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Research Article

A COMPARATIVE ANALYSIS ON CALCIUM RELEASE FROM ENAMEL AFTER TREATMENT WITH VARIOUS REMINERALIZING AGENTS AT A PH 4.5 : AN ATOMIC ABSORBTION SPECTROMETRIC ANALYSIS

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ABSTRACT

Focus of this study was to compare the amount of calcium released from enamel treated with different remineralizing agents at pH 4.5 using atomic spectrophotometric analysis. The present study was designed to assess the calcium release from enamel when subjected to an acid challenge. Enamel samples were divided into four groups of which intact enamel served the purpose of control group and other three groups were based on the remineralizing agents used (CPP-ACP [GC Tooth Mousse], CPP-ACPF [GC Tooth Mousse Plus], 0.044 % Sodium Fluoride [Phosflur]). All the groups of enamel samples were initially demineralized and followed up with remineralisation by adopting the pH cycling model. Acetate buffer was prepared at a specific pH of 4.5. The enamel samples were subjected to acid challenge in presence of a magnetic stirrer and buffer solution were pipetted at specific time intervals for pH 4.5. The solutions collected were transferred into sterile containers and subjected to atomic spectrometry analysis.

Keywords: Calcium, Critical pH, Atomic Absorption spectrometry, CPP-ACP, CPP-ACPF

INTRODUCTION

Dental caries is a disease of primary concern in the present day. The factors responsible for the cause of caries being multiple, it makes for a challenging task to recognize or target a specific aspect to help prevent or even reduce the rate of occurrence of caries. Nevertheless a number of promising advances have been made over the years and helped in the better understanding of the carious process. The aspect we focus here is the response of Enamel at a specific pH. Critical pH is the point at which a solution is just saturated with a mineral and the solution we refer to here is saliva with the mineral being enamel. The reason for the existence of tooth in the oral cavity without undergoing dissolution is due to the fact that the pH of saliva and plaque are more often than not greater than the critical pH. The critical pH is inversely proportional to the amount of calcium and phosphate ions in saliva of an individual and as concentration of these ions inevitably vary from each individual the critical pH isn't a constant value.[1] Research now suggests that in individuals with low calcium and phosphate concentration levels the critical pH is 6.5 and in those with higher levels of these minerals the critical pH is 5.5.[2] It's a known fact that plaque in its liquid phase contains ion concentrations of calcium and phosphate at higher levels when in comparison with saliva and its critical pH may be as low as 5.1.[3] All this research now will bring on doubts whether to accept the critical pH as a constant value.

MATERIALS AND METHODS

Method of Collection of Data

Inclusion criteria: Freshly extracted human Maxillary and Mandibular first molar teeth extracted on periodontal grounds from individuals below the age of 40. Teeth were selected based on randomized sampling method.

Exclusion criteria: Teeth with caries, hypoplastic lesions, stains, white spots, cracks, erosion, developmental anomaly or any other deformity were excluded.

Infection Control Protocol

The teeth were cleansed of visible blood and gross debris and were maintained in a hydrated state during storage. Extracted teeth were placed in sodium hypochlorite solution diluted with saline in a ratio of 1:10 in container with a secure lid to prevent leaking and labelled with the biohazard symbol. Elimination of microbial growth was achieved by using an autoclave cycle for 40 minutes. Teeth that do not contain amalgam restorations were preferred because they can be safely autoclaved. However, Extracted teeth containing amalgam restorations, were immersed in 10 % formalin solution for 2 weeks.[4] Enamel Sample preparation: 4 Enamel slabs of 2 mm thickness were prepared from Buccal and Lingual surface of molars by using diamond discs under water cooling at a speed of 25,000 RPM using Micromotor with a contra angled hand piece. The surface of enamel on the buccal and lingual surface was first made flat and then polished to get a fine gloss using 3 M SofLex polishing discs. The samples were prepared following all infection control protocols and barrier techniques.

Demineralisation Solution to Produce Caries like Lesions

The Demineralizing solution provides a partial demineralization of the enamel, leaving the enamel softened, yet structurally intact; which is morphologically similar to the human caries condition. The Demineralizing solution used for the study was proposed by Featherstone in 1992.[5]

Composition:

Acetate 0.1 mol / L
Calcium 0.1 mmol / L
Phosphate 0.1 mmol / L
Fluoride 0.1 mg / L (5.3µmol/L)
pH was adjusted to 5.0

Demineralising Solution for pH Cycling

Composition:

Calcium 2.0 mmol / L
Phosphate 2.0 mmol / L
Acetic acid 75.0 mmol / L

The pH was adjusted to 4.4 using 50 % NaOH after all the ingredients were dissolved completely.[6]

Groups

Enamel slabs were randomly divided into 4 Groups based on the type of remineralising agent to be used.

Table 1

Table 1: Showing the Division of Groups

Group I	Sound Enamel (No treatment)
Group II	Demineralised and treated with slurry of Caesinephosphopeptide - Amorphous calcium phosphate (GC Tooth Moouse) CPP-ACP
Group III	Demineralised and treated with slurry of Caesinephosphopeptide - Amorphous calcium phosphate with Fluoride (GC Tooth Moouse plus) CPP-ACPF
Group IV	Demineralised and treated with Sodium fluoride solution

Remineralisation Solution for pH Cycling

Remineralising solution simulates saliva which approximates the mineral ion composition and super saturation of saliva and promotes the remineralisation process. The remineralisation solution used for this study was originally reported by Ten Cate and Duijsters.

Composition:

Calcium 1.5 mmol / L
Phosphate 0.9 mmol / L
KCl 130.0 mmol / L
Sodium cacodylate 20.0 mmol /

Calcium and Phosphate at a known degree of saturation to mimic the remineralising properties of saliva, 130 -150 mMKCl to provide background ionic strength and 100 mM TRIS or 20 mM Cacodylate buffer at pH 7.0.[6]

Preparation of Acetate Buffer for Further Acid Challenge

Acetate buffer of 0.2 M was prepared for pH 4.5.(SYSTRONICS, micro pH system 361)

Composition:

Acetic acid 11.5 mL / L
Sodium acetate 16.4 g / L
The above solution was made to 200 ml in distilled water.

Acid Challenge

The samples of Intact Enamel and enamel treated with CPP-ACP, CPP-ACPF and fluoride were divided into 4 groups of three samples each based on the pH of Acid buffer. Individual specimen of every subgroup was immersed in 50 ml of acetate buffer. 5 ml of the solution was poured to a sterile centrifuge tube of 15 ml (Corning[R] 15 ml centrifuge tubes) at time intervals of 0, 0.5, 1, 5, 10, 15, 30 and 60 minutes using a micro pipette (Eppendorf,10 ml). All the centrifuge tubes were marked for future identification. The solution was continuously stirred using a magnetic capsule on a magnetic stirrer (REMI Magnetic Stirrer, 2 ML) at RPM of 100. The experiment was almost simultaneously conducted for all the 4 groups at the same time, so that the same buffer solution was used for all the samples.

Atomic Spectrometry

The acid buffers of all the groups for all time intervals were evaluated for calcium, using Atomic spectrometry (GBC 932 Plus atomic absorption spectrophotomter). A known standard of calcium was first measured and the values obtained were used to plot a standard curve. The range was set at 1-4 PPM of calcium. The values obtained from the test solutions were compared with the standard curve and the amount of calcium in the solution was calculated based on the formula:

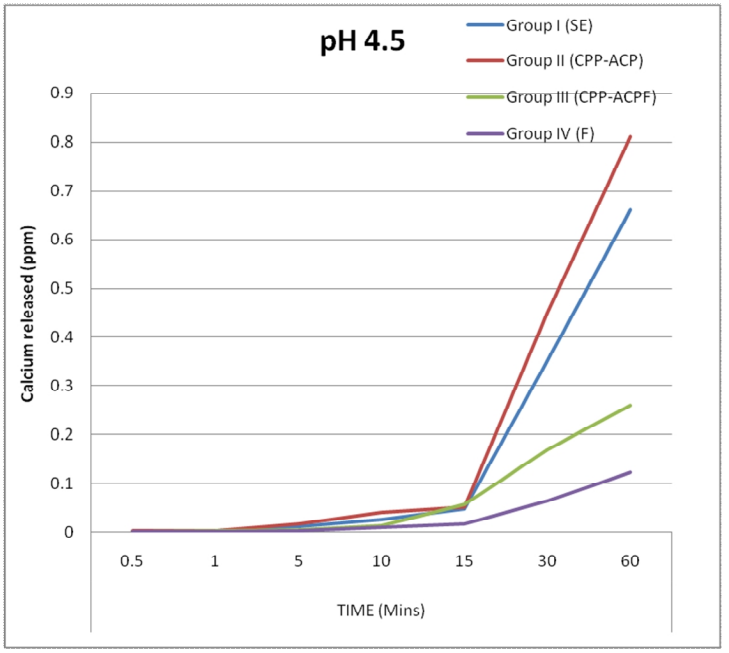
$$\text{Concentration of calcium} = \frac{\text{Absorbance of sample X conc. of Standard}}{\text{Absorbance of Standard}}$$

Statistical Analysis

All the Experimental data were analyzed using one way ANOVA. One way ANOVA and TukeysPosthok tests were conducted for all the values obtained.

Table 2: Showing Tukeys Posthok Analysis between Groups

Time Intervals For Acid Buffer At pH 4.5		Sum of Squares	Df	Mean Square	F	Sig.
0.5 M	Between Groups	.000	3	.000	51.503	.000
1 M	Between Groups	.000	3	.000	19.428	.000
5 M	Between Groups	.001	3	.000	50.137	.000
10 M	Between Groups	.003	3	.001	46.091	.000
15 M	Between Groups	.006	3	.002	90.268	.000
30 M	Between Groups	.547	3	.182	182.80	.000
60 M	Between Groups	1.900	3	.633	170.29	.000



Graph 1: Showing Calcium Released at ph 4.5 by al the Groups at Different Time Intervals; X axis- Time in Minutes, Y Axis – Calcium Released in PPM

RESULTS

- At pH 4.5, there was a marked difference in the calcium released between group IV and III when compared to Group I and II. There was also a substantial difference between Group III and IV. This graph shows that fluoride is more resistant to acid attack than ACP-CPPF at pH 4.5.
- Group II released the maximum amount of calcium followed by group I and Group III. Group IV released the least amount of calcium.
- At 60 and 30 minutes, significant difference was found between all the groups.
- At 15 and 1 minutes, significant difference was noticed between all the groups except between group I and II and Group II and III.
- At 10 and 5 minutes, significant difference was noticed between all the groups except between Group III and IV.
- At 0.5 minutes, significant difference was noticed between all the groups except between Group I and IV.

DISCUSSION

The results of our study infer that enamel treated with fluoride is more resistant to acid attack in comparison to enamel treated with CPP-ACPF at pH 4.5. Overall we can conclusively comprehend that at pH 4.5; maximum calcium release was observed in enamel treated with CPP-ACP followed up by intact enamel and enamel treated with CPP-ACPF respectively. One more key observation was that there was significant difference in the amount of calcium release at time intervals of 30 minutes and 60 minutes for pH 4.5. Research is now focused on remineralizing early enamel lesions. Unlike salts like sodium chloride the dissolution of hydroxyapatite crystals is determined by the pH of solution in which it's dissolving and not the volume of the solution. When hydrogen ion from the acid attacks the hydroxyapatite crystal calcium is released. Calcium and Phosphate ions continue to release until a state of super saturation is achieved. Solubility of hydroxyapatite depends on the pH of the solution in which it dissolves. Fluoride is necessary for the formation of subsurface caries. Caries forms between pH range of 4.0-5.5 at pH below 4.0, erosion occurs.[7] A study done to determine the ability of CPP-ACP to increase the incorporation of fluoride into plaque and to promote enamel remineralization in randomized, double blind trials, cross over study involving dentifrices and mouth rinses containing CPP-ACP and fluoride. The mouth rinses were used for 60 seconds four times per day for 14 days in an in situ model. The results showed that 2 % CPP-ACP produced a level of remineralization similar to 2800 ppm fluoride.[8] Fluoride in sub-ppm concentrations is effective in promoting mineral deposition and inhibiting mineral dissolution. The latter phenomenon is mostly likely attributable to the concomitant precipitation of a fluoride rich mineral phase which inhibits further dissolution. These fundamental processes result in an inhibition of enamel demineralization and an enhancement of enamel lesion remineralisation.[9] The rate of mineral dissolution with respect to enamel is significantly reduced in acidic solutions with presence of low fluoride levels.[10] This is in relation to the solution fluoride effect where in the fluoride in saliva as the solution aid in inhibition of dissolution of tooth material.[11] A study showed that measurements of pH have made it possible to examine dental plaque as a metabolic unit and to identify the Stephan pH response as an important indicator of caries activity. High reproducibility of results with the antimony electrode can aid in quantifying factors such as substrate concentration, substrate duration, substrate retention and ability of a foodstuff to stimulate saliva.[12]

REFERENCES

1. Colin Dawes. What is critical pH and why does a tooth dissolve in acid? J Can Dent Assoc 2003; 69(11): 722-724. PMid:14653937
2. Ericson Y. Enamel-apatite solubility; Investigations into the calcium phosphate equilibrium between enamel and saliva and its relation to dental caries. Acta Odont Scand 1949; 8(suppl 3): 1-139.
3. Dawes C, Guggenheim B. editor. Inorganic constituents of saliva in relation to caries. Cariology today. Basel: Karger; 1984. p. 70-74.
4. Pantera EA, Schuster GS. Sterilisation of extracted human teeth. J Dent Educ 1990; 54: 283-285. PMid:2335661

5. Featherstone JDB, Zero DT. An in situ model for simulation Assessment of inhibition of Demineralisation and enhancement of Remineralisation. Journal of Dent Res 1992; (71) (spec issue): 804-810.
6. George K Stookey, John DB Featherstone, Marcia Rapozo Hilo, Bruce R Schemehorn, Ross A Williams, Robert A Baker *et al*: The Featherstone laboratory pH cycling model: A prospective multi-site validation exercise. American Journal of Dentistry 2011; 24(5): 322-328.
7. JM Ten Cate, MJ Larsen, EIF Pearce, O Fejerskov. Chemical interactions between tooth and oral fluids, O Fejerskov, EAM Kidd, editors, Dental Caries and its clinical management; 2003. p. 649-669.
8. EC Reynolds, F Cai, NJ Cochrane, P Shen, GD Walker, MV Morgan *et al*. Fluoride and Casein Phosphopeptide-Amorphous Calcium Phosphate. J Dent Res 2008; 87(4): 344-348. http://dx.doi.org/10.1177 154405910808700420 PMid:18362316
9. Amir Azarpazhooh and Hardy Limeback. Clinical efficacy of casein derivatives. JADA 2008; 139: 916-924.
10. Margolis HC, Moreno EC, Murphy BJ. Effect of low levels of fluoride in solution on enamel demineralization *in vitro*. J Dent Res 1986; 65: 23-29.http://dx.doi.org/10.1177/00220345860650010301 PMid:3455694
11. Wong L, Cutress TW, Duncan JF. The influence of incorporated and adsorbed fluoride on the dissolution of powdered and pelletized hydroxyapatite in fluoridated and non- fluoridated acid buffers. J Dent Res 1987; 66: 1735-1741. http://dx.doi.org/10.1177/00220345 870660120801 PMid:2824586
12. I Kleinberg, GN Jenkins, R Chatterjee and L Wijeyeweera. The antimony pH electrode and its role in the assessment and interpretation of dental plaque pH. J Dent Res 1982; 61(10): 1139-1147. http://dx.doi .org/10.1177/00220345820610100601 PMid:6749924

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