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ORIGINAL RESARCH

SHORT- AND LONG-TERM BOND STRENGTHS OF A GOLD STANDARD TWO-STEP SELF-ETCH ADHESIVE SYSTEM TO DENTIN: A PRELIMINARY STUDY

Altın Standart İki Aşamalı Self-Etch Bağlayıcı Sistemin Kısa Dönem ve Uzun Dönem Dentine Bağlanma Dayanımları: Bir Ön Çalışma

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ABSTRACT

Purpose: *The aim of this study was to investigate the micro tensile bond strength of a self-etch adhesive system following 1 year storage in water.* ***Materials and Methods:*** *10 sound human molar teeth were used for micro tensile bond strength test. Two-step self-etch dentin adhesive (Clearfil SE Bond®) was applied to the flat dentin surfaces according to the manufacturer's instructions. Composite blocks (Z-250; 3M ESPE) of 5 mm in height have been prepared by using layering technique. Teeth were stored in water for 24 hours at 37°C and longitudinally sectioned to obtain dentin sticks of 1 mm^2.Randomly selected samples from half of the teeth were immediately subjected to micro tensile test and. Remaining specimens were tested after 1 year storage in water. Bond strengths were calculated in megapascal (MPa).* ***Results:*** *Means and standard deviations of the Clearfil SE Bond® micro tensile bond strength values were, respectively, 37.31 ± 13.77 MPa and 24.78 ± 2.99 MPa after 24 h and 1 year of storage in water. The difference was statistically significant (p=0.031).* ***Conclusion:*** *Long-term storage in water decreased the micro tensile bond strength values of the two-step self-etch adhesive which has been accepted as the gold standard in bond strength tests.*

Keywords: *Microtensile bond strength test; two-step self-etch adhesive; degradation; water aging*

ÖZ

Amaç: *Bu çalışmanın amacı, iki aşamalı bir self-etch dentin bağlayıcı sistemin 1 yıl suda bekletme sonucunda bağlanma dayanım değerlerindeki değişimi incelemektir.* ***Gereç ve Yöntem:*** *Mikro-gerilim bağlanma dayanım testi için 10 adet gömük üçüncü molar dişi kullanılmıştır. Düz dentin yüzeylerine iki aşamalı self-etch (Clearfil SE Bond; Kuraray) sistem uygulamasını takiben tabakalama yöntemi ile 5 mm yüksekliğinde kompozit bloklar (Z-250; 3M ESPE) oluşturulmuştur. Restore edilen dişler 24 saat 37°C suda bekletildiken sonra uzun akslarına paralel olarak kesilmiş ve 1 mm^2'lik dentin çubukları oluşturulmuştur. Rastgele seçilen 5 dişe ait çubuklar 24 saat sonra, kalan 5 dişten hazırlanan çubuklar ise 1 yıl 37°C suda bekletildikten sonra çekme testine tabi tutulmuştur. Elde edilen bağlanma dayanımı değerleri megapascal (MPa) olarak kayıt edilmiştir.* ***Bulgular:*** *Bağlanma dayanım değerleri 24 saat sonunda ortalama 37.31 ± 13.77 MPa, 1 yıl suda bekletme sonunda ortalama 24.78 ± 2.99 MPa olarak tespit edilmiştir. Suda bekletme sonucunda Clearfil SE Bond bağlanma dayanım değerlerindeki azalma istatistiksel olarak anlamlılık göstermiştir. (p=0.031).* ***Sonuç:*** *Bağlanma testlerinde altın standart olarak kabul edilen iki aşamalı bir self-etch bağlayıcının suda uzun dönem bekletilmesi, bağlanma dayanım değerlerinde azalmaya neden olmuştur.*

Anahtar kelimeler: *Mikro-gerilim bağlanma dayanım testi; iki aşamalı self-etch bağlayıcı; yıkım; suda yaşlandırma*

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Introduction

The most important factor that determines the clinical performance of dental restorations is their resistance to biodegradation. Biodegradation concept is related to resin matrix, filler composition and the interface. Chemical and mechanical degradations occur in the components of the interface over time (1, 2). Generally these morphological changes in the adhesive structure are seen in the long term. During chemical degradation, components of the interface hydrolyze due to the effects of saliva, bacterial enzymes or water content in dentin (3, 4), which results in plasticizing of the resin (2, 5, 6). Short- and long-term bond strength tests should be performed to evaluate time-dependent degradation of the dentin adhesives (7). To determine long-term efficiency of the adhesive, its short-term efficiency must first be established (2, 7). Although bond strengths of dentin adhesives are usually evaluated in the short-term or during the first 24 hours, more clinically relevant studies or those that include sample aging protocols are also required. Findings of experimental studies which have been designed to measure the short-term bond strength of the adhesive does not always correlate with real clinical observations. Therefore, in addition to short-term experiments, long-term studies that bear more resemblance to clinical conditions must also be carried out (2, 7).

In self-etching adhesive systems, inorganic phase of the dentin is dissolved by the acidic monomer instead of the phosphoric acid. As a result, monomer infiltrates the collagen network with the help of the solvent and hydrophilic components. When collagen network is completely surrounded by the monomer, it is protected against the degradation. Also, chemical reaction of the functional monomer has additional positive effects on the bonding. However, inadequate infiltration of the resin causes nanoleakage. Incomplete removal of water from the environment is associated with the presence of hydrophilic resin monomers. Water-tree formation is observed after an average of 1 year of water aging. The formation of the water trees can occur with slow absorption of water at the bonding interface. Aging of the interface depends on the water intake and the increase of porous regions in the hybrid layer (5, 8).Clearfil SE Bond® is a self-etch adhesive which demonstrates high performance in in vitro and in vivo studies (9-12). It is considered as the gold standard for studies on adhesion research and is therefore frequently used as a control group (9, 13-15). The aim of this study was to compare bond strength of the Clearfil SE Bond® system after 24 hours and 1 year of water storage.

Materials and Methods

Sample characteristics and preparation

10 impacted third molar teeth were used in this study. Periodontal tissues were cleaned using a scalpel and teeth were stored in 0.5% Chloramin T solution (Merck KGaA, Darmstadt, Germany) for 1 week. At the end of this period, solution was replaced with distilled water, stored in a refrigerator at 4°C and renewed periodically to prevent bacterial growth. All extracted teeth were used in the experiments within 6 months. Specimens were randomly allocated into two groups of equal size, which are described as the short-term (24 hours in water storage) and the long-term (1 year in water storage) groups. Teeth were glued to acrylic resin blocks with sticky wax and the occlusal enamel surface of each tooth was removed using a slow-speed diamond saw (Isomet, Buehler Inc., Lake Bluff, IL, USA) under water lubrication to expose flat superficial dentin, which was then polished with wet 600-grit silicon carbide paper to create a uniform surface and smear layer.

Clearfil SE Bond® (Kuraray Medical Inc., Okayama, Japan) was applied to dentin surfaces of teeth in each group according to the manufacturer's instructions. Next, teeth were completely encircled with tofflemire matrix, a composite core buildup was made in three layers of maximally 2-mm thickness to a height of 5 mm, using a microhybrid resin composite (Filtek Z 250, A2 Shade, 3M ESPE, St. Paul, MN, USA) (Table 1).Each increment was light cured with a quartz-tungsten halogen curing unit (Bisco VIP Dental Curing Light, Schaumburg, IL, USA) for 20 seconds. Light intensity output was checked with a dental radiometer (Hilux® Ledmax Light Curing Meter, Benlioğlu Dental Inc., Ankara, Turkey) After completing the composite blocks, teeth were placed in a drying oven at 37°C for 24 hours and glued to acrylic blocks parallel to their long axis by using sticky wax. Teeth were then sectioned with above mentioned precision saw to obtain 1 mm thickness dentin slices. In the next step, acrylic block was removed from the instrument, tooth axis was rotated 90° and again fixed to the block for cutting. Thus, 1 mm^2 sticks were obtained, which consisted of 5 mm of dentin and 5 mm of composite material. Same bonding and cutting procedures were followed for teeth in 1 year of water aging and 24 hours groups. The sticks from 5 teeth were stored for 1 year in water which changed periodically.

Table 1. *Brand names, manufacturers, contents and operating instructions of the composite filling material and two-step dentin bonding agent which were used in this study. MDP; 10-methacryloyloxydeoyl dihydrogenphosphate, HEMA; hydroxyethylmethacrylate, bis-glycidyl methacrylate; Bis-GMA, urethane dimethacrylate; UDMA, glycol dimethacrylate; BIS-EMA, CQ; Camphorquinone.*

Material	Manufacturer	Contents	Operating instructions
Clearfil SE Bond® (two-step,self-etch dentin bonding)	KURARAY Medical Inc., Okayama, Japan	**Primer:** MDP, HEMA, hydrphilicdimethacrylate, N-Diethenol p-toluidine, water. **Bond:** MDP, BisGMA, HEMA, hydrophobicdimethacrylate, CQ, N, N-Diethenol p-toluidine, Silanatecooloidalsilica.	Apply one layer of primer. Wait for 20 s. Dry with mild air and apply bond. Remove excess bond with gentle air flow. Polymerize with light for 10 s.
Filtek Z 250® (Universal restorative system)	3M ESPE Inc., St. Paul, MN, USA	BIS-GMA, UDMA, and BIS-EMA resins 60 % by volume, 0.01-3.5µm diameter zirconia/silica filler.	Apply material into the cavity in 2 mm layers. Polymerize with halogen light device for 20 s.

Bond strength testing

Bonded sticks were attached to a modified device for microtensile testing with cyanoacrylate resin (Zapit Dental Ventures of North America, Corona, CA, USA) and subjected to a tensile force in a microtensile testing machine (Micro Tensile Tester Bisco Inc., Schaumburg, IL, USA) at a crosshead speed of 0.5mm/min. until fracture occurs. Values obtained from the samples at fracture points were recorded separately for short- and long-term groups and expressed as MegaPascal (MPa).

Fracture surfaces were examined under stereomicroscope (Olympus® SZ61, Munster, Germany) at X30 magnification. Fracture surfaces were classified separately as:

- Adhesive/Mix, (fracture from resin/dentin interface or any amount of fracture from surrounding tissues (composite or dentin) in addition to adhesive surface
- Cohesive dentin
- Cohesive composite (16-18).

Statistical Analysis

SPSS Statistics 21 for Windows® (IBM Inc., Chicago, IL, USA) and G*Power version 3.1.9.2 (Kiel University, Kiel, Germany) softwares were used to compare variables and to determine study power, respectively. Since Kolmogorov-Smirnov normality test showed that the data did not meet the requirements of normal distribution, non-parametric Mann Whitney U test was used to compare medians of study groups. The proportions of the categorical variables for each fracture type in short- and long-term groups were compared with Fisher's and Yates' Chi Square tests. The confidence interval was set to 95% and $p < 0.05$ was considered as statistically significant.

Results

46 sticks were obtained from 5 teeth which were treated with Clearfil SE Bond® and stored in water for 24 hours. Number of sticks per tooth and minimum, maximum and mean bond strength values obtained from these sticks for each tooth are presented in Table 2.Median (minimum-maximum) bond strength of 46 sticks which were obtained from teeth treated with Clearfil SE Bond® agent was found to be 33.59 (26.70-60.40) MPa (Table 3).

Table 2. *Number of sticks, minimum, maximum, median and mean values of bond strengths for each tooth after 24 hours and 1 year of water storage which were obtained from 5 teeth treated with Clearfil SE Bond®.Statistical analysis.*

Duration	Tooth number	n	Mininum value (MPa)	Maximum value (MPa)	Median value (MPa)	Mean value (MPa)
24 hours	1	9	22.2	51.6	44,0	38.5
	2	7	35.5	77	65,5	60.4
	3	13	12.9	53.2	41,1	33.6
	4	9	11.7	46	24,6	26.7
	5	8	12.9	40	32,5	27.6
1 year	1	11	7.7	46.8	22,6	27.5
	2	8	21	34.7	25,1	27.3
	3	11	6	37.1	24,2	22.4
	4	12	7.7	57.7	22,8	25.9
	5	14	8.9	41.5	19,8	20.9

Table 3. *Means, standard deviations (SD), medians, minimum and maximum bonding strength values of the samples after 24 hours and 1 year of water storage.*

	24 hours	1 year
Mean ± SD (MPa)	37.31±13.77	24.78±2.99
Median (MPa)	33.59	25.88
Minimum (MPa)	26.70	20.90
Maximum (MPa)	60.40	27.50

56 sticks were obtained from 5 teeth which were treated with Clearfil SE Bond® and stored in water for 1 year. Number of sticks per tooth and minimum, maximum and mean bond strength values obtained from these rods for each tooth are presented in Table 2. Median (minimum-maximum) bond strength of 56 dentin rods which were obtained from teeth treated with Clearfil SE Bond® agent was found to be 25.88 (20.90-27.50) MPa (Table 3). Comparison between the micro tensile bond strengths of teeth which were stored in water for 24 hours and 1 year revealed that the bond strength values after 24 hours were significantly higher and there was a significant time-dependent decrease in bond strength (p=0.031). When values obtained for 24 hours and 1 year (for all sections) groups are considered together, the study power was calculated as 0.95. Adhesive/Mix type fractures constituted the highest percentage of failures in sticks which were prepared from samples treated with Clearfil SE Bond® after 24 hours and 1 year. Adhesive/Mix type fractures were observed in 52.2% of the samples stored in water for 24 hours and in 82.2% of the samples stored in water for 1 year (p=0.002) (Table 4).

Table 4. *Percentage distributions of fracture types in 24 hours and 1 year water storage groups.*

Duration	Adhesive/Mix	Dentin/Cohesive	Composite/Cohesive
24 hours	24/46 (52.2%)	6/46 (13%)	16/46 (34.8%)
1 year	46/56 (82.2%)	5/56 (8.9%)	5/56 (8.9%)
p	**0.002**	0.538	**0.003**

Discussion

Clearfil SE Bond® is one of the most frequently used adhesives in laboratory studies and are considered as gold standard in terms of bonding capability. Shirai *et al.* (19) and Toledano *et al.* (20) have reported that the functional monomer10-methacryloyloxydeoyldi hydrogenphosphate (10-MDP) in Clearfil SE Bond® has the highest chemical bonding potential with hydroxyapatite and hydrolytic stability among other monomers. Toledano *et al.* (20) also associated the high bond strength of Clearfil SE Bond® to the high percentage of monomer 10-MDP content found in this material. In our study, mean micro tensile bond strength value for Clearfil SE Bond® was 37.31 ± 13.77 MPa after 24 hours. Our findings were in accordance with those of Van Landuyt *et al.* (21) who had reported dentin bond strength of Clearfil SE Bond® as 40.5 MPa after 24 hours, Proença *et al.* (22) 42.7 MPa, Sadek *et al.* (23) 40.4 MPa, Inoue *et al.* (24) 40.8 MPa, Kenshima *et al.* (25) 40.7 MPa, Lodovici *et al.* (26) 33.8 MPa, Yeşilyurt *et al.* (27) and Van Landuyt *et al.* (28) 35.1 MPa. Fillers are added to adhesive systems to improve bond strength. Presence of filler in the material enables the application of relatively thick adhesive layer. According to a hypothesis, this relatively thick adhesive layer acts as stress breaker and it tolerates shrinkage stresses (16, 19). High bond strengths observed with Clearfil SE Bond® in our study could have been influenced by its MDP content, as well as being an adhesive resin which contains fillers (particle-filled). 24 hours bond strength of Clearfil SE Bond® was found to decrease from 37.3 MPa to 24.7 MPa after 1 year of water storage and this difference was statistically significant (p=0.031). In accordance with our findings, Toledano *et al.* (7) have reported that the micro tensile bond strength of Clearfil SE Bond® at 24 hours had decreased from 40.8 MPa to 20.6 MPa after 1 year of water storage. In our study, significant decrease in the bond strength of Clearfil SE Bond® after 1 year could be related to the storage conditions, such as sectioning samples to form sticks. Preparing samples as sticks could have shortened the water diffusion distance which, in turn, could have led to direct contact of water with the interface. Such conditions could result in rapid decline in bond strength. Armstrong *et al.* (29) also showed that the bond strength values of samples prepared with Clearfil SE Bond® at 1 month decreased significantly from 47.7 MPa to 21.6 MPa after 15 months of water storage. Akın *et al.* (30) reported that the mean bond strength of samples at the end of 24 hours was 30.05 MPa which decreased to 26.13 MPa after 6 months of water storage. Clearfil SE Bond® is a two-step (primer and bond) self-etch system with hydrophilic material properties. Torkabadi *et al.* (31) related the decrease in the bond strength values of hydrophilic adhesive systems after 1 year of water storage to the water absorption of 2-hydroxyethyl methacrylate (HEMA) which diminishes mechanical strength of the resin.

Hashimoto *et al.* (32) suggested the hydrolysis process as the main cause of damage at the resin/dentin interface. Hydrolysis is the water absorption of resin from inter-fibrillar spaces in the hybrid layer. Water absorbed in resin can be found in two forms such as free water between polymer chains or as water attached to polymer chains (31). Water penetrating the interface by hydrolysis disrupts covalent bonds and creates empty spaces in hybrid layer by causing detachment of filler particles from the resin. Thus, the mechanical properties of the resin start to decline and the polymer swells (5, 6). Clinical relevance of this reaction is the time-dependent decrease in the resin-dentin bond strength (33). Water intake of the polymer depends on hydrophilic properties of the bonding agent. Water absorption capability of the structure is proportional to the hydrophilic characteristics of the material. Hydrophilic components such as HEMA, increase water uptake of the bonding agent and enables water molecules to migrate from dentin layer to the adhesive layer (31). Methacrylate monomers modified with carboxylic or phosphoric acid groups can also increase water uptake. Hydrophobic monomers such as bis-glycidyl methacrylate and methyl methacrylate are the main components which decrease the water uptake (34). Hashimoto *et al.* (32) reported that they did not observe time-dependent decrease in the bonding strength values when samples are stored in mineral oil instead of water. Yiu *et al.* (6) investigated 5 experimental bonding agents that have different hydrophilic characteristics. After preparation, authors have stored their samples either in water or mineral oil for 1, 3, 6 and 12 months. They reported the hydrophilicity of the resin as the most important factor that determines the percentage of water absorption and have found that long-term water storage declined bonding strength of 5 experimental bonding agents. In addition, they also suggested that long-term storage of samples in mineral oil instead of water could stabilize, or even increase, the tensile strength of hydrophilic resin mixture over time (6).

Most frequent type of failure on the sample surfaces in our study was adhesive/mix fracture both in 24 hours and 1 year groups. Also, percentage of such mechanical failures was observed to increase drastically after 1 year water storage, when compared to that of 24 hours group. In many studies, failures at the fracture surfaces of stick shaped samples have been classified under three main categories, namely the adhesive/mix, cohesive dentin and cohesive composite type of fractures (16-18, 26, 35, 36). Reis *et al.* (35) reported that it would be very difficult to distinguish between adhesive and mix types with naked-eye observation, as well as stereomicroscope. Authors have therefore suggested to combine adhesive and mix fracture types. Same approach was also used in this study. Samples having adhesive or mix fracture surfaces were grouped and analyzed as a single entity (35, 36). Cohesive fractures and pre-test fractures were not included in statistical analysis (37). Meerbeek *et al.* (13) have suggested that pre-test fractures should be excluded from statistical analysis. Otherwise, pre-test fracture values, usually accepted/assumed as 0 or 5 MPa, would decrease the mean bond strength of the dentin bonding system and such conditions could not reflect the real performance of the adhesive agent.

Conclusion

Long-term water storage of the two-step self-etch dentin bonding system which is considered as the gold standard in mechanical tests, have caused significant decrease in the bond strength values.

Therefore, there is a need to develop new dentin bonding agents which provide more mechanical stability in long-term bonding strength tests and can be used as gold standard.

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None declared

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