ISSN: 2581 – 3633
PubMed - National Library of Medicine - ID: 101745081

International Journal of Medical Science and Advanced Clinical Research (IJMACR)

Available Online at:www.ijmacr.com

Volume – 7, Issue – 3, June - 2024, Page No. : 165 – 171

Comparative Evaluation of Remineralsing Efficiency of Nanohydroxyapatite Particle, CPP-ACP And Sodium Fluoride on Artificially Induced Caries In Dentin: An In Vitro Study

[1]Dr.Rimpy Mehra, Post Graduate Student, Department of Conservative Dentistry and Endodontics, H.P.G.D.C, Shimla, India

[2]Dr.Ashu K. Gupta, Principal, H.P.G.D.C, Shimla, India

[3]Dr.Neha Sharma, Senior Lecturer, Department of Conservative Dentistry and Endodontics, H.P.G.D.C, Shimla, India

Corresponding Author: Dr. Rimpy Mehra, Post Graduate Student, Department of Conservative Dentistry and Endodontics, H.P.G.D.C, Shimla, India.

How to citation this article: Dr. Rimpy Mehra, Dr.Ashu K. Gupta, Dr.Neha Sharma, "Comparative Evaluation of Remineralsing Efficiency of Nanohydroxyapatite Particle, CPP-ACP And Sodium Fluoride on Artificially Induced Caries In Dentin: An In Vitro Study", IJMACR- June- 2024, Volume – 7, Issue - 3, P. No.165 – 171.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Introduction: Dental caries is one of the most prevalent chronic diseases. In this study, use of remineralising agents like nanohydroxyapatite, CPP-ACP and sodium fluoride was done to check their remineralising efficiency using SEM-EDX, ATR/FTIR and Vickers hardness test.

Aim and objective: To compare the remineralizing efficiency on artificially induced caries in dentin by Nanohydroxyapatite particles (dent 91), CPP-ACP (GC tooth mousse) and NaF (colgate) using surface hardness test, SEM EDX analysis and FTIR.

Materials and methodology: 40 extracted premolars were taken and 2mm dentin slices were made then these slices were treated with demineralising solution subsequently remineralising agents were applied and ph cycling was done for 21 days to evaluate remineralising efficiency. measurements were taken to quantify calcium and phosphate levels by SEM-EDX, hardness was evaluated using Vickers hardness test and phosphate to amide ratio was checked using ATR/FTIR.

Results: The calcium and phosphate content, hardness value and phosphate to made ratio in the nanohydroxyapatite containing samples was highest as compare to other groups.

Conclusion: Within the limitation of present study, it can be concluded that all three remineralising agents exhibited significant remineralising potential with dent 91 toothpaste containing nanohydroxyapaptite displaying best result in mineral gain and surface hardness followed by cpp acp containing toothpaste (GC

tooth mousse) and then fluoride containing paste (colgate).

Keywords: remineralsiisation, surface microhardness, nanohydroxapatite paste, CPP-ACP, sodium fluoride.

Introduction

Dental caries is one of the most prevalent chronic diseases[1.] It is defined as a multifactorial microbial infectious disease characterized by demineralisation of inorganic and destruction of the organic substance of the tooth.

Permanent enamel is an acellular tissue composed chiefly of minerals, 85% in volume. On the other hand, permanent dentin contains (by volume) 47% apatite, 33% organic components and 20% water. Therefore, the ratio of surface area/crystallite volume is larger, which makes the mineral phase more reactive. As a result, dentin surfaces are more susceptible to caries attack than enamel surfaces. The organic matrix is mainly composed of collagen (90%), but there are many non- collagenous components that determine the properties of the matrix and interfere with de- and remineralization reactions.

In the past fluoridated dental products were considered utmost to prevent caries but excessive amount of fluoride intake can lead to fluorosis and inordinate fluoride application can cause occult or hidden caries.

In recent years new materials have been introduced that could remineralize dentin, one of them is casein phosphopeptide amorphous calcium phosphate (CPP-ACP). Casein phosphopeptide forms nanoclusters with amorphous calcium phosphate thus providing a pool of calcium and phosphate which can maintain the super saturation of saliva and it also has antibacterial properties.

Today, the science of engineered nanoparticles (NPs) offers alternative strategies for the remineralization of acid-affected enamel and dentin.The Nano-hydroxyapatite has a strong ability to bond with proteins, as well as with fragments of plaque and bacteria, when contained in toothpastes.

In this study we aim to compare the remineralising efficiency of three different remineralising agent (Nanohydroxyapatite, casein phosphopeptide-amorphous calcium phosphate and sodium fluoride) by evaluating changes in inorganic and organic composition of artificially induced caries in dentin.

To compare the changes in inorganic content EDX was done and to check organic content ATR/FTIR was done and to check microhardness Vickers test was done.

Materials and method

Study design: cross sectional comparative study**.**

Study population: 4o intact, mature premolars are selected.

Inclusion criteria: Morphologically intact non carious premolars extracted for orthodontic and periodontal reasons will be used in this study.

Exclusion criteria: Fractured teeth, Eroded teeth, Cracked teeth, Carious teeth, Restored teeth, Teeth with altered morphology (crown or root)

Data analysis: Data will be entered in MS Excel and analysed using epi info V7 software.

Qualitative variables will be expressed in frequency and % while quantitative variables will be expressed in mean and SD.

Statistical evaluation of difference in Collagen degradation and changes in calcium and phosphate content of the dentin disk after use of remineralizing agent will be performed by ANOVA test.

P value of less than 0.05 will be considered as statistically significant.

Method

Forty freshly extracted premolars of age group less than 16 years were collected within the period of three months. All the selected teeth were washed properly stored in 0.1 % thymol solution at room temperature.

Then all the extracted teeth were cut into eighty dentin slices above the level of CEJ of 2mm thickness using Carborundum disc. All these slices were free of coronal enamel (coronal enamel was removed before cutting the dentin slices with the help of Carborundum disc).

The coronal side of dentin surface was roughened using silicon carbide papers and then washed with distilled water while the opposite side was coated with acid resistant nail polish.

The demineralising solution was made by adding 2.2 mM Cacl2, 2.2 mM potassium dihydrogen orthophosphate and 0.05 mM acetic acid in a beaker and mixed with the help of stirrer then ph strips were used to check ph of the solution. The remineralising solution was made by adding 1.5 mM Cacl2, 0.90 mM potassium dihydrogen orthophosphate and 130 mM potassium chloride in a beaker and all solutions were mixed with the help of stirrer then ph of solution was adjusted to 7 which was monitored by ph strips.

All the prepared dentine slices were immersed in demineralizing solution for 72 hours at room temperature and then washed with distilled water.

The demineralized dentin slices were randomly divided into four groups each containing twenty samples.

Group 1: Distilled water

Group 2: NaF (Colgate)

Group3: Nanohydroxyapaptite particles (Dent 91)

Group 4: Casein phosphopeptide-amorphous calcium phosphate (GC tooth mousse)

After dividing into 4 groups the remineralizing agent was applied and then these dentin slices were put into remineralizing solution.

The application of remineralizing agent on the dentin slices from the above groups was done with the help of toothbrush for 2 minutes and then these dentin slices were rinsed thoroughly by distilled water. It was done to mimic the real situation.

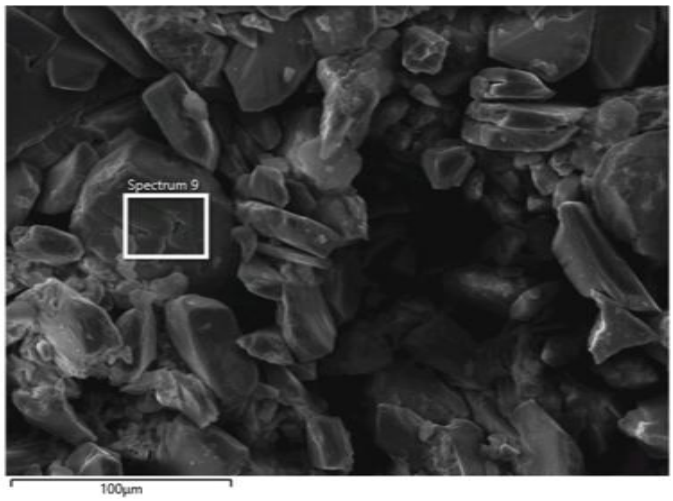
After the above procedure, dentin slices were put again in demineralizing solution for 8 hours followed by remineralizing solution for 16 hours. In between changing of solutions the dentin slices were treated with remineralizing agent.

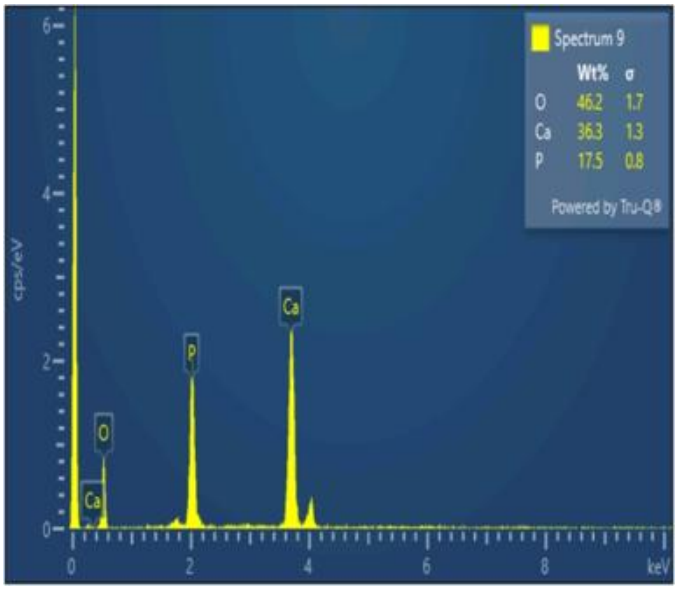
This procedure of putting dentin slices in alternate manner in demineralizing and remineralizing solution is called ph cycling and it was done for 21 days.

This ph cycling model was used to simulate the dynamic variation in mineral saturation and ph altering with natural caries process, which refers to exposure of dentin to combination of demineralization and remineralization.

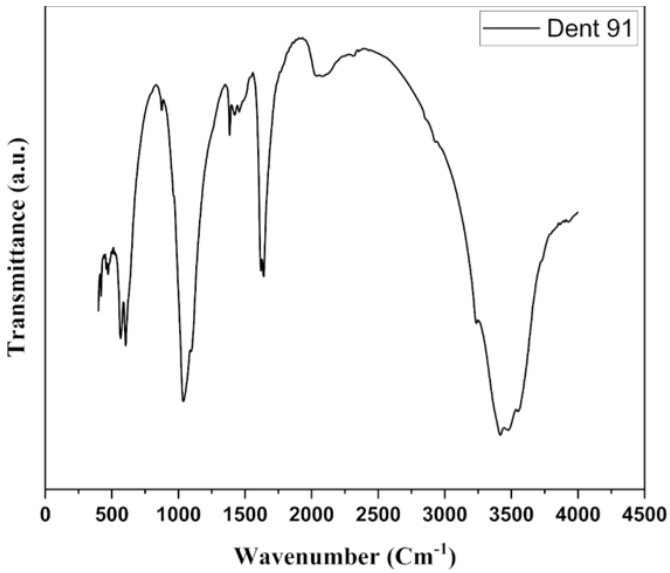
After completion of procedure sample were evaluated using SEM-EDX, ATR/FTIR and Vickers microhardness test.

Figure 1:





Graph: 1



Graph: 2

Statistical Analysis

The statistical analysis was done using Statistical Package for the Social Sciences (SPSS for Windows, Version 19.0). Descriptive statistics were calculated as mean and standard deviation. Prior to analysis, the normality testing of data was done using Shapiro-Wilk test which showed that the data were normally distributed (P>0.05). Thereafter, the comparison of study parameters among the study groups was done using Analysis of Variance (ANOVA). In case of statistical significance, Tukey's post-hoc test was used for multiple comparisons. The level of significance for the present study was fixed at a p-value of less than 0.05.

Treatment of entire surface with nanohydroxyapatite i.e., (group 3) produced highest surface mean hardness value and mineral deposition. The hardness was significantly more than fluoride group (group 2), control group (group1) as well as cpp–acp group (group 4). cpp-acp also shows increased mineral content of calcium, phosphate.

The mean calcium and phosphorus level of group 4 is higher than group 1 and 2 but not greater than group 3 that is dent 91 containing nanohyroxyapatite. This comparison is shown in table 1 and 2 respectively. And also surface hardness value of group 4 is also greater than group 2 but not greater than group 3 and group 1.

The group 2 has lower calcium values than group 3 and 4 but has higher value than group 1.

The ATR- FTIR result shows that after the dentin slices were demineralised the peak appeared at 3312 cm-1, which was the stretching peak of –OH bond. The characteristic peak at 1467 cm-1 was the amide I, the one at 1550 cm -1 was the characteristic peak of amide II, the one at 1239 cm -1 was the characteristic peak of amide III band, and the peaks at 1374 and 1465 cm-1 were the vibration peaks of CH.

After the demineralised dentin slices were treated with cpp-acp the peak of 1077 cm-1 was increased which indicates that sample was phosphorylated and it indicates that slices were remineralized. In group 2 peak is shown at 1018cm-1 which has hydroxyapatite peak, this indicates that crystals formation has occurred.

Discussion

Dental caries is a dynamic process of continual changes of pathological demineralization and physiological protective remineralization of dental hard tissue which is mainly composed of hydroxyapatite (HA), collagen and non-collagenous matrix proteins.[2]

The majority of the ongoing research on enamel and dentin remineralization focus on

1. increasing the availability of calcium ions to foster apatite precipitation in deeper areas of the demineralized tissue, and
2. The use of biomimetic analogues for ion transport and to guide mineral deposition in order to restore the tissues original organization and functionality.

An inherent challenge in evaluating a material's ability to remineralize dentin lies in the fact that an increase in mineral content after treatment is not necessarily associated with improved physical properties like elastic modulus, hardness, and strength. [3]Apatite mineral in dentin needs to be bound to the collagenous matrix or ideally be incorporated into the collagen fibrils to mechanically reinforce the tissue.[4]Remineralization that leads to improved tissue mechanics is called functional remineralization (FR).

Over the years, clinical and scientific evidence have shown the benefits of minimally invasive dentistry .One of the cornerstones of this concept is the possibility of remineralizing initial enamel lesions or the caries-affected dentin with application of various remineralising agents available.

Besides fluorosis in children, surface only remineralisation that often occurs in the presence of high topical fluoride concentrations can increase the incidence of occult caries (fluoride syndrome) across all age groups (Ball, 1986).

So, there is a need for new age remineralisation technology with an ability to complement fluoride, and effect a fuller consolidation of carious lesion.

First parameter checked was Vickers hardness test because the diagonals in the square indenter are easily detected. The tooth samples were impressed by a diamond indenter at load of 100gm for 15 seconds. After load removal diagonals of indentation were measured with an optical microscope.

Second parameter checked in our study for evaluation of remineralization was high resolution scanning electron microscopy with energy dispersive X ray analysis. This test is used for evaluation of mineral loss or gain in experimentally induced caries lesions and is used for quantification of various ions such as Ca, P, F, Si, O etc.

Third parameter checked in our study was attenuated total reflection-fourier transform spectroscopy (ATR-FTIR) which uses mid infrared region of wavelength 400 to 4000 cm-1. The mid infrared region including characteristic infrared bands for the mineral phase (phosphate and carbonate ions) and organic phase (collagen amide I and amide III).

In our study cpp- acp also shows increased mineral content of calcium, phosphate. CPP is a bioactive peptide extracted from casein in milk by trypsinization and shows excellent biocompatibility. [5-7]

CPP–ACP is formed by the complexation of casein phosphopeptide (CPP) with amorphous calcium phosphate (ACP) via a phosphorylated peptide chain (containing sequence SPSPSPEE). [8]Since CPP has a space compartment effect, which prevents the formation and growth of calcium phosphate crystals, calcium phosphate is probably complexed with CPP in an

amorphous state to form a stable CPP–ACP nanocomposite.The demineralising solution for preparation of artificial dentin caries contained 2.2 mM Cacl2, 2.2 mM potassium dihydrogen orthophosphate and 0.05 mM acetic acid the specimens were soaked in freshly prepared solution for 72 hours. The ph of this solution is 4.4 which was monitored using ph strips.

After demineralisation application of remineralising agents was done and these specimens were then immersed in remineralising solution for 16 hours. This procedure is repeated in an alternate manner for 21 days. This is called ph cycling. The ph-cycling procedure, standardized by Marquezan, et al (2009), is one of the best methods to simulate affected dentin caries.In our study group 3 and group 4 shows higher hardness value than group 1 and group 2 and mineral content of all remineralising agent was higher than group1.Nano HAP containing toothpaste had highest remineralising efficacy among all the remineralising agents.

Conclusion

Within the limitation of present study, it can be concluded that all three remineralising agents exhibited significant remineralising potential with dent 91 toothpaste containing nanohydroxyapaptite displaying best result in mineral gain and surface hardness followed by cpp acp containing toothpaste (GC tooth mousse) and then fluoride containing paste (colgate).

References

1. Rocha Gomes Torres C, Borges AB, Torres LM, Gomes IS, de Oliveira RS. Effect of caries infiltration technique and fluoride therapy on the colour masking of white spot lesions. J Dent. 2011;39(3):202–207.
2. Zhou XD. Dental caries: principles and management. China: Springer; 2015.
3. Burwell AK, Thula-Mata T, Gower LB, Habelitz S, Kurylo M, Ho SP, et al. Functional remineralization of dentin lesions using polymer-induced liquid-precursor process. PLoS One. 2012;7(6): e38852.
4. Bertassoni LE, Habelitz S, Marshall SJ, Marshall GW. Mechanical recovery of dentin following remineralization in vitro–an indentation study. J Biomech. 2011;44(1):176–81. H
5. Ranjitkar S, Narayana T, Kaidonis JA, Hughes TE, Richards LC, Townsend GC. The effect of casein phosphopeptide-amorphous calcium phosphate onerosive dentine wear. Aust Dent J. 2009;54(2):101–7.
6. Grychtol S, Basche S, Hannig M, Hannig C. Effect of CPP/ACP on initial bioadhesion to enamel and dentin in situ. ScientificWorldJournal. 2014;2014:512682.
7. Lenton S, Nylander T, Teixeira SC, Holt C. A review of the biology of calcium phosphate sequestration with special reference to milk. Dairy Sci Technol. 2015;95:3–14.
8. Dashper SG, Catmull DV, Liu SW, Myroforidis H, Zalizniak I, Palamara JE, Huq NL, Reynolds EC. Casein phosphopeptide-amorphous calcium phosphate reduces streptococcus mutans biofilm development on glass ionomer cement and disrupts establishedbiofilms. *PLoS*ONE. 2016;11(9):e016232 2.

Legends Tables

Table 1: Comparison of calcium levels among the study groups

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		P value
					Lower Bound	Upper Bound	
Control	7	20.9857	.82952	.31353	20.2185	21.7529	<0.001*
Colgate	7	23.9143	1.86497	.70489	22.1895	25.6391	
Dent 91	7	37.7857	1.92044	.72586	36.0096	39.5618	
GC Tooth Mousse	7	26.7857	3.63613	1.37433	23.4229	30.1486	
Total	28	27.3679	6.82360	1.28954	24.7219	30.0138	

Table 2: Comparison of phosphorus levels among the study groups

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		P value
					Lower Bound	Upper Bound	
Control	7	14.9143	1.75635	.66384	13.2899	16.5386	<0.001*
Colgate	7	15.0571	1.01465	.38350	14.1187	15.9955	
Dent 91	7	19.5286	1.85806	.70228	17.8102	21.2470	
GC Tooth Mousse	7	18.1143	1.64664	.62237	16.5914	19.6372	
Total	28	16.9036	2.52197	.47661	15.9257	17.8815	