AL-AZHAR
Dental Journal
For Girls

The Official Publication
of The Faculty of Dental
Medicine For Girls,
Al-Azhar University
Cairo, Egypt.

Print ISSN 2537-0308 • Online ISSN 2537-0316

ADJ-for Girls, Vol. 10, No. 1, January (2023) — PP. 29:39

Effect of Different Biomimetic Materials on White Spot Lesions in Enamel (A split-mouth clinical trial)

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Codex : 1-04/23.01

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http://adjg.journals.ekb.eg

DOI: 10.21608/adjg.2022.100061.1422

Restorative Dentistry
(Removable Prosthodontics, Fixed Prosthodontics, Endodontics, Dental Biomaterials, Operative Dentistry)

ABSTRACT

Purpose: To assess the remineralizing effect and clinical effectiveness of different biomimetic materials (Zinc carbonate hydroxyapatite microparticles (Zn CHA), bioactive glass nanoparticles (BAG) and Poly-amido amine (PAMAM)) on white spot lesions in enamel (WSLs). **Materials and Methods**: A number of 84 teeth (21 patients) were assigned into 3 groups of 28 teeth (7 patients) in accordance to the time of assessment (immediately B1, after one-month B2 and after two months B3). According to the tested material; every patient had a split mouth (four quadrants). Zn CHA was used in the first group A1, BAG was used in the second group A2, PAMAM was used for the third groupA3, whereas in the fourth group A4; no treatment was carried out (control). Standardized periapical radiographs were obtained for assessment of re-mineralization using measurements of radiodensity (%). Standardized digital photography was obtained for colour change assessment. **Results:** The change between the study groups has statistical significance (pvalue=0.00) at all times grouping (for Radiodensity % and the colour changes). Moreover; results revealed nonsignificant change between BAG and PAMAM groups (at all time groups). Also; difference between Zn CHA and BAG was not statistically significant at two months only (Radiodensity %). **Conclusions**: All the tested materials have a remineralizing effect and improved the appearance of the WSLs. Zn-CHA needs longer time (2 months) as a biomimetic remineralizing agent. Time is an important issue for biomimetic re-mineralization. Biomimetic re-mineralization can be achieved by regular use of biomimetic toothpaste.

KEYWORDS

PAMAM, Bioactive glass, Hydroxyapatite.

- Paper extracted from Doctor thesis titled “Effect of Different Biomimetic Materials on White Spot Lesions in Enamel”

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INTRODUCTION

Chalky white spots due to mineral losing underneath superficial enamel, on clinical examinations are recognized as white spot lesion (WSL). It is mostly occurred at buccal surface of upper lateral incisors, premolars, canines, and central incisors, in the descendant arrangement of their incidence. Subsequently, these lesions resulted in esthetic challenges; influencing self-esteem, confidence and treatment satisfaction received by the patients [1,2].

Many techniques had been suggested to decrease severity and incidence of WSLs. Preservation of optimum oral hygiene measures, fluoride-containing (re-mineralizing) agents dentifrices, toothpaste, varnishes, anti-bacterial mouthwash-xylitol gum, chlorhexidine and derivative of Casein -casein phosphor-peptide-amorphous CaP are effective measures used in decreasing prevalence and inhibition of WSLs. Other involved managements options comprise bleaching, resin infiltrations and micro-abrasion [3,4].

Lately some agents had been presented that help in re-mineralization via incorporating a biomimetic remineralizing material in commercially available products aiming for obtaining optimum re-mineralization with a very simplified techniques provided by patients at home under supervision of their dentists [5,6].

Although Fluoride is considered the gold standard in re-mineralization strategies, it lacks the potential to arrange mineral crystals in an orderly way (hierarchical structure of enamel). Moreover, chronic swallowing high doses of fluoride products may cause dental fluorosis in children, skeletal fluorosis, where fractures and calcification of tendons and ligaments may occur, causing reduced joint mobility and serious problems [7].

Management of carious lesions according to the medical model is highly desirable and important method of minimal invasive dentistry. Re-mineralization procedure is a natural repairing mechanism which happens underneath near-neutral pH condition. Minerals deposition from saliva within the carious lesion results in formations of larger HAP crystals that are insoluble and much more resistance to dis-solution by acids [8].

It is likely to advance a bio-mimetic re-mineralizing protocol by imitating the bio-mineralizing process. Crystallization by non-classical pathway proposed that amorphous progenitors of hydroxyapatite (HAP) are engaged in the natural re-mineralization procedure of hard tissue by transforming into HAP crystals [9].

Zinc carbonates hydroxyapatite microparticles are considered as the most important and suitable biomimetic remineralizing agents for WSLs. HAP is most steady, and the smallest soluble, form of CaP in nature. Biorepair total protection plus tooth paste is a commercially available tooth paste in the market with no need for special preparations in labs containing Zinc Hydroxyapatite 20 wt. % (microRepair). All patients can apply it at home with no fear of toxicity even children under 6 years old can use Biorepair without adults' supervision due to Fluoride absence [10,11].

The invention of bioactive glasses (Bioglass) by Dr. L. Hench caused a revolution in conservative dentistry. It aids the precipitation of new hydroxyapatite by improving the body's own mechanism. Calcium, phosphate, sodium, and silicate ions existing in BAG are reactive in existence of body liquids, which cause the precipitations of CaP. Resultant surface changes and pH alteration contributed to the establishment of nucleation locations for CaP precipitation and enamel re-mineralization. BioMin C is a tooth paste commercially available Fluoride free with added Chloride ion for more reactivity and can be used with the patient at home [12].

Poly (amido amine) (PAMAM) dendrimers, recognized as "non-natural proteins" for their protein-like building, are very branched polymer. PAMAM dendrimer acts as nucleation template which stimulate crystal construction with orientations, structures and mineral phases analogous to enamel. Amine-terminated PAMAM (PAMAM-NH2) G3.0 [NH2(CH2)2NH2] :(G=3); PAMAM(NH2)32 has

32 surface amino groups, 1g in 20 wt. % ethanol for easy application as the raw material is very sticky and cannot be applied to enamel surface [13].

Accordingly, the aim of the current work was to estimate the remineralizing effect and clinical effectiveness of different biomimetic materials (Zinc carbonate hydroxyapatite microparticles, bioactive glass nanoparticles and Poly-amido amine (PAMAM)) on WSLs of enamel.

MATERIALS AND METHODS

Trial registration and ethical approval:

The trial was registered online in the Clinical Trial. gov (PRS). Data regarding the objective of the study, inclusion and exclusion criteria, number of patients enrolled, primary outcome, centers, starts, and estimated research completion dates were provided. The identification number for the registry was (NCT05001035).

The research protocol, patient information sheet as well as consent form were analyzed and accepted via the "Ethical Research committee" of faculty of Dental Medicine for girls; Al-Azhar University, Cairo, Egypt after looking for rights, safety and dignity of clinical trial (Protocol Code: OP-P-018-001). (Final Code: REC-OP-21-06)

Trial design and sample size calculation:

A randomized, controlled and split-mouth clinical trial with 1:1 allocation ratio. This trial was conducted at the restorative dental clinic at The Faculty of Dental Medicine for girls; Al-Azhar University. This clinical trial was written following the CONSORT 2010 guidelines (Consolidated Standards of Reporting Trial) to guarantee clear and transparent reporting.

Based on the study done by S. Sivaranjani et al., 2018 and Menglin Fan et al., 2020 who matched the effect of different re-mineralizing agents on enamel, via α-levels of (5 %) and β-levels of (20 %) i.e., power = 80%; the least counted sample size was 7 teeth for each group which was obtained from 21 patients (total 84 teeth). The sample size has been determined via the G-power 3.1.2 software, Germany.

Patient selection:

A number of 21 patients involved in this work were selected having at least 4 1st premolars (one on each quadrant) which will be extracted for orthodontic treatment at The Faculty of Dental Medicine for girls; Al-Azhar University. These patients were selected to fulfil the following inclusion (age groups between 15-25 years old, good oral hygiene and free of destructive habits for dental tissues as Bruxism, acid regurgitation, soft drinks consumption. Free of any cracks, malformations, dysplasia or caries. Good periodontal status with no pain on percussion.). Exclusion criteria (Rampant uncontrolled caries, Bad oral hygiene / periodontal disease, Heavy smoker, Pregnant/lactating females, Patients with destroying habits for teeth, Patients with significant tooth wear or hyposalivation) [14].

Patient risk assessment, Informed consent:

Patients were assessed for their caries risk at the beginning of the study using caries risk assessment form (Age >6) provided by the American dental association 2011 (ADA). According to this form all the patients enrolled for this trial have been evaluated to be in the low-risk class.

All procedures, purpose, risks, benefits, number of visits, and expected duration for the clinical trial were explained to participants in colloquial language. All participants' signatures were obtained before starting the study on written knowledgeable agreement in accordance to the guideline of the Ethics Committee of Human Research.

Preparation of patients and the artificial White Spot lesion on Enamel:

All Patients were given written oral hygiene instructions: regulating frequency of sugar and sugary drinks consumption, emphasizing eating a healthy diet, identical measures of teeth brushing i.e., 2 times per day with toothpaste, proper technique of brushing. The teeth were scaled using ultra sonic scaler* to remove any hard calculus. A low-speed hand piece (NSK-EC-Japan) with pumice paste (Alpha-Pro prophylaxis paste- medium grit 29µ, PM3995/REV003- Dental Technologies, Inc., USA) was used to remove any soft deposits before initial treatment and each application and assessment according to its time table. Buccal surface was demineralized to simulate aggressive white spot lesion in enamel with 34 % phosphoric acid gel (pH less than 1; Dentsply, USA) for 30s[15]. The size of this artificial white spot lesion was determined using a standard window of 3mm×3mm diameter which is already made in a matrix band.

Patient grouping:

Twenty-one patients (total 84 teeth) have been allocated into 3 key groups in accordance to the time of assessment (n=7 patients); (immediately (Group B1), after one month (Group B2) and after 2 months (Group B3)). Three main groups were used for standardization within the same group and not to disrupt the time of extraction of premolars for orthodontic treatment.

Each patient had split mouth for each quadrant (upper right premolar tooth received Zn CHA microparticles (Group A1), upper left premolar tooth received BAG nanoparticles (Group A2), lower right premolar tooth received Poly-amido amine (PAMAM) (Group A3) while the lower left premolar tooth received no treatment i.e.; negative control (Group A4)).

Biomimetic materials application:

After obtaining the aggressive white spot lesions, biomimetic materials were applied. Biorepair plus Total Protection was applied in accordance to producer's instruction. Pea sized amount of the paste was applied to the white spot lesion by a micro brush, left in pose for 30 sec., rinsed with water. BioMin C was applied in accordance to producer's instruction. Pea sized amount of the paste was applied to the white spot lesion by a micro brush, brushed gently for two minutes, swirl the foamy paste for 30 sec. Patient was asked to spit out excess foam with no rinsing. PAMAM Dendrimer Generation 3.0 was applied according to manufacturer's instructions. One coat of PAMAM using micro brush, left for 60 seconds and the surface was dried. Eating, drinking and teeth brushing were not allowed directly after the treatment has been completed for 30 minutes. The application was repeated weekly for each biomimetic agent till the time of assessment.

Digital radiography records assessment (Radiodensity %):

According to the time of assessment (immediate, after one month and after two months); standardized reproducible periapical radiographs for each treated tooth were obtained using a digital image plate, held in a film holder for optimizing parallel technique with standard image. The image plate was exposed by the x-ray machine at 70 Kilovolt and 0.5 Ma dose for 0.04 seconds. Assessment of re-mineralization was done using measurements of radiodensity (%). Vista Scan system and DBSWin software was utilized for performing these measurements. The mineral density mean value was calculated from the equation (average density = average intensity / profile); the profile was determined directly in the utilized software.

Standardized digital photography assessment:

Standardized professional digital photography (standard: professional camera; Canon EOS Rebel T5i/700D camera -Japan-, light conditions, focus and zoom of the lens, the shots were on a vertical dimension to the long access of the teeth, 20cm distant and with camera holder for optimum standardization) was obtained for every single tooth before application and according to their time grouping (colour change assessment with the corresponding scores). Tow observers were scoring the colour change the first one was the main investigator (not blinded) and the second one was blinded completely for the work. Each change in colour took a code as following; (no improvements

* *WOODPECKER ULTRASONIC UDS-K SCALER, China*

= score 0, mild improvements = score 1, moderate improvements = score 2, excellent improvements = score 3 and complete disappearance of the artificial white spot = score 4).

Statistical analysis

Quantitative variables have been introduced as (mean±SD), confidence intervals and range. Data have been tested for normal distribution via the Kolmogorov-Smirnov testing. The findings of the Kolmogorov-Smirnov testing showed that almost all of the data have normal distribution (parametric data), so parametric test was utilized. The percent change in radiodensity has been determined by the following formula (Values after- before X100 / the before Value). This was trailed by Tukey's post hoc testing for pairwise comparisons. Paired t testing has been utilized for comparison among observation times (effect of time). Qualitative data (digital photography scores) were presented as number and percentage. Chi square test has been utilized for inter (between) and intra (within) group comparisons for qualitative data. The result was judged significant at p value ≤0.05. The SPSS 18.0 has been utilized for statistical analysis.

RESULTS

Digital radiography results (Radiodensity%):

Comparison of mean average change in radiodensity % in different assessment times between different groups:

Results of the effect of treatment agents on percentage of change of radiodensity of demineralized enamel (Table 1 and Fig. 1) revealed that, a significant change was found among all groups (except between A2 & A3 at all times and between A1& A2 at two months only). At B1 (immediate), PAMAM (A3) group yielded the highest mean percentage of change of radiodensity, followed by BAG (A2) group, then Zn CHA (A1) group, whereas the control group showed the lowest mean percentage of change radiodensity.

Table (1) *Descriptive statistics and comparison of mean average change in radiodensity % in different groups (ANOVA test):*

		Mean	Std. Dev	Std. Error	95% Confidence Interval for Mean		Min	Max	F	P
					Lower Bound	Upper Bound				
Immediate	Biorepair plus total protection	3.69^{b}	1.36	0.52	2.43	4.95	2.10	5.20	14.705	0.000*
	BioMin C	7.54^{a}	3.24	1.23	4.54	10.54	3.80	10.70		
	PAMAM G3.0	7.87^{a}	3.39	1.28	4.73	11.01	3.90	11.10		
	Negative control	0.39^{c}	0.19	0.07	0.21	0.56	0.20	0.70		
One month	Biorepair plus total protection	8.27^{n}	3.02	1.14	5.48	11.07	4.70	11.50	38.384	0.000*
	BioMin C	14.63^{m}	2.71	1.02	12.12	17.13	11.30	19.50		
	PAMAM G3.0	15.03^{m}	3.53	1.33	11.77	18.29	10.70	20.90		
	Negative control	1.63^{Q}	0.45	0.17	1.22	2.04	1.10	2.20		
Two months	Biorepair plus total protection	16.73^{y}	3.50	1.32	13.49	19.97	10.90	21.30	31.794	0.000*
	BioMin C	$18.51^{x,y}$	2.78	1.05	15.95	21.08	15.20	21.20		
	PAMAM G3.0	21.64^{x}	3.47	1.31	18.44	24.85	16.30	25.10		
	Negative control	5.91^{z}	3.02	1.14	3.12	8.71	2.90	10.20		

*At p value≤0.05, * result was significant*

Tukey's post hoc testing: Within the same comparing, meaning that it was sharing the same superscript letter have nonsignificant change

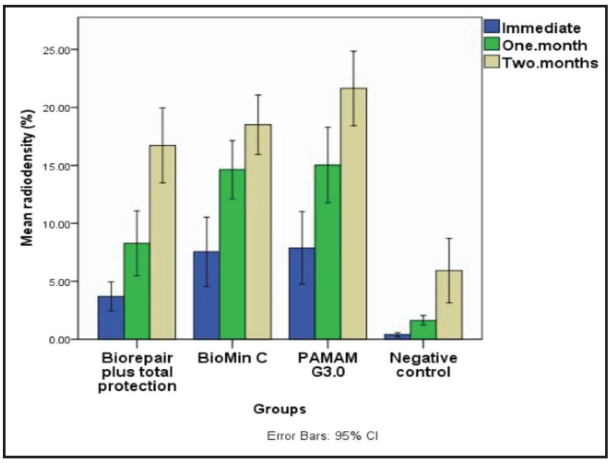

Figure (1) Bar chart illustrating radiodensity % in different groups at different observation times.

At B2 (after one month), PAMAM group produced the highest mean percentage of change of radiodensity, followed by BAG group, then Zn CHA group, while control group showed the lowest mean percentage of change of radiodensity. At B3 (after two months), PAMAM group displayed the highest mean percentage of change of radiodensity, followed by BAG group, followed by Zn CHA group, then control group which showed the lowest mean percentage of change of radiodensity.

Results of the effect of evaluation time on percentage of change of radiodensity within each group revealed statistically significant difference in percentage of change of radiodensity between different evaluation times within each group. Percentage of change of radiodensity in all groups at B3 (after 2 months) was significantly higher as compared to B1 (immediate) and B2 (after 1 month).

Digital photography analysis:

Comparison of mean average change in colour in different assessment times between different groups:

Results of the effect of treatment agents on colour change of clinical photography of demineralized enamel (Table 2 and Fig. 2,3) showed that, a significant change was between all groups (except between A2 & A3 at all times). At B1 (immediate), PAMAM (A3) group yielded the highest mean of colour improvement, followed by BAG (A2) group, then Zn CHA (A1) group, whereas the control group showed the lowest improvement of colour.

Table (2) *Descriptive statistics [No. (%)] and comparison of change in colour scores in different groups at different observation times (Chi square test):*

		Biorepair plus total protection (ZnCHA)	**BioMin C (BAG)**	**PAMAM G3.0**	**Negative control**	P value (between groups)
Immediate	Score **0**	3 (43%)	0	0	7 (100%)	0.0003*
	Score 1	4 (57%)	3 (43%)	3 (43%)	0	
	Score 2	0	4 (57%)	4 (57%)	0	
One month	Score **0**	0	0	0	3 (43%)	0.0025*
	Score 1	3 (43%)	0	0	4 (57%)	
	Score 2	4 (57%)	2 (29%)	2 (29%)	0	
	Score 3	0	4 (57%)	4 (57%)	0	
	Score 4	0	1 (14%)	1 (14%)	0	
Two months	Score **0**	0	0	0	0	0.0071*
	Score 1	0	0	0	4 (57%)	
	Score 2	1 (14%)	0	0	3 (43%)	
	Score 3	4 (57%)	3 (43%)	2 (29%)	0	
	Score 4	2 (29%)	4 (57%)	5 (71%)	0	
P value (within the same group)		0.0007*	0.015*	0.007*	0.026*	

*At p value≤0.05, * result was significant*

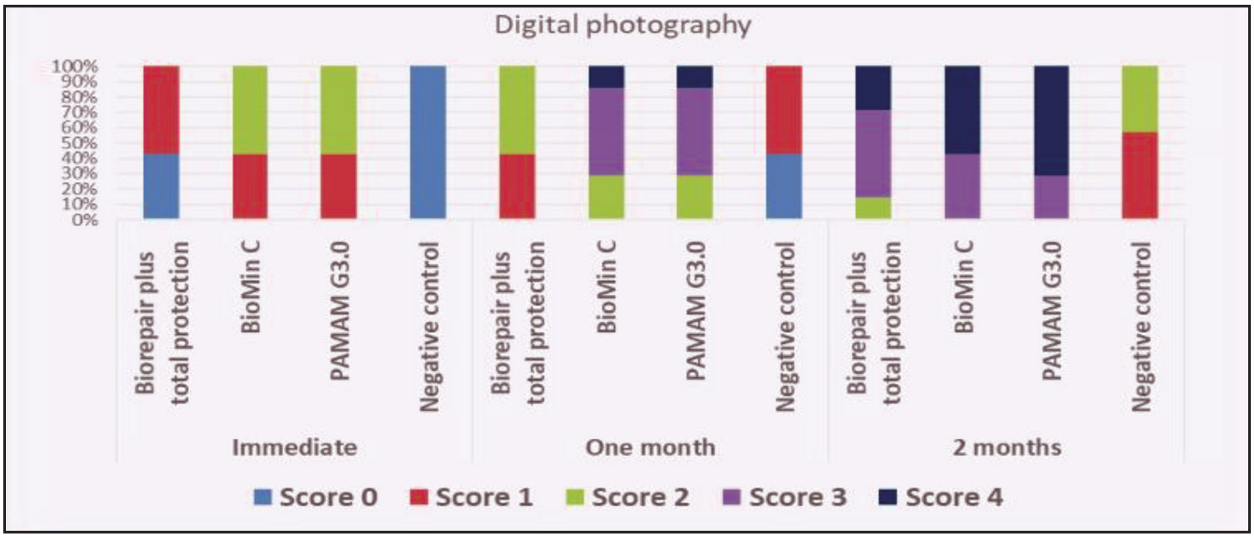

Figure (2) Bar chart showing variation in colour scores in different groups at different observation times

Figure (3) Colour improvement with different stages of WSLs.

At B2 (after one month), PAMAM group produced the highest colour improvement of WSLs, followed by BAG group, then Zn CHA group, while control group showed the lowest colour improvement. At B3 (after two months), PAMAM group displayed the highest colour improvement, followed by BAG group, followed by Zn CHA group, then control group which showed the lowest colour improvement.

Effect of evaluation time on colour improvement of WSLs within each group:

Results of the effect of evaluation time on colour improvement within each group (Table 2 and Fig.2,3) revealed statistically significant difference in colour improvement between different evaluation times within each group. Colour improvement in all groups at B3 (after 2 months) was significantly higher as compared to B1 (immediate) and B2 (after 1 month).

DISCUSSION

Dental caries is a dynamic procedure of pathological de-mineralization of teeth. By regulating the mineral balance favourably towards the re-mineralization, the initial caries lesion (WSL) can be stopped or repaired.

Biorepair total protection plus tooth paste was used at this study. The action mode is a mixture of lessening the de-mineralization impact of the acidic challenges [11], bacterial inhibition of Zn dopped particles and a re-mineralization/repairing impact brought about by the further establishment of CaP.

BioMin C tooth paste also was used at this study as it is reactive in existence of body liquids, causing the precipitations of Ca & P. CaP releases are started by a solution intermediated dis-solution. Resultant surface variations and pH changes contributed to the formations of nucleation locations for CaP precipitations and enamel remineralization. It is also fluoride free with no fear of fluoride toxicity[12].

PAMAM-NH2 generation 3.0 was utilized in this study because of the analogous structure and self-assembly behaviour of PAMAM make it potential to mimic the structure and functions of amelogenin, that have a significant function in modulating enamel formations. PAMAM dendrimers act as nucleating patterns and induce crystal formations with structures, orientations and mineral phases analogous to enamel [13].

The WSLs were monitored at three periods of time; B1: immediately after application for monitoring spot effect of the remineralizing agents on WSLs, B2: short term evaluation one month and B3: longer-term evaluation two months were selected to evaluate progression or regression of the WSLs based on mineral deposition [16].

According to the radiodensity % change results at this study, the highest percentage of was recorded after treatment with PAMAM followed by BAG then Zn CHA. This could be explained by PAMAM-NH2 are identical to HAP (Ca/P 1.67) in natural tooth enamel. PAMAM-NH2 showed brilliant ability to persuade enamel superficial re-mineralization, and this re-mineralization attributed by PAMAM dendrimers smoothened the enamel superficial and decreased S. mutans adhesions. This comes in accordance with literature studies which revealed that PAMAM-NH2 have improved re-mineralization capability than PAMAM-OH. PAMAM-NH2 can efficiently restore the sub-surface enamel lesions to a depth of 20-100 μ m [17].

Zinc carbonates hydroxyapatite microparticles treated WSLs group showed significant increase in radiodensity % change. This could be due to nano-structured Zn-CHA micro-crystals, made in lab, representing the active element of the investigated Zn-CHA toothpaste. This matter of repair by promoting carbonate substitutions to the phosphate and/or hydroxyl groups in hydroxyapatite. This replacement repair is gradually increasing over time reaching a good level after two months. Moreover; throughout the CHA solubilizations, P, Ca ions, and Zn ions are liberated, permitting the Zn to act as a powerful anti-bacterial influence, that interfered with plaque formations, avoiding more degradation of the anew deposited Zn-CHA coating. This is in agreement with previous studies which revealed that this synthetic CHA depositions mostly happens on the enamel zones marked by enamel losses and/or damages (perhaps because of erosion influences), consequently considered as a real enamel repairing[18]. Another study concluded, the layer shaped by Zn- CHA tooth-pastes may contribute a re-mineralizing influence on the dental superficial, and a great benefit for further bacterial attacks [19].

Bioactive glass nanoparticles treated WSLs group revealed significant increase in radiodensity %, this can be explained by induction of apatite formations on exterior superficial of the enamel in existence of saliva or other physiological liquids. Ca, P, Na, and silicate ions found in BAG are responsive in the existence of body liquids and cause the depositions of CaP. CaP releases are started by a solution-intermediated dis-solution. As the procedure of dis-solution continues, the dissolution products cause a variation in the chemical compositions and pH. Resultant surface variations and pH variation contribute to the creation of nucleating locations for CaP [20]. These findings are in accordance with previous reports that evaluated the remineralizing effect of BAG on demineralized enamel and suggested that BAG may be measured an effective re-mineralizing agent for white spot Lesion [21]. Another study stated that BAG treated WSLs exhibited greater possibility to promote re-mineralization of de-mineralized human enamel [22].

However, the current results are in contrary with literature which revealed that the efficacy of Zn-CHA toothpastes in remineralizing enamel, this impact can be affected immediate or after only 3-days of treatments (23). Other studies said that the hydroxyapatite-containing dentifrice has highest remineralizing potential followed by bioactive glass paste (24,25).

As for effect of time, results revealed a significant increase at radiodensity % in all groups after one month, and the highest percentage of recovery was recorded after two months. This could be because of the synergistic impact of artificial saliva in enhancing the remineralizing process by increasing Ca and P ions precipitation together with the remineralizing effect of each treatment agent in the experimental groups, thus increasing enamel radiodensity% (26).

Although radiodensity % of the control group increased by time, it was lower than other treatment groups. This may be explained by that the saliva had a buffer capacity and plays as a holder for vital ions, that could bring a remineralizing effect on enamel, encouraging re-mineralization. The buffer ability of saliva plays an important function in preserving levels of pH in plaque and saliva. The buffering systems existing in the saliva are phosphate system, carbonic acid/bi-carbonate system and protein system (27).

The result of radiodensity % was in the same direction with clinical photography observations which demonstrated that, colour improvement of WSLs appearance was clinically significant with all treatments. Clinical photography results revealed the highest colour improvement, which confirms its significant effect on promoting enamel re-mineralization was recorded for PAMAM treated teeth.

Zinc carbonates hydroxyapatite microparticles treated teeth showed significant improvement in colour of WSLs over the negative control (no treatment) but, the least recorded mean improvement in colour in comparison to other interventions. Toothpaste containing HA caused a significant improvement in WSL extension after 6-mths. This decrease from 1 - 6 months speculates that repeated application of toothpaste could lead to a higher reduction over time. HA performs better over time with subsequent applications with tendency to disappear WSLs over 6 months (28). Another study stated that biomimetic hydroxyapatite induced a visible enamel re-mineralization after 2 months of application. HA failed to improve white spot lesion appearance in the short-term trial (7days).

Bioactive glass nanoparticles treated WSLs group revealed that colour change was significantly more than Biorepair plus total protection. Bioactive glass was found to be more effective versus WSL because of its immediate capability for enhancement of enamel superficial, high biocompatibility and low cytotoxicity. This interacting layer transformed from microscopic brushite crystals to a hydroxy-apatite layer within only 14 days (29).

CONCLUSIONS

Within the limitations of this work, all the tested materials have a remineralizing effect and improved the appearance of the WSLs. Zn-CHA needs longer time (2 months) as a biomimetic remineralizing agent. Time is an important issue for biomimetic re-mineralization. Biomimetic re-mineralization can be achieved by regular use of biomimetic toothpaste.

ACKNOWLEDGEMENT

We would like to sincerely thank Dr Sarah Shahin, Lecturer in Operative Department, Faculty of oral and dental medicine, Saini University and Dr Mohamad Mokhtar, orthodontic specialist at Benha Teaching hospital, for assisting throughout the study.

RECOMMENDATIONS

Further clinical studies are recommended.

CONFLICT OF INTEREST

There is no conflict of interest.

FUNDING

No funding presented to the current work.

REFERENCES

1. Heravi F, Ahrari F, Tanbakuchi B. Effectiveness of MI Paste Plus and Remin Pro on re-mineralization and color improvement of post-orthodontic white spot lesions. Dent Res J (Isfahan) 2018;15: 95–103.
2. Temel SS, Kaya B. Diagnosis, prevention and treatment of white spot lesions related to orthodontics. Int J Oral Dent Health 2019; 5:085.
3. Kakkar S, Singh G, Tandon P. Comparison of various white spot lesion preventing medicaments: an in vitro study. J Indian Orthod Soc 2018; 52:94–9.
4. Lopatiene K, Borisovaite M, Lapenaite E. Prevention and treatment of white spot lesions during and after treatment with fixed orthodontic appliances: a systematic literature review. J Oral Maxillofac Res 2016;7: e1.
5. Mohanty P, Padmanabhan S, Chitharanjan AB. An in vitro evaluation of re-mineralization potential of novamin on artificial enamel sub-surface lesions around orthodontic brackets using energy dispersive X-ray analysis (EDX). J Clin Diagn Res 2014;8: ZC88–91.
6. Rahimi F, Sadeghi M, Mozaffari HR. Efficacy of fluoride varnish for prevention of white spot lesions during orthodontic treatment with fixed appliances: a systematic review study. Biomed Res Ther 2017; 4:1513–26.
7. Aidaros N, Mosallam R, Farouk H. Effect of green tea, black tea and moringa oleifera on re-mineralization of artificially demineralized enamel and dentin: An in-vitro microhardness analysis. Adv Dent J. 2021; 3:24–34.
8. Arifa MK, Ephraim R, Rajamani T. Recent advances in dental hard tissue re-mineralization: A review of literature. Int J Clin Pediatr Dent. 2019; 12:139–44.
9. Zhang X, Li Y, Sun X, Kishen A, Deng X. Biomimetic re-mineralization of demineralized enamel with nano-complexes of phosphorylated chitosan and amorphous calcium phosphate. J Mater Sci Mater Med 2014; 2619-28.
10. Antoniac, I., Miculescu, F., Cotrut, C., Ficai, A., Rau, J.V., Grosu, E., et al. Controlling the Degradation Rate of Biodegradable Mg-Zn-Mn Alloys for Orthopedic Applications by Electrophoretic Deposition of Hydroxyapatite Coating. Materials 2020, 13, 263.
11. Balhuc, S.; Campian, R.; Labunet, A.; Negucioiu, M.; Buduru, S.; Kui, A. Dental Applications of Systems Based on Hydroxyapatite Nanoparticles—An Evidence-Based Update. Crystals 2021, 11, 674.
12. Nagarathana C, Sakunthala BK, Naveena Preethi P. An update on current remineralizing agent. OHDM. 2015; 14:183-7.
13. Abedi-Gaballu F, Dehghan G, Ghaffari M, Yekta R, Abbaspour-Ravasjani S, Baradaran B, et al. PAMAM dendrimers as efficient drug and gene delivery nanosystems for cancer therapy. Appl Mater Today 2018; 12:177–90.
14. Ebrahimi M, Mehrabkhani M, Ahrari F, Parisay I, Jahantigh M. The effects of three remineralizing agents on regression of white spot lesions in children: A two-week, single-blind, randomized clinical trial. J Clin Exp Dent. 2017;9 641-8.
15. Chung H. Y. and Li C.C. Microstructure and nanomechanical properties of enamel remineralized with asparagine–serine–serine peptide. Materials Science and Engineering C 2013; 33, 969 - 73.
16. Hu H, Feng C, Jiang Z, Wang L, Shrestha S, Yan J, et al. Effectiveness of remineralizing agents in the prevention and reversal of orthodontically induced white spot lesions: a systematic review and network meta-analysis. Clin Oral Investig. 2020; 24:4153-67.
17. Menglin Fan, Min Zhang, Hockin H. K. Xu, Siying Tao, Zhaohan Yua, et al. Re-mineralization effectiveness of the PAMAM dendrimer with different terminal groups on artificial initial enamel caries in vitro. Dental Materials 2020; 36:210-20.
18. Butera A, Pascadopoli M, Gallo S, Lelli M, Tarterini F, Giglia F, et al. SEM/EDS Evaluation of the Mineral Deposition on a Polymeric Composite Resin of a Toothpaste Containing Biomimetic Zn-Carbonate Hydroxyapatite (microRepair®) in Oral Environment: A Randomized Clinical Trial. Polymers (Basel). 2021; 13:2740.
19. Supontep Teerakanok, Minglei Zhao, Russell Giordano, Yuwei Fan, Interaction of doped magnesium, zinc and fluoride ions on hydroxyapatite crystals grown on etched human enamel, Journal of Crystal Growth, 2021,571, 126262-8.
20. Omran, T., Mostafa, M., Abd El-Raouf, E. 'Assessment of Remineralizing Effect of Bioactive Glass Based Toothpastes: An In-Vitro Comparative Study', ADJG, 2021,8, 491-9.
21. Bakry, A.S.; Abbassy, M.A.; Alharkan, H.F.; Basuhail, S.; Al-Ghamdi, K.; Hill, R. A Novel Fluoride Containing

Bioactive Glass Paste is Capable of Re-Mineralizing Early Caries Lesions. Materials journal 2018, 1636-46.

22. Ali, Saqiba Farooq, Imrana, Al-Thobity, Ahmad M.b Al-Khalifa. An in-vitro evaluation of fluoride content and enamel re-mineralization potential of two toothpastes containing different bioactive glasses. Journal of Bio-Medical Materials and Engineering, 2020, 30, 487-96.
23. Orsini G, Procaccini M, Manzoli L, Sparabombe S, Tiriduzzi P, Bambini F, et al. A 3-day randomized clinical trial to investigate the desensitizing properties of three dentifrices. J Periodontol. 2013;84: e65-73.
24. Geeta RD, Vallabhaneni S, Fatima K. Comparative evaluation of re-mineralization potential of nanohydroxyapatite crystals, bioactive glass, casein phosphopeptide-amorphous calcium phosphate, and fluoride on initial enamel lesion (scanning electron microscope analysis) - An in vitro study. J Conserv Dent. 2020; 23:275-9.
25. Khandelwal JR, Bargale S, Dave BH, Poonacha K S, Kariya PB, Vaidya S. Comparative evaluation of remineralising efficacy of bioactive glass agent and nano-hydroxyapatite dentifrices on artificial carious lesion in primary teeth: An in vitro study. Adv Hum Biol 2020; 10:129-33
26. Ishikawa, Rina; Nagasaki, Ryota; Kawamura, Naohiko. Characterization and Application of Bioactive Glass and Chitosan Nanoparticles for Tooth Enamel Re-mineralization. Journal of Biomaterials and Tissue Engineering, 2021, 11, 1236-43
27. Farooq I, Bugshan A. The role of salivary contents and modern technologies in the re-mineralization of dental enamel: a narrative review. F1000Res. 2020; 9:171.
28. Badiee M, Jafari N, Fatemi S, Ameli N, Kasraei S, Ebadifar A. Comparison of the effects of toothpastes containing nanohydroxyapatite and fluoride on white spot lesions in orthodontic patients: A randomized clinical trial. Dent Res J (Isfahan). 2020; 17:354-9.
29. Linjawi AI. Sealants and White Spot Lesions in Orthodontics: A Review. J Contemp Dent Pract. 2020; 21:808-14.