The effect of dentin desensitizers and Nd:YAG laser pre-treatment on microtensile bond strength of self-adhesive resin cement to dentin

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PURPOSE. The purpose of this study is to evaluate if pre-treatment with desensitizers have a negative effect on microtensile bond strength before cementing a restoration using recently introduced self-adhesive resin cement to dentin. MATERIALS AND METHODS. Thirty-five human molars’ occlusal surfaces were ground to expose dentin; and were randomly grouped as (n=5); 1) Gluma-(Glutaraldehyde/HEMA) 2) Aqua-Prep F-(Fluoride), 3) Bisblock-(Oxalate), 4) Cervitec Plus-(Clorhexidine), 5) Smart protect-(Triclosan), 6) Nd:YAG laser, 7) No treatment (control). After applying the selected agent, RelyX U200 self-adhesive resin cement was used to bond composite resin blocks to dentin. All groups were subjected to thermocycling for 1000 cycles between 5-55°C. Each bonded specimen was sectioned to microbars (6 mm × 1 mm × 1 mm) (n=20). Specimens were submitted to microtensile bond strength test at a crosshead speed of 0.5 mm/min. Kolmogorov-Smirnov, Levene’s test, Kruskal–Wallis One-way Analysis of Variance, and Conover’s nonparametric statistical analysis were used (P<.05).

RESULTS. Gluma, Smart Protect and Nd:YAG laser treatments showed comparable microtensile bond strengths compared with the control group (P>.05). The microtensile bond strengths of Aqua-Prep F, and Cervitec Plus were similar to each other but significantly lower than the control group (P<.05). Bisblock showed the lowest microtensile bond strength among all groups (P<.001). Most groups showed adhesive failure.

CONCLUSION. Within the limitation of this study, it is not recommended to use Aqua-prep F, Cervitec Plus and Bisblock on dentin when used with a self-adhesive resin cement due to the decrease they cause in bond strength. Beside, pre-treatment of dentin with Gluma, Smart protect, and Nd:YAG laser do not have a negative effect. [J Adv Prosthodont 2014;6:88-95]
tubules. It reacts with the serum albumin in the dentinal fluid, causing its precipitation. HEMA forms deep resinous tags and occludes the dentinal tubules. Oxalate, which is another desensitizing agent; reacts with calcium ions of liberated dentin and forms calcium oxalate crystals. These oxalate crystals block dentinal tubules. Similarly fluorides decrease the dentin permeability by precipitation of calcium fluoride crystals inside the dentin tubules. Yet other desensitizing agents nowadays in use are the chlorhexidine and triclosan containing ones. Chlorhexidine, due to its antibacterial action, has been used as a cavity disinfectant and triclosan is an anti-inflammatory agent.

Another desensitizing treatment for dentin hypersensitivity is laser applications. The lasers used for the treatment of dentin hypersensitivity are divided into two groups; these are either low or middle output power lasers. For the treatment of dentin hypersensitivity are divided into two groups; these are either low or middle output power lasers. For the latter; carbon dioxide laser (CO₂), neodymium- or erbium-doped yttrium aluminum garnet lasers (Nd:YAG, Er:YAG lasers) and erbium, chromium doped: yttrium, scandium, gallium and garnet (Er,Cr:YSGG) lasers are classified. Nd:YAG, Er, Cr: YSGG, and CO₂ lasers can occlude dentinal tubules partially or totally, through their ability to melt peritubular dentin and therefore reduce patients’ hypersensitivity symptoms. The mechanism of Nd:YAG laser effect on dentin hypersensitivity is also by direct nerve analgesia. It has been noted that this type of laser is more effective than the Er:YAG and CO₂ lasers.

Self-adhesive resin cements have been recently introduced to simplify the technique sensitive pretreatment steps and to prevent application errors of cementation procedures in fixed prosthodontics. These partially hydrophilic cements are used without any pretreatment, and results in literature stating their good bond strength to dentin were reported. The adhesion obtained is claimed to rely on micromechanical retention and chemical interaction between monomer acidic groups and hydroxyapatite. These cements’ initial property of hydrophilicity and moisture tolerance provide improved adaptation to the tooth structure.

However in clinical practice, desensitizing agents are frequently used with resin cementation of fixed partial dentures. Although desensitizers are helpful by reducing the patient’s discomfort, their effects on bonding performance of adhesive cementation to dentin cannot be ignored. There is lack of literature reporting the bond strengths of different kinds of desensitizers when used with the newly developed self-adhesive resin cement. Therefore, the present study aimed to compare the effect of five major chemically different groups of desensitizing agents and Nd:YAG laser application on microtensile bond strength (MTBS) between tooth and a recently introduced self-adhesive resin cement. The null-hypothesis was that pretreatment of dentin with different types of desensitizers and Nd:YAG laser application has no effect on bond strengths.

**MATERIALS AND METHODS**

Dentin desensitizing agents and methods used in the study are shown on Table 1. Dentin surface with no treatment acted as the control group.

The project was approved by the Institutional Review Board of Baskent University, Ankara, Turkey (D-DA14/02). To determine MTBS, 35 caries-free human molar teeth were used. The teeth were stored in distilled water with 0.1% thymol solution at 4ºC for a maximum of 6 months. The root surfaces were cleaned from tissue residue with a scaler. All teeth were then embedded in custom made rectangular molds using autopolymerizing acrylic resin (Steady-Resin; Scheu-Dental GmbH, Iserlohn, Germany) exposing the teeth 3 mm from the acrylic resin surface. The specimens were then grinded until the dentin surfaces were exposed with a coarse grit diamond rotary cutting instrument (6856 L-016 Gebr. Brassler GmbH & Co. KG, Leimog, Germany) under water. To standardize the dentin surface texture, the occlusal surface of each tooth was ground off in a mechanical grinder (MetkonGripo 2V Grinder Polisher, Metkon Instruments Ltd, Bursa, Turkey) with 600-grit silicon carbide abrasive paper under running water for 30 sec. The exposed dentin surfaces were inspected under an optic microscope (M3M, Wild, Heerbrugg, Switzerland) to ensure that no enamel was left. All specimens were left in distilled water until bonding application to moist dentin surfaces. The specimens were randomly divided into 7 groups.

Composite resin (Filtek Z250; 3M ESPE; St Paul, MN, USA) rectangular blocks (10 mm in length × 3 mm in thickness × 5 mm in width) were prepared in plastic molds and light cured with an light emitting diode divice (Hilux LEDMAX-550, Benlioglu Dental Ankara, Turkey) at 60 seconds at 4 different locations with an intensity of 1000 mw/cm². The dentin specimens were secured in a custom-made metallic holding tool to keep the bonding surfaces parallel to the bench. Desensitizer agent was applied to dentin surfaces according to the manufacturer’s introductions (Table 1). Resin blocks were cemented with RelyX U200 self-adhesive resin cement (3M ESPE; St. Paul, MN, USA) on the dentin surfaces and loaded by custom-made metallic holding devices, which carried out axial constant load of 2 kg for 60 seconds to standardize the bonding pressure. Excess cement was removed, and the bonded specimens were polymerized at 4 different locations of 60 seconds each.

All groups were subjected to thermocycling for 1000 cycles between 5-55°C with a dwelling time of 20 seconds in each bath and transferring time of 10 seconds according to the standards of the International Organization for Standardization (ISO).

Each bonded specimen was sectioned into microbars (6 mm × 1 mm × 1 mm) using a micromet (Metkon Micromet 175 Precision Cutter, Metkon Instruments Ltd, Bursa, Turkey). The microbars were inspected under a stereomicroscope (Leica mz 21, Bensheim, Germany) and intact 20
microbars were selected for each group for MTBS tests. Each microbar was bonded to the attachment jaw using cyanoacrylate adhesive system (502 Super Glue, Evabond Group, New Taipei City, Taiwan) Special care was shown to center the composite-dentin interface at the free space between the jaws of the attachment unit. A crosshead speed of 0.5 mm/min was applied using microtensile tester machine (Bisco, Schaumburg, IL, USA) and load at failure was recorded for each specimen. MTBS values were calculated as force to failure (N)/ bonding area (mm²).26

Failure modes were recorded by one operator under an optical microscope (M3M, Wild, Heerbrugg, Switzerland) at a magnification of ×25. These modes were classified as: adhesive failure, no cement remnants left on dentin surface; mixed failure, cement remnants partially left on dentin exposed and cohesive failure, dentin surface completely covered with cement or failure in dentin tissue itself.

Data analysis was performed by using the statistical software (Statistical Program for Social Sciences, ver 11.5; SPSS, Inc., Chicago, IL, USA). Whether the distributions of continuous variables were normal or not was determined by using the Kolmogorov-Smirnov test. Homogeneity of variance was evaluated with the Levene test. Kruskal–Wallis One-way Analysis of Variance was used to confirm the difference among the groups at a \( P = .05 \) significance level. When the \( P \) value of the Kruskal-Wallis was significant, to determine which group differed from which other, the post hoc Conover’s nonparametric statistical analyses was used.

A total specimen size of 133 (n=19) was required to detect a microtensile bond strength difference of at least 2.17 MPa between the 2 test material groups, and with a power of 90% at the 5% significance level. The 2.17 MPa value difference was taken from the study of Akca et al.27 Specimen size estimation was performed by using software (NCSS and PASS 2000; NCSS, Inc., Kaysville, UT, USA).

Table 1. The brand names, batch numbers, chemical compositions, application steps and manufacturers of the materials used in the study

| Brand Name (Batch No.) | Chemical composition | Application steps as recommended by the manufacturer | Manufacturer |
|------------------------|----------------------|------------------------------------------------------|--------------|
| Gluma 10096            | Glutaraldehyde (5%) distilled water HEMA (35%) | • Apply on dried dentin and leave for 30 to 60 sec. • Apply air until the fluid film has disappeared. • Rinse with water. | Heraeus Kulzer, Hanau, Germany |
| Aqua-Prep F 1100010772 | HEMA (<20%) Sodium fluoride (<3%) | • Etch dentin for 15 sec with 32% phosphoric acid. • Rinse for 5 sec, air dry for 2-4 sec, do not desiccate. • Apply on dried dentin and leave for 20 sec. • Excess was removed with 5 sec light air-drying until a shiny surface | Bisco Inc., Schaumburg, IL, USA |
| Bisblock 1000012460    | Oxalic Acid (<5%) | • Etch the tooth for 15 sec, and rinse with water. • Gently air dry 2-3 sec. • Apply on dried dentin and leave for 30 sec. • Rinse with water. | Bisco Inc., Schaumburg, IL, USA |
| Cervitec Plus 123      | Ethanol, water, acrylate copolymer, vinylacetate copolymer and chlorhexedinediacetate, thymol | • Apply on dried dentin and leave for 30 sec. • Do not rinse the tooth surface. | Ivoclar Vivadent AG, FL-9494 Schaan, Liechtenstein |
| Smart protect 140201   | Glutaraldehyde, triclosan, olaffur, 2- propanol (HEMA free) | • Apply on dried dentin and massage in for 10 sec. • Allow to take effect for a further 20 sec. • Do not rinse the tooth surface. | Detax, GmbH & Co. KG, Ettlingen, Germany |
| Nd:YAG laser N/A       | The dentin surface was irradiated with a pulse 10 Hz-1 W, with a total irradiation time of 60 sec to simulate clinical manipulation. | Fotona Fidelis Plus II, Fotona d.d., Ljubljana, Slovenia |
| RelyX U200             | Base paste: Methacrylate monomers containing phosphoric acid groups, methacrylate monomers, silanated fillers, initiator components, stabilizers rheological additives Catalyst paste: Methacrylate monomers, alkaline (basic) fillers, silanated fillers initiator components, stabilizers, pigments, rheological additives | Dose the two pastes simultaneously in 1:1 ratio from the clicker, mix the catalyst and base, apply on dentin | 3M ESPE; St. Paul, MN, USA |
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RESULTS

The MTBS values are presented in Fig. 1 and at Table 2. The horizontal lines in the middle of each box indicates the median microtensile bond strength, while the top and bottom borders of the box mark the 25th and 75th percentiles, respectively. The whiskers above and below the box mark indicate the maximum and minimum microtensile bond strength levels. The experimental groups Gluma, Smart Protect, and Nd:YAG Laser showed comparable results with the control group (P>.05). Although there was no statistically significant difference between Gluma and the control group, Gluma showed higher MTBS values. The experimental test groups treated with Aquaprep F (P=.006), Bisblock (P<.001) and Cervitec Plus (P=.020) showed significantly lower MTBS values in comparison to the control group. The experimental test group treated with Bisblock showed the least MTBS among all tested groups (P<.001).

Adhesive failures were predominant for all of the groups. 85% of the control group specimens showed adhesive failure, and 15% showed mixed failure. For the Gluma group, 80% of the failures were adhesive, and 20% mixed. For the Cervitec Plus group, 80% of the failures were adhesive, and 20% mixed. 95% of Aquaprep F group specimens showed adhesive failure, and cohesive failure was found in 5% of the specimens. Nd:YAG Laser and Bisblock group specimens showed all adhesive failures.

DISCUSSION

In the present study, Gluma, Smart Protect and Nd:YAG laser did not affect the MTBS, whereas Cervitec Plus, Aquaprep F and Bisblock decreased the MTBS of self-adhesive resin cement to dentin. Therefore, the null hypothesis was partially rejected.

A limited number of studies have evaluated the impact of Gluma on the self-adhesive resin-dentin interface.23, 28-30 Sailer et al.29 showed that glutaraldehyde-containing dentin desensitizer and bonding agent (Gluma and Syntac) tended to positively influence the shear bond strength of the self-adhesive resin cement (RelyX U200) to dentin. Additionally, Sailer et al.28 showed statistically increased shear bond strength values when glutaraldehyde/HEMA containing desensitizer was applied before self-adhesive cement luting. Moreover, Stawarczyk et al.23 showed increased shear bond strength values even after aging procedures and also it has been revealed that Gluma had a positive effect on the tensile bond strength of self adhesive resin cements even with chewing simulations.9 These results are in agreement with the findings of the current study. Although pretreatment of dentin surface with Gluma tends to increase MTBS of self-adhesive resin cement to dentin, the difference was not statistically significant in the present study. Previously, it has been revealed that the bond strength of resin cement was highly dependent on the HEMA concentration, with a maximum at 35%, and nearly independent of the glutaraldehyde concentration when greater than 3%.31 Additionally Qin et al.32 showed that glutaraldehyde in Gluma cannot cross-link mineralized dentin. Actually; HEMA depresses the surface tension of water and enhances monomer diffusion into dentin.33 Arrais et al.34 showed the presence of a thin layer of resinous structure which was penetrating and occluding dentinal tubules. Moreover, they did not show the septa formation in tubule lumen in contrast to another study.35 Apart from this, glutaraldehyde/HEMA products

Table 2. Microtensile bond strength values (MPa)

| Groups       | Microtensile bond strength Mean (min-max) |
|--------------|------------------------------------------|
| Control      | 7.79 (5.38-10.85)AB                      |
| Gluma        | 8.75 (7.06-11.80)A                       |
| Aquaprep F   | 5.50 (4.36-6.28)C                        |
| Bisblock     | 2.40 (2.24-2.58)D                        |
| Cervitec Plus| 5.57 (4.38-6.70)CE                       |
| Smart Protect| 6.54 (5.58-8.95)BE                      |
| Laser        | 6.68 (4.46-7.71)BC                      |
| Test statistic| $\chi^2 = 67.775$                       |
| P-value      | <.001                                    |

The same upper cases indicate no statistically significant difference between groups (P>.05).
also contain water; therefore, they may act as rewetting agents. Although there is scarce information on the effect of glutaraldehyde/HEMA on the smear layer and bonding capacity of the self-adhesive resin cement, HEMA might be responsible for increased bond strength. On the other hand, Stawaczky et al.35 stated that a reaction between glutaraldehyde and phosphate may lead to very strong and stable bonding of Gluma desensitizer in combination with self adhesive resin cement. 

In the current study, pretreatment of dentin surface with AquaPrep F decreased the MTBS of self-adhesive resin cement. Dündar et al.36 reported increased shear bond strength values with 2 different brands of adhesive cement, when the same desensitizing agent was used. The researchers have attributed the increased bond strength values to HEMA induced rehydration mechanism allowing time for the penetration of the primer into dentin.36 This finding is contrary to the present study, the differences were in the resin cement types used and the primer used in the mentioned study.36 According to manufacturer’s instruction AquaPrep F applied after 15 seconds etching with 32% phosphoric acid, and contains HEMA-including hydrophilic monomers intend to rehydrate the collapsed collagen matrix caused by air drying. Therefore, it is hard to explain the decreased MTBS value for this desensitizing agent. One possible reason may be the precipitates occluding dentinal tubules, and also funnel shaped dentinal tubules which have been showed by Arrais et al.34 They determined the function of fluoride introduced to the desensitizer as obstruction of dentinal tubules.34

Previous studies document that as a result of the reaction between potassium oxalate and ionized calcium in dentin or dentinal fluid, calcium oxalate crystals form. These crystals, including the tubule orifice, cover the dentin surface thoroughly, therefore; adhesive resins do not bond well to oxalate-treated dentin.37,38 In the present study, Bisblock was applied after 15 seconds of acid etching according to the manufacturer’s instructions. According to Ashley et al.39, potassium oxalate gel application on etched dentin caused the crystal formation inside the tubules rather than on the surface and it is also stated that the crystal formation inside the tubules did not compromise the formation of typical hybrid layer. Tay et al.40 showed that when oxalates were used after acid-etching, MTBS values were comparable to the non-treated dentin as well. However in the present study, Bisblock decreased the MTBS of self-adhesive resin cement to dentin. The current study confirms the need for cleaning the surface of all calcium oxalate crystal as also stated by Aranha et al.41 On the other hand, etching dentin surface after oxalate application to deplete the crystals from the surface as suggested in a previous study,39 might hinder the formation of sufficient hybrid layer for bonding self-adhesive resin cement to dentin. However; it has been shown that phosphoric acid treatment before self-adhesive cementation may have comparable or increased bond strength results over non treated dentin.16,42

The present study also evaluated the bond strength of desensitizing agents containing antibacterial and anti-inflammatory agents. Smart Protect showed comparable MTBS values with the control group. Although triclosan may create low surface free energy and therefore impair the adhesion of resin-based cements due to reduced wettability43 and glutaraldehyde cannot crosslink dentin, the application of this luting cement did not compromise bond strength to dentin. On the other hand, in a previous study,43 reaction between glutaraldehyde and phosphate has been proposed to be responsible for the enhanced shear bond strength values of self-adhesive resin cement to dentin. Similarly, it has been shown that triclosan containing desensitizing agent did not influence the bonding strength of adhesive cementation; however the adhesive cement of the previous study was used with the primer of the system.36

In a recent study45 using chlorhexidine digluconate as additional primer with acid-etched resin-bonded dentin has been supported, and also; it has been declared that chlorhexidine may partially reduce the degradation of the resin-dentin bonds, when incorporated into hydrophilic dental adhesives. Additionally Santos et al.46 showed that the use of chlorhexidine digluconate as a dentin-cleaning agent has comparable results with non-treated dentin on bond strengths of self-adhesive resin cement, and Lin et al.47 showed improvement on the durability of the bond to dentin when chlorhexidine was applied before self-adhesive cementation. On the other hand pretreatment of dentin surface with varying concentrations of chlorhexidine (0.2%, 2.0%) decreased MTBS of self adhesive resin cement in previous studies.24,26 The current study revealed the negative influence of Cervitec Plus pretreatment on MTBS of this acidic and hydrophilic, new category of resin cement. The previously mentioned study26 speculated that the precipitates originated from chlorhexidine pretreatment might act as a barrier, limiting the resin cement’s interaction with the surface, diminishing the potential for bonding. In the light of these findings,26 it is possible to explain the decreased MTBS when Cervitec Plus desensitizing agent was used.

Another desensitizing treatment evaluated in the present study was Nd:YAG laser. When this type of laser irradiation is used, reduction or complete obliteration of the dentinal tubule lumen46 and closure of exposed dentin tubules47 have been shown in many of scanning electron microscope inspections. Generally, decreased bond strength values were reported when self-etch, and etch-and-rinse systems were used after Nd:YAG laser treatment.27,48 Reduced bond strength values might be due to tubule obliteration, when self-etching primer was used on the interface of dentin and composite resin,27 because closed tubules may hinder the penetration of resin monomer and resin tag formation may not occur. In the current study Nd:YAG laser irradiation (1 W, 10 Hz) did not influence the MTBS values.

A predominance of interfacial debonding between dentin and adhesive cement was noted for both control and dentin desensitized groups in the present study. Additionally it has been revealed that self-adhesive resin cements with or
without desensitizer presented mainly adhesive failures after water storage and entirely adhesive failure after thermocycling. Similarly Hitz et al. showed only adhesive failure of specimens, where self-adhesive resin cement was directly applied on dentin surfaces. However, Di Hipólito et al. showed predominance of cohesive failure when self-adhesive resin cement bonded to smear layer covered dentin, and adhesive failure for chlorhexidine pretreated dentin, on specimens not subjected to thermocycling. In a similar way, results of Sailer et al. revealed cohesive failure alone for freshly ground dentin and Gluma pretreated dentin when self-adhesive resin cement was applied, without the thermocycling process. Hitz et al. showed the negative impact of thermocycling on bond strength of dentin and self-adhesive resin cement interface. Contrary to these findings, Sailer et al. showed predominantly cohesive failure despite the absence of the thermocycling process; indicating the cement itself was the weakest link and Stawarczyk et al. also showed cohesive failure before and after aging procedures.

Adhesive cementation may be more technique sensitive than conventional cementation and the clinical success may be compromised by the technical challenges imposed on the dentist. These drawbacks were resolved with the introduction of self-adhesive resin cements. RelyX U200 used in the presented study is newly introduced self-adhesive resin cement with an additional monomer and a new rheology modifier added to the mixture with the processing of the filler particles optimized, which led to a formulation with increased mechanical properties.

Although this study was performed under in vitro conditions, the results provide guidance for clinical trials. The differences among materials and methods used make the results difficult to compare. According to the authors’ opinion, different study protocols may hence cause conflicting results. In the presented study, effects of 5 different desensitizing agents (of all kinds of contents in the market today) and Nd:YAG laser pretreatment on the bonding compatibility of newly introduced self-adhesive resin cement were compared. Hence, this protocol is believed to make a more conclusive decision in which desensitizing material to use when necessary with the new generation self-adhesive resin cements. Also, to the authors’ knowledge, there is no study conducted with all kinds of desensitizing agents on this newly developed cement.

On the other hand, the present study did not simulate pulpal pressure and dentinal fluid; however water is formed in dentinal tubules when dentin is exposed. Normally, water complicates the bonding mechanism of the conventional resin cements to dentin. However, self-adhesive resin cement’s initial characteristic of hydrophilicity and moisture tolerance provides improved adaptation to the tooth structure. Thus, inherent hydrophilicity of no treatment applied dentin and surface dryness of tubule occlusion succeeded dentin may affect the bonding ability of self-adhesive resin cement.

This study investigated MTBS values of a newly introduced self-adhesive resin cement; the bond strengths might be brand dependent hence, there is need for comparison of MTBS using different kinds of self-adhesive resin cements. In addition, scanning electron microscopy evaluation is needed to determine the adaptation between self-adhesive resin cement and dentin when different desensitizers are used.

**CONCLUSION**

Within the limitations of the presented in vitro study, Gluma and Smart Protect desensitizing agents and Nd:YAG laser irradiation may be viable options in terms of bond strength when using a self-adhesive resin cement as a luting agent.

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