Remineralization effect of case in phosphopeptide-amorphous calcium phosphate on enamel white spot lesions. A quantitative energy dispersive X ray elemental analysis: An in vitro study

Fabrizio Guerra[1], Marta Mazur[1]*, Gianna Maria Nardi[1], Cristina Chelariu[2], Maurizio Bossù[1], Umberto Romeo[1], Livia Ottolenghi[1]

[1] Department of Oral and Maxillo-facial Sciences, Sapienza University of Rome, P.le A. Moro, 5 - 00185 Rome, Italy
[2] Chemistry engineer, Dental hygienist, Private Practice, Rome, Italy

*Corresponding author: Dr. Marta Mazur, Department of Oral and Maxillo-facial Sciences, Sapienza University of Rome, P.le A. Moro, 5 - 00185 Rome, Italy, Tel.: +39-0649976636; e-mail: martamazur@live.it

Article history
Received: May 30, 2014
Accepted: June 15, 2014
Published: June 30, 2014

Abstract

Background: *The objective of this study was to evaluate, by means of elemental analysis the mineral density, calcium, and phosphorus weight percent of sound enamel, demineralized and CPP-ACP treated enamel. Elemental analysis allows elemental and isotopic composition of a biologic sample. It can be qualitative (determining what elements are present), and quantitative (determining how much of each are present). INCA Energy 250, Oxford Analytical Instruments Ltd. (UK), energy-dispersive X-ray spectroscopy system for elemental analysis was performed on random assigned samples.*

Methods: *12 sound premolars were extracted for orthodontic reason. Each tooth was sectioned by using a double-faced diamond microtome under water cooling into three section for a total of 36 samples and randomly assigned to three groups: Group 1 (control), Group 2 (WS: white spot) , Group 3 (WST white spot treated) of 12 samples each. Samples (Group 2 and Group 3) underwent equally to 24 h and 48 h of acid bath duration. Then all the treated samples (Group 3) were coated with CPP-ACP for 5 min before immersion into water twice a day. Group 2 served as control for enamel damage evaluation. Inca Point & ID, an analytic platform software for SEM was used for elemental analysis on samples from Group 1 (C), 2 (WS) and Group 3 (WST) in order to determine the weight % and atomic % presence of Ca and P.*

Results: *The results of the samples analysis from the three Groups show different weight % and atomic% of Ca and P, and clearly reflect the different mineralization rates.*

Conclusions: *10% Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) complex, promotes remineralization in vitro. The results of this in vitro study completely agree with this statement. Clinical studies to investigate the intraoral effectiveness of topical applications of CPP-ACP on white spot lesions are required to confirm these results.*

Keywords: *Demineralization, Remineralization, CPP-ACP, White spot*

1.Introduction

Dental caries progression or reversal depend upon demineralization and remineralization process. The "Caries Balance" is determined by the weight of pathological and protective factors [1]. Minimally invasive dentistry goals are: avoid any removal of enamel or dentin, reduce pathological factors and enhance enamel remineralization. The caries risk assessment should be based upon the "Caries Balance" and the treatment plan should be personalized on patient risk assessment [2]. The balance between pathological and preventive factors can be swung in the direction of prevention by the active role of the dentist and the dental

staff. The repair process-remineralization occurs when the pH rises again and calcium and phosphate from saliva together with fluoride enter the subsurface region of the lesion and form a new layer on the existing crystal remnants in the lesion [3-6].

White spot enamel lesion are defined by International Caries Detection and Assessment System (ICDAS II) [7]. This system records lesions at the surface level, after they have been cleaned and dried, and allows the detection of early white spot caries with a good degree of reproducibility.

Lesions are staged using the criteria summarised below:

0. No lesion
1. Lesion seen only when dry
2. Lesion seen wet
3. Localized enamel breakdown
4. Localized enamel with dentin shadow
5. Distinct cavity with dentin shadow
6. Extensive distinct cavity

Early intervention on white spot enamel lesion is possible with casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) complex, a milk protein derivative, that has been proven to have anticariogenic effects [8-13]. It prevents enamel demineralization and promotes remineralization through buffering free calcium and phosphate ion activities, thereby incorporating ACP into plaque and onto tooth surface and maintaining a state of supersaturation with respect to tooth enamel [14-16]. Remineralized enamel has an increased resistance to subsequent acid challenge than untreated enamel [8, 9]. The purpose of this in vitro study is to qualitatively assess, by means of scanning electron microscopy (SEM), the differences exhibited on enamel surfaces in sound teeth and in exposed and not exposed to CPP-ACP teeth after being immersed in an artificial demineralizing solution. It was hypothesized that topical applications of CPP-ACP would have a remineralizing effect on enamel once exposed to an acid solution.

2. Material and Methods

12 sound premolars extracted for orthodontic reason were collected after an adequate informed consent has been obtained. The samples were stored in 4° C phisiological solution water (NaCl 0.9% a pH between 4,5 and 7) for 48 h and dehydration was performed in alcohol (90%) for 30 days. Teeth with cracks visible under 4X magnification, hypoplasia, white spots, caries, or reconstruction were not included in this study.

Each tooth was sectioned using a double-faced diamond microtome under water cooling into three sections for a total of 36 samples and assigned randomly to three groups: Group 1 (control), Group 2 (WS: white spot) and 3 (WST: white spot treated). Samples (Group 2 and Group 3) underwent equally to 24 h and 48 h of acid bath duration. Then all the treated samples (Group 3) were coated with CPP-ACP for 5 min before immersion into water twice a day. Group 2 served as control for enamel damage evaluation. Group 2 served as control for enamel damage evaluation, in order of 100-110 µm depth in the enamel layer, with no evident damage on the surface.

INCA Energy 250, Oxford Analytical Instruments Ltd. (UK), energy-dispersive X-ray spectroscopy system for elemental analysis was performed. Elemental analysis is a process where a sample is analyzed for its elemental and isotopic composition. Elemental analysis can be qualitative (determining what elements are present) and quantitative (determining how much of each are present). Energy dispersive X-ray spectroscopy (EDS) allows chemical analysis of a biological tissue surface.

Samples from Group 2 (WS) and Group 3 (WST) underwent to elemental analysis, in order to determine the atomic % and weight % presence of Ca and P.

The elemental analysis was performed on random assigned points on the observed surfaces: the data were elaborated by Inca Point & ID software. The results of the samples analysis from the three Groups show different weight % and atomic % of Ca and P, and clearly reflect the different mineralization rates. The presence of Au (used for the mineralization coat), Cl and Na are due to the handle sample manipolization.

3. Results

The present study evaluated the remineralization potential of CPP-ACP paste on induced enamel white spot lesions using SEM-EDX. Energy dispersive X-ray analysis was used to determine the mineral content of calcium and phosphorus in % weight of sound, demineralized, and remineralized enamel in each group. The calcium and phosphorus content was then converted into Ca/P ratios for each group from the obtained data. Figure 1, 2, 3, 4 demonstrates the elemental analysis of

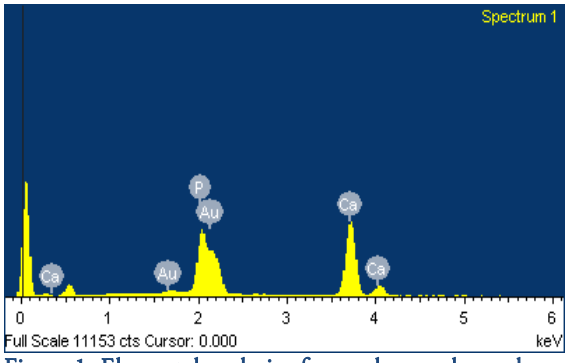

Figure 1. Elemental analysis of sound enamel sample.

study groups for different periods of treatment time with

CPP-ACP.

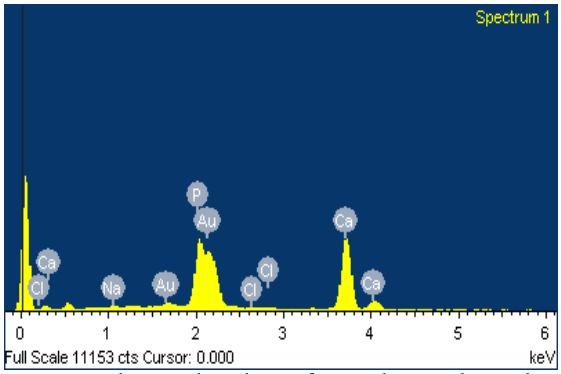

Figure 2. Elemental analysis of sound enamel sample, after 24 h of demineralization.

Figure 5, 6 illustrates comparison of mean Ca/P ratios of Sound, Demineralized, Remineralized enamel samples.

4. Discussion

This in vitro study support the evidence that CPP-ACP can be effective in enhancing in vitro enamel remineralization [14]. The CPP form clusters of amorphous calcium phosphate (ACP) in metastable solution and prevent their nucleation and precipitation.

The CPP-ACP anticariogenicity activity is due to localizing ACP in dental plaque, which buffers the free calcium and phosphate ion activities, helping to maintain supersaturation and depressing demineralization while enhancing remineralization [15-16].

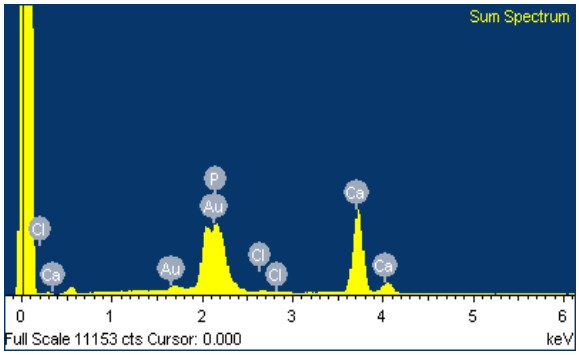

Figure 3. Elemental analysis of 24 h demineralized enamel, after 30 days of remineralization.

CPP-ACP incorporation into plaque resulted in a 144% increase in the plaque calcium and a 160% increase in plaque phosphorus. After the diffusion into the White Spot body of lesion, $CaHPO^4$ (and associated species) would increase the degree of saturation with respect to HA by the formation of Ca^{2+} and other ions. HA formation in the lesion would form acid and phosphate, including H^3PO^4, which would diffuse out of the lesion following a concentration gradient. The study results indicate that CPP-ACP acts as a reservoir of calcium phosphate ions, including the neutral ion pair CaHPO4, which are formed in acid environment. At low pH the CPP- bound ACP would buffer plaque pH thus dissociating into calcium phosphate ions, including $CaHPO^4$. The increase in plaque calcium and phosphate ions and ion pairs would offset any fall in pH, preventing enamel demineralization.

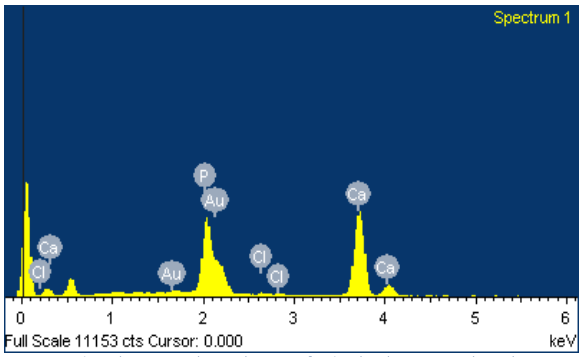

Figure 4. Elemental analysis of 48 h demineralized enamel, after 30 days of remineralization.

Plaque pH decrease even during enamel lesion remineralization. Therefore, this could explain why the CPP-ACP remineralizing solutions are so efficient, since they would maintain $CaHPO^4$ concentration gradient into the lesion by buffering capacity. Schupbach et al demonstrated that CPP incorporation into acquired pellicle in vitro would not only increase its remineralization potential but also inhibit the adherence of Streptococcus mutans and Streptococcus sobrinus [17]. Several phosphoproteins interact with calcium phosphates. The proposed functions of these proteins are: (A) the stabilization of calcium phosphate in solution, preventing spontaneous precipitation; and (B) biomineralization, where the protein, cross-linked to a collagen matrix, has been proposed to act as a nucleator/promoter of crystal growth. These functions depend on concentration and conformation of the protein and the composition and degree of saturation of the aqueous phase.

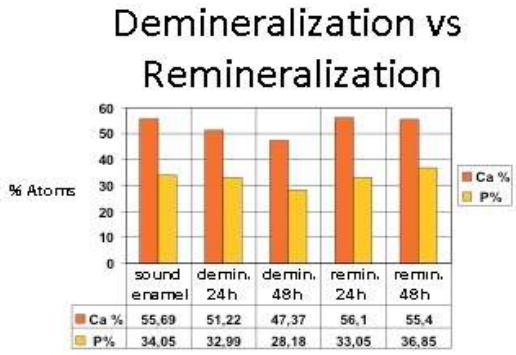

Figure 5. Ca/P atomic ratios of demineralized and remineralized specimens in study groups.

The CPPs are an excellent delivery vehicle for the colocalization of Ca, F, and phosphate at the tooth surface in a slow-release amorphous form, producing superior anticaries efficacy.

Strength of this study is the high technological asset, using the elemental analysis at the ultrastructural level. This microanalytical technique is used with SEM investigation. When the sample is bombarded by the electron beam of the SEM, electrons are ejected from the atoms on the specimen's surface (secondary electrons).

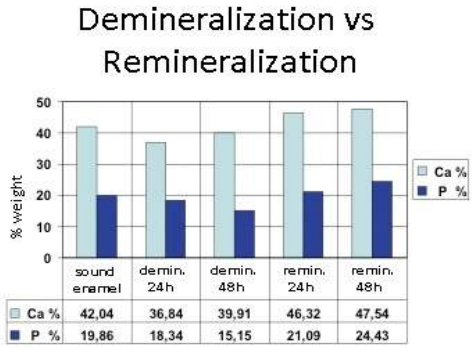

Figure 6. Ca/P weight ratios of demineralized and remineralized specimens in study groups.

The electron vacancy is filled by an electron from a higher shell, and an X-ray is emitted (characteristic X-rays) to balance the energy difference.

The EDX X-ray detector measures the number of emitted X-rays vs. their energy. The energy of the X-ray is characteristic of the element from which the X-ray was emitted. A spectrum of the energy vs. relative counts of the detected X-rays is evaluated for qualitative and quantitative determinations of the elements in the specimen using a computer-based program [18-19]. Limitations are mainly in the in vitro setting, although validated, that can be not completely consistent with the salivary environment at the plaque/enamel interface. The elemental analysis is one of the important indicators for caries development, but the multifactorial process must be considered.

5. Conclusion

Taking into account the limitations of this in-vitro study, the following conclusions can be drawn:

10% Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) complex, prevents enamel demineralization and promotes remineralization through buffering free calcium and phosphate ion activities, thereby incorporating ACP into plaque and onto tooth surface and maintaining a state of supersaturation with respect to tooth enamel. The results of this in vitro study completely agree with this statement. Clinical studies to investigate the intraoral effectiveness of topical applications of CPP-ACP on white spot lesions would be required to confirm these in vitro results [20].

References

1. Featherstone JDB. Prevention and reversal of dental caries: role of low level fluoride. Community Dent Oral Epidemiol 1999;27:31-40.

2. John DB. Featherstone, Adair SM, Anderson MH, et al. Caries Management by risk assessment: consensus statement, April 2002. CDA Journal 2003; 31: 257-269.

3. Kidd EAM, Fejerskov O. What constitutes dental caries? Histopathology of carious enamel and dentin related to the action of cariogenic biofilms. J Dent Res 2004;83: C35- C38.

4. Featherstone JDB, The Caries balance: contributing factors and early detection. J Calif Dent Assoc 2003;31:129-33.

5. Featherstone JD. The caries balance: the basis for caries management by risk assessment. Oral Health Prev Dent. 2004;2 Suppl 1:259-64.

6. Young DA, Featherstone JD, Roth JR, Anderson M, Autio-Gold J, Christensen GJ et al. Caries management by risk assessment: implementation guidelines. J Calif Dent Assoc 2007;35(11):799-805.

7. International Caries Detection and Assessment System (ICDAS) Coordinating Committee. Criteria Manual International Caries Detection and Assessment System (ICDAS II). Scotland: Dental Health Services Research Unit; 2005. http://www.icdas.org.

8. Reynolds EC, Cain CJ, Webber FL, Black CL, Riley PF, Johnson IH. Anticariogenicity of calcium phosphate complexes of tryptic casein phosphopeptides in the rat. Journal of Dental Research 1995;74:1272–9.

9. Reynolds EC. Remineralization of enamel subsurface lesions by casein phosphopeptide-stabilized calcium phosphate solutions. Journal of Dental Research 1997;76:1587–95.

10. Reynolds EC. Anticariogenic complexes of amorphous calcium phosphate stabilized by casein phosphopeptides: a review. Special Care in Dentistry 1998;8:8–16.

11. Reynolds EC, Black CL, Cai F, Cross KJ, Eakins D, Huq NL, et al. Anticariogenic casein phosphopeptide-amorphous calcium phosphate. Journal of Clinical Dentistry 1999;10:86–8.

12. Shen P, Cai F, Nowiciki A, Vincent J, Reynolds EC. Remineralization of enamel sub surface lesions by sugar free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. Journal of Dental Research 2001;80:2066–70.
13. Cai F, Shen P, Walker G, Reynolds C, Reynolds EC. Remineralization of enamel subsurface lesions in situ using sugar free lozenges containing casein phosphopeptide-amorphous calcium phosphate. Australian Dental Journal 2003;48:240–3.
14. Kumar VLN, Itthagarun A, King NM. The effect of casein phosphopeptide-amorphous calcium phosphate on remineralization of artificial caries-like lesions: an in vitro study. Australian Dental Journal 2008;53:34–40.
15. Iijima Y, Cai F, Shen P, Walker G, Reynolds C, Reynolds EC. Acid resistance of enamel sub surface lesions remineralized by a sugar free chewing gum containg casein phosphopeptide-amorphous calcium phosphate. Caries Research 2004;38:551–6.
16. Reynolds EC, Cai F, Shen P, Walker GD. Retention in plaque and remineralization of enamel lesions by various forms of calcium in a mouthrinse or sugar-free chewing gum. Journal of Dental Research 2003;82:206–11.
17. Schiupbach P, Neeser JR, Golliard M, Rouvet M, Guggenheim B. Incorporation of caseinoglycomacropeptideand caseinophosphopep tide into the salivary pellicle inhibits adherence of mutans streptococci. J Dent Res 1996; 75:1779-88.
18. Oshiro M, Yamaguchi K, Takamizawa T. Effect of CPP-ACP paste on tooth mineralization: An FE-SEM study. J Oral Sci 2007;49:115-20.
19. Russ JC. Electron probe x-ray microanalysis-principles. In: Erasmus DA, ed. Electron probe microanalysis in biology. London: Chapman & Hall, 1978: 5-36.
20. Alessandri Bonetti G, Zanarini M , Incerti Parenti S , Marchionni Silvia , Checchi L. In vitro evaluation of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) effect on stripped enamel surfaces. A SEM investigation. Journal of Dentistry 2009; 37:228–232.