Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 3001-3009 | ISSN:2251-6727

Effect of Calcium Phosphate-Based Desensitizers on Stain Resistance of Acid Eroded Enamel- an in Vitro Study.

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(Received: 04 February 2024 Revised: 11 March 2024 Accepted: 08 April 2024)

KEYWORDS
Desensitizers, Erosion, Predicta, Staining susceptibility, TMD, NaF

ABSTRACT:

Aim: To investigate the effect of two calcium-phosphate-based bioactive desensitizers, i.e., Teeth Mate (TMD) and Predicta, and compare them with gold standard NaF on the stain resistance of acid-eroded enamel.

Method and Material: From extracted human incisors, 150 polished enamel samples (4 x 4 x 1 mm) were made. For 15 minutes, each sample was submerged in 50 milliliters of 0.5% citric acid (pH 2.5) to facilitate erosion. A profilometer was used to measure the surface roughness [Ra] before and after erosion. Thirty samples were randomly assigned to five groups. Predicta, TMD, and NaF (0.21%) treatments were given to three groups for a total of five minutes. Desensitizer was not applied to one group that had deteriorated. The samples were not treated or deteriorated for the control group. All of the samples were maintained in artificial saliva (AS) with a pH of 7.2 for a full day at 370 °C. Every eight hours, fresh applications of the solutions were made. After that, samples were submerged in a tea solution and kept in an incubator for seven days to see if they would discolor. Both before and after therapy Digital photos were captured in order to assess stain resistance.

Results: Surface roughness was significantly increased by acid erosion, according to a one-way ANOVA analysis of variance. The groups treated with Predicta and TMD showed decreased mean color difference ΔE values compared to the groups treated with NaF and no desensitizer.

Conclusions: Surface roughness increased, and more stains were absorbed due to acid erosion. Nevertheless, the use of TMD and Predicta, bioactive desensitizers based on calcium phosphate, reduced the roughness and halted the development of extrinsic stains.

Introduction:

According to the American Association of Endodontic Erosion, “a loss of tooth substance by a chemical process without bacteria.” Dental erosion is defined by smooth, round-limited, shallow, flat, and silky concavities with smooth surfaces that are frequently free of stains and plaque (1). Both intrinsic and extrinsic acids can cause

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erosion, and variations in salivary flow and contents can alter erosion. (2,3). Intrinsic factors include regurgitation, vomiting, and rumination (2,4).Extrinsic causes include acidic beverages, work-related erosive materials, etc. (2).

G. V. Black estimated in 1908 that less than 0.1% of people had tooth erosion. In addition to listing more potential etiologies from developmental, systemic, or extrinsic origins, he conjectured that the origin might be hereditary (1). Epidemiological research conducted over the past 20 years has revealed that a significant portion of youth exhibit dental erosion symptoms. In a study by Bartlet et al., with participants ages 11 to 14, they discovered that a median of 12% of the subjects' surfaces showed surface wear, and 57% of the subjects had more than ten teeth worn down. The average worldwide prevalence rate of erosive tooth wear, according to a comprehensive review of children and adolescents, is estimated to be 30.4%, with a range of 7.2 to 74.0%. (1).

Enamel that has been damaged by acid may be more prone to stain absorption, which could result in surface discoloration and staining—a serious aesthetic issue (5). When it comes to treating dental erosion caused by citric acid, fluoride treatment is regarded as the gold standard (6, 7). Calcium fluoride-like deposits (CaF2), which function by reducing or lessening the contact between the acid and the underlying enamel, are often what cause their protective effect. (8) Fluoride-induced enamel remineralization raises surface hardness and lowers surface porosity, which can help prevent stains from being absorbed, per a study by Junko and colleagues.(9).

Because calcium phosphate mineral-based desensitizers are biocompatible and effectively obstruct dentinal tubules, reducing dentine permeability, there has been a notable surge in interest in these products in recent years. These substances, which contain calcium and phosphate, release phosphate and calcium ions. In order to remineralize, the mineral phase on the surface of the enamel or dentin becomes "supersaturated" with calcium and phosphate. (10,11). Thus, the minerals calcium and phosphate fill in the gaps left by the surface of worn enamel, increasing its hardness and decreasing its porosity, which reduces discoloration. (9).

In this study, the impact of acid-eroded enamel on staining susceptibility has been assessed using desensitizers based on calcium phosphate. The null hypothesis asserts that stain resistance on acid-eroded enamel does not alter after calcium phosphate-based bioactive desensitizers and sodium fluoride solutions are applied.

Materials and Methods:

This in-vitro study was carried out by the Conservative Dentistry and Endodontics department of the Government Dental College and Hospital in Ahmedabad.

Sample Preparation:

Seventy-five maxillary central incisors free of caries and restoration, freshly extracted for periodontal or prosthetic reasons, were selected. Tissue fragments and calculus were removed using an ultrasonic scaler. Teeth with stain, fluorosis, any discoloration, lesion, developmental defects, and any hypocalcification were excluded. The infection control protocol for the teeth collected for this study was followed as per Occupational Safety and Health Administration and Center for Disease Control and Prevention guidelines. 75 labial surfaces of human incisors were crushed using a diamond disc to create flat enamel surfaces that were about 1 mm thick. Under flowing water, 3-µm diamond paste and silicon carbide papers ranging in grit from 600 to 2000 were used to polish the enamel surfaces. A total of fifty (4 x 4 x 1 mm) flattened enamel surface samples were manufactured. To get rid of any last residues of the polishing chemicals, the polished enamel surfaces were ultrasonically cleaned in deionized water for five minutes.

An examination of pre-erosion surface roughness (Ra) was performed on all 150 specimens. A profilometer (Mitutoyo Surftest SJ 210) was used for it. On the surface of the sample, the roughness measurement spots were randomly marked. Scanning was performed with a diamond stylus with a 0.2 µm tip radius, perpendicularly to the samples, with a contact load of 1.90 mN. The movement speed of the stylus was 0.25mm/sec along the length of the measuring line, which was 0.5mm with a cutoff of 2.5mm.

The surface under investigation is mapped out by the scanning process. The arithmetic average height of roughness component irregularities from the mean line measured throughout the sampling length is known as the average surface roughness, and it is measured in microns (µm). Smoother surfaces are indicated by smaller Ra values.

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120 specimens were subjected to acid erosion for 15 minutes after a baseline assessment of surface roughness using 50 milliliters of a pH 2.5 citric acid solution at 0.5%. The surface unevenness of the samples was evaluated again to confirm acid erosion. 30 samples served as controls and were not eroded.

Surface Treatment with Desensitizers:

Details of three different dentin desensitizers used in the study is listed in Table 1

Table 1

Products and Manufacturer	**Composition**
PBD, Parkell, Edgewood, New York, USA: Predicta Bioactive Desensitizer gel	Phosphate, calcium, and nanohydroxyapatite
Teethmate Desensitizer (TMD) (Kuraray Noritake Dental, Tokyo, Japan)	Powder: Dicalcium phosphate anhydrate (DCPA) Tetra calcium Phosphate (TTCP) Liquid: aqueous and preserved
0.2% sodium fluoride (Prevident, Colgate)	FD&C Blue, sodium benzoate, benzoic acid, glycerine, poloxamer 338, water, and 0.2% sodium fluoride

150 specimens with varying surface treatments were split into 5 groups at random.

Group 1: Erosion + Predicta desensitizer. It was applied by rubbing with an applicator for 30 seconds, washed with deionized water after five minutes.

Group 2: Erosion + TMD. Using a microbrush, After 15 seconds of combining the powder and liquid, a slurry was created, which was then applied to the enamel surface and rubbed in for 30 seconds. Deionized water was used to clean the region after five minutes.

Group 3: Erosion +0.2% NaF. Specimens were immersed in 0.2% sodium fluoride solution for five minutes.

Group 4: Erosion + No desensitizer treatment.

Group 5: No Erosion + No desensitizer treatment (Control Group)

After treatment all specimens were stored in an incubator for 24 hr, in artificial saliva (Nanochemazone, Canada). Predicta, TMD, and 0.21% NaF were reapplied at eight and sixteen hours, thus three times in 24 hrs. For all the samples artificial saliva was changed every eight hours to keep the solution's freshness intact. All samples were taken out and allowed to air dry gently after 24 hours.

Stain Susceptibility Assessment:

Following incubation, all samples were photographed using a macro lens on a digital camera Canon 1500 D (Shinagaa, Japan), both before and after staining with tea solution. The camera was set to auto-white balance, no flash, ISO 200, shutter speed 125, and aperture size 3.5. In order to create a stain, two tea bags (Wagh Bakri, India) were boiled in 100 milliliters of water for five minutes. The samples were submerged in a tea solution and maintained at 370 °C for seven days in an incubator. On the fourth day, a fresh staining solution was added. Following a seven-day soaking period, the specimens that were stained were carefully dried, and the color was assessed once more.

The analysis of the image was done using Adobe Photoshop CS 6 software. A 2 x 2 mm grid was superimposed on all photographs to standardize size and point for evaluation of color. One point was selected for each sample. At this stage, CIE L*a*b* values and values for the x and y coordinates were recorded. The following formula was used to determine the color difference (Δ E) between the values obtained at baseline and after staining.

$$\Delta E = \left[(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2\right]^{1/2}$$

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The statistical analysis was conducted using SPSS version 23. A post Hoc test was used for pairwise comparisons, and a one-way ANOVA was used to examine color differences (Δ E) overall.

Results

The average surface roughness (Ra) before and after citric acid erosion was 0.214 µm and 0.635 µm, in that order. (Table 2). Thus, all the samples showed increased roughness after immersion in citric acid.

Tables 3 and 4 display the statistical data for the color difference (ΔE) for each group and the intergroup comparison, respectively. The observed order of the Mean Color Difference (ΔE) was: Group 3: NaF, 38.69 > Group 2: TMD, 19.84 > Group 1: Predicta, 10.69 > Group 4: Erosion, 46.75 > Control Group 5, 7.10. The group with the least color difference, the control group (group 5, ΔE-7.10), was significant when compared to group 1 ($p < 0.05$) and statistically significant when compared to groups 2, 3, and 4. When compared to groups that underwent surface treatment with desensitizers, such as Groups 1, 2, and 3, Group 4 (Erosion without treatment) had extremely substantial staining. ($p < 0.001$). When compared to TMD (Group 2, ΔE = 19.84) and NaF (Group 3, ΔE = 38.69), groups treated with desensitizers from Group 1 (Predicta, ΔE = 10.69) displayed a reduced color difference after staining, and the results were statistically highly significant for both ($p < 0.001$). But when it came to stain resistance, the TMD group (Group 2, ΔE = 19.84) outperformed the NaF group (Group 3, ΔE = 38.69, $p < 0.001$).

Discussion:

In clinical language, "dental erosion" refers to a pathologic, persistent, localized, painless loss of dental hard tissue that is physically caused by acid and/or chelation chemically scraping away from the tooth surface without bacterial involvement. There are three types of tooth erosion: extrinsic, intrinsic, and idiopathic. Eccles (1979) classified erosion into the subsequent categories: Class II: localized lesion including dentin and l/3 of the surface; Class I: superficial lesion involving just enamel. Class III: Over 1/3 of the dentin surface is affected by a generalized lesion. (3).

Prism sheath regions in enamel are where the lesion mostly appears, and prism core breakdown follows. The interprismatic zones eventually suffer the same fate. In comparison to prismatic enamel, aprismatic enamel erodes much more unevenly and is thus less vulnerable to erosive disintegration (12). Enamel erosion causes the bulk material to be centripetally etched away, leaving a partially demineralized surface layer that results in a roughened surface (12).

With currently available remineralizing treatments, remineralization is now conceivable in first-embedding erosive lesions. Artificial saliva, fluoride, calcium phosphate-based desensitizers (including amorphous and crystalline calcium phosphate), desensitizers incorporating bioactive glass, and agents based on nanohydroxyapatite are other remineralizing agents that are utilized for erosion. In our investigation, 0.2% NaF and calcium phosphate-based desensitizers, such as Predicta and TMD, were contrasted.

In order to minimize rapid decomposition and get around the drawbacks and challenges of using genuine saliva, samples used in this investigation were preserved in artificial saliva. Samples were maintained at a temperature of 370 °C in an incubator (13).

For fifteen minutes, the greatest roughness was produced by using citric acid. Since it is frequently present in orange and lemon juice and is a crucial component of many acidic drinks, It could deteriorate the hard tissue of teeth (14). The demineralization pattern of enamel erosion results in a roughened surface that makes staining agent diffusion easier. It expands the surface area where discoloring pigments can be retained.

Since deionized water doesn't contain any minerals, no remineralization was seen when samples were washed in it after being treated to remove desensitizer for this investigation (15).

Tea has little calcification or decalcification potential; hence, the staining process used a tea beverage to generate discoloration on the tooth (9).

In our investigation, samples were stored for seven days, and staining was examined to assess intra-observer reliability. A week later, the same observer reevaluated the data (16). Since the tetra calcium and dicalcium-based sensitizers needed five minutes to set, the samples were desensitized and then left (17).

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In profilometry, the sample is scanned using a diamond stylus loaded with a 1.90 mN-specific force and having a 0.2 µm tip radius. The process of scanning builds a map of the examined surface, and as a result, the stylus tip replicates the surface roughness of the surface under analysis (18). In samples treated with citric acid, profilometer examination revealed increasing surface roughness from pretreatment (0.211 micrometers) to posttreatment evaluation (0.635 micrometers). (Refer to Table 2). (19)

There are several techniques for determining stain susceptibility.

- Digital photography; spectrophotometer; spectrocolorimeter; spectroradiometer

The current study used digital photography to assess stain susceptibility. The ideal camera choice for dentistry photography is a DSLR because of its integrated flash and automatic settings, which yield excellent overall results, color accuracy, and picture quality.

Benson et al. came to the conclusion that this approach is more accurate and reproducible when used to quantify enamel demineralization or remineralization using digital photographs (20). The Commission International de l'Eclariage L*a*b* system was used to measure the color change of the lesion. Photoshop (Adobe Inc., San Jose, Calif.) was used to compute ΔE and observe the value of L*a*b*. The brightness degree is shown on the L* axis. The red/green axis is represented by the a* value, where a higher number indicates a greater red color component. The yellow/blue axis is represented by the b* number; an increase denotes a greater yellow tint. (21).

The order of color difference (ΔE) observed after staining was: erosion > NaF > TMD > Predicta > Control.

The mean color difference ΔE of Group 1 (Predicta) was found to be significantly lower ($p < 0.001$) than that of Group 2 (TMD -19.84), Group 3 (NaF - 38.69), and Group 4 (Erosion - 46.75). Group 1's ΔE (Predicta = 10.69) was notably lower than group 5's (Control = 7.10, $p < 0.05$). (Table 4) Nano HAP, which is found in Predicta (Group 1), is said to be the most biocompatible and bioactive substance (22). Enamel porosity might be simultaneously repaired by well-sized nanoapatite particles (23). According to a number of studies, nano-HAP may be able to fill up voids and gaps on demineralized teeth or even replace damaged enamel through deposition onto natural tissue (24). The presence of water-insoluble silica, which has the outstanding capacity to restore demineralized enamel to its previous state by depositing a new crystalline phase epitaxially on the existing one, may be the cause of the bioactive desensitizer's lower stain susceptibility (23). Tschoppe et al. used nanoHAP consecutively in mouthwash, toothpaste, and chewing gum. It was concluded that all of these materials had the potential to remineralize dental enamel.(25)

Group 2-TMD (ΔE = 19.84) had a considerably lower mean color difference (ΔE) than Group 3 (NaF, ΔE = 38.69), Group 4 (Erosion, ΔE = 46.75), and Group 5 (Control, ΔE = 7.10) ($p<0.001$). This is because TMD forms a hydroxyapatite layer on the surface. However, TMD showed higher staining than Predicta (Group 1), as Predicta contains both nano-hydroxyapatite and bioactive glass. TMD is an aqueous solution of two products: calcium-deficient hydroxyapatite and calcium hydroxide, leading to incomplete porous coverage of enamel (24, 26). The findings of this study correlate with the study done by Hamba et al., which showed a lower effectivity of TMD than a bioactive desensitizer. The fluoro-alumino-calcium silicate glass component was found to be a more stable fluorapatite crystal. (27)

Sodium fluoride (Group 3) showed significantly higher staining (ΔE -38.69) compared to the TMD and Predicta groups. This is because, according to a study by Kyaw KY et al., fluoride can only induce remineralization in saliva that contains enough calcium and phosphate ions. This means that the enamel needs to be exposed to saliva for a sufficient amount of time in order for the ions to get saturated and create fluorapatite or fluorohydroxyapatite on the enamel surface. (28). In the current investigation, immersion in artificial saliva for 24 hours is not long enough to achieve the concentration of phosphate and calcium ions needed to create fluoroapatite.

Group 4 (the erosion without treatment group) showed highly significant (ΔE = 46.75, $p < 0.001$) staining among all the groups. Saliva is one of the most crucial components in the restoration of weakened enamel, according to Buzalaf and 41 et al., since it contains phosphates and calcium that promote spontaneous remineralization (29). Fluorides and calcium phosphate

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technologies are used to speed up the remineralization process because saliva alone does not accelerate natural remineralization very quickly. Therefore, even though the samples in our investigation were kept in fake saliva to assess the remineralization impact of saliva, the 24-hour immersion period was insufficient to allow for the occurrence of natural remineralization.

Group 5 showed the lowest staining (ΔE = 7.10) of all the groups. This is because samples were neither eroded nor treated with any desensitizers, thus the normal enamel crystallization was preserved.

Our study correlates with the study done by Nakata T et al., which reported that calcium phosphate-based desensitizers contribute to the protection of enamel surfaces against acidic challenges. (30)

Thus, the null hypothesis is partially rejected as the newly developed nanohydroxyapatite-based bioactive desensitizer, Predicta, showed a significant difference from TMD and NaF in preventing staining of acid-eroded enamel surfaces.

While we made every effort to replicate the intra-oral environment in the current investigation, there were certain limitations. For example, erosion in the oral cavity is never a standalone process; rather, it is typically accompanied by abrasion from brushing teeth or eating. Digital photography was also employed to assess color disparity. Other sophisticated techniques, nevertheless, might also be applied. To improve the evaluation, more research with larger sample numbers and in vivo environments is needed.

Conclusion:

Within the limitations of the present study, it can be concluded that:

• All desensitizers used in the present study—Predicta, TMD, and 0.21% NaF—showed remineralization effects on acid-eroded enamel surfaces.

• Among all desensitizers Predicta bioactive desensitizer proved to be the most effective in stain prevention of acid-eroded enamel, followed by TMD and 0.21% NaF.

Future Scope and Clinical Significance

Predicta, a newly developed nano-HAP based bioactive desensitizer, can be used for the management of erosive enamel surfaces in order to prevent staining.

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Legends of Tables:

GROUP 1: Predicta desensitizer [EROSION+PREDICTA]

Group 2: Teethmate desensitizer [EROSION+ TMD]

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Group 3: 0.21% NaF solution [Erosion+NaF]

Group 4: Erosion +without treatment

Group5: No erosion+No treatment

Table 1

Products and Manufacturer	**Composition**
PBD, Parkell, Edgewood, New York, USA; Predicta Bioactive Desensitizer gel	Phosphate, calcium, and nanohydroxyapatite
Teethmate Desensitizer (TMD) (Kuraray Noritake Dental, Tokyo, Japan)	Powder: Dicalcium phosphate anhydrate (DCPA) Tetra calcium Phosphate (TTCP) Liquid: water, preservative
0.2% sodium fluoride (Prevident, Colgate)	FD&C Blue, sodium benzoate, benzoic acid, glycerine, poloxamer 338, water, and 0.2% sodium fluoride

Table 2 Surface Roughness (Ra) Before and After Erosion with Profilometer

	MEAN
PRETREATMENT	0.214
POSTTREATMENT	0.635

Table 3: Colour Difference (ΔE) for different Groups

Group	n	Minimum	Maximum	Mean	Std. Deviation	F value	P value
Group 5	30	4.69	9.70	7.1	1.16	799.462	<0.001**
Group 4	30	40.29	51.95	46.75	3.06		
Group 3	30	35.01	46.92	38.69	2.92		
Group 2	30	17.21	22.56	19.84	1.57		
Group 1	30	6.56	17.46	10.69	2.68		

**-Highly significant (p<0.001)

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Table 4: Inter Group comparison of Color Difference (ΔE)

Comparison between		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Group5	Group 4	-39.65	.87995	<0.001**	-41.8864	-36.9923
	Group 3	-31.59	.87995	<0.001**	-33.7660	-28.8720
	Group 2	-12.74	.87995	<0.001**	-14.9870	-10.0930
	Group 1	-3.59	.87995	0.004*	-5.6595	-.7655
Group4	Group3	8.06	.87995	<0.001**	5.6733	10.5674
	Group2	26.91	.87995	<0.001**	24.4523	29.3464
	Group 1	36.06	.87995	<0.001**	33.7798	38.6739
Group3	Group2	18.85	.87995	<0.001**	16.3320	21.2260
	Group1	28	.87995	<0.001**	25.6595	30.5535
Group2	Group1	9.15	.87995	<0.001**	6.8805	11.7745

**-Highly significant ($p<0.001$), *-significant ($p<0.05$)