ORIGINAL RESEARCH

Chemomechanical Caries Removal with Enzymatic Technology: A Comparative *In Vitro* Study

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Abstract

Purpose: This study aims to compare the efficiency of caries removal between the chemomechanical caries removal agent Brix 3000 and the conventional method using a laser fluorescent device (DIAGNOdent Pen), caries detection dye, microbial culture, and real-time polymerase chain reaction (PCR) methods.

Materials and methods: The study involved 64 primary molars with dentinal caries between March and June 2022. Standardization was achieved based on International Caries Detection and Assessment System (ICDAS-II) criteria (score 6) and DIAGNOdent Pen measurements (30 and above). Caries were removed using the conventional method (32) and Brix 3000 (32). Following this, dentin samples were collected, and measurements were made with the DIAGNOdent Pen to evaluate the presence of residual caries in both groups. After the measurements, the presence of residual caries was evaluated in both groups using caries detection dye. The decrease in values obtained by microbial culture and real-time PCR methods of dentin samples taken before and after caries removal was evaluated.

Results: When DIAGNOdent Pen and caries detection dye were evaluated, the caries removal efficiency of the conventional method was found to be higher ($p < 0.05$). As a result of the microbial culture evaluation for *Streptococcus mutans* and total bacteria, the caries removal efficiencies of both methods were found to be similar ($p > 0.05$). The caries removal efficiency of Brix 3000 was found to be higher as a result of the evaluation with real-time PCR for *S. mutans* ($p < 0.05$).

Conclusion: It is thought that Brix 3000 is an effective agent for caries removal and may be an alternative agent to the conventional method, especially considering its advantages in children.

Keywords: Cariology, Chemomechanical caries removal, Laboratory research, Microbiology, Pediatric dentistry.

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Introduction

Dental caries is defined as a biofilm-mediated, noninfectious, and multifactorial disease.[1] The ecological shift of the bacteria in the dental biofilm brings about an imbalance between demineralization and remineralization.[2] As a result of this process, the caries lesion develops.[1] Conventional caries removal methods use burs and rotary instruments. The most severe problems encountered during caries removal are anxiety, fear, and pain. In spite of pain control with local anesthesia,[3] noise and vibration of mechanical handpieces, and fear of the needle still cause discomfort.[4] In addition, the removal of healthy tooth structure leads to unnecessary weakening of the tooth, harmful thermal effects on the pulp, and iatrogenic pulp exposure.[5] Minimally invasive caries removal methods have been developed to overcome these problems.[6]

The chemomechanical caries removal method is a minimally invasive method that removes infected dentin, preserves the healthy structures, prevents pulp irritation, and patient discomfort.[5] Chemomechanical caries removal agents are characterized by solutions or gels that can dissolve carious tissue to aid in removing caries.[7] These agents, which facilitate caries removal by softening the carious tissue, are sodium hypochlorite (NaOCl) or enzyme-based.[3]

Brix 3000® is a papain-based gel formulation introduced in Argentina.[8] Unlike other papain-based agents, Brix 3000® contained a high concentration of papain (3000 U/mg in each 10%), and the papain was bioencapsulated by encapsulation buffer emulsion (EBE) technology.[7] Thus, it can remove the carious tissue without causing damage or pulp cytotoxicity.[9] It also has bactericidal, bacteriostatic, and anti-inflammatory properties.[10]

In the literature, various studies had been managed to evaluate the efficacy of other chemomechanical agents.[11–15] However, there are limited studies on Brix 3000®.[3,8,16,17] In addition, there are very few studies investigating the chemomechanical agents' caries removal efficacy microbiologically,[18–21] and there is one study which evaluated the caries removal efficacy with real-time polymerase chain reaction (PCR) technique.[22]

The aim of this study was to evaluate and compare the caries removal efficacy of chemomechanical caries removal agent Brix 3000® with conventional caries removal method using a laser

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Conflict of interest: None

fluorescent device (DIAGNOdent™ Pen), caries detection dye, microbial culture, and real-time PCR.

Materials and Methods

Selection of the Samples

This study is an *in vitro* study conducted between March and June 2022. About 64 primary molars with open access dentin caries lesions, recently exfoliated or freshly extracted for orthodontic purposes, were used in this study. This study was accepted by the Gazi University Clinical Research Ethics Committee (E-21071282-050.99-186377).

International Caries Detection and Assessment System (ICDAS-II) and laser fluorescent device (DIAGNOdent™ Pen, KaVo, Biberach, Germany) were used to maintain adequate standardization for dentin caries lesions. Primary molars selected according to the ICDAS-II 6 score[23] and DIAGNOdent™ Pen value of 30 and above[11] were included in the study. Teeth with restorations, developmental anomalies, and pulp exposure after caries removal were excluded.

DIAGNOdent™ Pen is a laser-based instrument developed for detecting and quantifying dental caries. DIAGNOdent™ Pen was calibrated before the teeth were examined using a ceramic mounting provided by the manufacturer. For secondary calibration, the fluorescence of a sound spot on the smooth tooth surface was measured. The initial carious dentin laser fluorescence value was measured with a conical tip (suitable for the occlusal and approximal surface) and recorded following the manufacturer's recommendations. All surfaces of the cavity were scanned with the conical tip perpendicular to the lesion, and the highest value was recorded. An experienced pediatric dentist performed all readings. Three measurements were obtained from the same cavity site, and the mean value was calculated.

Study Design and Sample Size

The sample size was calculated using G*Power 3.1 (Franz Faul, Universität Kiel, Kiel, Germany) software and was decided to be at least 64 teeth (32 for each group). The study sample was randomly (coin flip) assigned into two groups: group I—conventional method and group II—chemomechanical caries removal method (Brix 3000®).

After extracting the teeth, the roots were cleaned with 70% ethyl alcohol in sterile boxes to prevent contamination. The teeth were fixed in self-curing acrylic resin blocks, with the roots inside the blocks and the crowns entirely outside.

Caries Removal Procedure

Brix 3000® Group

Brix 3000® gel was applied to the cavity with a blunt spoon excavator and left for 2 minutes to interact with infected dentin according to the manufacturer's recommendations. Initially, Brix 3000® gel was clear but became turbid due to decomposition. The softened carious tissue was removed with a blunt spoon excavator without pressure. This procedure was repeated until the gel was no longer turbid and healthy dentin was obtained. No washing or rinsing was done between the applications. After that, the cavity was examined using visual-tactile criteria to assess complete caries removal. Visual criteria are based on the nonturbid appearance and the gel remaining clear. Tactile criteria are based on the hard dentin surface and the absence of a catch or a tug-back sensation.

Conventional Group

Carious tissue was removed using a low-speed handpiece with a sterile round steel bur compatible with the cavity size. In order to facilitate the adhesion of dentin particles to the bur, it was soaked with sterile water. The cavity was frequently washed with sterile water to prevent temperature rise during the procedure. After that, the cavity was controlled using the same criteria as above, but visual criteria are based on the absence of discoloration. Then, the cavity in both groups was rinsed with sterile water and dried with a sterile cotton pellet.

Assessment of Residual Caries

Following the caries removal process, the laser fluorescence value of each tooth was measured with a cylindrical tip (suitable for flat surfaces) and recorded again using the DIAGNOdent™ Pen as above. The cutoff value for sound tissue was considered 0–13. The residual caries was also evaluated using a caries detection dye (Reveal, Prevest Denpro, Jammu, India). The caries detection dye was applied to the cavity using sterile cotton pellets and left in for 10 seconds according to the manufacturer's recommendations. Then the cavity was rinsed with sterile water and dried with sterile cotton pellets. The cavity was examined by two different observers under reflector light. All observers were calibrated according to the color of stained dentin. Infected dentin will show noticeable (dark) staining, whereas noninfected dentin will only absorb a small quantity of dye (light). The cavity was considered sound if there was light or no staining and having residual caries if there was dark staining.

Microbial Assessment

The methodology was to evaluate *Streptococcus mutans* and total bacteria colony-forming units (CFU) per dentin sample. For the microbiological analyses, dentin samples were taken before and after caries removal. Before sampling, the outermost carious dentin layer was removed with a sterile spoon excavator to prevent contamination. In order to standardize the amount of dentin samples collected, new and the same ISO size (no. 16) sterile burs were utilized in the conventional method and the same size sterile sharp spoon excavators (no. 19) were utilized in the chemomechanical method. Since the instruments used to collect dentin samples were different in both methods, the collected dentin samples were weighed for standardization. The dentin samples were placed into 500 µL phosphate-buffered saline (PBS) medium contained in a sterile Eppendorf tube and labeled.

Microbial Culture

Dentin samples of both groups were transported to the microbiology laboratory within 2 hours to be analyzed. The samples were vortexed for 60 seconds to dislodge the dentin samples from burs to homogenize. Then, the bur was removed from the tubes. After homogenization, a serial dilution of 10^{-4} of the homogenized suspension in sterile saline was prepared (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}). Using a calibrated pipette, a 50 µL volume of the homogenized suspension was inoculated into a trypticase yeast extract cysteine sucrose bacitracin (TYCSB) agar and blood agar (added with 5% sheep blood). Then, TYCSB agar plates were incubated at 37°C for 24–72 hours in an environment containing 5% carbon dioxide (CO_2), and blood agar plates were incubated at 37°C for 48 hours.

After incubation, *S. mutans* and total viable colonies were counted based on their morphology in all dentin samples. The identification and colony counts were made macroscopically (10×) from the appropriate dilution and corrected for the dilution factor.

The viable colonies of the homogenized suspension were expressed as total CFU per milliliter (CFU/mL). For ease of comparison, all values of CFU were transformed to $\log_{10}$. After that, the samples were stored at −80°C until further analysis.

Real-time Polymerase Chain Reaction

The real-time PCR analyses were performed on all dentin samples. Frozen samples were thawed and vortexed for 60 seconds to homogenize. DNA was isolated from samples using the DiaRex® (Bacterial DNA isolation kit, Diagen, Ankara, Turkey) according to the manufacturer's recommendations. The DNAs obtained from the samples were evaluated by spectrophotometer and electrophoresis for concentration and purity. The SYBR Green real-time PCR method was performed for detection and quantification. For *S. mutans* and total bacteria PCR analysis, primers were obtained from the NCBI (https://www.ncbi.nlm.nih.gov/) database. The following reaction mixture was used: SYBR Green master mix, forward and reverse primer, H_2O, and DNA template. Using eight strip tubes, amplification and detection were performed with a real-time PCR device (LightCycler 480, Roche Diagnostics, Rotkreuz, Switzerland). The reaction conditions included an initial denaturation at 95°C for 1 minute, followed by 40 cycles of denaturation at 95°C for 10 second, annealing at 60°C for 30 second, and extension at 72°C for 30 second, where the fluorescence is acquired.

The amount of DNA was calculated by comparing the standard curve's threshold cycle (Ct) values. The total bacteria and *S. mutans* were expressed as the number of gene copies and the number of cells per milligram of dentin, respectively.

Statistical Analysis

Data analysis was conducted using IBM SPSS version 25 software (IBM Corporation, Armonk, NY, USA). For the evaluated groups, descriptive results including minimum, maximum, mean, and standard deviation were calculated. The normality of distribution was determined using the Shapiro–Wilk test. The data were analyzed by the independent sample *t*-test and Mann–Whitney *U* test.

For each technique, differences in laser fluorescence value before and after caries removal were analyzed by the Wilcoxon signed rank test. The Mann–Whitney *U* test was used for comparison between the chemomechanical caries removal and conventional methods. For caries detector dye and DIAGNOdent™ Pen, the Chi-squared test was used to determine significant differences concerning residual caries.

In the statistical analysis, a *p*-value of 0.05 was accepted as the significance threshold.

Results

There was no difference among the groups in the initial DIAGNOdent™ Pen values ($p > 0.05$). Statistically significant differences were observed between initial and final DIAGNOdent™ Pen values within each group. Each group showed a significant drop in DIAGNOdent™ Pen value after caries removal ($p < 0.05$). No significant difference was found between the conventional method and Brix 3000® ($p > 0.05$) (Table 1).

The caries detector dye showed no caries in 28 teeth with the conventional method, whereas 21 teeth were caries-free with Brix 3000®. A statistically significant difference was found between staining with caries detection dye after treatment with the conventional method and Brix 3000® ($p < 0.05$). The number of teeth stained after treatment with the conventional method was less than in Brix 3000®. A statistically significant difference was found between DIAGNOdent™ Pen readings obtained after treatment with Brix 3000® and the conventional method ($p < 0.05$). It was determined that readings between 0 and 13 (no caries) were mainly in the conventional method, and readings of 14 and above (residual caries) were mostly in the Brix 3000® group (Table 2).

Microbial culture analysis showed statistically significant reductions in total viable bacteria and *S. mutans* counts in both groups ($p < 0.05$) (Table 3).

Real-time PCR analysis showed statistically significant reductions in *S. mutans* counts in both groups ($p < 0.05$). A statistically significant decrease in total bacterial count was observed in the conventional method ($p < 0.05$). A decrease in Brix 3000® also occurred; but the difference did not reach statistical significance ($p = 0.970$) (Table 4).

Brix 3000® showed a greater reduction in *S. mutans* compared to the conventional method in real-time PCR analysis ($p < 0.05$). Also, Brix 3000® showed a greater reduction in *S. mutans* compared

Table 1: DIAGNOdent Pen mean fluorescence values before and after caries removal procedure

			Initial		*Final*		*Change*		
	Groups	*n*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*p-value*
DIAGNOdent Pen	Chemomechanical caries removal (Brix 3000®)	32	97.8	2.9	17.7	5.5	80.1	6.5	0.000*
	Conventional caries removal	32	97.8	2.8	14.6	6.9	83.2	6.2	0.000*

*$p < 0.05$ (statistically significant); SD, standard deviation

Table 2: DIAGNOdent Pen values and caries detector dye staining status in detecting residual caries after treatment with conventional method and Brix 3000®

		Group		
		Conventional caries removal	*Chemomechanical caries removal (Brix 3000®)*	*p-value*
DIAGNOdent Pen	No caries (0–13)	15	7	0.035*
	Residual caries (≥14)	17	25	
Caries detector dye	No caries (no stain)	28	21	0.036*
	Residual caries (stain)	4	11	

*$p < 0.05$ (statistically significant)

Table 3: Comparison of total viable bacterial count and *S. mutans* CFU/mL values before and after treatment between two study groups: group I—(Brix 3000®) and group II—(conventional caries removal) by microbial culture analysis

	Group		*Mean*	*SD*	*p-value*
S. mutans	Conventional caries removal	Before	3.16	1.65	0.000*
		After	1.24	1.11	
	Chemomechanical caries removal (Brix 3000®)	Before	3.65	1.68	0.000*
		After	1.44	1.23	
Total bacteria	Conventional caries removal	Before	7.16	1.79	0.000*
		After	4.78	2.05	
	Chemomechanical caries removal (Brix 3000®)	Before	6.12	2.14	0.000*
		After	3.16	1.37	

*$p < 0.05$ (statistically significant); SD, standard deviation

Table 4: Comparison of total bacterial count and *S. mutans* CFU/mL between two study groups: group I—(Brix 3000®) and group II—(conventional caries removal) by real-time PCR analysis

	Group		*Mean*	*SD*	*p-value*
S. mutans	Conventional caries removal	Before	6.19	1.09	0.000*
		After	4.82	1.18	
	Chemomechanical caries removal (Brix 3000®)	Before	7.41	1.56	0.000*
		After	6.13	1.65	
Total bacteria	Conventional caries removal	Before	6.22	0.47	0.000*
		After	5.22	1.10	
	Chemomechanical caries removal (Brix 3000®)	Before	6.52	0.64	0.970
		After	6.38	0.63	

*$p < 0.05$ (statistically significant); SD, standard deviation

Table 5: Comparison of mean difference total bacterial counts and *S. mutans* CFU/mL between two study groups: group I—(Brix 3000®) and group II—(conventional caries removal)

		Group	*Mean difference*	*SD*	*p-value*
S. mutans	Microbial culture	Conventional caries removal	3.11	1.67	0.134
		Chemomechanical caries removal (Brix 3000®)	3.63	1.69	
	Real-time PCR	Conventional caries removal	5.92	1.56	0.000*
		Chemomechanical caries removal (Brix 3000®)	7.32	1.55	
Total bacteria	Microbial culture	Conventional caries removal	7.14	1.79	0.088
		Chemomechanical caries removal (Brix 3000®)	6.10	2.16	

*$p < 0.05$ (statistically significant); SD, standard deviation

to the conventional method in microbial culture analysis, but the difference did not reach statistical significance ($p = 0.134$). The conventional method group showed a greater reduction in total bacterial count compared to the Brix 3000® group in microbial culture analysis, but the difference did not reach statistical significance ($p = 0.088$). There was no comparison between the two groups' total bacterial counts in real-time PCR analysis, since the difference did not reach statistical significance before and after the removal of carious dentin tissue (Table 5).

Discussion

Brix 3000® is the only chemomechanical caries removal agent that uses the EBE technology to date. It immobilizes and causes higher proteolysis to remove degraded collagen fibrils from infected dentin, thus providing lower dissolution of active substance by oral fluids.[24] On the contrary, the conventional method is fast and efficient, but it can lead to unnecessary removal of sound and affected dentin, which affects the ability of remineralization.[16]

This study aimed to evaluate and compare the caries removal efficacy of Brix 3000® and the conventional method. In this study, the efficacy of caries excavation was evaluated by caries-detecting dye and DIAGNOdent™ Pen. The microbial flora of caries serves as the primary etiological factor, and it needs to be decreased to manage the disease process.[8] Therefore, microbiological analysis was added to this study to evaluate the efficacy of caries removal.

A statistically significant difference was found between DIAGNOdent™ Pen readings and staining with caries detection dye after treatment with both groups ($p < 0.05$). It was determined that readings between 0 and 13 (no caries) were mainly in the conventional method, and readings of 14 and above (residual caries) were mostly in the Brix 3000® group. The number of teeth stained after treatment with the conventional method is less than Brix 3000®. As far as we know, there is no study in the literature comparing the caries removal efficacy of Brix 3000® and the conventional method using DIAGNOdent™ Pen and caries detector dye.

Previous studies have documented that the efficacy of the conventional method by DIAGNOdent™ Pen was significantly higher

than chemomechanical caries removal agents.[11,25,26] These studies were in accordance with our study. On the contrary, a study stated no significant difference between the caries removal efficacy of the conventional method and chemomechanical caries removal agents.[27] It has been reported that the color in residual dentin showed significantly higher laser fluorescence measurements.[28,29] Based on this information, it was thought that Brix 3000®, which only removed infected dentin, would exhibit higher fluorescence values due to the color of the affected dentin. In the conventional method, lower fluorescence values were obtained because the affected dentin was removed.

Previous studies have documented that the number of teeth stained after treatment with the conventional method was significantly lower than with the chemomechanical caries removal method.[5,14,30] These studies were in accordance with our study. On the contrary, a study stated no significant difference between staining of the cavities with caries detection dye after treatment with the conventional method and chemomechanical caries removal agents.[31] Caries detection dyes can stain the organic matrix of less mineralized dentin in the circumpulpal area and sound dentin at the enamel-dentin junction. It cannot adequately discriminate infected dentin from sound dentin. It has been reported that objectivity is low in the studies conducted.[32,33] Based on this information, it was thought that the reason for less staining with the conventional method was the removal of both infected and affected dentin due to excess tissue removal.

The reduction in total viable bacteria and *S. mutans* count by microbial culture analysis before and after caries removal was statistically significant in both groups. But no significant difference was observed when comparing both groups ($p < 0.05$). This finding is consistent with the study conducted by Ismail and Al Haidar.[16] The decrease in bacterial count shows the bacteriostatic and bactericidal potential of Brix 3000®. The reduction in *S. mutans* count obtained by real-time PCR analysis before and after caries removal was statistically significant in both groups ($p < 0.05$). The decrease in total bacterial count obtained by real-time PCR analysis was statistically significant in the conventional method ($p < 0.05$). A decrease also occurred in Brix 3000®; however, the difference did not achieve statistical significance ($p = 0.970$).

No study in the literature has compared the caries removal efficacy of Brix 3000® and the conventional method using real-time PCR and microbial culture methods. These methods serve as conventional microbiological approaches for detecting total bacterial count and the specific cariogenic strain *S. mutans*. Earlier studies reported no significant difference in the reduction of total viable bacteria and *S. mutans* count when comparing conventional methods to chemomechanical caries removal agents.[5,15,34] These studies were in accordance with our study. On the contrary, some studies stated a significant reduction when conventional methods were compared to chemomechanical caries removal agents. The reduction of the bacterial count was higher in the chemomechanical method; therefore, the caries removal efficacy was also higher.[13,19,35,36]

The microbial culture method is considered to be the gold standard for detecting microorganisms. However, real-time PCR analysis offers a highly sensitive and specific detection method for detecting specific cariogenic bacteria.[32,37] Although there was a reduction in *S. mutans* count obtained by real-time PCR analysis after caries removal with Brix 3000®, no significant difference could be found in the total bacterial count ($p > 0.05$). This is because real-time PCR analysis has a higher ability to detect specific bacteria, such as *S. mutans*. The real-time PCR analysis can detect the total amount of bacterial DNA in a given mass and does not consider cell viability.[22,38,39] While a reduction in the total bacterial count was observed through microbial culture analysis after caries removal with Brix 3000®, no significant reduction in total bacterial count was found through real-time PCR ($p > 0.05$). The reason for this is that the real-time PCR analysis also showed dead bacteria and bacterial residues. Although the reduction in *S. mutans* obtained by real-time PCR analysis after caries removal with Brix 3000® was found to be significantly higher than the conventional method ($p < 0.05$), the bacterial count of *S. mutans* remaining in the cavity after caries removal with Brix 3000® was found to be higher. The reason for this is that the real-time PCR analysis also showed dead bacteria and bacterial residues and uncontrolled caries removal in the conventional method. Cavity disinfectants are used in dental treatments to reduce the bacterial count residue in the cavity.[40,41] Based on this information, it was thought that after caries removal with Brix 3000®, the bacteria remaining in the cavity could be removed using cavity disinfectants.

The limitations of this study are that it was *in vitro*, it was performed only in primary teeth, and the cavities where the chemomechanical agent could be directly applied were selected. Dentists should note that chemomechanical caries removal methods might require high-speed hand instruments when there is no direct access to the carious lesion. Long-term clinical studies comparing Brix 3000® with conventional methods are recommended.

Conclusion

- According to the findings of this study, Brix 3000® is an effective chemomechanical agent for caries removal, and its success will be higher with sealed restoration after using cavity disinfectants.
- Brix 3000® has been shown to be a promising treatment option for children, especially those anxious about the dental procedure. This agent shows promise due to additional advantages such as patient comfort and preservation of sound tooth structure compared to the conventional method.

Clinical Significance

The use of chemomechanical caries removal agents preserves sound tooth structure, unlike the burs used in conventional caries removal methods. These agents are also very important in preventing patient discomfort.

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