure types were predominantly adhesive in all groups, and in the groups with chitosan, the number of mixed-type failures was increased.

Discussion

The clinical success of indirect restorations depends on the adhesion strength and durability of the bonding of resin cements to the teeth (27,28). To enhance the bond strength and adhesion sustainability of self-adhesive cements to dentin, different conditioning methods were suggested (29,30). This study was conducted in order to investigate the effect of 1% chitosan solution on the micro-shear bond strength of adhesive resin cements to dentin.

The results of this study demonstrated that the use of 1% chitosan solution for 1 *min* before the application of cement significantly increased the micro-shear bond strength of self-adhesive cements. Therefore, the null hypothesis of the study was refuted. Most previous studies confirmed the results of the current study (9,16,31,32) while in some other studies, it has been stated that the use of chitosan solution does not affect the change in bond strength of cement to dentin (10,19-22).

Chitosan is a natural polysaccharide obtained from the deacetylation of chitin in shrimp or crab shells. Recently, chitosan has attracted attention in dental research owing to its biodegradability, bioadhesion, biocompatibility, antibacterial properties, and lack of toxicity. Chitosan is similar to EDTA with two nitrogen atoms and a pair of free electrons, responsible for ionic changes and the mechanism of chelation with calcium. Chitosan has hydrogen and hydroxyl group bridges in the polymer chain, which can be bonded with a covalent bond to substrates containing calcium, such as dentin, by chelating. With this mechanism, chitosan is combined with existing calcium ions, which form a complex that precipitates on the surface of the dentin. This complex increases the surface energy of the dentin substrate, increases the wettability, and ultimately the bond strength of the resin cement to dentin (22,32).

Some previous studies have shown that, due to an acidic pH and the presence of amino acids (-NH3) in the bipolymer, chitosan can reduce minerals substances and the thickness of the smear layer (33). The use of chitosan solution on the dentin surface by removing the smear layer and surface demineralization can increase the penetration of resin monomers and improve the quality of the hybrid layer by self-adhesive resin cements (23,34,35).

In addition, regarding the effect of chitosan solution on increasing the bond strength of cement to dentin, some studies have reported that chitosan can be effective on cross-linking of collagen fibers and increasing bonding durability to dentin (31,36,37). Cross-linking and collagen reinforcement by the combination of chitosan biopolymers improves the biological and mechanical properties of collagen fibers and the quality of the hybrid layer (37,38).

Some studies reported that the dentinal fluid in dentin acts as an oxide and oxygen-rich reservoir, which may interfere with the resin bonding to dentin and prevent polymerization of the resin. Chitosan exhibits potent antioxidant properties due to its unique molecular structure, particularly the presence of hydroxyl and amino groups that allow it to scavenge free radicals through hydrogen donations and electron pair-sharing antioxidant and matrix-preserving effects make it a valuable material for enhancing the durability of dental restorations by inhibiting oxidative damage and degradation at the dentin-adhesive interface (39–41).

Saker *et al* in 2016 studied about effectiveness of

EDTA, PAA, and chitosan on two self-adhesive cements types. It was reported that chitosan solution can remove the smear layer and improve the bond strength similar to other chelators (42). It was revealed that the effect of chitosan on bond strength to dentin depends on its concentration (22,43).

In a study conducted by Akha *et al* in 2020, the impact of different pretreatments on the microtensile bond strength of universal adhesives in the self-etch mode was investigated. Their findings indicated that 1% nano-chitosan pretreatment had no significant effect on bond strength (19). Considering that universal adhesive systems usually have low pH and viscosity and exhibit superior capabilities in dissolving the smear layer and infiltrating the underlying dentin compared to self-adhesive cements, this factor may account for some of the differences observed in our studies. The chelating effect of chitosan can be effectively employed when using self-adhesive cements to modulate the smear layer, enhance dentin penetration, and facilitate the formation of a stable hybrid layer.

Study results of Elska *et al* in 2012 (22) and Diolosa *et al* in 2014 (20) also differed from findings of the current study. These researchers used adhesive systems incorporated with chitosan at various concentrations. Their methodology and direct incorporation of chitosan into adhesive formulations led to results that differed from those of the present study.

Despite the results obtained in this study on the positive effect of chitosan on self-adhesive cements, it may suggest that more laboratory and clinical studies be conducted with different formulations of this substance.

Conclusion

Within the limitations of the study, it was shown that dentin substrate pretreatment with chitosan solution 1% prior to application of self-adhesive resin cements would significantly improve the micro-shear bond strength to dentin.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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