ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

International Journal of Dental Science and Innovative Research (IJDSIR)

IJDSIR : Dental Publication Service

Available Online at: www.ijdsir.com

Volume – 4, Issue – 3, June - 2021, Page No. : 104 - 113

Comparative evaluation of remineralization potential of two agents on artificially demineralized human enamel using laser fluorescence (DIAGNOdent): An in vitro study

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Citation of this Article: Dr. Moksha Nayak, Dr. Sarath Das K, Dr. Lakshmi Pandey, "Comparative evaluation of remineralization potential of two agents on artificially demineralized human enamel using laser fluorescence (DIAGNOdent): An in vitro study", IJDSIR- June - 2021, Vol. – 4, Issue - 3, P. No. 104 – 113.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

The aim of this study was to evaluate the remineralization potential between chicken egg shell cream (CESC) and calcium sucrose phosphate(CaSP) paste on artificially demineralized human enamel using laser fluorescence (DIAGNOdent).

In this in vitro study 30 human non carious premolars were selected and classified into 3 groups of 10 premolars in each; Group 1**:** (n=10) Calcium sucrose phosphate (CaSP) Group 2: (n=10) Chicken egg shell powder cream(CESP) Group 3: (n=10) Control (artificial salaiva). All the group samples were assessed using laser fluorescence (DIAGNOdent) at the base line, after demineralization and remineralization. The values obtained were statistically analyzed using paired 't' test for intragroup comparison and one way ANOVA test and bonferroni test was used for intergroup comparison.

All the two experimental groups showed a statistically significant amount of remineralization (P<0.001) , Significant amount of remineralization was produced by CaSP and CESC after the 15th day, the remineralization produced by CESC was slightly lesser than the remineralization produced by CaSP.

Keywords: Laser fluorescence (DIAGNOdent), Remineralization, Calcium sucrose phosphate (CaSP), Chicken egg shell powder cream(CESP), Demineralized human enamel.

Introduction

Dental caries is a complex multifactorial disease caused by an imbalance in physiologic equilibrium between tooth

mineral and biofilm. It is one of the most prevalent disease affecting humans with a prevalence of 81.5% among 5-6 year old and 59.6% among 12-13 year old[1,2]. The prevention of dental caries and the remineralization of enamel subsurface lesions before restorative intervention is the major challenge and goal of modern dentistry.

White-spot lesions are the earliest macroscopic findings of enamel caries[3]. When enamel surface layer stays intact during subsurface demineralization, but, without treatment, it will eventually collapse into a fully formed cavity[4].

The demineralization process can be prevented by creating an suitable environment conducive for remineralization by various remineralizing agents. The process of restoring lost mineral ions to the tooth structure and strengthening the porous lattice work is known as remineralization[5].

Various remineralizing agents have been suggested in the past with promising efficiency in enamel remineralization. Studies have also shown the remineralizing potential of casein phosphopeptide-amorphous calcium phosphate(CPP-ACP), calcium sucrose phosphate(CaSP), tricalcium phosphate and bioactive glass containing calcium sodium phosphosilicates as an adjunctive to fluoride therapy in the non- invasive management of early carious lesions [5,6,7].

The minimal invasive treatment of incipient carious lesions with the novel preventive formulations like calcium sucrose phosphate (CaSP) have been shown successful enamel remineralization. Calcium Sucrose Phosphate (CaSP) is a mixture of calcium

sucrose mono and diphosphate, disucrose monophosphate and inorganic calcium phosphate that contains 11% calcium, 9.5% organic phosphate and 2.5% inorganic phosphate[6]. It reduces the rate of dissolution of hydroxyapatite in acid buffers and decreases enamel demineralization[7].

Egg shell calcium is considered the best natural source of calcium. Chicken egg shell cream (CESC) has been investigated in various fields due to its rich calcium source in bone substitution[9,10] treatment of osteoporosis[9] and remineralization of early enamel lesions. It contains 94% calcium carbonate, 1% calcium phosphate, 1% magnesium carbonate, and 4% organic matter[12]. It contains about 39% elemental calcium with a bioavailability as high as from calcium carbonate $CaCO_3$)[13]. Egg shells contains not only calcium but also other elements such as fluoride and strontium -despite the low concentration - and have a positive effect on bone and remineralization of early enamel carious lesions[12].

Laser fluorescence (LF) is introduced as an early approach for identifying dental caries. It is useful for early detection of hidden caries in non-cavitated teeth through a

Non-invasive method. The DIAGNOdent device is based on the principle that carious tooth tissue fluorescence more strongly than sound tissue when irradiated with light with a wave length of 655nm. The process of remitted florescence shows various scales, higher the scale deeper the caries invasion [14].

The utilization of the remineralizaing potential of chicken egg shell cream (CESC) is the new naturally available, economical, readily available, alternative for the commercially available remineralizing products. To date very few studies have been conducted concerning the remineralizing potential of CESC. Evaluation of remineralization potential of CESC along with calcium sucrose phosphate by using a laser fluorescence is still limited. Hence this study aimed to investigate the remineralizing potential of CESC and comparing its potential with the commercially available calcium sucrose phosphate formulation (EnaFix) using laser fluorescence.

Materials and methods

Maxillary and mandibular caries free premolar teeth, freshly extracted for Orthodontic reasons from young adults were obtained for the present study from the Department of Oral and Maxillofacial Surgery at K.V.G Dental College and Hospital, Sullia, Dakshina Kannada, Karnataka.

The collection, storage, sterilization and handling of the sample teeth were followed according to Occupational Safety & Health Administration (OSHA) and the Centre for Disease Control & Prevention recommendations and guidelines.

Occupational Safety & Health Administration (OSHA) and the Centre for Disease Control & Prevention recommendations and guidelines

1. The teeth were cleaned of visible blood and debris.
2. Selected teeth were kept in 3% sodium hypochlorite for 15 minutes for disinfection and stored in normal saline with 0.2% thymol.
3. The teeth were kept in a well-constructed container with a secure lid to prevent leaking during transport.
4. The containers were labelled with the biohazard symbol.

Inclusion Criteria

- Caries free teeth.
- Teeth extracted for orthodontic reasons only.

Exclusion criteria

- Extracted teeth with any restorations.
- Extracted teeth with developmental anomalies
- Extracted teeth with intrinsic stains.
- Extracted teeth with any wasting diseases like attrition, abrasion and erosion.
- Extracted teeth with laser fluoresces (DIAGNOdent) score more than 7.

Enamel specimen preparation and grouping

The teeth were thoroughly cleaned of its debris, calculus, and soft tissues. The buccal surfaces of all the teeth were polished using micromotor, contra-angled handpiece, polishing brush, polishing cup, and polishing paste. The polished extracted teeth were randomly grouped into three groups depending on the materials used for the study using simple randomized sampling.

Group 1: (n=10) Calcium sucrose phosphate (CaSP)

Group 2: (n=10) Chicken egg shell cream (CESC)

Group 3: (n=10) Control (no agent used)

Each extracted teeth were coated with nail varnish, leaving an enamel window of 3 mm × 3 mm on the buccal surface in the middle one-third of the crown. All the samples were examined using DIAGNOdent® (KaVo) to assess for any surface changes present on the labial window. As recommended by the manufacturer, prior to every measurement session, the DIAGNOdent pen were calibrated against its own ceramic standards.

The labial window area were carefully scanned using the type B probe by holding the tip in close contact with the tooth surface and tilting the tip around the measuring area in order to collect the fluorescence from all directions.

Demineralizing solution preparation

Demineralizing solution was prepared in the department of biochemistry, K.V.G dental college and hospital, Dakshina Kannada, Karnataka.

- 2.2 mM calcium chloride ($CaCl_2.2H_2O$)
- 2.2 mM monosodium phosphate ($NaH_2PO_4.7H_2O$)
- 0.05 M lactic acid

The final pH was adjusted to 4.5 with 50% sodium hydroxide (NaOH).

Artificial saliva preparation

- 2.200 g/L gastric mucin,
- 0.381g/L sodium chloride (NaCl)
- 0.213 g/L calcium chloride ($CaCl_2.2H_2O$)

- 0.738 g/L potassium hydrogen phosphate ($K_2HPO_4.3H_2O$)
- 1.114 g/L potassium chloride (KCl).

The final pH was adjusted to 7.00 at 37 C° with 85% lactic acid.

Production of egg shell powder

The CESC were obtained by the process of calcination following the protocol given by World Property intellectual organization (WO/2004/105912: Method of producing egg shell powder)[15]. Twenty chicken eggs were obtained from a local hatchery, the inner contents were removed and the eggshells were cleaned in distilled water. The egg shells were then kept in hot water bath at 100°c for 10 minutes and followed by removing the membrane. These egg shells then crushed using a sterile mortar and pestle. The crushed particles then heated at 1200˚c in a muffle furnace and powdered to small particles.

The CESP was obtained by the process of calcination. This Calcination process was done to obtain pure powder free of pathogens and to increase the alkalinity of powder.

The CESP contains 95% calcium carbonate, which converts to basic calcium oxide on calcination, which is responsible for the increase in alkalinity[16].

Production of egg shell powder cream

One gram of chicken eggshell powder mixed with one gram of xanthan gum powder and one ml of 4% acetic acid were mixed until uniform creamy consistency were achieved.

The samples were then immersed into the glass container containing 50 ml of demineralizing solution for a period of 48 hours at 37˚C inside the universal incubator. After 48 hours of incubation the samples were washed with deionized water, dried with the help of an air syringe. They were evaluated with DIAGNOdent and samples showing value of 9 and above on the digital display were taken for further evaluation (Picture 1). Samples were rubbed with representative remineralizing agents at every 24 hours with the help of polishing cup attached to a contra angle micromotor hand piece for 5 minutes for a period of 15days. The samples were washed with deionized water and placed in the universal incubator at 37˚C between each remineralizing cycle (Picture 2). In control group(group 3), samples were washed with deionized water and placed in artificial saliva. Artificial saliva were replenish every 24 hour just before immersion of freshly treated samples. The samples were exposed intermittently to demineralizing solution for 2 hour followed by artificial saliva in every 24 hours in order to generate the daily acid challenges in the oral cavity. After 15 days all samples were then assessed using DIAGNOdent to record the values.

The DIAGNOdent values obtained were tabulated and statistically analyzed using paired‘t’ test for intragroup comparison and one way ANOVA test and Tukeys Post hoc test for intergroup comparison.

Observations and Results

Statistical analysis was done for intragroup and intergroup for DIAGNOdent score. By applying paired‘t’ test a highly significant decrease in the mean values of DIAGNOdent score of enamel from baseline to after demineralization was observed .There was a highly significant decrease in the mean values of DIAGNOdent score of enamel from demineralization to 15 days after remineralization in group 1 and group 2. This shows that CESC and CaSP has a potential for remineralizing artificial enamel carious lesions. Comparative evaluation of relative percentage improvement in DIAGNOdent score at each stage of the study was done by applying one-way ANOVA (Table 1). Pairwise comparisons was done by Tukeys post hoc test (Table 5).

Figure 1: evaluation of tooth with DIAGNOdent pen

Figure 2: Specimens in universal incubator

Observations from this study proved that two remineralizing agents used in this study were effective in remineralizing artificially demineralized human enamel. Remineralization of demineralized human enamel lesion samples were assessed using laser fluoresces after 15 days showed a considerable reduction in the DIAGNOdent score compared to the base line scores. Group 1 (CaSP) showed considerable reduction in the DIAGNOdent score after remineralization compared with group 2(CESC) but there was no change in the base line score to group 3 were only artificial saliva was used.

Complete remineralization of the artificially demineralized human enamel was not seen in any of the group during 15 days remineralization protocol.

Table 1: Descriptive statistics

Groups		DIAGNOdent Evaluation Before Remineralization	DIAGNOdent Evaluation After Remineralization
Group 1	Mean	11.5000	7.2000
	N	10	10
	Std. Deviation	1.35401	1.22927
Group 2	Mean	11.6000	9.8000
	N	10	10
	Std. Deviation	1.07497	1.22927
Group 3	Mean	11.6000	11.6000
	N	10	10
	Std. Deviation	1.17379	1.17379

One way anova; Group 1: Calcium sucrose phosphate (CaSP) paste; Group 2: Chicken egg shell cream (CESC); Group 3: control (artificial saliva); N= number of sample evaluated.

Table 2: Comparison of mean DIAGNOdent values at baseline

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.067	2	.033	.023	.977
Within Groups	39.300	27	1.456		
Total	39.367	29			

One way anova; df = degree of freedom; F = homogeneity in variance. At baseline there was no statistical significant difference in difference in DIAGNOdent values between the three groups.

Table 3: Comparison of mean DIAGNOdent values after remineralization

	Sum of Squares	df	Mean Square	F	Sig.
Inter-group comparison	97.867	2	48.933	33.364	.000
Intra-group comparison	39.600	27	1.467		
Total	137.467	29			

One way anova; df = degree of freedom; F = homogeneity in variance. There is a statistically significant difference in DIAGNOdent values between the three groups after intervention.

Table 4: Intergroup comparison of the study groups

Study groups	Study groups	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Group 1	Group 3	-4.40000*	.54160	.000	-5.7429	-3.0571
	Group 2	-2.60000*	.54160	.000	-3.9429	-1.2571
Group 2	Group 3	-1.80000*	.54160	.007	-3.1429	-.4571

	Group 1	2.60000*	.54160	.000	1.2571	3.9429
Group 3	Group 2	1.80000*	.54160	.007	.4571	3.1429
	Group 1	4.40000*	.54160	.000	3.0571	5.7429
*. The mean difference is significant at the 0.05 level.						

Post Hoc Test (Tukey HSD) Group 1: Calcium sucrose phosphate (CaSP) paste; Group 2: Chicken egg shell cream (CESC); Group 3: control (artificial saliva).

The difference in DIAGNOdent values between control and chicken egg shell cream group were statistically significant.(p=0.007)

The difference in DIAGNOdent values between control and Calcium sucrose phosphate paste group were statistically significant. (p=0.000)

The difference in DIAGNOdent values between chicken egg shell cream group and Calcium sucrose phosphate paste group were statistically significant. (p=0.000)

Table 5: Comparison of pre and post intervention among remineralizing agents

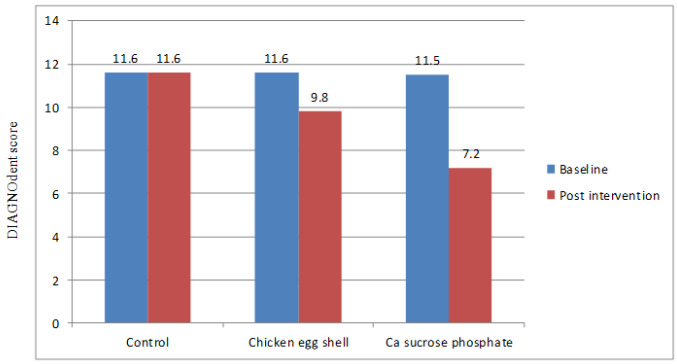
		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	CESC pre	11.6000	10	1.07497	.33993
	CESC post	9.8000	10	1.22927	.38873
Pair 2	CaSP pre	11.5000	10	1.35401	.42817
	CaSP post	7.2000	10	1.22927	.38873

Paired T-Test; N= number of sample evaluated.

In both chicken egg shell cream group and Calcium sucrose phosphate paste group, there is a statistically significant decrease in DIAGNOdent values following intervention (p=0.000)

At the end of 15 days remineralization cycle highest reduction in the DIAGNOdent score was observed in group 1 followed by group 2. There was no reduction in DIAGNOdent score in group 3 after the complete remineralization cycle.

Graph 1: Comparison of mean reduction in DIAGNOdent score among the study groups



Group 1:Calcium sucrose phosphate(CaSP) paste; Group 2:Chicken egg shell cream(CESC); Group 3: control(artificial saliva)

Discussion

Prevention, control and treatment are the major steps in the management of dental caries. Although it is a challenge to diagnose and manage dental caries, a wide range of newer technologies, materials and research have made possible the management in more predictable manner.

The major difference between caries and diseases of other tissues and organs is that the hard substance of the tooth cannot be regenerated (enamel), or it can only be actively regenerated by cells to a slight degree (dentin). ‘‘Healing’’ occurs primarily through mineralization processes in which cells do not directly participate. Remineralization can only occurs where there are crystal nuclei[17]. The present trend of managing is more towards concept of

prevention and minimal intervention rather than traditional approach of “drill & fill”.To permanently arrest caries progression by using exclusively noninvasive means, the tooth surface needs to be sufficiently accessible for cleaning and remineralizing agents[18].

S. Thabitha Rani et al done an in vitro study to evaluated the remineralizing potential of Calcium Sucrose Phosphate (CaSP) and Caesine PhosphoPeptide-Amorphous Calcium Phosphate (CPP-ACP) on early enamel lesions. The study result concluded that mean surface micro hardness recovery with CaSP was significantly higher than with CPP-ACP and CaSP paste was effective in remineralizing early enamel lesions than CPP-ACP[6]. Gade V done an in an vitro study investigated the remineralization efficacy of two different remineralizing agents on enamel caries like lesions. The study concluded that Enafix being a cost effective material as compared to GC Tooth mousse plus, it can be used as an alternative for better remineralization of enamel caries[21].

The present study evaluated the remineralizing potential of two remineralizing agents, CaSP and CESC using DIAGNOdent. Among the tested materials CaSP showed the higher remineralization capacity on artificially demineralized human enamel followed by CESC. Calcium phosphate-based remineralization tech- nologies look promising as adjunctive treatments to topical fluorides in the non-invasive management of early caries lesions. calcium phosphate-based remineralization tech- nologies look promising as adjunctive treatments to topical fluorides in the non-invasive management of early caries lesions.

The calcium phosphate-based remineralization technologies like Calcium Sucrose Phosphate (CaSP) is a mixture of calcium sucrose mono and diphosphate, disucrose monophosphate and inorganic calcium phosphate that contains 11% calcium, 9.5% organic phosphate and 2.5% inorganic phosphate look promising as adjunctive treatments to topical fluorides in the non-invasive management of early caries lesions[6,7]. Absorption of sucrose phosphate ions rapidly on the enamel surface there by reducing the rate of acid dissolution of hydroxyapatite and quick remineralization by calcium and phosphate ions by common ion effect[19].

Calcium plays an active role in remineralisation of enamel and CESC has a very high percentage of bio-available calcium. Recent chicken egg shell studies using X-ray fluorescence spectroscopy revealed that it contains 98% calcium[13]. The pH of a CESPC solution evaluated by pH meter is 11.7. The increased pH of a remineralising cream is favourable, as it increases the ion activity of anions such as phosphate and hydroxyl ions in the cream. At low pH, there will be more availability of H+ ions, which will combine with these anions making them less available for remineralization[20]. Similar observation of the present study also reported by Mony B et al, that chicken egg shell solution along with the rich bioavailable calcium content of chicken egg shell has the potential to favored remineralisation[13]. Haghgoo R et al in an in vitro study compared the efficacy of nano-hydroxyapatite (NHA) and eggshell for remineralization of enamel caries-like lesions by pH cycling. The study result concluded that eggshell solution can be used as a remineralizing agent for incipient enamel carious lesions[12].

Gangrade A et al evaluated the remineralization efficacy of different calcium and fluoride based delivery systems on artificially demineralized enamel surface. The study concluded that complete remineralization did not occur within 7 days. Hence this present study 15 days of remineralization cycle was chosen. SnF2 showed the highest potential for remineralization followed by CaSP and CPP-ACPF[22]. In the present study results showed fifteen days of remineralization cycle failed to produce

complete remineralization in artificially demineralized human enamel. Hence period of application which required for the remineralizing agents to completely remineralize the lesion is not determined by this study. Enamel remineralization was confirmed with the reduced DIAGNOdent value observed after the application of remineralizing agents compared to the previous demineralization values.

Bioavailabilities of calcium and phosphates ions with increase pH present in CESC are essential for tooth remineralization therefore using CESC is a novel approach for tooth remineralization. Contrastingly, restorative measures and microinvasive therapies (ie, fissure sealing and caries infiltration) are invasive approach to caries management. The present in vitro study showed CESC has remineralizing properties on artificialy demineralized human enamel but comparably less than CaSP preparations.

Conclusion

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

CaSP and CESC preparations used in this study could effectively remineralize the artificially demineralized human enamel.

CaSP preparations have better remineralizing potential than CESC preparations in remineralizing artificial enamel caries.

Complete demineralization did not occur by all the remineralizing agents in 15 days.

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