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A COMPARATIVE EVALUATION OF THE EFFICIENCY OF DIFFERENT ACIDS FOR REMOVAL OF SMEAR LAYER AFTER CAVITY PREPARATION. AN IN VITRO STUDY

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ABSTRACT

Introduction: To evaluate and compare the efficiency of different acids for removal of smear layer after cavity preparation.

Materials: 40 extracted molars stored in normal saline, fine grit straight fissure diamond abrasive(CPC, China), 17% ethylene diamine tetra acetic acid solution, 5% citric acid solution, 17% polyacrylic acid solution, 37% phosphoric acid gel.

Methods: 40 Extracted human intact second molar were selected, divided in 4 groups randomly n=10. After that,occlusal surface of human molar were prepared with standardized box of 3mm using diamond abrasive. Then prepared sample were studied under scanning electron microscope. After preoperative scanning electron microscopy, prepared sample in each group were treated with 17% EDTA, 37% phosphoric acid, 17% polyacrylic acid, 5% citric acid respectively for 60 seconds, then irrigated with 5ml of distilled water and after which specimen were dried and studied under scanning electron microscope.

Results: Scanning electron microscope evaluation of dentin surface etched with 17% ethylene diamine tetra acetic acid for 60 seconds revealed maximum number of opened dentinal tubules with reduced number of smear plug. However, higher percentage of number of opened dentinal tubules with no smear layer plugs were observed with 17% polyacrylic acid as compared to other acids. But it is found to be very aggressive on dentin surface indicating loss of calcium ions.

Conclusions: Within the limitation of the study 17% EDTA and 37% phosphoric acid revealed maximum number opened dentinal tubules with less smear plug and simultaneously less surface deterioration.

KEYWORDS: Smear Layer, EDTA, Phosphoric Acid, Polyacrylic Acid, Citric Acid, Dentinal Tubules

INTRODUCTION

Dentin is a hydrated biologic composite with distinct and variable morphology. The peritubular dentin is largely apatite and lines the lumen of each tubule. Intertubular dentin is a composite that consists mainly of 2 distinct materials: a collagen matrix and an apatite crystal reinforcement. In bonding techniques, the permeability of dentin to adhesive agents is of critical importance. The opportunity exists for resin to infiltrate both dentinal tubules and intertubular dentin. Resin

penetration into tubules can effectively seal the tubules and contribute to bond strength if the resin bonds to the tubule wall.[44] However resin infiltration into intertubular dentin can occur only if the mineral phase of dentin is removed by acid conditioner.[1-10] Prepared dentin surfaces create a loosely bounded smear layer, reported between 0.05 and 15 µm thick, with a composition similar to the structures from which they were created.[11-18].

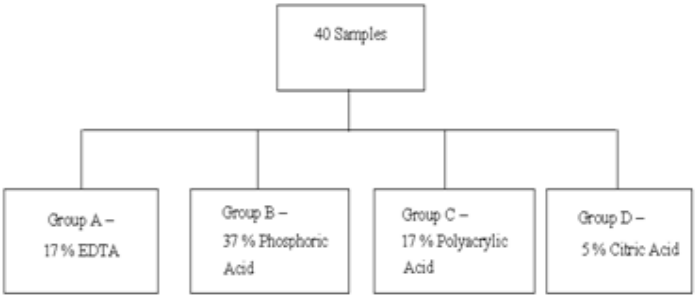
Early animal studies indicated that acid etching caused moderate-to-severe pulpal reactions, but there is a high probability that pulpal irritation may have been due to microleakage of bacteria and their by-products.[19,20] Acid etching of dentin is used as a surface preparation step to eliminate wide variation in tooth surface structures after cutting and to improve adhesion for a variety of procedures in restorative and preventive dentistry.[21] Furthermore, acid etching has been reported to reduce microleakagearound restoration margins and provide substantial cuspal reinforcement.[22-24]

Most current adhesive systems remove the smear layer from the cut dentin surface, demineralize the dentin to a certain depth, and leave behind a collagen rich network for the interaction with adhesive resins; this process results in the formation of a hybrid layer, or resin-dentin interdiffusion zone.[25-28] Different acid etchants have been recommended for removing the smear layer and demineralizing the dentin surface. The recommended uses of acid etchants allow for penetration of binding resins and result in the formation of tags.[29,30] The permeability of dentin to adhesive agents is of crucial importance. Such penetration may contribute to the increased bond strength of restorative material that use acid etching.

MATERIALS AND METHODS

Approval of Ethic Committee was taken for this study. The teeth were randomly selected from known patients. All patients signed an informed consent document to take part in this research. Their age ranged from 45 to 73 years old.

40 extracted human intact second molar were debrided to remove remnants of periodontal ligaments. The teeth were stored in normal saline at room temperature. Then the roots of teeth were removed by separating disk. Subsequently, teeth were randomly divided into four groups (n = 10). Group A – 17 % EDTA, Group B – 37% phosphoric acid, Group C – 17 % polyacrylic acid, Group D- 5% citric acid.



Then the teeth were mounted in wax, occlusal surface of teeth were prepared to expose the dentin to the standardized depth of 3mm by rotary instrument with diamond abrasive. A new fine grit straight fissure diamond abrasive was used for each specimen. Then the samples prepared were used for evaluation of dentin surface by SEM. After pre-SEM evaluation, the teeth (n=10) were subjected to different acids (17 % EDTA, 37 % phosphoric acid, 17 % polyacrylic acid, 10 % citric acid). Acids were applied for 60 seconds to the dentin with different applicator tips for each new sample. Toileting of cavity was done with normal saline (30N) using needle and syringe for 10 seconds to remove acid. Specimens then were carried for SEM evaluation.

SCANNING ELECTRON MICROSCOPY

After reducing occlusal surface to 3mm the specimen were studied under scanning electron microscope. For observing and confirmatory formation of smear layer after using rotary instrument simultaneously EDAX (Energy Dispersive X-Ray Spectroscopy) analysis was also carried out to evaluate mineral contents.

Scanning of specimen was carried out at 1000x magnification. Then after treating with acids, teeth were irrigated with 5ml distilled water.

Scanning of specimen was carried out at most representative area of each third of each tooth and was magnified at 4000x.

SEM EVALUATION

To evaluate the degree of smear layer removal, the scoring system described by Takeda et al[45] was used but with modification. Briefly score 1 = no smear layer, with all tubules cleaned and opened; score 2 = few areas covered by smear layer, with most tubules cleaned and opened; score 3 = smear layer covering almost all the surface, with few tubules opened; and score 4 = smear layer covering all the surfaces. It was a blinded evaluation performed by three independent observers.

NUMBER OF OPENED DENTINAL TUBULES

Evaluation of number of opened dentinal tubules was carried out with the help of grid made using Adobe Photoshop software, with the box of cross section of 2cms. It was a blinded evaluation performed by three Independent observers. Table 1 Shows the Percentage of Opened Dentinal Tubules among all the Groups

Table 1: The Descriptive Statistics of Percentage of Opened Dentinal Tubules across Four Solutions

Solution	Percentage of Opened Dentinal Tubules				
	Mean	Standard Deviation	Median	Minimum	Maximum
EDTA (n=10)	94.2	1.8	94.3	90.3	96.6
Phosphoric(n=10)	87.1	1.6	86.9	84.6	89.7
Polyacrylic(n=10)	98.1	1.4	98.3	96.6	96.3
Citric (n=10)	80.2	2.3	80.3	75.8	84.5

Percentage of opened tubules is calculated using following formula:

$$\frac{No.of\,opened\,dentinal\,tubules\,seen}{Total\,no.of\,dentinal\,tubules}\ x\ 100$$

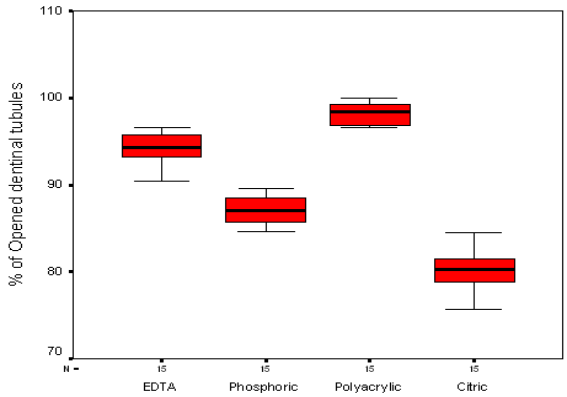


Figure 1: The Distribution of Percentage of Opened Dentinal Tubules across Solutions

EDAX ANALYSIS

Energy dispersive X-ray spectroscopy is an analytical technique used for the elemental analysis or chemical characterization of a sample. It is one of the variants of X-ray fluorescence spectroscopy which relies on the investigation of a sample through interactions between electromagnetic radiation and matter, analyzing X-rays emitted by the matter in response to being hit with charged particles. Its characterization capabilities are due in large part to the fundamental principle that each element has a unique atomic structure allowing X-rays that are characteristic of an element's atomic structure to be identified uniquely from one another.[50] EDAX analysis was carried out for calculating percentage of mineral before and after treating with acid.

Figure 2 and Figure 3 illustrate the reduction in calcium and phosphorus content after treating the specimens by each solution respectively. EDAX analysis was carried out in order to calculate the reduction in mineral contents.

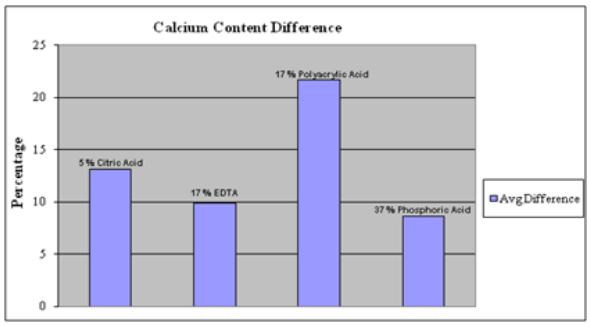


Figure 2: Percentage Reduction in Calcium Content for Each Solution

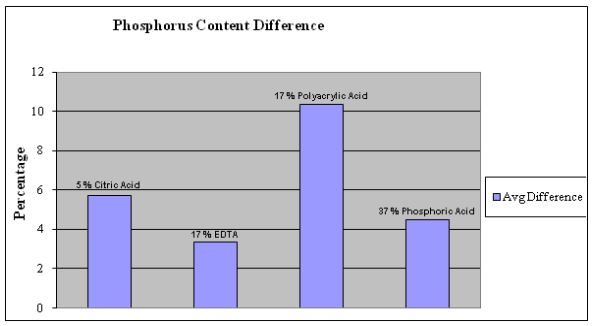


Figure 3: Percentage Reduction in Phosphorus Content for Each Solution

RESULTS

Table 1 show that after SEM evaluation the maximum numbers of opened dentinal tubules were seen with 25-40% smear plug when treated with 17% EDTA solution for 60 seconds on surface dentin. Whereas, less number of opened dentinal tubule with negligible smear plug i.e. 5-10% were observed when treated with 17% polyacrylic acid. 37% phosphoric acid gel also gave similar results as 17 % EDTA but with more number of smear plugs. 5% citric acid found to be less efficient to open dentinal tubules. The statistical comparison between the four groups can be seen in Table 2

Table 2: The Statistical Comparison of Percentage of Opened Dentinal Tubules across Four Solutions

Comparison between Solutions	P-Value
EDTA v/s Phosphoric	0.001
EDTA v/s Polyacrylic	0.001
EDTA v/s Citric	0.001
Phosphoric v/s Polyacrylic	0.001
Phosphoric v/s Citric	0.001
Polyacrylic v/c Citric	0.001

P-values are obtained using one-way analysis of variance with Bonferroni's correction for multiple group comparisons.

When elemental analysis was carried out it is found that there was maximum loss of calcium and phosphorus ion when dentin surface was treated with 17% polyacrylic acid as compared to 17% EDTA and 37% phosphoric acid.

DISCUSSIONS

It is noteworthy that the literature describes a variety of chemicals with a broad range of concentrations and different irrigation regimens to remove the smear layer. This study used EDTA, a well-known chelating agent widely used to remove inorganic components of the smear layer[46,47], citric acid, a weak organic acid with relatively low cytotoxicity used as an aqueous acidic solution[48]; and remove the smear layer and smear plugs formed during coronal cavity preparation. [49]The present study has compared the action of 37% phosphoric acid with well-established solutions, such as 17% EDTA and 5% citric acid at experimental periods of time in which these chemicals are known to be effective. As per review of available literature comparing EDTA, citric acid, phosphoric acid and polyacrylic acid has not been at the same concentrations as those used in the present study.

From the above results of study it is concluded that 17% polyacrylic acid for 60 seconds is very effective for opening maximum number of dentinal tubules and to remove smear plugs. But at the same time it causes more demineralisation on dentin surface as compared to 17% EDTA and 37% phosphoric acid.

17% EDTA for 60 seconds was found to be less aggressive to dentin surface but smear plugs were seen.37% phosphoric acid for 60 seconds removed smear layer moderately and was comparatively less harmful to dentin surface as compared to 17% polyacrylic acid and 17% EDTA.

In Vivo application of such protocol will enhance the predictable bonding requirement for adhesive restorative application.

CONCLUSIONS

Within limitation of study it is concluded that 17% EDTA and 37% phosphoric acid for 60 seconds on dentin surface were moderate in removing the smear layer and opening of dentinal tubules while contributing less for removal of calcium and phosphorus ion from dentin surface and hence they cause less demineralisation on dentin surface.

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