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Effect of Nanohydroxyapatite with and without Chlorhexidine on Remineralization of Carious Dentine at Different Intervals: An in vivo study

Samaa A. Ibrahim[1], Maha A. Niazy [2], Mohammad A. El- Yasaky [3] *and* Naglaa S. El- Kilani [4]

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dentaljournal.forgirls@yahoo.com

ABSTRACT

This invivo study was carried out to evaluate the effect of nanohydroxyapatite (n-HAp); applied with or without prior application of chlorhexidine (CHX), on remineralization of caries- affected and caries infected dentine types. Forty molars in eight patients having class I caries and no pulpal involvement were selected. In each selected molar, caries was removed except from the pulpal floor. Thereafter, molars were divided into two groups (n= 20) according to the type of carious dentine left on the pulpal floor; caries- affected or infected dentine. Each group was further subdivided into two subgroups (n=10) according to the material that was applied on carious dentine; n-HAp with or without 2% CHX. All cavities were finally sealed with glass ionomer restoration. Assessment of mineral density was performed at base line, one and three months using digitized radiography. The results showed increased mineral density in caries- affected dentine group; with no significant difference neither n-HAp was applied with or without CHX, nor was the application time increased more than one month. In caries- infected dentine group, however, mineral density was decreased. Such decrease was significantly evident at the third month; when n-HAp was applied without CHX. It was concluded that n- HAp could only promote remineralization of caries-affected dentine. Additionally, although application of CHX prior to n-HAp showed no considerable influence on remineralization, yet, it could be recommended in case of caries- infected dentine as an antiseptic.

KEYWORDS

Carious Dentine, Chlorhexidine, Mineral Density, Nanohydroxyapatite, Remineralization.

1. Assisstant Lecturer of Operative Dentistry, Faculty of Dental Medicine, Al- Azhar University (Girls' Branch).
2. Professor of Operative Dentistry, Faculty of Dental Medicine, Al- Azhar University (Girls' Branch).
3. Professor of Operative Dentistry and Dean of Faculty of Dental Medicine, Sinai University.
4. Associate Professor of Oral and Dental Medicine, Periodontology, Radiology and Oral Diagnosis, Faculty of Dental Medicine, Al- Azhar University (Girls' Branch).

INTRODUCTION

Dental caries still remains a major public health problem. Traditional treatment of carious teeth, involving removal of all carious tooth structure prior to placement of the restorative material, often sacrificing more structure than necessary [1]. The first author who suggested that carious dentine could be rehardened in a similar manner to enamel was Head in 1912. Calcium hydroxide was employed as the possible remineralizing agent.

Nanohydroxyapatite (n- HAP) is considered one of the most biocompatible and bioactive materials and has gained wide acceptance in medicine and dentistry in the recent years. Nanosized particles have similarity to the apatite crystals of tooth enamel in crystal structure [2]. Additionally, an increasing number of reports have shown that n- HAP has the potential to remineralize artificial carious lesions; when added to tooth pastes, mouth washes, etc.[3, 4].

Incomplete removal of caries- infected dentine during cavity preparation, results in the trapping of active bacteria; that may later affect the pulp. So, the use of antibacterial agent is useful in eliminating these harmful effects[5]. Accordingly, dentine of the inner carious layer can be preserved and remineralized if the bacteria are removed or killed[6,7].

Chlorhexidine (CHX) has a broad spectrum of action against both Gram + ve and Gram – ve microbes. Additionally, it was found that chlorhexidine seemed to be effective in promoting the remineralization of demineralized dentine [8].

This study was, therefore, conducted to evaluate the effect of n- HAp with and without CHX on remineralization of caries- affected and caries- infected dentine types. Assessment of remineralization was performed at base line, one month and three months using digitized radiography.

MATERIALS AND METHODS

1. Case selection:

Eight healthy female patients in the age range of 25- 45 years were selected for this study. Each patient should have one or more molars (with a total of forty molars) containing occlusally active caries with no previous operative procedures, no spontaneous pain, no mobility and no tenderness on percussion. Radiographically, no internal or external resorption, no periapical or furcation radiolucency and no widening of periodontal ligament space [9]. All patients were asked to sign a written consent.

2. Cavities preparation and grouping of selected teeth:

In all selected molars, caries was removed using round bur # 023 (Komet, Lemgo, Germany) (1 bur/ 5 cavities) under water coolant. Caries was removed from everywhere in the cavity except for that on the pulpal floor. Where, only the overlying soft biological material was removed then, caries indicator (Sable ™ Seek ᴴ, Ultradent, USA) was utilized to identify the type of carious dentine at the pulpal floor: either affected or infected Teeth were divided into two groups (n= 20) according to the type of carious dentine (A) on which the materials under investigation would be applied; either affected (A_1) or infected (A_2). Each group was subdivided into two subgroups (n=10) according to the material that would be applied on the carious dentine (B); n- HAp with CHX (B_1) or n- HAp without CHX (B_2). Assessment of mineral density was performed at intervals (C) of: Base line (C_1), one month (C_2) and three months.

3. Application of 2% CHX solution:

2% chlorhexidine gluconate solution (Consepsis, Ultradent, USA) was applied on the pulpal floor in half of samples, left for 1 minute as recommended by Kim et al. [8], followed by a blast of compressed air for 5 seconds. Caution was taken not to over dry the preparation.

4. Application of n- HAp and sealing of cavities:

N- HAp (Nano Tech, Egypt) powder was mixed with saline as reported by Swarup et al. [10] using cement spatula. The mix was then transferred, by amalgam carrier, to half of the prepared cavities after CHX application, while n- HAp was applied without prior application of CHX in the other half, then condensed with amalgam condenser. Soon after, all cavities were sealed with glass ionomer (Medifil IX, Promedica, Germany).

5. Imaging of teeth:

A posterior parallel kit was utilized for aim of image standardization, the image plate was inserted at one end of the parallel kit, while the other end was connected to the X- ray cone. An X- ray machine (Kodac 2200, France) adjusted at 70 kV and 0.63 mA was used for imaging. The image plate was scanned by Durr Vista scan and the image was stored. These procedures were followed at 0 (base line), 1 month and 3 months intervals.

6. Assessment of remineralization:

DBSWin software was utilized for performing the measurements (Figure 1). In each sample a line was drawn along the CEJ to act as a reference, while another parallel line was drawn at the bottom of the cavity. The distance between both lines was standardized (for each sample) by measuring the length of a vertical line connecting them. The length of the line centralized at the bottom of the cavity was determined (in pixels) directly via the previously mentioned software. Such length was also, fixed for each sample throughout the assessment intervals.

Three points on this line (at its start, middle and end) were determined. The intensity at each of these points was identified by taking the corresponding three readings from the DBSWin software and recording it to calculate the average intensity. These procedures were done at base line (C1) then repeated after one month (C2) and three months (C3) intervals. The mineral density mean value was calculated from the equation (average density= average intensity/ profile); (the profile was determined directly in the utilized software). The change in density, for each sample, at different intervals were therefore, easily calculated. Results were calculated, tabulated and statistically analyzed.

Fig. (1) An image showing the way of measurement using DBSWin Programme.

RESULTS

Caries- affected dentine showed significant increase in mineral density compared to caries-infected dentine (P value< 0.001). Caries- affected dentine subgroups showed increased mineral density, with no significant difference neither n-HAp was applied with or without CHX, nor the application time was increased more than one month (P value= *0.114&0.229 respectively)*. In caries- infected dentine, however, mineral density was decreased. Such decrease was significantly evident at the third month of assessment; when n-HAp was utilized without CHX (P value= *0.005) in contrary to when n-HAp was used with CHX (P value= 0.873)*. Results of mineral density (mean values± SDs) for both carious dentine types as function of the remineralizing materials applied and different assessment intervals are listed in table (1) and graphically illustrated in figure (2).

Three way ANOVA revealed significant influence (P< 0.05) of type of carious dentine on remineralization (caries- affected dentine > caries infected dentine). The type of the remineralizing material applied, however, was statistically non significant (n- HAp with CHX > n- HAp without CHX). The influence of the assessment interval was revealed also to be statistically non-significant (P > 0.05). (Table 2).

Table (1) *Mineral density (mean values± SDs) for both carious dentine types as function of the remineralizing materials applied and different assessment intervals.*

	Caries- affected treated with n- HAp with CHX	Caries- affected treated with n- HAp without CHX	Caries- infected treated with n- HAp with CHX	Caries- infected treated with n- HAp without CHX
Density (base line)	69.82±3.53	65.89±6.64	62.45±6.66	58.08±2.53
Density (after 1 month)	70.71±7.30	66.14±6.62	62.10±9.69	57.56±5.90
Density (after 3 months)	75.43±3.46	71.43±6.35	60.14±9.97	51.20±1.27
P- value	0.114	0.229	0.873	0.005*

** Superscript means statistically significant at P≤ 0.05.*

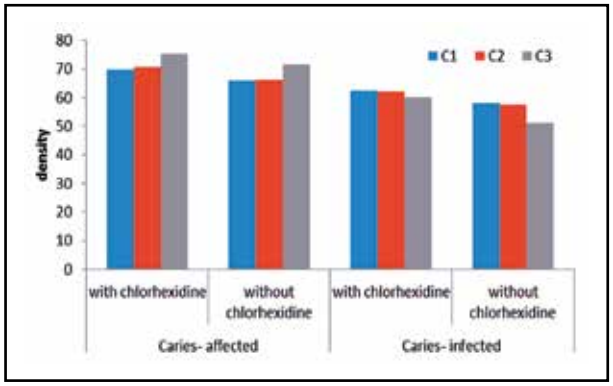

Fig. (2) Multiple column chart of mineral density mean values for both carious dentine types as function of the remineralizing materials applied and different assessment intervals.

Table (2) *Three way ANOVA of significance comparing variables affecting mineral density mean values.*

Variable	Sum of Squares (SS)	DF	Mean Square (MS)	F	P value
Type of carious dentine	2689.324	1	2689.324	66.358	<0.001*
Remineralizing material	537.750	1	537.750	13.269	0.067
Assessment interval	3.955	2	1.977	.049	0.952

** Superscript means statistically significant at P≤ 0.05.*

DISCUSSION

Dentine demineralization (other than that caused by acid etching for resin- dentine bonding) could result from an acid attack by bacteria and / or a food source [8]. This study adopts the remineralization of carious dentine rather than the surgical model of treatment.

The remineralizing material selected for this study was n- HAp with or without CHX. The former; (n- HAp), provides an external source of calcium and phosphorous. The latter; (CHX), however, was selected due to its well- known anti-bacterial activity, beside its action as MMPs (matrix metalloproteinases) inhibitor [11], therefore, CHX was utilized to protect the demineralized exposed collagen matrix from degradation accompanying the caries process.

Results of the in vivo part of this study showed that, for caries- affected dentine: the subgroup treated with n- HAp with CHX showed increased mineral density than n- HAp without CHX. No significant difference, however, was found between both at neither one month nor three months of assessment. The increase in mineral density mean values in both sub groups; either n- HAp was applied with or without CHX, can relatively refer to n- HAp remineralizing ability. Such ability was attributed to the similarity of n- HAp to the chemistry and size of natural tooth mineral component. Additionally, the nanosize increases its reactivity and penetrability into the lesion.

The statistically insignificant difference either n- HAp was used with or without CHX came in contrary to the results obtained by Kovtun et al. [12], who claimed that this combination has provided both remineralizing and antibacterial actions. The difference from this study is that they used HAp loaded with CHX in a single particle in the nanometric size. Also, the nanoparticles were placed in rinsing solution in contrary to the full concentration n- HAp powder used in this study. Additionally, substrates of both studies were different; in their study the tested material was applied on root lesion, in this study however, they were applied on coronal carious dentine.

Regarding caries- infected dentine, mineral density was decreased in both subgroups of n- HAP; either with or without CHX. This could be probably due to the heavy load with microorganisms, high degree of dentine degradation, and loss of much of mineral seeds. Therefore, remineralization was hindered and instead, demineralization continued. Oppositely, Mertz- Fair hurst reported mostly bacteriologically inactive lesions after sealing occlusal dentinal lesions [13]. It should be emphasized that; their study included shallower lesions exhibiting demineralized but not heavily infected dentine. This study, however, included advanced lesions in which high degree of infection and dentine degradation were expected.

Decrease in mineral density mean values was less in the subgroup treated with CHX than that with no CHX which can be illustrated on basis of CHX antibacterial and anti MMPs actions. The difference, however, was not statistically significant after one month either due to the heavy bacterial load and/ or variety of included microorganisms. The latter may be resistant to CHX especially that the CHX application time in this study was one minute; which is long enough to allow additional microorganisms to reach the cavity during application. This necessitates longer time for CHX to exert its antibacterial action than one month, however, the effect of CHX was significantly evident on the third month.

CHX was however, unable to completely prevent decrease in mineral density, this may be due to that lactobacillus species are predominant in deep carious lesions more than mutans streptococci. CHX was proven by several studies to show more efficacy on mutans streptococci than lactobacilli[14]. Additionally, it is worth to mention that CHX anti- collagenolytic activity; that was evidenced in studies of Carrilho et al.[15] and Brackett et al.[16], was consistent with sealing of CHX into the

dentinal tubules with resin restoration, accordingly, the formed resin tags minimize CHX removal by the outflow of dentinal fluids, which is not the case in the present study. Also, they used non carious teeth; in contrary to the present study.

There was a significant difference in mineral density between caries- affected and caries- infected dentine recorded at all assessment intervals. This would be explained on basis that; caries- affected dentine with partial demineralization has more –ve charge than caries- infected dentine, so, its surface can attract more calcium ions (+ve charge) and thereby, the nucleation ability of its surface is greater than caries infected dentine [17].

CONCLUSION

N- HAp could promote remineralization of caries- affected dentine in contrary to caries- infected dentine. One month interval, however, seemed sufficient for such remineralizing effect. Additionally, although CHX showed no considerable influence on remineralization. Yet, it can be recommended for use with infected dentine for its antiseptic role.

REFERENCES

1. Yoshiyama M, Doi J, Nishitani Y, Itota T, Tay F, Carvalho R, Pashley D. Bonding ability of adhesive resins to caries affected and caries infected dentine. J Appl oral Sci 2004; 12(3): 171- 6.
2. Tschoppe p, Zandim D L, Martus P, Kielbassa A M. Enamel and dentine remineralization by nanohydroxyapatite tooth pastes. J Dent 2011; 430- 7.
3. Lu K L, Zhang J X, Meng X C, Li X Y. Remineralization effect of the nano-HA tooth paste on artificial caries. Key Eng Mater. 2007; 2: 267- 70.
4. Kim M Y, Kwon H K, Choi C H, Kim B L. Combined effects of nano- H A and NaF on remineralization of early carious lesion. Key Eng Mater. 2007; 330- 32:1347- 50.
5. Hiraishi N, Yiu C K Y, King N M, Tay F R. Effect of 2 % chlorhexidine on dentine microtensile bond strength and nanoleakage of luting cements. J Dent 2009; 440- 8.
6. Cury J A, Rebello M A, Del Bel CA, Derbyshire M T, Tabchoury C P. Biochemical composition and cariogenicity of dental plaque formed in the presence of sucrose or glucose and fructose. Caries Res 2000; 34: 491- 7.
7. Lima J P, Sampaio de Melo M A, Borges F M, Teixeira A H, Steiner- Oliveira C, Nobre- dos- Santos M et al. Evaluation of the antimicrobial effect of photodynamic antimicrobial therapy in an in situ model of dentine caries. Eur J Oral Sci 2009; 117: 568- 74.
8. Kim D S, Kim J, Choi K K, Kim S Y. The influence of chlorohexidine on the remineralization of demineralized dentine. J Dent 2011; 39(12): 855- 62.
9. Niazy MA, El- Marsafy SMA and Nour El- Deen MM. Clinical and radiographic evaluation of two tissue engineering materials(Bio Gen and Bioaggregate) and direct pulp capping agent in human tooth. Cairo Dent J 2011; 27(1): 17- 26.
10. Swarup S, Rao A and Suprabha B. S. Pulp regeneration using n- HAp as scaffold in an immature central incisor: A 10- month follow up. J Interdisiplinary Dent 2014; 4(2): 89- 92.
11. Komori PC, Pashley DH, Tjäderhane L, Breschi L, Mazzoni A, de Goes MF et al. Effect of 2% chlorhexidine digluconate on the bond strength to normal versus caries-affected dentine. Oper Dent. 2009; 34(2): 157- 65.
12. Kovtun A, Kozlova D, Ganesan K, Biewald C, Seipold N, Gaengler P et al. Chlorhexidine- loaded calcium phosphate nanoparticles for dental maintenance treatment: Combination of mineralizing and antibacterial effects. RSC Advances 2012; 2: 870- 5.
13. Mertz- Fairhurst E J. Arresting caries by sealants: Results of a clinical study. J Am Dent Assoc 1986; 112: 194- 7.
14. Wicht M J, Haak R, Schütt-Gerowitt H, Kneist S, Noack M J. Suppression of caries- related microorganisms in dentine lesions after short-term chlorhexidine or antibiotic treatment. Caries Res 2004; 38: 436–41.
15. Carrilho M R, Geraldeli S, Tay F, de Goes M F, Carvalho R M, Tjaderhane L et al. In vivo preservation of the hybrid layer by chlorhexidine. J Dent Res 2007; 86: 529- 33.
16. Brackett M G, Tay F R, Brackett W W, Dib A, Dipp F A, Mai S et al. In vivo Chlorhexidine stabilization of hybrid layers of an acetone- based dentine adhesive. Oper Dent 2009; 34: 379- 83.
17. Yang B, Flaim G and Dickens S. H. Remineralization of human natural caries- like lesions with an experimental whisker- reinforced ART composite. Acta Biomater 2011; 7: (5): 2303- 9.