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EFFECT OF REMINERALIZATION ON METROLOGY OF SURFACE FEATURES OF INDUCED ACID ERODED TOOTH ENAMEL

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ABSTRACT

Aim: To evaluate the remineralization potential presented by surface metrology, of two remineralizing agents: CPP-ACPF (MI paste plus) and fluoride containing mouthwash (Aquafresh extra care mouthwash) on the induced acid erosion bovine enamel.

Materials and Methods: Ten freshly extracted bovine permanent incisors were selected. The incisal one third of their crowns were sectioned by a slow speed diamond disc. The labial surface of each sample was examined under atomic force microscope (AFM) to record its surface roughness (Ra values). All samples were then demineralized by citric acid solution and reexamined under AFM .The demineralized samples were randomly divided into two equal groups I and II (5 samples each), according to the remineralization regimen used; samples in group I were treated with MI Paste plus while those in group II were treated with Aquafresh extra care mouthwash. All samples were reexamined under AFM to evaluate Ra values.

Results: Acid erosion showed an increase of surface roughness value (72.85 nm) compared to that recorded for the original surface (38.45 nm). After remineralization, there was a decrease of the mean Ra value in group I (52.78nm) and group II (59.54 nm). ANOVA test revealed a statistical significant difference among base line data (original enamel surface), demineralized and remineralized samples in both groups. Scheffe`s test revealed a statistical significant difference between mean Ra value of demineralized samples versus that of base line data as well as that of remineralized samples in both groups.

Conclusion: The tested CPP-ACP based compound offered a significant remineralization potential of initially eroded bovine enamel compared to the tested fluoride containing mouthwash as detected by reduced surface roughness.

Keywords: Dental erosion, enamel remineralization, bovine enamel.

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INTRODUCTION

The prevalence of dental caries has shown a decline in recent years due to the use of fluoride products, education and preventive dental programs [1]. However, the lifestyle and eating habits of modern society where exposure of teeth to an acid environment is becoming common have contributed to the onset of other disorders such as erosion [2].

Dental erosion is defined as the chemical dissolution of dental enamel without bacterial involvement. It is classified into two distinct phases; (initial phase), in which there is only softening and partial demineralization, first seen clinically as white spots and (advanced Phase), with tooth surface loss due to the successive erosive attacks with a remaining softened surface [3,4], this is clinically observed as a combination of the surface demineralization by an erosive agent and the abrasion of the demineralized surface [5].

However it was reported by many investigators [2,6-9], that dental erosion is an irreversible loss of tooth structure by chemical processes of acid dissolution without bacterial involvement. Does not begin as a subsurface enamel lesion that is conducive to remineralization, as in the caries process, but rather as a surface softening lesion that is susceptible to wear and resistant to remineralization by conventional therapies [10]. While Zhou and Zheng [11] and Tyagi et al [12] reported that the erosive enamel loss is a dynamic process with demineralization and remineralization.

Furthermore Amaechi and Higham [13] have determined the possible remineralization of initial erosive lesion by human saliva in their in vitro study, comparing its effect with that of artificial saliva and a remineralizing solution.

Some attempts have been carried out to find a useful way to repair the erosive lesions of human teeth enamel [14]. More attention has been paid to the investigation of remineralization of softened surface enamel. Several in vitro studies have shown that it is capable of rehardening and that its abrasion resistance can be improved [15].

Therefore, research work on the repair methods of tooth lesions due to acid attack have been increasingly recognized to be necessary in clinically dealing with tooth erosion making remineralization of enamel subsurface lesions before restorative intervention a major challenge and goal of modern dentistry [16].

Casein phosphopeptide amorphous calcium phosphate complex (CPP–ACP) has been introduced as a supplemental source of calcium and phosphate ions in the oral environment [17]. Tooth crèmes using CPP ACP such as MI Paste recognize the importance of the neutral ion species, gaining access to the sub surface lesion through a porous enamel surface [18].

Surface metrology is the study of surface geometry (surface texture or surface roughness) and measurement of small scale features on surfaces. Primary form, waviness and roughness are the parameters most commonly associated with the field [19].

Atomic force microscope (AFM) has been used to measure the effect of an exposure to a remineralizing solution on the nano-mechanical properties of previously softened enamel, and was also reported as a very useful tool to investigate the morphology of demineralized and remineralized enamel surface [20].

Considering the controversy found regarding the remineralization repair of the initial erosion lesions, the aim of this in vitro study was to evaluate the remineralizing potential of two remineralizing agents CPP-ACP (MI paste plus) and fluoride containing mouth wash (Aquafresh extra care mouthwash) on induced acid eroded bovine enamel as investigated by AFM.

MATERIALS AND METHODS

Teeth selection and samples preparation

Ten freshly extracted bovine permanent incisors aged between 18-36 months free of cracks or stains as confirmed under a light microscope were selected from slaughterhouse. The selected teeth were thoroughly scaled using hand scaler to remove remaining tissue debris. Roots were separated at cemento-enamel junction using a slow speed diamond disc. Pulps were extirpated. The incisal one third of the crowns were sectioned by a slow speed diamond disc under water cooling and stored in distilled water kept in a refrigerator until use [21].

Sample testing

All samples were embedded in self-cure acrylic resin blocks 10 mm in diameter keeping labial surfaces exposed to be examined under atomic force microscope (AFM) utilizing a surface area of 500 nm^2 to investigate surface roughness of the original enamel surface. The labial surface of each sample was analyzed for at least 10 different sites. The collected data were used as baseline data of the original enamel surface for comparison [22].

After examining all samples, they were stored in artificial saliva at 37°C in an incubator throughout the following steps of the study that was replenished daily [23].

Demineralization step

All samples were demineralized by immersion in 50 ml 0.001M citric acid solution for 10 minutes at room temperature, then carefully rinsed with distilled water to remove any residual acid on its surface and dried by an intermittent oil free air spray [24].

Each sample was reexamined under AFM to investigate surface roughness of the demineralized enamel surface [20]. The samples were then divided randomly into two equal groups I and II (5 samples each), according the remineralization regimen to be used.

Remineralization regimen

In group I, MI Paste plus** was applied to the enamel surface using a gloved finger according to manufacturer's instructions for 5 minutes 10 times daily with an interval 30 minutes in between the applications for 7 days. The paste was rinsed with distilled water and dried with an oil free air spray for 10 seconds after each application [23].

While in group II, samples were immersed in Aquafresh extra care mouthwash*** according to manufacturer's instructions for 1 minute 10 times daily with an interval of 30 minutes in between the applications for 7 days then rinsed with distilled water and dried with an oil free air spray for 10 seconds after each application [25].

Samples of both groups were reexamined under (AFM) to investigate surface roughness of the remineralized enamel surface

Statistical analysis

The collected data were tabulated and statistically analyzed using one way ANOVA, Scheffe's and T test.

RESULT

The mean ± standard deviation of surface roughness (Ra) values in nanometers (nm) were calculated for baseline data (original enamel surface) and demineralized samples to be compared with those calculated after remineralizing samples in group I using MI paste plus and the corresponding values of group II remineralized samples using Aquafresh extra care mouthwash as shown in table (1,2).

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Regarding group I: ANOVA test revealed a statistical significant difference among the untreated, demineralized and remineralized samples, the lowest mean value was recorded in the untreated samples, while demineralized samples recorded the highest mean value denoting increased surface roughness. However after remineralization using MI paste plus there was a decrease in the mean value as illustrated in table (1).

A pair wise Scheffe`s test was then performed; and revealed a statistical significant difference between untreated original surface versus both demineralized samples and remineralized samples as well as between demineralized versus remineralized samples as shown in table (1).

Similar findings were found in group II where a statistical significant difference was found among the values recorded in the untreated original surface, demineralized nm) and remineralized samples using aqua fresh extra care mouthwash). Scheffe's test revealed a statistical significant differences between untreated versus both demineralized and remineralized samples) as well as between demineralized versus remineralized samples as illustrated in table (2).

Comparing The effect of both tested methods of remineralization, T-test revealed a statistical significant difference between mean surface roughness value recorded in group I versus that recorded in group II denoting a significant improvement of surface roughness using MI paste plus compared to Aqua fresh extra care mouthwash, as illustrated in table (3).

Atomic force microscope images

Representative atomic force microscope images of the original, demineralized, and remineralized samples in both groups are shown in (Fig.1-4).

TABLE (1): Statistical analysis of the Mean ± SD surface roughness values of baseline data, demineralized and remineralized samples in group I

Treatment / Test	Baseline data Mean ± SD	Demineralized data Mean ± SD	Remineralized data Mean ± SD	ANOVA	
				F	P-value
Among all recorded values	38.451.90±	72.858.06±	52.787.14±	0.732	0.001*
Baseline vs Demineralized	0.359 (0.000*)				
Baseline vs Remineralized			0.232(0.005*)		
Demineralized vs Remineralized		-1.083(0.000*)			

TABLE (2): Statistical analysis of the Mean ± SD surface roughness values of baseline data, demineralized and remineralized samples in group II

Treatment / Test	Baseline data Mean ± SD	Demineralized data Mean ± SD	Remineralized data Mean ± SD	ANOVA	
				F	P-value
Among all recorded values	38.451.90±	72.858.06±	59.5410.25±	0.732	0.001*
Baseline vs Demineralized	0.359 (0.000*)				
Baseline vs Remineralized			0.232(0.000*)		
Demineralized vs Remineralized		0.320(.019*)			

TABLE (3): Statistical analysis of the Mean ± SD surface roughness values of remineralized samples of both groups

Remineralized Samples	Mean ± SD	T- test	P- value
Group I MI paste plus	52.78±7.14	0.320	0.000*
Group II Aqua fresh extra care mouth wash.	59.54±10.25		

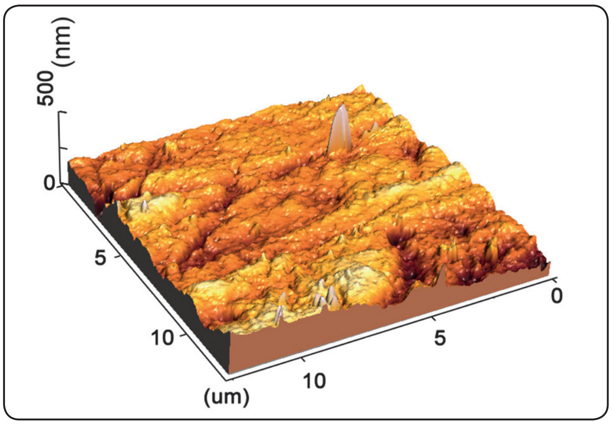

Fig. (1) Three dimensional AFM image of original untreated enamel surface.

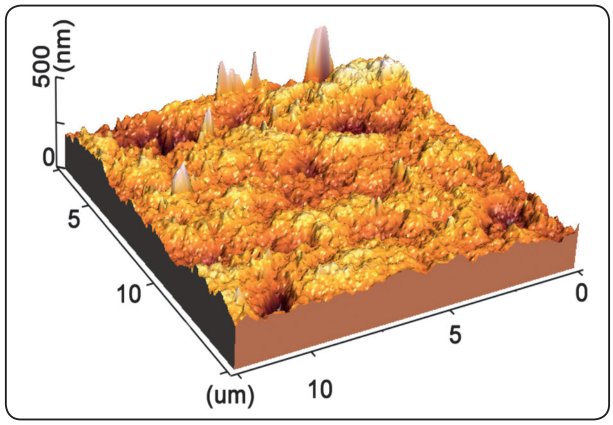

Fig. (2) Three dimensional AFM image of demineralized enamel surface.

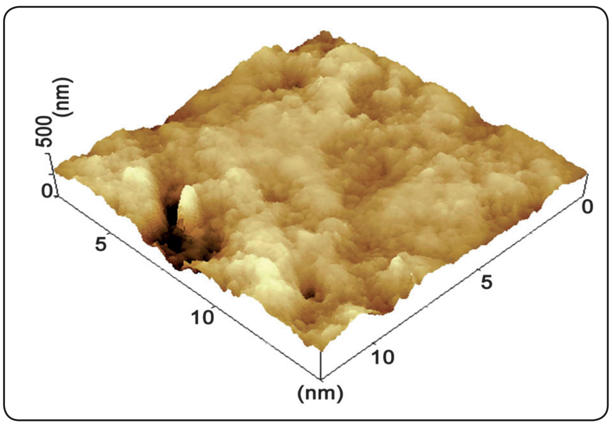

Fig. (3) Three dimensional AFM image of remineralized enamel surface in group I using MI paste plus.

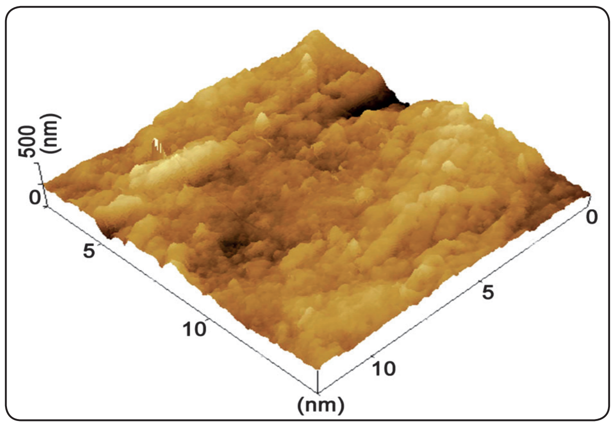

Fig. (4) Three dimensional AFM image of remineralized enamel surface in group II using Aqua fresh extra care mouth wash.

DISCUSSION

Despite a reduction in the prevalence and severity of dental caries in recent decades, a significant increase in other pathological entities such as dental erosion has been observed [26]. Dental Erosion is predominantly characterized by surface demineralization with a very shallow subsurface lesion, while incipient carious lesions have a relatively intact enamel surface and a much deeper subsurface lesion. Given the differences in the depths of subsurface lesions, their nature of remineralization is probably different [27].

Remineralization has been a major area of investigation involving prevention of initiation and interruption in progression of erosive lesions as desirable modes of erosion management Padmini et al [28].

So it was the aim of the present study to evaluate the remineralization potential presented by surface metrology of two remineralizing agents: CPP-ACPF (MI paste plus) and a fluoride containing mouthwash (Aquafresh extra care mouthwash) on induced acid eroded bovine enamel using AFM images.

Freshly extracted permanent bovine teeth were used in the current study as a representative substitute of human teeth, following many authors[13,29-35] who utilized bovine enamel for studies of adhesion, demineralization and remineralization reporting that the chemical structure of bovine enamel as well as its response to erosive challenges is comparable to that of human enamel. To standardize the samples only the incisal portion of the crown was utilized according to Vieira et al [36].

Artificial saliva was selected as a storage medium simulating the oral environment. This selection was to overcome the limitations and difficulties of using natural saliva, avoiding quick decomposition. In addition it will easy to obtain comparable results with other studies [37].

Samples were kept in an incubator to resemble oral cavity temperature. The storage medium was replaced periodically every 24 hours to minimize deterioration and bacterial growth [38,39].

Citric acid is a common ingredient in beverages, and, its potential to erode dental hard tissue is an increasingly growing health concern for dental personnel. It has been used by many researchers with no consensus regarding the ideal concentration and application time [40]. Therefore, a concentration of 0.001 M citric acid, a level commonly found in fruit juice drinks was used to induce erosion in this study [24].

It has been reported that in the initial stage of erosion when a scaffold of mineral crystals is still present, the lesions could be repaired by remineralization, however once the surface was completely lost, erosion process could not be reversed, and that 10 minutes application time to induce acid erosion did not remove this scaffold that's why this application time was selected currently [13].

(AFM) was proved as a wide accepted tool for surface roughness characterization providing high contrast, high resolution images. In addition, samples viewed by AFM do not require any special treatments that would irreversibly change or damage the sample, and does not typically suffer from charging artifacts in the final image [41].

The current increased surface roughness of demineralized samples was similar to those reported by others [20,24,35,41-44] recording a statistically significant increase in the surface roughness of enamel following exposure to citric acid and attributed this to its demineralizing ability recording an increased dimension and number of pores of enamel surface.

An explanation of the current result might be the use of distilled water to rinse the samples after acid application that could remove the residual reagents, free calcium and phosphorus ions, and some part of softened enamel where it was speculated that the free calcium and phosphorus ions that can interact with fluoride ions were removed from the enamel surfaces during the washing process [45].

Several studies [22,42,45-50] explained the current finding where a considerable decrease in Ra value was found after remineralization with CPP-ACPF (MI paste plus) and attributed the protective effect of CPP-ACPF to the fact that it provides a reservoir of bioavailable Ca and P that maintain supersaturated levels, thus inhibits enamel demineralization. Furthermore [51] reported that CPP-ACPF decrease demineralization, reduce the erosion depth, promote enamel remineralization and improve surface roughness probably by deposition of mineral into the surface porous zone of the eroded enamel

In addition, Reynolds et al [46] reported that CPP-ACPF responsible for rehardening the softened enamel where ACP located at enamel surface probably buffered the free calcium and phosphate ion activities, causing the super saturation of ions which depressed demineralization and enhanced remineralization.

Furthermore the addition of 900 ppm F in MI paste plus, showed better remineralization potential [52]. It is likely that a combination of CPP–ACP and fluoride resulted in co-localization of calcium and phosphate ions with fluoride ions at the enamel surface, presumably as CPP–ACPF nano-complexes [53].

While others [54,55] disagreed with the current results reporting no significant reduction in enamel surface roughness after twice a day application of MI Paste Plus during a 6-day denoting that the period and frequency of application of this topical cream has an important role in its efficacy and a factor to be considered comparing results of different studies.

Regarding the effect of the tested Aqua fresh extra care mouthwash, there was a decrease in Ra value compared to that recorded after demineralization and this could be attributed to the formation of CaF2 as a major product formed when enamel is treated with topical fluoride to react with calcium and phosphate ions. The final product of these reactions may be fluoroapatite, which inhibits demineralization and enhances the remineralization of crystals [56].

This current result came in agreement with various studies [20,24,35,43,57-59] using citric acid as an erosive agent, analyze mineral loss and surface roughness by profilometry and found that Aqua fresh extra care mouthwash reduced the enamel erosion.

However others [33,60] used Cola soft drink as an erosive agent on bovine incisors and examined surface roughness and mineral loss by AFM and revealed that fluoride containing mouthwash increased hardness, but did not reduce roughness as the pH of fluoride containing mouthwash is high and can influence the fluoride concentration on the enamel surface and the deposit of CaF2 layer [58].

Comparing group I utilizing MI paste plus versus group II utilizing aqua fresh extra care mouthwash, it was found that the remineralization potential of MI paste plus was better than aqua fresh extra care mouthwash. This might be explained by the nature of CPP-ACP which was determined to be an amorphous electroneutral nanocomplexes with a hydrodynamic radius [53,61]. This nanomeasurment enables calcium and phosphate ions released from the remineralizing agent to enter surface and subsurface enamel porosities. These ions would be thermodynamically driven and they have a high binding affinity for apatite; hence, on entering the lesion, they would bind to the more thermodynamically favored surface of an appetite crystal face [62].

CONCLUSION

Taking into consideration the limitations of this in vitro study, it could be concluded that CPP-ACPF past has a good remineralization potential of enamel erosion.

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