Mansoura University
Faculty of Dentistry
Pediatric Dentistry and Dental Public Health Department

Remineralizing Effect of Nano-Eggshell, Casein Phosphopeptide Amorphous Calcium Phosphate (CPP-ACP), and Sodium Fluoride on Initial Enamel Caries: Randomized Controlled Trial.

Protocol of Thesis

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By

Ghada Ezzat Taha Elhindawy

Assistant Lecturer of Dental Public Health and Preventive Dentistry

B.D.S (2012) Mansoura University

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Supervisors

Dr. Rabab Ibrahim Salama

Professor of Dental Public Health and Preventive Dentistry, Faculty of Dentistry, Mansoura University.

Dr. Salwa Adel Hegazy

Professor of Dental Public Health and Preventive Dentistry, Faculty of Dentistry, Mansoura University.

Dr. Rasha Ibrahim Ramadan

Assistant Professor of Dental Public Health and Preventive Dentistry, Faculty of Dentistry, Mansoura University.

Introduction

Tooth structure is exposed to intermittent periods of demineralization and remineralization. If these periods are imbalanced, it will lead to advanced demineralization and deterioration of the tooth. [1] Extended multiple contacts with cariogenic dietary substrates can lead to an imbalance and formation of an initial enamel lesion, which can easily develop into dentin caries. [2]

Dental caries is considered one of the most prevalent chronic diseases affecting populations. Demineralization occurs due to fermentation of dietary carbohydrates by bacteria over time which forms acid byproducts and results in localized destruction of dental hard tissues. [3]Untreated dental caries leads to discomfort, pain and may end with functional limitations. Many diagnostic approaches to detect initial caries include visual examination, tactile inspection using dental probe, radiography, electronic monitoring and DIAGNOdent. [4]

The recent agreement in caries control is that it should be detected and treated in its initial stages so that it can be reversed. [5] After detection, it can be managed by minimal invasive repair, including remineralization treatment, using fluoride-containing products and behavioural changes.[6] Remineralization is the process which promotes calcium and phosphate ions that are delivered from an external source to deposit into the crystal voids in the demineralized enamel. Remineralization process can be classified into fluoride and non-fluoride remineralization.[7]

Fluoride is a significant protective agent; its topical application can arrest or reharden dental carious lesions through inhibiting destruction of dental minerals by adsorbing onto the mineral surfaces. Fluoride also can encourage remineralization at these surfaces which results in formation of

a fluorapatite layer that is more resistant to acid challenge. Fluoride ions can affect the balance between demineralization and remineralization which is an important role. [8]

Fluoride varnishes such as sodium fluoride and stannous fluoride extend the contact time between fluoride and the tooth surface. Six- monthly applications have been recognized to be efficient in decreasing the initial caries in both children and adolescence.[9] As well as, professional application of fluoride during orthodontic treatment can prevent initial caries formation around the orthodontic brackets.[10]Despite many fluoride varnishes are presented, only sodium fluoride varnish was widely studied to be effective in children and adolescence.[9]

Sodium fluoride varnish has presented in numerous studies [9-11] to assess its remineralizing and preventing efficacy on initial enamel caries among children and adolescence which prove its effectiveness as a remineralizing agent. On the other hand, there was limitation on the use of fluoride in children, due to the risk of fluorosis if swallowed. So, there was a need for new approaches and active ingredients for preventive oral health products which could be safe if swallowed. [12]

Non–fluoride remineralization involves the use of calcium phosphates as active ingredients in oral care products. It is a promising biomimetic method to enhance the remineralization and prevent enamel caries. [12] It includes phosphate-based agents such as β tri calcium phosphate, amorphous calcium phosphate (ACP), sodium calcium phosphosilicate (bioactive glass), Nano-hydroxyapatite and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP). [13]

Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) is based on the enzymatic breakdown of milk casein and formation of casein

phosphopeptides (CPP). Because of its higher content of phosphoseryl remnants, CPP preserves calcium and phosphate ions in an amorphous and non-crystalline state. In this state, these ions can enter the tooth enamel and precipitate on the initial caries enhancing remineralization. [13]

Eggshell powder (ESP) is a rich source of calcium and important elements. It contains around 94% calcium carbonate, 1% calcium phosphate, and 1% magnesium carbonate. Hence, ESP is considered to be effective in remineralization of the enamel surface. Particularly when using it as a solution, because its minerals could easily diffuse in to the enamel surface porosities. [14]

Nano sized particles have been integrated in many dental materials. The properties of nanoparticles are relatively different from their equivalent normal sized materials. They have a large surface area, high surface energy, and easy deposition in irregular spaces, so the solubility and reactivity of these particles are higher. Many studies have investigated the effect of eggshell powder as a remineralizing agent for treating the initial carious lesion and compare its effect with other remineralizers. [15-18]

Research was conducted by Elbahrawy E and El –Dosoky A, in 2019[16] to evaluate and compare the enamel remineralization potential of chicken eggshell powder (CESP) solution or slurry on initial enamel caries in permanent teeth. This study assessed the mineral content and surface microhardness of all samples using EDX and Vickers microhardness tester respectively. It found a significant increase in both Ca and PO4 weight % after remineralization in all samples compared with their values after demineralization. The results showed the remineralization using CESP solution has the same efficacy as topical application of CESP slurry using finger rubbing. The study concluded that CESP has a promising future in treating initial enamel surfaces lesion due to its natural source and easy

bioavailability.

Another in-vitro study was performed by Anusha et al in 2020 [17] to evaluate and compare the remineralizing effect of Nano-Eggshell and GC Tooth Mousse on initial caries like lesions in young permanent teeth. The study evaluates the effect of particle size of Nano-Eggshell particles on remineralization using X-Ray diffraction Analysis-Diffractometer. The result revealed that, samples which were remineralized with Nanoeggshell on day 7 and 14 showed a mean micro hardness that was relatively higher as compared to those samples of GC Tooth Mousse.

A clinical study was carried out by Sarhan et al in 2021[18] to assess the remineralizing effect of eggshell powder (ESP) compared to Casein Phosphopeptide- Amorphous Calcium Phosphate (CPP-ACP) in carious post orthodontic white spot lesions. In this study, patients were divided into two groups according to the treatment they used. White spot lesions were assessed at baseline, 1 month, 3 months and 6 months by DIAGNOdent. The results showed that there was no statistically significant difference in the overall DIAGNOdent mean readings between (ESP) and Casein Phosphopeptide Amorphous Calcium Phosphate (CPP-ACP).

In Egypt, there is lack of studies to our knowledge carried out through clinical trials to evaluate the efficacy of Nano-eggshell varnish as a remineralizing agent. Therefore, this study will be conducted to assess and compare the remineralizing effect of Nano- eggshell, Casein Phosphopeptide Amorphous Calcium Phosphate (CPP-ACP), and sodium fluoride on initial enamel carious lesions.

The null hypothesis will be; there is no difference in the remineralizing effect of Nano-Eggshell, Casein Phosphopeptide Amorphous Calcium Phosphate (CPP-ACP), and sodium fluoride on initial enamel carious lesions.

Aim of this study

This study will be conducted to investigate:

1. The effect of Nano-eggshell varnish , CPP-ACP (GC Tooth Mousse), and sodium fluoride varnish on the remineralization of initial enamel caries among a group of adolescent patients aged 15-20 years using DIAGNOdent.

2. The influence of the previous three materials on caries activity and dental caries experience among the same group of adolescent patients using the criteria of International Caries Classification and Management System (ICCMS) and Decayed, Missing and Filled Surfaces (DMFS) index, respectively.

Martials and method

1. Study design:

This study will be a randomized controlled clinical trial.

2. Materials:

1. Nano-eggshell varnish prepared by (Nano Gate, Nasr city, Cairo, Egypt).
2. Casein Phosphopeptide- Amorphous Calcium Phosphate (CPP-ACP) (GC Tooth Mousse), commercially available.
3. Sodium fluoride varnish (5%), commercially available.
4. Disposable examination kit for each patient.
5. Cotton rolls and gauze for moisture control and removal of plaque.

3. Devices:

DIAGNOdent (Kavo Germany) device for detecting the initial caries.

4. Conduction of the study

4.1 Ethical consideration:

- First the approval of the ethical committee of the Faculty of Dentistry, Mansoura University on the study protocol will be obtained.
- The objective of the study will be explained to the patients and informed consent will be taken after ensuring confidentiality of data.

4.2 Infection control measures

The researcher will be responsible for infection control measures by:

1. Wearing personal protective equipment during the clinical procedures.

2. Using disposable and sterilized instruments for each patient.

3. Disinfectants and surface barriers will be used and changed between patients.

4.3 Sample size

For defining sample size in the clinical part, data from previous studies based on the remineralization ability will be used [18] sample size calculations will be used in an α- error of 5%, 15% drop out and power of 80%. The effect size of 0.534, F test is used for the three tested groups. The total sample is 75 and will increase to 90 to increase the sample power and allow equal distribution of both genders in each group.

4.3.1 Sample selection

- This part of the study will be conducted on a group of adolescent patients aged from 15-20 years attending to Faculty of Dentistry, Mansoura University.
- Patients will be selected from outpatient clinic, Faculty of dentistry, Mansoura University according to the following inclusion and exclusion criteria.
- *Inclusion criteria:*

1. Patient age should be (15-20) years with equal numbers of males and females.

2. Patient should have at least three to four teeth with ICDAS II scores 1-2 as described in table (1) [19]

Table (1) ICDAS II Criteria

ICDAS II Score	Criteria
0	Sound tooth surface.
1	First visual change (opacity or discoloration) in enamel clearly visible after air drying but hardly visible on the wet surface.
2	Distinct visual change (opacity or discoloration) in enamel, visible without air drying.
3	Localized enamel breakdown without visible dentin.
4	Underlying dark shadow from dentin without cavitation.
5	Distinct cavity with visible dentin.
6	Wide distinct cavity with visible dentin.

3. Patients should have moderate to high caries risk according to the American Dental Association (ADA) caries risk assessment form. [20]
4. Patients should have good oral hygiene measures which include tooth brushing at least once a day with fluoridated dentifrices [21]

• *Exclusion criteria*

1. Known hypersensitivity to one of the components of the material under study.
2. Systemic disorders or medication interfering with normal salivary flow such as Sjogran Syndrome, radiotherapy or drugs causing xerostomia.

4.4 Clinical Procedures

1. Patients who selected according to the inclusion and exclusion criteria of the study will be re-examined.
2. The teeth are going to be examined wet and dry after drying their surfaces for 5 seconds with a dental air–water syringe.
3. All stages of caries ranging from initial (non-cavitated) to cavitated lesions will be identified visually on buccal, lingual and occlusal

surfaces of each permanent tooth, using the ICDAS II criteria [19] and confirmed by DIAGNOdent.

4. *Randomization:*

- To ensure random allocation of the selected patients into three groups, randomization procedure will be done using random computer application (http://www.graphpad.com/quickcalcs/index.cfm) to assign the three groups by block randomization.
- To ensure allocation concealment, the selected block will be kept in closed envelopes.
- The allocated patients will be divided into three groups (30 patients, 15 males and 15 females in each group) according to the material used.

- Group I: Nano-eggshell varnish.
- Group 11: CPP-ACP (GC Tooth Mousse).
- Group 111: Sodium fluoride varnish (5%). (As a control group)

5. *Conduction of the study:*

- All the patients will be instructed to use fluoridated tooth paste contains 1450 ppm fluoride once daily at the bed time. A well trained, calibrated examiner will apply varnishes materials in group I and group III every 3 months according to the manufacture instructions. In group II, the participants will be instructed to use the CPP-ACP (GC mousse crème) daily after brushing their teeth at bed time and keep the GC mousse crème over their teeth surfaces for a minimum of 3 minutes before rinsing.
- Patients in the three groups will be instructed to avoid drinking or eating anything for at least 30 minutes after the application of all materials. No

other fluoride supplements (mouthwash and fluoride tablets) will be allowed for use.[17, 18]

6. *Assessment of the outcome:*

- Caries activity will be measured at baseline and after 3, 6, 9, 12 months by the criteria of International Caries Classification and Management System (ICCMS) [22], as the following:
  - *Active caries*: Enamel surface is whitish/yellowish, opaque with loss of lustre, feels rough when the tip of the ball ended probe is moved gently across the surface. Lesion is in the plaque stagnation area and may be covered by thick plaque prior to cleaning.
  - *Inactive caries*: Enamel surface is whitish, brownish or black. Enamel may be shiny, feels hard and smooth when the tip of the ball probe is moved gently across the surface. For smooth surfaces, the caries lesion is typically located at some distance from the gingival margin. Lesion may not be covered by thick plaque prior to cleaning.
- The remineralizing status of the initial carious lesion will be measured at base line and after 3, 6, 9, 12 months using DIAGNOdent (Kavo-Germany) device.
- The Decayed, Missing, Filled Surfaces (DMFS) index for all participants will be measured at the baseline and after 12 months to evaluate the caries experience.

5. Statistical analysis:

Data will be arranged, tabulated and analysed statistically; using Statistical Package of Social science (SPSS) program for windows (standard

version 24). Suitable tests will be used for comparison of the results. The results will be considered significant when ($p \leq 0.05$).

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