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Original Article

Scanning Electron Microscopic Evaluation of Residual Dentin Surface in Primary Teeth After Using Two Chemo-mechanical Caries Removal Agents: An *in vitro* study

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Abstract

Objective: The objective of the study was to evaluate and compare the residual dentin surface after chemo-mechanical caries excavation with Carie-careTM and Brix-3000 by scanning electron microscopy in primary molars.

Materials and Methods: Extracted sixty deciduous molars with occlusal caries were collected and randomly assigned into two groups with thirty each. Group 1; samples were treated with Carie-careTM, Group 2; samples were treated with Brix-3000. Following caries excavation, the tooth specimens were subjected to scanning electron microscopic evaluation.

Results: Scanning electron microscopic evaluation showed that the two agents were equally effective in removing the infected dentin and smear layer in primary teeth. However, on statistical comparison using Chi square test and Mann Whitney showed no significant differences in two groups.

Conclusion: Both the chemo-mechanical caries removal agents were effective in caries removal in primary molars. Hence, Brix-3000 can be effectively used in primary teeth in clinical practice.

Keywords: Brix-3000, Carie-care, chemo-mechanical caries removal, dental caries, primary teeth, scanning electron microscope

Introduction

Dental caries is an irreversible disease of microbial origin affecting the mineralized tissues of the teeth, it is characterized by demineralization of the inorganic part and destruction of the organic portion of the tooth and it often leads to cavity formation.[1] Generally caries removal is done by drilling.[2] Despite a drill's proven efficacy in removing carious tissue, the procedure is associated with unpleasant

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patient experiences.[3] Therefore, a painless non-invasive technique is essential to manage fearful and uncooperative children.[4] Hence, chemo-mechanical caries removal (CMCR) was introduced.[3] The objective of this technique is to remove the most external portion of caries (infected layer) and leave the deeper affected dentin which is capable of being demineralized and repaired.[5] Chemo-mechanical caries removal systems act by causing degradation of the partially degraded collagen in the outer layer of infected dentin.[6,7] CMCR technique is a minimally invasive technique, it is inexpensive and has good patient compliance. Local anesthesia is not required, minimal dentin removal and maximum tissue preservation is achieved in this procedure.[8]

Carie-careTM (Innovation- Hub 5, Bengaluru, India) is a natural gel based CMCR agent introduced in India. Carie-careTM consists of an extract of papaya (papain) 100mg, chloramines, clove oil 2mg, blue colored gel, sodium methyl paraben and sodium chloride.[9] Many studies are published on the clinical efficacy and residual dentin surface using Carie-careTM. However, Brix-3000 (Brix SRL, Argentina) is a newer enzymatic gel composed of Papain 3000U/mg (10%). In which papain is bio-encapsulated by using encapsulating buffer emulsion technology (EBE), exclusive technology that immobilizes and confers stability, which increases the enzymatic activity of the final product exponentially with respect to current technology.[10] The adhesion between dentine and restorative material depends not only on the adhesive system used but also on the residual dentin substrate after carious excavation. Therefore, the purpose of this in-vitro study was to evaluate and compare the residual dentin surface after caries excavation with Carie-careTM and Brix-3000 using SEM in primary teeth.

Materials and Methods

The present in-vitro study was conducted in the Department of Pediatric and Preventive Dentistry. The prior ethical approval for the study was obtained from institutional ethical committee. Extracted sixty deciduous molars with occlusal caries invading dentin were collected. These teeth were obtained from children aged 6-10 years with their parents' consent based on the following inclusion and exclusion criteria;

Inclusion criteria

- Extracted primary molars with active occlusal carious lesion involving dentin.
- Extracted primary molars with active occlusal carious lesion involving dentin with external or internal resorption of roots.

Exclusion criteria:

- Primary molars with deep dentinal caries clinically approaching or involving pulp.
- Medically compromised children's teeth.

Immediately after the extraction, the teeth were stored in a container with distilled water (Fig. 1a). Later, the teeth were cleaned and randomly allotted into two groups with thirty each (Fig. 1b).

Control Group: 30 tooth samples, caries excavation done with Carie-careTM (Fig. 1c).

Experimental Group: 30 tooth samples, caries excavation done with Brix-3000 (Fig. 1d).

The caries excavation was done according to the manufacturer's instructions (Fig. 2, 3). The CMCR gel was applied on the carious lesion of tooth and left there undisturbed for 30-60 seconds. This produces softening of carious dentin, which was removed with a blunt excavator. This step was repeated 2-3 times, caries excavation was stopped when the applied CMCR gel remained non-turbid, and this stage can be considered as complete removal of carious dentin. The residue of gel was removed; cavity was immediately rinsed with water and then wiped with a sterile moist cotton pellet. The visual test for assessment of complete caries excavation was based on unchanged color and non-turbid appearance of the CMCR agent used. The tactile assessment of healthy dentin was performed with an exploratory probe.

SEM evaluation of the tooth samples [8]: The SEM evaluation was done in Mechanical Engineering Department using TESCAN-VEGA3LUM (4.5x to 100000x) equipment (Fig. 4). The tooth specimens were immersed in distilled water and subjected to ultrasonic bath for five minutes. Later the specimens were overnight dried in a desiccator until the residue of water was removed. Thus, prepared tooth samples were later sputtered coated in a gold sputtering unit and introduced into vacuum chamber of SEM (Fig. 4). The deepest part of the cavity was observed under SEM. Five different areas were checked, and a series of micro-photographs were taken at a magnification of 5000x and 10,000x for viewing the surface morphology. All the microphotographs were taken at same working distance (WD=19.5 micron) for the analysis. SEM ultra-morphologic evaluations were done based on residual dentin in terms of dentinal tubule patency, surface irregularities smear layer,

Figure 1. (a) Armamentarium (b) Sixty extracted primary molars in two groups with 30 in each (c) Carie-care (d) Brix-3000

exposed dentinal tubules, inter tubular micro-porosities, collagen network. The presence of smear layer was graded based on the grading system introduced by Rome et al.[11]

Smear layer;

Score 0- Absence of smear layer.

Score 1- Moderate smear layer.

Score 2- Dense smear layer with visible dentinal tubules.

Score 3-Dense smear layer with no visible dentinal tubules.

Similarly, the following parameters were scored as follows:

Patency of dentinal tubules;

Score 0- Absence of patent dentinal tubules.

Score 1- Minimal number of patent dentinal tubules.

Score 2- Moderate number of patent dentinal tubules.

Score 3- All the dentinal tubules were patent.

Surface irregularities;

Score 0- Smooth surface.

Score 1- Partially irregular surface.

Score 2- Complete roughened surface.

Score 3- Roughened surface with globular projections.

Intertubular micro-porosities;

Score 0- Absence of intertubular micro-porosities.

Score 1- Minimal micro-porosities.

Score 2- Moderate micro-porosities.

Score 3- Abundant micro-porosities.

Exposed dentinal tubules;

Score 0- Absence of dentinal tubules.

Score 1- Minimal number of exposed dentinal tubules.

Score 2- Moderate number of exposed dentinal tubules.

Score 3- All the dentinal tubules were exposed.

Collagen network: Because of the presence of minimal collagen network, only presence or absence was evaluated.

Score 0- Absent.

Score 1- Present.

The SEM microphotographs (Fig. 5-8) were evaluated and then compared by two blinded investigators

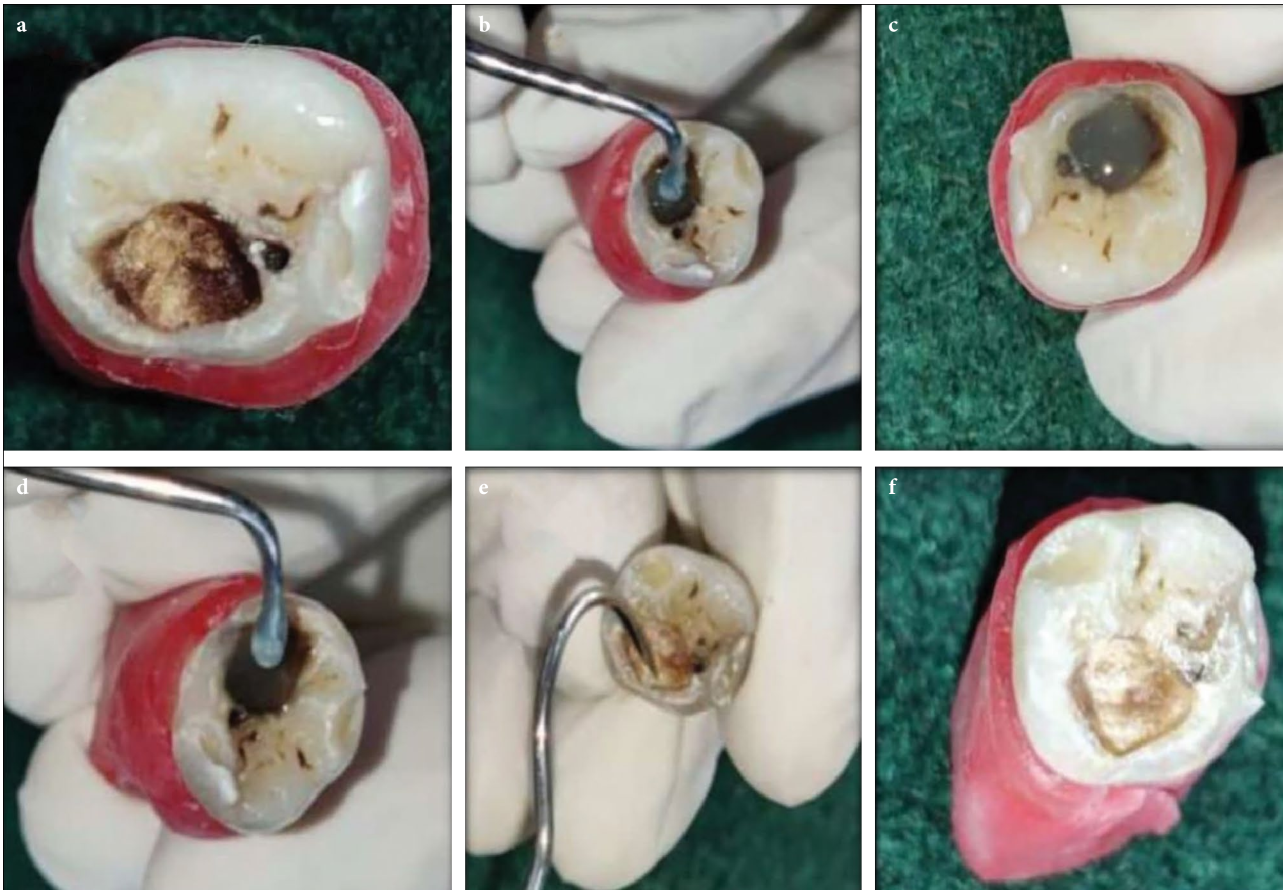

Figure 2. Chemo-mechanical caries removal using Carie-care (a) Preoperative image of the carious tooth (b) Application of Carie-care agent (c) Turbidity noted (d) Removal of carious lesion (e) Checking the surface with the probe (f) Post-operative image showing clean cavity

independently. Data collected were entered in Microsoft Office Excel 365 and were subjected to statistical analysis using statistical package for social science (IBM SPSS, Version 20.0. Armonk, NY: IBM Corp, USA) software. Data was presented by means of descriptive statistics (mean, standard deviation) and comparison was done using Chi Square Test and Mann Whitney Test.

Results

Table 1 shows comparison of different parameters related to ultra-morphology of residual dentin at 5000x magnification between 2 study groups using Chi Square Test. Table 2 shows comparison of different parameters related to ultra-morphology of residual dentin at 10000x magnification between 2 study groups using Chi Square Test. Table 3 shows comparison of mean values of different parameters related to ultra-morphology of residual dentin at 5000x magnification between 2 study groups using Mann Whitney Test. Table 4 shows comparison of mean values of different parameters related to ultra-morphology of residual dentin at 10000x magnification between 2 study groups with Mann-Whitney test.

The residual dentine surfaces in group 1 and group 2 samples showed the presence of smear layer which was partially occluding the tubules of dentine. Only slight differences were observed between the two groups among the various parameters. The differences were statistically not significant. The results of this in-vitro study showed that both the CMCR agents are equally effective in removing the infected dentine and smear layer in primary molars.

Discussion

Scanning electron microscope (SEM) is a tool used in research. It produces images of tooth samples by scanning it with a focused beam of electrons. These images provide significant information about a sample's composition and surface topography.[12] Hence, SEM evaluation of residual dentin surface after caries excavation with two different CMCR agents was considered in this

Figure 3. Chemomechanical caries removal using Brix-3000 (a) Preoperative image showing the carious tooth (b) Application of Brix-3000 agent (c) Turbidity noted (d) Removal of carious lesion (e) After caries removal clear liquid seen (f) Postoperative image showing clean cavity

Figure 4. (a) Gold sputtering unit (b) Scanning electron microscope (TESCAN-VEGA3 LMU 4.5x to 1000000x)

study. The dentinal substrate remaining after caries excavation is very important to maintain pulp vitality, for the adhesion of restorative material and to achieve bio-mimetic restoration.[8,13,14] The remaining dentinal substrate is a hydrated biological complex. It shows variations with different physiological processes, age changes and diseases. Drastic changes are observed in

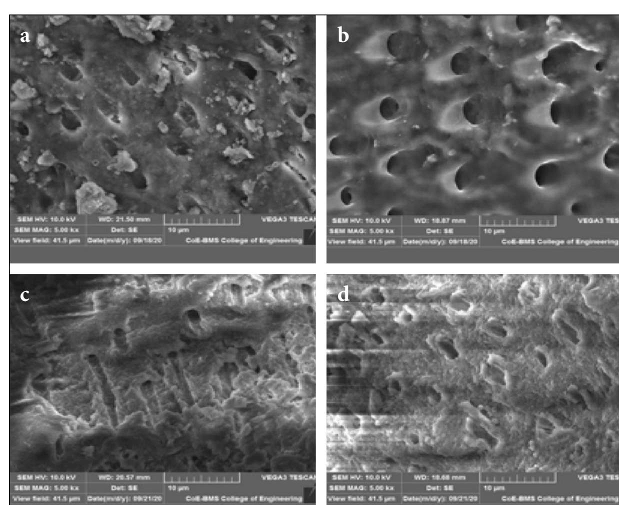

Figure 5. SEM microphotograph of Group 1 sample under 5000x magnification showing (a) Smear layer with visible dentinal tubules (b) Exposed dentinal tubules with absence of smear layer (c) Patent dentinal tubules and collagen network (d) Intertubular microporosities with surface irregularities

its structure according to the depth of cavity preparation, response to previous caries irritations, cavity cut-

Table 1. Comparison of different parameters related to ultra-morphology of residual dentin at 5000x magnification between 2 study groups using Chi Square test

Parameters	Grading	Group 1		Group 2		χ^2 value	p
		n	%	n	%		
Smear layer	Score 0	7	23.3%	4	13.3%	1.028	0.79
	Score 1	14	46.7%	16	53.3%		
	Score 2	6	20.0%	7	23.3%		
	Score 3	3	10.0%	3	10.0%		
Patency of dentinal tubules	Score 0	5	16.7%	6	20.0%	1.098	0.58
	Score 1	17	56.7%	13	43.3%		
	Score 2	8	26.7%	11	36.7%		
	Score 3	0	0.0%	0	0.0%		
Surface irregularities	Score 0	9	30.0%	4	13.3%	2.620	0.27
	Score 1	20	66.7%	24	80.0%		
	Score 2	1	3.3%	2	6.7%		
	Score 3	0	0.0%	0	0.0%		
Inter tubular micro porosities	Score 0	4	13.3%	2	6.7%	3.167	0.37
	Score 1	14	46.7%	18	60.0%		
	Score 2	10	33.3%	10	33.3%		
	Score 3	2	6.7%	0	0.0%		
Exposed dentinal tubules	Score 0	2	6.7%	2	6.7%	4.007	0.26
	Score 1	6	20.0%	7	23.3%		
	Score 2	7	23.3%	13	43.3%		
	Score 3	15	50.0%	8	26.7%		
Collagen network	Absent	23	76.7%	21	70.0%	0.341	0.37
	Present	7	23.3%	9	30.0%		

P-value<0.05: Statistically significant

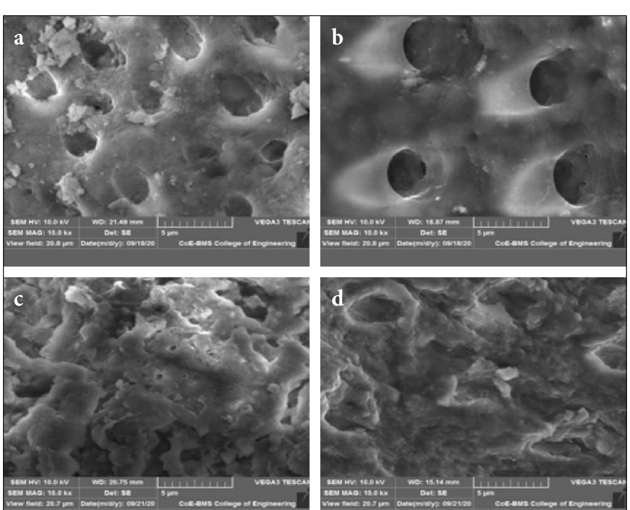

Figure 6. SEM microphotograph of Group 1 sample under 10000x magnification showing (a) Smear layer with visible dentinal tubules (b) Exposed dentinal tubules with absence of smear layer and patent dentinal tubules (c) Collagen network (d) Intertubular microporosities with surface irregularities

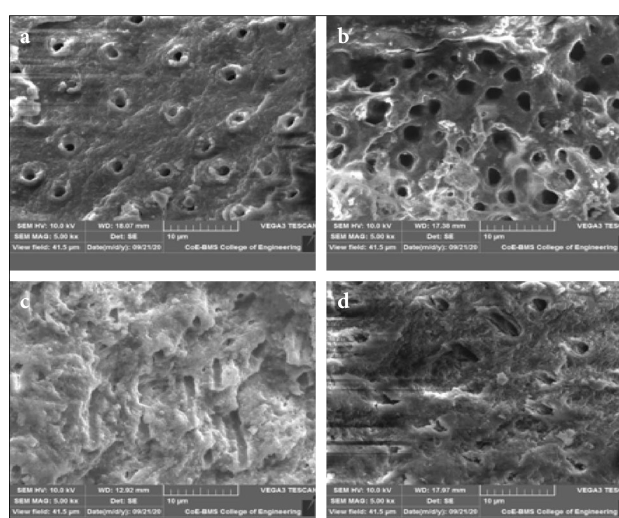

Figure 7. SEM microphotograph of Group 2 sample under 5000x magnification showing (a) Smear layer with visible dentinal tubules (b) Exposed dentinal tubules (c) Patent dentinal tubules and collagen network (d) Intertubular micro-porosities with surface irregularities

Table 2. Comparison of different parameters related to ultra-morphology of residual dentin at 10000x magnification between 2 study groups using Chi Square test

Parameters	Grading	Group 1		Group 2		χ^2 value	p
		n	%	n	%		
Smear layer	Score 0	9	30.0%	7	23.3%	0.583	0.90
	Score 1	14	46.7%	14	46.7%		
	Score 2	5	16.7%	7	23.3%		
	Score 3	2	6.7%	2	6.7%		
Patency of dentinal tubules	Score 0	5	16.7%	3	10.0%	0.643	0.73
	Score 1	13	43.3%	15	50.0%		
	Score 2	12	40.0%	12	40.0%		
	Score 3	0	0.0%	0	0.0%		
Surface irregularities	Score 0	13	43.3%	8	26.7%	3.434	0.18
	Score 1	17	56.7%	20	66.7%		
	Score 2	0	0.0%	2	6.7%		
	Score 3	0	0.0%	0	0.0%		
Inter tubular micro porosities	Score 0	5	16.7%	1	3.3%	5.572	0.13
	Score 1	12	40.0%	17	56.7%		
	Score 2	11	36.7%	12	40.0%		
	Score 3	2	6.7%	0	0.0%		
Exposed dentinal tubules	Score 0	2	6.7%	2	6.7%	3.505	0.32
	Score 1	3	10.0%	5	16.7%		
	Score 2	7	23.3%	12	40.0%		
	Score 3	18	60.0%	11	36.7%		
Collagen network	Absent	24	80.0%	21	70.0%	0.800	0.37
	Present	6	20.0%	9	30.0%		

P-value<0.05: Statistically significant

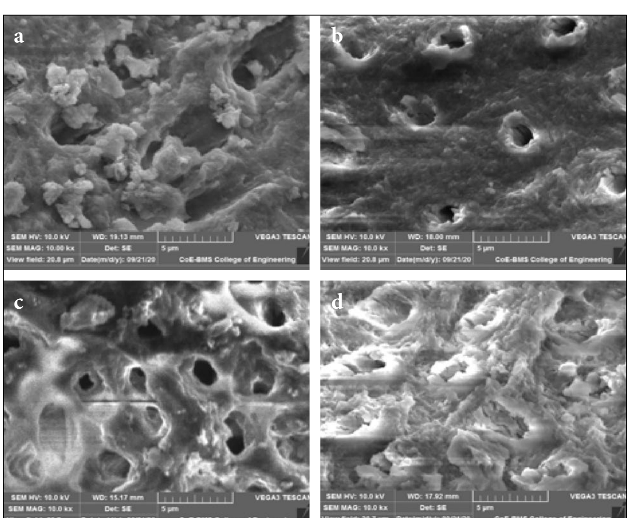

Figure 8. SEM microphotograph of Group 2 sample under 10000x magnification showing (a) Moderate smear layer (b) Exposed dentinal tubules with absence of smear layer (c) Patency of dentinal tubules and collagen network (d) Intertubular micro-porosities with surface irregularities

ting technique and restorative materials used.[8,15] A variety of factors influence the quality of adhesion between dentinal surface and restoration, including the presence of a smear layer produced by the process of caries excavation.[14]

In the present study in both the groups inter-tubular micro-porosities and surface irregularities were seen, which can be a contributing factor for better adhesion to the restorative materials (Figs. 5d, 6d, 7d, 8d). According to Tonami KI et al, open dentinal tubules present in the remaining dentinal substrate after caries removal with CMCR agents was due to the initial high pH of the gel used.[16] This is usually due to the presence of the component chloramine in CMCR agents. It produces opening of dentinal tubules in the outermost layer of remaining dentinal substrate (Figs. 5b, 6b, 7b, 8b). Collagen fiber structure undergoes dissociation when chloramine reacts with collagen and proteins. It decomposes degenerated collagen and softens the dentin which in turn removes the smear layer and exposes

Table 3. Comparison of mean values of different parameters related to ultra-morphology of residual dentin at 5000x magnification between 2 study groups using Mann Whitney test

Parameters	Groups	n	Mean	SD	Mean ranks	Sum of ranks	p
Smear layer	Group 1	30	1.17	0.91	29.12	873.50	0.51
	Group 2	30	1.30	0.84	31.88	956.50	
Patency of dentinal tubules	Group 1	30	1.10	0.66	29.62	888.50	0.67
	Group 2	30	1.17	0.75	31.38	941.50	
Surface irregularities	Group 1	30	0.73	0.52	27.73	832.00	0.11
	Group 2	30	0.93	0.45	33.27	998.00	
Inter tubular micro porosities	Group 1	30	1.33	0.80	31.10	933.00	0.77
	Group 2	30	1.27	0.58	29.90	897.00	
Exposed dentinal tubules	Group 1	30	2.17	0.99	33.28	998.50	0.19
	Group 2	30	1.90	0.89	27.72	831.50	
Collagen network	Group 1	30	0.23	0.43	29.50	885.00	0.56
	Group 2	30	0.30	0.47	31.50	945.00	

P-value<0.05: Statistically significant

Table 4. Comparison of mean values of different parameters related to ultra-morphology of residual dentin at 10000x magnification between 2 study groups using Mann Whitney test

Parameters	Groups	n	Mean	SD	Mean ranks	Sum of ranks	p
Smear layer	Group 1	30	1.00	0.87	29.10	873.00	0.51
	Group 2	30	1.13	0.86	31.90	957.00	
Patency of dentinal tubules	Group 1	30	1.23	0.73	29.90	897.00	0.77
	Group 2	30	1.30	0.65	31.10	933.00	
Surface irregularities	Group 1	30	0.57	0.50	27.43	823.00	0.11
	Group 2	30	0.80	0.55	33.57	1007.00	
Inter tubular micro porosities	Group 1	30	1.33	0.84	30.18	905.50	0.88
	Group 2	30	1.37	0.56	30.82	924.50	
Exposed dentinal tubules	Group 1	30	2.37	0.93	33.75	1012.50	0.12
	Group 2	30	2.07	0.91	27.25	817.50	
Collagen network	Group 1	30	0.20	0.41	29.00	870.00	0.38
	Group 2	30	0.30	0.47	32.00	960.00	

P-value<0.05: Statistically significant

the dentinal tubules. The essential therapeutic oil which is one of the components of Carie-careTM is rich in phenolic compounds namely eugenol and eugenol derivatives which are precursors of flavones, isoflavones and flavonoids. They have antioxidant, anti-inflammatory, DNA-protective, analgesic and antimicrobial properties. These will provide further protection to the remaining dentinal substrate.[8]

Remineralization occurs in the presence of cross banded collagen, since the apatite crystals can attach to these collagen molecules.[15] Similar findings were observed in the teeth treated with Carie-careTM in the inner affected dentin.[17] In the present study, Carie-careTM group had 6 samples showing presence of collagen network under 5000x (Fig. 5b) and 7 samples under 10000x (Fig. 6c) and Brix-3000 group had 9 samples showing collagen network under 5000x and 10000x.

The presences of open dentinal tubules were due to the explosive nature of tooth structure removal in CMCR. Also, the protruding of peri-tubular dentin was seen under SEM. The surface irregularities in the cavity floor without any smear layer may help in bonding of composite resins.[18] A clinical study on Carie-careTM CMCR technique showed that it was as effective as mechanical caries removal in primary molars of school children. It also showed that CMCR technique was less time consuming, had more efficacy and better patient acceptance both in clinical and community settings.[19]

Limitations of the study:

- Only primary molars were evaluated in this study.
- Residual bacterial deposits after caries removal were not evaluated.

Further recommendations

- SEM evaluation of residual dentine surface on permanent teeth can be undertaken.
- Residual bacterial deposits after caries removal can be evaluated.
- All the new chemo-mechanical caries removal agents should be evaluated under SEM for their efficacy.

Conclusion

The following conclusions were drawn from this *in-vitro* study;

1. SEM evaluation of remaining dentinal substrate after caries removal using Carie-careTM and Brix-3000 showed that they are effective in primary molars.
2. No statistically significant differences were observed between the 2 groups in caries removal.
3. Brix-3000 can be used as an effective CMCR agent for primary teeth.

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Conflict of Interest: None declared.

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