Effectiveness of Different Methods in Removing Dentin Caries of Primary Teeth: Micro-CT and SEM Evaluation

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Objectives: *With the recent improvements in technology, the expectation of minimal invasion and maximal comfort in caries removal techniques is increasing. This study aims to examine the effectiveness of six caries removal methods in primary teeth.* ***Study design:*** *Sixty primary molars (10 teeth in each groups) were used. The groups were: Group I (Tungsten Carbide Bur), Group II (Sono abrasion), Group III (Air abrasion), Group IV (Carisolv), Group V (Er:YAG Laser), Group VI (ART). In micro-CT scanning, mineral density at the cavity floor was examined before and after caries removal. After caries removal, the patency of the dentinal tubules was examined in two teeth from each group on SEM images. Statistical analyses were performed using Kruskal-Wallis, Wilcoxon tests.* ***Results:*** *For six different caries removal methods, tooth mineral (inorganic, total) densities at cavity floors were compared among the groups after the procedures, and no statistically significant difference was found ($p>0.05$). On the SEM images, it was seen that the dentinal tubules were exposed and no smear layer was formed in the Carisolv group. Significant rough surfaces were exposed in the laser group.* ***Conclusion:*** *It was observed that alternative caries removal methods are at least as effective as the traditional method in primary teeth for clinical applications.*

Keywords: *Caries removal techniques, Micro-CT, mineralization, SEM, smear, tubule.*

INTRODUCTION

Tooth decay is a diet-glucose-dependent disease that results in the destruction of the hard tissues of the tooth and cavitation formation [1]. It is the most common preventable disease of childhood with pandemic characteristics. It affects 60% to 90% of school-age children in most countries. The resulting pain and discomfort can adversely affect the quality of life. Moreover, the management of this situation creates a great financial burden on society and individuals [2, 3].

Primary teeth are important for the continuation of growth and development in children within normal limits. These teeth have functions such as guiding permanent dentition and helping the relations between the lower / upper jaws to develop in a normal position [2]. For this reason, decayed primary teeth are preferred to be kept in the mouth until the physiological age of falling off.

However, in children, the treatment of these teeth with traditional methods may not always be possible due to the lack of patient cooperation. Various techniques have been developed to remove decayed tooth tissue. With the technological improvements, alternatives have emerged in cavity preparation methods, tools, and techniques in the systems used for caries removal [4].

Protection from infectious diseases and the prevention of cross-contamination are of great importance for dental health professionals and patients [5]. Aerosols are released into the air by the preparation of teeth with high-speed handpieces, applications with ultrasonic devices, or the use of air-water spray during cleaning [6].

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These aerosols may contain microorganisms and viruses [5, 7]. Especially due to the Covid-19 pandemic in recent years, the popularity of non-aerosol applications has also increased among dentists.

Some techniques used in the removal of caries are thought to distinguish between the demineralized areas. However, some techniques cannot make this distinction. These are known to have the potential to fully remove the softened pathological area or to damage the intact tooth area [4].

Conventionally used rotary devices and alternative systems cause stress, fear, and anxiety in patients due to their noise and vibration. They are also known to produce significant levels of aerosols. The devices and systems planned to be alternative aim not only to eliminate these disadvantages but also to protect healthy tissues while removing infected tooth tissues. There are still studies on how much from which layer of decayed tooth tissue should be removed to achieve a successful mechanical and biological restoration [1]. Alternative systems aim to remove soft, infected dentin.

Expected features of optimal caries removal techniques are; ease of use in the clinical environment, ability to distinguish and extract pathological tissues only, minimum pressure requirement for painless and optimum use, no heat and vibration during operation, affordable price, and easy maintenance [4].

This study aimed to evaluate the effectiveness of six different caries removal techniques used in dentin caries of primary teeth in terms of mineral density at the cleaned cavity floor with Micro Computed Tomography (Micro-CT) and to show the condition of the smear layer and dentinal tubules using Scanning Electron Microscope (SEM).

The null hypothesis (H_0) of this study is that there is no statistically significant difference among the caries removal techniques used in dentin caries of primary teeth in terms of mineral density at the cleaned cavity floor in the Micro-CT examination.

MATERIALS AND METHOD

According to the power analysis, in order to calculate the statistical differences among the groups, the estimated number of samples was nine per group, with an alpha level of 0.05 and a power of 0.80 [8]. The approval of this study was obtained from the ethics committee of İnönü University (2015/57). In this study, the decayed primary molar teeth with the indication of the extraction due to the physiological root resorption of the patients who applied to İnönü University, Faculty of Dentistry, Department of Pediatric Dentistry and Oral and Maxillofacial Surgery were used.

Standardization of the study design

In this study, 60 primary teeth with dentin caries (10 teeth in each group) were used. Before the teeth were included in the study, periapical radiography was taken using the parallel technique. The distance between the pulp chamber ceiling and the floor of the carious lesion was calculated for each tooth. In order to standardize the decayed teeth to be included in the study, the radiological evaluation criteria presented in Pieira et al.'s study [9] was used. The decayed teeth with D3 and D4 scores that were found to reach the dentin radiologically were included in the study.

The radiological assessment was made by two pediatric dentists (GD, EK) with at least five years of experience in their field in a darkened room with a radiographic illuminator to ensure contrast enhancement of the tooth images. The assessments were done double-blinded. The examiners were trained and calibrated prior to the study. The assessments were made at two different times on radiographs. The initial and repeated whole assessments performed by the examiners showed that the intra- and inter-examiner agreement ranged from 0.968 to 0.985 and 0.975 to 0.991, respectively according to Cohen's Kappa (κ>0.75 is evaluated as a good agreement). The study included primary teeth with enamel-dentin caries and teeth with a distance of at least 2 mm between the pulp chamber ceiling and the floor of the carious lesion. After cleaning the tissue residues on the teeth, the teeth were randomly divided into six groups and kept at +4°C in distilled water until the study period.

The teeth were randomly divided into 6 groups as group 1: Tungsten carbide, group 2: Sono-abrasion, group 3: Air-abrasion, group 4: Carisolv gel, group 5: Erbium:Yttrium-Aluminum-Garnet (Er:YAG) laser and group 6: Atraumatic restorative treatment (ART). The decay removal and cavity preparation techniques are presented in Table 1.

Table 1. Caries removal and cavity preparation techniques

Method	Technique
Mechanical rotary systems	Drills + Rotary tools
Mechanical, non-rotating systems	Excavators, Air-abrasion, Airpolishing, Ultrasonic methods, Sono-abrasion
Chemo-mechanical systems (Caries removal methods only)	GK-101, GK-101E Caridex™, Carisolv™, Enzymes
Photoablation systems	Lasers

Caries removal procedures

To prevent inter-examiner variations, all study samples were prepared by an examiner (GD). Before caries removal, the examiner was trained and calibrated for the caries removal application.

The dentin caries removal procedure was performed in all groups according to tactile (obtaining a hard surface in dentin depending on whether a blunt probe does not get stuck while rolling over the cavity floor or there is no retraction feeling) and visual (no discoloration) criteria until a "no caries" score was given [10].

Detailed information on the procedures applied to the teeth included in the study is presented in Table 2. Carisolv gel application was made in accordance with the manufacturer's recommendations, and 200 mJ, 2 Hz, non-contact 12 mm distance, 2.94 μm tip, water 25 mL/min device setting was used in the preparation of the cavity with Er:YAG laser [11].

Micro-CT examination

The samples were placed in the SkyScan 1172 (Bruker, Kontich, Belgium) micro CT device for scanning, and a total of 500-550 cross-section images were taken from each sample at approximately 8.89 micron thickness with the help of an 11Mp camera using 0.5 mm aluminum and copper filters with 100 kV power, 100 mA current. While 360-degree rotation was used for scanning the teeth, the rotation step was determined as 0.40 degrees. DICOM (Digital Imaging and Communications in Medicine) compliant images taken from the sections were converted to BMP (Bit Map Picture) format. Resolution of each section image was 2000 x 2000 pixels and pixel size was 8.89 micron.

Table 2. Caries removal techniques applied to groups

Groups	Technic	Procedure	Device And Materials
Group 1	Bur (Tungsten Carbide)	After the enamel was removed with a diamond rond bur , caries was removed using brand-new burs used in a slow-speed handpiece, according to the size of the lesion. For each tooth a new polymer bur was used. The visual and tactile criteria of the operator were based on to finish the process.	Steel round burs (ISO 12-14)
Group 2	Sono abrasion	The enamel was removed with a diamond rond bur and the preparation was continued with the sonic system. The hemispherical diamond-coated tip was selected for cavity enlargement. 30 ml/min water spray cooling was used during the lateral movements of the instrument. The visual and tactile criteria of the operator were based on to finish the process.	Sonicflex 2000N,Kavo Dental, Biberach, Germany
Group 3	Air abrasion	Cavities were prepared with the handpiece of the air abrasive system, with using a 27 µm aluminum oxide particle stream at 201 psi air pressure for 60 seconds on dentin specified in the air-abrasion device's user manual, at a distance of approximately 5 mm at a 90° angle with the surface. After air abrasion, the dental surface was thoroughly rinsed for 20 seconds. The visual and tactile criteria of the operator were based on to finish the process.	EMS Swiss, CA, USA
Group 4	Carisolv	Carious dentine was covered with Carisolv gel for 30 seconds and the softened carious dentin tissue on the surface was removed with the specially designed hand instrument. When the gel became cloudy, it was replaced with a new one. The procedure was repeated until the gel became clear. After the cavity was free of caries, the remaining gel was wetted with water and removed with a cotton pellet. The cavity was washed with water and dried.	CarisolvTM Gel Multimix, MediTeam Dental AB, Gothenburg, Sweden
Group 5	Laser (Er:YAG)	Caries removal took place with the non-contact mode of handpiece, the working distance was approximately 12 mm. Cavities were prepared with an emission wavelength of 2.94 µm, 200 mJ pulse energy, under 2,5ml/min water spray cooling and frequency 2 Hz. The visual and tactile criteria of the operator were based on to finish the process.	Er:YAG Laser Fotona, Ljubljana, Slovenia
Group 6	ART (Atraumatic Restorative Treatment)	The cavities were prepared only with hand tools. Than cavity was enlargement with an enamel chisel and carious dentine was removed with a spoon excavator. Dentine excavation was stopped when hard dentine was detected according to the visual and tactile criteria of the operator.	Straight spoon excavator (Ash, G5-Claudius Ash Ltd, Potters Bar, Herts,UK)

Digital sectional images were acquired under the following conditions: 100 mA beam current, 100 kV accelerating voltage, 0.5-mm aluminum and copper filter, 9.9-mm pixel size at 2000 x 2000 resolution dpi, and 360 rotation at the 0.5 step [8, 12].

The positional errors of the processed radiological images in sagittal, transversal and vertical ways were corrected by SkyScan Dataviewer 1.5.0 64 bit program, (Fig 1). The new data series was uploaded to the CTAn (version 1.13.5.1, SkyScan, Kontich, Belgium) program, and the active examination area was limited by determining the cross-sectional area within the carious lesion in the cervico-occlusal direction in transversal sections. The carious lesion was revealed by separating the lesion area from the surrounding intact dentine tissue and the air space on the outer surface with the Region of Interest function in the CTAn program (Fig 2).

In the second stage, the lesion area was switched to the black-and-white image (Binary Page) page and the working range (Thresholding) suitable for the lesion density was determined.

All teeth samples were examined for mineral density of the 1 mm thick area on the floor of the carious lesion/ cleaned cavity using the micro-CT before and after caries removal. The mineral density of dentin tissue within the cavity boundaries was calculated in gr/cm^3 using the mineral density function on the black-and-white image page after selecting the lesion area with the Region of Interest function in the CTAn program (Fig 3).

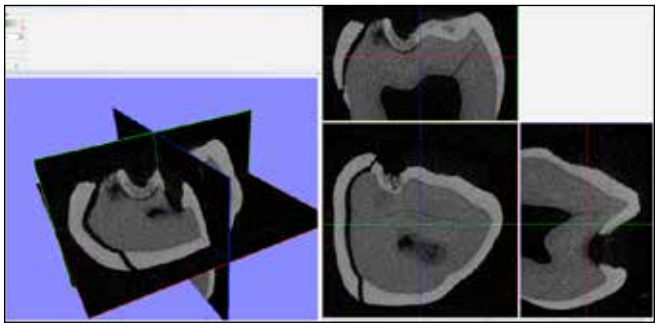
Mineral density was determined as both "inorganic" and "inorganic+organic (total)" values.

Initial inorganic values were named as 'I_0', inorganic values after caries removal procedures as 'I_1', initial total values as 'T_0', total values after caries removal procedures as 'T_1'. After the decayed tissue was removed by the specified methods, the measurements were repeated as I_1 and T_1.

SEM analysis

The teeth from which the carious lesion was removed, a total of 12 teeth, two from each group were fixed on the stubs using double-sided adhesive carbon disc (Agar Scientific). Subsequently, the specimens were dried in a vacuum of 10−2 mbar provided by a Sputter

Figure 1. Removing of pollution and image artifacts with Dataviewer 1.5.0 64 bit (SkyScan, Kontich, Belgium) program



Coater (Bal-Tec, SCD 050; Liechsteinstein). A 45mA sputtering current was applied for 30 sec to obtain a 15 nm gold-palladium layer on the upper surface of the specimens in this equipment. The samples were examined in a Scanning Electron Microscope (SEM, LEO-Evo 40; Cambridge, United Kingdom) at magnifications of ×2500 and ×1000 operating at an accelerating voltage of a 20 kV under high vacuum (10−5 mbar). A secondary electron detector was employed to observe the micro-morphological characteristics of the specimens. (Fig 4, Fig 5)

Statistical analysis

Data analysis was performed using the statistical package IBM SPSS Statistics 21 (SPSS Inc., Chicago Illinois, USA). Since the results were not normally distributed according to the Shapiro-Wilk test, they were expressed as mean±SD and median (IQR Q25-75).

Figure 2. CTAn 1.15.4.0 (Skyscan, Kontich, Belgium) software, isolation of caries lesion from the dentin tissue .

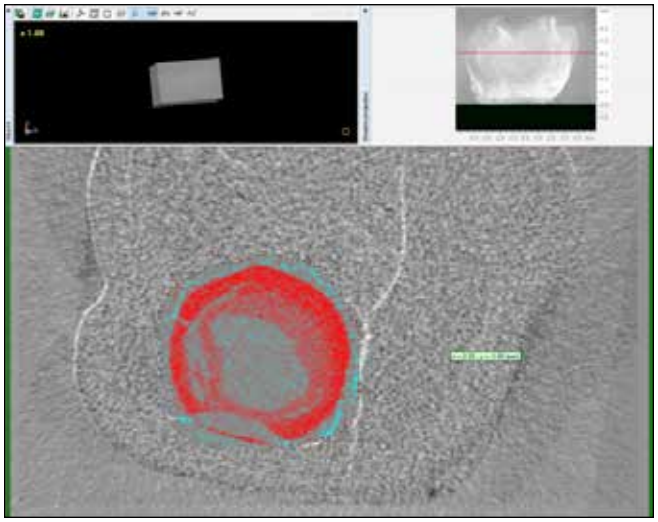
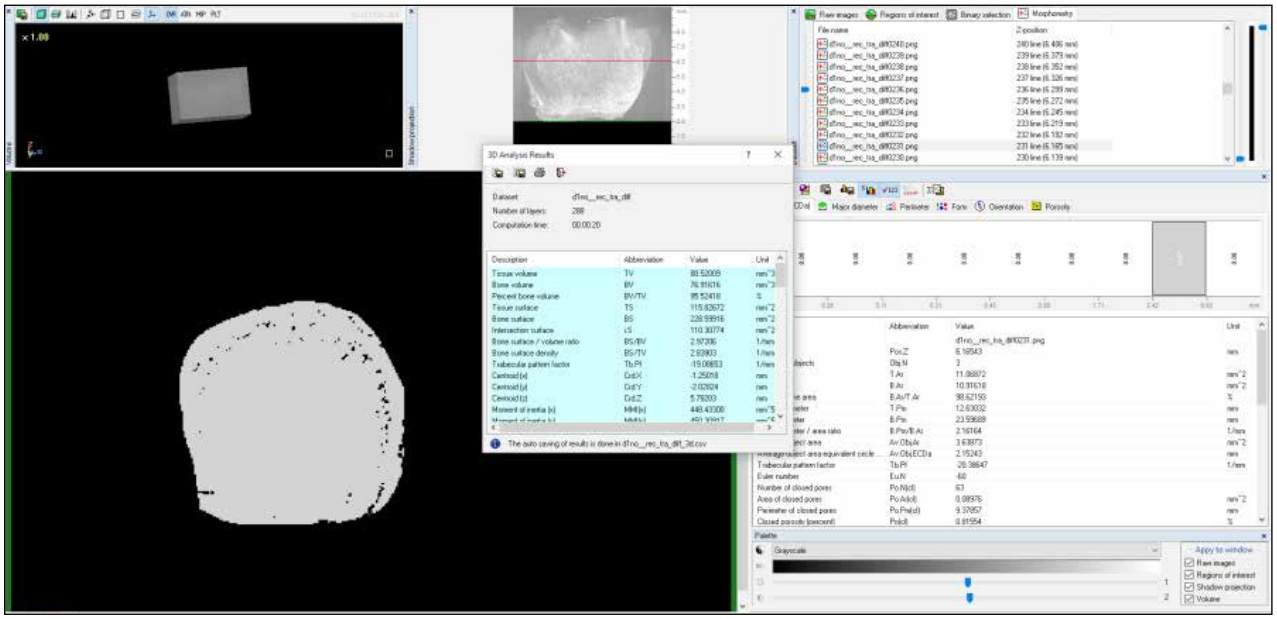


Figure 3. Calculation of mineral density of dentin tissue within the cavity boundaries using CTAn.



The Kruskal-Wallis test was used for comparison among the groups, and the Wilcoxon signed-rank test was used for comparison between the micro-CT values before and after the caries removal procedures. Statistical significance was set at $p<0.05$.

RESULTS

The details of inorganic and total values (gr/cm^3) measured with the micro-CT before and after the caries removal procedures are given in Tables 3 and 4, respectively. The values are presented as mean ± SD and median (IQR Q25-75).

There were statistical differences among the groups in both I_0 and T_0 ($p<0.05$). The median values of I_1 were not statistically significant among the groups ($p>0.05$). In all of the groups, it was detected that there were statistical differences between the median values of I_0 and I_1 ($p<0.01$) (Table 3). The median values of T_1 were not statistically significant among the groups ($p>0.05$). In all of the six groups, it was detected that there were statistical differences between the median values of T_0 and T_1 ($p<0.01$) (Table 4).

SEM images showing the tubular structure and smear layer at the cavity floor after caries removal procedures are shown in Figures 4 and 5 and summarized in Table 5.

In the SEM analysis, the dentinal tubule mouths were found to be more open in Group 4 (Carisolv gel) than in other groups. After the laser application, it was seen that although the tubule mouths were not open, distinctly rough surfaces were exposed.

Figure 4. SEM images of the cavity floor dentin after removing carious dentin at 2500 magnifications.

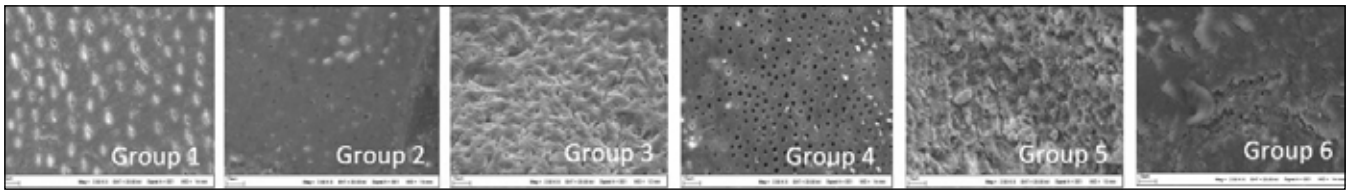


Figure 5. SEM images of the cavity floor dentin after removing carious dentin at 1000 magnifications.

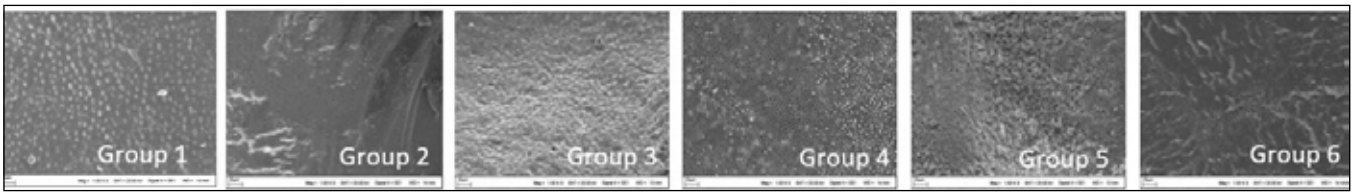


Table 3: Pre and post-procedure inorganic values (gr/cm³) and their statistical comparisons

Group	I_0		I_1		I_1- I_0	
	Mean (SD)	Median (IQR Q25-75)	Mean (SD)	Median (IQR Q25-75)	Mean differences (SD)	**p- value
1	0.17 (0.03)	0.17 (0.16-0.20)[a]	0.21 (0.01)	0.21 (0.21-0.22)	0.038 (0.032)[a]	0.007
2	0.18 (0.02)	0.19 (0.17-0.20)[a]	0.21 (0.02)	0.21 (0.20-0.24)	0.030 (0.026)[a]	0.007
3	0.13 (0.05)	0.14 (0.09-0.16)[ab]	0.21 (0.01)	0.21 (0.21-0.22)	0.083 (0.047)[ab]	0.005
4	0.16 (0.05)	0.16 (0.11-0.20)[a]	0.21 (0.01)	0.21 (0.21-0.22)	0.057 (0.048)[ab]	0.005
5	0.12 (0.07)	0.12 (0.06-0.20)[ab]	0.22 (0.02)	0.22 (0.21-0.22)	0.097 (0.063)[ab]	0.005
6	0.07 (0.05)	0.07 (0.02-0.12)[b]	0.22 (0.01)	0.21 (0.20-0.23)	0.143 (0.061)[b]	0.005
*p- value		0.001		0.941	<0.001	

*Kruskal-Wallis Test, **Wilcoxon Signed Ranks Test [a,b] different letters show the statistical differences in the same column

Table 4: Pre and post-procedure total values (gr/cm³) and their statistical comparisons

Group	T_0		T_1		T_1- T_0	
	Mean (SD)	Median (IQR Q25-75)	Mean (SD)	Median (IQR Q25-75)	Mean differences (SD)	**p- value
1	0.11 (0.07)	0.09 (0.05-0.15)[ab]	0.23 (0.05)	0.25 (0.21-0.27)	0.130 (0.060)	0.005
2	0.17 (0.07)	0.20 (0.15-0.21)[a]	0.26 (0.06)	0.25 (0.20-0.29)	0.083 (0.101)	0.005
3	0.06 (0.03)	0.06 (0.03-0.09)[b]	0.22 (0.05)	0.22 (0.20-0.24)	0.162 (0.067)	0.005
4	0.09 (0.08)	0.09 (0.03-0.17)[ab]	0.25 (0.05)	0.27 (0.20-0.28)	0.157 (0.074)	0.005
5	0.08 (0.07)	0.06 (0.03-0.13)[ab]	0.23 (0.06)	0.22 (0.20-0.23)	0.150 (0.066)	0.005
6	0.06 (0.04)	0.07 (0.02-0.09)[b]	0.23 (0.05)	0.24 (0.21-0.26)	0.164 (0.061)	0.005
*p- value		0.020		0.469	0.035	

*Kruskal-Wallis Test, **Wilcoxon Signed Ranks Test [a,b] different letters show the statistical differences in the same column

Table 5. In SEM examination, the condition of the smear layer and dentinal tubules formed after caries removal

Group	Technique	SEM Examination	
		Smear layer	Dentin tubules
1	Drill	thick smear layer	partially open
2	Sono-abrasion	uniform smear layer	partially open
3	Air-abrasion	thin smear layer	partially open
4	Carisolv	thin smear layer	almost all open
5	Laser	non-uniform smear layer	almost entirely closed
6	ART	thin smear layer	partially open

DISCUSSION

Nowadays, with the development of technology, the expectation of minimal invasion and maximum comfort in caries removal and cavity preparation techniques is increasing. Especially during the Covid-19 pandemic process, dentists have shown a growing interest in non-aerosol-forming techniques. Therefore, studies evaluating the efficiency of the techniques are needed.

The use of rotary instruments in the treatment of carious lesions can cause significant loss of tooth structure [13], resulting in weakening of the tooth structure and injuries to the pulp [14]. The use of rotary instruments also requires local anesthesia, especially in children, since it causes vibration, pressure, pain, heat, and sound [15]. For these reasons, there is a trend in dentistry towards less invasive methods [13]. Today, there are many decay removal methods in dentistry. Alternative caries removal methods such as manual excavators, sono-abrasion, air-abrasion, laser, chemo-mechanical methods, and enzymes have been proposed to avoid the disadvantages of rotary instruments and to minimize other disadvantages [1, 16].

This study used the micro-CT technique to compare the efficiency of six different (Bur, Sono abrasion, Air abrasion, Carisolv, Laser, ART) caries removal techniques in cleaning pathological tissues. It was concluded that each of these techniques was effective enough in caries removal and the methods did not have an advantage over one another. Therefore, the null hypothesis of this study is accepted.

Micro-CT is an imaging technique that can provide both qualitative and quantitative data before and after specific treatments applied to teeth. This technique is becoming widespread in research on dentistry [17]. It is preferred because of both its ability to make precise measurements in the examination of the mineral concentration of the teeth and its ease of use. Mitropolus *et al* reported that although the micro-CT diagnostic value showed a strong correlation with visual diagnosis, it remained weaker in dental caries studies than histological examination [18]. Similarly, some existing studies have indicated that the micro-CT is reliable in defining caries before and after cavity preparation and is valuable in examining mineralized and carious dentin tissue [8, 19, 20]. In this study, the micro-CT method, which can measure tooth mineral density with a high degree of precision, was preferred to measure dentin mineralization values before and after the application of six different caries removal methods. SEM examination was also included in the method in addition to the micro-CT evaluation.

At the beginning of our study, according to the micro-CT data taken before the caries removal procedure, significant differences were found among the groups in the inorganic measurement values and the total measurement values. Although standardization was achieved in the selection of the carious teeth to be included in the study through two-dimensional radiography, the density of the carious lesion area still differed among teeth. The teeth were randomized to the groups, and the statistical difference among the groups was not significant in the course of the study.

After the caries removal techniques were applied, the values of teeth mineral densities (both the inorganic and total densities) were similar in each group ($p>0.05$), and there was a statistically significant difference between these values and the values before the caries removal procedure ($p<0.05$). This result shows that the applied techniques are equally effective in removing caries from the dentin surface and that they do not cause tooth mineral loss or damage, which is consistent with the literature. In a similar study, Thomas *et al* [21] compared the minimal invasivity potential of caries removal techniques. They evaluated the effectiveness of chemo-mechanical methods and standard mechanical methods on caries removal and compared their effects on healthy tooth tissue by volumetric measurements with the micro-CT. The authors found that the caries removal rates were "similar" and the invasion to the healthy tooth tissue was "less" by chemical methods [21]. In a clinical study conducted on young permanent teeth, Sontake *et al* [22] compared the efficacy of chemomechanical and traditional methods. They did not observe a difference in caries removal potential and showed the advantages of chemical methods in terms of patient comfort. In this study, since it was an *in vitro* study, the parameters such as the duration of the dental procedure and the patient's sense of pain could not be evaluated.

The damage potential of mechanical techniques with rotary devices to intact dental tissue has been demonstrated in previous similar study [1]. However, there is no study that directly examines the effect of the chemical methods on primary tooth intact tissue in terms of cavity floor density. In another similar study, there was no difference between the chemical and sono techniques in terms of reducing pathological microflora in carious tissue in primary teeth [4]. In this study, there was no difference between the chemical and traditional methods in terms of cavity floor density. Since the histochemical structure of the primary tooth is different from that of the permanent teeth, there is a need to carry out equivalent studies to achieve standardization of the effectiveness of the techniques.

In this study, SEM analysis was performed to evaluate the smear layer and dentinal tubules after caries removal. The smear layer remained in almost all of the groups, but there were more exposed dentinal tubules in the Carisolv gel group. Besides, defects like laser ablation craters were encountered in the laser group.

Considering the studies comparing Carisolv gel and other rotary devices in the literature, some have reported that there is no difference in decay removal [1, 23, 24], while some have indicated that the Carisolv gel technique has better caries removal sensitivity and complementarity than the mechanical methods [25, 26].

The studies have also reported that the Carisolv gel technique creates a smear layer, closes most dentinal tubules, and has less destructive effects on sound dentin tissue [24, 27, 28]. In this study, no smear layer was observed in the Carisolv gel technique, and the dentinal

tubules were open. There are a limited number of studies evaluating the removal of carious dentin tissue with different techniques by SEM. Papacarie Duo and Carie Care were compared in a study examining the smear layer and tubular structure after caries removal with two chemical agents [29]. It has been reported that a thin smear layer was formed in both of them, but a cleaner tubule structure and a more compatible anatomical structure remained after the Carie Care application.

Tsanova and Tomov [30], in their study examining the changes caused by three different caries removal methods on tooth morphology, stated that the smear layer was not observed on the dentin surface after the Er: YAG laser application, the tubule mouths were open, and there was excessive surface roughness.

In their study, Al-Batayneh *et al* [31] investigated the effect of the Er:YAG laser on caries removal in human primary and permanent teeth and observed that deep defect-like laser ablation craters were formed in dentin. The higher ablation rates for enamel and dentin in primary teeth showed that the laser could remove decayed tissue more effectively in primary teeth. De Oliveira *et al* [11] stated that the Er:YAG laser used in primary tooth dentin with high energy (300 mJ) and pulse rate (4 Hz) caused excessive melting on the dentin surface. They reported that the changes were caused by the deterioration of the composition of the proteins and the decrease in the Ca/P ratio. In this study, defect-like laser ablation craters were encountered as a result of SEM analysis, and this finding supports the results of Al-Batayneh *et al* [31]. However, contrary to the findings of Tsanova and Tomov [30], it was observed that the dentin surface was covered with a smear layer. This situation is thought to be caused by the obstruction of the dentinal tubules by the ablation effect of the Er:YAG laser.

Although this study compared the effectiveness of dental caries removal techniques, the findings are still insufficient to answer the question "How do we get more standardized results?" This *in vitro* study evaluated and compared the success of caries removal techniques only in terms of their mechanical aspects. It is important to compare caries removal techniques under *in vivo* conditions and evaluate them in terms of aspects such as patient-technical comfort, time, pain, and short-long-term complications in order to choose the optimal technique in clinical practice.

Despite all these limitations, the strength of the study is that it is a comprehensive study comparing almost all caries removal techniques (except enzyme-based technique) within the same study protocol. To the best of our knowledge, there is no study in the literature showing the change in mineral density at the cavity floor using the micro-CT and the structure of the dentinal tubules with SEM imaging technique after applying the different caries removal techniques. Being a pioneering study in this regard is another strength of the study.

CONCLUSION

In this study, it was observed that six different caries removal techniques were sufficient to remove pathological tissue, and the densities at the cavity floors where the caries were removed were not found to be superior to each other as a result of the micro-CT evaluation. In the SEM examination, Carisolv gel revealed the dentinal tubule orifices. Although the tubule orifices are not open after laser application, it is thought that the exposure of distinctly rough surfaces may have an effect on the bond strength of the filling.

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Ethics approval and consent to participate

Ethical approval was obtained from Clinical Research Ethics Committee in Turkey: Inönü University School of Medicine (ethic number: 2015/57). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The written consent was obtained from all the the parents confirming that they would provide their children's extracted primary teeth for the research, and they would allow it to be published.

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REFERENCES

1. Banerjee A, Watson TF, Kidd EA. Dentine caries excavation: a review of current clinical techniques. Br Dent J, 2000; 188: 476-82.
2. Law CS. Management of premature primary tooth loss in the child patient. J Calif Dent Assoc, 2013; 41: 612-8.
3. Pakkhesal M, Riyahi E, Naghavi Alhosseini A, Amdjadi P, Behnampour N. Impact of dental caries on oral health related quality of life among preschool children: perceptions of parents. BMC Oral Health, 2021; 21: 68.
4. Hassan AF, Yadav G, Tripathi AM, Mehrotra M, Saha S, Garg N. A Comparative Evaluation of the Efficacy of Different Caries Excavation Techniques in reducing the Cariogenic Flora: An in vivo Study. Int J Clin Pediatr Dent, 2016: 9: 214-7.
5. Volgenant CMC, de Soet JJ. Cross-transmission in the Dental Office: Does This Make You Ill? Curr Oral Health Rep, 2018; 5: 221-8.
6. Kedjarune U, Kukiattrakoon B, Yapong B, Chowanadisai S, Leggat P Bacterial aerosols in the dental clinic: influence of time, position and type of treatment, Int Dent J, 2000: 50: 103–7.
7. Zemouri C, de Soet H, Crielaard W, Laheij A. A coverage review on bio-aerosols in healthcare and dental environments. PLoS One, 2017; 12: e0178007.
8. Neves Ade A, Coutinho E, De Munck J, Van Meerbeek B. Caries-removal effectiveness and minimal-invasiveness potential of caries-excavation techniques: a micro-CT investigation. J Dent, 2011; 39: 154-62.
9. Pereira AC, Eggertsson H, Martinez-Mier EA, Mialhe FL, Eckert GJ, Zero DT. Validity of caries detection on occlusal surfaces and treatment decisions based on results from multiple caries-detection methods. Eur J Oral Sci, 2009; 117: 51-7.
10. Kidd EA, Ricketts DN, Beighton D. Criteria for caries removal at the enamel-dentine junction: a clinical and microbiological study. Br Dent J, 1996; 180: 287-91.
11. de Oliveira Ortolan AS, Torres CP, Gomes-Silva JM, de Menezes-Oliveira MA, Pécora JD, Palma-Dibb RG, Borsatto MC. Effect of erbium-doped yttrium aluminium garnet laser parameters on ablation capacity and morphology of primary dentin. Photomed Laser Surg, 2009; 27: 885-90.
12. Kucuk EB, Malkoc S, Demir A. Microcomputed tomography evaluation of white spot lesion remineralization with various procedures. Am J Orthod Dentofacial Orthop, 2016; 150: 483-90.
13. Divya G, Prasad MG, Vasa AA, Vasanthi D, Ramanarayana B, Mynampati P. Evaluation of the Efficacy of Caries Removal Using Polymer Bur, Stainless Steel Bur, Carisolv, Papacarie–An Invitro Comparative Study. J Clin Diagn Res, 2015; 9: 42-6.
14. Corrêa FN, Rocha Rde O, Rodrigues Filho LE, Muench A, Rodrigues CR. Chemical versus conventional caries removal techniques in primary teeth: a microhardness study. J Clin Pediatr Dent, 2007; 31: 187-92.
15. Celiberti P, Francescut P, Lussi A. Performance of four dentine excavation methods in deciduous teeth. Caries Res, 2006; 40: 117-23.
16. Flückiger L, Waltimo T, Stich H, Lussi A. Comparison of chemomechanical caries removal using Carisolv or conventional hand excavation in deciduous teeth in vitro. J Dent, 2005; 33: 87-90.
17. Soviero VM, Leal SC, Silva RC, Azevedo RB. Validity of MicroCT for in vitro detection of proximal carious lesions in primary molars. J Dent, 2012; 40: 35-40.
18. Mitropoulos P, Rahiotis C, Stamatakis H, Kakaboura A. Diagnostic performance of the visual caries classification system ICDAS II versus radiography and micro-computed tomography for proximal caries detection: an in vitro study. J Dent, 2010; 38: 859-67.
19. Nogueira Rocha Clementino Luedemann, T. Micro-computed tomography in caries research. Doctoral Dissertation, lmu. 2007
20. Erpaçal B, Adıgüzel Ö, Cangül S. The use of micro-computed tomography in dental applications. Int Dent Res, 2019; 9: 78-91.
21. Thomas AR, Nagraj SK, Mani R, Haribabu R. Comparative evaluation of the efficiency of caries removal using various minimally invasive techniques with conventional rotary instruments using cone beam computed tomography: An in vitro study. J Int Oral Health, 2020; 12: 253-9.
22. Sontakke P, Jain P, Patil AD, Biswas G, Yadav P, Makkar DK, Jeph V, Sakina BP. A comparative study of the clinical efficiency of chemomechanical caries removal using Carie-Care gel for permanent teeth of children of age group of 12-15 years with that of conventional drilling method: A randomized controlled trial. Dent Res J (Isfahan), 2019; 16: 42-6.
23. Kitsahawong K, Seminario AL, Pungchanchaikul P, Rattanacharoenthum A, Pitiphat W. Chemomechanical versus drilling methods for caries removal: an in vitro study. Braz Oral Res, 2015; 29: 1-8.
24. Corrêa FN, Rodrigues Filho LE, Rodrigues CR. Evaluation of residual dentin after conventional and chemomechanical caries removal using SEM. J Clin Pediatr Dent, 2008; 32: 115-120.
25. Hamama HH, Yiu CK, Burrow MF, King NM. Chemical, morphological and microhardness changes of dentine after chemomechanical caries removal. Aust Dent J, 2013; 58: 283-92.
26. Maru VP, Shakuntala BS, Nagarathna C. Caries Removal by Chemomechanical (Carisolv™) vs. Rotary Drill: A Systematic Review. Open Dent J, 2015; 31: 462-72.
27. Jawa D, Singh S, Somani R, Jaidka S, Sirkar K, Jaidka R. Comparative evaluation of the efficacy of chemomechanical caries removal agent (Papacarie) and conventional method of caries removal: an in vitro study. J Indian Soc Pedod Prev Dent, 2010; 28:73-7.
28. Reddy MV, Shankar AJ, Pentakota VG, Kolli H, Ganta H, Katari PK. Efficacy of antimicrobial property of two commercially available chemomechanical caries removal agents (Carisolv and Papacarie): An ex vivo study. J Int Soc Prev Community Dent 5, 2015; 183-9.
29. Thakur R, Patil SDS, Kush A, Madhu K. SEM Analysis of Residual Dentin Surface in Primary Teeth Using Different Chemomechanical Caries Removal Agents. J Clin Pediatr Dent, 2017; 41: 289-93.
30. Tsanova STs, Tomov GT. Morphological changes in hard dental tissues prepared by Er:YAG laser (LiteTouch, Syneron), Carisolv and rotary instruments. A scanning electron microscopy evaluation. Folia Med (Plovdiv), 2010; 52:46-55.
31. Al-Batayneh OB, Seow WK, Walsh LJ. Assessment of Er:YAG laser for cavity preparation in primary and permanent teeth: a scanning electron microscopy and thermographic study. Pediatr Dent, 2014; 36: 90-4.