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Original research

The effects of caries removal techniques on volume-loss percentage and microtensile bond strength*

Özlem Kanar[1], Dilek Tağtekin[1], Bora Korkut[1], Funda Yanıkoğlu[2]

Purpose
This study aimed to calculate the volume loss percentages (VLP) regarding the ICDAS II system and various caries removal techniques (CRT) and to assess the microtensile bond strength (µTBS) in terms of VLP following CRT.

Materials and Methods
Three-dimensional data of human extracted molars were acquired with an extraoral dental scanner (Ineos x5, Dentsply Sirona) before and after the caries removal. Each ICDAS score group (0,3,4 and 5) was divided into four subgroups according to the CRT: stainless steel bur (Group S), ceramic bur (Group C), tungsten carbide bur (TCB) and air abrasion (AA) with bioactive-glass (Group TB), and TCB and AA with Al_2O_3 (Group TA). Pre and post-caries removal data were overlaid in a 3D modeling software and were volumetrically measured (n=10). Following the restoration, samples were prepared with non-trimming technique and subjected to microtensile testing.

Results
ICDAS II scores were found related the VLP (p< 0.001) and the µTBS (p<0.001). CRT was not effective on VLP (p=0.110), whereas CRT type was significant on µTBS (p<0.001). In Group TA, a strong negative correlation was observed between the µTBS and the VLP for ICDAS 5 score (r=-0.919; p=0.027).

Conclusion
ICDAS II can provide a preliminary indication for the amount of VLP and reduction in µTBS following caries removal. The use of 29 µm Al_2O_3 with air abrasion in deep caries removal may improve µTBS while potentially reducing the VLP.

Keywords: *Air abrasion, caries removal technique, dental scanner, microtensile bond strength, volume loss*

Introduction

The ICDAS II system is a modification of the caries classification system in which the diagnosis of caries lesions in permanent teeth is visually graded regarding the depth with reasonable accuracy and reproducibility. It enables precise clinical decisions for the identification of dental caries and also facilitates the epidemiological research field (1). Studies have demonstrated the correlation between the ICDAS II scores for caries classification and histologic, microscopic, radiologic, and fluorescence alterations (2, 3).

The concept of minimally invasive dentistry recommends an approach that respects tissue integrity by removing only the infected layer of the caries lesion (4). In a systematic review, it was reported that the use of complete caries removal (CCR) did not provide any advantage over the removal of soft dentin (5). Selective caries removal (SCR) is known to cause fewer postoperative complications than CCR (6). While caries progression, one of the clinical success criteria, is higher in CCR (7), some studies men-

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ORCID IDs of the authors: Ö.K. 0000-0002-9701-5860; D.T. 0000-0002-2675-1764; B.K. 0000-0001-6360-9436; F.Y. 0000-0001-5708-1030

[1]Department of Restorative Dentistry, Faculty of Dentistry, Marmara University, Istanbul, Türkiye

[2]Department of Restorative Dentistry, Faculty of Dentistry, Istanbul Kent University, Istanbul, Türkiye

Corresponding Author: Özlem Kanar

E-mail: ozlem.kanar@marmara.edu.tr

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tioned no difference between SCR and CCR (8).

Rotary instruments and bur systems are frequently used in clinical routines and are among the most useful methods for caries removal. Recently, ceramic burs have been introduced to protect intact dental structures and to remove soft and carious dentin with increased efficiency (9). Air abrasion systems have also been recommended to improve the bond strength (10-12). The application of aluminum oxide particles to the dentin surface has been suggested to contribute to adhesion by forming micro-retentive structures, but there are also studies with contradictory outcomes (11). Aluminum oxide abrasive particles, frequently used to remove tooth structure, have an average size of 27, 29, and 30 µm.

Bioactive glass particles (BAGs), which have found a place in different fields of medicine and dentistry, have recently been used in restorative dentistry to treat dentin hypersensitivity, enamel remineralization, and direct pulp capping. Small particles of bioactive glasses are available for these devices for application by air abrasion. BAGs can reach smaller areas within the body due to their small size. Especially in cases where dentin regeneration is the main objective, using active ingredients with a diameter of 2-3 µm is advantageous for easier penetration through the tubules (13, 14). Some studies have reported that BAGs selectively remove caries lesions on the enamel surface without damaging the sound structures (15, 16). However, limited research shows whether BAG is effective on dentin bonding positively or negatively (17, 18).

Fracture of restoration is one of the most common causes of tooth failure, with excessive loss following caries removal. On the other hand, the larger and deeper the restoration volume, the more difficult adhesion results due to less inter-tubular dentin on the surface, forming an effective hybrid layer (19).

Digital three-dimensional systems, which accelerate clinical workflow by eliminating unnecessary procedures, have become important in digital dentistry (20). Intraoral scanners process the images in dental arches in a short time, allowing data to be processed and recorded using appropriate digital software. CAD/CAM systems have also been provided to quantitatively calculate the volume loss of teeth using three-dimensional methods without radiation in a short time (21, 22).

Because substantial tissue loss will affect the fracture strength and bonding to dentin (23, 24), the method clinicians prefer for caries removal might be effective on the volumetric loss and the dental adhesion. Considering that the depth of caries may also affect the clinician's restorative approach (25), it might be better to evaluate all these factors with the ICDAS scoring. Bond strength was previously examined in deep, caries-affected (26) or superficial dentin (18) tissues but was never correlated with the volume loss. Moreover, no previous study has evaluated how volume loss percentages affect the dentin bond strength after caries removal.

Therefore, the present study was aimed to (1) investigate the effect of CRTs on VLPs, (2) investigate the effect of CRTs on µTBS, (3) investigate the correlation between the quantitative VLPs and the µTBS, (4) investigate the correlation of ICDAS II with VLPs and µTBS. The null hypotheses of the study were considered as follows: there is no relation between ICDAS II scores and the VLPs after caries removal, the type of CRT is not effective on VLP, there is no relation between ICDAS II scores and the µTBS, the type of CRT is not effective on µTBS, and there is no relation between the VLP and the µTBS, respectively.

Materials and Methods

Preparation of experimental groups

This project has been reviewed and approved by the Ethical Committee of Marmara University, Faculty of Medicine (Date: 16.04.2021 Protocol no: 09.2021.494). Permanent human first and second molars, which were extracted within the last six months for periodontal and restorative reasons, were involved in the study and kept in 0.1% thymol solution. The teeth selected had no former restoration, abrasive lesions, fractures, or cracks on the surface, and were absent of caries on the buccal, cervical, and proximal surfaces. A total of 132 teeth with ICDAS scores of 0, 3, 4, and 5 were included in the study and were classified according to the ICDAS II system after cleaning the surfaces. The ICDAS criteria are defined in Table 1. All the teeth were taken into square molds, and cold acrylic was applied 2 mm below the cementoenamel junction. For initial volumetric measurements, teeth with significant cavitation were modeled with pink wax to represent intact teeth.

The study consists of two parts. For the VLP calculation, 120 teeth having ICDAS scores 3,4 and 5 were included in the study. Details of the study design are schematized in Figure 1. The teeth with ICDAS scores of 3, 4, and 5 were divided into four subgroups (n=10 for each) according to the caries removal techniques: stainless steel bur (Group S), ceramic bur (Group C), tungsten carbide bur and air abrasion with bioactive glass particles (Group TB), and tungsten carbide bur and air abrasion with 29 µm Al_2O_3 particles (Group TA), (n=10 for each).

Initial scanning and data acquisition

Regarding the initial data acquisition (before caries removal), the samples were coated with CAD/CAM spray (CRM Matte Spray, CRM Kimya, Türkiye) for scanning with Ineos X5 (Dentsply Sirona, Germany). The arm of the scanning device was inclined at 45-70 degrees to scan the entire tooth surface. All scans were performed in "single die" mode.

Caries removal techniques

Occlusal caries lesions were removed for the teeth in the score groups ICDAS 3, 4, and 5. An access cavity was prepared with a coarse grit diamond bur (Sorensen, Cotia, SP, Brasil) in all groups to reach the center of the lesion. Four different techniques performed removal of the dentin caries lesions. The burs used in each group were changed for every 5 teeth to ensure standardization.

Regarding Group S, stainless steel round burs (Meisinger, Germany) with size 14 or 16 and 6-8 blades were used to remove the caries lesions, depending on the width of the lesion. All infected tissues were removed. Visual and tactile examination was used to ensure the complete removal of caries (27). Regarding Group C, infected dentin was removed at a slow

***Table 1**. ICDAS scoring for occlusal surfaces.*

Code	Description
0	Represents a sound occlusal surface. After air drying for 5 seconds, no change in the translucency of the enamel.
1	The tooth appears intact when moist, but after 5 seconds of air drying, a carious opacity or discoloration (white or brown lesion) limited to pits and fissures is observed on the occlusal surface.
2	Opaque lesion (white spot lesion) and/or brown discoloration is observed on the occlusal surface whether moist/dry conditions, which are wider than fissure/fossa.
3	Localized enamel breakdown with no visible dentin or underlying shadow; surface enamel has lost continuity and fissures are widened.
4	Characterized by the dark appearance on the occlusal enamel tissue, due to the dark reflection from the dentin. Localized breakdown of the enamel is present or absent.
5	There is an exposed dentin with the enamel cavitation. Less than half of the occlusal tooth surface is affected by caries.
6	There is a significant cavity with visible dentin. More than half of the crown is affected by caries.

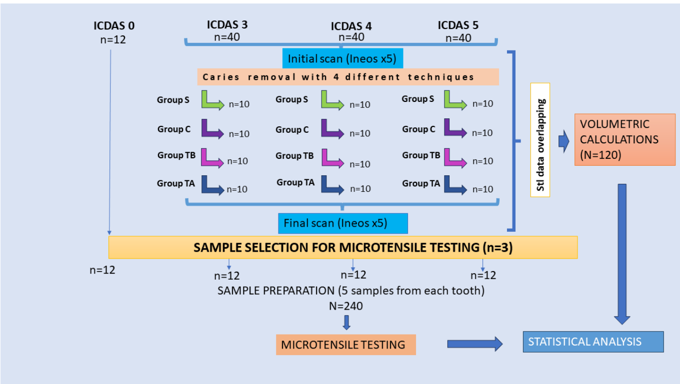

***Figure 1.** Flowchart of the study: Group S: Stainless steel bur, Group C: Ceramic bur, Group TB: Tungsten carbide bur and air abrasion with bioactive glass particles, Group TA: Tungsten carbide bur and air abrasion with 29 µm Al_2O_3 particles.*

speed with a size #14 or #16 ceramic round burs (Cerabur, Komet, Germany) according to minimally invasive principles. The point at which the ceramic bur stopped while removing the caries was considered the endpoint of the preparation. Regarding Group TB, caries removal was performed with tungsten carbide round burs (Frank Dental, Germany) numbered 16, 18, and 21 (Frank Dental, Tegernsee, Germany) at a slow speed. Then, an air abrasion system (Aquacare Single, Velopex, UK) was used to deliver bioactive glass particles (Slyc, Velopeks, UK) with ethanol. The 0.6 mm diameter nozzle was located approximately 5 mm from the dentine surface with a pressure of 0.4 MPa. The application time was set to 5-20 seconds. Regarding the Group TA, caries removal was performed with tungsten carbide burs number 16, 18, and 21 (Frank Dental Gmbh, Tegernsee, Germany) at a slow speed like the previous group. The remaining lesions were air abraded with 29 µm Al_2O_3 particles (Aquacare, Velopex, UK). The application was performed similarly to the last group, between 5-20 seconds, at a pressure value of 0.4 MPa and a distance of 5mm.

Secondary scanning and data acquisition

All samples were again coated with CAD/CAM spray, and the scans were performed using the same angles and scanning mode as the initial. Both scan data of each tooth were exported in .stl format and recorded with particular scores and numbers.

Volumetric calcutations

The .stl data of all samples at baseline and after caries removal were transferred to a 3D modeling software program (Meshmixer 3.5, Autodesk, USA). The primary and secondary 3D models (in mm^3) of each sample were overlapped regarding the tubercle cusps.

Microtensile testing

For microtensile testing, 36 samples were collected from ICDAS 3, 4, and 5 scores and CRTs subgroups (n=3 for each). Twelve teeth with an ICDAS score of 0 were also included in the study to be used as a control group in the microtensile testing. Thus, 48 teeth were subjected to microtensile testing including the Group control. Tubercle cusps of the teeth were removed with a precision cutting device to obtain a flat surface. Smooth and intact dentin surfaces were roughened by the mentioned four caries removal techniques to mimic the caries removal procedure. The cavity surfaces were flattened using the selected method regarding the deepest point. Cavity borders were expanded to 5-7 mm in the bucco-lingual direction and 6-8 mm in the mesio-distal direction.

A universal adhesive agent (Prime&Bond Universal, Dentsply Sirona, USA) was applied to the cavity by the manufacturer's instructions and polymerized with a led light-curing device (Smart-Lite Pro, Dentsply Sirona, USA) with an irradiation of 1200 mw/cm^3 for 20 seconds. Then, the restorations were completed by curing the resin composites (Neospectra ST, Dentsply Sirona, USA), which increased in thickness by 2 mm. Then, the teeth were aged by soaking in water at 37 °C for 24 hours in an incubator (ZWYR-240, Labwit, Australia)

First, acrylic blocks were placed in the precision cutting device (Isomet 1000, Buehler, USA). 1 mm wide blade incisions were made in the mesiodistal and buccolingual directions. Resin-dentin bars with a surface area of 1 mm^2 were then obtained. From each tooth, five samples of appropriate size were selected for the micro tensile testing, and a total of 240 samples were obtained in terms of ICDAS 0, 3, 4, and 5 score groups (n=15 samples in each subgroup. Each sample was fixed to the micro tensile testing machine (Microtensile Tester, Bisco, Schaumburg, USA) with cyanoacrylate adhesive agent (Pattex, Henkel, Dusseldorf, Germany) and subjected to tensile force at a crosshead speed of 0.5mm/min until the sample is broken. Photos of the dental scanner, software interface, and microtensile bond strength test are shown in Figure 2.

After µTBS testing, dentin and resin test surfaces were investigated with a stereo-light microscope (Leica Microscopy Systems, Wetzlar, Germany) at 10X magnification. The dentin surfaces of the debonded samples were also investigated to determine the failure modes. The failure modes were classified as adhesive failure if 100% of the failure was between dentin and bonding resin, cohesive failure if 100% was in the composite resin or dentine, or mixed failure if the failure was partially adhesive and partially cohesive.

Scanning electron microscope analysis

Two samples from each group were processed for scanning electron microscope (SEM) examination. Samples were coated with gold in a vacuum cold sputter (SC7620, Laughton, Sussex, UK). The adhesive interfaces were examined under 1000x and 3000x magnifications. SEM images are given in Figure 3.

Statistically analysis

Data were analyzed with IBM SPSS V23 (IBM SPSS, Armonk, NY, USA). Compliance with normal distribution was examined by the Shapiro-Wilk test. Generalized linear models were used to compare bonding values according to the caries removal technique and ICDAS scores, and multiple comparisons were examined with the Tukey HSD test. Pearson's correlation coefficient was used for normally distributed data, and Spearman's rho correlation coefficient was used

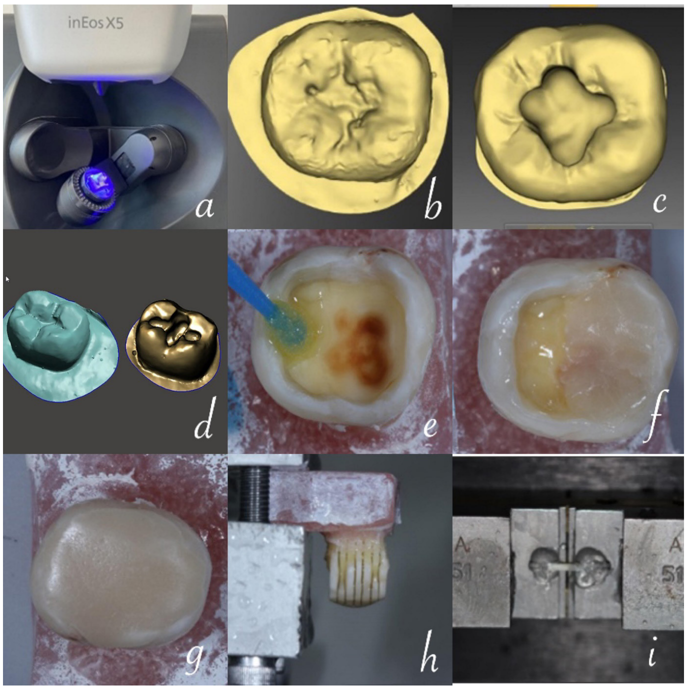

Figure 2. *a) Ineos X5 scanning device b) initial occlusal view of the 3D model c)secondary occlusal view of the 3D model d) transfer of initial and secondary data to Meshmixer modeling programme e) adhesive application before restoration f) composite layering g)final occlusal view of the restored sample h)sample preparation for the microtensile testing i) fixing the sample to the microtensile tester.*

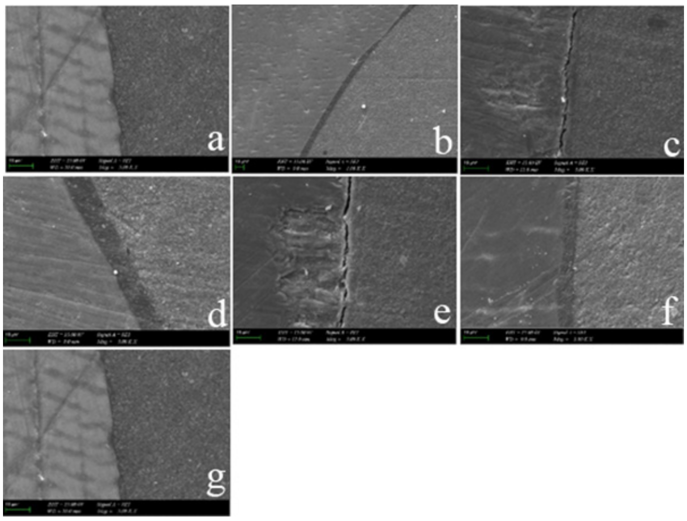

Figure 3. *SEM examination of dentin and adhesive interface: The right of each picture shows the restorative material, and the left shows the dentin. a)Group S: 1000X magnification, b)Group S: failure in dentin-adhesive interface, 3000X magnification, c)Group C: thicker adhesive interface, 3000X magnification, d) Group C: failure in dentin-adhesive interface, 3000X magnification, e)Group TB: Uniform but thicker adhesive interface, 3000X magnification, f) Group TB: Irregularity in dentin surface and thinner adhesive layer, 3000X magnification, g) Group TA: air abraded caries affected dentin-thin layer of adhesive interface.*

for non-normally distributed data. Analysis results were presented as mean ± standard deviation for quantitative data and frequency (percentage) for categorical data. Deem significance was set at $p<0.050$.

Results

According to the Q Robust Anova test, the caries removal technique was not considered significant on the median values of VLP ($p=0.110$), while the ICDAS II scoring was significant on VLP ($p<0.001$) (Table 2). The median VLP was 5% for ICDAS score 3, 11% for ICDAS score 4, and 14% for ICDAS score 5 (Table 3), and all of the scores showed significant differences. The interaction of CRT and ICDAS II scoring was statistically significant ($p=0.018$).

The maximum median VLP was 18.5% in the Group S and ICDAS 5 score (Group 5S) and in the Group S and ICDAS 5 score (Group 5C). The lowest median VLP was obtained as 4% in Group TB and in the ICDAS 3 score (Group 3TB).

The CRT was considered significant among all groups regarding the µTBS ($p<0.001$). (Table 4) The mean µTBS was 19.94 MPa in Group S, 16.4 MPa in Group C, 15.03 MPa in Group TB, and 25.17 MPa in Group TA, respectively (Table

Table 2. *Comparison of volume loss percentage (VLP) values according to caries removal techniques (CRT) and ICDAS scores: Q Robust Anova Test*

	Q	p
Caries removal technique	2,012	0,110
ICDAS scoring	9,615	**<0,001**
Caries removal technique * ICDAS scoring	15,257	**0,018**

5). ICDAS scoring was also considered significant on µTBS ($p<0.001$). While ICDAS 0 and 3 groups and ICDAS 4 and 5 groups showed similar µTBS, µTBS was superior in ICDAS 0 and ICDAS 3 groups to ICDAS 4 and 5 groups.

The CRT and ICDAS score interaction on µTBS was also considered significant ($p<0.001$). The highest mean value for the ICDAS 5 score was obtained in Group TA, with 26.45 MPa, while the lowest was observed in Group C, with 14.29 MPa (Table 5).

According to the correlation analysis between µTBS and VLP, the relation between µTBS and VLP was not significant in Groups 3S, 3C, 3TB, 4S, 4C, 4TB, 5S, 5C, 5TB, 3TA and 4TA, respectively ($p>0.05$). In Group 5TA, a statistically significant negative and very high-level correlation was observed between µTBS and VLP ($r=-0.919$; $p=0.027$) (Table 6).

Regardless of CRT, there was no significant correlation between ICDAS 3 µTBS and VLP ($p=0.366$). In the ICDAS 4 score, a statistically significant positive moderate correlation was observed between µTBS and VLP ($r=0.543$; $p=0.013$). In the ICDAS 5 score, a significant negative moderate relation was found between µTBS and VLP ($r=-0.498$; $p=0.026$) (Table 6).

The distribution of failure modes among the samples is given in Table 7 as percentages (%) and numbers. All groups had adhesive failures, while the highest number of adhesive failures was observed in Group C. Group TA presented fewer adhesive failures than all groups.

Discussion

The extent of the lesion may, in many cases, influence the clinician's approach to caries removal. Therefore, when removing a deep caries lesion, clinicians may need to leave some affected dentin in the center and create a peripheral seal zone to minimize microleakage (28). The design of our study, based on the ICDAS scoring of caries removal techniques, was born from this approach.

In the present study, teeth with ICDAS 1 and 2 scores were excluded, for which there was no indication for interventional treatment and teeth with ICDAS 6 scores in which more than half of the crown was destroyed, and the pulp was generally involved. Regarding proximal caries, the extent and progression of these lesions are more variable. In addition, removing proximal caries also requires removing intact marginal ridges to reach the center of the carious lesion. Moreover, some susceptible tissue should be removed following the caries removal before the restorative phase. Because proximal caries result in a greater volume loss than occlusal caries, the authors considered that the difference between caries removal methods might not be significant. Consequently, proximal and cervical caries were excluded to ensure standardization in volumetric measurements.

Table 3. *Descriptive statistics of VLP according to Caries removal techniques.*

Caries removal technique	ICDAS Score			Total
	3	4	5	
Group S	7 (2-19)^{ABC}	14,5 (9 - 18)^{A}	18.5 (12-39)^{ABC}	14.5 (2-39)
Group C	5 (3- 20)^{BC}	10.5(4 - 13)^{ABC}	18.5 (5- 31)^{AB}	10.5 (3-31)
Group TB	4.5 (2 - 7)^{C}	8.5 (3 - 13)^{ABC}	11(5 -21)^{ABC}	7 (2-21)
Group TA	4 (2-7)^{C}	9.5 (3-21)^{ABC}	10.5 (8- 23)^{ABC}	8 (2-23)
Total	5 (2-20)^{a}	11(3-21)^{b}	14 (5-39)^{c}	9 (2-39)

Median (minimum-maximum); a-c: No difference between main effects with the same letter; A-C: No difference between interactions with the same letter

Table 4. *Comparison of caries removal techniques and ICDAS scoring according to µTBS*

	SS	DF	MS	F	p	PES
Caries Removal Technique (CRT)	3.652.700	3	1.217.560	151.510	**<0.001**	0.670
ICDAS score	233.900	3	77.970	9.700	**<0.001**	0.115
CRT x ICDAS score	334.900	9	37.210	4.630	**<0.001**	0.157

F: Analysis of variance test statistic. SS: Sum of squares. DF: Degrees of freedom. MS: Mean squares. PES: Partial eta squared. R2 = 70.11%. Adjusted R2 = 68.10%

Table 5. *Descriptive statistics of ICDAS scoring and caries removal techniques on µTBS*

Caries Removal Technique	ICDAS score				Total
	ICDAS 3	ICDAS 4	ICDAS 5	ICDAS 0	
Grup S	22.23 ± 2.02^{F}	17.47 ± 2.6^{F}	17.75 ± 4.23^{F}	20.71 ± 4.43^{EF}	19.54 ± 3.95^{d}
Group C	19.05 ± 3.56^{AB}	15.27 ± 2.7^{AB}	14.29 ± 2.26^{A}	16.98 ± 2.78^{A}	16.4 ± 3.34^{a}
Group TB	14.45 ± 3.95^{CDE}	14.69 ± 2.56^{F}	14.33 ± 2.05^{F}	16.64 ± 2.84^{EF}	15.03 ± 3.01^{c}
Group TA	23.89 ± 1.73^{BC}	24.19 ± 1.73^{DEF}	26.45 ± 1.69^{DEF}	26.13 ± 2.04^{BCD}	25.17 ± 2.1^{b}
Total	19.91 ± 4. 64^{a}	17.9 ± 4.48^{b}	18.2 ± 5.68^{b}	20.12 ± 4.92^{a}	19.03 ± 5.02

A-F: No difference between interactions with the same letter. a-d: No difference between main effects with the same letter. Mean ± s. Deviation

Table 6. *Examination of the correlation between μTBS and VLP, regarding and regardless of the caries removal techniques and ICDAS scoring.*

Caries removal technique	ICDAS scoring	r	p
Group S	ICDAS 3 μTBS – VLP	-0,135[a]	0,829
	ICDAS 4 μTBS – VLP	-0,710[a]	0,179
	ICDAS 5 μTBS – VLP	0,669[a]	0,217
Group C	ICDAS 3 μTBS – VLP	0,024[a]	0,696
	ICDAS 4 μTBS – VLP	0,679[a]	0,208
	ICDAS 5 μTBS – VLP	-0,283[a]	0,644
Group TB	ICDAS 3 μTBS – VLP	-0,188[a]	0,762
	ICDAS 4 μTBS – VLP	0,263[a]	0,669
	ICDAS 5 μTBS – VLP	0,770[a]	0,128
Group TA	ICDAS 3 μTBS – VLP	0,346[a]	0,568
	ICDAS 4 μTBS – VLP	-0,142[a]	0,820
	ICDAS 5 μTBS – VLP	-0,919[a]	**0,027**
Total	ICDAS 3 μTBS – VLP	0,214[a]	0,366
	ICDAS 4 μTBS – VLP	0,543[b]	**0,013**
	ICDAS 5 μTBS – VLP	-0,498[a]	**0,026**

[a]Pearson's correlation coefficient, [b]Spearman's rho correlation coefficient

Table 7. *Distribution of the failure modes among the caries removal techniques*

Caries removal technique	Adhesive failure	Cohesive failure	Mixed failure
Group S	57 (24%)	3 (13%)	0 (0%)
Group C	60 (25%)	0 (0%)	0 (0%)
Group TB	52 (21.6%)	6 (2.5%)	2 (0.8%)
Group TA	51 (%21.25)	8 (3.3%)	1 (0.4%)

Another procedure we performed to ensure standardization in volumetric measurements was to create a notch at the level of the cementoenamel border after embedding them in cold acrylic. In this way, the crown heights of all teeth were standardized. The first 3D model (before caries removal) was cut at the marked point and then fixed to the coordinate plane during the overlapping process. After taking the tubercle cusps of the first model as a reference, the overlapping was performed, and since the coordinate plane where the model was cut was already fixed, the second model was also cut from the same plane, thus avoiding errors in volume measurement.

In cases where microtensile bond strength is evaluated, two cavity preparation methods have been used. The first is to obtain a flat dentin surface by removing the tubercles with a precision cutting blade and placing the restoration into the crown body using a mold (29). The second method is to create a standard inlay cavity preparation. Since we assessed the effect of caries removal techniques and ICDAS scores on VLP in our study, we used the second method. In addition, to the best of our knowledge, there has been no previous study on ICDAS scoring and μTBS. Therefore, we performed cavity preparations concerning the deepest point of each tooth sample to standardize ICDAS scores.

Two techniques are used in sample preparation for microtensile testing: trimming and non-trimming techniques (30). In the trimming technique, a cross-section is usually taken from the tooth sample, and then the area around the point to be examined is removed using a bur. Grinded samples are prepared in an hourglass shape. However, defects can easily form at the interface if sample preparation is not done carefully. With this technique, the time taken for sample preparation is even longer, and cracks may appear at the interface during trimming, which affects the test result (30). Therefore, it may not be possible to calculate the μTBS accurately. In the non-trimming technique, a large number of samples can be prepared from one tooth, and the sample preparation process is relatively easy compared to the trimming technique. As a result, in the present study, the samples prepared using the non-trimming technique.

Our findings regarding the VLP measurements revealed significant differences between ICDAS scoring. Our first hypothesis was rejected as VLPs increased gradually with the increase in scoring. Previously, Yanıkoğlu *et al*. (21) calculated the caries-related volume loss with increasing ICDAS scores in premolars, following caries removal. Similarly, they concluded that there was a VLP consistent with an increase in ICDAS score. However, they reported a volume loss of 12% for the ICDAS 3 score, 14% for the ICDAS 4 score, and 30% for the ICDAS 5 score. These VLPs seem higher than in our study. It is thought that this result may be due to our groups only consisting of occlusal caries lesions to provide standardization in our research. Besides, the researchers were senior dental students, and using caries detection dyes may have resulted in the excessive removal of caries lesions, contrary to our study.

Various methods have been recommended to determine the endpoint of caries removal. Although such caries detection dyes are claimed to be effective as a clinical assessment to distinguish between infected and caries-affected dentin, they seem to stain demineralized organic matrix rather than bacteria. For example, McComb *et al*. (31) suggested that the dye had a low specificity and recommended using other clinical assessment methods, such as visual and tactile examinations, instead. A commonly used in some studies to determine the cutoff point of carious tissue removal is laser fluorescence (DIAGNOdent Pen) measurements (29, 32). Nevertheless, Neves *et al*. (27) reported that laser fluorescence measurements from the base of the cavity were affected by the discoloration of the residual dentin, and therefore, their use in the endpoint of caries removal was questionable. The visual and tactile examination was used in this investigation since it is the most extensively used clinical criteria to decide the caries removal endpoint. As a result, the visual and tactile examination was used in this assessment since it is the most extensively used clinical criteria to decide on finishing the caries removal and preventing eventual excessive volume loss.

As a result of the present study, VLPs among the caries removal techniques were not considered significant (p=0.110) (Table 2). Therefore, our second null hypothesis is accepted. However, the results shown in Table 2 are interesting regarding the distribution of VLPs. When examining the results, it should be noted that the teeth used are not standard plastic teeth. Since the statistical analysis was calculated based on the VLP, differences in the initial volume of the teeth might have affected the results. Moreover, the data are not normally distributed. A numerical decrease in VLPs was observed when tungsten carbide burs were combined with air abrasion methods. In addition, the interaction between CRT and ICDAS scoring is considered significant (p=0.018). A statistically significant difference between Group 4S and Group 3TB was observed. Significant volumetric changes above one score might indicate that CRT may be selected based on the ICDAS scoring.

Şeker *et al*. (33) compared the caries-related volume loss using stainless steel round bur and ceramic bur. Conversely, they reported a significantly higher VLP in Group S compared to Group C. In this case, the approaches to caries removal should be clarified. The present study aimed to measure CRT abrasiveness and dentin destruction in all study groups using a minimally invasive approach. However, in the study, as mentioned above, the complete caries removal technique was used in the group using SSRB, and all colored layers of caries were removed. This contrary result might be due to the difference in our approach to caries lesions. In addition, the fact that Şeker *et al*. (33) used an intraoral scanner for volume calculation and did not standardize the scanning conditions might have caused this difference.

In previous studies, quantitative assessments of caries removal were performed by using micro-computed tomography or cone beam computed tomography (34, 35). Although micro CT also offers advantages such as assessing mineral density and calculating enamel and dentin volume separately, the cost of these devices is quite high and difficult to access. In addition, considering that it does not contain ionizing radiation and can be calculated in a shorter time than Micro CT, it can be said that it is useful to use scanners to measure volume loss. Furthermore, an extraoral scanner, which records at a constant speed, distance, and certain angles and whose accuracy is superior to intraoral scanners (36), was used in our study. Although several studies in the literature use intraoral scanners to calculate volume loss (21, 33), the use of an extraoral scanner for volumetric assessment of caries removal is the novelty of our study.

Regarding the µTBS, ICDAS 0 and ICDAS 3 groups revealed superiority to ICDAS 4 and 5 groups. However, there was no significant difference between ICDAS 0-3 and ICDAS 4-5. Hence, hypothesis 3 might be partially rejected. Since score 3 is an enamel caries in the initial stage, most of the tested samples consist of intact dentin as in ICDAS score 0, and therefore, this result is reasonable. Samples obtained from ICDAS 4 and 5 groups mainly consist of caries-affected and deep dentin. Considering the difficulty of bonding to deep and caries-affected dentin in previous studies, it can be concluded that this part of our study is compatible with general literature knowledge.

Our present study showed a significant difference among the CRT regarding the µTBS (p<0.001). Among the CRTs, the maximum µTBS was observed in the Group TA, followed by the Group S, while the lowest µTBS was obtained in the group TB. Therefore, our fourth null hypothesis should be rejected. Based on the VLP results of Group S, it is thought that it exposed more sound dentin, and consequently, the bonding was found to be higher than Group C. Superiority in Group TA might be since removing the smear layer with Al_2O_3 particles and creating micro-retentive structures on the dentin surface.

Regarding the SEM evaluation, in most samples, Group TB presented a smoother dentin surface than Group TA, while some samples showed gap formations between the adhesive and dentin interface. Both groups, TA and TB, exhibited good adhesive integrity in most evaluated samples. Additionally, group TA showed a thinner adhesive layer in sound dentin while causing more irregularities. Furthermore, no gap formation was observed at the caries-affected - alumina abraded dentin surface and adhesive interface. Although the SEM evaluation explains the higher µTBS of Group TA, the decreased µTBS of Group TB, despite the excellent interface integrity in its overall distribution, might be considered that chemical factors come into play and affect the adhesive material differently. Spagnuolo *et al*. (18) evaluated the µTBS of application of BAG and Al_2O_3 particles to intact dentin surface using air abrasion and bonding to a universal adhesive in self-etch mode. After 24 hours of artificial saliva storage, the group Al_2O_3 showed higher µTBS than the group BAG. They suggested that this was due to the alkalinity of BAGs. Similarly, there is a significant difference between Group 0TA and Group 0TB (in ICDAS 0 score), which is taken as a control. As Spagnuolo *et al*. (18) assumed, this might be due to the higher pH of BAGs affecting the immediate performance of the universal adhesive while used in self-etch mode. However, they reported that after prolonged artificial saliva storage, BAG maintained the interface integrity, while the µTBS significantly decreased in the group Al_2O_3. Therefore, considering its less cytotoxicity than Al_2O_3 particles (18), it might be concluded that more studies on the long-term performance of BAGs are needed.

Banerjee *et al*. (37) offered air abrasive BAG for producing negotiate preparations because the air-abrasive stream cutting from the tip can follow a much narrower path through the enamel than the narrowest rotary bur. However, the use of BAG may be limited to demineralized enamel due to its lower microhardness than alumina particles, and its use in removing deep caries may require the creation of an access cavity. Since our study design aimed to remove less intact structures and improve µTBS, this method, which is reported to be an effective method for removing demineralized enamel, was modified for caries-affected dentin. A limitation of air abrasion with BAG was that, during our research, caries removal took longer due to its less aggressive cutting properties than alumina.

One of the most important findings of our study is in Group 5TA, which presented increased µTBS with decreased volume loss (Table 6). Therefore, our fifth null hypothesis might be rejected. Although volumetric assessment revealed no difference among the caries removal techniques, the significant decrease of VLP in Group TA accompanied an increase in µTBS. This result might provide a clue to clinicians in managing deep dentin caries. Following removing the soft dentin with a tungsten carbide bur, it might be helpful for clinicians to benefit from an air abrasion device to deliver the alumina particles to reduce volume loss and strengthen µTBS. Considering improved µTBS in Al_2O_3 application by air abrasion, both Group Control (ICDAS 0) and the caries-affected dentin might indicate the utility of alumina abrasion not only in occlusal cavities but also in any clinical condition where additional bond strength is required.

The present study is not devoid of limitations. A single type of universal adhesive was used in all groups and performed in self-etching mode. Understanding how acid etching affects the adhesive-air abraded dentin interface with various particles may improve clinicians' management of deep caries and enhance their prediction of outcomes at clinical follow-up. Therefore, further studies should be performed, including different adhesive systems and etching modes.

Conclusion

Within the limitations of this *in vitro* study, caries scoring with the ICDAS II system may provide clinicians with preliminary information about the cavity depth according to the structural volume remaining after caries removal. Due to the increased volume loss, significant decreases might occur in dentin bonding. However, to overcome this disadvantage, clinicians might prefer using air abrasion systems following the rotary instruments, especially alumina particles. This approach might reduce potential postoperative complications, especially in deep caries lesions. Further studies should be carried out on using bioactive glasses in adhesive procedures and their effect on the dentin surface and the adhesive systems.

Türkçe öz: *Çürük uzaklaştırma yöntemlerinin hacim kaybı ve bağlanma üzerindeki rolü. Amaç: Bu çalışmada, ICDAS II sistemi ve çeşitli çürük uzaklaştırma yöntemlerine (CRT) ilişkin hacim kaybı yüzdelerinin (VLP) hesaplanması ve CRT sonrası mikrogerilim bağlanma kuvvetinin (µTBS) VLP açısından değerlendirilmesi amaçlanmıştır. Gereç ve Yöntem: İnsan çekilmiş büyükazı dişlerinin, çürük uzaklaştırma öncesinde ve sonrasında ağız dışı dental tarayıcı (Ineos x5, Dentsply Sirona) ile üç boyutlu kayıtları alındı. Her bir ICDAS skor grubu (0,3,4 ve 5) CRT'ye göre dört alt gruba ayrıldı: paslanmaz çelik frez (Grup S), seramik frez (Grup C), tungsten karbit frez (TCB) ve biyoaktif cam ile air abrazyon (Grup TB), ve TCB ve* Al_2O_3 *ile air abrazyon (Grup TA). Çürük uzaklaştırma öncesi ve sonrası kayıtlar 3D modelleme yazılımında (Meshmixer, Autodesk, ABD) çakıştırıldı ve hacimleri hesaplandı (n=10). Restorasyonun ardından non-trimming tekniğiyle hazırlanan numuneler mikrogerilim testine tabi tutuldu. Bulgular: VLP ve µTBS, ICDAS II skor gruplarına göre anlamlı farklılıklar gösterdi. (p<0.001) . CRT tipi VLP üzerinde etkili olmazken (p=0.110), µTBS üzerinde ise CRT etkisi anlamlıydı (p<0.001). Grup TA'da µTBS ile ICDAS 5 skor grubundaki VLP arasında güçlü negatif korelasyon görüldü (r=-0,919; p=0,027). Sonuç: ICDAS II sistemi, çürüğün uzaklaştırılmasını takiben hacim ve bağlanma dayanımındaki değişiklikleri öngörmeyi sağlayabilir. Derin çürüklerin uzaklaştırılmasında air abrazyon ile 29 µm* Al_2O_3*'ün kullanılması bağlanmayı arttırırken hacim kaybını azaltabilir.Anahtar Kelimeler: air abrazyon, çürük uzaklaştırma yöntemi, hacim kaybı, mikrogerilim bağlanma kuvveti.*

Ethics Committee Approval: This project has been reviewed and approved by the Ethical Committee of Marmara University, Faculty of Medicine (Date: 16.04.2021 Protocol no: 09.2021.494).

Informed Consent: Participants provided informed consent.

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Author contributions: OK, DT participated in designing the study. OK participated in generating the data for the study. OK participated in gathering the data for the study. OK, DT participated in the analysis of the data. OK wrote the majority of the original draft of the paper. OK, DT, BK, FY participated in writing the paper. OK has had access to all of the raw data of the study. OK has reviewed the pertinent raw data on which the results and conclusions of this study are based. OK, DT, BK, FY have approved the final version of this paper. OK guarantees that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

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