The Effect Of Adding Chitosan Nanoparticles To Casein Phosphopeptide Amorphous Calcium Phosphate (CPP-Acp) In Tooth Remineralization: A Sem Study

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Abstract : With the advent of new materials, caries treatment is now carried out in contemporary approach. Based on the principle that caries can be remineralized, non-invasive intervention has been launched on caries lesion that has not formed into cavities, using therapeutic agents to heal the lesion by replacing the lost mineral in tooth structure. This study was aimed to compare the effect of CPP-ACP alone and the combination of CPP-ACP and chitosan nanoparticles in remineralizing tooth enamel by observing the morphological changes in enamel surface with Scanning Electron Microscope (SEM). Twenty four enamel samples from extracted molars were assigned into four groups. The first group was incubated in artificial saliva, the second was immersed in demineralizing solution, the third was immersed in demineralizing solution and applied with CPP-ACP gel, while the fourth group was immersed in demineralizing solution and applied with the combination of CPP-ACP gel and chitosan nanoparticles. The application of CPP-ACP and combination of CPP-ACP and chitosan nanoparticles were performed for 7 days. SEM was utilized to observe the morphological changes on enamel surface. The combination of CPP-ACP and chitosan nanoparticles resulted in little morphological enamel changes as compared to CPP-ACP group, and so it was concluded that both have the same ability to increase tooth enamel remineralization.

Keywords: CPP-ACP, nanoparticle chitosan, enamel remineralization.

INTRODUCTION

The treatment of caries nowadays is done using contemporary approach. The non-invasive intervention for caries lesion that has not turned into cavities is carried out using therapeutic agents to heal the lesion by replacing the minerals lost due to demineralization process. One of the methods to reduce demineralization and increase enamel remineralization is fluoride application, but excessive doses can cause fluorosis during ages when tooth development is occurring and in large enough quantities can even be toxic. Taken together the safety issues and dosage limitations are considered an important limitation of fluoride therapies thereby opening the window for the development of new anti-cariogenic products with fewer possible side-effects or that are potentially more effective than fluoride.

It is now known that the penetration of calcium and phosphate ions is essential in repairing deeper damage in tooth structure. The newest technology in
remineralization process is developed based on phosphopeptides from casein protein of milk.\textsuperscript{1} Casein phosphopeptide (CPP) is comprised of multiphosphoseryl clusters with the ability to stabilize calcium phosphate by forming colloidal casein-phosphopeptide amorphous-calcium-phosphate nanocomplexes (ACP). Through multiple phosphoseryl clusters, CPP binds to ACP into a metastable solution which prevents the destruction of calcium and phosphate ions.\textsuperscript{1}

CPP-ACP also acts as a bioavailable reservoir of calcium and phosphate ions, helping to maintain a state of supersaturation of these ions, thereby enhancing remineralization.\textsuperscript{1}

The use of natural products is gaining popularity in dentistry. Chitosan is one of the biomaterials which recently being further developed due to its medical benefits and safe-to-human use. Chitosan has special features such as good biocompatibility, it is biodegradable, non toxic, and does not cause immunologic reaction or cancer. With these features, chitosan and its modification with other agents can be applied clinically as a biomaterial.\textsuperscript{2}

The present study aimed to compare the effect of CPP-ACP and the combinations of CPP-ACP and chitosan nanoparticles in remineralizing tooth enamel using Scanning Electron Microscope (SEM) to observe the morphological changes in enamel surface.

**MATERIAL AND METHODS**

Chitosan gel was created by dissolving 1gr chitosan from the shell of *blangkas* (Tachypleus gigas) in 50 ml weak acid (acetic acid 1%), and then stirred in jar test at 200 rpm for ±30 minutes until the gel was formed. Whilst stirred, 20 drops of tripolyphosphate solution was added into the gel to achieve smooth appearance. The gel continued to be stirred in jar test for another 30 minutes. The gel was subsequently placed into ultrasonic bath to break the chitosan particles into nanoparticles sizing 180 nm. This residue of nano chitosan was then added to CPP-ACP (GC-Tooth Mousse) in 1:1 measurement.

In this study six molar teeth were used. The roots were removed using disc burr with water flow. Each tooth was sectioned into four pieces in mesiodistal and buccolingual/buccopalatal direction, resulting in 24 samples which then were randomly assigned into four groups. Buccal/lingual side was mounted on paralon pipe of 1 cm in diameter with acrylic resin, exposing an open area of 2x2 mm. Samples were numbered 1 to 24 and randomly assigned to four groups; six samples for each group. The first group was only incubated in artificial saliva at 37°C. The second group was immersed in demineralizing solution for 4 days and then incubated in artificial saliva at 37°C. The third group was immersed in demineralizing solution for 4 days, applied with CPP-ACP gel once daily for 5 minutes, and then incubated in artificial saliva at 37°C. The last group was immersed in demineralizing solution for 4 days, applied with the combination gel of CPP-ACP and chitosan nanoparticles for 5 minutes once a day, and then incubated in artificial saliva at 37°C. The whole procedure took seven days. The SEM observation of samples was done on the eighth day.

**RESULTS**

Scanning Electron Microscope (SEM) was utilized to observe the morphological features on enamel surface of every treatment groups. The enamel under observation in this study was a clinically and macroscopically intact surface which did not...
undergo polishing. The results were depicted in the following pictures: (A) morphological appearance of enamel surface which was only incubated in artificial saliva, (B) morphological appearance of enamel surface which underwent demineralization, (C) morphological appearance of enamel surface which underwent demineralization and application of CPP-ACP gel, and (D) morphological appearance of enamel surface which underwent demineralization and application of CPP-ACP-chitosan gel.

**Fig.1:** The morphological appearance of enamel surface under SEM. a) The enamel was only incubated in artificial saliva; b) The enamel was immersed in demineralizing solution; c) The enamel was applied with CPP-ACP gel; d) The enamel was applied with the combination gel of CPP-ACP and chitosan (magnification 1000X).

### DISCUSSION

The samples in the present study were crown of molars extracted from patients aged 18-35 years, in good and intact condition clinically and macroscopically. Each crown was divided into four parts, mesiobuccal, mesiolingual, distobuccal and distolingual, and then assigned to group I, II, III and IV, respectively. All mesiobuccal part would be in group I, mesiolingual in group II, distobuccal in group III, and distolingual in group IV. This was done based on the author’s assumption that enamels parts from the same teeth would inhibit the same appearance before treatment.

The enamel analyzed in this study was macroscopically intact enamel which did not undergo polishing. Qualitative analysis was done using SEM to observe morphological appearance of each group.

In the first group which was incubated in artificial saliva (Figure (a)), it was found that the enamel surface was not entirely smooth and enamel tip was observed as a normal variety of enamel.

Figure (b) illustrated the appearance of enamel which was immersed in demineralizing solution. Porosity and wavy surface were noted, indicating demineralization process that had taken place.

Figure (c) showed enamel surface which underwent demineralization and application of CPP-ACP gel. Less porosity and smoother surface was seen compared to enamel in the second group (Figure (b)). Several studies have reported that CPP-ACP possess the ability to penetrate further into enamel to replace calcium and phosphate ions lost to demineralization. The deeper penetration resulted in more optimum ion replacement, hence maximizing enamel structure repair.\(^3\)\(^-5\)

Figure (d) displayed enamel surface applied with the combination of CPP-ACP and chitosan. The enamel surface became much smoother compared to enamel applied with CPP-ACP only (Figure C). It was assumed to be related with the ability of chitosan to prevent the loss of calcium and phosphor during enamel demineralization process.\(^6\)

The nano-sized chitosan particles increase the surface area up to hundred times compared to micro-
sized particles, therefore increasing the efficacy of chitosan in binding with other chemical clusters. The result of the study showed that CPP-ACP and the combination of CPP-ACP and chitosan have the same ability to increase tooth enamel remineralization.

REFERENCES


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