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Scanning Electron Microscope Analysis of Resin Bonding Characteristics Using Mechanical and Chemo Mechanical Caries Removal Techniques-An *In Vitro* Study

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Abstract

Aim: To evaluate and compare the efficacy of three caries removal techniques namely Hand Excavation, Smart Burs and Cariecare on resin bonding characteristics of dentine using SEM analysis.

Materials and Methods: In-vitro, randomized, prospective study was done on 30 extracted permanent molars by randomly dividing samples into 3 groups, namely Spoon excavator, Cariecare and Smart Bur on the basis of method of caries excavation. Following caries removal by the three methods, the samples were restored using composite resin and sectioned into two halves for SEM analysis.

Results: Cariecare removed the caries significantly more efficiently and showed better resin tags and hence bonding characteristics than both mechanical methods (p value .000).

Conclusion: Cariecare can be used as an alternative method followed by acceptable bonding material for treatment of carious permanent teeth in children while bonding with total etch resin technique.

Keywords: Cariecare; chemomechanical; Resin bonding; Total etch; Smart bur; Polymer bur; Excavation

Abbreviations: RPM: Rotation Per Minute; SEM: Scanning Electron Microscope; TEM: Transmission Electron Microscopy.

Introduction

Pit and fissure caries affects 95% of children with newly erupted permanent molars. Immature status of enamel

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and wide dentinal tubules lead to rapid progression and gross destruction of occlusal surface at a greater rate in children. Non- invasive and painless method of caries removal is needed in day to day practice of pediatric dentistry. Latest innovations in adhesive dentistry promote remineralization and sealing at tooth- material interface. Present non- invasive method of caries removal in young permanent teeth has been developed over time with the aim of pulp protection, minimal pain and most of all requiring patient's cooperation in terms of efficiency and time needed.

The ultimate goal of any restorative procedure is efficient caries removal and minimal pulp injury with efficient coronal seal. Dental caries excavation has been the most primitive method in practice which is carried out by using the hand instruments. Later, rotary instruments were introduced in the year 1871, when Morisson introduced the first commercially manufactured foot engine which was heavy and had low speed. In recent years, equipment having a speed of approximately 3, 00,000 rpm is being used. Despite the efficiency, the vibration and noise associated with these instruments is often an unpleasant experience to patients, especially children causing an aversion and therefore avoidance of the dental treatment further worsening the carious process. Hence, there has been growing interest in developing non-rotary, non-invasive techniques in an effort to make the process comfortable to the patient while preserving the healthy dental tissues.

Habib et al. in 1975 was the first to report the system of chemo-mechanical caries removal as the GK-101 system which used the pharmacodynamics action of sodium hypochlorite for the removal of caries. The chemo-mechanical removal technique is easy to use and overcomes many of the disadvantages associated with the use of bur including pain induction. It involves the application of a chemical solution which softens the outer infected, non-remineralizable carious dentin, which, can be removed with blunt instruments, leaving behind the affected demineralized dentin that is capable of remineralization and repair. It has gained high acceptance especially among children as it helps to avoid administration of local anesthesia in 82-92% of the patients during caries excavation.

With time, various systems like Caridex and Carisolv were introduced. In 2003, a Brazilian formulation named Papacarie was introduced, which is a gel based on papain, a proteolytic cysteine enzyme that has antibacterial and anti-inflammatory properties. Thus, the Carie-Care system was introduced in India by Uni-Biotech Pharmaceuticals Pvt. Ltd., which consists of papaya extract (papain) 100 mg, clove oil 2 mg, colored gel (blue), chloramines, sodium chloride, and sodium methyl paraben, with similar property as that of Papacarie. Cariecare also contains chloramines which have the potential of dissolving carious dentin by means of chlorination of the partially degraded collagen, soften it and facilitate its removal.

In an attempt to develop a selective caries-removal rotating instrument, a "plastic" bur was made of a polyamide/imide (PAI) polymer, possessing slightly lower mechanical properties than sound dentin. The commercial version of these burs (SmartPrep, SSWhite Burs; Lakewood, NJ, USA) consisted of a polymer (PEKK – polyether-ketone-ketone) with a particular hardness of 50 KHN, which was higher than the hardness attributed to carious dentin (0 to 30 KHN), but lower than that of sound dentin (70 to 90 KHN) [1]. These plastic burs removes only the insensitive, soft, and necrotic carious dentin (caries-infected dentin), leaving the demineralized, non-infected sensitive layer (caries-affected dentin) reducing the induction of pain.

Efficacy of caries removal methods have been studied through various techniques including microhardness testing, micro CT evaluation as well as light microscopy and SEM evaluation. The bonding efficiency of carious affected dentin and composite interface is finally responsible for success of restorative procedure and depends on various factors like dentin permeability, presence of smear layer, opening of dentinal tubules, internal and external dentin wetness. Ferrari et al qualitatively evaluated the bonding of tooth surface and resin material by evaluating the resin tags using a four-step (0-3) scale method on the basis of their morphology and density [2]. Therefore the present study is planned to evaluate the microscopic structure of carious dentin after caries removal using conventional Hand Excavation, Smart Bur and Cariecare followed by composite resin bonding with respect to observed microscopic structure in permanent teeth using Scanning Electron Microscope.

Materials and Methodology

This in-vitro, randomized, prospective study was conducted in Department of Pedodontics and Preventive Dentistry, Sudha Rustagi College of Dental Sciences and Research, Faridabad in collaboration with Advanced Instrumentation Research Facility, Jawahar Lal Nehru University Campus, and Delhi to assess the resin tag formation in dentin following caries removal by 3 techniques namely; Hand Excavation, Smart Burs and Cariecare. Based on the pilot study, the sample size was

estimated at 42 samples. For convenient distribution of samples, 54 samples (27 teeth) were considered.

Selection of teeth

30 freshly extracted permanent first molars with occlusal dentinal caries which met the inclusion criteria were selected for the trial. The teeth with carious lesion that have extended till the dentin, without extensive coronal destruction were included. Teeth with incipient carious lesion on the enamel or deep lesions close to the dental pulp or involving pulp were excluded. Radiographs were taken to verify the caries involvement. The extracted teeth were rinsed with sterile water immediately after extraction and were kept frozen in-200C until further procedures in a small container. The teeth were thawed at room temperature overnight before the experiment. The teeth were mounted before starting the procedure. Information regarding the tooth number, time needed for caries removal and completeness of caries removal by each technique was recorded in a proforma.

Inclusion criteria:

- Only permanent molars were included
- Teeth with large occlusal, single surface caries involving enamel and dentin
- Caries not extending beyond 2/3rd dentin

Exclusion criteria:

- Deep caries lesions
- Teeth with pulpal exposure
- Multisurface caries

Random division of samples

30 teeth with single surface carious lesions which fulfilled the inclusion and exclusion criteria were then selected. The teeth were designated as samples and were randomly divided into three basic groups (Group I to Group III) based on the caries excavation employed. The division of samples is further explained as below.

Group I (n=18)	Caries Removal with Spoon Excavator
Group II (n=18)	Caries Removal with Smart Bur
Group III (n=18)	Caries Removal with Cariecare

Procedure

The procedure involved the removal of caries in the selected teeth by employing any of the three carious removal techniques.

- In Group I, caries removal was performed using Spoon Excavator. Superficial softened layer was loosened from the margins of the lesion, followed by bulk removal of caries with small size spoon excavator.
- In Group II, caries removal was performed under coolant with a round Smart Bur attached to a low speed (500-800 rpm) hand piece until all the softened dentin has been removed.
- In Group III, caries removal was performed using Cariecare gel. The carious cavity was filled completely with Cariecare gel, as per manufacturer's instructions and was allowed to act for 60 seconds. The softened carious dentine was then removed using a spoon excavator until a hard surface is attained.

The complete removal of caries was confirmed by application of Basic Fuchsin dye and assessment was done. Caries removal procedure was repeated if need be. The cavity of all groups was dried using three way syringe and etched with 34% phosphoric acid for 20 seconds. The etchant was delivered directly to the carious cavity to fill it completely, followed by rinsing, drying, application of an adhesive (Prime & Bond NT, DENTSPLY) and completely restoring with composite resin (Ceram X, Nano ceramic, DENTSPLY) using increments that were light cured for 45 seconds each. The tooth samples were coded according to the group and sectioned vertically through the centre with the help of a diamond disc under running water into two halves (mesial and distal) to visualize the resin dentin interface. The sections were then subjected to SEM Analysis.

Sample preparation for sem analysis

The samples were mounted on aluminium stubs with double sided carbon tape, placed in a vacuum chamber, sputter-coated with Polaron SC7640 and observed under a scanning electron microscope using Carl Zeiss EVO 40 used at 20 kV.

Evaluation criteria

a. Evaluation of caries removal efficacy of all the three methods will be done by visual analysis of following criteria SEM micrographs by two trained blinded examiners which are not associated with the study:- (1) Number of dentinal tubules, (2) Lumen of dentinal tubules, (3) Presence of smear layer.

b. Evaluation of composite bonding efficacy of all the three methods will be done by visual analysis of following criteria SEM micrographs by two trained blinded examiners which are not associated with the study:- (1) Presence of hybrid layer, (2) Occlusion of dentinal tubules.

c. Resin Tag Scoring: Resin tags were evaluated quantitatively by measuring the length of the resin tags according to a scale given on the photographs. Five different measurements were performed on each photograph and the mean was taken as the representative value for that specimen. For qualitative evaluation of the tags, a four- step (0-3) scale method according to Ferrari et al. (2002) will be used for evaluation of resin-dentine interfaces.

- Score 0--no resin tag formation
- Score 1--few and short resin tags
- Score 2--when long resin tags were visible
- Score 3--dense resin tags with numerous lateral branches.

The scores will be tabulated, analysed and conclusions will be drawn.

- In order to compare the score of the different groups under the three variables i.e. No. of Dentinal tubules, Lumen of Dentinal tubules and Smear Layer, One Way ANOVA with post hoc Tukey 't' test at 5% level of significance was used.
- In order to compare the score of the different groups under the three variables i.e. Hybrid Layer, Occlusion of Dentinal tubules and Resin Tag Score, we shall consider using One Way ANOVA with post hoc Tukey 't' test at 5% level of significance.

Results

Present study was an in vitro study in which 54 samples were equally distributed in 3 groups, Group I which involved use of spoon excavator, Group II which involved the use of Smart Bur and Group III which involved use of Cariecare were subjected to bonding and restoration with Light cure composite (3M ESPE) after caries removal with respective agents in Group I, II, III. Efficiency of caries removal by different agents used was visually measured in specified area of micrographs obtained after SEM examination by parameters 1) number of open dentinal tubules present, 2) lumen of dentinal tubules and 3) presence or absence of smear layer. (Table 1) Further, parameters for quality of bonding were measured quantitatively by 1) presence or absence of hybrid layer, 2) occlusion of dentinal tubules and qualitatively by a scale which is a four step assessment of the morphology and density of resin tags. (Table 2) Data obtained was subjected to statistical analysis (Figure 1 and 2).

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Score Of No Dentinal Tubules	Spoon Excavator Vs Smart Bur	.66667*	.18078	.002	.2303	1.1031
	Spoon Excavator Vs Cariecare	-.83333*	.18078	.000	-1.2697	-.3969
	Smart Bur Vs Carie Care	-1.50000*	.18078	.000	-1.9364	-1.0636
Score Of Lumen Of Dentinal Tubules	Spoon Excavator Vs Smart Bur	.83333*	.15481	.000	.4596	1.2070
	Spoon Excavator Vs Cariecare	-.33333	.15481	.089	-.7070	.0404
	Smart Bur Vs Carie Care	1.16667*	.15481	.000	.7930	1.5404
Score Of Smear Layer	Spoon Excavator Vs Smart Bur	.50000*	.14003	.002	.1620	.8380
	Spoon Excavator Vs Cariecare	-2.00000*	.14003	.000	-2.3380	-1.6620
	Smart Bur Vs Carie Care	2.50000*	.14003	.000	2.1620	2.8380

Table 1: Multiple Comparisons Descriptive Table for Caries Removal.

Multiple Comparisons						
Tukey HSD						
Dependent Variable	(I) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Score Of Hybrid Layer	Spoon Excavator Vs Smart Bur	.83333*	.15125	.000	.4682	1.1984
	Spoon Excavator Vs Cariecare	-1.00000*	.15125	.000	-1.3651	-.6349
	Smart Bur Vs Carie Care	-1.83333*	.15125	.000	-2.1984	-1.4682
Score Of Occlusion Of Dentinal Tubules	Spoon Excavator Vs Smart Bur	.33333	.16502	.118	-.0650	.7317
	Spoon Excavator Vs Cariecare	-.83333*	.16502	.000	-1.2317	-.4350
	Smart Bur Vs Carie Care	-1.16667*	.16502	.000	-1.5650	-.7683
Score Of Resin Tag	Spoon Excavator Vs Smart Bur	.66667*	.13202	.000	.3480	.9854
	Spoon Excavator Vs Cariecare	-2.00000*	.13202	.000	-2.3187	-1.6813
	Smart Bur Vs Carie Care	-2.66667*	.13202	.000	-2.9854	-2.3480

Table 2: Multiple Comparisons Descriptive Table for Bonding Characteristics.

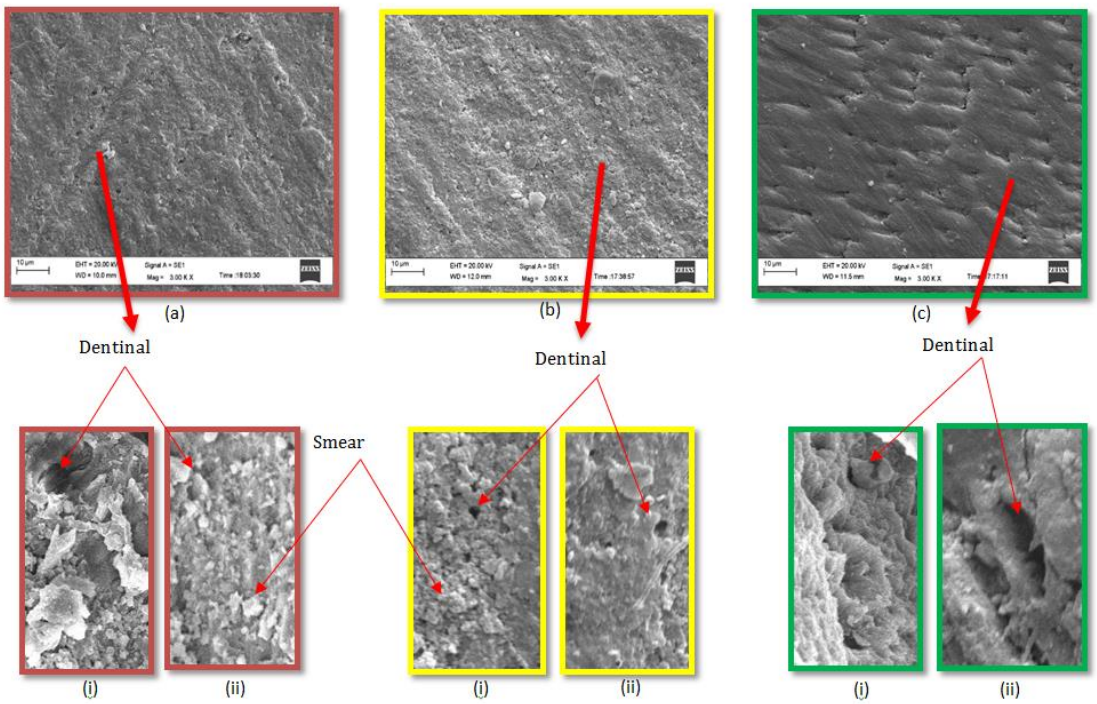

Figure 1: Scanning Electron Microscope microphotographs showing residual dentin morphology after caries removal by the three groups seen at X3.0K magnification (a) Spoon Excavator (Group I); (i) Open dentinal tubule at X5.0K; (ii) Partially occluded dentinal tubule at X2.K , (b) Smart Bur (Group II)); (i) Partially occluded dentinal tubule at X2.0K; (ii) Open dentinal tubule at X2.K, (c) Cariecare (Group III)); (i) Partially occluded dentinal tubule at X2.0K; (ii) Open dentinal tubule at X5.K.

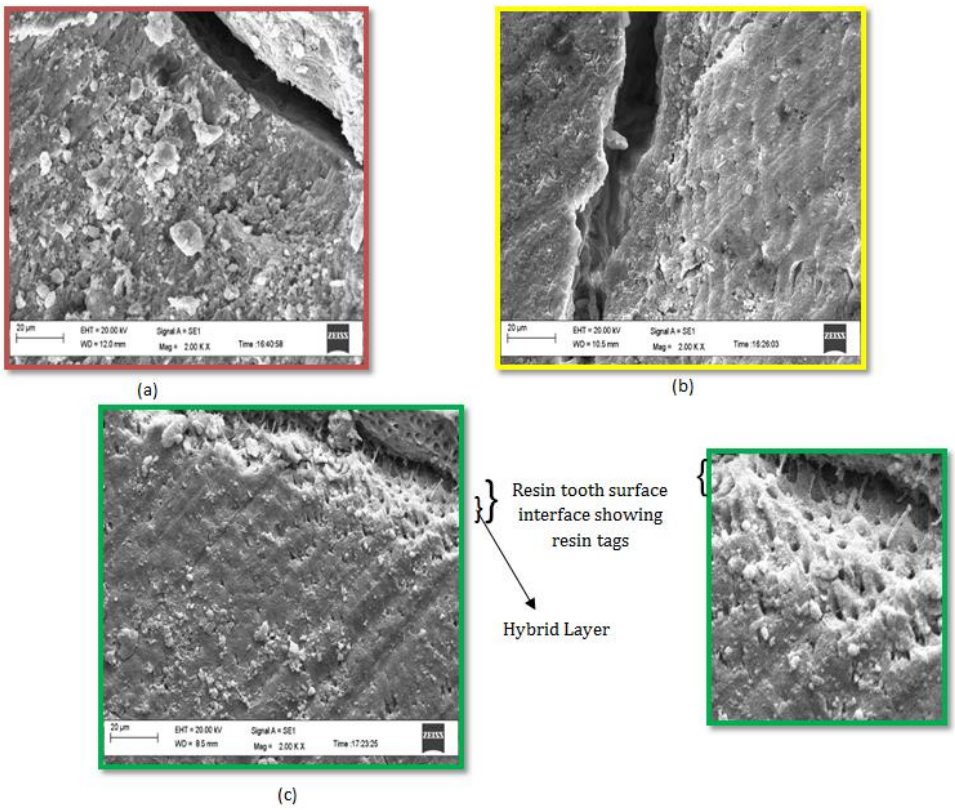

Figure 2: SEM microphotographs showing resin tag morphology and density after composite restoration in the three groups seen at X2.0K magnification (a) Spoon Excavator (Group I), (b) Smart Bur (Group II), (c) Cariecare (Group III).

Discussion

Caries removal by minimally invasive methods followed by achieving perfect coronal seal is the most needed procedures in pediatric dentistry. Mechanical methods for caries removal like air rotor and carbide bur remove carious dentin aggressively and large amount of affected dentin is also removed. All latest inventions support minimal removal of caries to prevent any harm to underlying young pulp. In recent methods of minimally invasive caries removal, smart bur is the newest addition (2003) along with advanced established methods like chemo mechanical and only excavation. Highly prevalent pit and fissure caries of permanent molars in children spread rapidly as dentinal tubules are larger in size and have more organic content. Polymer burs were introduced in year 2000 and Smart Bur, newer in this series consisted of a polymer (PEKK-polyether-ketone-ketone) with a particular hardness of 50 KHN, which was higher than the hardness attributed to carious dentin (0 to 30 KHN), but lower than that of sound dentin (70 to 90 KHN) [3]. Its mechanism of action has been said to allow a "self-limiting," less invasive/less destructive dentin caries excavation, selectively removing only the softened, infected, non-remineralizable dentin and thus conserving tooth substance and based on Fusayama's idea, where carious dentin is regarded as consisting of an outer layer in which the organic material is substantially degraded, and therefore not remineralizable, and an inner layer with limited collagen degradation, which is capable of being remineralized [4]. Further chemo mechanical was introduced long time back and has been studied extensively. It promises better success with invention of new dentin bonding technology these days. Hand excavation and removal of selective carious dentin with tactile stimulation is most commonly used method where other means are not available. Total etch composite resin are always preferred over self-etch resin for achievement of better bonding strength in especially young dentin and enamel after minimally invasive caries removal.

Present study was planned to evaluate of efficacy of caries removal by three minimally invasive methods namely, Hand Excavation, Smart Burs and Cariecare on resin

bonding characteristics of dentin using visual parameters for SEM micrographs. The bonding was also checked as per SEM analysis with parameters of hybrid layer, occlusion of dentinal tubules and quality of resin tags. Parameters for caries removal were number of open dentinal tubules present, lumen of dentinal tubules and presence or absence of smear layer. Further, parameters for quality of bonding were measured quantitatively by presence or absence of hybrid layer, occlusion of dentinal tubules and qualitatively by a scale which is a four step assessment of the morphology and density of resin tags.

The micrographs were visually examined by blinded examiners for quantitative and qualitative parameters of caries removal and bonding characteristics Normal Dentin on SEM evaluation at ultrastructural level shows dentinal tubules, smear layer, specifically arranged collagen fibres, intertubular and peritubular dentin [5]. This in-vitro, randomized, prospective study was conducted in Department of Pedodontics and Preventive Dentistry in collaboration with Advanced Instrumentation Research Facility, Jawahar Lal Nehru University Campus, and Delhi to assess the resin tag formation in dentin following caries removal by 3 techniques namely; Hand Excavation, Smart Burs and Cariecare in 42 samples among mechanical methods of caries removal i.e. smart bur and hand excavation, Hand excavation was found to be more efficient in caries removal and resin bonding as compared to smart bur (p value .000). Same results have been achieved by Prabhakar A et al. [6] in previous studies. On comparison of chemo mechanical and mechanical caries removal methods, chemo mechanical agent (Cariecare) removed the caries significantly more efficiently and showed better resin tags and hence bonding characteristics than both mechanical methods (p value .000). Similar results were reported by Hafez MA et al. [7].

Caries causes disruption of these normal structures both at microscopic and macroscopic level. Extent of caries removal affects final bonding characteristics of total etch composite resin with affected dentin. The quality of favourable bonding for longevity of restoration depends upon extent of caries removal and preparation of residual affected carious dentin after application of minimally invasive methods like hand excavation, smart bur and chemo mechanical agents. Cariecare performed the best followed by hand excavation and smart bur in caries removal Cariecare works on the principle that partially degraded collagen in carious dentine will be chlorinated by chemo mechanical caries removal solutions. It acts by breaking the partially degraded collagen molecules, contributing to the degradation and elimination of the fibrin “mantle” formed by the carious process. The attack causes cleavage of the polypeptide chains and hydrolyses the cross-links of collagen fibrils. Right after the degradation, oxygen is freed, and this explains the appearance of bubbles on the surface and the blearing of the gel during the clinical procedure. These signs demonstrated that the removal process has been started [7]. It has been shown that the dentinal surfaces formed after biochemical caries removal are very irregular with many overhangs and undercuts. In addition, the biochemical method removes the smear layer completely and exposes dentinal tubules [8]. Also in children, Cariecare is the most painless method, though it takes more chairside time Kochhar et al. [9]. concluded that chemo mechanical removal of caries were found to be effective measures of caries removal and could be considered as viable alternatives to painful procedures like Air rotor in management of dental caries especially in children [10].

Various studies have shown that a greater amount of residual caries was observed with polymer bur and also, the time needed is more due to low rotational speed of the bur (approx. 800 rpm), layer by layer removal of caries and less hardness of the bur material [6]. Studies that used caries excavation techniques that tended to over-prepare cavities and form a thick smear layer [11]. In our study, Cariecare showed the best bonding when compared with both Hand excavation and Smart Bur with p value (.000). Cariecare promotes better bonding and better tag formation as observed in SEM micrographs after composite bonding, probably because of the presence of a minimal smear layer, open dentinal tubules and also the complete removal of the gel after acid etching and water rinsing [12]. Studies have reported that the micro tensile bond strength to carious dentin excavated with SmartPrep burs was lower for both etch-and-rinse and self-etching adhesives, transmission electron microscopy (TEM) of the bonded interfaces disclosed remnants of carious tissue at the excavated dentin/composite interface. Banerjee et al demonstrated that the use of conventional hand excavation appears to weaken the bond strength of self-etch adhesives to the remaining dentine and they attributed this finding to the presence of an infected smear layer [11].

Carious dentin behaves differently than caries affected dentin when it comes to bonding with total etch technique [13]. Dentin bonding strength, stability are affected negatively when the dentin is carious as compared to non-carious dentin. Efforts have been made to achieve ideal bonding, strength and clinical performance close to normal dentin. In this endeavour, total etch composite technique has proven itself the best during various research studies [14] as after mechanical preparation of a cavity with a bur or excavators, an amorphous smear

layer including organic and inorganic debris that occludes the tubules is formed on the surface of dentine [15]. The smear layer adheres firmly to the dentine surface and cannot be removed by ordinary water spray. The presence of the smear layer prevents adhesion of resin to dentine [16]. The use of phosphoric acid on dentine may remove the smear layer and partially dissolve the surrounding peritubular dentine, allowing more resin to infiltrate into the dentine tubule [15].

The results of the current study show that caries removal as well as resin bonding is statistically significantly better in terms of efficacy of caries removal parameters like in case of chemo-mechanical method as compared to mechanical methods (hand excavation and smart bur). Subsequently resin tag formation, formation of hybrid layer is better established in Cariecare and least in Smart Bur. In addition, chemo mechanical methods also avoid over preparation of dentin which is seen in case of hand excavation and hence, agree with the concept of minimal invasive dentistry.

Conclusion

Cariecare can be used as an alternative method for treatment of carious permanent teeth in children while bonding with total etches resin technique. Smart burs cannot substitute conventional or chemo mechanical caries removal techniques. Hence, use of chemo mechanical caries removing agent can be incorporated in pediatric practice following careful case selection since it favors minimal invasive dentistry.

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