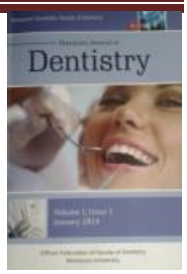

Comparison of the Effect of Three Remineralizing Agents on Surface Microhardness and Surface Roughness of Primary Teeth Enamel

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Abstract:

Background: Prevention of initial dental caries has an essential role instead of the treatment especially in children.

Aim: to evaluate the effect of nano-bioactive glass paste, CPP-ACP paste and fluoride varnish on the artificially induced carious lesion of the primary teeth enamel regarding surface microhardness and surface roughness.

Materials and methods: sixty extracted primary second molars were divided into four groups (n=15) as follow; group A (Bioactive glass paste), group B (CPP-ACP paste- GC tooth mousse), group C (fluoride varnish- Fluor Protector), group D (control group). The surface microhardness (Vickers Microhardness machine) and surface roughness (Stylus Profilometer) were measured for the control group D as a baseline and for the other three groups after demineralization. After that, different remineralizing agents for 10 days were applied. Then another surface microhardness and roughness tests were done for all groups. The data were collected and statistically analyzed using One Way ANOVA test with Post Hoc Tukey test.

Results: Both BAG paste and CPP-ACP paste have a highly significant recovery in compared to fluoride varnish after remineralization in microhardness and SEM evaluation but there was no significant difference between the different study groups in the surface roughness results

Conclusion: All of three agents BAG, CPP-ACP and fluoride varnish are capable to remineralize early carious lesions while BAG is more effective than CPP-ACP but eventually both have similar remineralization potential which is higher than the fluoride.

Keywords:Remineralization, bioactive glass, microhardness, surface roughness.

Introduction

Dentalcaries is a chronic, multifactorial, infectious disease initiated by acids from bacterial metabolism diffusing into enamel and dentine [(1)]. The caries development is thought to be active during demineralization periods more than periods of remineralization. To restore the natural equilibrium, either remineralization must be improved, or demineralization must be inhibited [(2)]. The early enamel lesions have a potential for remineralization with an increased resistance to further acid challenge, mostly with the use of enhanced remineralization treatments [(3)].

The use of fluoride (F) agents in various forms is a verified method that strengthens the enamel structure, making it less liable to demineralization by the formation of stronger fluorapatite crystals and enhancing enamel remineralization[(4)].

Casein phosphopeptide (CPP) is a phosphopeptide obtained from milk protein casein that contains phosphoseryl sequences and stabilizes the calcium phosphate in nanocomplexes. CPP prevents dissolution of calcium and phosphate ions by binding with amorphous calcium phosphate (ACP) [(5)]. It has been shown in both in vivo and in vitro studies that CPP-ACP can remineralize the enamel subsurface lesion [(6, 7)].

Recently, bioactive glass materials have been introduced in many fields of dentistry. This unique material has several novel features, most significant of which are its ability to act as a biomimetic mineralizer matching the body's own mineralizing behavior while also affecting cell signals in a way that benefits the restoration of tissue structure and function [(8)].

Bioactive glass is considered to be a breakthrough in re-mineralization technology [(9)], it is a multi-component inorganic compound made up of elements such as silicon, calcium, sodium and phosphorus. The active ingredient is amorphous calcium sodium phosphosilicate. This compound in aqueous environment release bioavailable calcium, sodium and phosphate ions contributing to the remineralization process [(10)].

Therefore, the present study was conducted on different remineralizing agents to assess the effect of using them on the remineralization, microhardness and roughness of primary teeth induced enamel caries.

followed by corresponding changes in the stroma, such as angiogenesis [(17,19,13)].

Mean vascular density (MVD) is a quantitative analysis of angiogenesis, which has been evaluated by using various molecules including: CD31, CD34 and CD105 (endoglin) [(20,21)]. CD105 (endoglein) is a homodimeric cell membrane glycoprotein and is a component of TGF-β receptor complex. This marker is an indicator of endothelial cell proliferation and is up-regulated during angiogenesis [(22,23)]. Moreover, the expression of CD105 is one of the most conspicuous characteristics of newly formed blood vessels; Hence, it is more appropriate to determine MVD [(24)]. Several types of cells are associated with the development of cysts and tumors [(16)]. Among inflammatory cells, mast cells have been considered in growth and expansion of cysts. Mast cells are

one of the defense cells of immune system with metachromatic cytoplasmic granules [25,26].

Recently,mast cells were recognized in the pathogenesis of more aggressive pathologic lesions [27]. Mast cells have an inhibitory role on the development of pathological lesions. However, stimulatory role of mast cells in the growth of pathological lesions is more prevalent and obvious than their inhibitory effect [28]. With respect to several roles of mast cells such as participation in inflammation, degradation of extracellular matrix and bone resorption[29], previous studies have identified mast cells in odontogenic cysts, but there were limited studies about the role of mast cells in the pathogenesis of odontogenic cysts [30]. There is a hypothesis that the more aggressive behavior of odontogenickeratocysts is related at least, partly, to distribution of mast cells. However, their pathogenesis and mechanism of expansion and enlargement have not been evaluated [31].

The aim of this study was to determine the density of microvessels and MCs in odontogenic cysts. Correlate the microvessel density with their corresponding mast cells density in the three types of cysts, in order to detect their possible role in the variable behavior of these odontogenic cysts.

Material and method :

Materials:

1) Bioactive glass (BAG) nano-paricles paste, Nanostreams Company, Cairo.
2) Casein phosphopeptide – Amorphous calcium phosphate (CPP-ACP) paste (GC tooth mousse) GC Company.
3) Fluoride varnish (Fluor Protector) IvoclarVivadent.
4) 37% phosphoric acid gel.

Methods:

A total of sixty primary molar teeth extracted for orthodontic reasons or naturally exfoliated with sound buccal surfaces were selected forming sample of the study. The teeth were cleaned of soft-tissue debris with distilled water and highly polished. The teeth were stored in 0.1 % thymol until further use.

The radicular part of each tooth was removed. The coronal part was sectioned mesiodistally into two halves using low speed diamond tipped disc. The buccal surfaces were mounted in an acrylic resin molds with the enamel surface exposed. Then samples were randomly distributed into four groups. The specimens were divided according to the remineralizing agents into four equal groups:

1) Group A: Bioactive glass paste (n=15).
2) Group B: CPP-ACP (n=15).
3) Group C: Fluoride varnish (n=15).
4) Group D: Control group (n=15).

The control group (n=15) was subjected to a baseline microhardness testing and surface roughness. Then the rest of experimental specimens were subjected to artificial caries like lesions formation before the treatment by the application of 37% phosphoric acid gel on the whole buccal surface. It was applied for 20 minutes to demineralize the enamel surface to simulate early enamel lesions. Then the specimens were rinsed with water and dried. The buccal surface was divided into two halves by a marker. A microhardness test was applied after demineralization on one half of the sample. Surface roughness test was applied on the other half.

The BAG paste, CPP-ACP paste and fluoride varnish were applied on the whole demineralized tooth surface every day for 10 days. After each application, samples were washed under distilled water and stored in artificial saliva. Another final microhardness test and surface roughness were recorded on the two halves of the specimens.

humidifying chamber, followed by incubation with secondary biotinylated antibodies and streptavidin for 15 min each. Di-aminobenzidine was applied to produce brown staining followed by counterstaining with Mayer's hematoxylin. After each step, the slides were put in phosphate-buffered solution (PBS). For the negative control, the primary antibody was eliminated and replaced with PBS.

For immunohistochemical and histochemical counting using the microvascular count technique, according to the method suggested by Weidner et al. [33]. MVD and MCD was assessed as the mean number of microvessels and MCs per high power field. The field size for 400 magnification (40 objectives and 10 ocular) was approximately 0.18 mm^2. Scores of overall CD105 and Touilidine blue expression were represented as mean density/mm2 ± SD for quantitative variables using SPSS (Statistical package for Social Siences) software. Comparisons among the experimental groups were done using One-way analysis of variance test (ANOVA) ($p<0.05$). To compare the number of microvessels and MCs between inflamed and non-inflamed DCs and OKCs, independent t-test was used. Pearson correlation coefficient test was used to determine the correlation between MVD and MCD. A p-value of <0.05 was considered statistically significant

Results:

Microhardness test	Group A	Group B	Group C	Group D	Test of significance
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Demineralization	279.94 ± 16.88^{ab}	286.50 ± 12.14^{cd}	255.87 ± 16.29^{ace}	362.33 ± 33.78^{bde}	F=55.31 P<0.001* P_A<0.001* P_B<0.001* P_C<0.001*

After remineralization	356.79±18.66[a]	362.28±26.45[b]	306.25±17.06[abc]	362.33±33.78[c]	F=14.35 P<0.001* P_A=0.589 P_B=0.99 P_C<0.001*
Test of significance between demineralization and remineralization	t=19.68 P<0.001*	t=11.32 P<0.001*	t=10.79 P<0.001*		

Regarding the mean values and standard deviations for enamel microhardness (Kgf/mm^2) of demineralized and remineralized samples, it was found that the highest mean at demineralization stage was obtained for group B (286.50) then group A (279.94) with the least was for group C (255.87) while the control group was (362.33) as shown in **Table (1).** After remineralization, group B (362.28) had the same value as control group (362.33) and the mean for group A showed increased mean value than the demineralized value which almost equivalent to the control group. The least recovery in the microhardness value was with Fluoride group (306.25).

Table (1): Comparison of the mean surface microhardness values of the studied groups during demineralization and after remineralization.

The mean values for enamel surface roughness after demineralization and after remineralization as shown in **Figure (1)**, that the highest mean value at the demineralization stage was obtained for group A then group C with the least was for group B while the control group had the least roughness value. There were no statistical differences between the groups.

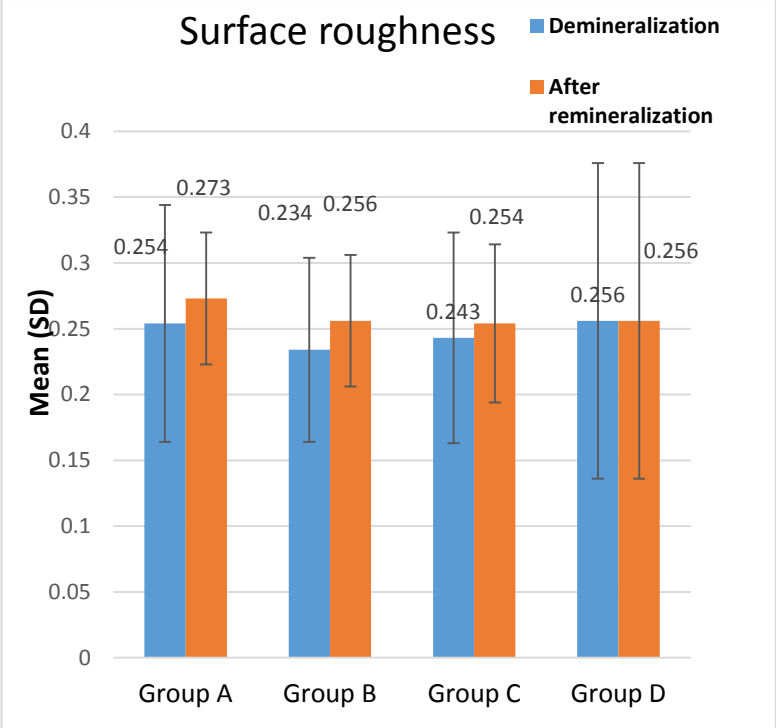

Figure (1): Bar chart showing mean surface roughness after demineralization and after remineralization among studied groups.

Discussion:

Minimal intervention dentistry depends on the least invasive treatment options possible to reduce tissue loss and patient discomfort. Focusing mainly on prevention and early interfering with caries, as its first basic principle is to remineralize early carious lesions, enhance a biological or therapeutic method instead of the traditional surgical way [(11)].

In this study, primary molars were selected because they are different histologically than permanent teeth. Also they are more susceptible to caries as the children tend to eat more sugars in their dietary habits [(12)]. As well as remineralization of the primary teeth is more easy and less stressful than restoration due to difficult management of the children in the dental clinics [(13)].

In the present study, 37% phosphoric acid was used for 20 minutes to demineralize the enamel surface simulating an early carious lesion. As a result of that, adequate microhardness and surface roughness variations were obtained so that the following remineralization could be well differentiated and compared. This was agreed by Palaniswamy et al. [(14)] and Vashisht et al. [(15)] who used the same duration to get demineralized enamel surface.

Regarding the result of the present study, there was statistically significant difference between studied groups and the control group after demineralization. While after remineralization, the results showed that both BAG and CPP-ACP have a highly significant recovery and an increase in the microhardness values compared to fluoride varnish. But the result of all the studied groups revealed increased values when compared with results after

demineralization. These results was supported by Mehta et al [16], Palaniswamy et al [14] while .Esfahani et al [17] suggested that there was improvement in the microhardness of CPP-ACP and fluoride varnish due to long term and repeated application for a month.

The results of surface roughness in this study showed no significant difference between the different study groups when compared to the control after demineralization. This result was in acceptance with China et al [18], on the other hand, Mathias et al [19] found that the surface roughness decreased after the application of CPP-ACP. This would be explained as they used abraded surfaces by bleaching agents for roughened enamel.

Conclusion:BAG is more effective than CPP-ACP but eventually both have similar remineralization potential which is higher than the fluoride regarding the surface microhardness with no or minimal effect in surface roughness for the three agents.

References

1. Selwitz RH, Ismail AI, Pitts NB. Dental caries. Lancent. 2007;369:51-59.

2. Kawasaki K, Ruben J, Tsuda H, Huysmans MC, Takagi O. Relationship between mineral distributions in dentine lesions and subsequent remineralization in vitro. Caries Res 2000; 34:395-403.

3. Hicks J, Garcia-Godoy , Flaitz C. Biological factors in dental caries: Role of remineralization and fluoride in the dynamic process of demineralization and remineralization (part 3). J Clin Pediatr Dent 2004; 28:203-214.

4. Chu CH, Mei ML, Lo EC. Use of fluorides in dental caries management. Gen Dent. 2010;58:37–43.

5. Reynolds EC. Additional aids to rernineralization of the tooth structure. In: Mount GJ, Hume WR, editors. Preservation and Restoration of Tooth Structure. 2nd ed. Brisbane, Australia: Knowledge Books and Software; 2005. pp. 111–8.

6. Reynolds EC. Remineralization of enamel subsurface lesions by casein phosphopeptide-stabilized calcium phosphate solutions. J Dent Res. 1997;76:1587–95.

7. Cai F, Shen P, Morgan MV, Reynolds EC. Remineralization of enamel subsurface lesions in situ by sugar-free lozenges containing casein phosphopeptide-amorphous calcium phosphate. Aust Dent J. 2003;48:240–3.

8. Salonen JI, Arjasmaa M, Tuominen U, Behbehani MJ, Zaatar EI. Bioactive glass in dentistry. J Minimum Interv Dent 2009;2:208-18.

9. Wefel JS. NovaMin: Likely clinical success. Adv Dent Res 2009;21:40-3.

10. . Madan N, Sharma V, Pardal D, Madan N. Tooth remineralization using bio-active glass - A novel approach. J Acad Adv Dent Res 2011;2:45-9.

11. Rao A, Malhotra N. The role of remineralizing agents in dentistry: A review. Compendium of continuing education in dentistry. 2011; 32(6):26-33.

12. Chou R, Cantor A, Zakher B. Prevention of dental caries in children younger than 5 years old: Systematic review to update the U.S. Agency for health care research and quality (US). 2014;104:1.

13. Gao SS, Zhang S, Mei MI, Chin-man Lo E, Chu CH. Caries remineralization and arresting effect in children by professionally applied fluoride treatment – a systematic review. BMC Oral Health. 2016;16:12.

14. Palaniswamy UK, Prashar N, Kaushik M, Lakkam SR, Arya S, Pebbti S. A comparative evaluation of remineralizing ability of bioactive glass and amorphous calcium phosphate casein phosphopeptide on early enamel lesion. Dental Research Journal. 2016; 13(4):297-302.

15. Vashisht R, Kumar A, Indira R, Srinivasan MR, Ramachandran S. Remineralization of early enamel lesions using casein phosphopeptide amorphous calcium phosphate: An ex-vivo study. Contemp Clin Dent. 2010;1(4):210-213.

16. Mehta AB, Kumari V, Jose R, Izadikhah V. Remineralization potential of bioactive glass and casein phosphopeptide amoprphous calcium phosphate on intial carious lesion: An in vitro pH-cycling study. Journal of Conservative Dentistry. 2014;17(1):3-7.

17. Esfahani KS, Mazaheri R, Pishevar L. Effects of treatment with various remineralizing agents on the microhardness of demineralized

enamel surface. Journal of Dental Research, Dental Clinincs and Dental Prospects. 2015;9(4):239-245.

18. China ALP, Souza NM, Gomes YSBL, Alexandrino LD, Silva CM. Effect of fluoride gel on microhardness and surface roughness of bleached enamel. The Open Dentistry Journal. 2014; 8:188-193.

19. Mathias J, Kavitha S, Mahalaxmi S. A comparison of surface roughness after micro abrasion of enamel with and without using CPP-ACP: An in vitro study. J Conserv Dent. 2009 Jan; 12(1):22-25.