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INFLUENCE OF ABRASIVE AND NON-ABRASIVE TOOTHPASTE ON THE SURFACE PROPERTIES, STAIN REMOVAL AND STAINABILITY OF THE TEETH ENAMEL (AN IN VITRO STUDY)

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ABSTRACT

Objectives: This study was conducted to evaluate the surface roughness, microhardness, stain removal effectiveness, and stainability of abrasive and non-abrasive toothpaste on the tooth enamel. **Subjects and** methods: The sample were categorized into three groups; Group I; Enamel specimens without toothpaste brushing (control group), Group II; Enamel specimens with abrasive toothpaste brushing, Group III: Enamel specimens with non-abrasive toothpaste brushing.. The enamel specimens for the different tested groups were evaluated for morphologic surface changes by scanning electron microscopy (SEM), for surface roughness by atomic force microscopy (AFM), for surface microhardness by using a Vickers microhardness tester, for colour changes by spectrophotometer. **Results** the use of abrasive and non-abrasive toothpastes resulted in significantly increase in surface roughness, and stainability, of enamel when compared to control group, while, the abrasive toothpaste resulted in significant higher surface roughness, stainability and stain removal when compared to the non-abrasive one. the surface microhardness showed that the use of abrasive and non-abrasive toothpastes resulted in significantly decrease in surface microhardness of enamel when compared to control group, while, the abrasive toothpaste resulted in higher significant decrease in surface microhardness when compared to the non-abrasive one. **Conclusion:** The use of abrasive and non-abrasive toothpastes can increase the surface roughness, decrease the surface hardness of enamel, increase the susceptibility of enamel surface to stains, can remove the stains. The use of abrasive toothpaste has higher harmful roughness effect on the enamel, decreases the enamel hardness, increases the susceptibility of enamel surface to stains, has the higher ability to remove the stains from the enamel surface.

KEYWORDS: Abrasive and Non-Abrasive Toothpaste, stains, roughness.

INTRODUCTION

The loss of hard tooth structure due to erosion and/or abrasion has increased interest in preventive public health, as it affects the tooth surfaces leaving a tooth with a demineralized and soft surface layer[(1)]. The erosive softened enamel has been shown to have increase susceptibility to abrasion layer by layer due to the further abrasive forces. Furthermore, the severity and progression of these defects are influenced by modifying host factors such as the composition and pH value of the saliva [(1,2)].

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The cleaning potential of toothpaste dependent mainly on their abrasive potential and its ability to remove the dental stains from brushed surfaces[3,4]. However, the toothpaste with abrasive particles may cause better cleaning efficacy, but their higher abrasive potential may lead to a harmful erosive effect on hard tooth structure and led to tooth sensitivity [5]. Additionally, the toothpaste with abrasive particles that harder than enamel may damage the tooth enamel and lead to enamel erosion [1]. So, the hardness abrasive particle of the toothpaste must be lesser than the hardness of the tooth enamel and higher than the hardness of the tooth stains [5].

Additionally, concerning the esthetic, the roughened tooth surface could be able to gain the stain easier than the smooth surface and serious esthetic problems might occur . So, to avoid or reduce the erosive effect of the toothpaste on the tooth enamel, non-erosive toothpaste has been developed as a preventive measure against wear of the hard tooth structure [6]. However, there are little studies that compare the effect of the different toothpaste with a different erosive ability on the hard tooth structure. Therefore, the aim of this study was to investigate erosive/abrasive enamel wear of the tooth enamel while using an abrasive and non-abrasive toothpaste with standard toothbrushes.

SUBJECTS AND METHODS

Material

No.	Material	Specification	Manufacturer
1	– Abrasive toothpaste	- Colgate Total Whitening. - With hydrated silica as abrasive system. - PVM/MA copolymer.	- Colgate-Palmolive, Colgate, New Jersey, USA
2	– Non-abrasive toothpaste	– Colgate Cavity Protection. – Di-calcium phosphate. – Tetra-sodium pyrophosphate.	- Colgate-Palmolive, Colgate, New Jersey, USA
3	– Soft toothbrush		Colgate-Palmolive, Colgate, New Jersey, USA

Study design:

– An experimental in vitro controlled study.

Study setting:

– The study was carried out in Pediatric Dentistry and Public Health Department, Faculty of Dentistry (Boys, Cairo), Al-Azhar University.

Sample size:

The findings of an earlier investigation were used to compute the sample size using a freeware tool (G*Power 3.1.9.3 for Mac OS X) [7]. This number was 12 each group[3]. a=0.05 and a power of 0.95, assuming a normal distribution, the effect size (dz=0.4229) and necessary sample size were determined.

Sample grouping:

A total of 108 enamel specimens' blocks were used in this study and were divided into three equal main groups (n= 36) according to the type toothpaste used in this study as follow:

- **Group I:** Enamel specimens without toothpaste brushing (control group).
- **Group II:** Enamel specimens with abrasive toothpaste brushing.
- **Group III:** Enamel specimens with non-abrasive toothpaste brushing.

Then, the samples of each main group were subdivided into three equal subgroups (n=12) according to the type of test:

A. Surface morphology and roughness.

B. Surface hardness.

C. Stain removal and stainability.

- **Intervention:**

A. Sample selection:

– A total of 144 specimens were collected from sound permanent mandibular molars and were used in this in vitro study.

– The teeth were examined visually to rule out those that had stains, fractures, or cracks on the labial surfaces of the enamel [6,8].

– Also excluded from the research were teeth with cavities, hypomineralization, or inadequate root development [6].

– Light microscope was used as an adjunct to the visual detection [9].

B. Sample preparation:

The removed teeth were cleaned with pumice and water, then kept in synthetic saliva at 5°C until usage [6,8].

Enamel disks of 4 mm in height, 4 mm in width, and 2 mm in depth were cut from the buccal surfaces of each tooth at the midway using a water-cooled diamond precision saw.

The discs spent 10 minutes being cleaned in an ultrasonic bath of distilled water.

The blocks were separately embedded in clear acrylic resin using a metal mold with dimensions of (6 × 6 × 5 mm), leaving the enamel surface exposed

– The enamel surfaces were ground flat using a model trimmer and were smoothed on wet emery paper numbers 800, 1500, and 2000 grit until all grinding marks were removed [10].

– After that the flattened surface was highly polished with emery paper.

– The finished specimens were examined under a light microscope, and discarded if surface imperfections were observed [9]

- **Brushing Protocol**

Each enamel specimen in each group was brushed with either abrasive or non-abrasive toothpaste along with the allocated group.

The toothpastes were weighed with digital scales that had each been given a base. Initial equalization was done by pressing the "tare" button until it displayed a number in the form of "0, 00 gr. Toothpaste was then applied above each specimen until the scale showed 0.10 gr, then mixed with 0.1 ml of distilled water. After that, the solution of toothpaste was applied to the surface specimen. The brushing of enamel specimens was done for about 2 minutes, twice daily for 15 days with a soft toothbrush, in one direction which was assumed to be the equivalent of brushing for one year. After brushing the specimens rinsed with water.

All brushing procedure was done by the same operator.

Staining protocol:

The staining of the enamel specimens was carried out by immersion of the enamel blocks into specially prepared staining broth, which prepared by adding (17.5g) instant coffee and (30.g) tea to sterilized *trypticase soy* broth (TSB).

Then, the specimens were inoculated with *Micrococcus luteus* bacterial culture and incubated in the incubator at 37°C for a 24-hour to simulate oral cavity medium. The enamel specimens attached and the staining broth in the trough, was incubated at 37°C with the specimens rotating continuously through the staining broth and air. The broth was replaced once daily for 10 consecutive days.With each broth change, the trough and specimens were rinsed and brushed with water to remove any loose deposits.

1. Evaluation of surface profilometry:

a. Evaluation of morphologic surface changes:

- The enamel specimens for the different tested groups were evaluated for morphologic surface changes by scanning electron microscopy (SEM) before and after the use of different kinds of toothpaste [(4)].
- The specimens were suitably gold-sputter-coated and chosen for SEM inspection at magnifications of 1000, 2000, and 4000 [(4)].

b. Evaluation of morphologic surface roughness:

- The enamel specimens for the different tested groups were evaluated for surface roughness by atomic force microscopy (AFM) before and after the use of different kinds of toothpaste**.**
- The scanned area was 5μm X 5 μm for each enamel specimen.

Average roughness value (Ra), measured in nanometers, is the arithmetic mean of the distance between peaks and valleys from a mean line.

- Measurements were made three times on each specimen surface in one specimen[(123)].
- Then, the total measurement results were then averaged to define the surface roughness for each test specimen.

2. Evaluation of surface micro-hardness:

- The surface microhardness of the enamel specimens for the different groups were evaluated by using a Vickers microhardness tester (Digital microvickers hardness test Vexus MHV-1000Z) before and after the use of different kinds of toothpaste.
- 15 seconds were spent with the 0.25 N load applied
- At a distance of 100 m from one another, three indentations were produced on each surface.[(6)].
- Then, the total measurement was averaged to define the surface Vickers microhardness number (VHN) value for each test specimen [(6)].

3. Evaluation of stainability (color change):

- After ending the tooth brushing protocol, the color change before and after the staining process was evaluated by spectrophotometer (U.V-Vis. UV 3101 PC, Shimadzu scanning spectrophotometer.
- The enamel samples were evaluated using a spectrophotometer under controlled environmental circumstances in accordance with the Commission International de l'Eclariage (CIE) L*a*b* system.
- The observer angle of the device was set at 2°, and it was configured for a limited area view.
- The D65 standard light source was used, along with the reflectance mode and 100% ultraviolet. The stainability of the enamel blocks was estimated as follow;
- After the preparation of the enamel blocks and after using the different toothpastes for brushing

following the previously mentioned protocol, the specimen's surface in each group were evaluated by spectrophotometer.

- Then, the enamel specimens were stained by the specially prepared broth and the color change was evaluated again by spectrophotometer.
- The CIE system was used to measure the results of the colour change, which were expressed in terms of three coordinate values (L*a*b*), where the L* axis represents the degree of lightness and ranges from 0 (black) to 100 (white), the a* plane represents the degree of green/red colour, and the b* plane represents the degree of blue/yellow colour, both of which are present in the sample.
- The total colour change (E) is calculated using the following formula: E*ab equals [(L*)2 + (a*)2 + (b*)2] 0,5.

4. Evaluation of staining removal (color change):

- The color change after staining and stain removal processes of the enamel blocks were evaluated by spectrophotometer.
- The stain removal ability of the abrasive and non-abrasive toothpastes was estimated as follow;

- The color of the stained enamel specimens was evaluated by spectrophotometer.
- Then, the stained enamel specimens in each group was brushed using the tested toothpaste in the same protocol as mentioned before.
- After that, the color change was evaluated by spectrophotometer and estimated as the ability of the two tested toothpaste on stain removal.

The total colour change (E) was determined using the formula: E*ab = [(L*) 2+ (a*) 2+ (b*) 2]. 0,5.

Data management and analysis: Using SPSS version 22, the data that were gathered throughout the study were tabulated and statistically evaluated using the ANOVA test. The same group's findings before and after brushing were compared using a student t-test. To compare the outcomes of the tested groups, use the one-way analysis of variance (ANOVA) test. P-value 0.05 was the threshold for significance.

RESULTS

I. Surface profilometry:

Surface Roughness:

One-way ANOVA test (f=928.06, p0.00001) results for all tested groups showed that there was a statistically significant difference in the average surface roughness between all tested groups. Where the lower (mean ± SD) values of the average surface roughness were recorded with the enamel without any brushing procedure "control group" (group I) (65.02±1.36 **μm**), followed by enamel brushed with non-abrasive toothpaste (group III) (97.99±3.82 **μm**). While the highest (mean ± SD) value of average surface roughness was recorded with enamel brushed with abrasive toothpaste (group II) (144.54±5.92 **μm**).

II. Hardness:

There was a statistically significant difference in the surface hardness between all tested groups, as shown by the One-way ANOVA test (f=48.199, p0.00001), which was used to analyse the statistical data on the surface hardness of all tested groups. Where the higher (mean ± SD) values of the surface hardness were recorded with the enamel without any brushing procedure "control group" (group I) (63.63±1.32 N/mm^2), followed by enamel brushed with non-abrasive toothpaste (group III) (59.68±1.08 N/mm^2). While the lowest (mean ± SD) value of surface hardness was recorded with enamel brushed with abrasive toothpaste (group II) (57.08±1.95 N/mm^2).

III. Color change:

A. Stainability (color change):

The statistical analysis of color change of all tested groups revealed that; there was ***statistically significant*** difference in the color change between all tested groups as indicated by **One-way ANOVA** test (*f*=399.74, p<0.00001). Where the lower (mean ± SD) values of the color change was recorded with the enamel without any brushing procedure "control group" (group I) (5.99±0.32), followed by enamel brushed with non-abrasive toothpaste (group III) (8.30±0.80). While the highest (mean ± SD) value of color change was recorded with enamel brushed with abrasive toothpaste (group II) (13.86±0.68).

B. Stain-removal (color change):

1. Comparison of color change decrease percentage (%):

The statistical analysis of color change decrease percentage (%) between abrasive and non-abrasive toothpastes groups revealed that; there was ***statistically significant*** difference in the color change decrease percentage (%) between the two tested groups as indicated by unpaired **t-** test (t=3.839, p=0.0012). Where the lower (mean ± SD) values of the color change decrease percentage (%) was recorded with the enamel brushed with non-abrasive toothpaste (group III) (41.13±8.97). While the highest (mean ± SD) value of color change decrease percentage (%) was recorded with enamel brushed with abrasive toothpaste (group II) (54.33±6.13).

2. Comparison of color change in the abrasive toothpaste group before and after brushing:

The statistical analysis of color change decrease in the abrasive toothpaste group before and after brushing revealed that; there was ***statistically significant*** difference in the color change between the two tested groups as indicated by paired **t-** test (t=22.758, p=0.0001). Where the lower (mean ± SD) values of the color change was recorded with the enamel brushed with abrasive toothpaste after brushing (Δ E2) (6.31±0.72). While the highest (mean±SD) value of color change was recorded with enamel brushed with abrasive toothpaste before brushing (ΔE1) (13.86±0.68).

TABLE (1) Comparison among all tested groups.

Variable	Mean ±SD	*f*-ratio	p-value
Roughness			
Control	65.02 ± 1.36^{A}		
Abrasive	144.54 ± 5.92^{C}	928.06	<0.00001*
Non-abrasive	97.99 ± 3.82^{B}		
Vickers microhardness			
Control	63.63 ± 1.32^{A}		
Abrasive	57.08 ± 1.95^{C}	48.199	<0.00001*
Non-abrasive	59.68 ± 1.08^{B}		
Stainability (color change)			
Control	5.99 ± 0.32^{A}		
Abrasive	13.86 ± 0.68^{C}	399.74	<0.00001*
Non-abrasive	8.30 ± 0.80^{B}		
Color change decrease %			
Abrasive	54.33±6.13	3.839	0.0012*
Non-abrasive	41.13±8.97		
Stain removal ability (color change)			
ΔE1	13.86±0.68	22.758	0.0001*
ΔE2	6.31±0.72		
Stain removal ability (color change)			
ΔE1	8.30±0.80	15.886	0.0001*
ΔE2	5.48±0.53		

**; The results statistically at p<0.05.*

; ns= non-significant.

3. Comparison of color change in the Non-abrasive toothpaste group before and after brushing:

The statistical analysis of color change decrease in the non-abrasive toothpaste group before and after brushing revealed that; there was ***statistically significant*** difference in the color change between the two tested groups as indicated by paired **t-** test (t=15.886, p=0.0001). Where the lower (mean ± SD) values of the color change was recorded with the enamel brushed with non-abrasive toothpaste after brushing (ΔE2) (5.48±0.53). While the highest (mean ± SD) value of color change was recorded with enamel brushed with non-abrasive toothpaste before brushing (ΔE1) (8.30±0.80).

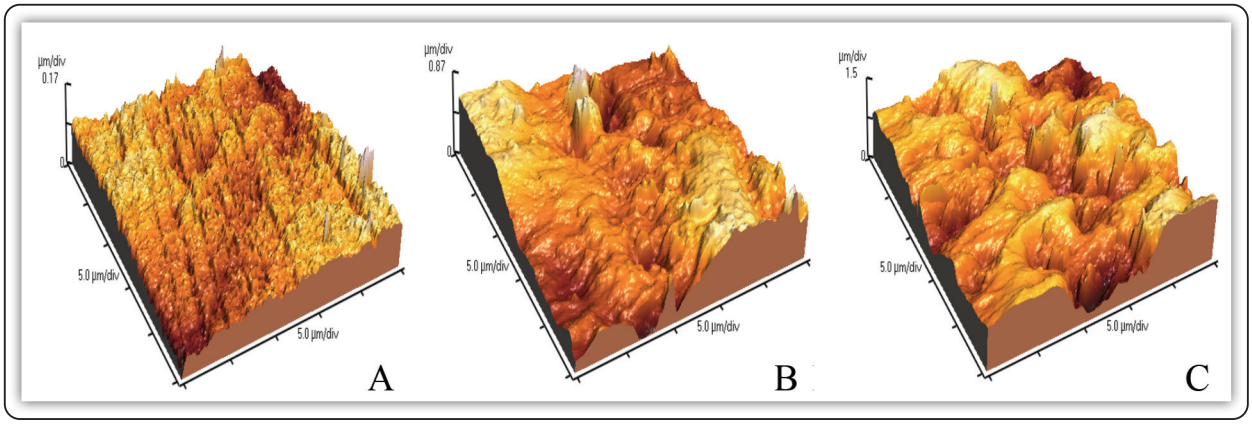

FIG (1) Atomic force microscope scan (A: Control group. B: Non-abrasive group. C: Abrasive group)

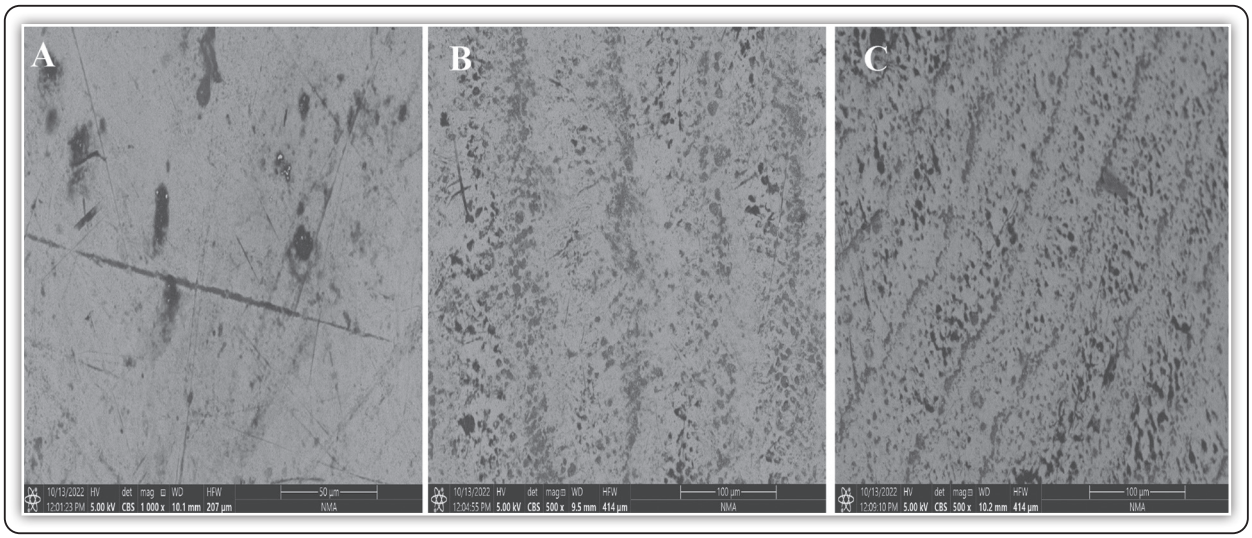

FIG (2) Electron microscope scan (A:Control group. B: Abrasive group. C: Non-abrasive group)

DISCUSSION

Surface roughness:

The current study's findings demonstrated that when toothpaste either "abrasive or non-abrasive" was applied to the enamel surface, the average surface roughness (Ra) parameter of the enamel considerably increased as compared to the control group. These findings might be explained by the inclusion of abrasive particles in both toothpastes examined in the current investigation[11]. These findings support Bolay et al. [12], who discovered that brushing techniques enhanced the enamel's surface roughness. Hilgenberg et al.[13] in addition, claimed that all toothpastes promoted changes to the enamel surface, most likely through the use of a bleaching agent. Increased enamel surface roughness is another side effect of using toothpastes with herbal components, albeit the results of Korsuwant et al indicate that this rise is not enough to induce bacterial buildup in a medical setting (enamel surface roughness should not exceed 0.2m). However, Abdel-Hamid et al. [14] observed that after a year of simulating dental brushing with toothpaste modified with 10% nano-hydroxyapatite, the modified toothpaste had no effect on the roughness of the enamel.

The study's findings, however, indicated that when compared to non-abrasive toothpaste, the usage of abrasive toothpaste had a more meaningful influence on the rise of enamel's average surface roughness. Since greater values were a symptom of changes in roughness, it may be because toothpaste's ash content and solid residues have the capacity to change the surface enamel [13]. Also, these results could be attributed to the type of abrasive material which present in each toothpaste tested in the present study. This because the abrasive toothpaste which used in the present study "Colgate Total Whitening" (represent abrasive tooth paste) has a hydrated silica in its composition as the main abrasive component, while the "Colgate cavity protection" (represent non-abrasive tooth paste) contain tetra-sodium pyrophosphate as usually incorporated abrasive agent.

According to earlier research, silica has powerful abrasive characteristics [13]. Also, it is known that silica has higher abrasion ability when compared to the other abrasive content and when compared to tetra-sodium pyrophosphate. When using dentifrice that contains abrasives, there is a risk of enamel microwear and a larger porosity on the enamel surface[15]. In order to promote a "new" surface with high roughness values, the employment of abrasives with enamel may remove the superficially damaged layer[16].

The hardness of the abrasive material is one of the elements affecting the improvement of the material's abrasion. Compared to tooth enamel, which has a score of 5, silica and hydrated silica have a Mohs hardness rating of 5-7[17]. The findings of the present study thus provide an explanation for how dental enamel may get scratched when it comes into contact with silica materials with higher Mohs hardness values, which would raise the Ra value [17]. Also, these results could be attributed to the irregularity of particle shape of the abrasive toothpaste which could affect the value of surface roughness on a tooth when compared to the round one in the non-abrasive toothpaste [4,86,118]. These outcomes support the findings of Worschech et al.[15], who discovered that the studied groups' enamel surface roughness increased following the use of abrasive dentifrices for surface cleaning. Also, the results of Hilgenberg et al. [13], showed that the whitening toothpastes promoted changes to the enamel surface.

Hardness:

Regarding the hardness loss of enamel following the toothbrushing, it was highly variable in the literature; Neves et al. [16] reported that the slight abrasion in this enamel may remove the superficial degraded layer and promote a "new" surface. This could explain the results of surface hardness in the present study. Where, the results of this study revealed that the toothpaste either "abrasive or non-

abrasive", which applied on the enamel surface significantly decreased the surface microhardness parameter of the enamel when compared to the control group. This could be attributed to the loss of enamel due to wear following the toothbrushing[15,16].

Also, it was reported that the abrasive content of toothpaste increasingly wears the enamel in proportion to the number of brush strokes [18]. This could also explain the results of the present study where the use of abrasive toothpaste has the significant higher reduction in the surface hardness of enamel when compared to the non-abrasive one. Also, according to research by Hara et al.[19] fluoride in dentifrice enhances the surface hardness of dentin, making it more resistant to brushing abrasion. This could explain the results of higher hardness of the non-abrasive toothpaste in the current study where the Colgate cavity which used in the present study had higher fluoride when compared to the whitening toothpaste.

Stainability and Staining removal:

Regarding the stain ability, the results of this study revealed that the toothpaste either "abrasive or non-abrasive", which applied on the enamel surface significantly increase the stainability of the enamel when compared to their control. This could be attributed to the increased roughness in tooth surfaces after tooth brushing with the two different tested toothpastes which may contribute to the increased staining caused by specially prepared stained solution [20,21]. However, the intrinsic roughness and porosities in tooth surfaces, which may contribute to the staining generated by the solution, may be to blame for the colour change of the control group in the current investigation after being exposed to the staining solution [21]. Moreover, the results of the current study revealed that the use of abrasive toothpastes resulted in significant higher color change when compared to the non-abrasive one. This may be connected to the relatively significant changes in the surface of the enamel caused by brushing with abrasive toothpastes, such as erosion and porosities, which may be the result of the prolonged contact time between the abrasive agent and the tooth structure [20].

However, regarding the stain removal, the results of this study revealed that the toothpaste either "abrasive or non-abrasive", which applied on the enamel surface significantly remove the stain of the enamel when compared to their base-line before brushing. This could be because of the presence of slight abrasion in the tested non-abrasive toothpaste as well as presence of higher amount of abrasive content in the abrasive type of toothpaste which able to remove the superficial pellicle layer and degraded superficial enamel layer and promote a "new" surface [15]. However, according to the results of the present study the abrasive toothpastes has the higher color change percentage i.e. the higher stain removal ability when compared to the non-abrasive one. This could be attributed to the abrasiveness of the tested toothpaste in each tested group, and hence its ability to remove staining, as Lima et al. [22], was reported that the stain removal ability is related directly to both the type and amount of abrasive which included in the toothpaste.

Also, after the use of brushing procedures in this study in both tested groups, the enamel surfaces may have had some irregularities and porosities. This might be a reason for the failure of both tested toothpastes to remove the all stains that caused by the prepared staining solution; because this staining agent probably diffuses through these structural defects more deeply because it is in liquid form [119]. This could also explain why the color of the tested enamel in both tested groups dose not return to their initial value before the staining. Additionally, the resistant to stain removal by tooth brushing in both tested groups in the present study could be also attributed to the cumulative staining ability of the rough surface. As it concluded that, the effect of tooth staining was cumulative [20].

CONCLUSION

The usage of both abrasive and non-abrasive toothpastes can change the surface roughness, surface hardness, and stain sensitivity of enamel. It can also be used to remove stains from the enamel. The use of abrasive toothpaste has higher harmful roughness effect on the enamel, decreases the enamel hardness, increases the susceptibility of enamel surface to stains, has the higher ability to remove the stains from the enamel surface.

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