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Original Research

24% Indigenously Prepared Ethylene Diamine Tetra Acetic Acid Compared to Self-Etching Adhesives and their Effect on Shear Bond Strength of Composites in Primary Teeth: An *In-vitro* Study

Priya Nagar[1], Yogesh L. Tandil[2], Chandru T. P.[3], Anamika Gupta[4], Devendra Kalaria[5], Prafful Kumar[6]

Contributors:
[1]Reader, Department of Pedodontics & Preventive Dentistry, Krishnadevaraya College of Dental Sciences, Bangalore, Karnataka, India; [2]Senior Lecturer,Department of Conservative and Endodontics, Yogita Dental College & Hospital, Khed, Ratnagir, Maharashtra, India; [3]Reader, Department of Pedodontics and Preventive Dentistry, Coorg Institute of Dental Sciences, Virajpet, Karnataka; [4]Senior Lecturer, Department of Community and Preventive dentistry, Teerthankar Mahaveer Dental College and Research Centre, Moradabad, Uttar Pradesh, India; [5]Reader, Department of Conservative and Endodontics, College of Dental Science & Hospital, Indore, Madya Pradesh; [6]Senior Lecturer, Department of Orthodontics, School of Dental Sciences, Greater Noida, Uttar Pradesh.

Correspondence:
Dr. Nagar P. Unit-GL, Orchard Green Villaments, #139/34 Domlur Layout 1st Phase, Bangalore - 560 071, Karnataka, India. Email: dr_priya_nagar@yahoo.com

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Abstract:
Background: Over the years, it has been known that 34% phosphoric acid is the benchmark in etchants with the best shear bond strength shown with composites in primary teeth. However, with latest technological advancements and innovations, in order to reduce the number of steps and less damage to the tooth structure, non-rinse conditioner (NRC) & Single-Etch and various other etchants have been tried and tested. These etchants have been found to have shear bond strength comparable to phosphoric acid. In this study, indigenously prepared 24% ethylenediaminetetraacetic acid (EDTA) has been compared with established etchants, as to prove if their shear bond strength was closely related. As it is a well-known fact that EDTA could be less damaging to the enamel during etching and hence can be an alternative for etching of primary teeth.
Materials and Methods: For the study 60 caries-free primary molars were used, they were sectioned in the middle, after making area for bonding; the marked area was then etched using different etchants for 30 s. Each of the teeth was then rinsed and bonded with composite resin and thermocycling was done. Shear bond strength testing was done on the composite using Universal Testing Machine.
Results: Results of the study showed that phosphoric acid showed the highest bond strength, closely followed by Single Etch (Adper Prompt) and NRC, then by EDTA.
Conclusions: About 24% EDTA can be another comparable replacement for phosphoric acid if used with a Single Etch Primer, like Prime and Bond NT on primary teeth. 34% phosphoric acid has the highest bond strength values with composite resin. Single etch followed by NRC has the second and third highest bond strength values, which are comparable to phosphoric acid.

Key Words: Bond strength, composites, etchants, indigenously prepared 24% ethylene diamine tetra acetic acid, primary teeth

Introduction

Buonocore[1] proposed the acid-etch technique in 1955 for bonding acrylic resin to enamel etch with 85% phosphoric acid, a basic principle was established for adhesive and preventive dentistry: Acids could be used to change the surface of enamel to make it more receptive to adhesion, as recommended by the use of phosphoric acid to obtain better adhesion of resin and paint coating to metal surface. The formation of a tag like resin extensions into the enamel micro-porosities was considered the predominant mechanism of bonding the resin to phosphoric acid-etched enamel. This is actually known as acid-etch technique, and it has remodeled the way restorative dentistry is practiced.[2] The aptitude of clinicians to bond restorative materials to enamel has basically changed concepts of cavity preparation, caries prevention, and esthetics.

Although the use of phosphoric acid was not widely accepted, until 1970s, it is presently used in dentistry as an etching agent for enamel and dentin.[3] Shear bond strengths of resin composite to enamel etched with 30-40% phosphoric acid normally vary from 18 to 25 MPa.[2,4,5] Such bond strengths provide successful retention of resins for a variety of clinical situations, including direct resin restorations, porcelain and composite veneers and inlays, bonded prostheses, orthodontic brackets, and pit and fissure sealants. Besides, etching reduces leakage around restoration margins in enamel.[3]

More recently, another family of acidic conditioners was introduced in Japan: The self-etching primers. These acidic primers include a phosphonated resin molecule that performs two functions concurrently – conditioning and priming of dentin and enamel. The new conditioner is not rinsed off, unlike conventional etchants; they are based on the use of non-rinsed acidic polymerizable monomers. They were developed to simplify the bonding procedure to work as conditioner, primer, and resin. Except from simplification, the justification

behind these systems is to de-mineralize the surface of the dentin and at the same time infiltrate it with monomers, which can be further be polymerized in the same place.

Following the trend, two non-rinsing self-etching have been introduced for use with polyacid-modified resin composites – Adper Prompt (ESPE) and Non Rinse Conditioner (NRC) (Dentsply DeTrey). The first-mentioned is a self-etching adhesive while the later is a non-rinsing conditioner with a one-bottle adhesive. Adper Prompt according to its manufacturer, is an "All-in-one" adhesive system that combines etching, priming, and adhesive potentials in only one solution, whereas NRC requires the application of an adhesive, Prime & Bond NT (Dentsply DeTrey) after NRC is applied.

Another important aspect of adhesives is presence or absence of smear-free dentin surface.[6] Some group of researchers had shown that higher bond strength was a result of the formation of a hybrid layer and resin tags that protrude into the dentin tubules. The hybrid layer forms when the mineral phase of dentin is dissolved and its collagen matrix is exposed, enabling infiltration of this matrix with resin monomer. Formation of the hybrid layer was first described by Nakabayashi and is today regarded as a prerequisite for successful dentin bonding. Etchants such as phosphoric acid and ethylenediaminetetraacetic acid (EDTA) has been shown to remove most of the smear layer and leave the dentinal tubules open. In addition, they have been shown to expose collagen fibrils to varying degrees of intertubular dentin as well as on the walls of dentinal tubules. Phosphoric acid at 34% is a relatively strong acid with a pH of 1, while 24% EDTA is a gentler chelating agent that removes Ca2+ at neutral pH. In the present study, indigenously prepared 24% EDTA is used.

The present study was done to compare the shear bond strength of 34% phosphoric acid, 24% EDTA, Adper Prompt (Self-Etching), and NRC with composite on primary teeth.

Materials and Methods

Materials used

About 34% phosphoric acid, dentsply, detrey, 24% EDTA, indigenously prepared, NRC, dentsply, detrey, Adper Prompt, 3M, ESPE, light cured composite resin, charisma, Heraeus Kulzer, Germany, Prime & Bond NT, Dentsply, Detrey.

Accessories

Acrylic resin (DPI-RR cold cure acrylic repair material, Mumbai), 0.9% isotonic saline solution, 60 primary molars.

Equipment

Diamond Disc, Mounting jig 4 mm × 4 mm × 4 mm, Bonding Jig, Light Curing Unit, Universal Hounsfield Testing Machine (Hounsfield, U.K.), Transparent Teflon Cylinder 2 mm × 3 mm, Scanning Electron Microscope, Thermocycling Unit.

Armamentarium for bonding procedure

Plastic instrument, condenser, tweezers, probe, kidney tray, dampen dish, tissue paper, varnish, stopwatch, distilled water.

Indigenous preparation of EDTA

The chemical formula for EDTA is $[CH_2N(CH_2COOH)CH_2COONa]_2.2H_2O$. It is a disodium salt with a molecular weight of 372.24. 2.4 g of EDTA salt were mixed with 10 ml of distilled water, and was filtered. About 5 ml of the filtrate was taken and 100 g of sodium carboxymethylcellulose (Na CMC), which is a gelling agent, was added to it. Sodium paraben was added as a preservative. The pH was checked to be neutral with the pH-testing strip. The gel was transferred to a sterile 2 ml syringe and stored.

Methodology

Following methods were used during the different phases of the study.

Preparation of teeth

About 60 primary molars extracted for the purpose of serial extractions were used. All teeth were caries-free and none were root filled. It was made sure that the teeth had a minimum of one-third of their roots left for the proper anchorage in the acrylic block. The teeth collected were those which were extracted just 3 months before the study. After extraction teeth were rinsed in tap water and the attached soft tissue was removed. All teeth were stored in 0.9% saline in the refrigerator. The saline solution was changed every day.

Teeth were sectioned longitudinally using a diamond disk and under the continuous supply of saline dropping on the disc while sectioning. This exposed the middle half of the tooth with the outer 1 mm enamel layer and the inner dentin. After sectioning the teeth were stored in distilled water, which was changed every day.

Mounting of the tooth in an acrylic block

For the purpose of bond strength testing, each tooth was mounted in an aluminum jig measuring 4 mm × 4 mm × 4 mm. The standardization of the placement and alignment of the tooth was taken care off.

Bonding area selection

For the bonding procedure the tooth surface was cleaned using pumice slurry, the area of bonding was selected (2 mm × 2 mm) on the lingual cusp covering 1 mm of the enamel and 1 mm of dentinal surface and was marked using a round patch. Rest of the surface was coated with nail polish varnish to restrict the placement of etchant on the surrounding areas. Just before the bonding procedure the patch was removed and the area was again washed with pumice water.

Etching procedure

The teeth were randomly divided into four groups and the bonding procedures were performed:

1. Phosphoric acid: The bonding site was etched with 34% phosphoric acid gel (Dentsply DeTrey), for 30 s and then rinsed for 15 s. The dentin was carefully blot dried while still presenting a shiny moist aspect. Prime & Bond NT was applied vigorously and cured for 10 s.
2. EDTA: The bonding site was etched with an indigenously prepared 24% EDTA, for 30 s and then rinsed for 15 s. The dentin was carefully blot dried while still presenting a shiny moist aspect. Prime & Bond NT was applied vigorously and cured for 10 s.
3. NRC: After the application of NRC the surface was left undisturbed for 30 s and gently air-dried for 3-5 s. Prime & Bond NT was applied vigorously and cured for 10 s.
4. Adper Prompt: Both the bottles of Primer and Bond were shaken vigorously. One drop each was taken and mixed was applied on the bonding site for 30 s and then light-cured for 10 s.

Bonding procedure

The specimens were mounted and secured on the bonding jig exposing the bonding site. A transparent cylinder (2 mm-diameter 3 mm-length) was placed on the selected area. The cylinder was filled with composite, Charisma, and light cured for 20 s. The cylinder was carefully removed.

Thermocycling procedure

For the thermocycling procedure all the specimens were stored in distilled water for 24 h at 37°, following which they were thermocycled for 500 cycles at 5° and 55°, with a dwell time of 30 s and transfer time of 10 s.

Bond strength testing

Shear bond strengths were measured on a Universal Testing Machine (Hounsfield) using a compressional mode with a cross-head speed of 1 mm per minute. The data were subjected to one-way ANOVA, "*t*"-Test, Duncan's Test. All statistical analysis was carried out with the SPSS software.

Results

All the data were subjected to one-way ANOVA followed by a *post-hoc* test using SPSS for Windows (Statistical Presentation System Software).

The mean bond strength values of various etchants are 19.59 Mpa for Single Etch, 17.02 MPa for NRC, 14.02 MPa for EDTA, and 22.35 MPa for phosphoric acid (Table 1).

Bond strength values of various etchants were significantly different from each other ($F = 981.382$, $P < 0.001$) with 3° and 56° of freedom (Table 2).

The test revealed that each treatment group differed significantly from other group, having EDTA treatment least and phosphoric acid highest, whereas the mean bond strength value of NRC treatment was found to be second highest followed by single etch treatment (Table 3).

Intergroup comparisons of various test groups were performed, and significant results were found (Table 4).

Discussion

The use of self-etching primers and adhesives that do not require rinsing and serve simultaneously as conditioner and primer (NRC) or conditioner, primer and adhesive (Adper Prompt) is a recent approach to the simplification of the bonding techniques specially in pediatric setup whereby eliminating the separate step of rinsing, it gets easier for the dentist and is less time consuming for the patient too.

Single etch (Adper Prompt) is a solution containing methacrylated phosphates and water in a unique application unit.[7] NRC is composed of two organic acids (maleic acid and itaconic acid) dissolved in water. Maleic acid acts as a

Table 1: Bond strength of various etchants with composite on primary teeth.

Etchants	Test samples	Mean (Mpa)± Std. deviation	Std. error
Single etch	15	19.59±0.293	7.56E-.02
NRC	15	17.02±0.463	0.119
EDTA	15	14.02±0.530	0.137
Phosphoric acid	15	22.35±0.436	0.112
Total	15	18.24±3.13	0.404

NRC: Non-rinse conditioner, EDTA: Ethylenediaminetetraacetic acid

Table 2: One Way ANOVA.

Source of variation	Bond strength				
	Sum of squares	df	Mean square	*F*	Significance
Between groups	569.23	3	189.74	981.38	0.001
Within groups	10.823	56	0.193		
Total	580.06	59			

Table 3: Duncan's multiple range test.

Etchants	Test samples	Subset for alpha=0.05			
		1	2	3	4
EDTA	15	14.03			
NRC	15		17.02		
Single etch	15			19.59	
Phosphoric acid	15				22.35

NRC: Non-rinse conditioner, EDTA: Ethylenediaminetetraacetic acid

Table 4: Intergroup comparisons of the bond strength of various etchants.

Comparisons of various etchant groups	"*t*" value	"*P*" value	Inference
Single etch – NRC	18.05	0.001	S
Single etch – EDTA	35.53	0.001	S
Single etch – Phosphoric acid	20.35	0.001	S
NRC – EDTA	16.43	0.001	S
NRC – Phosphoric acid	32.41	0.001	S
EDTA – Phosphoric acid	46.90	0.001	S

NRC: Non-rinse conditioner, EDTA: Ethylenediaminetetraacetic acid, S: Significant

conditioning agent while itaconic acid behaves as a priming agent with the ability of copolymerizing with Prime & Bond NT.[8] Recent research indicates that a combination of conditioning with EDTA and application of a self-etching primer results in a clinically acceptable bond strength.[9]

EDTA being a gentle chelator, with a neutral pH can be an ideal replacement for phosphoric acid as these qualities make its action on pulp less harmful, also it will cause less demineralization as compared to phosphoric acid. In the present study, indigenously prepared EDTA has been used because of the relatively unavailability of 24% EDTA in the market easily.

The rationale behind the action of these self-etching agents is a formation of a continuum between tooth surfaces and adhesive material, which is accomplished by the concurrent demineralization and penetration of resin in enamel and dentin surfaces.

This study evaluated the shear bond strength of four of the etchants to enamel and dentin of primary teeth. All materials which were experienced achieved adequate bond strengths to primary teeth's enamel. In permanent teeth, bond strengths between 17 and 24 MPa are vital to successfully defy the polymerization contraction forces of composite resin.[10] The prismless external layer present in enamel of the primary teeth is said to reduce the efficacy of acid etching and the consequence of this is a superficial etching pattern. It is therefore recommended that etching times for enamel of primary teeth with phosphoric acid have therefore been wide-ranging.[10] With a 15-30 s application of 34% phosphoric acid a comprehensive etch pattern can be exhibited. That's the reason in the present study 30 s etching time is used with all the etchants.

Although few of the studies like Blomloff[11] have used 3 min as the etching time for EDTA, but still in the present study for standardization the etching time for EDTA has been kept 30 s only. The acrylic PENTA with a hydrophobic and a hydrophilic group is the base for Prime & Bond NT. The molecule in the hydrophilic group is a phosphoric group, which resembles phosphoric acid. It is able to de-mineralize the dentin, but the result is not sufficient to etch the enamel, which is similar to a self-etching primer. In the present study, primary teeth were used which were extracted for the reason of serial extraction, as non-carious teeth with a minimal of one-third roots present were used. The teeth were grinded longitudinally to achieve two halves from a single tooth because of relatively less availability of deciduous non-carious teeth.[12]

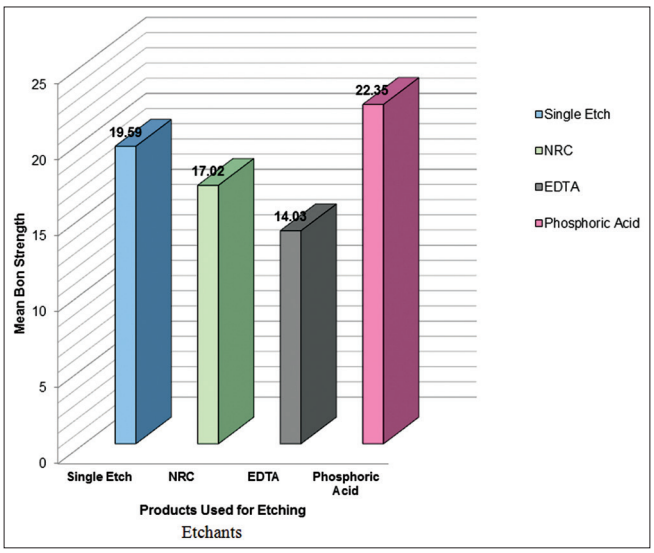
Primary dentin in comparison with permanent dentin has reduced mineral content hence a diverse effect of acid conditioning was observed on primary dentin. Nor *et al*. suggested that the hybrid layer produced in the primary teeth was 25-30% thicker than in permanent teeth and concluded that primary dentin was more reactive to acid conditioner, using 10% phosphoric acid, two diverse bonding systems, and 10% maleic acid.[13,14]

Since primary teeth have lesser number dentinal tubules in comparison to permanent teeth, the dilution of acidic conditioners may not as effectively and effortlessly achieved.[15] However, when primary teeth and permanent teeth are compared, it is found that numbers of dentinal tubules with a larger diameter in dentin is present in the earlier. It is also found, due to more number of dentinal tubules with larger diameter is found in primary teeth and seepage of acids is seen farther into the dentin along these tubules. Hence, demineralization caused is substantial.[15] Etching the primary teeth for a shorter duration may result in total diffusion of the resin and produce a thinner hybrid layer.[14]

Even though the etching pattern of Single Etch (Adper Prompt) and NRC have been described as well defined,[7,8] the enamel shear bond strengths obtained with them were of high magnitude only when they were combined with a compomer. A viable explanation would be the low viscosity of the compomer and the fact that these conditioners are water based materials and hence they are chemical makeup is well-suited for materials used for restorations, which have improved hydrophilic character like in case of compomers, unlike resin composites which have hydrophobic character.[9]

Bond strength comparisons of various etchants on primary teeth with composite

The mean bond strength values of various etchants were phosphoric acid (22.35 MPa), single etch (Adper Prompt) (19.59 MPa), NRC (17.02 MPa), and the lowest by EDTA (14.02 MPa). (Table 1 and Graph 1).



Graph 1: Comparison of mean bond strength values of various etchants

Though the mean values of all the four groups were very near to each other, but they were highly significant from each other.

Results of one-way ANOVA (Table 2) shown that the mean of squares between the groups and within the groups were 189.74 and 0.19 at the level of significance of 0.001 (Table 4).

And these bond strength values were significantly different from each other correlating the values shown by Rosa,[16] Henning,[17] and Blomloff.[11] Since in this study only composite material was used, this might also have contributed to the highest bond strength shown by phosphoric acid.

Although the etching pattern of Single Etch (Adper Prompt) and NRC have been described as well defined.[7,8] The enamel shear bond strengths obtained with them were of high magnitude only when they were combined with a compomer. A viable explanation would be the low viscosity of the compomer and the fact that these conditioners are water based materials and hence they are chemical makeup is well-suited for materials used for restorations, which have improved hydrophilic character like in case of compomers, unlike resin composites which have hydrophobic character.[9]

Conclusions

1. EDTA 24% can be another comparable replacement for phosphoric acid if used with a single etch primer like Prime & Bond NT as it showed comparable bond strength values.
2. Phosphoric acid has the highest bond strength values with composite resin on primary teeth.
3. Single etch followed by NRC has the second and third highest bond strength values, which are comparable to phosphoric acid and hence can be used as a valuable replacement for the conventional technique of phosphoric acid, as it eliminates the step of rinsing and thus makes the procedure less time consuming for the operator and is convenient for the patient.

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