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DENTINAL OCCLUSION OF TWO DESENSITIZING TOOTHPASTES USING MANUAL VERSUS ELECTRIC TOOTHBRUSHES

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ABSTRACT

Objective: To investigate dentinal occlusion of two desensitizing toothpastes using manual versus electric toothbrushes.

Methods: A total of 40 sound human molars were used in this study. From each tooth, one dentin disc specimen was prepared 2 mm in thickness. Specimens were divided randomly into 2 main groups (n=20), according to the type of toothpaste used; Arginine-based toothpaste and Nanohydroxyapatite-based toothpaste. Each main group was subdivided into 2 subgroups (n=10) according to the type of toothbrush; manual toothbrush and electric toothbrush. Each specimen was brushed twice daily, 1 min each, for 4 days and subjected to acidic challenge using grapefruit juice. Specimens were examined under scanning electron microscope to determine degree of dentinal occlusion before and after acidic challenge. Degree of dentinal occlusion were determined using occlusion scoring system on a scale of 1-5. Data were tabulated and statistically analysed.

Results: Hydroxyapatite toothpaste revealed significantly higher dentinal occlusion score than arginine toothpaste in all experimental groups. Electric toothbrush recorded higher dentinal occlusion score than manual toothbrush with arginine toothpaste before and after acidic challenge. However, no significant difference was recorded between both toothbrushes with hydroxyapatite toothpaste. Hydroxyapatite toothpaste was not affected by the acidic challenge in contrast to arginine toothpaste which was negatively affected.

Conclusion: Hydroxyapatite toothpaste was effective in dentinal tubules occlusion and able to resist acidic challenge whether brushed using manual or electric toothbrush. Use of electric toothbrush with arginine toothpaste was beneficial in improving dentinal tubules occlusion before and after acidic challenge.

KEY WORDS: Dentinal occlusion, Manual toothbrush, Electric toothbrush, Arginine, Hydroxyapatite.

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INTRODUCTION

Dentin hypersensitivity (DH) is one of the most prevalent dental problems arising from opened dentinal tubules and manifested as short sharp pain upon exposure to thermal, evaporative, tactile, osmotic and/or chemical stimuli [(1)]. Hydrodynamic theory has been hypothesized as an explanation for dentin hypersensitivity. The theory stated that provoking stimuli resulted in increasing dentin permeability and movement of the dentinal fluid inside the dentinal tubules, leading to deformation of odontoblastic processes along with adjacent nerve fibers [(2)]. Hypersensitivity has several aetiologic factors such as gingival recession, dental erosion, abrasion, attrition and abfraction [(3)]. In addition, dietary acids could result in exposure of dentinal tubules as it could easily remove the smear layer protecting the dentin surface [(4)].

Management of dentin hypersensitivity starts with patient education in order to eliminate risk factors as decrease uptake of dietary acids and avoidance of aggressive toothbrushing [(3)] and ends with different treatment modalities. Dentin hypersensitivity could be treated either by desensitizing the nerve or occluding the exposed dentinal tubules. Occlusion of dentinal tubules is achieved by deposition of the desensitizing material on the dentin surface and/or precipitation inside the opened dentinal tubules [(5)]. Deposition of the desensitizing material on the exposed dentin surface could provide a short-term relief of pain as it can be easily removed by routine toothbrushing or dissolved by dietary acidic beverages [(5)]. However, intratubular deposition of desensitizing material inside the exposed dentinal tubules is considered more efficient as the deeper the penetration, the longer the durability of the treatment [(6)]. Use of desensitizing agents is a common practice for treatment of dentin hypersensitivity using stannous fluoride [(7,8)], potassium oxalates, sox dium fluoride [(6)], using adhesives and resins [(9)], iontophoresis [(10)] and laser application [(11)].

Presence of desensitizing materials with bioactive properties is a highly efficient and durable treatment for occlusion of exposed dentinal tubules and reduction of dentinal fluid flow [(12)]. Arginine is a common amino acid that presents naturally in saliva. Mode of action of arginine proposed that soluble arginine adsorbed on the surface of insoluble calcium carbonate particles, forming positively charged agglomerates that bind with negatively charged dentin occluding exposed dentinal tubules. Association of arginine and calcium carbonate creates an alkaline environment which helps in deposition of calcium and phosphate ions present in saliva on dentin surface [(13,14)].

Nanohydroxyapatite (n-HAp) is considered a promising active ingredient used for treatment of dentin hypersensitivity due to its high biocompatibility and bioactivity [(15,16)]. Hydroxyapatite is the major inorganic constituent of natural teeth. It has been shown that nanohydroxyapatite particles are similar in morphology and structure to natural tooth enamel apatite crystals [(16)]. It enhances surface remineralization by forming a biomimetic apatite coating which closely resemble enamel and dentin composition [(17)]. Resistance of desensitizing toothpastes to acidic challenge during clinical service is of great importance as consumption of acidic beverages has been markedly increased specially, highly acidic soft drinks and fruit juices [(4)].

Most of the patients suffering from dentin hypersensitivity prefer to use desensitizing toothpastes because toothbrushing is an easy procedure and a routine daily practice to perform oral hygiene measures. Manual toothbrush was the only available type of brushes for decades until powered toothbrushes have been introduced to the market in 1960s. Different types of electric toothbrushes from different brands are currently available as oscillating–rotating, sonic and ultrasonic ones [(18)]. Previous studies compared manual and electric toothbrushes regarding their efficiency in plaque removal and improvement of gingival health [19], effect of their brushing forces on abrasion of enamel and dentin tissues [20] and their effect on plaque composition [21]. However, no studies have investigated their effect on dentinal occlusion of desensitizing toothpastes in treatment of dentin hypersensitivity.

Hence, this study was conducted to investigate dentinal occlusion of two desensitizing toothpastes using manual and electric toothbrushes before and after acidic challenge.

MATERIALS AND METHODS

Specimens' Preparation and Grouping

A total of 40 sound human molars were used in the current study. The study was approved by Fayoum University Ethical Committee for Scientific Studies and Research (FU-ECSSR) with a research code EC 2115. The teeth were washed under running water, scaled from adhering soft tissue, plaque and calculus. They were stored in saline at 4°C for not more than one month. From each tooth, a dentin disc specimen, 2mm in thickness, was prepared. A total of 40 specimens were prepared and divided randomly into 2 main groups (n=20), according to the type of toothpaste used; Arginine-based toothpaste (Sensitive Pro-Relief ™, Colgate-Palmolive Manufacturing, Poland) and Nanohydroxyapatite-based toothpaste (BioRepair plus, Coswell Farma, Itlay). Each main group was subdivided into 2 subgroups (n=10) according to the type of toothbrush used; manual toothbrush (Oral-B complete deep clean manual toothbrush, 40/medium- Procter & Gamble, Ireland) and electric toothbrush (Oral-B Vitality, cross action, Braun GmbH, Germany).

Preparation of Dentin Disc Specimens

Teeth roots were mounted in self-cured acrylic resin till the level of the cemento-enamel junction using cylindrical molds (1.5 cm internal diameter and 2 cm length). Occlusal enamel was wet ground using 80 grit sandpaper discs till reach flat dentin surface. Dentin discs, 2 mm thickness, were cut using low speed diamond disc (BesQual, NY 11373, USA) under sufficient water coolant. Thickness of each dentin disc was checked using a digital micrometer (Tri circle, Shanghai, China). Dentin disc specimens were etched using 6% citric acid for 2 min and then rinsed thoroughly under running water for at least 30 sec to achieve patent dentinal tubules. Representative specimens were examined under scanning electron microscope (FEI Company, Netherland, Model Quanta 250, Field Emission Gun (FEG) at 4000X magnification to ensure complete removal of smear layer and opening of dentinal tubules before application of the desensitizing toothpastes.

Brushing Procedure

Each specimen was brushed for 1 min, twice a day (morning and evening) with total of 2 min per day. This procedure was repeated for 4 days by the same operator to ensure standardization. In between brushing cycles, samples were stored in artificial saliva. Artificial saliva was prepared according to guidelines assigned by *Rodrigues et al, 2007* [22]. The prepared artificial saliva is formed of: 50 mmol/L potassium chloride, 1.5 mmol/L calcium, 0.9 mmol/L phosphate, 20 mmol/L Trihydroxyl methyl-amino methane (Tris) diluted in one litre of distilled water.

Acidic Challenge

Specimens of each group were subjected to acidic challenge on days 3 and 4 of treatment with desensitizing toothpastes. They were immersed in 20 ml grapefruit juice twice a day (2 min for each cycle) after 60 min of toothbrushing with the tested desensitizing toothpastes [4,23].

Environmental Scanning Electron Microscopic Assessment

Specimens were examined using scanning electron microscope (FEI Company, Netherland, Model Quanta 250, Field Emission Gun (FEG) to assess degree of dentinal tubules occlusion before and after acidic challenge. Environmental scanning electron microscopic model was used as it is characterized by its low vacuum mode which allows investigation of samples without the need for coating. Tested specimens were mounted on metal stubs and images were obtained at 4000X magnification. The SEM images were assessed for level of dentinal tubule occlusion, using occlusion scoring system, (on a scale of 1–5) by two reviewers in accordance with the following ranking system: 1 = Occluded (100% of tubules occluded), 2 = Mostly occluded (75% of tubules occluded), 3 = Equally occluded/unoccluded (50% of tubules occluded), 4 = Mostly unoccluded (25% of tubules occluded) and 5 = Unoccluded (0%: no tubule occlusion) and the mean score was calculated for each sample.

Statistical Analysis

Numerical data was represented as mean and standard deviation (SD) values. They were explored for normality by checking the data distribution using Shapiro-Wilk test. Data showed non parametric distribution, so they were analyzed using Kruskal–Wallis test followed by pairwise comparisons utilizing Dunn's post hoc test with Bonferroni correction for intergroup comparisons and Wilcoxon signed-rank test for intragroup comparisons. The significance level was set at $p<0.05$ within all tests. Statistical analysis was performed with R statistical analysis software version 4.1.1 for Windows*.

RESULTS

Descriptive statistics for dentinal tubules occlusion score values for different experimental groups were presented in table (1).

Results of dentinal occlusion of different toothpastes are presented in table (2). Nanohydroxyapatite toothpaste had a significantly higher dentinal tubules occlusion score than arginine toothpaste with a p value <0.001 with both types of toothbrushes and before and after acidic challenge.

TABLE (1): Descriptive statistics for dentinal tubules occlusion scores of different experimental groups

Acidic challenge	*Toothpaste*	*Tooth brush*	*Mean*	*95% CI*		*SD*	*Median*	*IQR*
				Lower	*Upper*			
Before acidic challenge	***Arginine***	***Manual***	2.90	2.55	3.25	0.57	3.00	0.00
		Electric	2.30	2.00	2.60	0.48	2.00	0.75
	Nanohydroxyapatite	***Manual***	1.20	0.94	1.46	0.42	1.00	0.00
		Electric	1.10	0.90	1.30	0.32	1.00	0.00
After acidic challenge	***Arginine***	***Manual***	3.70	3.40	4.00	0.48	4.00	0.75
		Electric	2.80	2.41	3.19	0.63	3.00	0.75
	Nanohydroxyapatite	***Manual***	1.40	1.08	1.72	0.52	1.00	1.00
		Electric	1.20	0.94	1.46	0.42	1.00	0.00

95%CI= 95% confidence interval for the mean; SD=standard deviation; IQR=interquartile range

* R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Effect of toothbrushes revealed that electric toothbrush recorded higher dentinal tubules occlusion score in comparison to manual toothbrush with arginine toothpaste before and after acidic challenge. However, no significant difference was found between electric and manual toothbrushes with hydroxyapatite toothpaste in any of the tested groups as presented in table (3).

Regarding the effect of acidic challenge, results showed that arginine toothpaste with both toothbrushes had a significantly lower dentinal tubules occlusion score after acidic challenge. Nanohydroxyapatite toothpaste showed no significant difference between dentinal tubules occlusion scores before and after acidic challenge using either manual or electric toothbrushes (table 4).

TABLE (2): Intergroup comparison of dentinal tubules occlusion scores for different toothpastes

Toothbrush	*Acidic challenge*	*Dentinal tubules occlusion score (Mean±SD)*		*p-value*
		Arginine toothpaste	*Nanohydroxyapatite toothpaste*	
Manual	**Before acidic challenge**	2.90±0.57	1.20±0.42	**<0.001***
Electric		2.30±0.48	1.10±0.32	**<0.001***
Manual	**After acidic challenge**	3.70±0.48	1.40±0.52	**<0.001***
Electric		2.80±0.63	1.20±0.42	**<0.001***

*; *significant (p<0.05)*

TABLE (3): Intergroup comparison of dentinal tubules occlusion scores for manual and electric toothbrushes

Toothpaste	*Acidic challenge*	*Dentinal tubules occlusion score (Mean±SD)*		*p-value*
		Manual toothbrush	*Electric toothbrush*	
Arginine	**Before acidic challenge**	2.90±0.57	2.30±0.48	**0.027***
Nanohydroxyapatite		1.20±0.42	1.10±0.32	**0.583**
Arginine	**After acidic challenge**	3.70±0.48	2.80±0.63	**0.005***
Nanohydroxyapatite		1.40±0.52	1.20±0.42	**0.366**

*; *significant (p<0.05)*

TABLE (4): Intragroup comparison of dentinal tubules occlusion scores before and after acidic challenge

Toothpaste	*Toothbrush*	*Dentinal tubules occlusion score (Mean±SD)*		*p-value*
		Before acidic challenge	*After acidic challenge*	
Arginine	*Manual*	2.90±0.57	3.70±0.48	**0.006***
	Electric	2.30±0.48	2.80±0.63	**0.037***
Nanohydroxyapatite	*Manual*	1.20±0.42	1.40±0.52	**0.346**
	Electric	1.10±0.32	1.20±0.42	**1**

*; *significant (p<0.05)*

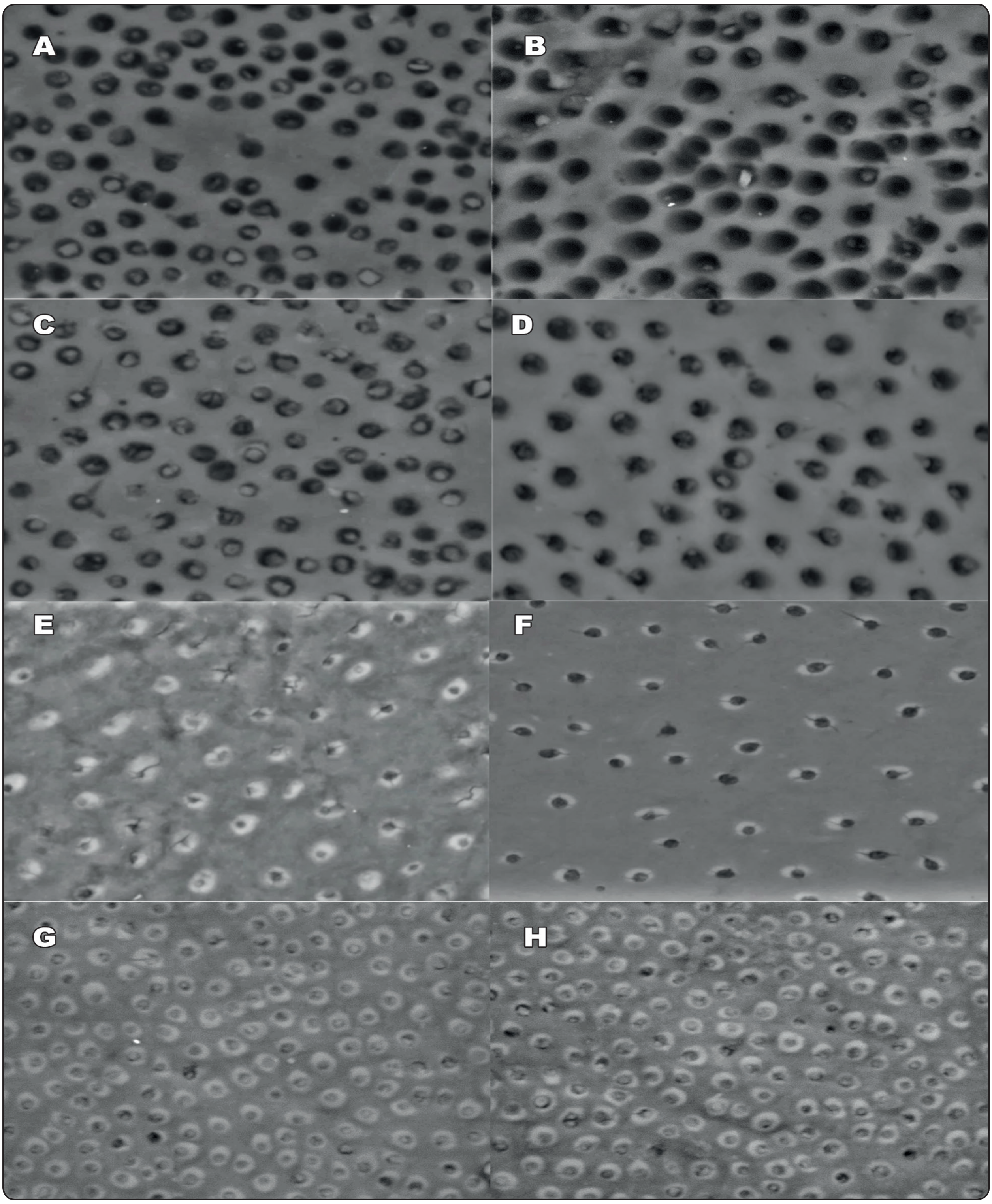

Fig. (1): SEM images of dentin specimens of different experimental groups at 4000X magnification. (A,B) Arginine-based toothpaste using manual toothbrush before and after acidic challenge, (C,D) Arginine-based toothpaste using electric toothbrush before and after acid challenge, (E,F) Nanohydroxyapatite-based toothpaste using manual toothbrush before and after acidic challenge, (G,H) Nanohydroxyapatite-based toothpaste using electric toothbrush before and after acidic challenge.

DISCUSSION

Nowadays, dentifrices are routinely used in treatment of dentin hypersensitivity by occluding the opened dentinal tubules using different desensitizing agents aiming to relief pain and resist future acidic challenge [4]. The current study has demonstrated an acidic challenge using grapefruit juice to mimic the situation that occurs when a soft acidic drink is consumed. The brushing time of two minutes was chosen based upon Public Health England recommendations for the whole mouth [24].

From the results of the current study, the use of arginine-based toothpaste led to immediate occlusion of opened dentinal tubules however upon acidic challenge, reduction in dentinal tubules occlusion was recorded.

It has been suggested that the mechanism of arginine/calcium carbonate toothpaste in dentinal occlusion is based on naturally occurring biological process by salivary glycoproteins. Saliva transports calcium and phosphate in close proximity to dentinal tubules to induce occlusion and formation of a protective coat of salivary glycoprotein with calcium and phosphate which occurs under alkaline pH conditions [25]. In the current study, dentin specimens were stored in artificial saliva in between brushing cycles as *Hiller et al, 2018* [26], demonstrated that arginine results in better dentinal occlusion in the presence of saliva.

This result was supported by *Lavender et al, 2010* [13], who demonstrated an increase in calcium and phosphorus levels after treatment with arginine-based toothpaste in comparison to previously etched dentin specimens using electron spectroscopy for chemical analysis.

It has been reported that arginine and calcium carbonate form positively charged agglomerations which bind to negatively charged dentin and plug within patent dentinal tubules about 2 μm depth [27]. This charge could be lost upon exposure to acidic conditions and this may lead to dissociation of the formed agglomerations uncovering dentin surface [28]. This might explain the low acid tolerance of arginine. This was in accordance with *Davies et al, 2011* [14], who recorded high concentrations of calcium and phosphate over the occluded dentinal tubules using EDX analysis upon use of arginine-based toothpaste, which dramatically decreased after acidic challenge.

This finding was in agreement with other studies [4,28] which reported that arginine-based dentifrices have a great ability for immediate sealing of opened dentinal tubules with subsequent reduction of dentinal fluid flow. However, they showed low tolerance upon exposure to acidic challenge. In contrary, previous researches [13,29] reported resistance of arginine-based toothpastes to acidic challenge for 2 min in cola drink. This demonstrates the influence of the used protocol of acidic challenge regarding type of acidic beverage on the revealed findings.

In the current study, hydroxyapatite-based toothpaste recorded higher dentinal occlusion in comparison to arginine-based toothpaste with high resistance to acidic challenge in all experimental groups. This might be attributed to the use of nanoscaled hydroxyapatite-based toothpaste in this study. The commercial product "Biorepair" toothpaste used in this study was analyzed in a previous study using high resolution scanning electron microscopy and reported the presence of nanoscale sized hydroxyapatite particles in the range of 50-100 nm [30].

The use of nanosized materials is a promising and efficient method of delivering active therapeutic constituents into dentinal tubules [31] which enables them to act as fillers that can easily occlude and penetrate into exposed dentinal tubules [16]. Also, the fact that the diameter of the dentinal tubules is about 2.4 -3 μm [32] supports the idea of ease and efficient occlusion of patent dentinal tubules by nano-scaled therapeutic particles.

In addition, nanohydroxyapatite particles might act as reservoir for calcium and phosphate ions creating an environment which is supersaturated with these ions in respect to tooth minerals [33].

During remineralization process, nanohydroxyapatite attracts large amounts of calcium and phosphate ions from the remineralization solution to tooth tissue, thus promoting crystal integrity and growth [34]. Precipitation of hydroxyapatite onto the exposed dentinal tubules does not inhibit spontaneous remineralization potential via further precipitation of calcium phosphate ions [35]. This was in accordance to *Amaechi et al, 2015* [1], who found that the use of nanohydroxyapatite-based toothpaste revealed a larger number of totally obliterated dentinal tubules and higher surface deposits. Also, *Kulal et al, 2016* [36], revealed a significantly higher percentage of dentinal tubule occlusion for nanohydroxayapatite crystals when compared to arginine. From the SEM images of the current study, it appears that nanohydroxyapatite-based toothpaste results in substantial and consistent level of dentinal occlusion (fig 1 E,F,G,H) which might be due to its remineralization potential while for arginine-based toothpaste, particles are rather deposited on dentin surface or inside dentinal tubules (fig 1 A,B,C,D) without being attached to dentin.

Regarding results of acidic challenge, hydroxyapatite-based toothpaste showed satisfactory acid resistance which might be attributed to their similar inorganic composition to tooth tissues that allowed their blending to dentinal tubules after obliterating them [37]. In addition, its ability to penetrate the opened dentinal tubules not just cover the dentin surface might be another explanation for this result as *Ishikawal et al, 1994* [38], reported deep penetration of apatite mineral inside the dentinal tubules up to 15 µm. Further explanation might be due to the buffering effect of the phosphate ions released during acidic challenge which results in neuetralization of such acids [39]. On the other hand, the released calcium and phosphate ions could promote remineralization which consequently balance hydroxyapatite solubility [39]. *Colombo et al, 2017* [40], found that BioRepair toothpaste was able to protect enamel against erosive acidic challenges.

Regarding the effect of toothbrush type on dentinal occlusion scores, it was found that electric toothbrush resulted in higher dentinal occlusion than manual toothbrush with arginine-based toothpaste before and after acidic challenge. This might be attributed to the brushing force applied using manual versus electric toothbrushes. Forces applied during tooth brushing which are transmitted from brush handle to tooth tissues could be highly detrimental [41]. *Wiegand et al, 2013* [20], found that manual toothbrush reported higher brushing forces with an average force 1.6 ± 0.3 N associated with higher dentin abrasion in comparison to electric toothbrush which recorded 0.9 ± 0.2 N brushing forces. The brushing force may have acted more strongly and interfered with crystal formation and dentinal tubule occlusion. *Weijden et al, 2011* [42], reported that the use of powered toothbrushes is associated with lower force during usage in comparison to manual toothbrushes, which result in less tooth-surface loss, taking into consideration that new brands are provided by pressure sensor. In the current study, the used electric toothbrush was supplied by pressure sensor which causes stoppage of brushing upon application of high pressure. *Sehmi and Olley, 2015* [41], compared three brushing forces; 100g, 200g and 400g regarding their effect on dentin tubule patency. They found that 400g brushing forces resulted in less dentinal tubule occlusion while at 100g brushing forces, more tubule occlusion was recorded even after acidic challenge. An in vitro study showed that manual toothbrushes produced higher dentin wear than powered toothbrushes [43].

Another possible explanation of the superior performance of electric toothbrush might be related to the fact that powered toothbrushes rely in its work on the principle of acoustic microstreaming due to its oscillating and rotating movement [19,44] in which hydrodynamic forces are generated by rapid vibration of the bristles in a liquid medium[19]. This acoustic streaming might promote infiltration of sub-micron particles into opened dentinal tubules as reported by *Vyas et al, 2017* [31].

No differences were recorded between electric and manual toothbrush used with hydroxyapatite-based toothpaste before and after acidic challenge. This might be attributed to the nature of hydroxyapatite and its great similarity to natural tooth structure which could allow its blending with tooth tissues as previously mentioned and hence, the type of toothbrush was not effective.

CONCLUSION

Within the limitations of the current study, it could be concluded that hydroxyapatite toothpaste was effective in dentinal tubules occlusion and able to resist acidic challenge whether brushed using manual or electric toothbrush. Use of electric toothbrush with arginine toothpaste was beneficial in improving dentinal tubules occlusion before and after acidic challenge.

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