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Resistance to demineralization of remineralizing agents on dentin – An Atomic Absorbtion spectrophotometric study

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ARTICLE INFO

Article history:
Available online 2 January 2015

Keywords:
Beta tricalcium phosphate
Casein phosphopeptide-Amorphous calcium phosphate
Remineralization
Atomic absorbtion spectrophotometer

ABSTRACT

Objectives: To evaluate the effect of various Remineralizing agents on Intact Human dentin surface exposed to a demineralizing agent quantitatively using Atomic Absorption Spectrophotometer (AAS).

Materials and methods: 10 Freshly extracted intact human premolars from patients of age group 14–18 yrs extracted for orthodontic purposes were used for the study. 40 dentinal sections of size measuring 4 × 4 × 2 mm were prepared from the surfaces of teeth. All the specimens were then divided into 4 groups of 10 specimens. **Group I (Control Group)** – No Surface treatment, stored in artificial saliva only. **Group II (Control Group)** – Demineralization only, No surface treatment with remineralizing agent. **Group III** – Demineralization followed by CPP-ACFP [4 mins twice daily for 14 days]. **Group IV** – Demineralization followed by β-TCP [4 mins twice daily for 14 days]. Specimens were stored in artificial saliva throughout the study. Artificial saliva was then evaluated for calcium dissolution using Atomic Absorption Spectrometer (AAS). Data obtained were compared and statistically analysed using one way ANOVA and Tukey's HSD test.

Results: Both the Remineralizing agents showed resistance to release of calcium and phosphate from dentin. Group 3 (CPP-ACPF) Showed lesser release of calcium and phosphate when compared to group 4 (β-TCP) which was not statistically significant.

Conclusion: Both the remineralizing agents tested showed resistance to release of calcium and phosphate from dentin on simulation of oral conditions. Among the Remineralizing agents tested, Fluoride Enriched Casein Phosphopeptide-Amorphous calcium Phosphate (CPP-ACPF) showed marginally better remineralization potential on dentin than Beta tricalcium phosphate (β-TCP).

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1. Introduction

In the oral cavity demineralization of the both enamel and exposed dentin is occuring as a consequence of acidic environment can be mainly attributed to dental caries and dental erosion by acids from intrinsic and extrinsic sources. In recent decades, researchers have shown a marked interest in studying not only the cause, process and management of

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http://dx.doi.org/10.1016/j.jpfa.2014.12.001
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demineralization, but also various means and methods of remineralization of tooth structure.[1]

Traditionally fluoride based topical remineralizing agents such as 1.23% acidulated phosphate fluoride gel (APF), Sodium fluoride [NaF] were used in the management of demineralized lesions occurring due to acidic challenges such as dental caries, erosion, white spot lesions as well as developmental defects such as hypoplasia. In the past decade several newer formulations have been introduced as alternatives to fluoride based remineralizing agents. A Casein Phosphopeptide – Amorphous calcium phosphate (CPP-ACP) based remineralizing agent was introduced followed by a fluoride-enriched Casein Phosphopeptide-amorphous calcium phosphate (CPP-ACPF) as a therapeutic agent for the management of demineralized lesions. This was followed by introduction of another new advanced formulation based on β-tricalcium phosphate for remineralization of tooth.[1]

Dentin remineralization is important for improving dentin bonding stability and controlling primary and secondary caries.[2] The presence of phosphoproteins in dentine, its complicated structure and greater water content make dentine a rather more challenging substrate.[2] The function of collagen in the remineralization of dentin did not seem to contribute either positively or negatively to embedding fluoride ions into the hydroxyapatite structure. Recently collagen has been shown to be critical for dentin remineralization, in the presence of fluoride. Various clinical studies have shown that the introduction of fluoride shifts the balance from demineralization to remineralization or lesion arrest.[2]

Several studies have been reported on the use of remineralizing agents in the management of demineralisation arising from dental caries and erosion on dental enamel; however, there are only a few reports on their effect on dentin.[1] Hence, the aim of this study was to evaluate and compare the effect of two remineralizing agents on intact human dentin surface exposed to a demineralising agent quantitatively using Atomic Absorption Spectrometer (AAS).

2. Methodology

The present study was conducted in the Department of Conservative Dentistry and Endodontics, A.B. Shetty Memorial Institute of Dental Sciences, Mangalore and Department of Chemical Engineering, NITK, Suratkal, Mangalore.

10 Freshly extracted intact human premolars extracted for orthodontic purposes were collected from the Dept of Oral & Maxillofacial Surgery and stored in 0.9% isotonic saline for less than four weeks. Teeth were disinfected and handled according to the recommendations and guidelines laid by OSHA and CDC.[3,4] Teeth with caries, restorations, erosions, surface discolorations and cracks were excluded from the study.

Forty dentinal sections of size measuring 4 × 4 × 2 mm were prepared from the surfaces of maxillary and mandibular first premolar teeth using diamond cutting disc with slow speed hand piece under water cooling. Except the front surface all the other surfaces were covered using an impermeable surface coating material like nail varnish. All the specimens were then divided into 4 groups of 10 specimens each. Except group 1 all the Specimens were immersed in 40 ml of demineralising solution consisting of Acetate 0.1 Mol/L, Calcium 0.1 Mol/L, Phosphate 0.1 Mol/L, Fluoride 0.1 mg/L at pH 5.0 for a period of 4 days at a constant temperature of 37 °C. Specimens of all the groups were subjected to surface treatment as follows:

- Group I (Control group) – No Surface treatment, stored in artificial saliva only.
- Group II (Control group) – Demineralization only, No surface treatment with remineralizing agent
- Group III – Demineralization followed by CPP-ACFP [4 mins twice daily for 14 days].
- Group IV – Demineralization followed by β-TCP [4 mins twice daily for 14 days].
- Specimens were stored in artificial saliva throughout the study.

Artificial saliva was then evaluated for calcium dissolution using atomic absorption spectrometer. (AAS). Data obtained were compared and statistically analysed using one way ANOVA and Tukey's HSD test.

3. Results

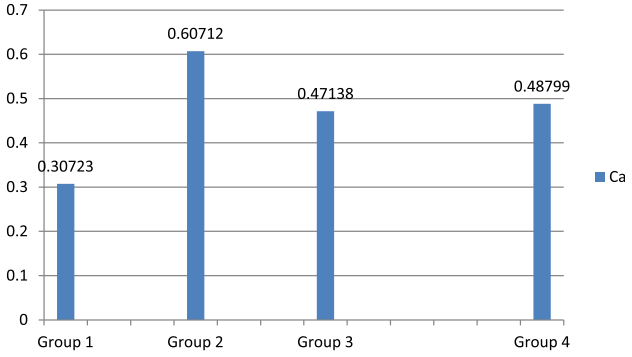
The highest mean value of calcium dissolution was shown in group 2 (demineralized) whereas lowest was seen in group 1 (control group) according to Graph 1.

Amongst the experimental groups, Group 3 surface treated with CPP-ACPF showed lower calcium dissolution [0.40 ppm] compared to Group 4 surface treated with beta tricalcium phosphate [0.48 ppm] according to Graph 1 (Tables 1 and 2).

When comparing group 1 (control) and group2 (demineralized) there was statistically significant difference found between these groups according to Table 3.

When comparing group3 (surface treatment with CPP-ACPF) and group 4 (Surface treatment with β-TCP) there was no statistically significant difference found according to Table 3.

When comparing group 2 (demineralized) and group 3 (surface treatment with CPP-ACPF) there was statistically significant difference found between these groups according to Table 3.



Graph 1 – Bar diagram showing mean values of calcium dissolution (AAS).

Table 1 – Mean and standard deviation of calcium in all the four groups (AAS).

Groups	Mean (Calcium)	Std deviation
Control	0.332	0.081
Demineralized	0.567	0.043
CPP-ACFP	0.401	0.123
Betatricalcium phosphate	0.48	0.148

Table 2 – Calcium dissolution in all groups using Atomic Absorption Spectrophotometer [ANOVA].

	Sum of Squares	df	Mean Square	F	Sig.
Between groups	0.378	3	0.126	15.627	0.000
Within groups	0.290	36	0.008		
Total	0.668	39			

When comparing group 2 (demineralized) and group 4 (surface treatment with β-TCP) statistically significant differences was found between these groups. However it is not as significant when compared to group 3 according to Table 3.

4. Discussion

Atomic Absorption Spectrophotometer (AAS) is a technique used for measuring quantities of chemical elements present within the samples by measuring the absorbed radiation by chemical element of interest.[5] In this study, AAS has been used to quantitatively evaluate the calcium dissolution in the artificial saliva used to store the prepared samples. The highest calcium content was seen in group 2 (demineralized group) and lowest in group1 (control –Intact group) which was statistically significant. Amongst the two experimental groups, both the groups showed resistance to demineralization on remineralization of dentin with Group 3 (CPP-ACPF) showing lesser calcium levels than Group 4 (β-TCP) which is statistically not significant. This can be attributed to the fact that fluoride in sub-ppm concentrations is effective in promoting mineral deposition and inhibiting mineral dissolution. The latter phenomenon is most likely attributable to concomitant precipitation of fluoride rich mineral phase which inhibits further dissolution[6] which is in relation to the solution fluoride effect, where fluoride in saliva aids in inhibition of dissolution.[7] Calcium dissolution in saliva from dentin surface was seen in all the groups as reported by Pashley who suggested that host derived proteinases in the form of different types of MMP's (Matrix Metallo-Proteinases) released from the dentin matrix are capable of degrading the organic matrix of dentin after demineralization under different physiological and pathological conditions. Various MMP's which are a family of zinc dependent proteolytic enzymes, including at least MMP-2, 8, 9 and 20 have been found to present in human dental caries lesions.[8] In this study artificial saliva composed of NaCl, $MgCl_2$, K_2HPO_4, $CaCl_2$, CMC-Na and Ion Exchanged Water has been used to simulate the salivary components in the oral cavity.[9,4] However, in the intra oral environment natural saliva offers greater protection during the remineralization–demineralization cycle than artificial saliva due to its ability to form salivary pellicle that restricts acid diffusion and transport of ions. The advantage of an in-vitro model is that it provides well-controlled experimental conditions to induce demineralization-remineralization; but in-vivo factors such as pH cycling and salivary influence could not be directly simulated in this experiment, hence remineralizing agents could be used in clinical situation for remineralization of dentin.

5. Conclusion

Within the limitations of this in-vitro study, the following conclusions can be drawn:

1) Both the remineralizing agents tested showed resistance to release of calcium and phosphate from dentin on simulation of oral conditions and this could be applied to clinical situations like root caries, dentine hypersensitivity and cervical dentin wear.
2) Among the remineralizing agents tested, Fluoride Enriched Casein Phosphopeptide-Amorphous calcium Phosphate (CPP-ACPF) showed marginally better remineralization potential on dentin than Beta tricalcium phosphate (β-TCP)

Table 3 – Intergroup comparison of all the four groups using Tukey's HSD test [AAS].

(I) Group	(J) Group	Mean difference (I–J)	Std. error	Sig.	95% Confidence Interval
					Lower Bound
Control	Demineralization only	0.27415	0.04016	0.000	0.3823
	CPP-ACPF	0.13841	0.04016	0.008	0.2466
	BETA-TCP	0.15502	0.04016	0.002	0.2632
Demineralization only	Control	0.27415	0.04016	0.000	0.1660
	CPP-ACPF	0.13574	0.04016	0.009	0.0276
	BETA-TCP	0.11913	0.04016	0.026	0.0110
CPP-ACPF	Control	0.13841	0.04016	0.008	0.0302
	Demineralization only	0.13574	0.04016	0.009	0.2439
	BETA-TCP	0.01661	0.04016	0.976	0.1248
BETA-TCP	Control	0.15502	0.04016	0.002	0.0469
	Demineralization only	0.11913	0.04016	0.026	0.2273
	CPP-ACPF	0.01661	0.04016	0.976	0.0916

Conflicts of interest

All authors have none to declare.

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