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Impact of Remineralization Potential of Biphasic Calcium Phosphate Powders on Incipient Enamel Caries

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ORIGINAL ARTICLE

Impact of Remineralization Potential of Biphasic Calcium Phosphate Powders on Incipient Enamel Caries

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Abstract

Objectives: **To evaluate and compare the remineralizing effect of biphasic calcium phosphate (BCP) powder compared with nanohydroxyapatite (nano-HA) and beta-tricalcium phosphate (β-TCP) powders using laser fluorescence.**

Materials and methods: **Fifteen sound human premolars and molars were subjected to infection control measures. Then, the roots were removed and crowns were sectioned to provide blocks from the buccal and lingual surfaces. Each specimen underwent pH cycling to produce white spot lesions. The specimens were divided into three groups according to the treatment used. The total sample size for all groups was thirty specimens (10 specimens for each group). The change in mineral content was evaluated by laser fluorescence using a DIAGNOdent pen at baseline, after demineralization, and after remineralization for 14 and 30 days. The mean value of each specimen reading was calculated.**

Results: **BCP showed lower laser fluorescence results compared with HA and β-TCP (recorded by DIAGNOdent pen) after application for 14 days (8.04 ± 0.55) and 30 days (6.08 ± 0.65). However, the results were not statistically different from the results of other groups (HA group 8.18 ± 0.87 and 6.36 ± 0.922 and β-TCP group 8.36 ± 1.17 and 6.93 ± 0.81). The remineralizing potential of the three materials increased with increasing the time of application.**

Conclusions: **BCP powder could be used for enamel remineralization. Its potential for the remineralization of incipient enamel caries is similar to HA and β-TCP.**

Keywords: Enamel remineralization, Biphasic calcium phosphate, Laser fluorescence

1. Introduction

Dental caries is a continuous process of alternating cycles of demineralization and remineralization. Demineralization causes white opaque areas called white spot lesions or incipient enamel caries which represent subsurface enamel porosity.[1] Remineralization is defined as the natural repair process for uncavitated lesions to rebuild new crystals on the demineralized surface.[2] It was suggested that initial carious lesions could regress or nearly disappear by the use of topical remineralizing agents.[3]

Various remineralizing materials could be used such as bioactive glass, fluoride-releasing materials, arginine bicarbonate, and Portland cement.[4] Topical fluoride has been considered the gold standard for remineralization.[5] Nowadays, new calcium and phosphate[6] materials such as hydroxyapatite (HA),[7] beta-tricalcium phosphate (β-TCP),[8] amorphous calcium phosphate,[9] and casein phosphopeptide amorphous calcium phosphate[10] has been used. These materials are biocompatible, chemically stable, and similar in structure to enamel HA. But, the low solubility and low biodegradation are their main limitations.[11]

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The studied materials HA, biphasic calcium phosphate (BCP), and β-TCP have similar compositions of calcium and phosphorous but, differ in the ratio between calcium and phosphorous. HA has a Ca/P ratio of 1.67. β-TCP has a Ca/P ratio of 1.45 and BCP (70 HA–30 β-TCP) has a Ca/P ratio of 1.62.[12] Also, BCP formed of the two phases so these three materials were chosen for the study. The nature of the material depends on the ratio of HA to β-TCP. This could control the solubility, degree of degradation, and mechanical properties. Increasing the HA ratio in comparison to the β-TCP ratio makes the material more insoluble, decreases the degree of degradation, and improves mechanical properties such as compressive strength. The effect of β-TCP on the strength of BCP composite had shown that mechanical properties decrease with increasing β-TCP ratio.[13]

HA has always been considered a model of human enamel due to its crystalline and chemical similarity. Nanohydroxyapatite (nano-HA) has been used for remineralization of initial lesions with different forms, concentrations, and shapes.[14,15] A previous study concluded that HA nanoparticles can self-assemble to form enamel-like structures in aqueous solutions.[16] Also, HA induces remineralization by directly filling micropores in demineralized enamel.[15]

β-TCP is considered a precursor to HA formation. Hemagaran and Neelakantan[17] reported that using β-TCP increases calcium levels in saliva and promotes the remineralization of the tooth surface. β-TCP is more soluble than HA and is more rapidly resorbed. The resorption rate of β-TCP is nearly the same as normal cancellous bone.[18]

BCP is formed from the two phases HA and β-TCP with various ratios. So, by controlling the HA/β-TCP ratio, the bioactivity, and mechanical properties could be controlled.[19] In a study that evaluated the tubule occlusion capacity of BCP with three HA/β-TCP ratios, results confirmed that BCP promotes the blocking of open dentinal tubules and dentin remineralization.[20]

Laser fluorescence is a noninvasive method that is used for early diagnosis of noncavitated tooth decay. It emits red light (655 nm) which is absorbed by inorganic and organic tooth components. The process of remitted fluorescence has variable scales between 0 and 99. A value of 20 or 25 and higher indicates a caries lesion; the higher the scale, the deeper the lesion.[21] The mechanism could be explained as when the red light meets a porosity due to demineralization or hypomineralization, it stimulates remitted fluorescent light of different wavelengths.[22] Also, this may be due to some bacterial metabolites, protoporphyrin, that result in the red fluorescence of carious teeth.[23] The reproducibility of laser fluorescence measurements has been high in both *in vitro* and clinical studies by Costa et al.[24] and Lussi and Francescut.[25]

This study was designed to evaluate the enamel remineralization potential of BCP powders on enamel incipient caries compared with HA and β-TCP powders using laser fluorescence.

2. Materials and methods

HA, β-TCP, and BCP powders were prepared by wet chemical precipitation from analytical grades calcium hydroxide and phosphoric acid.[12]

2.1. Teeth collection and storage

Fifteen sound human premolars and molars extracted for periodontal reasons were collected from the outpatient clinic of the Faculty of Dentistry, Mansoura University, and were subjected to infection control measures. The study protocol was approved by the Faculty of Dentistry, Mansoura University Research Ethical Committee Board with code (M11160321). The Research Ethics Committee approved the informed consent dismissal.

2.2. Preparation of enamel blocks

To prepare the specimens, the roots were first removed by using a microsaw (PICO155; Pace Technologies, AZ, USA) at 2500 rpm speed under copious water spray coolant. Then the crowns were sectioned mesiodistally into two halves. Blocks of average dimensions 6 mm × 4 mm × 2 mm were cut from the buccal and lingual surfaces of each molar.[26]

2.3. Induction of artificial caries by pH-cycling

A demineralizing solution with pH 4.8 was prepared from 2.2 mM (2.2 mol of calcium chloride for each liter divided by 1000) calcium chloride, 2.2 mM sodium dihydrogen phosphate, and 50 mM acetic acid.[27] Also, a remineralizing solution with pH 7.0 was prepared from 1.5 mM calcium chloride, 0.9 mM sodium dihydrogen phosphate, and 0.15 M (molar) potassium chloride.[28]

Each specimen was immersed individually in a plastic tube for 8 h in 1 ml of the demineralizing solution and 16 h in 1 ml of the remineralizing solution. Both solutions were refreshed daily for 14 days at room temperature without agitation.[29]

2.4. Grouping of specimens

The specimens were divided into three groups (10 each) according to the type of remineralizing agent used.

Group A: HA; a 10 % w/v HA aqueous slurry was applied to enamel blocks. Group B: BCP group; a 10 % w/v aqueous slurry of BCP (HA/β-TCP 70/30) was applied to enamel blocks. Group C: β-TCP group; a 10 % w/v aqueous slurry of β-TCP was applied to enamel blocks. The slurries were put in the falcon tubes containing enamel specimens for 24 h daily and changed every day. The remineralizing agents were applied for 14 days then the time was extended to 30 days.

2.5. Assessment of changes in enamel mineral content using laser fluorescence

To evaluate the changes in enamel mineral content due to the application of the remineralizing agents, a DIAGNOdent pen (DDPen; KaVo, Biberach, Germany) was used. First, specimens were dried with an air syringe for 10 s before measuring. After attaching the device probe, calibration was done using a ceramic reference block before testing each specimen. Then the probe was applied perpendicular to the middle part of each specimen to collect the fluorescence from all directions and determine the maximum value. This step was repeated three times for each specimen and the mean value of the readings was calculated.[30] Measuring was done at baseline, after demineralization, and after remineralization for 14 and 30 days. The results were assessed according to the scale developed by the manufacturer, 0–13 sound, 14–20 enamel caries, 21–29 caries in dentin–enamel junction, and more than 29 dentin caries.[31]

2.6. Statistical analysis

Data analysis was performed by SPSS software, version 25 (SPSS Inc., PASW Statistics for Windows version 25; SPSS Inc., Chicago, Illinois, USA). Quantitative data were described using mean ± SD for normally distributed data after testing normality using the Shapiro–Wilk test. The significance of the obtained results was judged at less than or equal to 0.05 level. Repeated measures analysis of variance (ANOVA) was used to compare the differences in laser fluorescence between the groups and the effect of time. Then one-way ANOVA was used to analyze the differences between the groups and the post-hoc Tukey test was used for pair-wise comparison.

3. Results

The results of repeated measures ANOVA showed that the type of remineralizing material did not affect the enamel remineralization evaluated using laser fluorescence ($P > 0.05$). However, the enamel remineralization was significantly affected by the time of application of the mineralizing material ($P < 0.01$) as shown in Table 2. At baseline, the β-TCP group showed the highest mean value of laser fluorescence readings (3.39 ± 0.91) while the BCP group had the lowest (2.71 ± 1.27), however, there was no significant difference between the three groups ($P > 0.05$) as shown in Table 1 and Fig. 1. After demineralization, laser fluorescence values significantly increased in all groups ($P < 0.001$) as shown in Table 2 and Fig. 2. The HA group showed the lowest mean value (13.22 ± 1.02) while the BCP group had the highest (14.05 ± 1.85). However, there was no significant difference between the three groups ($P > 0.05$) as shown in Table 1 and Fig. 1. After remineralization for 14 days, laser fluorescence values significantly decreased in all groups ($P < 0.001$) as shown in Table 2 and Fig. 2. The β-TCP group had the highest mean value (8.36 ± 1.17) while the BCP group had the lowest (8.04 ± 0.55). Nevertheless, there was no significant difference between the three groups ($P > 0.05$) as shown in Table 1 and Fig. 1. After remineralization for 30 days, laser fluorescence values significantly decreased in all groups ($P < 0.001$) as shown in Table 2 and Fig. 2. The β-TCP group again showed the highest mean value (6.93 ± 0.81) while the BCP group had the lowest (6.08 ± 0.65). But, there was no significant difference between the three groups ($P > 0.05$) as shown in Table 1 and Fig. 1.

4. Discussion

The concentration of remineralizing powder in distilled water was 10 % w/v as it is the most frequently utilized.[32] The amount of nano-HA aggregation increased with higher concentrations due to the increase in the deposition of phosphate and calcium ions which can affect the amount and depth of entering the lesion.[33]

The remineralizing effect of β-TCP can be explained by proper solubility that leads to an increase in calcium levels after its application.[34] Presence of free calcium and phosphorus ions provided a suitable remineralizing solution to facilitate the remineralization of initial carious lesions. These results are in agreement with the results of Bhat et al.[35] who evaluated the remineralizing potential of three remineralizing agents and their effect on microhardness. TCP provided better results than fluoride.

Table 1. Descriptive statistics of surface enamel laser fluorescence results at different time points of the investigated remineralizing agents.

Laser fluorescence results	Hydroxyapatite group	Biphasic calcium phosphate group	Beta tricalcium phosphate group	Test of significance
Baseline	3.25 ± 0.68	2.71 ± 1.27	3.39 ± 0.91	$F = 0.889$ $P = 0.429$
After demineralization	13.22 ± 1.02	14.05 ± 1.85	13.62 ± 0.81	$F = 0.682$ $P = 0.519$
After remineralization for 14 days	8.18 ± 0.87	8.04 ± 0.55	8.36 ± 1.17	$F = 0.193$ $P = 0.826$
After remineralization for 30 days	6.36 ± 0.922	6.08 ± 0.65	6.93 ± 0.81	$F = 1.87$ $P = 0.184$

Parameters described as mean ± SD.
F, one-way analysis of variance test.

The effect of nano-HA reported in this study may be related to the high uptake of nano-sized particles of hydroxyapatite which can act as a filler for the small holes of demineralized tooth surface as well as binding of nano-HA particles to the enamel.[36] This finding was consistent with the results obtained by Pepla et al.[37] and Sharma et al.[38] Also, Huang et al.[39] reported that HA is not soluble enough to provide the needed P and Ca ions to prevent demineralization.

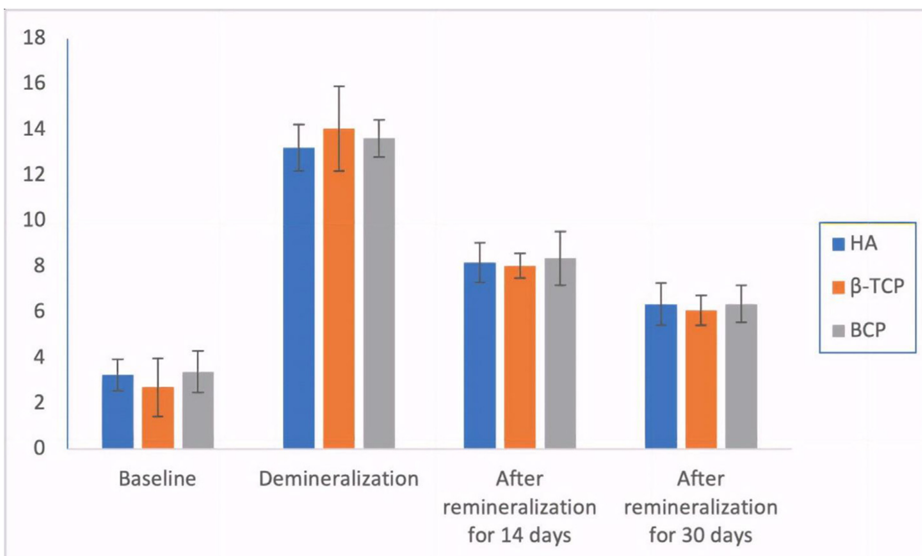

Fig. 1. Mean laser fluorescence values measured by DIAGNOdent pen among studied groups.

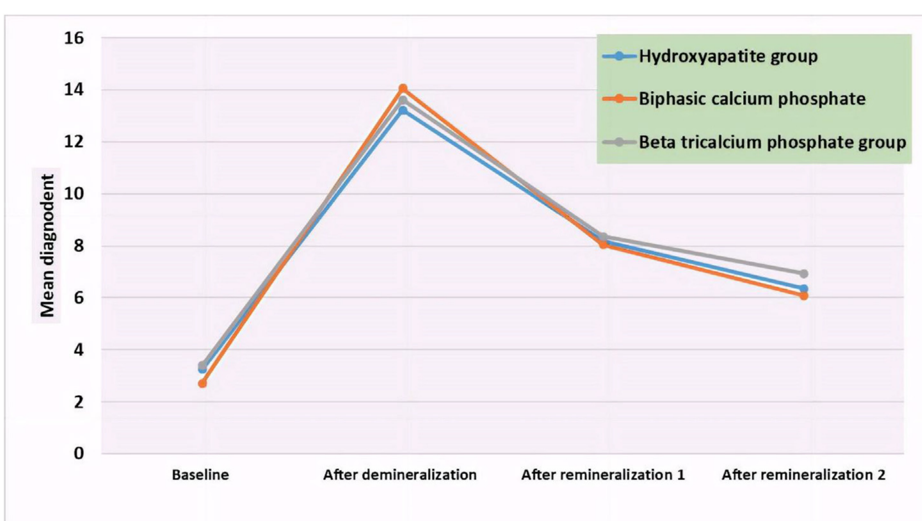

Fig. 2. Means of laser fluorescence results at baseline, after demineralization, and remineralization for 14 and 30 days for each studied group.

Table 2. Descriptive statistics of surface enamel laser fluorescence of HA, β-TCP and BCP at different time points.

Laser fluorescence results	Baseline	After demineralization	After remineralization for 14 days	After remineralization for 30 days	Test of significance	Within group significance
Hydroxyapatite group	3.25 ± 0.68	13.22 ± 1.02	8.18 ± 0.87	6.36 ± 0.922	$F = 6.93$ $P < 0.001$[a]	$P1<0.001$[a] $P2<0.001$[a] $P3<0.001$[a]
Biphasic calcium phosphate group	2.71 ± 1.27	14.05 ± 1.85	8.04 ± 0.55	6.08 ± 0.65	$F = 5.98$ $P < 0.001$[a]	$P1<0.001$[a] $P2<0.001$[a] $P3<0.001$[a]
Beta-tricalcium phosphate group	3.39 ± 0.91	13.62 ± 0.81	8.36 ± 1.17	6.93 ± 0.81	$F = 7.93$ $P < 0.001$[a]	$P1<0.001$[a] $P2<0.001$[a] $P3<0.001$[a]

Parameters described as means ± SD.
F, one-way analysis of variance test.
P1: difference between baseline and after demineralization.
P2: difference between demineralization and after remineralization for 14 days.
P3: difference between remineralization after 14 days and remineralization after 30 days.
[a] Statistically significant.

The remineralizing effect of BCP could be explained by both mechanisms of HA and β-TCP. HA has poor solubility so, precipitation of nano-crystals of HA mostly takes place rather than dissolution. β-TCP is soluble so, dissolution into Ca and P ions takes place then these ions re-aggregate into Ca/P materials such as HA in other words it could be considered as precursor to HA formation.

The results of laser fluorescence of demineralized enamel agreed with the studies by Baeshen et al.[40] and Ciftci et al.[41] Juntavee et al.[32] compared remineralization potential of nano-HA toothpaste, functionalized tricalcium phosphate toothpaste, and fluoride toothpaste on carious lesions. In agreement with this study, they found that the tested materials were capable of remineralization of demineralized enamel. However, there was no significant difference among them in remineralization.

In an in-vivo study that compared the remineralizing effect of TCP and HA, results revealed that both were indeed effective in the treatment of incipient caries. In agreement with this study, their results concluded that nano-HA provided better results than TCP. However, there was no significant difference between them. Laser fluorescence readings by DIAGNOdent pen after demineralization of smooth surface caries for TCP and HA were 13.3 and 14.4 and after remineralization for 4 weeks were 9.4 and 6.67. No significant differences were found for the smooth surface remineralization similar to this study.[42]

4.1. Conclusions

BCP powder could be used for enamel remineralization. Its potential for the remineralization of incipient enamel caries is similar to HA and β-TCP.

Conflict of interest

There are no conflicts of interest.

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