1% phytic acid- novel alternative to the conventional etching agent- 37% ortho phosphoric acid – An invitro study

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ABSTRACT:

Objective: To evaluate and compare the effect of phytic acid and phosphoric acid on bond strength, etch depth and etch pattern in primary teeth

Methodology: 60 extracted non carious primary teeth were randomly divided into 2 groups (Group I: 1% phytic acid and group II: 37%phosphoric acid) with each containing 30 teeth. Dentin surfaces were sectioned and etched with 1% Phytic acid for 15 seconds; in control group 37% Phosphoric acid was used. Etch-and-rinse adhesive followed by composite build-ups was done. The specimens were subjected to tensile testing to evaluate bond strength. The Sectioned buccal surfaces were retained and etched for 15 seconds respectively, rinsed for 15 sec and air dried for 30 seconds. Study surfaces were analyzed by using Scanning electron microscopy (SEM) to estimate etch depth and pattern.

Result: The results demonstrated, no differences in etch patterns in both the groups irrespective of etching agent. 1% phytic acid etchant etched deeper than 37% phosphoric acid but there was no statistical significance. Phytic acid produced bond-strength values that were extremely significantly than that of the Phosphoric acid with the mean values was 22.4 ± 3.6 Mpa for phytic acid group and 14.4 ± 4.1 Mpa for 37% phosphoric acid.

Conclusion: Phytic acid etching showed increased etch depth and similar etch pattern when compared to phosphoric acid and produced significantly higher bond strength 37% phosphoric acid.

Keywords: Phytic acid, IP6, etchant, primary teeth

Introduction:
Micromechanical retention of adhesives to the tooth surface is based on infiltration of monomers through the etched nanometric spaces. Acid etching selectively erodes the tooth surface, thereby increasing the retention. Phosphoric acid (PA) etching technique facilitates an improvement in the marginal adaption and surface area that is suitable for the retention of resin composites.

Regardless of the fact that phosphoric acid etching produces greater durability and stronger adhesion to enamel, application of PA to dentine reveals a dense filigree of collagen fibers, leaving an area at the bottom of the demineralized zone with residual hydroxyapatite crystals. The surface of hydroxyapatite is of high-energy, unlike the outermost layer of collagen which is low. As a result of the acidic conditioning of dentin, the dense network of collagen fibers which is fragile does not aid in dentin adhesion and may even interfere with bonding mechanisms, resulting in post-operative sensitivity and collagen degradation by dentinal matrix metalloproteinases, which may jeopardize the long-term bond to the tooth surface. These demerits of phosphoric acid aid in search for newer acid agents that will meet the needs of ideal etchant.

Wettability is one of the most crucial aspects of adhesion. To form an adhesive contact film layer, there must be intimate contact between the adhesive and the partly deproteinized dentin.[2] The adhesive's surface tension must be as low as possible, while the substrate's surface energy must be high. Changes in the mineral content affect dentin's surface-free energy. The surface of hydroxyapatite is high-energy, whereas the surface of collagen is low-energy. [2] The dense web of collagen fibers becomes a low-surface energy substrate as a result of dentin's acidic conditioning. In this case, an increase in dentin's critical surface tension is highly desirable

because there is a direct correlation between dentin's surface energy and shear bond strength. [3]

In recent years, research has focused on techniques that alter the chemical composition of dentin by removing collagen from dentin in order to improve resin bonding. The removal of unsupported collagen fibers may improve primer and adhesive spreading and diffusion through dentin. Researchers believe that removing collagen fibers results in a dentin surface that resembles etched enamel.

IP6's significant negative charge, has the potential to chelate positively charged minerals like calcium and create complexes that dissolve in acidic condition but precipitate at neutral pH levels. [8] According to literature, IP6 has an anticariogenic action by lowering the solubility of enamel. Due to its strong affinity for hydroxyapatite, it also has an antiplaque action and inhibits bacteria from penetrating tooth surfaces.

Phytic acid–a novel dentine etching agent efficiently dissolved the smear layer. It precisely enhanced the bond strength, with reduced detrimental effect on pulpal cells when used as an etchant in permanent teeth. Considering the paucity of research on the use of phytic acid in primary teeth, the objective of the study is to evaluate and compare the impact of 1% IP6 and 37% phosphoric acid on primary teeth's Microtensile bond strength (μTBS), etch pattern and etch depth.
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Materials and methods

Sample size calculation:

The sample size was calculated using G-Power Software version 3.1.9.2, and it was calculated using the following parameters: Effect size, d = 0.6, α err prob = 0.20, Power (1-β err prob) = 0.80. The estimated sample size was 60 with 30 samples in each group. Institutional Scientific and Ethical Review Board clearance was obtained before commencing the study (IEC approval: 2825/IEC/2021).

Sample preparation for analysing Microtensile Bond strength

The microtensile bond strength for 20 samples of human primary teeth was assessed. Using a slow-speed diamond saw with water lubrication, flat dentin surfaces were produced perpendicular to the tooth's longitudinal axis. Depending on the etchant used to condition the dentinal surface, the specimens were divided at random into two groups with 10 samples each.

Dentin surfaces in group I (the control group) were etched for 15 seconds with 37% phosphoric acid (pH 0.6) (PRIME, Etching liquid, India), followed by a 10 second rinse. In group II, dentin surfaces were treated for 15 seconds with 1% IP6 solution (pH 1.2) (TCI CHEMICALS, JAPAN), followed by a 10 second rinse. Subsequent blot drying of the dentin surfaces, an etch and rinse adhesive (Te- Econom Bond; Ivoclar Vivadent) was applied as directed by the manufacturer and light-cured. The bonded surfaces were gradually layered with Tetric N-cream resin composite (Ivoclar Vivadent), up to a thickness of 5mm, and each layer was light-cured.

For 24 hours, the bonded specimens were kept in distilled water at 37°C. Then, each specimen was divided into serial slabs using a slow-speed diamond saw and water irrigation, perpendicular to the bonded interface. These later ones were further divided into 0.85 mm 0.85 mm composite-dentin beams.

Evaluation of Microtensile Bond Strength test

The Universal Testing Machine was used to apply tensile loading at a cross-head speed of 1 mm min-1 till failure to each beam after fixing it individually to a testing jig with cyanoacrylate adhesive.

Sample preparation for analysing Etch pattern and Etch depth

Fourty human non-carious primary teeth were used. Prior to the test procedures samples were stored in saline. At the cemento-enamel junction, the teeth was sectioned using a slow speed diamond saw in a mesio-distal direction to retain the buccal surfaces under water irrigation. The teeth were then randomly divided into 2 groups (I-II) of twenty teeth each.
The etchant was applied using 00 paint brush. The etchant used was 37% phosphoric and 1% phytic acid for group I & II respectively. The surfaces were then rinsed and air dried for 30 seconds.

Estimation of etch depth and etch pattern

The etched tooth surfaces were sputter coated and mounted on aluminium stubs. The mounted samples were analysed using various range of magnification from 6000-25000x to estimate etch depth and etch pattern.

Statistical analysis

Statistical evaluations were performed with SPSS for windows 17.0 (statistical package for the social sciences, SPSS Chicago, Ill. the USA). Kolmogorov-Smirnov and Shapiro-Wilks tests were done for intra group comparison. The significance level was considered as $p < 0.05$.

Results

The scanning electron microscopy images showed all 3 types of etch pattern described by Silverstone et al. [1] Type 1 etching pattern was most commonly seen in both the groups, and phytic acid showed similar etch patterns when compared to 37% phosphoric acid. Fig. 1 (A) depicts the scanning electron photomicrograph of a primary teeth with type 1 etch pattern, when subjected to 37% phosphoric acid with preferential removal of prism core material, leaving the periphery intact. Fig. 1

(B) exhibits Type 2 etch pattern when subjected to 1% phytic acid, with preferential removal of periphery material, leaving the prism core intact.

Fig. 2 (A)shows scanning electron microscopy images of etch depth in primary teeth after etching with 37% phosphoric acid (B) shows etch depth of primary teeth subjected to 1% phytic acid.

The result of μTBS testing and etch depth, are shown in Tables 1 and 2, Statistical analysis showed significant differences between the tested groups (P = 0.0002). The mean and SD values of bond strength were 14.44 ± 4.1 Mpa and 22.4 ± 3.6 Mpa for 37% Phosphoric acid and 1% Phytic acid respectively. Phytic acid group showed significantly higher micro tensile bond strength than phosphoric acid group. The mean etch depth values obtained for 37% PA group is 268 ±109.32 μm and 308 ± 106 μm for 1% phytic acid. There was no statistical difference in etch depth (P= 0.4163) between the tested groups.

Discussion

The two substrates of dentine are collagen, which has a low surface energy and hydroxyapatite, which has a high surface energy. The exposure of collagen fibers and loss of mineral components reduce the surface energy after acid etching. Because the wetting of the substrate is the first step in the interaction between an adhesive and a substrate.

In spite of the fact that phosphoric acid improved enamel bonding significantly, etching dentin with PA is considered aggressive. When phosphoric acid is applied to dentine, it illustrates collagen fibrils that are devoid of hydroxyapatite. These fragile collagen are more prone to collapsing, preventing optimal infiltration of resin and resulting in diminished bonding and sensitivity post-operatively [8]. Various methods have been studied to reduce the enzymatic reactions of PA on dentin. Furthermore, the use of crosslinking agents, such as glutaraldehyde, has been proposed to strengthen the susceptible collagen network.[9]

IP6 exhibited etch patterns similar to those described by Silverstone [10] in this study. The better the etch pattern, the higher the surface energy of the enamel, and thus the better the adhesive penetration, resulting in a stronger bond. Poole and Johnson proposed that dissimilarities in crystalline alignment may account for variable etch patterns on enamel surfaces. [2] According to Johnson et al., another key variable influencing etch pattern variance is shifts in the measurements of demineralising radicals and their electric charge.[3] The distinctive Type 1 and Type 2 etch patterns were dubbed "the ideal etch patterns" and were found to be common. The patterns were speculated to be significant in the micromechanical interlocking mechanism of enamel adhesion.

The quantity of acid used also influences the intensity of dentine demineralization. Redford and Leglar investigated the effect of different etching times on etch depth using 37% phosphoric acid and discovered mean values of 9, 12, 14, and 50 m in 15, 30, 60, and 120 seconds repectively. Increasing etching time results in more significant etch depth. This increased etch depth could be due to etching the enamel subsurface, which is more soluble than the surface enamel.[7,11] When etch depth was moderate, flat-surfaced prisms delimited by unique prism sheaths and enclosed by an interprism whose crystals highlighted an orientation clearly different from prism crystals resulted in the clearest distinction between prims and interprism. Three traits characterized the increased etch depth with increasing acid concentration and etching time: (a) Prisms tended to evolve from flat to cone-shaped, (b) prism sheaths were emphasized, and (c) the topographic difference between prisms and interprisms increased.[7,11]

In the present study, the etch depth of IP6 treated teeth showed increased depth of demineralization when compared to 37% of phosphoric acid. Legler discovered that the quantity of surface enamel stripped away during etching is ascertained by the acid type, etching duration, and chemical properties of the enamel. [12]

Investigations utilizing IP6 as a dentin etching agent in permanent teeth found an immense spike in bond strength when compared to phosphoric acid; similar results were found in our study, where 1% IP6 treated dentin surfaces showed an extremely significant increase in bond strength when compared to 37% PA. [4] Two possible events were postulated as the underlying mechanism for the enhanced resin-dentine bonding. The potential of phytic acid to form undissolved complexes with calcium at pH levels higher than 4 prompted the first mechanism. The pH of IP6 tends to increase when nullified with dentine due to the high dentin buffering capacity, allowing the formation of the irresolvable complex, which may provide strength for the susceptible collagen. [13]

The second mechanism proposed involved IP6's collagen crosslinking activity. pH influences IP6 protein interaction; at pH levels below the protein's isoelectric point, electrostatic interaction between IP6's anionic phosphate groups and the protein's cationic groups forms insoluble binary protein-IP6 complexes that dissolve only below pH 3.5. [13] At low pH, the IP6 binding sites within the protein are the α-NH2 terminal group, the α-NH2 of lysine, the imidazole group of histidine, and the guanidyl group of arginine. The competitive action of multivalent cations influences the stability of binary complexes. Dentinal collagen has a positive net charge after being exposed to acidic solutions such as IP6, establishing the formation of a binary interactions between IP6 and dentinal collagen. Since both IP6 and protein have a net negative charge at high pH, cations such as calcium bridge the IP6 to

protein, forming a soluble ternary protein-cation-IP6 complex. The imidazole group and carboxyl group are the important key targets in these complexes. [13]

A third, more current mechanism for IP6-protein interplay has been proposed, in which IP6 performs as a Hofmeister anion through its six anionic groups, resulting in protein strengthening and decrease in solubility by direct interaction with water in the surrounding medium.[13] Dentinal collagen subjected to IP6 also was less vulnerable to air-drying failure and collagenase deterioration when equated to the fragile collagen network achieved by using phosphoric acid.

In a study conducted by Nassar et al., the lower concentrations of IP6 used and IP6's ability to reduce the degree of oxidative stress through iron chelation were attributed to IP6's lower impact on pulpal cells when compared to phosphoric acid. This reduces iron's ability to catalyze the formation of hydroxyl radicals via the Fenton reaction. [4,5,6]

Matrix metalloproteinases are valuable in deterioration of dentin organic matrix, which is largely comprised of collagen, resulting in breakdown of resin-dentin bonding or progression of the caries. The use of matrix metalloproteinases modulators in dentistry has received a lot of attention as a method to enhance adhesive bonding to dentine, and there is presently a lot of research going on to develop them. The first-generation matrix metalloproteinases inhibitors work by chelating zinc and calcium ions, which are essential for maintaining optimal ternary complex and functional binding sites of matrix metalloproteinases. IP6 is a powerful zinc chelator in addition to being an excellent calcium chelator. The zinc-IP6 complex is constant and insoluble, and these properties, together with the capacity to bind to collagen, recommend that IP6 could be used as a dentinal matrix metalloproteinase inhibitor. In light of this, future research should focus on a thorough analysis and correlation of these two acids' implication on physical and chemical properties of enamel, as well as bonding efficiency, including the effect on microleakage under various conditions that mimic clinical situations.

Conclusion

The study's limitations include the use of single bonding system. The outcomes could differ from those obtained in vivo and when other bonding systems are used. Within these constraints, it is essential to validate that IP6 can etch enamel and dentin concurrently with similar etch patterns and higher etch depth values than PA. The bond strength to dentin of 1% phytic acid was significantly greater than that of 37% phosphoric acid. Given the high level of interest in IP6 research since the discovery of its potential use as an etchant, additional in vitro and in vivo studies are required to verify such impacts on bonding to tooth structure.

References

1. Silverstone LM, Saxton CA, Dogon IL, Fejerskov O. Variation in the pattern of acid etching of human dental enamel examined by scanning electron microscopy. Caries Res 1975;9(5):373e87
2. Poole DFG, Johnson NW. The effects of different demineralizing agents on human enamel surfaces studied by scanning electron microscopy. Arch Oral Biol 1967;12(12)
3. Johnson NW, Poole DFG, Tyler JE. Factors affecting the differential dissolution of human enamel in acid and EDTA. A scanning electron microscope study. Arch Oral Biol. 1971;16(4).
4. Nassar M, Hiraishi N, Islam MS, Aizawa M, Tamura Y, Otsuki M, et al. Effect of phytic acid used as etchant on bond strength, smear layer, and pulpal cells. Eur J Oral Sci. 2013 Oct;121(5):482–7.
5. Kong K, Hiraishi N, Nassar M, Otsuki M, Yiu CKY, Tagami J. Effect of phytic acid etchant on resin–dentin bonding: Monomer penetration and stability of dentin collagen. J Prosthodont Res. 2017;1;61(3):251–8.
6. Kong K, Islam MS, Nassar M, Hiraishi N, Otsuki M, Yiu CKY, et al. Effect of phytic acid etchant on the structural stability of demineralized dentine and dentine bonding. J Mech Behav Biomed Mater 2015;48:145–52.
7. Ishwarya D, Victor Samuel A, Kavitha R. Effect of different etching time on etch pattern and etch depth using 1% phytic acid in primary teeth – A Scanning Electron Microscopic study, 2022;1;32(2):94–9.
8. Van Meerbeek B, Conn J, Duke ES, Eick JD, Robinson SJ, Guerrero D. Correlative Transmission Electron Microscopy Examination of Non demineralized and Demineralized Resin-Dentin Interfaces Formed by Two Dentin Adhesive Systems;75(3):879–88.
9. MacEdo G v., Yamauchi M, Bedran-Russo AK. Effects of chemical cross-linkers on caries-affected dentin bonding. J Dent Res 2009 ;88(12):1096–100.
10. LM S, CA S, IL D, O F. Variation in the pattern of acid etching of human dental enamel examined by scanning electron microscopy. Caries Res 1975;9(5):373–87.
11. Redford DA, Clarkson B, Jensen M. The effect of different etching times on the sealant bond strength, etch depth, and pattern in primary teeth. undefined. 1986
12. Legler LR, Retief DH, Bradley EL. Effects of phosphoric acid concentration and etch duration on enamel depth of etch: An in vitro study. American Journal of Orthodontics and Dentofacial Orthopedics. 1990;98(2):154–60.
13. Nassar M, Nassar R, Maki H, Al-yagoob A. Phytic Acid : Properties and Potential Applications in Dentistry. 2021;8:1–17.
14. Selle PH, Cowieson AJ, Cowieson NP, Ravindran V. Proteinphytate interactions in pig and poultry nutrition: a reappraisal. Nutr Res Rev 2012;25(1):1e17.
15. Redford DA, Clarkson BH, Jensen M. The effect of different etching times on the sealant bond strength, etch depth, and pattern in primary teeth. Pediatr dent 1986;8(1):11e5.

16. Galil KA, Oral D, Gerald S, Wright Z. Acid etching patterns on buccal surfaces of permanent teeth. Pediatr dent 1979;1(4):230e4.
17. Legler LR, Retief DH, Bradley EL, Denys FR, Sadowsky PL. Effects of phosphoric acid concentration and etch duration on the shear bond strength of an orthodontic bonding resin to enamel. An in vitro study. Am J Orthod Dentofacial Orthop 1989;96(6):485e92.
18. Torres-Gallegos I, Zavala-Alonso V, Patino-Marin N, Martinez-Castanon G, Anusavice K, Loyola-Rodriguez J. Enamel roughness and depth profile after phosphoric acid etching of healthy and fluorotic enamel. Aust Dent J 2012;57(2):151e6.
19. Zavala-Alonso V, Martinez-Castanon GA, Patino-Marin N, Terrones H, Anusavice K, Loyola-Rodriguez JP. Characterization of healthy and fluorotic enamel by atomic force microscopy. Microsc Microanal 2010;16(5):531e6.

TABLES

Table 1: Microtensile bond strength (μTBS) values of the experimental groups

Group	n	μTBS (MPa)
37% phosphoric acid	10	14.4±4.1*
1% phytic acid	10	22.42±3.6*

The μTBS values are given as mean ± SD
Groups with * indicates significant statistical difference (Kolmogorov-Smirnov and Shapiro-Wilks tests, $P < 0.05$

Table 2: Etch depth (μm) values of the experimental groups

Group	n	Etch depth (μm)
37% phosphoric acid	10	268 ± 109
1% phytic acid	10	308 ± 106

The Etch depth (μm) values are given as mean ± SD

FIGURES

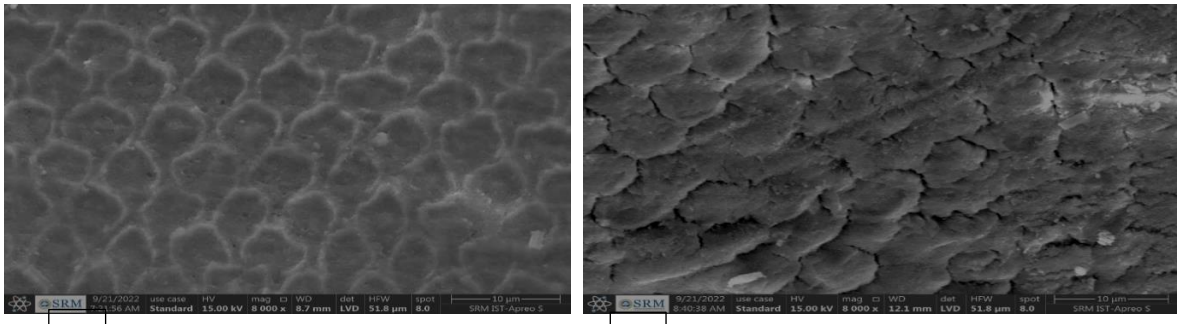

Fig 1: Scanning electron microscopy image of primary teeth enamel treated with A)37% phosphoric acid showing type 1 etch pattern B) 1% phytic acid showing type 2 etch pattern.

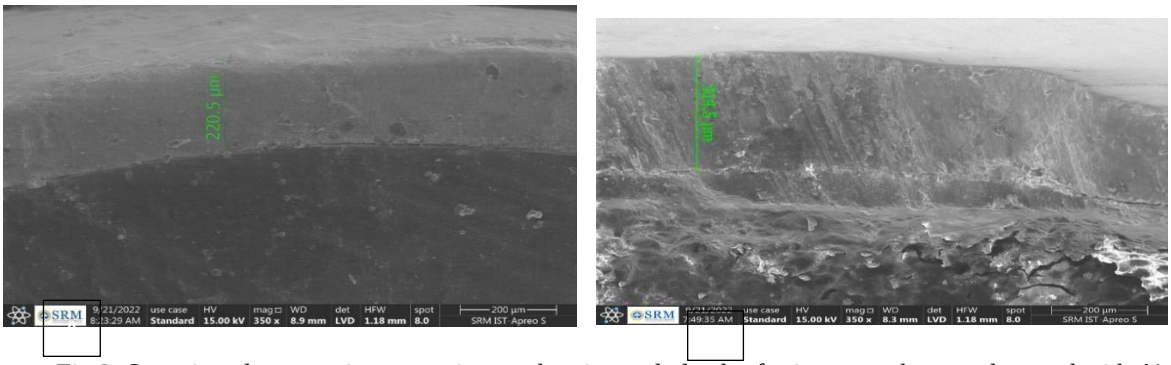

Fig 2: Scanning electron microscopy image showing etch depth of primary teeth enamel treated with A) 37% Phosphoric acid and B) 1% Phytic acid.