

Remineralizing Efficacy of Chitosan-Based Nanomaterials on Artificially Induced Incipient Carious Lesions

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Abstract:

Objective: Assessment of the remineralizing efficacy of Bioactive glass-chitosan and Hydroxy Apatite-Chitosan on artificial white spot lesions. **Materials and methods:** Sixty extracted human permanent teeth were selected for the study. Specimens were divided into four groups: group 1: fluoride varnish (Voco), group 2: Bioactive Glass-Chitosan varnish (Nano gate company), group 3: Hydroxy Apatite-Chitosan varnish (Nano gate company), group 4: control (treated with remineralizing solution). Artificial carious lesions were induced in all the specimens and treated with respective varnishes twice daily for 7 days. The DIAGNOdent pen (Kavo DIAGNOdent pen 2190) readings were taken, and the surface characteristics of the enamel specimens were evaluated by SEM at baseline, after demineralization and after remineralization. **Results:** SEM evaluation showed favourable surface changes in all the four study groups after remineralization therapy. Intragroup comparison of DIAGNOdent readings showed a highly significant difference between baseline, post demineralization, and post remineralization values. However, the intergroup comparison was statistically nonsignificant. **Conclusion:** All test agents were comparable in their remineralization potential.

Introduction

White spot lesions (WSL) can be defined as “a subsurface enamel porosity from carious demineralization that represents itself as a milky white opacity located on smooth surfaces.” They may be either active (rough and opaque) or inactive (smooth and shiny).¹

Remineralization may be as simple as the immediate repair of recently acid damaged enamel and occur on a need basis with no clinical evidence of a lesion. Conversely, the repair process may require prolonged mineral deposition to reverse a clinically detectable white-spot lesion (WSL). The fate of the lesion, whether it will progress to cavitate or remineralize, depends on biological factors in the plaque and saliva, the composition of enamel, oral hygiene, dietary habits, and exposure to preventive agents.²

Several methods in tooth surface remineralization have been studied. These methods include various forms of remineralizing agents. One of the most known and applied for caries prevention is fluoride (F). Nevertheless, fluorosis is claimed to be the most common side effect due to the total fluoride intake.³ As well, Oral care products containing fluoride are effective in remineralizing enamel but do not have the potential to promote the formation of organized apatite crystals. Therefore, fluoride alternatives have been proposed to avoid the previous concerns, including Nano-biomaterials such as Chitosan, Hydroxyapatite and Bioactive glass that offer a viable biomimetic option to the fluoride therapy.⁴

One of the favourable natural biomaterials that have a multitude of biomedical applications is Chitosan which is

derived from both plant and animal sources, by partial deacetylation of chitin under special conditions like a strongly alkaline environment. Chitosan has special properties such as perfect biocompatibility, high bioactivity, biodegradability, the reactivity of the amino group amino, antimicrobial activity, and the ability to form gel and film.⁴ A study has demonstrated its ability to penetrate the enamel carrying mineral ions deeper into the lesion as the amine group acts as a perfect template for calcium (Ca)²⁺ and phosphate (PO₄)³⁻ ions to regenerate new enamel-like mineralized tissue.⁵

The biomimetic Nano-Hydroxy apatite (nano-HAP) can protect the teeth with the creation of a new layer of synthetic enamel around the tooth, rather than hardening the existing layer with fluoride.⁶ The remineralizing mechanism of Nano-Hydroxyapatite depends on penetrating the enamel pores acting as a template in the precipitation process and attracting a large amount of Ca²⁺ and PO₄⁻ from the remineralization solution.⁷

Another impressive biomaterial is Bioactive glass (BAGs). When BAGs are brought into contact with body fluids, a rapid leach of (Na)⁺ and congruent dissolution of (Ca)²⁺, (PO₄)³⁻ and (Si)⁴⁺ take place at the glass surface. A polycondensed silica-rich layer is formed on the glass bulk, which then serves as a template for the formation of the calcium phosphate (Ca/P) layer at its outer surface.⁸ Moreover, the released ions can increase the pH and act as a buffer so it can inhibit loss from enamel. Enamel remineralization by the Bioactive glass occurred by the existence of an ion-enriched layer, firmly attached to the enamel surface. The basis of their bonding is the chemical reactivity of the glass in the presence of body fluids. The surface chemical reaction results in the formation of a hydroxycarbonate apatite (HCA) layer.⁹

Considering the existing concerns regarding the use of fluoride due to its potential side effects, attempts have been made to use synthetic remineralizing agents as an alternative to fluoride. Thus, this study aimed to assess and compare the remineralizing effects of nano-hydroxyapatite

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and bioactive glass combining them with chitosan on artificially created carious lesions in permanent anterior teeth using the DIGNOdent and the scanning electron microscope (SEM).

Materials and Methods:

Specimen preparation:

A total of sixty sound anterior teeth indicated for extraction were collected from the clinics of the Oral Surgery Department, Faculty of Dentistry, Mansoura University, according to the protocol approved by the Ethical Committee of Research (Ethics number m12010720). All teeth had been cleaned thoroughly to remove any deposits with a scaler and had been polished using pumice prophylaxis to remove debris and fluoride-rich layer. A polyvinyl stencil of 3x3 mm dimension was placed on the specimen surface, poured in stone modules and stored in distilled water until further use. Sixty teeth were divided into four groups (n=15). First group: Voco bifluorid 10 (5% sodium fluoride and 5% calcium fluoride) (Voco, Germany), group ii: Bioactive glass-chitosan complex (Nano Gate Company) (5ml, 10mg/ml), group iii: nano-hydroxyapatite composite with chitosan (Nano Gate Company) (5ml, 10mg/ml) and group iv: control. All teeth were examined by DIAGNOdent pen to determine the mineral content and selected surfaces were taken for the Scanning Electron microscope.

Lesion formation:

Each specimen was immersed in 8 mL of demineralizing solution for 4 days (96 hours). The solution was prepared using the formula 2.2 mM CaCl₂, 2.2 mM

KH₂PO₄, and 0.05 M acetic acid. KOH (1 M) was used to adjust pH to 4.4. The solutions were changed every day to avoid aggregation of demineralization products and pH change. After 96 hours, every specimen was washed thoroughly using deionized water and placed back in their test tubes immersed in distilled water until it was either measured by the DIAGNOdent pen or SEM

PH cycling model:

Each tooth was PH. cycled for 7 days using the demineralizing and remineralizing solutions. Each tooth was placed in 15 ml demineralizing solution for 3 hours twice daily and 15 ml remineralizing solution for 2 hours between these 2 times and overnight. The test materials were applied using a cotton applicator twice daily for 2 minutes each time: the first time before the first demineralization of the day and the second time after the second demineralization of the day. After the 7 days of pH-cycling, the specimens were then stored in distilled

water until examined. The solutions were changed daily, and their pH levels were measured before each cycle.

Specimen evaluation:

The evaluation was done using a DIAGNOdent pen (kavo DIAGNOdent 2190) for assessment of mineral content of teeth at baseline, after demineralization and after remineralization. The recording was taken according to the manufacturer's instructions. Reading 0-12 is normal enamel, 13-20 is initial enamel lesion and >20 is dental lesion.

Scanning electron microscope was used for the detection of surface morphological changes before demineralization, after demineralization and after remineralization. Samples were examined in SEM in conventional high-vacuum mode (20Kv). SEM images were obtained to evaluate the surfaces at 2000X magnification.

Statistical analysis and data interpretation:

Data were fed to the computer and analysed using IBM SPSS (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp). The significance of the obtained results was judged at the (0.05) level. Friedmann test was used to compare more than 2 studied periods with post Hoc test Wilcoxon signed-rank test.

Results:

DIAGNOdent® Pen:

Table 1 presented the median DIAGNOdent readings in all groups during the different stages of the study. It showed that all groups provided significantly higher differences after the remineralization compared to the mineral content at the baseline and after the demineralization (P<0.001). However, regarding the comparison between the group in each stage, there is no significant difference in any stage between them (P<0.05).

Scanning Electron Microscope:

The SEM observation at 2,000X magnification showed a smooth and intact surface at the baseline. After 96 h of demineralization, SEM images were taken. There was a loss of surface integrity in all the study groups. Enamel showed an irregular surface like a honeycomb pattern. After a 7-day remineralization regimen, samples were again subjected to SEM examination. The porous defects were all filled; thus, the surface integrity was re-established. (Figure 1)

Table 1: Comparison of DIAGNOdent pen (Kavo DIAGNOdent pen 2190) changes before and after demineralization, remineralization

	Baseline	After demineralization	After remineralization	Test of significance#	Within-group significance
G1	2(0-11)	15(12-19)	6(3-11)	P<0.001*	P1=0.001* P2=0.003* P3=0.001*
G2	2(0-6)	14(12-19)	4(2-13)	p<0.001*	P1=0.001* P2=0.001* P3=0.001*
G3	2(0-6)	13(12-16)	5(3-11)	P <0.001*	P1=0.001* P2=0.004* P3=0.001*
G4	0(0-5)	17(13-20)	5(2-15)	p<0.001*	P1=0.001* P2=0.002* P3=0.001*
P value	P=0.367	P=0.195	P=0.548		

Parameters are described as median (Range). P1: the difference between baseline and after demineralization. P2: the difference between baseline and after remineralization. P3: the difference between after demineralization and after remineralization. #Used tests Friedman test, with group significance done by Wilcoxon signed-rank test. G1: fluoride varnish. G2: nano- bioactive glass- chitosan complex. G3: nano-hydroxy apatite- chitosan complex. G4: control group.

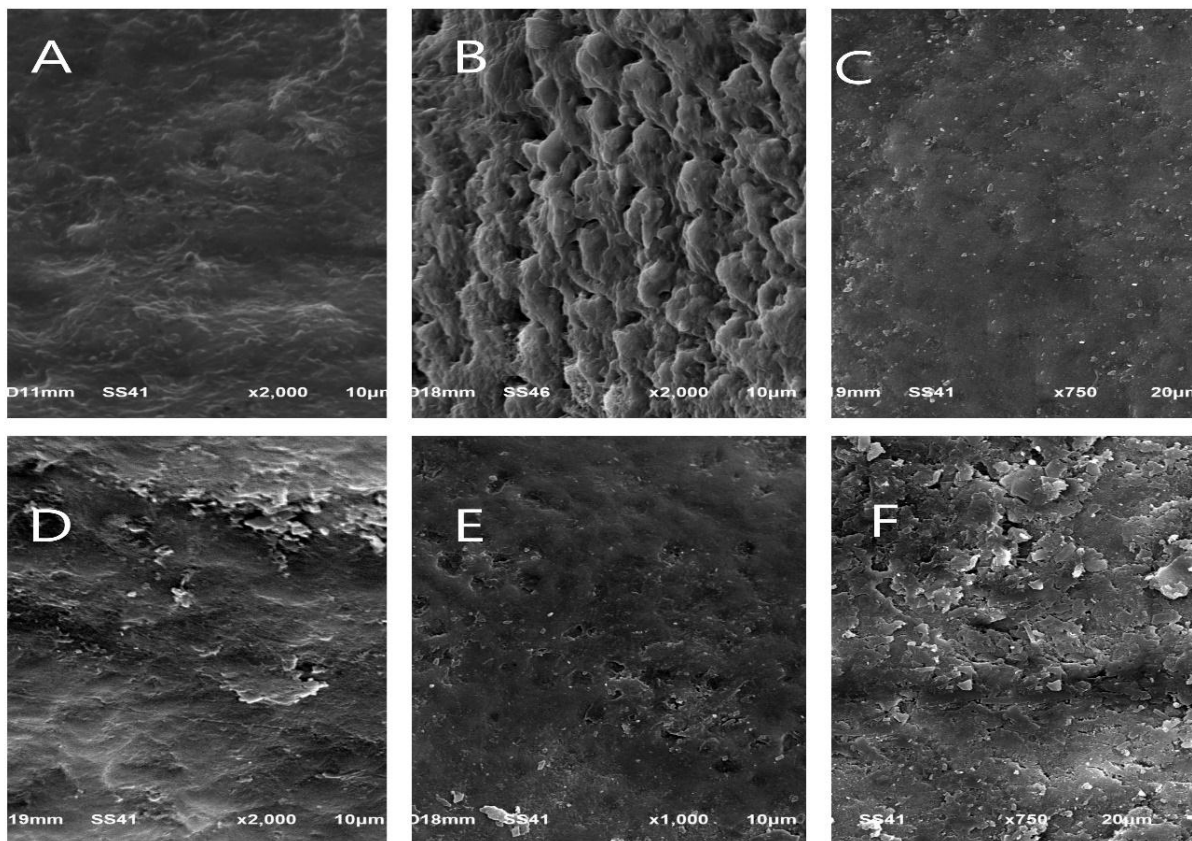


Figure1: SEM examination of A: sound enamel, B: demineralized enamel, C: fluoride group, D: BAG-Chitosan group, E: n-HAP Chitosan group, and F: control group

Discussion:

Enamel remineralization has been studied for about 100 years, and it has been suggested that “the noninvasive” treatment of early caries lesions by remineralization has the potential to be the major advance in the clinical management of the disease. White spot lesions (WSLs)

represent the first clinical observation of demineralization in enamel and their early diagnosis has been reported in the literature.¹⁰

Assessment of in vitro demineralization and remineralization can be done using different methods. In this study, the DIAGNOdent pen (Kavo DIAGNOdent pen 2190) was selected to assess the mineral gain after the

dynamic pH cycles and after the application of the test materials. Another method was the Scanning Electron Microscope (SEM) that was used to image the surface characteristic changes of the enamel before and after the remineralization.

In the present study, DIAGNOdent values increased post-demineralization and subsequently decreased post-remineralization. Similar results were demonstrated in studies conducted by Kamath et al.,¹¹ Daas et al.,¹² Taneja et al.,² who also used DIAGNOdent to assess remineralization.

After remineralization, the three materials and the with remineralizing solution had a great mineralizing effect no significant difference between them. The results affirmed that Nano-hydroxyapatite chitosan complex and Nano-bioactive glass chitosan complex had a similar effect to sodium fluoride and the remineralizing solution in remineralizing initial carious lesions. So, as a result, all agents have similar effectiveness in remineralization. This was in accordance with Manchery et al.,¹³ where they concluded that there was no statistical significance found between fluoride, nano-hydroxyapatite, and bioactive glass. But this was disagreeing with Geeta et al.,¹⁴ as they concluded that there was a significant difference between nano-hydroxyapatite, bioactive glass, and fluoride. This may be due to the use of the material in paste form and applying the materials three times/day, not two with taking into consideration using surface microhardness as an assessment method.

Scanning electron microscope (SEM) is one of the most sensitive, time-tested techniques to assess the demineralization and remineralization of the carious lesions in vitro as reported in earlier studies.^{15,16} Before demineralization, the SEM analysis revealed a homogeneous smooth appearance of the enamel surface. After demineralization, numerous depressions in a honeycomb pattern were revealed, which corresponded to the observations made by previous studies.^{2,11} Post remineralization, all the test specimens showed a re-establishment of surface integrity. The potential to induce remineralization of the test agents was demonstrated as seen by the increase in crystal size and occlusion of porous defects. However, there were minor differences in the surface morphology, which may be due to their different mechanisms for promoting remineralization.¹¹

In the fluoride varnish group, a post-treatment Scanning electron microscope (SEM) showed a reduction in the surface porosity with areas of the smooth enamel surface. Globular structures sedimented on the demineralized surface consisting mainly of calcium fluoride causing crystal.

The Bioactive glass Chitosan complex gave complete coverage and uniform enamel, suggesting that the bioactive glass particles were embedded in the treated enamel surface and effectively led to its remineralization.

A superior surface texture of enamel was noted in the present study on SEM evaluation post remineralization with nano-hydroxyapatite Chitosan complex compared to other groups. This could be attributed to the similarity of the nano-sized particles to the apatite crystals of tooth enamel in morphology, crystal structure, and crystallinity.

Surface enamel remineralization was also observed in the control group. Multiple porosities, irregular surfaces with slight amorphous surface precipitation were visible in the negative control group.

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