Original Article

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Dentistry Section

Effect of Chicken Egg Shell Powder Solution on Early Enamel Carious Lesions: An Invitro Preliminary Study

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ABSTRACT

Aim: To evaluate the remineralization potential of enamel surface lesion using chicken eggshell powder (CESP) solution.

Materials and Methods: Ten freshly extracted un-erupted third molars decoronated at cement-enamel junction (CEJ) used in this study. Each decoronated tooth was sectioned to get 4 samples of enamel blocks such that 40 blocks were obtained which were then subjected to demineralization protocol and grouped as: Group 1-untreated group, Group 2-subsurface demineralization, Group 3-subsurface demineralization + 7 days CESP immersion, Group 4- subsurface demineralization + clinpro application. The samples were evaluated for X-ray fluorescence spectroscopy analysis, microhardness testing and atomic analyses using energy dispersive X-ray spectroscopy. The data were statistically analysed by using one-way ANOVA and Tukey – Kramer multiple comparison test.

Results: X-ray Fluorescence spectroscopy shows Calcium concentration of 98% and 0.46% of phosphate. Group 4 (Clinpro) shows the highest efficacy in enhancing the remineralization followed by Group 3 (CESP). The results of atomic analyses showed that quantitative amounts of Ca weight % and P weight % is statistically greater for all the three groups except the demineralized group.

Conclusion: CESP with higher calcium content can remineralise enamel surface lesion.

Keywords: Calcium, Minimal invasive dentistry, Phosphate, X-ray flurorescence spectroscopy

INTRODUCTION

The early enamel carious lesion often referred as white spots results when the pH level at the tooth surface is lowered by the production of acids by cariogenic bacteria. The acid ions penetrate deeply into the prism sheath porosities leading to subsurface lesions leaving the surface intact because of the fact that remineralization occur preferentially at the surface due to increased levels of Ca and HPO4 ions, fluoride ions, and buffering by salivary products [1].

Bioactive agents like casein phosphopeptide–amorphous calcium phosphate CPP-ACP (RecaldentTM) based on milk products have been proven successful in the treatment of these early enamel carious lesions both in-vivo and in-vitro by replenishing the lost calcium and phosphate ions. Recently, another remineralising agent Clinpro Tooth Crème (3M ESPE, Saint Paul, MN,USA) was introduced that contains 0.21% w/w sodium fluoride and an innovative functional tri-calcium phosphate (fTCP) system and the manufacturer claims that it shows better remineralisation of white spot lesions than CPP-ACP [2].

Chicken eggshell powder (CESP) has been investigated in various fields regarding its potential use. CESP is a rather known Ca source. It contains about 39% (w/w) elemental Ca with a bioavailability as high as from $CaCO_3$. CESP has excellent antirachitic effects in rats and humans. It increases the bone mineral density in animal models of post-menopausal osteoporosis in ovariectomized female rats. CESP can stimulate chondrocyte differentiation and cartilage growth in-vitro. CESP reduces pain and osteoresorption in postmenopausal women and women with senile osteoporosis. It also increases bone mineral density in such patients making it suitable in the prevention and treatment of osteoporosis when taken as an oral supplement for 12 months [3,4]. CESP has the potential efficacy to act as an osteoconductive bone substitute in a rat calvarial defect model. However, no research has analysed the effect of CESP on remineralisation of early enamel carious lesions [5]. The aim of this in vitro study, therefore, was to evaluate the possible remineralization of enamel surface lesion using CESP by microhardness analysis and energy dispersive X-ray spectroscopy.

MATERIALS AND METHODS

Production of Eggshell Powder

This in-vitro study conducted in Chennai, India to determine the remineralizing potential of CESP. Being an in-vitro study no human or animal subjects were involved, hence ethical clearance wasn't needed. The CESP was obtained by the process of calcination following the protocol given by World Property intellectual organization (WO/2004/105912: Method of producing egg shell powder) [6]. This Calcination process was done to obtain pure powder free of pathogens and to increase the alkalinity of powder. Normally CESP contains 95% calcium carbonate, which converts to basic calcium oxide on calcination, and this is responsible for the increase in alkalinity [7]. Twenty chicken eggs obtained from a local hatchery (Suguna Poultry Farm, Chennai, India), the contents removed and the eggshells were cleaned in distilled water (India Chemicals, Pvt ltd. Mumbai, India). The eggshells then kept in hot water bath at 100°c for 10 minutes followed by removing the membrane. These eggshells then crushed using a sterile mortar and pestle. The crushed particles then heated at 1200°c in a muffle furnace (Jai Instrument & Co, Chennai, India) and powdered to small particles. Then the powder analysed for its individual elemental composition percentage by weight using X-ray fluorescence spectroscopy analysis (Spectrace 6000 USA).

Production of Eggshell Powder Solution

One gram of CESP dissolved in 20 ml of 4% acetic acid (India Chemicals, Pvt ltd. Mumbai, India) in a test tube. The clear fluid which is collected at the top was then transferred to a beaker and the pH of the solution was tested using a pH meter (Deluxe deep vision, model no: 101, California, USA) which was 11.7.

Sample Preparation

Ten freshly extracted unerupted third molars used for the study and were cleaned using distilled water. The teeth were decoronated at CEJ. Each decoronated tooth then sectioned longitudinally in a mesio-distal direction first and then in a bucco-lingual direction with diamond disc so that four samples obtained from a single tooth. Then from these samples, four enamel blocks of dimensions (4mm long, 4mm width and 2mm thick) made from each sample and embedded in acrylic blocks.

Demineralisation Protocol [8]

Carious lesions representing preliminary stage of subsurface enamel lesion were created by placing the tooth samples in 20ml of demineralization bath for 72 h ($CaCl_2$ = 2.2 Mm NaH_2PO_4 = 2.2 Mm Lactic acid = 0.05 M, Fluoride = 0.2 ppm, solution is adjusted with 50% NaOH to a pH of 4.5). The specimens kept in the demineralization solution ($CaCl_2$, NaH_2PO_4, Lactic acid and Fluoride) for 72 h at 37° C created a subsurface demineralization of approximately 150 microns width with an intact surface simulating an early enamel lesion [1]. The groups formed are as follows:

Study groups (n=40 enamel blocks)

Group 1 (n=10) - untreated group

Group 2 (n=10) - subsurface demineralization only.

Group 3 (n=10) –subsurface demineralization followed by suspending the tooth samples in chicken eggshell solution) for 21 h for seven consecutive days for remineralization. For every 24 h, fresh chicken eggshell solution was prepared and the samples washed twice with distilled water.

Group 4 (n=10)-subsurface demineralization followed by topical application of clinpro (3M ESPE, Saint Paul, MN,USA) and then suspending the tooth samples in artificial saliva for 7 days.

Microhardness Testing

The surface microhardness of all specimens was analysed by Vickers microhardness testing machine (Lieca, chu-linh, Japan). A load of 25g applied for 5 sec and five indentations made for each specimen with a spacing of 100 microns. The average value considered as the microhardness of corresponding specimen.

Atomic Analysis by EDX

Both calcium and phosphorus analysed in all samples by energy dispersive X-ray spectrometry (Quanta 200 FEG). Electron beams maintained at 2 x10–10 amp were used and X-ray intensities in counts per second were recorded. The accelerating voltage was 15 kV.

STATISTICAL ANALYSIS

Data were computerized and analysed using SPSS 11.0 software. The results were analysed using one way ANOVA and Tukey – Kramer multiple comparison test.

RESULTS

[Table/Fig-1] show the microhardness values for all the groups and [Table/Fig-2] depicts the calcium/phosphate ratio for all the groups.

X- Ray Fluorescence Spectroscopy Analysis

Chemical analysis of CESP using X-ray fluorescence spectroscopy shows highest calcium concentration of 98% and 0.46% of phosphate. It also reveals 0.53% of Magnesium, 0.18% of Strontium, 0.11% of Sulfur and 0.03% of Potassium.

The results of atomic analyses showed that quantitative amounts of Ca weight % and P weight % is statistically greater for all the three groups except the demineralized group, although there is no statistical difference among these three groups.

Groups	Vickers Hardness Number
Group 1	312±17.3
Group 2	196.8±7.6
Group 3	248.6±14.7
Group 4	291.5±17.5

[Table/Fig-1]: Microhardness values for all the groups
There was statistical difference among all the groups

Group	Ca%	P%	Ca/P molar ratio
Control	36.94	15.63	2.36
Demineralised	16.93	08.43	2
CESP	37.67	15.62	2.40
Clinpro	35.01	17.17	2.03

[Table/Fig-2]: Calcium/Phosphate ratio for all the groups

DISCUSSION

Calcium plays an active role in remineralisation of enamel and CESP has a very high percentage of bio-available calcium. In recent years, chicken eggshell has gaining importance in various fields. Our chemical analysis of CESP using X-ray fluorescence spectroscopy revealed that it contains 98% calcium. Recently many studies have evaluated the uses of eggshell such as a calcium oral supplement. In this study, calcination process is done to obtain pure powder free of pathogens and to increase the alkalinity of powder. This calcination process obtains pure powder free of pathogens. Furthermore, 10% acetic acid is added to ensure the eggshell powder is virtually free of pathogens [7]. For standardising the samples, all the sample groups was obtained from the same tooth respectively. This is a pilot study and the first of its kind to determine the remineralization potential of early enamel carious lesions by CESP solution. The CESP solution is applied to the tooth surface by means of different vehicles such as glycerin solution or methylcellulose gel in an appliance.

According to Lata et al., the demineralising solution creates a subsurface demineralisation of approximately 150 microns width with an intact surface simulating an early enamel lesion. The concentration of both calcium and phosphates in the demineralising solution was at 50% of saturation level, causing dissolution of only enamel subsurface. Addition of fluoride prevented surface demineralisation by forming fluorapatite at the surface [1]. The Clinpro Tooth Crème containing Tri calcium phospate with 950 ppm fluoride, as claimed by the manufacturer shows better remineralization effect in surface and sub surface lesions of enamel. During manufacturing process calcium fluoride, coexist in a protective barrier. This acts as a transport for tricalcium phosphate to the teeth. When it contacts saliva during brushing, the barrier breaks and makes the calcium, phosphate and fluoride readily available to the tooth. This terminates the demineralization and promotes remineralization [7].

Microhardness measurement is appropriate for a material having fine microstructure, non homogenous and prone to cracking like enamel. Surface microhardness indentation provides relatively a simple, non-destructive, rapid method. There is statistical difference among all the groups. Among the groups, clinpro showed statistically better remineralisation than CESP [Table/Fig-1].

When compared with other natural calcium sources, CESP has low levels of toxic metals like Pb, Al, Cd, and Hg. The N-terminal sequence (Met-Ala-Val-Pro-Gln-Thr-Met-Val-Gln) of eggshell matrix proteins has been suggested in the increased calcium transport and considered as a potential significance of eggshell calcium when used as calcium supplements [9,10]. Hence, CESP solution is used in this study.

The pH of a CESP solution evaluated by pH meter is 11.7. The increased pH of a remineralising solution is favourable, as it increases the ion activity of anions such as phosphate and hydroxyl ions in the solution. The ion activity corresponds to the concentrations of these ions in the solution. Therefore, there will be more availability of these ions for remineralisation. At low pH, there will be more H^+ ions, which will combine with these anions making them less available for remineralization. For example, when the pH of the solution is decreased from 7 to 5, the ion activity i.e. concentration of PO_4^{3-} decreases by 4500 fold and OH^- ion by 100 folds. In addition, the basic form of phosphate anion present in hydroxyapatite is PO_4^{3-} and these anions are present in higher concentrations only at a high pH of 11-12. For remineralization to occur, bioavailable calcium and phosphates are essential [11,12]. Therefore, the rich bioavailability of calcium along with the high concentration of phosphates [13] present in CESP solution [Table/Fig-2] coupled with its increased pH may be responsible for remineralization.

CONCLUSION

Within the limitations of this study, it can be concluded that the high pH of the of chicken egg shell solution along with the rich bioavailable calcium content of chicken egg shell has the potential to favoured remineralisation. Even though Clinpro showed more remineralization than CESP, the latter due to its easy bioavailability and natural source of calcium and phosphate can be the future in remineralising enamel carious lesions. Further, clinical studies regarding suitable vehicle for CESP is needed as this might increase the remineralization potential of CESP comparable to the commercially available agents.

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