ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

International Journal of Dental Science and Innovative Research (IJDSIR)
IJDSIR : Dental Publication Service
Available Online at: www.ijdsir.com
Volume – 7, Issue – 3, May – 2024, Page No. : 149 - 156

In Vitro Comparison of Enamel Remineralization in Primary Teeth using Polyamino Amine vs. Casein Phosphopeptide-Amorphous Calcium Phosphate Fluoride

[1]Sanka Sri Meghana, Post graduate, SRM Kattankulathur Dental College, SRM Institute of Science and Technology, Potheri, Kattankulathur, Chengalpattu District, Tamil Nadu, India

[2]Kavitha Ramar, Professor and HOD, SRM Kattankulathur Dental College, SRM Institute of Science and Technology, Potheri, Kattankulathur, Chengalpattu District, Tamil Nadu, India

Corresponding Author: Kavitha Ramar, Professor and HOD, SRM Kattankulathur Dental College, SRM Institute of Science and Technology, Potheri, Kattankulathur, Chengalpattu District, Tamil Nadu, India.

Citation of this Article: Sanka Sri Meghana, Kavitha Ramar, "In Vitro Comparison of Enamel Remineralization in Primary Teeth using Polyamino Amine vs. Casein Phosphopeptide-Amorphous Calcium Phosphate Fluoride ", IJDSIR- May – 2024, Volume –7, Issue - 3, P. No. 149 – 156.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Introduction: The most common oral disease is tooth decay, primarily caused by the demineralization of the hard structures in teeth. This occurs when acids produced by cariogenic bacteria increase, shifting the balance in favour of demineralization and resulting in net mineral loss, white spot lesions, and eventually cavitation. Increasing calcium (Ca) and phosphate (P) ions is essential to promote tooth remineralization.

Aim: This study aims to assess and compare the remineralization capabilities of casein phosphopeptide amorphous calcium phosphate with fluoride (CPP-ACP-F) and polyamino amine (PAMAM) on demineralized enamel in primary teeth.

Materials and Methodology: To create artificial carious lesions, thirty enamel specimens were produced and immersed in a demineralizing solution with a pH of 4.4 for ninety-six hours at 37°C. A 30-day remineralization phase was conducted with PAMAM and CPP-ACP-F. Using SEM-EDX, the specimens' calcium and phosphorus contents were assessed at three intervals: baseline, post-demineralization, and post-remineralization.

Results: According to Vickers hardness test images, the PAMAM-treated group exhibited hardness similar to normal tooth enamel, with the least hardness noted in the control group. SEM images indicated fewer micro porosities and greater amorphous deposits in the PAMAM group compared to the CPP-ACP-F group. One-way ANOVA statistical analysis, followed by multiple comparison tests, revealed a significant difference between the control group and both the CPP-ACP-F and PAMAM groups.

Conclusion: The investigation concludes that while CPP-ACP-F is effective in remineralizing early enamel caries, PAMAM is more effective. Hence, the topical application of PAMAM and CPP-ACP with fluoride-containing toothpastes plays a remarkable role in the remineralization of initial carious lesions.

Keywords: PAMAM, CPP-ACP, Demineralization-Remineralization, Fluoride.

Introduction

In the dynamic environment of the oral cavity, the continual processes of demineralization and remineralization play a fundamental role in maintaining the health and integrity of teeth. These processes involve a complex exchange of ions between the hard tissues of teeth and the oral environment, influencing the hardness and structure of the tooth.[1] The balance between demineralization and remineralization is crucial, as an imbalance favouring demineralization can lead to the formation of white spot lesions, which are early indicators of tooth decay. If these lesions are left untreated, they can progress into more severe cavities, requiring extensive intervention.[2]

However, the concept of minimal intervention dentistry emphasizes that even at its most basic level, there is still a possibility for the remineralization of white spot lesions. Remineralization, characterized by the reversal of the gradients of calcium and phosphate to promote inward diffusion, can effectively reverse the early stages of carious lesions. This process is seen as a significant breakthrough in the clinical treatment of caries, offering a conservative approach to managing early decay. [3]

Products containing bioavailable forms of calcium phosphate and fluoride have shown to be highly effective in promoting remineralization compared to products containing fluoride alone. These materials provide the necessary ions to support enamel remineralization, enhancing the tooth's natural defences against decay. [3]

Moreover, modern dental materials such as CPP-ACP-F have been developed to enhance enamel remineralization further. [4] These materials are designed to biomineralize enamel, making it more resistant to acid attacks and erosion. Amorphous calcium phosphate casein phosphopeptide with fluoride (CPP-ACP-F) and polyaminoamine are utilised in this study. The current study aims to assess and compare the remineralization capacity of CPP-ACP-F and polyamino amine (PAMAM) on demineralized enamel in extracted primary teeth.

Materials and Methods

The Institutional Ethics Committee approved the research project Institution Ethical Committee of SRM Institute of Science and Technology [IEC No.-8295] and this in vitro experimental investigation.

Sample size calculations- It was determined by utilizing data from a Varughese JM et al, Varghese NO et al, and Lata S et al research paper [5]. Thirty enamel specimens in total were taken into account. The assumptions used in this computation were the confidence interval of 95%, power of 80%, threshold of significance of 0.001, and exact difference between treatments, corrected to 4 units. SPSS software version 20 computations served as the basis for this.

Sample Preparation

To prepare the enamel specimens for analysis, an individual examiner trained in Scanning Electron Microscopy-Energy Dispersive X-ray (SEM-EDX) analysis performed the following steps:

Selection of Teeth

Thirty cavity-free enamel specimens were selected from the buccal surfaces of extracted human primary teeth. [6] The selection criteria excluded teeth with visible caries

lesions, including white spot lesions, hypoplastic lesions, developmental defects, or any other crown deformities. Teeth that had undergone any kind of therapeutic treatment were also excluded.

Cleaning and Examination

The soft tissue residues were meticulously cleaned using an ultrasonic scaler. Each tooth was then examined under magnification to identify and exclude any that showed signs of cracks, hypoplasia, or white spot lesions.

Sectioning of Teeth

At the level of the cementoenamel junction (CEJ), each tooth was horizontally sectioned using a diamond disc attached to a micromotor straight handpiece (NSK Japan) operating at 15,000 rpm. [7] This process separated the crown portion of the tooth. Subsequently, the crown's cusp and occlusal surfaces were removed using the same diamond disc and micromotor handpiece to obtain a flat surface for analysis.

Preparation of Enamel Windows

From each tooth, enamel windows measuring approximately 4 mm by 4 mm by 2 mm were created. These windows served as the specific areas for experimental analysis. [8] The remaining portion of each tooth specimen was carefully coated with nail varnish, leaving only the small rectangular enamel window exposed. This coating ensured that the exposed enamel was the only area subject to acid attack during the demineralization process. [9]

Baseline Analysis

Elemental analysis to determine the baseline calcium (Ca) and phosphorus (P) content was conducted using SEM-EDX. This initial analysis provided the control measurements for comparison after the demineralization and remineralization processes.

Creation of Artificial Carious Lesions

To simulate carious lesions, the enamel specimens were submerged in a demineralizing solution (5 mL per specimen) with a pH of 4.4 for ninety-six hours at 37°C. [10] This acidic environment promoted the loss of minerals, creating artificial carious lesions in the enamel.

Post-Demineralization Analysis

Following demineralization, each specimen underwent SEM-EDX analysis to estimate the loss of calcium and phosphorus, confirming the extent of demineralization and providing data for subsequent comparison.

Group Assignment and Treatment: The thirty sectioned enamel specimens were randomized into three treatment groups, each containing ten specimens:

Group	Description
Group 1	Polyamino amine generation 0 liquid was used
Group 2	CPP-ACP-F: composition: xylitol, phosphoric acid, silicon dioxide, titanium dioxide, ethyl-p-hydroxybenzoate, magnesium oxide, guar gum, propyl p-hydroxybenzoate, butyl p-hydroxybenzoate, glycerol, 10% CPP-ACP (10,000 ppm), D-sorbitol, propylene glycol, phosphoric acid, and 0.2% sodium fluoride (900 ppm).
Control	No remineralization agent

Table 1: Samples grouping have been tabulated

Each treatment group's specimens were subjected to a 30-day phase of remineralization, with daily applications of their respective remineralizing agents using a cotton applicator tip. After each treatment, the specimens were rinsed with distilled water and stored in artificial saliva at 37°C to mimic the oral cavity environment. [10] The artificial saliva was replaced every 24 hours to maintain consistency.

Following the remineralization period, the specimens underwent further Vickers hardness test and SEM-EDX analysis to evaluate changes in mineral content and

surface structure, allowing for a detailed comparison of the effectiveness of each treatment method.

Vickers hardness test: Employing the window's centre, each specimen was divided in half lengthwise. The sliced surface was polished and left uncovered. Five indentations in a row were positioned around 100 microns below the enamel's surface. Cross-sectional microhardness (CSMH), which measures variations in microhardness at the subsurface level under the same load and time parameters, was assessed for each section. At that point, a formula was used to calculate the surface microhardness values' percentage of mineral recovery.

Specimen Preparation for SEM-EDX: A thin layer of silver foil was employed as a sputter to sputter the enamel specimens[10]. Following sputtering, EDX was used to assess the specimens' mineral content (mass/atomic percentage). The EDX results' digital outputs were mathematically interpreted as baseline Ca/P ratios following demineralization and remineralization.

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 16.0 was utilised to conduct a statistical analysis of the acquired values. The data was analysed using the One-way ANOVA and Turkey Multiple Comparison Test. At the $p<0.05$ threshold, the significance was demonstrated.

Results

The study aimed to assess the effectiveness of different remineralizing agents on tooth enamel hardness and microstructure following demineralization.

1. Hardness Assessment

Vickers hardness test images revealed that the PAMAM-treated group (Fig. *1D*) exhibited hardness similar to normal tooth enamel (Fig. *1B*), followed by the CPP-ACPF group (Fig. *1E*), while the control group (Fig. *1C*) showed the least hardness.

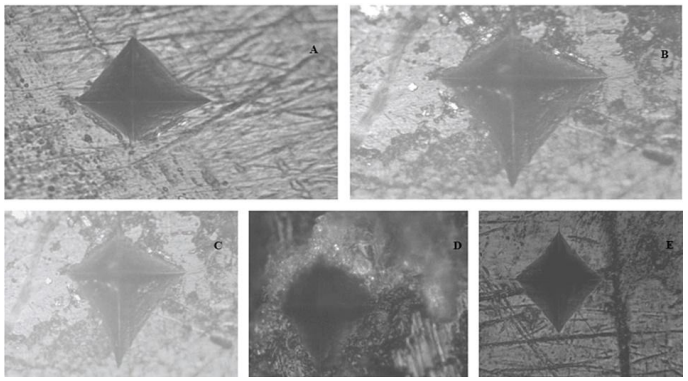

Figure 1: Vickers Hardness Test Results: 1A- Baseline Image 1B-Demineralized Tooth Image 1C- Control Group 1D- Pamam 1E-Cpp-Acp-F

Statistical analysis using the Tukey Multiple Comparison Test indicated that both the PAMAM and CPP-ACPF groups showed significantly higher microhardness recovery compared to the control group, with p-values of 0.001 or lower for both groups.[Table *2*] [Table *3*]

Groups	Baseline value (mean±S.D) HV	Demineralized value (mean±S.D) HV	Remineralized value (mean±S.D) HV
Group 1	304.81± 20.32	156.62 ± 35.23.	279.71±23.74
Group 2	295.54 ± 31.94	161 ± 32.76	235.87 ± 25.87
Control	285 ± 38.92	158.84 ± 31.74	151.74 ± 23.67

Table 2: Mean values of vickers hardness test

	Mean Diff.	95.00% CI	Significant	Summary	Adjusted P Value	
Control vs. Group 1	-128	-155.1 to -100.9	Yes	****	<0.0001	A-B
Control vs. Group 2	-84.13	-111.2 to -57.02	Yes	****	<0.0001	A-C
Group 1 vs. Group 2	43.84	16.73 to 70.95	Yes	**	0.0012	B-C

Table 3: Turkeys test of vickers hardness test

2. SEM Analysis

SEM images taken immediately after 96 hours of demineralization showed numerous micro porosities, less smooth enamel surfaces, and loss of aprismatic

enamel, indicating demineralization (Fig. *2B*). Following remineralization, SEM visuals showed fewer micro porosities and amorphous deposits on the enamel surface, indicative of remineralization. The PAMAM group (Fig *2D*) followed by exhibited more massive deposits compared to the CPP-ACPF group (Fig *2* C) .

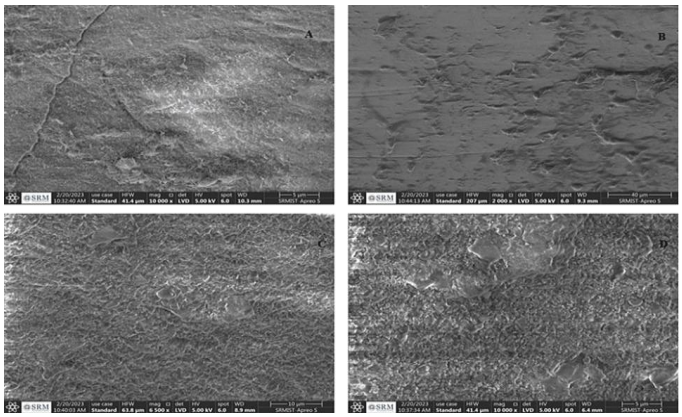

Figure 2: Scanning Electronic Microscopic Images 2A- Baseline Image 2B- Demineralized Tooth 2C -CPP ACP F Group 2D-PAMAM

3. EDX Analysis

Calcium-to-phosphorus (Ca/P) ratios were measured using digital outputs from EDX measurements at different intervals, including baseline,(Figure 3A) post-demineralization(Figure 3B), and 30 days post-remineralization (Figures 3C and 3D).The Ca/P mass ratio increased after remineralization compared to demineralization, indicating the restoration of mineral content in the enamel.

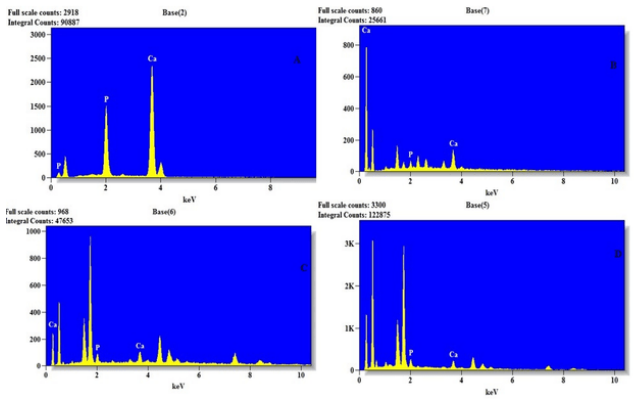

Figure 3: EDX images 3A- Baseline Image 3B- Demineralized Tooth 3C -CPP ACP F GROUP 3D-PAMAM

Overall, the results suggest that both PAMAM and CPP-ACPF treatments effectively enhanced enamel hardness and microstructure following demineralization. [Fig *4*] The SEM and EDX analyses further support the efficacy of these treatments in remineralizing enamel and restoring mineral content.

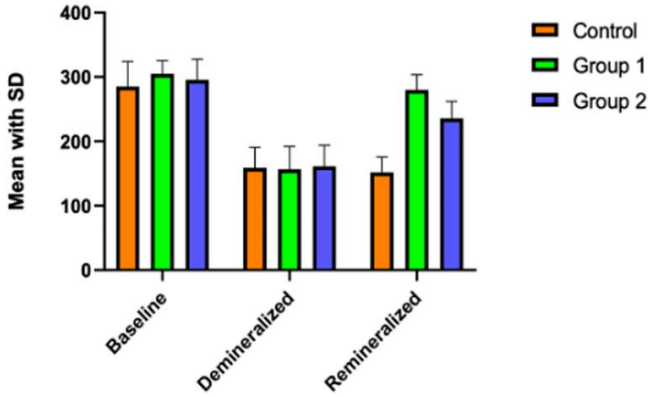

Figure 4: Graphical Representation Of Vickers Hardness Test

Discussion

The key to preventing cavities from happening remains managing the demineralization-remineralization balance. [11] Until recently, the idea of traditional carious lesion treatment was eradicating the caries and replacing it with a restorative substance. But after decades of research, the emphasis is now on early lesion detection and the application of non-invasive methods for efficacious carious lesion management. Early caries lesions can be effectively treated with non-invasive remineralization, which is a huge benefit in clinical management and bridges the conventional gap between surgical therapies and prevention. [12] The capacity to identify caries lesions at the earliest stages and accurately measure the amount of mineral loss is essential to achieving this objective and ensuring the right treatments are set in place. When there is an acid challenge, the joint advantage of supplying calcium phosphate in the surrounding media is

maintained by the synergistic anti-cariogenic activity of PAMAM and CPP-ACPF. These are great delivery systems for localizing fluoride, phosphate and calcium at the tooth surface that are offered in a slow-release amorphous form.[13] The kinetics of enamel demineralization and remineralization have been studied by caries-preventive agents using the pH cycling model extensively. [14-15] In this investigation, pH cycling was applied for 30 days to provide the demineralized enamel specimen enough time to undergo alterations. In this model, enamel specimens were demineralized for ninety-six hours using a pH-4.4 acetic acid buffer. According to Jayarajan J et al., there was a little increase in remineralization with the addition of fluoride (NaF 0.2%) to CPP-ACP as compared to CPP-ACP alone (Tooth Mousse). [16] When it came to the remineralization of simulated caries-like lesions on primary enamel, Bajaj M. et al. examined the effectiveness of CPP-ACP, Tri-calcium phosphate, and Hydroxyapatite. They found that Hydroxyapatite had a higher remineralization efficacy than both of these substances. [17]

In the current investigation, microhardness was measured. A quick, easy, and non-destructive technique for demineralization and remineralization research is surface microhardness indentation. As a result, each specimen's micro-hardness values were determined in three stages for this study: initially, following the production of a carious lesion (demineralization), and then following pH cycling. The initial baseline microhardness measurements in this investigation yielded values (VHN) between 254 to 363HV. This range of values satisfies the VHN of normal enamel tissue. [18] Following 72 hours of demineralization, the surface microhardness values for each set of enamel specimens were reduced to 162-183HV, in line with the findings of the study carried out by Maupome et al. [19]

The average CSMH (VHN) values that were acquired were: 151.74 ± 23.67 (CONTROL GROUP), 235.87 ± 25.87 HV (CPP-ACP F), and 279.71 ± 23.74 HV (PAMAM) [Table *1*]. This suggests that the PAMAM group had more remineralization than the CPP-ACPF group. S Lata demonstrated that CPP-ACP cream can remineralize early enamel caries at the surface, but less effectively than fluoride. [14] The combination of fluoride and ACP-CPP did not provide any more remineralization potential when compared to fluoride alone. Fluoride, ACP-CPP, or their combination cannot remineralize early sub-surface enamel caries. The values found in this study are significantly higher than those found in the S. Lata et al study , which is why they differ. [8]

Conclusions

PAMAM exhibited superior remineralization capacity in comparison to GC Tooth Mousse Plus™ (CPP-ACP with Fluoride). Therefore, toothpastes containing fluoride and topical application of PAMAM and CPP-ACP have a special function in the remineralization of early carious lesions. Compared to other agents, PAMAM provided the highest percentage increase of Ca and P after remineralization in the current investigation, according to EDX analysis. PAMAM had more massive amorphous deposits and less evidence of micro porosities, according to SEM examination, than CPP-ACP F.

Limitation

The lesions generated in the study to duplicate the demineralized surfaces might not accurately depict white spot lesions, which is one potential drawback of the current investigation. Comparing remineralization in vitro to a dynamic, complex biological system that often takes place in the oral cavity in vivo may reveal very different results. Therefore, one must proceed cautiously

when applying direct estimation to clinical circumstances.

References

1. Silverstone LM, Hicks MJ, Featherstone MJ: Dynamic factors affecting lesion initiation and progression in human dental enamel. II. Surface morphology of sound enamel and carieslike lesions of enamel. Quintessence Int. 1988, 19:773-85.
2. Marsh PD: The oral microflora and biofilm on the teeth. In: Fejerskov O, Kidd E, editors. Dental caries: The disease and its clinical management. Blackwell and Munksgaard: Oxford (UK. 20032947,
3. Larsen MJ: Dissolution of enamel. Scand J Dental Research. 1973, 81:518-22. 10.1111/j.1600-0722.1973.tb00358.x
4. Nanda K J, Naik S (May 02, 2020) An In-Vitro: Comparative Evaluation of Pre-treatment With Nano-Silver Fluoride on Inhibiting Secondary Caries at Tooth Restoration Interface. Cureus. 12:7934. 10.7759/cureus.7934
5. Indrapriyadharshini K, Madan Kumar PD, Sharma K, Iyer K: Remineralizing potential of CPP-ACP in white spot lesions - A systematic review. Indian J Dent Res. 2018, 29:487-496. 10.4103/ijdr.IJDR_364_17
6. Liang K, Gao Y, Li J, Liao Y, Xiao S, Zhou X, Li J: Biomimetic mineralization of collagen fibrils induced by amine-terminated PAMAM dendrimers-PAMAM dendrimers for remineralization. J Biomater Sci Polym Ed. 2015:963-74. 10.1080/09205063.2015.1068606. Epub 2015 Jul 27
7. Mendes AC, Restrepo M, Bussaneli D, Zuanon AC: Use of Casein Amorphous Calcium Phosphate (CPP-ACP) on White-spot Lesions: Randomised Clinical Trial. Oral Health Prev Dent. 2018:27-31. 10.3290/j.ohpd.a39749.
8. Lata S, Varghese NO, Varughese JM: Remineralization potential of fluoride and amorphous calcium phosphate-casein phospho peptide on enamel lesions: An in vitro comparative evaluation. J Conserv Dent. 2010, 13:42-6. 10.4103/0972-0707.62634
9. Aref, N.S., Alrasheed, M.K: Casein phosphopeptide amorphous calcium phosphate and universal adhesive resin as a complementary approach for management of white spot lesions: an in-vitro study. Prog Orthod. 23. 10:
10. Güçlü, Zeynep Aslı; Alaçam, Alev; Coleman, Nichola Jayne (2016: A 12-Week Assessment of the Treatment of White Spot Lesions with CPP-ACP Paste and/or Fluoride Varnish. BioMed Research International. 2016, 1:9. 10.1155/2016/8357621
11. Al-Batayneh, Ola B.; Jbarat, Reem A.; Al-Khateeb, Susan N. (2017: Effect of application sequence of fluoride and CPP-ACP on remineralization of white spot lesions in primary teeth: An in-vitro study. 83:236-240. 10.1016/j.archoralbio.2017.08.003
12. Alagha E, Samy AM: Effect of Different Remineralizing Agents on White Spot Lesions. Open Access Maced J Med Sci. 2021, 21:14-18. 10.3889/oamjms.2021.5662
13. Featherstone JD, Shariati M, Brugler S, Fu J, White DJ: Effect of an anticalculus dentifrice on lesion progression under pH cycling conditions in vitro. Caries Res. 1988, 22:337-41. 10.1159/000261133
14. Maupomé G, Aguilar-Avila M, Medrano-Ugalde H, Borges-Yáñez A: In vitro quantitative microhardness assessment of enamel with early salivary pellicles after exposure to an eroding cola drink. Caries Res. 1999, 33:140-7. 10.1159/000016508

15. Ten Cate JM, Duijsters PP. Alternating demineralization and remineralization of artificial enamel lesions. Caries Res. 1982:201-10. 10.1159/000260599
16. Thimmaiah C, Shetty P, Shetty SB, Natarajan S, Thomas NA: Comparative analysis of the remineralization potential of CPP-ACP with Fluoride, Tri-Calcium Phosphate and Nano Hydroxyapatite using SEM/EDX - An in vitro study. J Clin Exp Dent. 2019, 1:1120-1126. 10.4317/jced.55941
17. Bajaj M, Poornima P, Praveen S, Nagaveni NB, Roopa KB, Neena IE, Bharath KP: Comparison of CPP-ACP, Tri-Calcium Phosphate and Hydroxyapatite on Remineralization of Artificial Caries Like Lesions on Primary Enamel -An in vitro Study. J Clin Pediatr Dent. 2016;40. 5:404-9. 10.17796/1053-4628-40.5.404
18. Maurya A, Shashikiran N, Gaonkar N, et al.: Evaluation of Change in Microhardness by Application of MI Varnish on Primary Tooth Enamel, Affected by Use of Frequently Prescribed Paediatric Syrups: An In Vitro Study. Cureus. 12:6533. 10.7759/cureus.6533
19. Rajendran R, Antony D P (November 11, 2023: Development and Elemental Analysis of a Novel Strontium-Doped Nano-Hydroxyapatite Paste and Evaluation of Its Remineralization Potential: An In Vitro Study. Cureus. 15:48650. 10.7759/cureus.48650