Combined Effect of Enamel Deproteinization and Intermediate Bonding in the Retention of Pit and Fissure Sealants in Children: A Randomized Clinical Trial

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Objective: *This manuscript shows if enamel deproteinization along with an intermediate layer of bonding enhances the retention of pit and fissure sealants..* ***Study Design****: Two hundred six mandibular first permanent molars were allocated to Group I (n=103) and Group II (n=103). Group I underwent deproteinization, acid etching, bonding agent application and pit & fissure sealant placement while Group II treated with acid etching followed by pit & fissure sealant application only. Clinical analysis of all the teeth in the two groups was performed at 1, 3, 6, 9 and 12 months respectively. Pearson's chi – square test was utilized to evaluate the success of both treatment procedures (p<0.05).* ***Results:*** *At 12 months follow up the differences between the groups pertaining to Marginal integrity, Marginal discoloration and Anatomical form were statistically significant suggesting enhanced retention in Group I.* ***Conclusions:*** *Enamel deproteinization along with the use of intermediate bonding layer significantly enhances the retention of pit and fissure sealants in terms of enhanced marginal integrity, decreased marginal discoloration and preserving the anatomical form.*

Key Words*: Enamel deproteinization, Intermediate bonding, Pit and fissure sealant, Sodium hypochlorite*

INTRODUCTION

Dental caries is a major public health problem. Different tooth surfaces show variability in the susceptibility to dental caries, with the occlusal surfaces being more prone compared to the smooth surfaces.[1,2] The tendency of the occlusal surfaces of teeth to develop dental caries is related closely with the depth and morphology of pit and fissures which are considered generally as imperfections or faults in the cuspal odontogenesis.

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The battle of dentistry against decay in pits and fissures includes certain preventive innovations as Wilson[3] in 1895 determined early physical blocking of fissures with zinc phosphate cement and Hyatt[4] in 1923 had given the concept of prophylactic odontotomy, Bodecker[5] in 1929 proposed mechanical fissure eradication, Kline and Knutson[6] in 1942 had given the concept of chemical treatment of pits and fissures with silver nitrate. But the effort to prevent pit and fissure caries succeeded only after 1955, when the classical study of Buonocore[7] was published representing a pioneer method for mechanical bonding of acrylic resin to the enamel previously etched with phosphoric acid by sealing the pits and fissures.

The first paper on pit and fissure sealants was published by Buonocore and Cueto[8] in 1965. Authors utilized 50% H_3PO_4 buffered with 7% ZnO serving as etchant and along with it a mixture of methylmethacrylate monomer and grain from silicate cement as the sealant. They achieved reduction in caries upto 87% and complete retention of the material upto 71% after one year of observation. Influx of pit and fissure sealant was done by Buonocore for the first time on February 1971, under the name Nuva – Seal (L.D Caulk), accompanying its ultraviolet light source and curing initiator, the Caulk Nuva Lite.[9]

The required properties of an ideal fissure sealant includes biocompatibility, adequate bond strength, anticariogenecity, resistance to wear and abrasion, good marginal integrity and cost effectiveness. The success of sealant is determined through various factors like the optimal conditions under which the sealant is placed.[10]

Occlusal anatomy of the teeth also has the role in the success rates of pit and fissure sealants. Retention also depends on the conditioning of enamel, techniques of application, characteristics of the sealant material like viscosity, surface tension and proper adhesion.[11,12] Earlier, various pretreatment methods have been tried prior to acid etching to improve sealant retention like dry brushing with a rotary brush and paste,[13] prophy – jet air abrasion system,[14] enameloplasty sealant technique,[15] air abrasion with aluminium oxide,[16] preparation of tooth surface by bur[17] and mechanical removal of prismless layer of enamel.[18]

Acid etching technique works by removing contaminants, increasing the enamel surface energy, creating dissolution of prism cores which will result in microporosity where resin can flow and can form mechanical bond with enamel by polymerization.[19] Sealant retention has been recommended to be increased by the use of bonding agents. Their usage may change the rheology of material by increasing its flowability into the fissures and acid etched surface.[20] An *in vitro* study revealed that bonding agent reduces microleakage, increases the vertical penetration of the sealant and improves the shear bond strength when used under a sealant.[21]

Various studies suggested that organic material and salivary proteins presence in saliva, which are included normally and adsorbed on the superficial lesion zone, may create hindrance both in the conventional etching technique and in deepest penetration of resin. [22-25] The concept of enamel deproteinization was first introduced by Espinosa *et al* [26] in 2008. They showed that removing organic content from enamel surface with 5.25% sodium hypochlorite (NaOCl) as a deproteinizing agent prior to phosphoric acid etching doubles the enamel's retentive surface significantly from 48.8% to 94.47% and increased Type I and Type II etching patterns which is good for ideal bonding.

Since deproteinization concept has not been used on occlusal surface previously and there is no agreement regarding the role of intermediate bonding in the retention of pit and fissure sealants, this study was undertaken to evaluate the effect of enamel deproteinization and the use of intermediate bonding in the retention of pit and fissure sealants in children.

MATERIALS AND METHOD

The investigation protocol was approved through Institutional Ethics Committee prior to the beginning of study. A total of 337 children were screened, who reported to the out patient Department of Pedodontics and Preventive Dentistry, Teerthanker Mahaveer Dental College & Research Centre, Moradabad, India and 103 children who met the inclusion criteria were involved in the study using a stratified sampling method. Two hundred six mandibular first permanent molars in these 103 children were allocated into Group I (n=103) and Group II (n=103) using a split mouth design and based on randomization protocol.[27] The sample size was calculated following power analysis which was 85% for this study. Inclusion criteria for this study were, age group of 6 to 10 years (Both boys and girls), children having deep fissures and pits in recently erupted permanent first mandibular molars, the occlusal surface of tooth should be fully visible and if the guardians/parents of the patient agreed to make their child participate in the study. Patients with significant behavioral management problems, past negative dental experience, children suffering from any systemic disease, mentally compromised, teeth with the developmental defects, presence of carious lesions and previously filled teeth were excluded from the study. Rubber dam was used to isolate the selected tooth, followed by cleaning of fissures and pits of both side first permanent molars using fluoride free pumice powder mixed with glycerine followed by washing with distilled water.

Clinical procedure in Group I

The occlusal surface of the isolated tooth was treated with 3% sodium hypochlorite (Deepdent Products, New Delhi, India) as a deproteinization agent[28] for one minute, rinsed with distilled water followed by drying with oil free compressed air for 10 seconds. Afterwards, acid etching of the enamel was done with 37% phosphoric acid gel (Ammdent, Amrit chemicals and mineral agency, Mohali, India) for 15 seconds, the occlusal surface was then rinsed for 10 seconds using air water spray of the three – way syringe and dried using oil – free compressed air with a hand pump air pressure syringe. After ensuring a frosted appearance of the enamel, bonding agent (G-Bond, GC Corporation Tokyo, Japan) was placed with the help of applicator tip and was photo polymerized for 20 seconds and finally application of pit and fissure sealant (UltraSeal XT plus, Ultradent products, Jordan, USA) was done followed by it's photo polymerization for 40 seconds.

Clinical procedure in Group II

The occlusal surface of the isolated tooth was treated with 37% phosphoric acid gel for 15 seconds, followed by it's rinsing for 10 seconds using air water spray of the three – way syringe and dried using oil – free compressed air with a hand pump air pressure syringe. After ensuring a frosted appearance of the enamel at the fissure entrance, pit and fissure sealant was placed on the tooth surface followed by light curing of the sealant for 40 seconds.

Once the procedure was completed, rubber dam was removed followed by analysis of the occlusal discrepancies using articulating paper and finally the high points were removed with the help of bur mounted on airotor. Clinical analysis of all the teeth in the two groups was performed at 1, 3, 6, 9 and 12 months to assess the success of treatment procedures in terms of Marginal integrity, Marginal discoloration, and Anatomical form. The criteria for evaluation at periodic intervals was as described by Feigal *et al* [29] in 2000.

Marginal Integrity

0 Restorative material adjacent to the tooth and not detectable with an Explorer,

1 Margin detectable with the explorer,

2 Crevice along the margin of visible width and depth,

3 Crevice formation with exposure of central fissure.

Marginal Discoloration

0 No color change at the tooth-sealant interface,

1 Discoloration noted along the margin in one area,

2 Discoloration noted along the margin in multiple areas,

3 Severe discoloration with evidence of penetration and leakage.

Anatomical Form

0 Harmonious and continuous with occlusal form and structure,

1 Change in anatomical form but all pits and fissures covered,

2a Loss of sealant from one or two pits or accessory grooves (partial loss), but no need to repair or replace sealant,

2b Loss of sealant from pits or accessory grooves (partial loss), with a need for replacement or repair of the sealant,

3 Loss of sealant from all pits (total loss),

7 Partial loss due to occlusion,

9 Bubble (not connected with the margins).

Statistical analysis

The data thus obtained was tabulated and subjected to statistical analysis using statistical package for social sciences (SPSS Inc., Chicago, IL) version 17 for windows. Intergroup comparison was done using Pearson's chi-square test. The significance level was predetermined at $p < 0.05$.

RESULTS

The mean age of the children included in this study was observed to be 8.63±1.27 years. Table – 1 shows distribution of teeth available at different follow up intervals i.e. at 1, 3, 6, 9 and 12 months respectively. Tables–2, 3 and 4 represents the Marginal integrity, Marginal discoloration and Anatomical form scores at 1, 3, 6, 9 and 12 months for both the groups. Intergroup comparison revealed statistical significant differences pertaining to Marginal integrity and Anatomical form at 1, 3, 6, 9 and 12 months follow up interval. For marginal discoloration the results were statistically not significant at 1 month follow up but at 3, 6, 9, and 12 months the results were statistically significant ($p<0.05$).

DISCUSSION

Although, application of pit and fissure sealants causes reduction in occlusal caries, but the effectiveness of sealant may be associated with certain technical problems during its application that includes tissue management and salivary contamination.[30,31] Problems in the application of sealant can cause microleakage or limited or entire loss, which leads to the unsuccessful function of sealant with the rate of 5% to 10% a year.[29] Affluence of the sealant depends on the optimal conditions under which the sealant is applied.[7] The efficacy of sealant in the prevention of caries has been associated with the degree and duration of the retention of sealant.[11,32]

In 1955, Buonocore[7] came up with a new technique of acid etching to increase and improve the adhesion of restorative material to the enamel. In the underlying mechanism of bond, further research suggested that resin extensions mimicking tags were formed and were interlocked micromechanically with the microporosities of enamel created by etching.[33,34] Dental enamel consists of 96% of inorganic matter and organic matter is less than 1% out of which less than half part contains protein.[35] Phosphoric acid acts mostly on the mineralized part i.e inorganic portion of the enamel surface but it does not eliminate the organic material. Due to this outer organic layer, phosphoric acid is prevented to etch the surface of enamel efficiently, that results in variable pattern and a deceptive surface of enamel for bonding.[36] So, it is mandatory to remove the organic part from the enamel surface to improve the quality of pattern of etching, which gave rise to the enamel deproteinization concept.[26]

Since 1915 sodium hypochlorite solution has been used as wound irrigant and as early as 1920 acting as endodontic irrigant because of its proteolytic and bactericidal properties.[37,38] The role of sodium hypochlorite in detaching the organic content from the surface of enamel may prove to be considerable, so, sodium hypochlorite usage as a deproteinizing agent may be a feasible approach to optimize adhesion by removing the organic content of both the acquired pellicle and the structure of enamel.[26] Deproteinization of the surface of enamel before orthodontic bracket bonding was suggested firstly by Justus[39] et al using sodium hypochlorite.

Improved retention was seen in the studies where intermediate bonding layer was applied between the enamel and the sealant which increases the bond strength, reduces microleakage and increases the flow of resin into the deep fissures.[40,41] Hitt and Feigal[40] in 1992 proposed a modification of sealant application technique which included the bonding agent layer usage between the sealant and etched enamel. For the long term retention and effectiveness a low viscosity hydrophilic material bonding layer is recommended as a part of or under the actual sealant. In 1996 Symons *et al* [42] concluded that penetration of the sealant in deep pits and fissures was increased with the usage of Scotch – bond Multipurpose (3M Dental) and All – Bond 2 (Bisco) systems.

In the current study increased retention in Group I was observed and it may be because of sodium hypochlorite which was used as a deproteinizing agent as it removes the organic smear layer from the surface of enamel which cannot be achieved simply by acid etching with 37% phosphoric acid gel. The bonding agent layer which was applied on teeth under Group I also played an important role in enhancing the retention of the sealant.

The findings of the present study are comparable with those performed by Gomez *et al* [43] who reported that there was a significant increase in the degree of penetration of resin when sodium hypochlorite was used as a deproteinization agent for 1 minute prior to application of resin. Pithon *et al* [44] also concluded that deproteinization with 3% and 6% bromelain gel in association with papain gel increases the shear bond strength. A clinical trial by Swift et al[45] reported that ethanol or acetone based adhesive systems like Tenure primer may be more effective than water based adhesives or primer like Scotch bond Multipurpose. Grande et al[46] showed better clinical performance of the sealant when bonding agent (Optibond™) was used and Torres *et al* [47] suggested that when bonding agent layer is applied below the pit and fissure sealant then it significantly increases the shear bond strength.

The results of the present study are in contrast to the studies by Ahuja *et al* [48] who reported that acid etching remains the best method for pretreating the enamel surface and there is no need for enamel deproteinization. Rangel *et al* [49] also reported that deproteinization concept does not play any role in increasing the retention of the pit and fissure sealant. A clinical trial by Boksman *et al* [31] suggested that application of bonding agent before placement of pit and fissure sealant plays no role in increasing the retention rate of the sealant. Pinar *et al* [50] showed no significant differences among the sealants placed with and without bonding agents. The criteria for the selection of tooth, the operative technique performed, the use of isolation technique and the use of evaluation methods have been associated possibly with the differences found among studies.

Table 1:Distribution of teeth available at various follow up intervals (1, 3, 6, 9, 12 months).

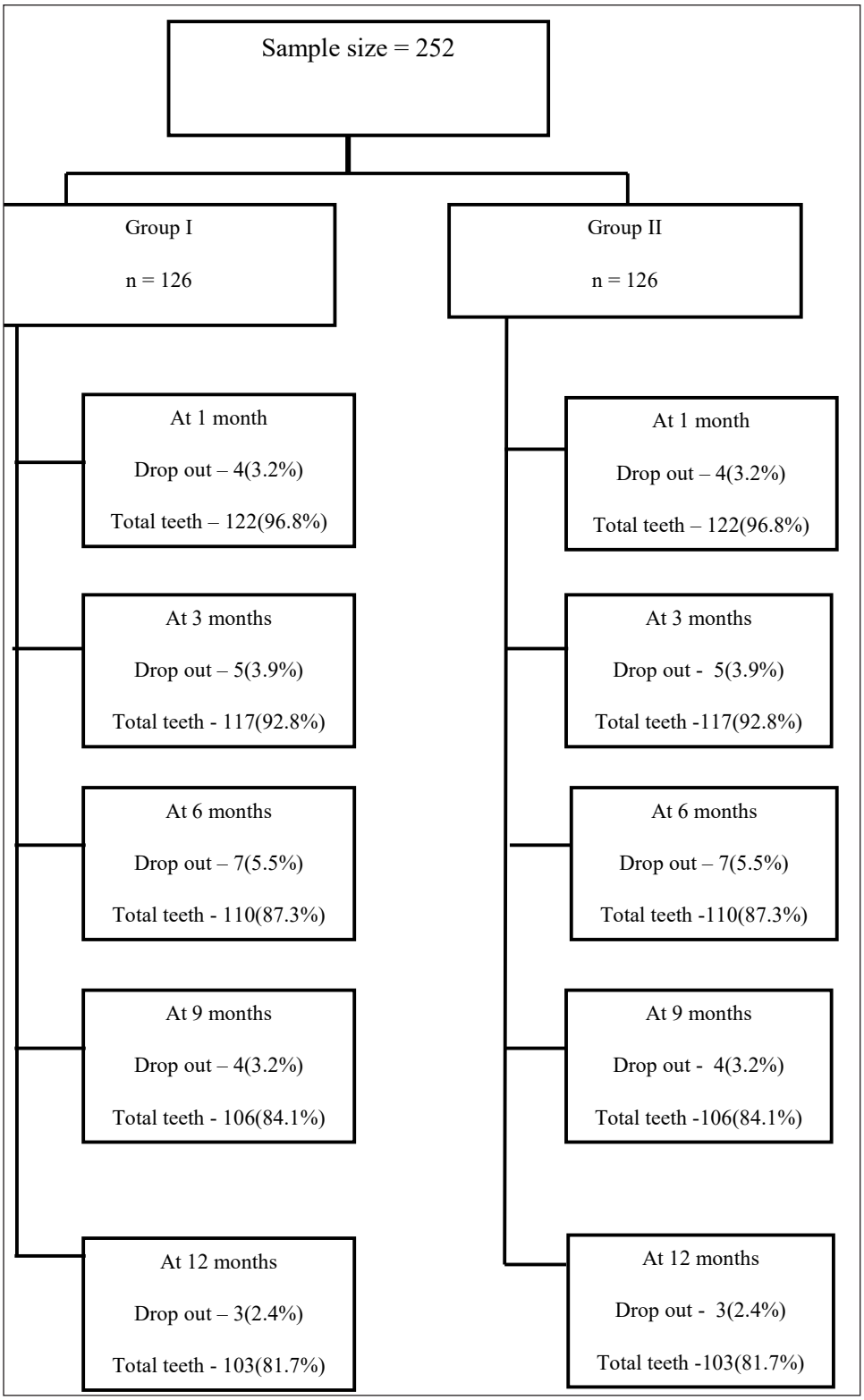

Table 2: Clinical assessment of Marginal integrity in both the groups at 1, 3, 6, 9, and 12 months.

Month	Scoring	Group I	Group II	Value	df	p-Value
1	0	100(97.1%)	73(70.9%)	26.305	1	<0.001
	1	3(2.9%)	30(29.1%)			
3	0	97(94.2%)	41(39.8%)	68.940	2	<0.001
	1	6(5.8%)	59(57.3%)			
	2	0(0%)	3(2.9%)			
6	0	85(82.5%)	16(15.5%)	93.791	2	<0.001
	1	17(16.5%)	69(67.0%)			
	2	1(1.0%)	18(17.5%)			
9	0	59(57.3%)	1(1.0%)	100.9	2	<0.001
	1	43(41.7%)	56(54.4%)			
	2	1(1.0%)	46(44.7%)			
12	0	31(30.1%)	0(0%)	136.0	2	<0.001
	1	70(68.0%)	20(19.4%)			
	2	2(1.9%)	83(80.6%)			

Table 3: Clinical assessment of Marginal discoloration in both the groups at 1, 3, 6, 9, and 12 months.

Month	Scoring	Group I	Group II	Value	df	p-Value
1	0	103(100.0%)	100(97.1%)	3.044	1	0.081
	1	0(0%)	3(2.9%)			
3	0	103(100.0%)	97(94.2%)	6.180	1	0.013
	1	0(0%)	6(5.8%)			
6	0	103(100.0%)	95(92.2%)	8.323	1	0.004
	1	0(0%)	8(7.8%)			
9	0	103(100.0%)	95(92.2%)	8.323	1	0.004
	1	0(0%)	8(7.8%)			
12	0	103(100.0%)	95(92.2%)	8.323	1	0.004
	1	0(0%)	8(7.8%)			

Table 4 : Clinical assessment of Anatomical form in both the groups at 1, 3, 6, 9, and 12 months.

Month	Scoring	Group I	Group II	Value	df	p-Value
1	0	101(98.1%)	74(71.8%)	27.699	2	<0.001
	1	2(1.9%)	28(27.2%)			
	2a	0(0.0%)	1(1.0%)			
3	0	97(94.2%)	41(39.8%)	68.906	3	<0.001
	1	5(4.9%)	50(48.5%)			
	2a	1(1.0%)	10(9.7%)			
	9	0(0.0%)	2(1.9%)			
6	0	85(82.5%)	16(15.5%)	94.205	3	<0.001
	1	16(15.5%)	60(58.3%)			
	2a	2(1.9%)	25(24.3%)			
	9	0(0.0%)	2(1.9%)			
9	0	59(57.3%)	1(1.0%)	103.9	3	<0.001
	1	42(40.8%)	49(47.6%)			
	2a	2(1.9%)	51(49.5%)			
	9	0(0.0%)	2(1.9%)			
12	0	31(30.1%)	0(0.0%)	142.5	4	<0.001
	1	69(67.0%)	16(15.5%)			
	2a	3(2.9%)	69(67.0%)			
	2b	0(0.0%)	16(15.5%)			
	9	0(0.0%)	2(1.9%)			

The present study is different from the previous studies[42,48,49,50] where they have used the concept of deproteinization or the usage of bonding agent alone. There is no study available in the literature that had evaluated the combined effect of enamel deproteinization along with the use of bonding agent in the retention of pit and fissure sealant. This study is first of its kind where the combined effect of these two concepts in increasing the retention of sealant was assessed. In the current study, split mouth design was utilized in which the procedures involving both the groups i.e Group I and Group II were performed in the same mouth, so that the direct comparison in both the groups can be done under identical environmental conditions, as the retention of the pit and fissure sealant might depend on factors such as oral hygiene, patient's behavior, diet, and other habits. Also, prior to the application of sealant all the samples were pretreated with fluoride free pumice powder mixed with glycerine using rubber cup mounted on contra angle handpiece. In the present study Feigal's[29] criteria for evaluation of the retention of sealant was selected, which is simple to follow and evaluation of the sealant was done at 1, 3, 6, 9 and 12 months to guard the sealant's retention at short regular intervals.

In future, certain *in vitro* studies should be carried out to observe the penetration of the sealant deep into the pits and fissures, as deeper penetration leads to intensification in the retention of sealant. In the present study the combined effect of enamel deproteinization and intermediate bonding was observed and it is impossible to differentiate whether the increased retention of the sealant is because of deproteinization or due to the presence of intermediate bonding layer. So, as to overcome this limitation, further split mouth studies are indicated with the teeth receiving deproteinization in one group and bonding agent application in the other. Despite the success obtained in the retention of pit and fissure sealants in deep pits and fissures that are prone to dental caries, other factors that may interfere in the etiology of caries were not considered in the present study. So, further longer term clinical studies involving the other factors involved in the retention of pit and fissure sealant should be planned.

CONCLUSIONS

1. At the end of 12 months follow up period, significant differences were observed between the two groups regarding marginal integrity, marginal discoloration and anatomical form of the pit & fissure sealants.
2. Enamel deproteinization along with the use of intermediate bonding layer significantly enhances the retention of pit and fissure sealants in children in terms of enhanced marginal integrity, decreased marginal discoloration and preserving the anatomical form.
3. The concept of enamel deproteinization can be applied to the occlusal surfaces of permanent molars that may lead to increase in the occurrence of etching patterns that are favorable for a good bond between the tooth and the sealant.
4. Further studies are recommended by including other factors that are responsible for increasing the retention of pit and fissure sealants.

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