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An in vitro evaluation of effect of remineralising potential of marine skeletal specie– meretrixmeretrix extract on human enamel

Running title: Remineralising potential of marine skeletal specie

ABSTRACT

Background: To test the effectiveness of a marine skeleton specie - Meretrix meretrix (cockle shell) powder extract on human tooth enamel to remineralize a subsurface defect.

Methods: The study included 5 freshly extracted human 3rd molar teeth that were sectioned at the cement-enamel junction (CEJ). Later, each tooth coronal region was separated into four pieces of enamel blocks, yielding twenty blocks that were demineralized and categorised as follows: Cockle shell powder extract from Group I-subsurface demineralization. Group II: subsurface demineralization + clinpro application for a thirty-day period in artificial saliva. The samples were evaluated using X-ray fluorescence spectroscopy, micro hardness testing, and energy dispersive x-ray spectroscopy for atomic analysis. The data was analysed using a one-way ANOVA and the Tukey Kramer multiple comparison test.

Results: In the cockle shell, X-ray fluorescence spectroscopy revealed calcium concentrations of 98.3 percent and 0.21 percent, respectively. Group II (Clinpro) has the greatest potential for speeding up the remineralization process, followed by Group I: CSEP (Cockle shell extract powder). Quantitative volumes of Ca weight percent and P weight percent are statistically bigger for both groups, according to the results of atomic evaluation.

The remineralisation efficiency of Group II (Clinpro) was higher than that of Group A. (mussel shell extract).

Conclusion: Enamel subsurface flaws can be remineralized using marine shells with a higher calcium content.

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

INTRODUCTION

Dental caries is a complex illness caused by a misalignment of pathogenic and protective factors in the oral cavity. Salivary dysfunction, cariogenic bacteria, and fermentable carbohydrates have all been scientifically proven to be significant pathogenic factors. The disparity created will inhibit physiological remineralization and demineralization processes, favouring demineralization [1.]As a result, understanding the role of remineralization in limiting caries progression and preserving healthy balance when the demineralization process occurs should be highlighted in dental caries therapy[2] . Human saliva contains supersaturated calcium and phosphate ions, making it effective at remineralizing enamel[3]. After all, if acid exposures prevent the body's natural remineralization process from taking place, alternate treatment procedures to promote remineralization are required. Several medicines that inhibit or stop the production of carious lesions have been bottled up or are currently being researched.

Fluoride (F) has recently become a popular remineralizing agent in clinical practise. Casein phosphopeptide–amorphous calcium phosphate (CPP-ACP), a remineralizing agent made up of 80 percent milk proteins, has surpassed fluoride as the most researched remineralizing agent in dentistry. Tricalcium phosphate (TCP) is a new remineralizing agent

that has been suggested to help raise the free calcium content in saliva & dental plaque [4]. Recently, another remineralizing agent, Clinpro Tooth Creme (3M ESPE, Saint Paul, MN, USA), with 0.21 percent w/w sodium fluoride and a new functional tri-calcium phosphate (fTCP) system, was investigated, with the producer claiming that it had a higher remineralizing capacity of initial subsurface lesions than CPP-ACP [5]. The potential utility of marine skeleton extract powder in the medicinal industry has been examined in a variety of ways. The cockle (Meretrixmeretrix) shell, a natural marine species, has been widely recognised for its exceptional value and pure calcium carbonate aragonite polymorph composition [6] .Cockle shell contains roughly 97 percent CaCO3, as well as organic compounds and oxides such as SiO2, MgO, and SO3 [6]. Aside from that, it has nearly identical mineral components to bone, with elevated calcium carbon (CaC) and no elements like mercury (Hg) or arsenic (As), making it ideal for biological applications [7] .However, no research has been done to see how mussel shell extract affects the remineralization of early enamel carious lesions [8,. 9, 10].

Objectives

The goal of this study was to see if Meretrixmeretrix (cockle shell) powder extract may help remineralize enamel surface lesions on human teeth.

METHODS

The in vitro quantitative observational research study was conducted for one month from June 20, 2020 to July 20, 2020 at the Department of Conservative Dentistry and Endodontics, Vinayaka Missions Sankarachariyar Dental College, Salem, Tamil Nadu state, India.

Synthesis of Calcium Carbonate Powder: The calcination procedure was performed to collect pure powder devoid of impurities and to elevate the alkalinity of cockle shell powder. Commonly cockle shell extract powder possesses of 98% calcium carbonate, which transforms to basic calcium oxide on calcination, and this promotes the boost in alkalinity [9].

The cockle shells were obtained from Malpebeach, Mangalore, India. The calcium carbonate powder was prepared from the cockle shells according to the method explained by Islam et al [10]. Cockle shells were dehydrated in an oven at 50^0 C for 7 days, and then crushed and amalgamated into a refined powder, which was filtered through a 90-m laboratory stainless steel sieve (Jai Instrument & Co, Chennai, India). X-ray fluorescence spectroscopy was used to determine the powder's discrete elemental makeup percentage by weight (Spectrace 6000 USA). ***Synthesis of Cockle Shell Powder Extract Solution:*** One gram of CSPE was mixed in 20 ml of 4% acetic acid in a test tube. The pure fluid which was found at the top was shifted to a beaker and the pH of the solution was checked with a pH meter which was found to be 11.8.

Preparation of Sample*:* Five recently extracted unerupted third molars were cleaned and decoronated at CEJ, then sectioned longitudinally in a mesio-distal and bucco-lingual manner with a diamond saw, dividing a single tooth into four portions (4mm width, 4mm long, and 2mm thick) and implanted in acrylic blocks. ***Demineralisation Protocol***[11]

Carious lesions displaying early subsurface enamel lesion was acheived by immersing samples in 20ml of demineralization bath for 72 hours (CaCl2 = 2.2 Mm NaH2PO4 = 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 samples kept in the demineralization solution (CaCl2, NaH2PO4, Lactic acid and Fluoride) for 72 hours at 37° C led to subsurface demineralization of almost 150 microns'diameter exhibiting an early enamel lesion [12].

Group I (n=10) –subsurface demineralization proceeded by placing the tooth samples in cockle shell extract solution for 24 hours for 30 successive days for remineralisation. For every 24 hours, fresh cockle shell extract solution was processed and the samples were purified with distilled water. Group II (n=10)-subsurface demineralization proceeded by topical application of clinpro (3M ESPE, Saint Paul, MN, USA) and then placing the tooth

samples in artificial saliva for 30 days. For every 24 hours, fresh artificial saliva was created and clinpro was applied topically.

Microhardness Testing: Vickers microhardness testing machine (Lieca, chu-linh, Japan) was used to test the surface microhardness. A mass of 25g was placed for 5 sec and five cycles made for every sample with a spacing of 100 microns.

Atomic Analysis by EDX

All specimens were tested for both calcium and phosphorus content by Energy dispersive X-ray spectrometry (Quanta 200 FEG). Electron beams kept at 2 x10–10 amps were used and X-ray dimension in counts per second were detected.

Statistical analysis

SPSS 11.0 software was used to analyse the obtained data using One-way ANOVA and Tukey – Kramer multiple comparison test.

RESULTS

Table 1 indicates micro hardness values for group I: Cockle shell Powder Extract (42.94 ± 0.04) and group II:Clinpro (59.25 ± 0.05). Table 2 indicates, calcium/Phosphate Ratio Mean ± S.D Value. group I: Cockle shell Powder Extract had Ca/P molar ratio of 2.58 and group B:Clinpro had Ca/P molar ratio of 2.35.

X- Ray Fluorescence Spectroscopy Analysis

Chemical examination of cockle shell powder extract using X-ray fluorescence spectroscopy revealed a maximum calcium concentration of 98 percent and a phosphate content of 0.21 percent. Magnesium 0.54 percent, Strontium 0.17 percent, Sulfur 0.13 percent, and Potassium 0.04 percent were also detected. The findings of atomic testing revealed that both groups had statistically greater quantitative levels of Ca weight percent and P weight percent.

DISCUSSION

The process of enamel remineralization is aided by the presence of calcium on the tooth surface. CSPE contains a high proportion of bio-available calcium. Cockle shell powder extract has been widely used in a variety of fields in recent years. X-ray fluorescence spectroscopy was used to examine the chemical composition of CSPE, which revealed that it contains 98 percent calcium. Various studies have recently revealed the use of cockle shell as a calcium oral supplement. The calcination technique was used in this study to gather pure powder free of microorganisms and to increase the alkalinity of the powder. The cockle shell powder was also treated with 10% acetic acid, which rendered it nearly pathogen-free[9] . This is the first study to use a CSPE solution to assess the remineralization capability of early enamel carious lesions. CSPE was applied to the surface of all samples using a glycerine solution.

According to Lata et al., subsurface demineralization of around 150 microns in width with intact enamel mimics a subsurface enamel lesion.

Only 50% of the calcium and phosphates in the demineralizing solution were saturated, resulting in the disintegration of only the enamel subsurface area. Fluoride is added to the surface to cause the formation of fluorapatite[12] . Clinpro Tooth Creme contains tri calcium phosphate with 950 ppm fluoride, which the manufacturer claims has good remineralization efficacy for surface and subsurface enamel defects. Calcium fluoride coexists in a protective barrier during the manufacturing process [13] .

This will assist in the transport of tricalcium phosphate to the tooth's surface. When it comes into contact with saliva during brushing, the barrier is broken, allowing calcium, phosphate, and fluoride to reach the tooth. This will halt the process of demineralization and encourage the process of remineralization[9] . Clinpro had a statistically superior remineralization property than CSPE, according to this study [Table-1]. When combined with other natural calcium sources, CSPE reduced harmful metal levels such as Pb, Al, Cd, and

Hg. The N-terminal sequence of cockle shell matrix proteins promotes calcium transport and is thought to be a possible benefit of cockle shell when used as a calcium supplement [14]. As a result, CSPE solution was used in this study.

The pH of a CSPE solution, which was predicted to be 11.8 by the pH metre, was tested. An rise in pH of a remineralising solution is recommended because it improves the ion activity of anions in the solution such as phosphate and hydroxyl ions. The concentrations of these ions in the solution are related to the ionic activity. As a result, these ions will be more readily available for remineralization. At low pH, there will be more H+ ions, which will combine with these anions, making them less available for remineralization. Furthermore, the basic form of phosphate anion found in hydroxyapatite is PO43-, and these anions can only be found in higher concentrations with a pH of 11-12.

Bioavailable phosphates and calcium are beneficial for remineralization [15]. As a result, remineralization may be caused by the enhanced bioavailability of calcium, as well as the increased concentration of phosphates present in CSPE solution [Table-2], in combination with the higher pH.

Limitations of the study: Because it was conducted in vitro, a perfect replica of the oral environment was not possible.

People who are allergic to marine items may not be able to use the marine shell paste.

CONCLUSION

Within the study's constraints, it was discovered that clinpro had superior remineralization than CSPE. Advanced clinical research is needed to determine the best vehicle for CSPE, since this could boost the remineralization capability of CSPE to levels comparable to commercially available remineralizing drugs.

Ethical Approval:

As per international standard or university standard written ethical approval has been collected and preserved by the author(s). The ethical clearance number was obtained (VMSDC/IEC/ Approval No.160).

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Legends for illustrations

Tables

Table 1: Microhardness test Mean ± S.D Value

S.NO	GROUP	Mean ± S.D
1	Group I (Cockle shell Powder Extract)	42.94 ± 0.04
2	Group II (Clinpro)	59.25 ± 0.05

Table 2: Calcium/Phosphate Ratio Mean ± S.D Value

S.NO	GROUP	Calcium	Phosphate	Ca/P molar ratio
1	Group I (Cockle shell Powder Extract)	37.31	14.26	2.58
2	Group II (Clinpro)	35.02	16.24	2.35