Acta Scientific Dental Sciences

Volume 2 Issue 6 June 2018

Research Article

Preparation and Characterization of Chitosan-Based Post-Bleaching Enamel Remineralizing Gel

Gihan H Waly*

Assistant Professor, Biomaterials Department, Faculty of Oral and Dental Medicine, Cairo University, Egypt

***Corresponding Author:** Gihan H Waly, Assistant Professor, Biomaterials Department, Faculty of Oral and Dental Medicine, Cairo University, Egypt.

Received: May 25, 2018; **Published:** May 30, 2018

Abstract

Objectives: The present study aimed to prepare a chitosan-based post-bleaching remineralizing gel and to compare it with a commercially available remineralizing agent regarding the ion release and remineralizing capacity.

Materials and Methods: The chitosan was purchased from Fluka BioChemika. One in-office bleaching product, Opalescence Boost PF 40%, Ultradent, was used. One commercially available remineralizing gel, MI Paste Plus, GC America, was used as a control. An experimental chitosan-based remineralizing gel was prepared using β-glycerol phosphate (β-GP) to neutralize the chitosan and hydroxyl ethyl cellulose (HEC) as a crosslinking agent. Sodium fluoride (NaF), nano amorphous calcium phosphate (NACP) and casein phosphopeptide (CPP) were added to the mixture and carboxymethyl cellulose (CMC) was used as a thickening agent. FTIR analysis was performed for both the pure chitosan and the prepared experimental gel. The release of calcium, phosphorus and fluoride ions from the control and experimental remineralizing agents was measured using Inductively Coupled Plasma emission spectrophotometer and Ion Selective Electrode (n = 6). To evaluate the remineralizing capacity, the crowns of eight sound upper central incisors were vertically sectioned in two halves. Each half of each tooth was assigned to one of the two groups (the control and the experimental group) (n = 8). The enamel surfaces of the teeth were subjected to three bleaching sessions (20 minutes each) using the in-office bleaching agent. Before starting the remineralization regimen, SEM analysis was done to assess the surface morphology and EDX surface elemental analysis was performed to obtain baseline ion content measurements. The enamel surfaces were then subjected to the remineralization treatment (10 minutes daily for two months period). At the end of the remineralization period, the teeth were reexamined with SEM and EDX. The remineralizing capacity was calculated as the change in mineral content from the baseline measurements. The measured ion contents were used for calculating the Ca/P ratios of the precipitated deposits.

Results: The experimental gel released comparable amount of calcium, less phosphorus and more fluoride compared to the control. After remineralization, the SEM and EDX examination revealed the formation of a calcified layer that occluded and covered most of the bleaching-induced enamel surface porosities. The formed precipitates had Ca/P ratio similar to that of human enamel. No significant difference was found in the surface calcium and phosphorus contents between the teeth of the two groups while the enamel treated with the experimental gel showed higher fluoride content than that treated with the control.

Conclusions: The experimental chitosan-based gel is as effective as the commercial remineralizing agent in promoting bleached enamel remineralization.

Keywords: Chitosan; post-bleaching; enamel remineralizing gel; amorphous calcium phosphate; casein phosphopeptide; remineralizing capacity; ion release

Introduction

With the growing concern about esthetics, tooth bleaching is becoming an increasingly popular procedure in the dental practice [1]. Bleaching can be accomplished using at home products (containing as low as 6% hydrogen or carbamide peroxide) or in-office products which contain higher concentrations (as high as 35 - 40%) of the bleaching agent and are to be used only by the dentist in the dental clinic [2]. Despite the esthetic advantage offered by tooth bleaching, the process has been accompanied by tooth sensitivity which was attributed to the changes that the bleaching agent causes in the composition and morphology of the tooth surface [3]. It has already been proven that bleaching agents cause a dissolution of the mineral content of both enamel and dentin. In case of enamel, this dissolution is reflected as an increased enamel surface porosity and loss of interprismatic substance [4]. In addition to the sensitivity issues, this increased surface porosity increases the enamel's susceptibility to bacterial adhesion and subsequent plaque formation [5]. A common practice that has been frequently used to reverse the unfavorable effects of bleaching is to use remineralizing agents. The remineralization process can be carried out either during bleaching by incorporat-

ing a remineralizing agent into the bleaching gel or through a separate post-bleaching remineralization procedure [5,6]. Generally, all commercially available remineralizing agents are based on several constituents, each playing a particular role in the remineralization process. For example, any remineralizing agent must contain mineral precursors that can supply calcium and phosphate ions to be used as building blocks during the process of new apatite deposition. Also, the presence of fluoride in the remineralizing gel assists the remineralization and allows the formation of more caries-resistant calcified phases [7]. Casein phosphopeptide (CPP) is another constituent of many of the commercially available remineralizing agents. It allows stabilizing high concentrations of calcium and phosphate ions at the tooth surface [8].

Chitosan is an attractive biomaterial with many biological merits which made it the material of choice for several biomedical applications. Chitosan is the deacetylated form of chitin, which is a natural polymer that forms the major component of the exoskeletons of crustaceans, insects and the cell wall of fungi [9]. Chitosan is biocompatible, biodegradable and has the tendency to adhere to biological surfaces. It also exerts a broad spectrum antimicrobial action against gram-negative, gram-positive bacteria and fungi [10]. All these properties made the chitosan a suitable material for drug delivery. Chitosan has been used in percutaneous drug delivery systems, topical eye drops formulations and wound healing ointments [11-13]. Inspired by these pharmaceutical applications, many researchers attempted using the chitosan, together with other ingredients, to formulate tooth remineralizing agents. Ruan., *et al.* prepared amelogenin-chitosan hydrogel for enamel remineralization and reported that the hydrogel benefited from the potential of amelogenin to control organized growth of apatite crystals and the antimicrobial activity of chitosan [14]. Chen., *et al.* prepared carboxymethyl chitosan/ amorphous calcium phosphate nanocomplexes and reported that they had promising effect on dentin remineralization which makes them potential indirect pulp capping materials during vital pulp therapy [15]. Xu., *et al.* used phosphorylated chitosan (P-chi) to mimick the nucleating role of the phosphorylated non-collagenous proteins in the biomineralization of dentin. The authors reported that the phosphorylated chitosan promoted the remineralization process and even enhanced the properties of the remineralized dentin surface [16].

Not only was chitosan reported to help in the remineralization process but was also credited to have a protective effect on enamel against demineralization. Enamel pretreated by chitosan lactate solution was reported to be more resistant to demineralization than untreated enamel when later exposed to cariogenic acid, acidic beverages and hydrogen peroxide bleaching [17].

Although promising, the use of chitosan in this particular dental field is still relatively new and requires further investigations to evaluate its potential usefulness. Therefore, the current study aimed to prepare post-bleaching chitosan-based remineralizing gel and to compare the ion release and remineralizing capacity of the prepared gel with those of a commercially available remineralizing agent as a control. The null hypothesis was that there was no significant difference between the prepared experimental gel and the commercially available agent regarding the ion release and the remineralizing capacity.

Materials and Methods

Materials

Chitosan (middle-viscous with degree of deacetylation ≈ 80%), β-glycerol phosphate disodium salt pentahydrate (β-GP) and hydroxyethyl cellulose (HEC) were purchased from Fluka BioChemika. Nano amorphous calcium phosphate (NACP) was obtained from Nano Tech, Egypt, and sodium fluoride (NaF) was purchased from El-Gomhouria Company, Egypt. Casein phosphopeptide (CPP) was obtained from Xi'an Lyphar Biotech Co, China. One in-office chemically-activated bleaching product was used, Opalescence Boost PF 40% Tooth Whitening System, Ultradent, USA, which is based on 40% hydrogen peroxide. One commercially available remineralizing agent, MI Paste Plus, GC America, was used as a control.

Methods

Preparation of the remineralizing gel

Chitosan solution (2% w/v) was prepared by dissolving 1600 mg chitosan flakes in 80 ml 0.15 M acetic acid aqueous solution using mechanical stirring. The pH of the resultant chitosan solution was 4.8. The chitosan solution was cooled to 4°C in an ice bath for 15 minutes before adding the other ingredients. An aqueous solution of β-glycerol phosphate (β-GP), prepared by dissolving 16 g β-GP in 24 ml distilled water, was cooled to 4°C and added dropwise to the chilled chitosan solution. This particular amount of β-GP was selected in order to raise the pH of the chitosan solution to 7.2. and to neutralize the positive charges on the chitosan chains thus allowing gelation while still preserving the bioadhesiveness as will be discussed later. The resultant mixture was vigorously stirred for five minutes to obtain a clear solution. An aqueous solution of hydroxyethyl cellulose (HEC) was prepared by dissolving 175 mg HEC in 16 ml distilled water. The HEC solution was then cooled and added dropwise to the ice-cooled chitosan/β-GP mixture where stirring was continued for additional five minutes. The β-GP and HEC were added to the chitosan gel in these particular concentrations because these ratios have already been investigated in previous author's research [18]. Sodium fluoride (NaF) (2%) and nano amorphous calcium phosphate (NACP) (20%) were added to the chitosan/ β-GP/ HEC mixture as sources of fluoride and calcium/phosphate ions respectively. These concentrations of NaF and NACP were selected as they were found effective in promoting enamel remineralization in previous researches [19,20]. Casein phosphopeptide (CPP) was added to the mixture at a concentration of 10%. Carboxymethyl cellulose (CMC) (50 mg) was dissolved in 15 ml deionized water then gradually added to the mixture as a thickening agent to obtain a suitable paste-like consistency. The prepared gel was then stored in a tightly sealed container in the refrigerator until used for testing.

Fourier Transform Infrared Spectroscopy (FTIR)

Attenuated Total Reflectance-FTIR spectra were obtained for the pure chitosan and for the prepared experimental gel using Bruker VERTEX 70 spectrometer, Germany, equipped with Dia-

mond crystal ATR system. Spectra were recorded from 4000 - 400 cm^{-1} with a resolution of 4 cm^{-1}. One of the objectives of the FTIR analysis was to ensure that certain important groups (namely NH_2), that are essential for the performance of chitosan, did not get fully consumed in chemical reactions with other ingredients of the gel.

Ion release measurement

The ion release test was performed on six samples from each of the two groups (the control and the experimental) (n = 6). A Specified volume (2 ml) of the remineralizing agent was dispensed at the bottom of a glass beaker with a diameter of 2.5 cm. The remineralizing agent was then left for 30 minutes to settle in the bottom of the beaker so that the area of the exposed surface of the gel was ≈5 cm^2. A volume of 15 ml of artificial saliva was then gently dispensed into the beaker on top of the remineralizing agent without disturbing it, yielding a "sample area/solution volume ratio" of 0.33 cm^{-1}. All beakers were stored in an incubator at 37°C for 10 minutes. At the end of the incubation period, 12 ml of the supernatant artificial saliva was aspirated from each beaker without disturbing the remineralizing agent lying underneath. The amount of calcium and phosphorus ions was measured using Inductively Coupled Plasma emission spectrophotometer (ICP) (JY plasma ICP Ultima 2, France). The ICP does not only measure free phosphorus ions but also the plasma has the ability to release the elemental phosphorus found in all phosphorus forms [21]. Since the experimental gel does not contain a source of elemental phosphorus ions, it may be inferred that the phosphorus ion amount measured by the ICP represents the phosphorus ions that the plasma has extracted from the phosphate groups of the ACP, β-GP and CPP. The fluoride ion release was measured using Ion selective electrode, InoLAB pH/ION 7320, WTW, Germany. In addition, the pH of the aspirated artificial saliva (the immersion medium) was measured using pocket-sized pH meter (Hanna Instruments, Italy). Before measurement, the pH meter was calibrated using standard buffer solutions of pH = 4 and pH = 7.

Morphological and quantitative assessment of the remineralizing capacity

Teeth preparation

Eight freshly extracted caries-free upper central incisors, obtained from the outpatient clinic, Surgery Department, Faculty of Oral and Dental Medicine, Cairo University, were used in the present study. The teeth were used after taking the patients' consents and the approval of the Ethics Committee. Before use, the teeth were hand-scaled to remove any hard deposits and soft tissue residues. Then, the teeth were disinfected by immersion in 10% formalin for 7 days (as recommended in previous studies) before separating the crowns from the roots [22]. To avoid any bias in teeth allocation between groups and to minimize the inter-group variations, the crown of each tooth was spilt in a buccolingual direction into two halves; one half was used in the test group and the other half was used in the control group (n = 8).

A small pit was drilled on the enamel surface of each tooth half in order to be used as a landmark to ensure that the elemental analysis that will be conducted later on is always carried out at the same spot. In addition, the location of this pit was particularly selected so that it lied close to the sectioning line. This ensured that the baseline mineral content of one specimen in the control group was very close to that of another specimen in the test group.

Scanning electron microscopic examination was performed for three representative random enamel specimens using Quanta 250 Field Emission Gun SEM (accelerating voltage 30 KV, FEI Company, Netherland) to be used as a reference for the morphology of the unbleached enamel surface for comparative reasons.

Teeth bleaching

The teeth were then subjected to three bleaching sessions, twenty minutes each, using the in-office Opalescence Boost PF 40% bleaching gel. Between each two consecutive sessions, the teeth were stored for seven days in artificial saliva (composition: 1.5 mmol/L $CaCl_2$, 8.2 mmol/L $NaHCO_3$, 4.8 mmol/L NaCl, 137 mmol/L KCl, and 4 mmol/L KH_2PO_4 in 250 mL) at 37°C [23].

Pre-remineralization morphological and quantitative assessment

The surface morphology of each of the eight bleached enamel specimens of each group was assessed by SEM examination. The elemental composition of each bleached enamel specimen was assessed using Energy Dispersive X-ray spectroscopy (EDX) to be used as a baseline measurement for assessing the remineralization capacity after treatment.

The remineralization regimen

The bleached teeth were subjected to a remineralization regimen where the test group specimens were treated with the experimental remineralizing gel while the control group specimens were treated with the MI paste. The remineralizing gel was applied to the enamel surfaces and the teeth, covered with the remineralizing agent, were carefully placed in plates containing artificial saliva and stored at 37°C for 10 minutes then were rinsed. This treatment was repeated daily for a period of two months. After each daily remineralization session, the teeth were stored in artificial saliva at 37°C until the next day's session.

Post-remineralization morphological and quantitative assessment

At the end of the two months remineralization period, the surface morphology of each enamel specimen was examined by SEM and the surface elemental composition was assessed using Energy Dispersive X-ray spectroscopy (EDX). The remineralizing capacity was assessed by calculating the difference in the surface content of the calcium, phosphorus and fluoride ions before and after remineralization for the same spot for each tooth (ΔCa, ΔP and ΔF). The change in ion concentration was used for assessing the rem-

ineralizing capacity rather than the absolute post-remineralization ion content in order to eliminate the effect of any variations in the original mineral content between the teeth before treatment.

Statistical Analysis

The data were presented as means and standard deviation values. Data were explored for normality using Shapiro-Wilk test and showed parametric distribution. Independent Sample T-Test was used to compare between the two groups. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

Results

FTIR

The FTIR analysis revealed that many of the characteristic bands of chitosan remained visible in the spectrum of the experimental gel with varying degrees of shifts (e.g. the band at 1422 cm^{-1} corresponding to the (CH_2) in CH_2OH group which shifted to 1431 cm^{-1} and the vibration band at 1656 cm^{-1} corresponding to the (C = O) in $NHCOCH_3$ group that shifted to 1649 cm^{-1}) [24]. The most important observation is the persistence of the 3445 cm^{-1} band that represents the (NH_2) group in primary amines which plays an important role in the gel performance as will be discussed later (Figure 1).

Ion release and pH Measurement

Regarding the calcium ion release, no significant difference was found between the control and experimental group. On the other hand, the experimental group released significantly less phosphorus and more fluoride compared to the control (Figure 2). The pH of the artificial saliva in which the two remineralizing agents were immersed ranged from 6.9 to 7.2 with no significant difference between the two groups (p = 0.007).

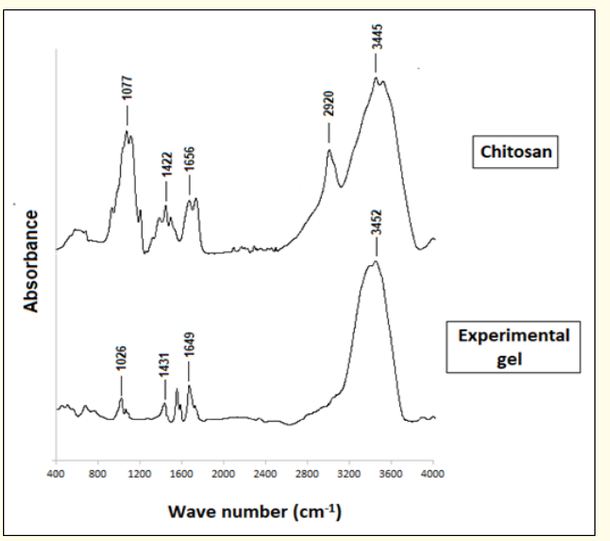

Figure 1: FTIR spectra of the chitosan and the experimental gel.

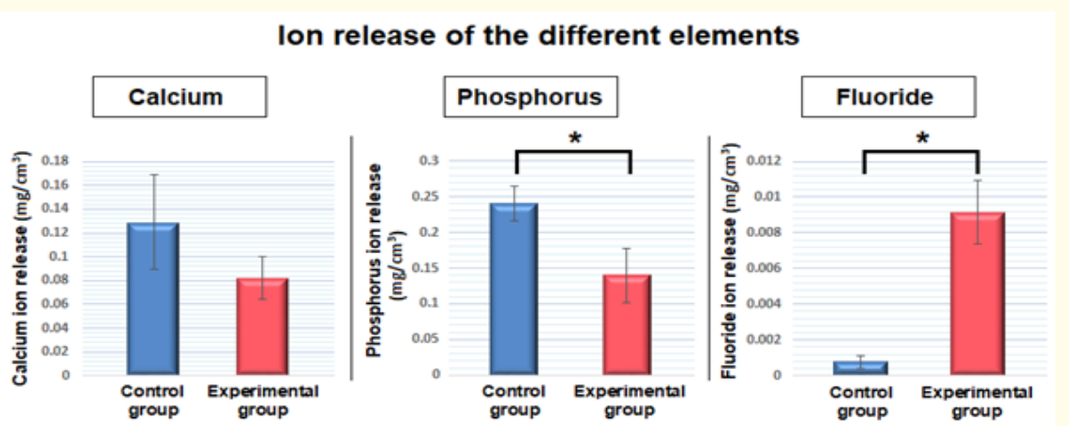

Figure 2: Comparison of the Ca, P and F ions release between the two groups.

Morphological and quantitative assessment of the remineralizing capacity

Morphological assessment

Microscopically, the unbleached enamel presented flat smooth surface with few scratches; a representative photo is shown in figure 3 just as a reference. The SEM examination of the bleached enamel (before remineralization) revealed changes in the surface morphology. Absence of the aprismatic enamel layer was observed and the surface showed signs of erosion with differential dissolution affecting the interprismatic substance and the peripheries of the prisms more than the prisms' cores leading to a highly porous surface (Figure 4).

After remineralization, the enamel surfaces of both groups (control and experimental) showed the precipitation of a layer of surface deposits that partially obscured the underlying surface roughness. At higher magnifications, the hard precipitates were not only observed covering the surface but were also seen occlud-

ing and clogging most the bleaching-induced surface porosities. No significant morphological differences were observed between the group treated by the control and that treated by the experimental gel (Figure 4).

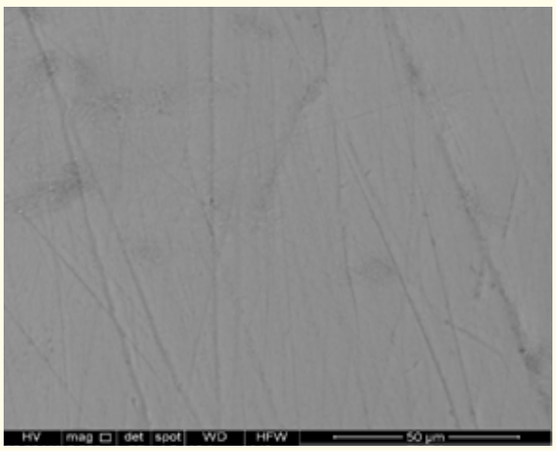

Figure 3: A representative SEM micrograph showing the flat surface of unbleached enamel.

Qualitative assessment (elemental analysis)

Regarding the change in the calcium (ΔCa) and phosphorous (ΔP) ions contents, no significant difference was found between the enamel treated with either the control or experimental remineralizing agent. However, the group treated with the experimental gel exhibited a significantly higher change in the fluoride ion content (ΔF) compared to the control (Figure 5). The mean calcium to phosphorus atomic molar ratio (Ca/P) of the treated enamel surfaces did not differ significantly between the two groups (Figure 6). The ratios for both groups lied within the range reported for human hydroxyapatite in previous researches [25-27].

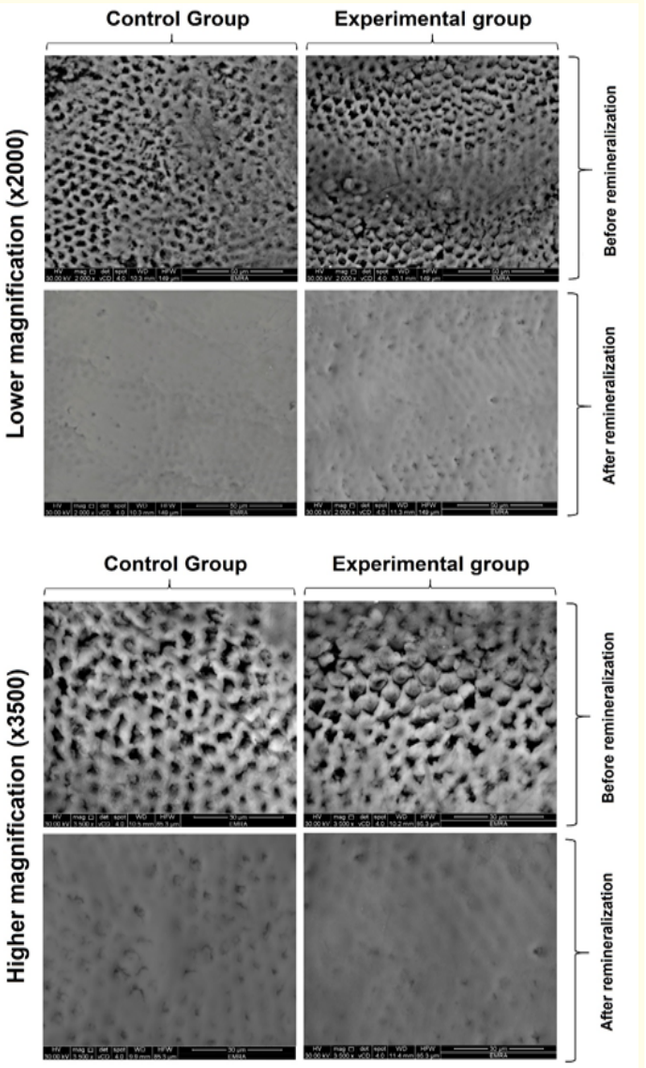

Figure 4: Representative SEM micrographs showing the surface topography of the control and experimental groups before and after the remineralization process at two different magnifications (x2000 and x3500).

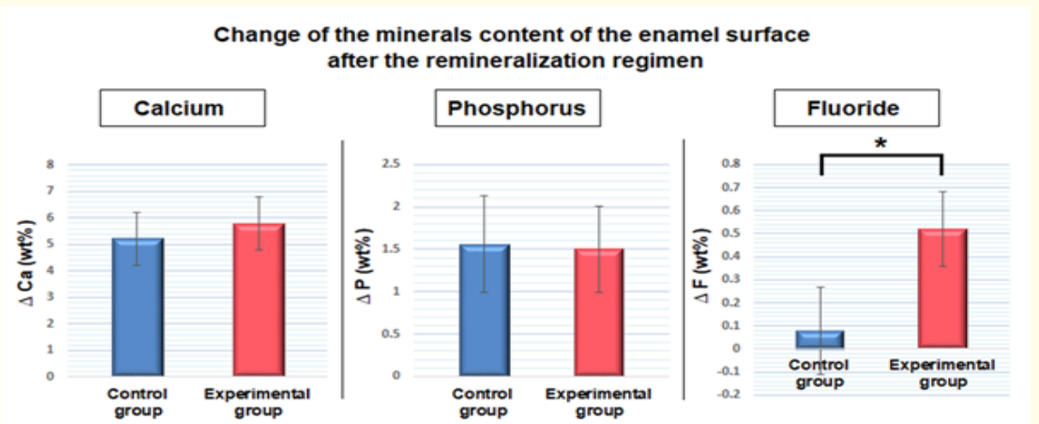

Figure 5: Comparison between the two groups regarding of the change of the calcium (ΔCa), phosphorus (ΔP) and fluoride ions (ΔF) content of the enamel after remineralization (using the mineral content of the surface before remineralization as a baseline).

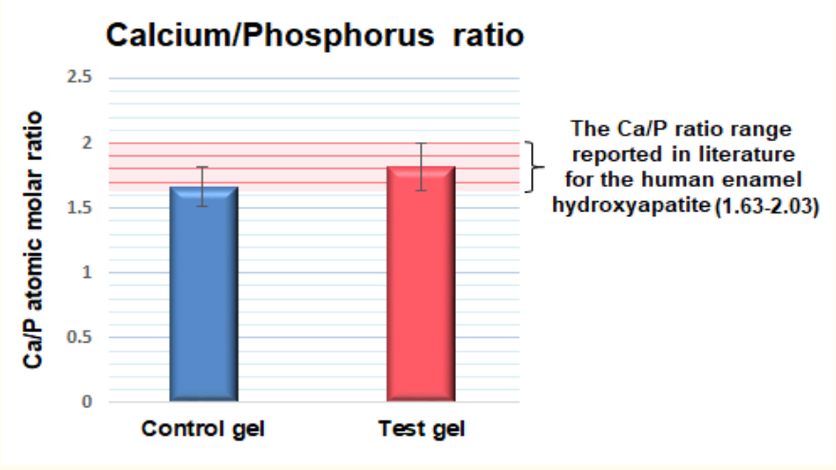

Figure 6: The Ca/P ratios as calculated from the EDX results of both groups.

Discussion

In an attempt to benefit from the unique favorable biological properties of chitosan, chitosan-based enamel remineralizing gel was prepared and compared with a commercially available remineralizing paste. Although the chitosan is the main ingredient of the experimental gel, some of the other ingredients that are added at much lower concentrations play a critical role in modulating the properties of the chitosan gel in order to successfully carry and deliver the minerals needed for efficient remineralization. That is why the performance of the gel is greatly influenced by finely tuning the concentrations of these ingredients. For example, in aqueous acidic media, the amine groups on the chitosan chains get protonated and acquire positive charges, the repulsion between which keeps the chains apart from each other and maintains the chitosan in solution. When the β-GP is added to the solution, it partially neutralizes these positive charges on the chitosan chains allowing the chains to come closer to each other. This chain approximation allows developing different inter-chain interactions like hydrogen bonding and hydrophobic interactions which eventually lead to gelation [18]. However, the positive charges on the chitosan chains are the key reason for the favorable bioadhesive properties of chitosan as they allow the chitosan to get electrostatically attracted to the negatively charged biological surfaces [28]. That's why the FTIR analysis was performed on the prepared experimental gel to ensure that the added amount of β-GP only reacted with and neutralized some of the protonated amine groups to allow for gelation but left some of the groups unreacted to maintain the bioadhesion. The FTIR spectra revealed that the bands of the amine groups were still present in the experimental gel which indicates that the proper concentration of β-GP has been used in the formulation.

Likewise, the concentration of the HEC crosslinking agent had to be properly adjusted such that it would allow the desirable degree of cross-linking that improves the coherence of the hydrogel while keeping its viscosity low enough to allow the gel to be extruded out of a tube or injected by a syringe [18].

The duration of the ion release test and the duration of the daily remineralization session (10 minutes for both) was estimated from the instructions of the manufacturer of the commercial remineralizing paste. The manufacturer recommends leaving the paste on the teeth undisturbed for a minimum of 3 minutes and recommends that after these minutes, the patient should only expectorate, but not rinse. The manufacturer also advises the patient to avoid eating or drinking for at least 30 minutes to keep the residues of the paste on the teeth for as long as possible. Accordingly, it was estimated that with the salivary flow washing out the remineralizing agent gradually, a 10-minutes period would be a reasonable estimate of the time in which there would be still enough amount of the paste on the tooth surface to promote remineralization.

Regarding the characterization of the prepared gel, the ICP results showed no significant difference in the calcium ion release between the two groups while the experimental group exhibited less phosphorus and more fluoride release than the control. Based on these results, the null hypothesis was rejected. To be able to interpret these data, it must first be emphasized that the starting amounts of the calcium and phosphate ions precursors incorporated in the two remineralizing agents were not the same from the beginning. This is because the manufacturer of the commercial agent (the control) only announces the names of the paste's ingredients without specifying their exact amounts in order to preserve the confidentiality of the formula. The situation is different for the fluoride, which is the only exception, where the manufacturer clearly declares that 0.2% NaF is incorporated in the MI Paste while 2% NaF was added to the experimental gel. This marked difference in the fluoride content was reflected on the results of the ion release and EDX elemental analysis where the experimental gel values for fluoride surpassed those of the control.

Based on the composition, it was expected that the phosphorus ion release values of the experimental gel would be very high because the gel contains many phosphorus-containing substances (namely the NACP, β-GP and CPP), but this was not the case. One reason that may decrease the release values of phosphorus ion from the experimental gel is the ion charge. The phosphorus element in the experimental gel is not present in the form of separate phosphorous ions. Instead, it is present as a part of the phosphate ions which are polyatomic anions that usually carry more than one negative charge. It is possible that the electrostatic attraction between these polyanions and the positively charged chitosan chains retarded or decreased the release of these negatively charged species. Although the fluoride ions are negatively charged as well, their release from the experimental gel was still relatively high and did not seem to be affected by the negative charge. This may be attributed to the difference in the negative charge density between the phosphate (PO_4^{3-}) and the fluoride (F^-) ions or the difference in the ionic size, a factor that may affect the rate of ionic diffusion [29].

The SEM examination revealed erosion of enamel and increased surface porosity and depressions as a result of the bleaching process. These morphological findings agree with those reported by Miranda., *et al* [30]. Treatment of the bleached enamel by either of the two remineralizing agents resulted in the precipitation of an almost continuous layer of mineralized deposits that covered the surface, sealed most of the surface porosities and depressions and decreased the surface roughness. The EDX results confirmed that these deposits were calcium phosphate compounds that had the same Ca/P ratio as biological hydroxyapatite found in human enamel [25-27].

The EDX analysis revealed that the calcium and phosphorus content of the enamel treated by either the control or experimental gel did not differ significantly from each other. This means that despite the fact that the control group released more phosphorus than the experimental group, this was not reflected as a difference in the precipitation of hard deposits on the enamel. This may be attributed to the fact that the performance of any remineralizing agent does not only depend on the absolute amounts of minerals contained in the paste but also on the bioavailability of these minerals [31]. This bioavailability is mainly governed by two factors: a) the presence of the calcium and phosphate precursor compounds in a soluble easily ionized form and (b) the ability of the remineralizing agent to remain in direct intimate contact with the tooth structure to allow for ionic diffusion. The experimental gel guarantees this bioavailability by a two-fold mechanism. First, the presence of the casein phosphopeptide (CCP) in the gel promotes remineralization by binding to the calcium phosphate nano clusters and inhibiting their growth beyond a critical size beyond which they cannot participate in the nucleation and deposition of new hydroxyapatite [8]. However, it is worth mentioning that this compositional merit is not only limited to the experimental gel since the CPP is also present in the control paste.

The second mechanism that ensures the minerals bioavailability is mediated by the bioadhesive properties of the chitosan that is attributed to its acquired positive charges as mentioned earlier [32]. It is well-established that the surface of the cleansed enamel is negatively charged under normal oral pH range due to the structure of its hydroxyapatite, in which the phosphate groups are packed close to the enamel surface [33]. The electrostatic attraction between these opposite charges allows the chitosan-based mineral-loaded experimental gel to attain and secure an intimate contact with the enamel. This intimate contact helps localize the free calcium and phosphate ions near the enamel surface creating a state of super saturation that prevents mineral dissolution from the enamel surface. In addition, this super saturation builds up a concentration gradient of calcium and phosphate ions across the enamel surface thus promoting inward ionic diffusion into the enamel which is an essential step for the remineralization process.

Based on the previously discussed findings, it's evident that the experimental chitosan-based gel prepared in the present study effectively promoted bleached enamel remineralization and gave comparable results to those of the control. Finally, the results of this *in vitro* study need to be strengthened by an *in vivo* investigation before being extrapolated for clinical application.

Conclusions

Within the limits of the present study, it may be concluded that the chitosan-based gel with the investigated composition is a suitable carrier that can release loaded minerals and promote efficient remineralization of bleached enamel surfaces.

Bibliography

1. De Geus JL., *et al*. "At-home vs in-office bleaching: A systematic review and meta-analysis". *Operative Dentistry* 41.4 (2016): 341-356.

2. Majeed A., *et al*. "Tooth bleaching: A review of the efficacy and adverse effects of various tooth whitening products". *Journal of the College of Physicians and Surgeons Pakistan* 25.12 (2015): 1-6.

3. Klarić E., *et al*. "Surface changes of enamel and dentin after two different bleaching procedures". *Acta Clinica Croatica* 52.5 (2013): 419-428.

4. Coceska E., *et al*. "Enamel alteration following tooth bleaching and remineralization". *Journal of Microscopy* 262.3 (2016): 232-44.

5. Attia RM., *et al*. "Changes in surface roughness of bleached enamel by using different remineralizing agents". *Tanta Dental Journal* 13 (2016): 179-186.

6. Furlan IS., *et al*. "Effect of high- or low-concentration bleaching agents containing calcium and/or fluoride on enamel microhardness". *General Dentistry* 65.3 (2017): 66-70.

7. Zenouz GA., *et al*. "Effect of fluoride, casein phosphopeptide-amorphous calcium phosphate and casein phosphopeptide-amorphous calcium phosphate fluoride on enamel surface microhardness after microabrasion: An in vitro study". *Journal of Dentistry, Tehran University of Medical Sciences* 12.10 (2015): 705-711.

8. Reynolds EC. "Calcium phosphate-based remineralization systems: Scientific evidence?". *Australian Dental Journal* 53.3 (2008): 268-273.

9. Elieh-Ali-Komi D., *et al*. "Chitin and chitosan: Production and application of versatile biomedical nanomaterials". *International Journal of Advanced Research* 4.3 (2016): 411-427.

10. Goy RC., *et al*. "A Review of the antimicrobial activity of chitosan". *Polímeros: Ciência e Tecnologi*a 19.3 (2009): 241-247.

11. Katas H., *et al*. "Chitosan nanoparticles as a percutaneous drug delivery system for hydrocortisone". *Journal of Nanomaterials* (2012): 372725.

12. Cheng YH., *et al*. "Thermosensitive chitosan-based hydrogel as a topical ocular drug delivery system of latanoprost for glaucoma treatment". *Carbohydrate Polymers* 25.144 (2016): 390-399.

13. Han SS., "Topical formulations of water-soluble chitin as a wound healing assistant". *Fibers and Polymers* 6.3 (2005): 219-223.

14. Ruan Q., *et al*. "Amelogenin-chitosan matrix promotes assembly of an enamel-like layer with a dense interface". *Acta Biomaterialia* 9.7 (2013): 7289-7297.

15. Chen Z., *et al*. "Biomimetic remineralization of demineralized dentine using scaffold of CMC/ACP nanocomplexes in an in vitro tooth model of deep caries". *PLoS One* 14.10 (2015): 1-19.

16. Xu Z., "Biomimetic deposition of calcium phosphate minerals on the surface of partially demineralized dentine modified with phosphorylated chitosan". *Journal of Biomedical Material Research B, Applied Biomaterials* 98.1 (2011): 150-159.

17. Visveswaraiah PM., *et al*. "Effect of water soluble carboxymethyl chitosan and chitosan lactate on enamel demineralization-An SEM study". *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 7.3 (2016): 427-433.

18. Waly GH. "Preparation of biodegradable scaffold for dental and medical tissue engineering". Doctor Thesis, Cairo University, Egypt (2007).

19. Ebrahimi M., *et al*. "The effects of three remineralizing agents on regression of white spot lesions in children: A two-week, single-blind, randomized clinical trial". *Journal of Clinical Experimental Dentistry* 9.5 (2017): 641-648.

20. Cardoso CA., *et al*. "Effect of calcium-phosphate nanoparticles' pastes on dentin erosion/abrasion in vitro". *Journal of Dental Research* 90 (2011).

21. Philips LB. "Phosphorus Primer for Maine's lakes". *Honors Thesis, Colby College, USA* (2008).

22. Kumar M., *et al*. "Sterilisation of extracted human teeth for educational use". *Indian Journal of Medical Microbiology* 23.4 (2005): 256-258.

23. Secilmis A., *et al*. "Effects of storage solutions on mineral contents of dentin". *Journal of Dental Sciences* 6.4 (2011): 189-194.

24. Zvezdova D. "Synthesis and characterization of chitosan from marine sources in Black Sea". *Annual proceedings, Association for the Advancement of Automotive Medicine* (2010).

25. Lelli M., *et al*. "Remineralization and repair of enamel surface by biomimetic Zn-carbonate hydroxyapatite containing tooth paste: A comparative in vivo study". *Fontiers in Physiology* 5 (2014): 333.

26. Li Z., *et al*. "A mineralogical study in contrasts: Highly mineralized whale rostrum and human enamel". *Scientific Reports* 5 (2015): 16511.

27. Zaichick V and Zaichick S. "The Effect of age and gender on calcium, phosphorus, and calcium-phosphorus ratio in the crowns of permanent teeth". *EC Dental Science* 5.2 (2016): 1030-1046.

28. Ways MM., *et al*. "Chitosan and its derivatives for application in mucoadhesive drug delivery systems". *Polymers* 10.267 (2018): 1-37.

29. Tsuzuki DS. "Factors Controlling the Diffusion of Ions in Ionic Liquids". Chemphyschem 13.7 (2012): 1664-1670.

30. Miranda CB. *et al*. "Evaluation of the bleached human enamel by scanning electron microscopy". *Journal of Applied Oral Science* 13.2 (2005): 204-211.

31. Walsh LJ. "Contemporary technologies for remineralization therapies: A review". *International Dentistry* SA 11.6 (2009): 6-16.

32. Freier T., *et al*. "Controlling cell adhesion and degradation of chitosan films by N-acetylation". *Biomaterials* 26.29 (2005): 5872-5878.

33. Bergem M. "The effect of brushing, acid etching and fluoride dentifrice on the surface of human enamel". *Master Thesis, University of Oslo, Norway* (2014).

Volume 2 Issue 6 June 2018