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Protective Effect of Different Remineralizing Agents on Artificially Induced Dental Erosion on Permanent Teeth: An In-Vitro Analysis

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Abstract

Aim: To assess the effect of Chicken Egg Shell Powder (CESP) solution and sea shell powder solution (SSP) on demineralized enamel surfaces, and to check the enamel surface microhardness using Vickers microhardness testing machine.

Methods and Material: 25 extracted permanent human central incisors specimens were cleaned and sectioned longitudinally resulting in 50 samples. These enamel sections were assigned to the following four groups. Group A: untreated teeth, Group B: demineralization by soft cola drink, Group C: demineralization by soft cola drink followed by 7 days' immersion in CPP-ACP, Group D: demineralization by soft cola drink followed by 7 days' immersion in CESP, Group E: demineralization by soft cola drink followed by 7 days' immersion in SSP. Enamel surface hardness was checked with Vickers microhardness testing machine. Statistical analysis was performed using one-way ANOVA and Tuckey post hoc test.

Result: CESP and SSP significantly ($p<0.05$) increases the microhardness of all treated teeth samples than CPP-ACP.

Conclusion: Both CESP and SSP can protect the tooth surface by preventing its erosive enamel loss and enhancing remineralization.

Keywords: Chicken Egg Shell Powder (CESP) solution, CPP-ACP, Enamel Demineralization, Sea Shell Powder (SSP), Vickers microhardness

Introduction

Dental Erosion is a procedure of chemical dissolution of tooth surfaces because of the acids delivered by extrinsic or intrinsic sources. Natural source to instigate this disintegration is mostly connected with gastric acid

in oral cavity pursued by spewing, regurgitation or gastro esophageal reflux.[1]

Even if the teeth are presented to persistent cycles of demineralization pursued by remineralization, this fragile balance can easily be disturbed because of broad utilization of low pH beverages like sodas, organic fruit juices, acidic drinks, wines and confections prompting acidic disintegration of the inorganic phase of tooth and consequent loss of tooth substance.[2] This ruinous procedure brings irreversible loss of tooth substrate in this way expanding its vulnerability towards scraped spot and may cause erosive wear of dental hard tissues eventually prompting tooth affectability and in serious cases pulpal exposure.[3] Many clinical confusions related with dental erosion are enamel crack, decrease vertical measurement dimension, tooth affectability, pulpal irritation and exposures generally in kids with huge pulps.[4,5]

The paradigm of present day dental practice is currently concerned with the idea of Minimal invasive dentistry which underlines upon idea of early discovery of carious injuries, remineralization of tooth surfaces and protection of surrounding tooth structure.[6,7] Enamel lacks inherent ability to repair itself whenever influenced by abrasion, fracture, caries, the rebuilding of early, superficial enamel layer loss depends on composition of saliva just as on tooth pastes and creams containing alloplastic materials, for example, calcium or fluoride.[8]

Demineralization coming about because of loss of calcium and phosphate ions can be re-established by utilizing non-invasive calcium phosphate delivery system. Present day tooth paste/creams/ gels containing sodium fluoride or tri calcium phosphate have now been considered as the best wellspring of in organic ions required for enamel remineralization.[9]

Generally, Casein phosphopeptide – amorphous calcium phosphate glues (CPP-ACP) has been utilized to remineralize subsurface enamel lesions. GC-TOOTH MOUSSE has a nano complex which gives anticariogenic properties. It has been connected to build enamel protection from caries.[10,11,12] CPP made out of a bunch of phosphoryl deposits that settled ACP nanocluster particles in metastable arrangement.[11] It is a sticky protein that bonds to phosphate and calcium particles and balanced out in an amorphous structure. It is just attached to plaque, enamel hydroxyapatite and pellicle and aides the free phosphate and calcium particles into enamel crystals, to frame the apatite crystals.[13]

Chicken egg is a known source of Ca containing about 39%(w/w) of basic Ca. It not just supports the chondrocyte separation in vitro yet additionally increases the bone mineral density in animal model of female rodent.[14] Chicken Egg Shell Powder (CESP) additionally increases the bone mineral density and lessens bone pain in postmenopausal ladies and ladies with senile osteoporosis.[15]

Seashell is one of the natural materials which is utilized in dental applications. Seashells are natural ceramics like our teeth and bones. Natural ceramic seashells have magnificent layers, otherwise called nacreous layers. They contain layers of calcium carbonate platelets and protein that are held together to give more quality and strength.[23]

Seashell and the eggshell are generally made up of calcium making them great applicants as wellsprings of Hydroxy appetite.[23] Previously no studies have been done comparing any remineralizing agents with sea shell powder.

Therefore, the aim of this study was to assess the remineralization of enamel surface hardness utilizing

CPP-ACP, CESP and SSP by microhardness test as no study till date has analysed their impact on remineralization.

Material and method

Preparation of Chicken Egg Shell Powder (CESP) solution: In this study twelve chicken eggs obtained from local hatchery were used. After cleaning with distilled water, they were placed in hot water bath for ten mins at 100°C to separate the membranes. They were crushed with sterile mortar-pestle and then kept in a muffle furnace (Neycraft Model JFF 2000) at 1200°C. To prepare the solution One gram of CESP was dissolved in 20ml of 4% acetic acid. The clear fluid at the top of test tube was transferred to a beaker. The pH of the solution measured was about 11.4 by using a pH meter. (Hannah)

Preparation of Sea Shell Powder (SSP) solution: The sea shells were thoroughly cleaned and scrubbed then were air dried. Shells were crushed using a metallurgical ring crusher and ground into powder. The solution was prepared same way as the CESP solution.

Sample Preparation: A total of 25 human permanent incisors were used. Teeth with visible crack, enamel caries and white spot lesion were excluded. All the extracted teeth were cleaned from soft tissue debris by ultrasonic scaler and disinfected by immersion in sodium hypo chloride solution (5%) for one hour.

The coronal portion of the collected teeth were separated from the radicular portion using a slow speed diamond saw with water irrigation. Then coronal portion were longitudinally sectioned so that 50 specimens were obtained from a total of 25 tooth samples such that each group had 10.

Samples were randomly divided into four groups:

Group A: untreated teeth, (control group)

Group B: demineralization by soft cola drink,

Group C: demineralization by soft cola drink followed by seven days' immersion in CPP-ACP,

Group D: demineralization by soft cola drink followed by seven days' immersion in CESP

Group E: demineralization by soft cola drink followed by seven days' immersion in SSP.

Demineralizing Protocol: Coca Cola drink was chosen as a demineralizing bath to induce dental erosion. The pH of the drink at 20°C was measured as 2.4. All specimens were randomly assigned to the following five groups (n=10). The enamel specimens in Group A were not subjected to any treatment. However, Group B, C and D specimens were exposed to four consecutive cycles of demineralization by 2 minutes immersion in six ml of soft drink carried out at 0,6,12 and 18 h intervals at room temperature.

Remineralizing protocol: The procedure was repeated twice daily for a period of seven days, followed by demineralization. Group C, GC Tooth Mousse was applied directly on the demineralized enamel surface for five minutes and then washed. Samples in Group D and E were exposed to 100 ml of CESP solution and SSP solution respectively for six hours and kept out of the solution for two hours, repeated till seven consecutive days. Before and after exposure to different remineralizing or demineralizing solutions all specimens were stored in a 100ml deionizing water. All the procedures were done at a room temperature of 24°C.

Surface Hardness Assessment: The surface micro hardness of all the samples were measured using Digital Display Vickers Micro Hardness Tester (Shimadzu, Japan) with a diamond shaped indenter. A load of 25g was applied to the surface of the specimens for 5 seconds. Five indentations were made with a spacing of 100 microns for each sample. The diagonal

length of the indentation made was then measured and Vickers values obtained were converted into micro hardness values.

Statistical analysis

Data were analyzed and presented as mean and standard deviation values by using SPSS 11.0 software. ANOVA was conducted to compare any difference in the surface micro hardness. In cases where a significant difference was found ($p<0.05$), a Post Hoc Analysis Tuckey test was conducted for inter group comparison.

Results

One-way ANOVA test showed significant difference in increase of surface micro hardness values after remineralization treatment in Group C, D and E. Post Hoc test showed statistical difference in Group D and E when compared Group C. (Table 1, Graph 1

Discussion

Dental Erosion remains an extraordinary test for clinician to perceive a significant reason factor of tooth structure loss, in connection to diagnosis, prevention and non-invasive treatment. If this condition stayed unchecked, it might continue to underlying dentin. Demineralized tooth surfaces can be remineralized whenever treated early.[1]

The severity of erosion is identified with a few components, including the chemical properties of the erosive medium and recurrence, contact of acid with tooth. It is identified with the adequacy of the protective mechanisms in the oral environment, for example, salivary creation, buffering limit, singular dental life systems.[1]

Microhardness estimation is appropriate for a material having fine microstructure, non-homogenous and inclined to cracking like enamel. Surface microhardness indentation gives generally a basic, non-destructive, fast technique.[16]

Different studies have utilized various techniques to evaluate the procedure of enamel remineralization. The generally used microhardness tests for assessing enamel remineralization are Vickers microhardness test and Knoop microhardness test.[11] As this study put the emphasis on assessment of surface microhardness of enamel, Vickers surface microhardness test was utilized.

The estimations of surface microhardness demonstrate the remineralization of enamel is more in CESP and SSP when contrasted and gathering other groups.

Accessibility of calcium and phosphate particles is fundamental for remineralization and increased pH of the arrangement alongside rich bioavailability of phosphate and calcium ions is primarily included for the procedure of remineralization.[24] Thus by creating supersaturation of these Calcium and phosphate ions advances remineralization and depresses the procedure of erosion on tooth surfaces.[22]

Calcium and phosphate ions initially enter the surface layer of enamel, to deposit minerals through the body of the lesion, which affirms the purpose behind the CPP metastable calcium phosphate solution of being productive remineralizing solution. Various demineralizing solution are present composing of calcium and phosphate with acetic acid or lactate. The fundamental variety lies in concentrations of components, which impacts the final pH and the sample exposure time. The pH utilized shifts from 3.5 to 5 and the time varies from 2 hours to 21 days.[2]

CPP-ACP paste increases the microhardness of demineralized enamel. A stable complex by casein phosphopeptide-nebulous calcium phosphate can be utilized for remineralization of essential enamel lesions. CPP-ACP keeps up a saturated condition of phosphate and calcium on the enamel surface, and thus,

diminishes demineralization and increases remineralization. CPP with the cluster sequence of Ser(p)- Ser(p)- Ser(p)- Glu-Glu can bond to ACP in the solution. This procedure holds a high level of phosphate and calcium particles on the surface of lesions, averts demineralization, and improves remineralization of enamel.[21]

CPP-ACP paste can attach to enamel, biofilm and soft tissues around the enamel and aids the free calcium and phosphate ions into enamel, bringing about the reformation of apatite crystals.[21] It improves the remineralization of carious lesions via saturation of enamel minerals. Previous studies have also demonstrated the role of CPP-ACP in preventing the process of demineralization. [21]

Calcium plays an active role in remineralizing enamel and CESP has a high level of bio-accessible calcium. Analysis of CESP utilizing X-beam fluorescence spectroscopy uncovered that it contains 98% calcium. Numerous studies have assessed the uses of eggshell, for example, a calcium oral supplement. Calcination procedure is done to get pathogen free powder and to build the alkalinity of powder. Besides, 10% acetic acid is added to guarantee the eggshell powder is for all intents and purposes free of pathogens.[16]

The shell structure has profoundly desirable mechanical properties that empower it to ensure the soft body of the animal against environmental harm and predators. Shells are brittle or semi weak materials of mineral components (~95%) and proteins (~5%). It is compositional mix that gives a shell its high level of hardness. [23]

A study by Bejoy Mony et al has shown similar results with the present study when remineralizing potential of Clinpro was compared with CESP. [22] However, further extensive clinical studies are needed comparing with the other commercially available remineralizing agents.

Conclusion

Within the limitations of this in vitro study, it can be concluded that remineralization and surface hardness of CESP and SSP showed no statistically significant difference on enamel. Whereas a significant difference was seen when compared with ACP-CPP on samples treated with demineralizing solution.

References

1. Feroz S, Moeen F, Haq SN, Protective Effect of Chicken Egg Shell Powder Solution (CESP) on Artificially Induced Dental Erosion: An in Vitro Atomic Force Microscope study; International Journal of Dental Sciences and Research. **2017**, 5(3), 49-55.
2. Poggio C LM, Dagna A, Chiesa M, Bianchi S. "Protective effect on enamel demineralization of a CPP-ACP paste: An AFM invitro study ". J Dent 37: 949-954. 2009.
3. Padma Gandi MS, Sangeeta Meena and Pallavi Waghmare. Comparative Study on remineralizing ability of Casein phosphopeptide Amorphous calcium phosphate and b-tricalcium phosphate on dental erosion: An invitro AFM Study. Gandi et al, Dentistry,2015;5:8
4. Lazarchik DA, Filler SJ. Effects of gastroesophageal reflux on the oral cavity. The American journal of medicine. 1997;103(5): 107-13.
5. Harley K. Tooth wear in the child and the youth. British dental journal. 1999;186(10).
6. Murdoch_Kinch CA MM. Minimally invasive dentistry. J Am Dent Assoc.134:87_95. 2003

7. R. PJO. Effects of potassium ions on action potential conduction in A- and C- fibers of rat spinal nerves J Dent Res 74(2): 634-641. 1995.
8. Oshiro M YK, Takamizawa T,Inage H,et al. "Effect of CPP-ACP paste on tooth mineralization: an FE-SEM study". J Oral Sci49: 115-20. 2007.
9. Kato MT LM, Sales-Pares SH, Buzalaf MA. "Preventive effect of commercial desensitizing toothpastes on bovine enamel erosion in vitro". Caries Res, 44: 85-9, 2010.
10. Reynolds EC; Remineralization of enamel subsurface lesions by casein phosphopeptide-stabilized calcium phosphate solutions. J Dent Res. 1997;76(9):1587-1595.
11. Chokshi K, Chokshi A, Konde S, et al. An in vitro Comparative Evaluation of Three Remineralizing Agents using Confocal Microscopy. J Clin Diagn Res. 2016;10(6):Zc39-42. doi:10.7860/jcdr/2016/18191.7984
12. Salehzadeh Esfahani K, Mazaheri R, Pishevar L. Effects of Treatment with Various Remineralizing Agents on the Microhardness of Demineralized Enamel Surface. J Dent Res Dent Clin Dent Prospects. 2015;9(4):239-245. doi:10.15171/joddd.2015.043
13. Reynolds EC. Anticariogenic complexes of amorphous calcium phosphate stabilized by casein phosphopeptides: a review. Spec Care Dentist. 1998;18(1):8-16.
14. Park JW BS, Suh JY, Lee DH, Kim SH, Kim H, et al. "Evaluation of bone healing with eggshell-derived bone graft substitutes in rat calvaria: a pliot study". J Biomed Mater Res A, 87(1). 203-14. 2008.
15. Rovensky J SM, Masaryk P, Svik K, Istok R. "Eggshell calcium in the prevention and treatment of osteoprosis". Int J Clin Pharmacol Res, 23(2-3). 83-92. 2003.
16. Mony B, Ebenezar R AV, Ghani MF, Narayanan A, Anand S, Mohan AG, Effect of Chicken Egg Shell Powder Solution on Early Enamel Carious Lesions: An Invitro Preliminary Study; Journal of Clinical and Diagnostic Research. 2015 Mar, Vol-9(3): ZC30-ZC32
17. A. L. Erosive tooth wear_ a multifactorial condition of growing concern and increasing knowledge. Monogr Oral Sci 20:1-8.2006.
18. Schaafsma A vDJ, Muskiet FA, Hofstede GJ, Pakan I, van der Veer E. "Positive effects of a chicken eggshell powder -enriched vitamin-mineral supplement on femoral neck bone mineral densityin healthy late post-menopausal Dutch women". Br J Nutr; 87(3): 267-75. 2002.
19. Park JW BS, Suh JY, Lee DH, Kim SH, Kim H, et al. "Evaluation of bone healing with eggshell-derived bone graft substitutes in rat calvaria: a pliot study". J Biomed Mater Res A, 87(1). 203-14. 2008.
20. Rovensky J SM, Masaryk P, Svik K, Istok R. "Eggshell calcium in the prevention and treatment of osteoprosis". Int J Clin Pharmacol Res, 23(2-3). 83-92. 2003.
21. Khamverdi Z, Kordestani M, Panahandeh N, Naderi F, Kasraei S. Influence of CO2 laser irradiation and CPP-ACP paste application on demineralized enamel microhardness. J Lasers Med Sci. 2018;9(2):144-148. doi:10.15171/jlms.2018.27.
22. Gaber AM, Dowidar KM, Talaat DM,The effect of ccp-acp on the surface microhardness of primary tooth enamel eroded by antihistamine syrup (in

vitro study) Alexandria Dental Journal. (2016);41 :86-91

23. Brundavanam RK, Fawcett D, Poinern GEJ. Synthesis of a bone like composite material derived from waste pearl oyster shells for potential bone tissue bioengineering applications. Int J Res Med Sci 2017;5:2454-61
24. Shetty S, Hegde MN,Bopanna TP, Enamel remineralization assessment after treatment with three different remineralizing agents using surface microhardness: an in vitro study. J Conserv Dent 2014;17 :49-52