



THE EFFECT OF BLOOD CONTAMINATION ON SHEAR BOND STRENGTH OF CALCIUM SILICATE-BASED PULP CAPPING MATERIALS

ABSTRACT

Objectives: The aim of this study was to examine the effect of different hardening times and blood contamination of MTA and Biodentine, which are widely used for pulp capping treatments in the market, on shear bond strength (SBS) with a self-etch adhesive resin, after different hardening times (24, 48, 72 and 96 hours).



Materials and Methods: Slots with a diameter of 5 mm and a height of 2 mm were prepared in 192 acrylic blocks for this study. Both ProRoot MTA and Biodentine were prepared according to the manufacturer's instructions, and half of the slots were filled with ProRoot MTA and the other half were filled with Biodentine. All the samples were divided into groups depending on four different hardening times and hardened. After the hardening process was completed, the group of each hardening time was divided into 2 subgroups (n:12) with and without contamination.

In the uncontaminated groups, a self-etch adhesive resin (Clearfil Liner Bond) and a resin-based composite (Filtek P60) were applied on the samples and polymerized. In the contaminated groups, the sample surfaces were contaminated with blood for 20 seconds. After washing and drying the samples, adhesive resin and composite were applied on them. After that, SBS tests were performed and the data were subjected to a 2-way ANOVA test analysis.

Results: In the uncontaminated groups, there was no significant difference in the SBS of each pulp capping material depending on different hardening times ($p>0.05$). ProRoot MTA showed statistically higher SBS than Biodentine in the 72 and 96 hour uncontaminated groups ($p<0.05$). Blood contamination caused a significant decrease in the SBS of ProRoot MTA and Biodentine ($p<0.05$).

Conclusions: In this study, it was determined that blood contamination reduces the SBS of pulp capping materials. Therefore, it is recommended to prolong the hardening times of the capping materials and to take clinical measures to prevent blood contamination as much as possible before restorative treatments are performed.

Keywords: Pulp Capping; MTA; Biodentine; Blood Contamination; Shear Bond Strength.

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INTRODUCTION

Pulp capping treatments (PCT) aim to maintain the vitality of the pulp by directly covering the pulp tissue exposed for various reasons with a capping material.^{1,2} Deep dentin caries, exposure of the pulp tissue due to mechanical or traumatic reasons are the most important factors that threaten the vitality of the pulp. In such cases, it is of great importance to allow the tooth to function in the mouth for a much longer time by maintaining the vitality of the pulp, in terms of contributing to phonation and aesthetics. It is aimed at maintaining the vitality of the pulp by ensuring the formation of tertiary dentin with the capping material applied over the exposed pulp tissue.^{2,3}

Various pulp capping materials have been introduced to the market for use in PCT so far. Although calcium hydroxide (CH) has been used as the gold standard in PCTs for many years,¹ it has disadvantages such as high solubility and lack of adhesion to dental tissues. In PCT with CH, it has been reported that the dentin bridge formed by CH has tunnel defects and porosities^{4,5}, and CH has an inability of adhesion to the dentin surface, so it causes microleakage in the long term and does not protect the pulp sufficiently.⁶

As a result of the search for different materials due to the disadvantages of CH, ProRoot MTA (Densply, Tulsa Dental, USA), developed by Torabinejad *et al.*^{7,8}, at Loma Linda University in 1993, is the first calcium silicate-based (CSB) cement used in dentistry. MTA has advantages such as providing a more homogeneous (containing less tunnel defect) dentin bridge formation in a shorter time compared to CH, causing less inflammatory reaction, hardening in the presence of moisture, meeting high chewing pressure after hardening, and being biocompatible.⁹ MTA is used for PCT, pulpotomy, repair of root perforations, bifurcation lesions, internal and external root resorption, apexification and as the apical plug material in endodontic surgery and deep cervical and radicular lesions.^{10,11} Although MTA currently continues to be used successfully in PCT, it has maintained a different material trend in this area due to its disadvantages such as having long hardening time and not being economical.^{7,12}

Biodentine (Septodont, France), developed in 2009, contains tricalcium silicate, calcium carbonate, calcium oxide and zirconium oxide in its powder and it is a CSB material containing modified polycarboxylate and calcium chloride components in its liquid. The main advantages of Biodentine are initial hardening time of approximately 12 minutes and enhanced physical properties according to the manufacturer's instructions.¹³ Biodentine, which is a biocompatible material, is used in the treatment of pulp capping, amputation, apexification and root perforation repair and as retrograde root filling.^{14,15} Biodentine stimulates odontoblast-like cells by forming mineralization nodules in dentin and provides dentin-like matrix formation.¹⁶ It stimulates mineralization by releasing calcium ions and creates a mineral infiltration zone at the dentin-cementum interface, providing better coverage and reducing microleakage.¹⁷ Biodentine is similar to MTA in terms of forming dentin bridge formation and causing lower inflammation in the pulp.¹⁸ According to the manufacturer's instructions, the shorter hardening time of Biodentine is an advantage, for the restorative procedures can be performed in a shorter time compared to MTA, which has an initial hardening time of 3-4 hours.⁷

In PCT, after the exposed pulp tissue is covered with pulp capping materials, temporary restorations are frequently made on them to complete their hardening, and the capping material is expected to harden. After the pulp capping materials harden for a certain time period, the temporary filling is removed and permanent restorations are completed with adhesive and composite resins.

There are different literature data on the different hardening times of capping materials, affecting bond strength to adhesive resins.¹⁹⁻²² In addition, it is often possible for the capping material to be exposed to oral fluids and blood contamination from the gingiva after hardening and prior to the construction of the upper restoration in clinical conditions. Moreover, an uncontaminated capping material has a different surface microstructure compared to the capping material exposed to blood contamination.²³ Therefore, blood contamination could affect the

shear bond strength (SBS) of the capping material to adhesive resins. However, to the best of our knowledge, a study on the effect of blood contamination of the capping materials on the SBS to restorative materials after hardening is not available in the literature.

For this reason, our first aim in this study was to investigate the SBS of ProRoot MTA and Biodentine, which are CSB capping materials widely used in PCT, to a self-etch adhesive resin after different hardening times (24, 48, 72, 96 hours). Our second aim is to investigate the effect of blood contamination in vitro of ProRoot MTA and Biodentine after different hardening times (24, 48, 72, 96 hours) on SBS to a self-etch adhesive resin.

Our initial hypotheses in this study were as follows:

1. There is no difference between the SBS of ProRoot MTA and Biodentine to a self-etch adhesive resin after different hardening times (24, 48, 72, 96 hours).
2. Blood contamination of ProRoot MTA and Biodentine after different hardening times (24, 48, 72, 96 hours) does not affect the SBS to a self-etch adhesive.

MATERIAL AND METHODS

Ethics

Ethical approval was obtained from the Health Ethics Committee of Karadeniz Technical University, Faculty of Medicine, Scientific Research Ethics Committee in Trabzon, Turkey (ID: 2019/294 and decision date 18.11.2019).

In this in vitro study, the effect of two CSB pulp capping materials, ProRoot MTA (Densply, Tulsa Dental, USA) and Biodentine (Septodont, Saint-Maurdes, France) on SBS to a two-step self-etch adhesive, Clearfil Liner Bond F (Kuraray Noritake Dental Inc, Japan) and a microhybrid composite (Filtek P60 Posterior Composite, 3M ESPE, Dental Products, USA) after different hardening times (24, 48, 72, 96 hours) was investigated. Furthermore, the effect of blood contamination of these pulp capping materials after these different hardening times on SBS was also investigated.

The materials used in the study, their contents, production numbers and application instructions are shown in Table 1.

Table 1. Materials, Contents, Production Numbers and Application Instructions

MATERIALS	BATCH NUMBERS	CONTENTS	APPLICATION INSTRUCTIONS
ProRoot White MTA (Densply, Tulsa Dental, USA)	0000249678	Powder: Tricalcium aluminate, tricalcium silicate, silicate oxide and tricalcium oxide, iron oxide, magnesium and bismuth oxide Liquid: distilled water, saline Ph = 12-13	Mix 1 pack of powder with 1 ampule of liquid for about 1 minute. After application, it is closed with damp cotton and temporary filling is made. After 96 hours, permanent filling is applied.
Biodentine (Septodont, Saint-Maur-desFosses, France)	B25453	Powder: Tricalcium silicate, dicalcium silicate, calcium carbonate, iron oxide, zirconium oxide Liquid: Calcium chloride, hydrosoluble polymer, water	The capsule is opened and 5 drops of liquid from the disposable liquid are dropped into the capsule. The capsule is closed and placed in the amalgamator and mixed for 30 seconds at 4500 rpm (rotation/min). The capsule is opened and the Biodentin material is applied with the appropriate handpiece.

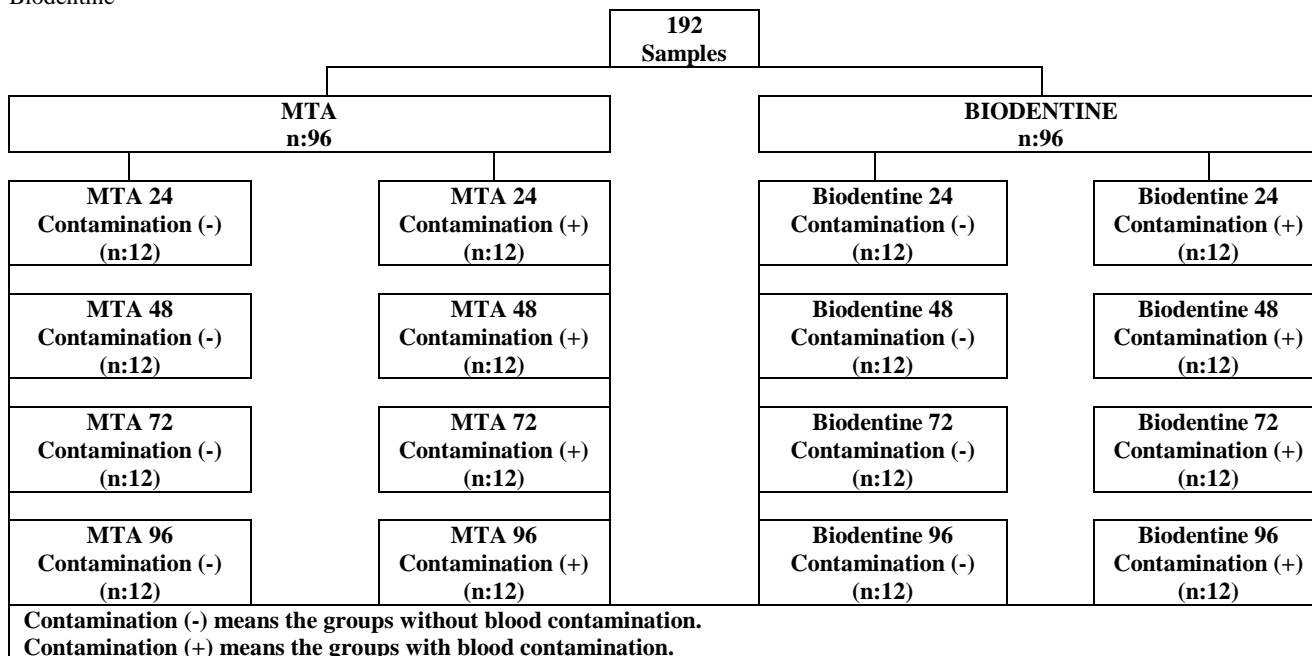
Clearfil Liner Bond F (Kuraray Noritake Dental Inc., Japan)	000019	Primer: 10-Methacryloyloxydodecyl dihydrogen phosphate (MDP), 2-hydroxyethyl methacrylate (HEMA), Hydrophilic aliphatic dimethacrylate, dl-camphorquinone, N,N-di-ethanol-p-tolidine, Water. Ph\approx 1.9 Bond: 10-Methacryloyloxydodecyl phosphate (MDP), 2-hydroxyethyl methacrylate (HEMA), Bisphenol A digicidyl methacrylate (Bis-GMA), Colloidal silica, Hydrophobic aliphatic dimethacrylate, dl-camphoroquinone, N,N-diethanol p-tolidine	1. The primer is applied to the tooth surface and waited for 20 seconds. Oil-free air is squeezed to allow solvents to evaporate and disappear. 2. Bond is applied to the tooth surface and oil-free air is gently squeezed to form a uniform layer. 3. The adhesive is cured with a dental light device that emits light at a wavelength of 400-515 nm for 10 seconds.
Filtek P60 Posterior Composite (3M ESPE, Dental Products, USA)	NA36198	It contains Zirconia / Silica and BiS-GMA, UDMA and BIS-EMA resin. The inorganic filler content is 61% by volume.	It is applied with the incremental technique in 2 mm masses and polymerized with a dental light device for 20 seconds.

Preparation of Test Samples

A total of 192 cylindrical acrylic blocks with a diameter of 30 mm and a height of 10 mm were prepared by forming 16 groups (n:12) for 4 different hardening times and contamination groups for each CSB capping material. Slots with a diameter of 5 mm and a height of 2 mm were

prepared in these 192 acrylic blocks. For each setting time of each pulp capping material, 24 samples were prepared and half of them were used in experiments with blood contamination and the other half in experiments without blood contamination (Table 2).

Table 2: Experimental Groups Prepared According to Different Hardening Times and Contamination Conditions of MTA and Biodentine



ProRoot MTA and Biodentine were applied to the slots prepared in acrylic blocks according to the manufacturer's instructions. The surface of the MTA samples was covered with moist cotton and all the prepared samples were hardened for the

planned times (24, 48, 72, 96 h) by storing them at 37°C and 100% humid environment.

In uncontaminated groups (without blood contamination), after certain hardening times, Clearfil Liner Bond F, a two-step self-etch

adhesive system, was applied on the pulp capping materials according to the manufacturer's instructions and polymerized with light (Elipar S10, 3M ESPE) for 20 seconds. After polymerization, a 3 mm high composite resin (Filtek P60, 3M ESPE) was applied on the capping materials in layers of 1.5 mm thickness each, using a mold and each layer was polymerized with light for 20 seconds again.

In the contaminated groups (with blood contamination), after certain hardening times, the surfaces of the pulp capping materials were contaminated with fresh blood for 20 seconds. After that, the surfaces were washed with water for 10 seconds and then dried by wiping once with a cotton roll. After the contamination and drying processes, adhesive resin and composite applications were performed and then all the samples were kept in a 100% humid environment at 37 degrees for 24 hours.

Shear Bond Strength Test

SBS test was applied to all the samples with a Universal Tension/Compression Testing Machine (Instron 3382, USA) at a speed of 0.5 mm/min. After the SBS test, the results were converted to Megapascals (MPa). The fracture surfaces of the

samples were examined with an optical microscope (Olympus Metallurgical Microscope) under X30 magnification, and different failure types (adhesive, cohesive and mixed) were determined.

Statistical analysis

SPSS for Windows 17.0 (SPSS Inc., Chicago, USA) program was used for statistical analysis. Normal distribution analysis of the data was performed with the Shapiro Wilk test. Two-way ANOVA test and Fisher's LSD test were used for repetitive samples and paired samples test was used for time-dependent variations. A $p < 0.05$ level was considered statistically significant in all the analyses.

RESULTS

Shear Bond Strength Test Results

Interactions are reported in Table 3. According to the interaction table, SBS test values were significantly influenced by both time ($p: 0.029$) and time x material ($p: 0.007$) whereas no effects were found for interactions (time x contamination), ($p: 0.341$) and (time x material x contamination), ($p: 0.274$).

Table 3. Two-way ANOVA interaction effects for shear bond strength

Source	Value	F	Hypothesis df	Error dF	Sig. p
Time	.192	3.3	3	42	.029
Time* Material	.249	4.6	3	42	.007
Time* Contamination	.076	1.1	3	42	.341
Time* Material* Contamination	.087	1.3	3	42	.274

df: degrees of freedom.

* $p < 0.05$; level was considered statistically significant.

1- Comparisons of SBS Results of Pulp Capping Materials Hardened at Different Times within Groups

Table 4 shows the mean and standard deviation values of SBS according to the different

hardening times (24, 48, 72, 96 h) and contamination status of the capping materials used in the study, as well as within-group differences.

Table 4. Mean and Standard Deviation Values of Shear Bond Strength (MPa) and Comparisons within Groups

PULP CAPPING MATERIALS / HARDENING TIME	24 HOURS	48 HOURS	72 HOURS	96 HOURS
MTA	20.46 ± 3.97 ^A	22.50 ± 4.52 ^A	23.75 ± 5.81 ^A	22.65 ± 4.11 ^A
CONTAMINATED MTA	10.43 ± 2.42 ^A *p<0.05 (with contMTA48) *p<0.05 (with contMTA72) *p<0.05 (with contMTA96)	14.92 ± 5.20 ^B	16.80 ± 2.52 ^B	17.14 ± 3.38 ^B
BIODENTINE	18.61 ± 5.51 ^A	20.32 ± 3.04 ^A	18.01 ± 3.46 ^A	18.55 ± 3.40 ^A
CONTAMINATED BIODENTINE	13.25 ± 3.74 ^A	11.72 ± 3.25 ^A	13.40 ± 6.06 ^A	12.26 ± 1.97 ^A

According to Bonferroni correction, a significant difference in shear bond strength of each pulp capping material at different hardening times (in the same row) is shown in superscript. Different letters indicate statistical differences in rows (p<0.05).

When the SBS values were examined statistically,

There was no statistically significant difference between the groups in terms of SBS after different hardening times (24, 48, 72, 96 hours) for the non-contaminated MTA group (p>0.05). Contaminated (Cont) MTA24 group showed statistically significantly lower bond strength compared to Cont MTA48, 72, 96 hour groups (p<0.05).

There was no statistically significant difference between the groups after different setting times (24, 48, 72, 96 hours) for the

uncontaminated Biodentine group (p>0.05). For the Contaminated (Cont) Biodentine group, there was no statistically significant difference between the groups after different setting times (24, 48, 72, 96 hours) (p>0.05).

2- Comparison of the Shear Bond Strength Test Results of Pulp Capping Materials Among the Groups within the Same Hardening Time

Comparison of the SBS values of contaminated and uncontaminated MTA and Biodentine groups with each other in the same hardening time group is given in Table 5.

Table 5. Comparison of the SBS Values Among the Groups within the Same Hardening Time

PULP CAPPING MATERIALS	Hardening Times			
	24 HOURS	48 HOURS	72 HOURS	96 HOURS
MTA	20.46 *p<0.01 (with Cont.MTA24)	22.50 *p<0.01 (with Cont.MTA 48)	23.75 *p<0.05 (with Cont. MTA 72) **p<0.05 (with BD72)	22.65 *p<0.01 (with Cont. MTA 96) **p<0.05 (with BD 96)
CONTAMINATED MTA	10.43	14.92	16.80	17.14 **p<0.01 (with Cont.BD 96)
BIODENTINE	18.61 * p=0.002; p<0.01 (with Cont.BD 24)	20.32 *p<0.01 (with Cont.BD 48)	18.01 *p<0.05 (with Cont.BD 72)	18.55 *p<0.01 (with Cont.BD 96)
CONTAMINATED BIODENTINE	13.25	11.72	13.40	12.26

* : It refers to the significant differences in the same hardening time (in the same column) of the contaminated and uncontaminated groups of the same pulp capping material.

** : It refers to the significant difference in the same hardening time of two different materials (MTA-Biodentine). Different letters indicate statistical differences in rows (p<0.05).

BD is used as an abbreviation for Biodentine.

When the shear bond strength values of MTA and Biodentine groups were compared with each other in the same hardening time,

There was no statistically significant difference between SBS at the end of the 24-hour and 48-hour hardening times of the uncontaminated MTA and Biodentine groups (p>0.05).

However, the SBS of MTA72 group (23.75 MPa) was found to be statistically higher than that of the Biodentine72 group (18.01 MPa) at the end of the 72 hour hardening period (p<0.05). Similarly, at the end of the 96 hour hardening time, the mean SBS of the MTA group (22.65 MPa) was statistically significantly higher than

the mean SBS of the Biodentine group (18.55 MPa) ($p < 0.05$).

When the Contaminated MTA and Contaminated Biodentine groups were compared,

At the end of the 24, 48, 72 hour setting time, there was no statistically significant difference between the SBS values of the Cont. MTA24, 48, 72 groups and those of the Cont. Biodentine 24, 48, 72 groups ($p > 0.05$). At the end of the 96-hour hardening period, SBS values of the Cont. MTA96 group were found to be significantly higher than the SBS values of the Cont. Biodentine96 group ($p < 0.05$).

The Bar Graphic showing the Contamination Status of the Pulp Capping Materials and the S values according to the different setting times is as in Figure 1.

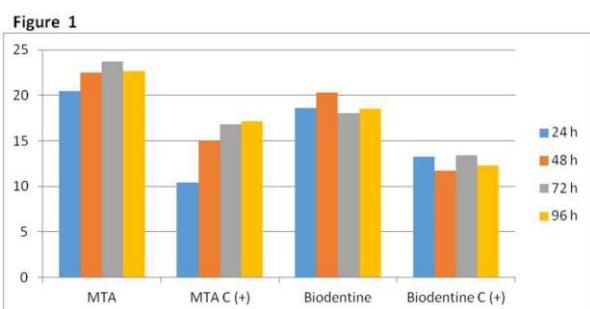


Figure 1. Bar graphic of shear bond strength values by capping material/contamination conditions and hardening time.

Evaluation of Stereomicroscope Images of Fractured Surfaces

In Figure 2, in the uncontaminated MTA groups, the failures were predominantly cohesive, but the adhesive failures increased as the hardening time increased.

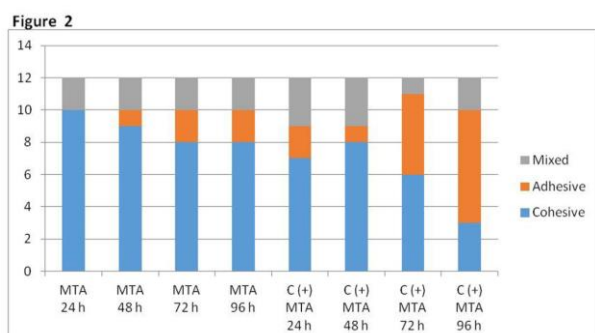


Figure 2. Distribution of failure types at different hardening times of MTA. C(+) means blood contamination.

In the Cont. MTA groups, the group with the highest rate of adhesive failure was the group hardening for 96 hours.

As seen in Figure 3, cohesive failures were mostly observed in the uncontaminated Biodentine groups. Adhesive failure type was more commonly seen in the Cont. Biodentine groups, particularly in the Cont. Biodentine 24 group.

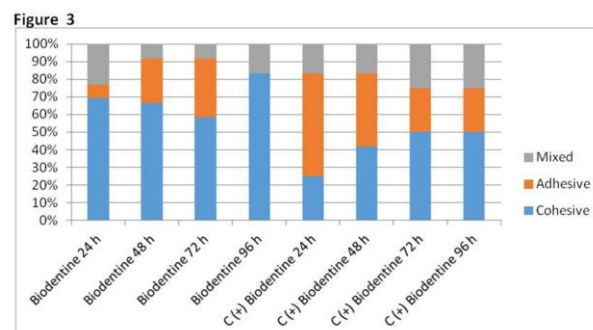


Figure 3. Distribution of failure types at different hardening times of Biodentine. C(+) means blood contamination.

DISCUSSION

Although different materials have been used in PCT in the literature to date, MTA and Biodentine are CSB materials preferred in PCT due to their advantages such as biocompatibility, high level of sealing properties, and forming reparative dentin formation depending on cell differentiation.^{14,15,18,24-26} Studies have shown that these materials stimulate the release of TGF- β 1 and that this growth factor provides reparative dentin regeneration from pulp stem cells and the dentin bridge, which is formed by the effect of both MTA and Biodentine, is less porous.^{27,28}

PCT should be followed by an impermeable upper restoration that prevents bacterial invasion at the restoration and dentin interface. That is because the prevention of microleakage and the high bond strength between the pulp coating material and the restoration are also factors that ensure the continuity of the vitality and function of the tooth.²⁹ For this reason, the upper restoration made in capping treatments is expected to show sufficient bond strength to the capping material used in the deepest part of the cavity, as well as to the tooth tissues.

Today, adhesive resins are the most preferred permanent restorative materials due to their aesthetic properties. Although there are many studies in the literature investigating the bond strength of direct pulp capping materials to dental

adhesives, different and contradictory results have been obtained in the bond strength of capping materials to adhesive resins after different hardening times in different studies.^{19,22,30,31}

However, there are different factors that can affect the bond strength of composite resins to capping materials in clinical conditions. During the restorative treatments in the clinical conditions, leakage of saliva and blood into the cavity during the different stages of restorative material applications will cause contamination of the surface and pose a risk for the bond strength of the restoration.^{32,33} Although there have been studies examining the bond strength of MTA and Biodentine to various adhesives, as far as we know, we have not come across any study on the effect of blood contamination of these materials on SBS.

Therefore, in this study, the effect of different hardening times (24, 48, 72, 96 h) and blood contamination of CSB capping materials (ProRoot MTA and Biodentine) on SBS to a two-step self-etch adhesive resin (Clearfil Liner Bond F) and a microhybrid composite resin (Filtek P60) was investigated in-vitro. According to the findings of this study, there was no statistically significant difference between the SBS values of uncontaminated ProRoot MTA at different hardening times ($p>0.05$). There was no statistical difference between the bond strength values of the uncontaminated Biodentine at different hardening times ($p>0.05$). The bond strength values of both MTA and Biodentine groups after all hardening times were found to be equal to or higher than the clinically expected bond strength (17-20 MPa) (Table 4).

When the uncontaminated MTA and Biodentine groups at the same hardening time were compared, no statistically significant difference was observed between the SBS at 24 and 48 hours, while the SBS of MTA groups hardening for 72 and 96 hours were found to be statistically significantly higher than the Biodentine72 and Biodentine96 groups ($p<0.05$), (Table 5). Therefore, the first hypothesis of our study, "There is no difference between the SBS of

ProRoot MTA and Biodentine at different setting times (24, 48, 72, 96 h)" was partially accepted.

Atabek *et al.*¹⁹ compared the bond strength of ProRoot MTA to adhesive systems after different hardening times (2h 45 min, 24h, 48h, 72h and 96h) using different adhesives and showed the highest bond strength with a 2-step total etch system (One-Step Plus) in 96 hours in an in vitro study. It was suggested that the acetone content of the adhesive increases the bond strength to MTA as a result of increasing monomer diffusion due to its high vapor pressure.^{19,34} It was also suggested that early bonding failure occurs during initial hardening, due to the possible high water content of MTA. When the bond strength values at all hardening times were examined, they stated that the performing of an adhesive restoration on MTA should be postponed for at least 96 hours in order for MTA to achieve sufficient physical properties.¹⁹

In our study, although the highest SBS values were obtained for ProRoot MTA at 72 and 96 hours, the bond strength values were found to be higher compared to this study. The reason for this difference may be different monomer systems in the adhesive used. The Clearfil Liner Bond F used in our study contains 10-MDP monomer in both the primer and the adhesive. It has been reported that self-etch adhesive systems containing 10-MDP monomer increase the bond strength by chemically binding the 10-MDP monomer it contains with Ca ions in MTA and Biodentine.^{21,35,36}

In another study, the SBS values of ProRoot MTA were found to be higher than Angelus MTA with a one-step adhesive system (Adhese One F) compared to total etch and self-etch systems.³⁷ The reason reported for this is that ProRoot MTA has fewer large particles and more homogeneous distribution compared to Angelus MTA. They assumed that these structures are calcium silicate and calcium silicate hydrate, and it was suggested that they form the basis of the binding phases in a hydrated Portland cement-based material.^{38,39} It was reported that acid application on the MTA surface has an erosive effect on the MTA surface and causes cracks on the surface, but self-etch

primer application does not create cracks on MTA surface.³⁷ In addition, it was suggested that the content with 5% filler ratio of Adhese One F, which is a one-step adhesive system, causes a lower shrinkage stress and increases the bond strength.⁴⁰ Furthermore, it was reported that potassium fluoride increases the surface strength of Portland cement in several engineering reports.^{41,42} An increase in surface strength can also result in reduced cohesive failure and increased bond strength. The fluoride content of Clearfil Liner Bond F may also contribute to its high bond strength in our study.

In a study by Odabaş *et al.*, no significant difference was observed between the SBS of different adhesive systems after Biodentine's hardening time of 12 minutes and 24 hours. In addition, the highest SBS was obtained with Clearfil SE Bond (19.5 MPa) at a hardening time of 24 hours, and Clearfil SE Bond was the only adhesive system that provided sufficient SBS among all adhesives used. There was no significant difference in SBS between Clearfil SE Bond and Clearfil S3 Bond groups at different hardening times. Furthermore, the bond strength value of Clearfil SE Bond (16.9 MPa) after the hardening time of 12 minutes was found to be higher than the other 12-minute groups, although there was no statistically significant difference.⁴³ In this study too, it was noted that 10-MDP monomer which formed chemical adhesion with the Ca ions in the structure of Biodentine increased the bond strength of Clearfil SE Bond.²¹

Moreover, Keleş and Şimsek examined the SBS of different adhesive systems to Biodentine at the end of the 12-minute hardening time. The SBS of Clearfil SE Bond (14.1 MPa), a two-step self-etch adhesive used in the study, was found to be higher than other adhesive systems (a single-step self-etch; Clearfil Universal Bond, a two-step total etch; Prime&Bond NT).³⁰ Çolak *et al.*³⁶ examined the SBS of different adhesive systems to Biodentine hardened for 9 minutes and 48 hours and reported that the hardening time did not affect the SBS values, but the adhesive containing 10-MDP (Clearfil S3 Bond) showed higher bond strength.

Aksoy and Ünal obtained the lowest SBS value in the group set for 12 minutes by applying different universal adhesive systems in both self-etch and total-etch modes at the end of five different setting times (12 min, 24 h, 48 h, 72 h, 96 h) of Biodentine. They also noted that there was no significant difference between self-etch and total-etch modes of universal adhesives in all the groups. They reported that the setting time of 24 hours is enough to obtain sufficient SBS and that the universal adhesives provide higher SBS regardless of the application mode of the adhesive systems used in the study.⁴⁴ In our study, Biodentine showed sufficient bond strength in 24 hours, and there was no significant difference between the groups in which the hardening time was increased.

On the other hand, Nekoofar *et al.* examined the bond strength of several different resin restorative materials with Biodentine, dividing Biodentine samples into groups according to three different hardening times (12 minutes, 1 week and 1 month). Biodentine samples hardening for 1 week were found to have significantly higher micro-SBS than those with 12-minute hardening time. In the study, it was noted that the bond strength of Biodentine, which is porous and low-resistant in the early stage, may decrease with the application of adhesive agent and polymerization shrinkage of the composite resin. It was reported that CSB materials that harden with hydration are not only weak in crystallized structures at the early stage, but also porous, but these materials complete their maturation after a certain period of time. However, in this study where the etch-rinse adhesive system was used, there was no significant difference in bond strength values between Biodentine samples hardened for one week and one month. The universal adhesive material (All Bond Universal) in self-etch mode applied to the Biodentine samples after 12 minutes of hardening exhibited significantly higher average micro-SBS compared to the other groups (Clearfil SE Bond, Adper Single Bond 2). In addition, it was reported that higher SBS values can be achieved with all adhesives if a longer waiting time can be planned after mixing

Bondentine.⁴⁵ In our study too, Bondentine showed sufficient SBS in four different setting times (24h, 48h, 72h, 96h). Differences in study findings may be due to differences in material method.

Hashem *et al.* investigated the bond strength of Bondentine in the early (0 min., 5 min., 20 min. and 24 hours) and late period (2 weeks, 1 month, 3 months and 6 months) with total etch and self-etch systems. In the study, the highest μ SBS in the early period was obtained in the 24-hour group.⁴⁶ However, in clinical applications, it has been suggested to extend the hardening time of Bondentine, since placing Bondentine in cavities with high C factor will increase polymerization stress. A 2-week waiting period is recommended for Bondentine to fully mature and reach maximum physicochemical properties.⁴⁷ As a result of the study, while there was no significant difference between all hardening times in the late period, it was noted that μ SBS values were significantly higher than the early period. Moreover, there was no significant difference between the 24-hour hardening time and the late period. In this study, ScotchBond Universal adhesive containing MDP monomer was used in self-etch mode.⁴⁶ Similarly, in our study, there was no significant difference between the SBS value of Bondentine in the 24-hour hardening time and the groups with the other hardening times (48h, 72h, 96h). The possible reason for obtaining similar results to this study may be the use of adhesive containing MDP monomer in our study too.

In the study performed by Tulumbacı *et al.*⁴⁸, the SBS of MTA was found to be higher than Bondentine using Prime&Bond NT after 72 hours of hardening time for both capping materials. In our study, however, there was no significant difference between SBS after 72 hours of hardening in both groups, and the highest bonding values were obtained. The use of different adhesive systems in this study and the differences in sample preparation for the SBS test may also have caused differences in the study results.

In the study where Cantekin and Avci hardened Bondentin and ProRoot MTA in two different periods of 15 minutes and 96 hours and

then All-Bond 2 total etch adhesive system was applied, the Bondentine group showed the highest SBS value (17.7 MPa). They noted that the reasons for the high SBS value were the low level of free radical monomer and polymerization shrinkage of the methacrylate-based composite resin (Aelite All Purpose Body, Bisco Inc) they used. In addition, it was suggested that the restorative treatment be delayed for 72 hours to 96 hours in order for MTA to reach the most suitable physical properties in the capping process with MTA.²² In our study, MTA showed sufficient SBS at all hardening times. Moreover, in our study, the highest SBS value was obtained in the MTA72 and then in the MTA96 groups, which is consistent with the recommendation in this study.

In a study by Abdel-Rhman *et al.*³¹, it was proposed that MTA provided the desired impermeability after at least 72 hours and recommended waiting for 72 hours before the restoration. It was also reported that although the initial setting reaction of Bondentin takes 12 minutes after mixing powder and liquid, Bondentin gains its full physical properties after 2 weeks.⁴⁹ In our study, however, there was no statistically significant difference between the SBS values of Bondentin at different hardening times, and it was observed that the SBS was similar in all setting times (24h, 48h, 72h, 96h). Again in our study, although there was no statistically significant difference in the MTA group, the highest bond strength was obtained after 72 hours.

Although there are different results regarding the bond strength of MTA and Bondentine to restorative materials, it is important for the durability of bond strength that these materials have reached sufficient maturation before restoration. Additionally, the bonding of capping materials to dentin as well as restorative materials is very important in terms of both sealing and durability of the restoration in the long term. As a matter of fact, there are many studies on the bond strength of these materials to dentin.⁵¹⁻⁵³

In the study by Kaup *et al.*, in which ProRoot MTA and Bondentine's bond strength to dentin was examined, the SBS of the groups with 2 days,

7 days and 14 days was evaluated and the SBS of MTA was found to be significantly lower than Biodentine in the groups hardened for 2 days. In the study, it was noted that the SBS values of MTA and Biodentine, which were hardened for 7 days and 14 days, increased significantly compared to the 2-day values. Biodentine was found to be superior to ProRoot MTA in terms of the SBS values.⁵⁰ In the study by Jantararat *et al.*⁵¹, the SBS of MTA and Biodentine to dentin was evaluated at the end of 60 minutes and 24 hours, and the SBS values of MTA and Biodentine hardened for 24 hours were found to be significantly higher. Atmeh *et al.*¹¹ reported that when Biodentine is applied on dentin, it forms alkaline bonding with its high pH value and forms Biodentine lattices in the dentinal tubules, which shows dentin-like adhesion-like structures.

Additionally, in clinical conditions, there is a high risk of contamination of capping materials with blood or saliva during the applying of adhesive resins. It is known that contamination reduces the bond strength of restorative materials to dentin.^{32,33,54} Contamination of capping materials with blood are mostly studies evaluating the bond strength to dentin.^{52,55-57}

To the best of our knowledge, there is no study in the literature on the effect of blood contamination of pulp capping materials on the SBS to adhesive resins before permanent restoration. Therefore, in this study, the effect of blood contamination on SBS after different hardening times was investigated.

According to the findings of this study, blood contamination decreased the SBS value at different hardening times in all MTA groups ($p < 0.01$ for Cont. MTA24, 48, 96; $p < 0.05$ for Cont MTA72). In the Biodentine groups too, blood contamination significantly decreased the bond strength at all hardening times ($p < 0.01$ for Cont Biodentine 24, 48, 96; $p < 0.05$ for Cont Biodentine72). Therefore, the second hypothesis of our study, "Contamination of ProRoot MTA and Biodentine with blood after different hardening times (24, 48, 72, 96 hours) does not affect the shear bond strength of a self-etch adhesive", was not accepted. We could not

discuss the findings of this phase of our study, as we could not find a study examining the SBS of capping materials to adhesive resins after contamination.

In the literature, there are studies on the bond strength of contamination to dentin tissue at different stages of restorations.^{33,54} Although the bond strength to dentin was not investigated in our study, it has been reported in the studies that blood contamination reduces the bond strength to dentin and that it especially adversely affects the bond strength of dentin before and after the application of adhesive systems.^{56,57} Procedures such as washing and drying with cotton to remove blood contamination during restorative treatments could also create effects that reduce bond strength. Therefore, since exposure to blood is inevitable when applying CSB capping materials to the exposure surface in clinical conditions, prolonging the hardening time to at least 72 to 96 hours could increase the bond strength of pulp capping materials to both dentin and composite restoration.

In this study, the SBS of both MTA and Biodentine to adhesive resins after different hardening times and after they were contaminated with blood was investigated, and a decrease in SBS bond strength was observed in all hardening groups compared to the uncontaminated groups. In addition, the SBS values of Cont MTA48, Cont MTA72, Cont MTA96 groups in contamination groups are between and very close to clinically acceptable bond strength values. The longest hardening time within the limits of this study was 96 hours.

Whether longer hardening times would affect the bond strength could be investigated in future studies. Moreover, studies in which saliva or both contamination materials are used in addition to blood contamination may be more clinically meaningful. In this study, the SBS of ProRoot MTA and Biodentine with only one self-etch adhesive was examined, and differences that may occur with different adhesive systems were not observed. Furthermore, in this study, the early SBS values of MTA and Biodentine were examined. In vitro and clinical studies, in which

different adhesive resins of these CSB capping materials, which are widely used in the clinic, are used and long-term study results and early-term bond strength values are compared, will also contribute to the literature.

CONCLUSIONS

In this in vitro study, the effects of different hardening times (24h, 48h, 72h, 96h) and blood contamination of CSB pulp capping materials (Biodentine, MTA) on the SBS to a self-adhesive (Clearfil Liner Bond F) were investigated. Although hardening MTA and Biodentine at different times did not have a negative effect on the SBS, contamination with blood negatively affected the SBS of both capping materials. However, SBS values increased with the prolongation of the hardening time of MTA (72 and 96 hours) in the contaminated groups.

Beyond this study, which examines the hardening times of different capping materials and the effect of contamination on the bond strength, there is a need for studies examining the effect of longer hardening times and different contamination agents on the bond strength of both restorative materials and dentin tissue with different adhesive systems.

Kan Kontaminasyonunun Kalsiyum Silikat Esaslı Kuafaj Materyallerinin Makaslama Bağlanma Dayanımına Etkisi

ÖZ

Amaç: Bu çalışmanın amacı piyasada yaygın olarak kullanılan kuafaj materyalleri olan MTA ve Biodentin'in farklı sertleşme süreleri (24, 48, 72 ve 96 saat) sonrasında kanla kontaminasyonunun ve sertleşme sürelerinin bir self etch adeziv rezinle makaslama bağlanma dayanımına (MBD) etkisinin incelenmesidir. **Gereç ve Yöntemler:** Bu çalışma için 192 adet akrilik bloğa 5 mm çapında, 2 mm yüksekliğinde yuvalar hazırlandı. Blokların yarısı üretici talimatlarına göre hazırlanan ProRoot MTA ve diğer yarısı ise Biodentin ile dolduruldu. Tüm örnekler 4 farklı sertleşme süresine göre gruplara ayrıldı ve sertleştirildi. Sertleşme süreci tamamlandıktan sonra, her bir sertleşme süresinin grubu kontaminasyonlu ve kontaminasyonsuz olarak 2 alt gruba (n:12) ayrıldı. Kontamine olmayan gruplarda, örnek yüzeylerine bir self etch adeziv rezin (Clearfil Liner Bond) ve bir rezin

esaslı kompozit (Filtek P60) uygulandı ve polimerize edildi. Kontamine olan gruplarda ise örnek yüzeyleri 20 saniye kanla kontamine edildi. Örnekler yıkayıp kurulandıktan sonra adeziv rezin ve kompozit uygulaması yapıldı. MBD testlerinden sonra elde edilen veriler 2-yönlü ANOVA testine tabi tutuldu.

Bulgular: Kontamine olmamış gruplarda her bir kuafaj materyalinin zamana bağlı olarak farklı sertleşme süreleri arasında bağlanma dayanım değerleri arasında anlamlı bir fark görülmedi ($p>0,05$). 72 ve 96 saatlik kontamine olmayan gruplarda ProRoot MTA, Biodentin'e göre istatistiksel olarak daha yüksek makaslama bağlanma dayanımı gösterdi ($p<0,05$). Kan kontaminasyonu, ProRoot MTA ve Biodentine'in MBD değerlerinde önemli oranda azalmaya neden oldu ($p<0,05$). **Sonuçlar:** Bu çalışmada kan kontaminasyonunun kuafaj materyallerinin makaslama bağlanma dayanımını azalttığı tespit edilmiştir. Bu nedenle restoratif tedavilerden önce, kuafaj materyallerinin sertleşme sürelerinin uzatılması ve kanla kontaminasyonu mümkün olduğu kadar önleyici klinik tedbirlerin alınması önerilir. **Anahtar Kelimeler:** Pulpa Kaplaması; MTA; Biodentin; Kan Kontaminasyonu; Makaslama Bağlanma Dayanımı.

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