Al-Azhar Journal of Dentistry

Manuscript 1629

Oral Medicine and Surgical Sciences Issue (Oral Medicine, Oral and Maxillofacial Surgery, Oral Pathology, Oral Biology)

Biomimetic Dentin Remineralization via Chitosan Hydrogel Versus Recaldent Paste. (An in Vitro Study)

Rana Helmy Abd el-baky

Heba Ahmed Adawy

Nehad Abdel Mawgoud Ahmed

ORIGINAL STUDY

Biomimetic Dentin Remineralization Via Chitosan Hydrogel Versus Recaldent Paste: An In Vitro Study

Rana H. Abd El-Baky [a,*], Heba A. Adawy [b], Nehad A.M. Ahmed [b]

[a] Department of Oral Biology, Faculty of Oral and Dental Medicine, Nahda University, Beni-Suef, Egypt
[b] Department of Oral and Dental Biology, Faculty of Dental Medicine for Girls, Al-Azhar University, Cairo, Egypt

Abstract

Purpose: This study involved treating demineralized dentin samples with either chitosan or Recaldent (GC MI Paste Plus) and then analyzing the degree of re-mineralization that occurred on the treated surfaces. **Patients and methods:** Eighty maxillary premolars were cut occlusally to expose the dentin surface, then classified into four equal groups (20 each): Negative control group (GI) the dentin was sound, demineralized group (GII) dentin surface was demineralized by acid etching, (GIII) dentin surface was re-mineralized by GC MI paste plus, and (GIV) dentin surface was re-mineralized by chitosan hydrogel. All groups were reserved in artificial saliva until examination at two different time intervals. Before the examination time, the prepared specimens were cut longitudinally. After 10 days, five specimens from each group were evaluated using a scanning electron microscope with energy-dispersive radiography spectroscopy. Fourier transforms Infrared spectroscopy examined the other five specimens from each group. These methods were repeated after 20 days. **Results:** The control group (GI) showed a smooth, regular dentin surface. GII (demineralized group) showed roughness, a non-homogenous dentin surface with complete surface irregularities. GIII (recaldent-treated) teeth showed average re-mineralization process features. GIV (chitosan-treated) teeth showed completely occluded dentinal orifices with overall masking of the surface with remineralizing material deposition. **Statistical results:** The statistical difference among the groups was significant. **Conclusion:** Chitosan is more effective as a re-mineralizing agent than GC MI paste plus.

Keywords: Biomimetic, Chitosan hydrogel, Demineralization, Recaldent, Remineralization

1. Introduction

Dentin is a complex tissue, the high percentage of type I collagen in dentin gives it its characteristic toughness and resilience, while hydroxyapatite (HAP) provides strength and hardness. The presence of water in dentine also plays a role in its mechanical properties and the diffusion of ions and other small molecules within the tissue [1]. Overall, the complex structure of dentin allows it to withstand the stresses of chewing and other mechanical forces while protecting the sensitive pulp inside the tooth [2].

Dentinal tubules are microscopic channels that run from the pulp to the tooth's outer surface. They contain processes of odontoblast cells, which are responsible for dentin formation [3]. The peritubular dentin is the mineralized tissue that immediately surrounds the dentinal tubules, and it is highly mineralized and more resistant to decay than the surrounding intertubular dentin. The intertubular dentin makes up the bulk of the dentin matrix between the tubules. The overall structure of dentin, with its tubules and surrounding mineralized and less mineralized tissues, provides a balance of strength and flexibility that allows the tooth to withstand the stresses of chewing and other forces [4].

The outermost zone of dentin in the crown, known as mantle dentin, has a different orientation of collagen fibers compared with the rest of the dentin [5]. In the root, the outermost peripheral zone of dentin has a granular layer with fewer minerals, followed by a hyaline layer that lacks tubules and helps to create a strong bond between the cementum and dentin [6]. Finally, the bulk of the dentin in both the crown and root comprises circumpulpal dentin, which includes intertubular and peritubular dentin [7].

Received 6 June 2023; accepted 29 October 2023.
Available online 16 September 2024

* Corresponding author. Department of Oral Biology, Faculty of Oral and Dental Medicine, Nahda University, Beni-Suef, 11728, Egypt.
E-mail address: drrnonhelmy@gmail.com (R.H. Abd El-Baky).

https://doi.org/10.58675/2974-4164.1629

As dentin is formed, the newly deposited dentin layer is initially unmineralized and referred to as predentin. Over time, the predentin becomes mineralized as more HAP is deposited [8], and it gradually matures into primary dentin, which is the dentin that existed before the tooth started to function. The development of secondary dentin then begins and will last the entire life of the tooth [5].

Dentin sensitivity is a common problem noticed in clinical practice. When the tooth's protective enamel layer is lost, or the underlying dentin becomes demineralized. This can expose the dentinal tubules, which are sensitive structures that can transmit sensations of pain or discomfort in response to various stimuli, such as cold, heat, sweetness, or acidity [9]. Dentin sensitivity can result from several causes, including tooth decay, gum disease, tooth wear, acidic drinks or foods, and aggressive brushing or tooth whitening. The prevalence of dentin sensitivity can vary widely, depending on the study's population and the criteria used for diagnosis, but it is generally estimated to affect between 8 and 57% of the population [3]. Treatment options for dentin sensitivity include desensitizing toothpaste, fluoride varnish, dental bonding, or restorative procedures, depending on the severity and underlying cause of the sensitivity.

Some of the pulp-damaging factors are attrition, erosion, abrasion, tooth decay, recession, and mechanical, chemical, and thermal variables [10]. Organic acids are a byproduct of bacteria that produce acids as they digest fermentable carbohydrates. A carious lesion occurs when these acids dissolve HAP [11]. Continuing in this manner can lead to the formation of a subsurface lesion with partial demineralization. However, if the demineralization is caught early before cavitation occurs, the lesion could be reversed and remineralized under proper conditions.

Reversal and remineralization of subsurface lesions can occur when the pH of the oral environment is maintained at a neutral or slightly alkaline level, and when there is an adequate supply of calcium, phosphate, and fluoride ions available to promote remineralization. Therefore, saliva is necessary for tooth remineralization because it provides the ions required to reassemble HAP blocks [12].

In a natural oral environment, the remineralization of dental tissues is the natural repair process that takes place at the tooth/saliva interface. However, natural remineralization can only overcome initial caries or mild demineralization of dentin, and it may not be sufficient to reverse or stop the caries process in more severe cases. In these situations, other interventions such as restorations or preventive measures may be necessary to prevent further damage to the tooth [13]. However, there is ongoing research into biomimetic remineralization techniques that aim to restore minerals to demineralized dentin using materials that mimic the natural mineral components of dentin. These materials may include solutions containing calcium, phosphate, fluoride, silica ions, or ultrafine bioactive glass particles that can release these ions over time [14]. These approaches hold promise for the development of new therapies that can promote the natural repair and regeneration of damaged dentin tissues.

2. Patients and methods

2.1. Teeth selection

Freshly extracted human maxillary premolars from orthodontic patients between the ages of 18 and 30 were used in this study. The teeth were carefully selected for the study based on certain criteria, such as being free of deformities, cracks, caries, attrition or erosion, and malformation. The study was carried out according to the recommendations of the Faculty of Dental Medicine for Girls at Al-Azhar University's Research Ethics Committee (REC-BI-23-01) Table 1.

Table 1. Showed the material used in the study.

Material	Brand name	Composition	Company
Artificial saliva	Artificial saliva	sodium dihydrogen phosphate 0.78 gm/lit, potassium chloride 0.4 gm/lit, calcium chloride 0.8 gm/lit, sodium sulfide 0.005 gm/lit, and urea 1 gm/lit are the various salt concentrations.	Nanogate company
37% phosphoric acid etched	Power etching	100 g of 37% phosphoric acid in aqueous solution form was formulated, into which 0.2 g of 1% methyl blue aqueous solution was added, and then 0.01 g of spearmint flavor was added.	Dentacart.com from USA, Dental store
Recaldent	MI Past plus	A mixture of amorphous calcium phosphate (ACP) and casein phosphopeptide (CPP).	Dentacart.com from USA, Dental store
Chitosan powder		Decetylated chitin, poly (D-glucosamine).	Loba-Chemie
Chitosan hydrogel		Chitosan powder was dissolved in 25 ml of acetic acid solution, then stirred at 80 °C for an overnight period.	Nanogate company

2.2. Teeth preparation

The selected teeth were immersed using a 10% sodium hypochlorite (NaOCl) solution to remove any organic material and debris from surfaces. After that, they were cleaned using water and a gentle brush. Finally, saline (0.9% NaCl solution) for storage of the teeth until they were needed to keep them hydrated and prevent them from drying out or becoming damaged.

Fig. 1. Shows the transverse section cutting method of the tooth.

2.3. Cross-sectioning of teeth

The occlusal enamel of all premolars was removed to expose the dentin surface by Isomet 4000 saw (Buehler UAS) under water-cooling with a 0.3 mm thick diamond disc as shown in Fig. 1, followed by ultrasonic bathing for 10 min to remove any debris or smear layer formed.

2.4. Specimens' grouping

A total of 80 prepared specimens were randomly assigned to four equal groups (20 each) as follows:

Group I — **Negative control** group: The specimens were immersed in saliva without any de-mineralizing or re-mineralizing agent till examination.

Group II — **Positive control** group: The specimens were demineralized for 30 seconds by 37% phosphoric acid from the exposed surface. The specimens were then submerged in an artificial saliva solution until evaluation.

Group III — The exposed surface was demineralized for 30 seconds using 37% phosphoric acid, followed by a wash with distilled water and drying. after which MI paste plus was applied to the etched surface, and it was left to dry for two hours at room temperature. The specimens were then submerged in an artificial saliva solution until evaluation.

Group IV — The exposed surface was demineralized with 37% phosphoric acid for 30 seconds, followed by washing and drying. After coating the etched surface with chitosan hydrogel, the hydrogel was applied with a bond brush and left to dry for two hours at room temperature, then submerged until evaluation in an artificial saliva solution.

All groups' application materials and solutions were refreshed every 2 days during incubation (10 and 20-day intervals) [15,16].

2.5. Longitudinal sectioning of the specimens

All specimens were split longitudinally after the incubation periods and before the examination, by cutting the tooth longitudinally by separating the prepared tooth mesiodistally by Isomet 4000 saw Buehler UAS with a 0.3 mm thick diamond disc under water-cooling as shown in Fig. 2, followed by ultrasonic bathing for 10 min to remove any debris or smear layer formed. The purpose is to examine the dentinal tubules horizontally and show the penetration of material into them using a scanning electron microscope (SEM).

2.6. Assessment methods

All specimens in each group were evaluated in 10 and 20-day intervals. The following procedure involved examining 10 prepared exposed dentin premolars from each group at each interval.

2.7. SEM-EDX test

Using a SEM, five specimens from each group were examined and characterized in transverse (T.S.) and longitudinal sections (L.S.). The SEM holder was prepared for imaging by coating it with double-sided carbon tape, with one side attached to the holder and the other side attached to the sample, as shown in Fig. 3. Then images were taken at 500x, 1000x, 2000x, and 3000x magnification to obtain an

Fig. 2. Photographs showing the prepared tooth's longitudinal section cutting method.

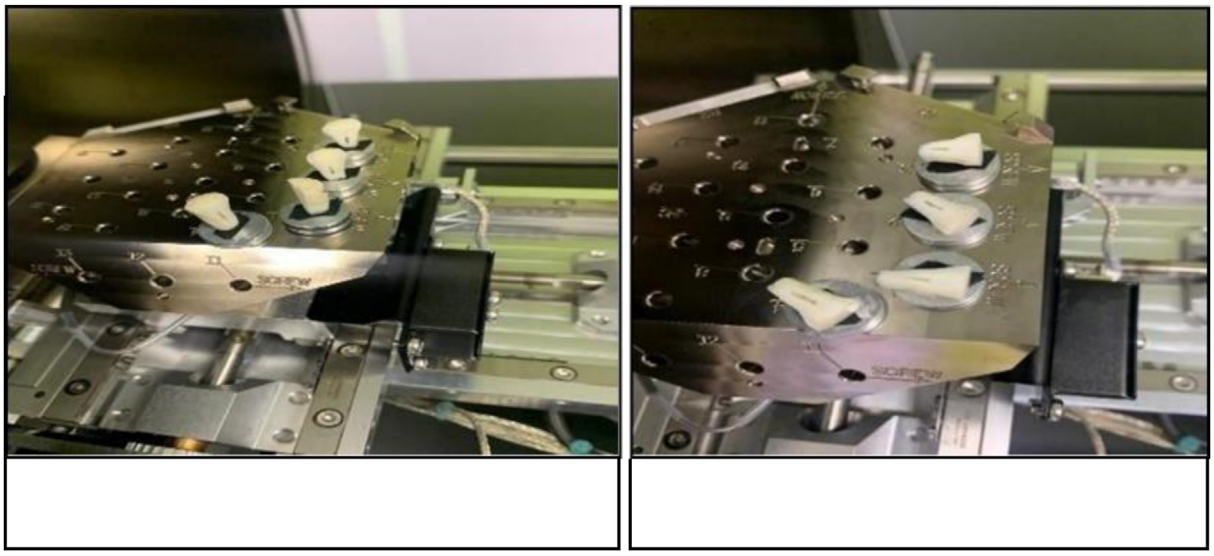

Fig. 3. Shows the longitudinal sections and transverse sections. Scanning electron microscope technique test.

overview of the general surface topography. With three analysis readings for each sample, energy-dispersive radiography (EDX) spectroscopy was coupled to SEM. After running the EDX program, the results were presented in the form of graphs and a table of the elemental percentage for assessment of mineral content before demineralization (group I), assessment of mineral content after demineralization (group II), and evaluation of re-mineralization (groups III and IV).

2.8. FTIR test

Five specimens from each group were examined by Fourier-transform infrared spectroscopy (FTIR), for chemical composition, providing structural information and an overview of functional groups by scraping the outer layer of prepared exposed dentin with a sharp excavator to be exposed to FTIR as a certain amount of powder was taken and evaluated. The powder used is 0.02 gm (90% kbr, 10% material).

3. Results

3.1. Scanning electron microscope (SEM)

Each dentin surface was scanned to obtain an overview of the surface, for evaluation of the morphological characteristics at different magnifications (500x, 1000x, 2000x, 3000x).

3.2. SEM results at the 10-day interval

3.2.1. SEM of GI (sound exposed dentin surface)

SEM examination of this group acts as a reference. At different magnifications: A T.S. (SEM) micrograph of the dentin surface at 3000X magnifications. Figure 4a, the surface of the dentin was smooth and regular, and an intact peritubular dentin layer appears as radiopaque areas that surround the orifice of dentinal tubules (black arrows), the orifices had a normal diameter (black circle), so the distance between them (intertubular dentin) was wide and more obvious (blue arrows).

The L.S. (SEM) micrograph of this group at 3000X magnifications. Figure 4b showed the slender shape of dentinal tubules, smooth surface dentin without irregularities (blue arrows), and dentinal tubes appearing in a normal size (red circle).

3.2.2. SEM of GII (demineralized dentin surface)

In the T.S. (SEM) micrograph at 3000X magnifications. Figure 4c, the surface of dentin was rough and showed complete surface irregularities with a severe non-homogenous surface than (group I), dentinal tubules orifices became wider (black circles), so the peritubular dentin layer relatively decreased and intertubular dentin decreased between orifices (blue arrows).

The L.S. (SEM) micrograph of this group at 3000X magnifications. Figure 4d, the (Blue arrows) showed severe surface roughness after acid etching application and showed widening of dentinal tubes (red circles).

3.2.3. SEM of GIII (MI paste plus)

The T.S. (SEM) micrograph of recaldent treated dentin surface as remineralizing agent (group III) at 3000X magnifications. Figure 4e represents the partially occluded dentinal tubules (red arrows), and (white arrows) showed the mineralized material deposited on the surface.

The L.S. (SEM) micrograph of (group III) at 3000X magnifications. Figure 4f, the (Blue arrows) showed slight material deposition on the dentin surface, and the (black circle) showed the extension of deposited MI material inside dentinal tubes.

3.2.4. SEM of GIV (chitosan group)

The T.S. (SEM) micrograph of (group IV) at 3000X magnifications. Figure 4g showed multiple opaque areas appearing around the dentinal tubules' orifices (peritubular dentin) (Black arrows), the (red square) showed partially occluded dentinal tubules and narrowing of dentinal tubules orifices and some other orifices were completely occluded by the remineralization process (black circles) and (blue arrows) showed the remineralized material molecules of chitosan hydrogel.

While the L.S. (SEM) micrograph of this group is at 3000X magnifications. Figure 4h, the (blue arrows) show the coated layer of minerals deposited on the dentin surface (blue arrows), and (the red circle) showed the extension of material inside tubules that occluded the dentinal tubes.

3.3. SEM results at the 20-day interval

3.3.1. SEM of GI (sound exposed dentin surface)

SEM examination of this group (G I) At 3000X magnification, a T.S. (SEM). Figure 5a showed a thicker intact peritubular dentin layer than in 10-day intervals (black arrows), in which more radiopaque areas surround the orifice of dentinal tubules (black circle) that appear orifices in normal size, and some become narrower (red circle).

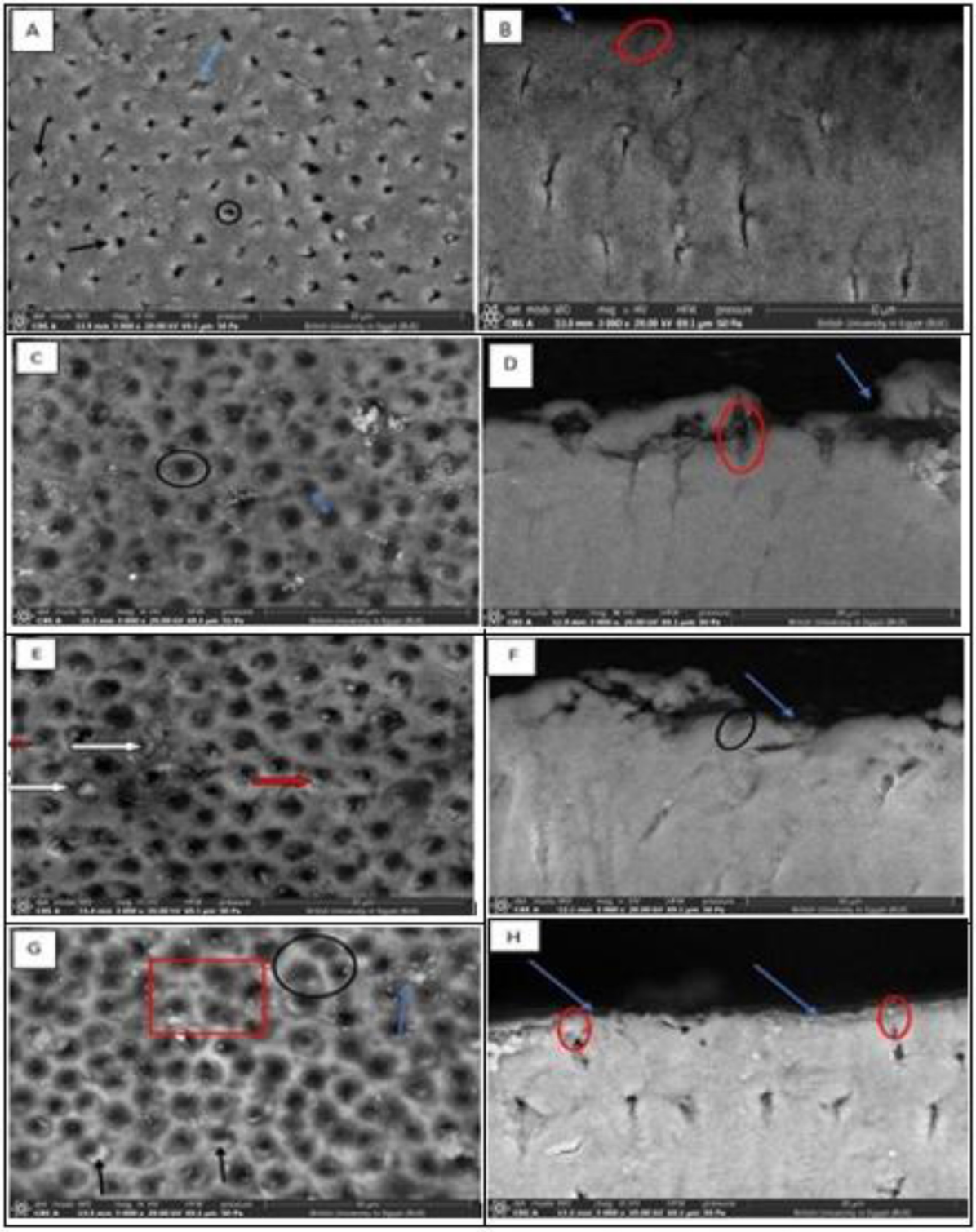

Fig. 4. Transverse sections. and longitudinal sections scanning electron microscope microphotographs for all groups at 3000x, at 10-day intervals showing; A) transverse sections of GI, B) longitudinal sections of GI, C) transverse sections of GII, D) longitudinal sections of GII, E) transverse sections of GIII, F) longitudinal sections of GIII, G) transverse sections. of GIV, and H) longitudinal sections of GIV.

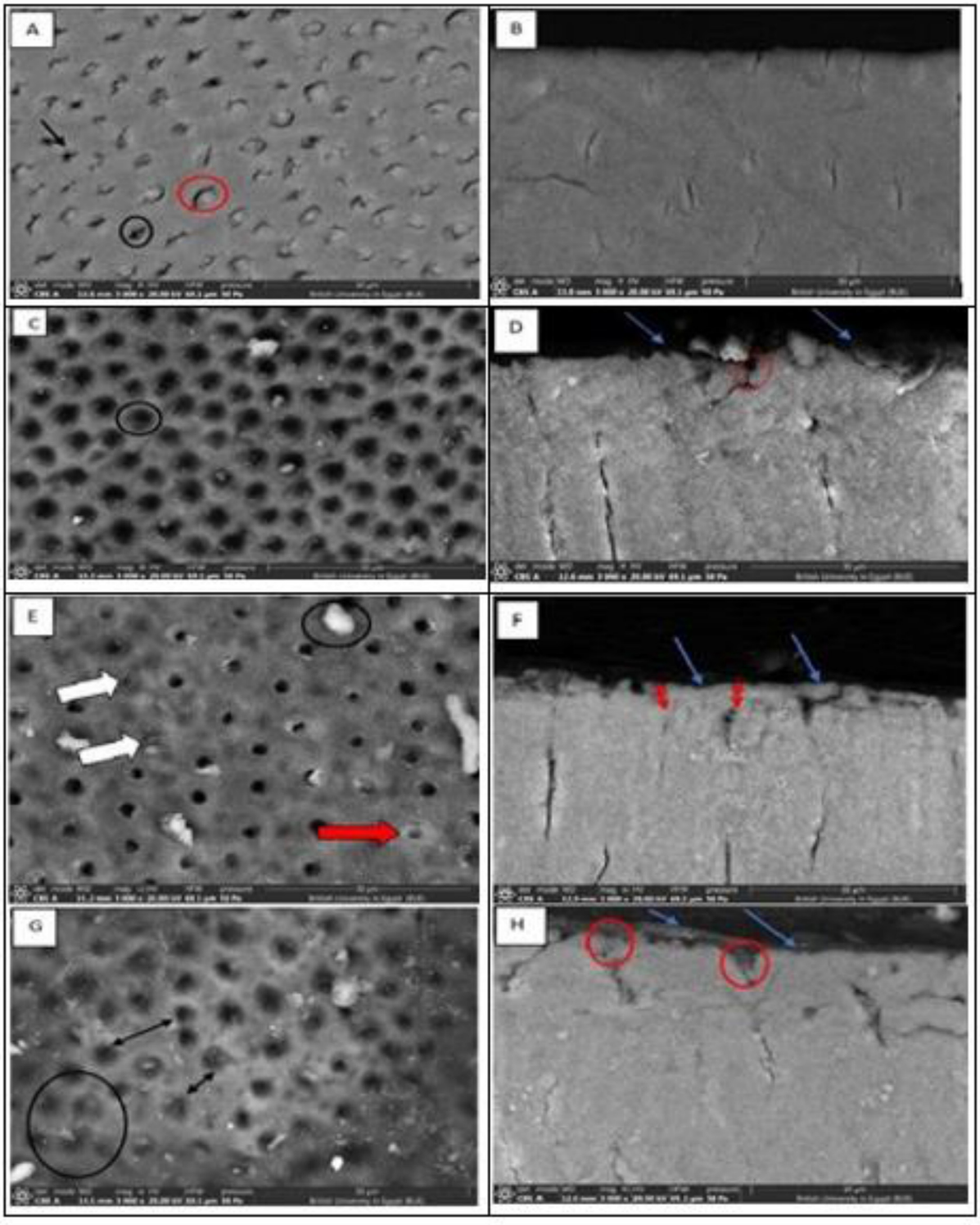

Fig. 5. Transverse sections and longitudinal sections scanning electron microscope microphotographs for all groups at 3000x, at 20-day intervals showing; A) Transverse sections of GI, B) longitudinal sections of GI, C) Transverse sections of GII, D) longitudinal sections of GII, E) Transverse sections of GIII, F) longitudinal sections. of GIII, G) Transverse sections of GIV, and H) longitudinal sections of GIV.

While an L.S. (SEM). Figure 5b showed the same results as the L.S. at 10-day intervals.

3.3.2. SEM of GII (demineralized dentin surface)

Scanning of this group at 3000X magnifications. Figure 5c, the T.S. (SEM) showed the dentin surface was rough with some smooth areas than 10-day intervals, (black circles) showed widening of dentinal tubules orifices.

The L.S. (SEM) micrograph of (group II) at 3000X magnifications. Figure 5d, the (blue arrows) showed a discontinued surface with roughness and irregularities after acid etching application but less than 10-day intervals, widening of dentinal tubules (red circles).

3.3.3. SEM of GIII (MI paste plus)

The scanning of GIII (MI paste plus), with 3000X magnifications, T.S. (SEM) micrograph. Figure 5e, the (white arrows) represented completely occluded dentinal tubules orifices than in 10-day intervals, and the (red arrows) showed partially occluded dentinal tubules and narrowing of them by deposition of peritubular dentin, some large, deposited material molecules appear on the surface (black circles). The L.S. (SEM) micrograph of (group III) at 3000X magnification, (blue arrows) showed a smoother dentin surface than the 10-day interval. Figure 5f, the (red arrows) show the extension of remineralized material inside the dentinal tubes.

3.3.4. SEM of GIV (chitosan group)

The SEM of this group at 3000X magnifications. The T.S. (SEM) micrograph. Figure 5g, the (black circles) showed multiple completely occluded dentinal orifices with other partially blocked orifices and increased intertubular dentin distances between the orifices (black arrows). The image showed the overall covering or masking of the surface with remineralizing material deposition.

The L.S. (SEM) micrograph at 3000X magnification. Figure 5h showed a thick coated layer of remineralized material deposition of chitosan gel on the dentin surface (blue arrows), and (red circles) showed an extension of material inside dentinal tubules that blocked the tubules.

3.4. Energy dispersive analysis radiography (EDX) results

A one-way analysis of variance test was used to analyze the calcium and phosphate levels, where HAP minerals were expected to form after remineralization.

Table 2. Shows the statistical result of the analysis of variance test for the Ca/P ratio of the different groups at different two-time intervals.

Groups	Mean	SD	*P* value
10-day			
GI	2.499	0.057	0.00001*
GII	2.179	0.079	
GIII	2.277	0.0459	
GIV	2.389	0.0442	
20-day			
GI	2.568	0.0278	0.00001*
GII	2.279	0.0327	
GIII	2.401	0.0276	
GIV	2.471	0.0294	

**P* less than 0.05.

Table 3. Shows the pair-wise comparison.

Groups	*P* value
10-day	
GI	
GII	<0.00001*
GII	
GIII	<0.000245*
GIV	<0.00001*
20-day	
GI	
GII	<0.00001*
GII	
GIII	<0.00001*
GIV	<0.00001*

**P* less than 0.05.

Within two intervals time (10, 20-days) intervals, Table 2 showed a statistically significant higher mean Ca/P ratio in G I, followed by G IV as compared with G II, and GIII ($P < 0.05$). Table 3 revealed a statistically significant difference between GI and GII; the difference between GII, GIII, GII, and GIV was statistically significant.

Figure 6 showed that chitosan came in second place in the mean of the Ca/P ratio and showed superiority over MI paste.

3.5. FTIR results

PO_4^{-3} functional groups represent the amount of minerals precipitated at different tested groups captured at wave numbers. (570, 608,1051) as shown in Fig. 7.

4. Discussion

This study evaluated and compared the remineralizing effect of chitosan hydrogel with a high

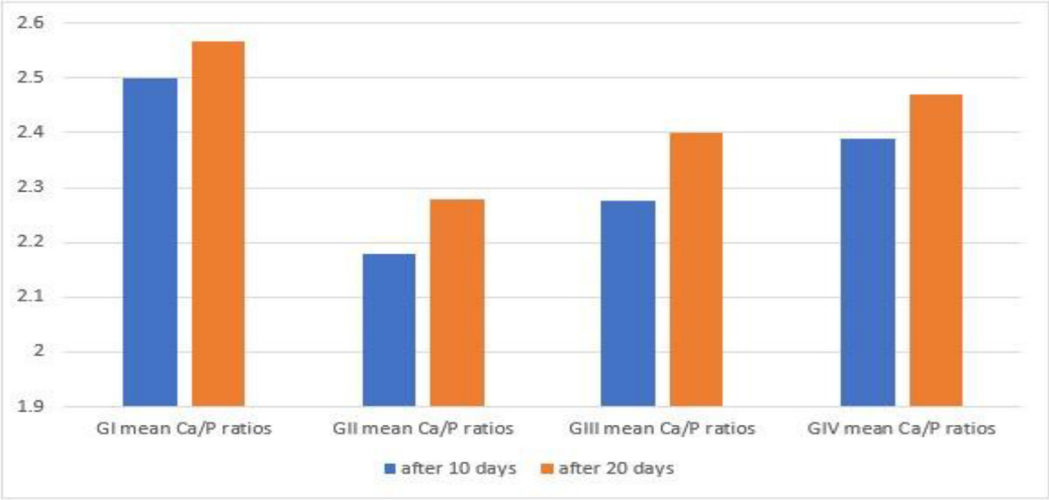

Fig. 6. A bar chart showing a comparison between all groups in two-interval time groups.

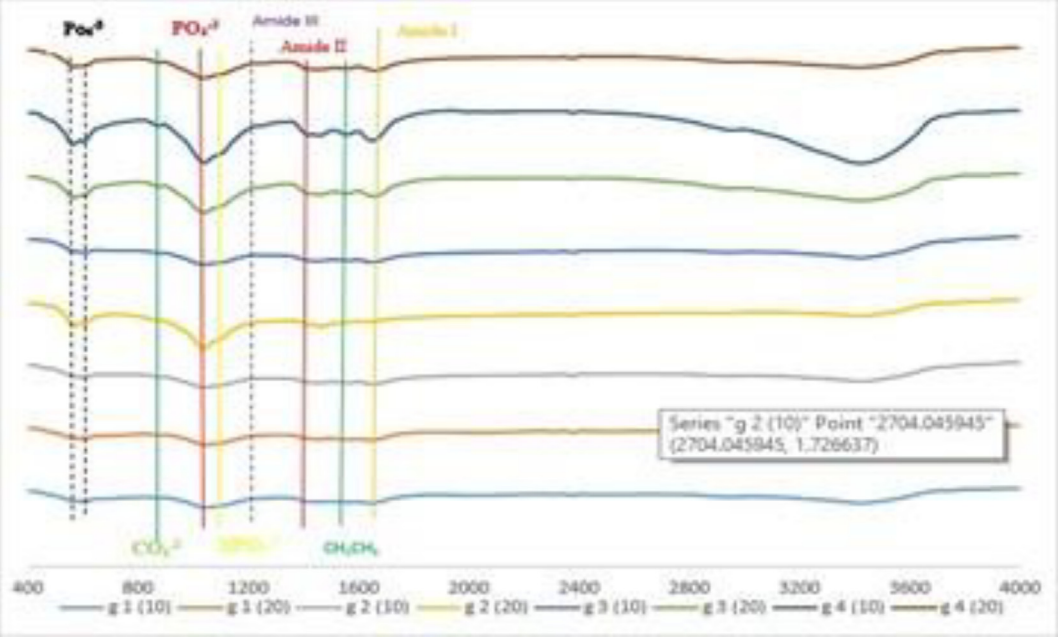

Fig. 7. Showed characteristic Fourier-transform infrared spectroscopy spectra for GI, GII, GIII, and GIV.

percentage of calcium and MI paste plus, its active ingredient CPP-ACPF on the demineralized dentin surface.

The selection of dentin tissue for this study because dentin remineralization is more difficult to achieve compared with enamel remineralization, both in a clinical setup and in the laboratory [17]. This is because dentin is a more complex and heterogeneous tissue, with a higher organic content and a more porous structure than enamel. As a result, there are fewer studies on dentin remineralization compared with enamel remineralization.

Fluoride-based and nonfluoride-based remineralization systems are two major categories that can be used to categorize remineralization systems [18]. Therefore, the selection of chitosan material as a nonfluoride-based natural product for the dentin biomimetic remineralization system as in group IV and compared with recaldent as a fluoride-based material as in group III.

The teeth used in this study were collected from orthodontic clinics, eighty maxillary premolars of patients with ages ranging from 18 to 30 years old, as chosen according to a previous study [19], which

confirmed that this age is the most interested in starting orthodontic treatment if needed. While the type of tooth extracted during orthodontic treatment depends on each case, the most relevant extracted is the first maxillary premolar.

All collected teeth will be cross-cut occlusally to expose the dentin surface and apply the selected material easily. A recent study used the same cutting technique to evaluate the effectiveness of different remineralization agents on primary teeth early smooth surface caries [20]. Therefore, a cross-section is the best way to place materials on the surface of the dentin, and expose the dentinal tubules orifices, to follow-up on the results.

After cutting the teeth, they were divided into four equal groups, group I was the control group which was immersed in saliva without any de-mineralizing or re-mineralizing agent till examination. Sound premolars showed smooth surfaces with normal dentinal tubules orifice, there were no surface irregularities. This group compared the demineralized results to confirm the process of demineralization and compare it with the results of the remineralization process.

The remaining 60 premolars were demineralized by 37% phosphoric acid from the exposed surface to produce artificial carious lesions. According to a recent study [3], the brushing action of the acid, which was used to demineralize dentin and expose a collagen matrix, would reactivate its impact, push it into the tubules to widen them even more, and demineralize the peritubular dentin (bigger diameter).

A study [21] assumed that it is better to etch dentin with phosphoric acid at concentrations of 37% since lower quantities produce dicalcium phosphate dihydrate, which sticks to the dental surface even after rinsing. While higher phosphoric acid concentrations may cause the early formation of monocalcium phosphate monohydrate, which is easier to remove by rinsing because of its altered solubility in water, some residue may still be present on the dentin surface.

Group II was kept in artificial saliva to mimic the natural remineralization of the oral cavity till examination without any re-mineralizing agent and it is considered a positive group, this group compared with the results of the remineralization process.

The remaining demineralized teeth were experimental specimens divided into two groups; group III where this group of demineralized dentin was treated with MI paste plus with the active ingredient (recaldent) acting as a re-mineralizing agent, and group IV where this group of demineralized dentin was treated using chitosan hydrogel as a re-mineralizing agent.

To mimic the conditions of the oral cavity, all specimens were preserved in artificial saliva. According to a study [22], artificial saliva performs better in vitro storage studies than deionized water. It could act as a chemical storage facility for calcium and phosphate ions, accelerating the remineralization procedure. To maintain PH and maintain ionic equilibrium during incubation, artificial saliva media was changed every 48 h [23].

All groups were cut longitudinally after the end of the specified incubation period and before the examination process to image the dentinal tubules horizontally and the extension of re-mineralizing material into them by SEM. According to a previous study [24], the authors sectioned the tooth perpendicular to its long axis at 4 and 6 mm from the anatomic apex to obtain one apical specimen from each root. Each specimen was examined under low magnification to obtain an overall view, and an area with a maximum density of sealer penetration was selected.

The SEM of group I (control group), showed a smooth surface with no irregularities from the top view and a normal diameter of dentinal tubules from the side view. These results for the two-time intervals are shown in Figs. 4 and 5a, b with no demineralization effects and EDX results confirmed the SEM results.

The Ca/P mean value equals 2.49 after 10 days of immersion which represent the baseline ratio before the beginning of the procedure, while the ratio reported 2.56 after 20 days showed increasing in Ca and P minerals which were gained from artificial saliva over time.

In group II (demineralized group), the top view scanning pictures at 10-day intervals showed irregularities, and a nonhomogenous surface than group I, which revealed widely opened dentinal tubules after acid etching application creating a spongy appearance as shown in Fig. 4c. The intertubular dentin distance decreased between orifices and some regions of peritubular dentin were entirely demineralized while other areas were partially demineralized.

From the side view, Fig. 4d showed surface roughness due to the loss of minerals after acid etching application and the widening of dentinal tubules' canals. The EDX results confirmed the SEM results and showed a decrease in the Ca/P ratio in group I, which dropped from 2.49 to 2.17. This drop confirmed the irregular surface because of the loss of minerals.

This group was preserved in artificial saliva that acts as natural remineralization. Therefore, by time, the SEM pictures at 20-day intervals, Fig. 5c showed some filling of some irregularities of a demineralized surface by inorganic components, some radiopaque areas of peritubular dentin appeared because of gaining some minerals from artificial saliva media, and variously shaped opening dentinal tubules, including some with circular forms and others with oval shapes and tiny aggregates inside the dentinal tubule. The EDX results reported an increased Ca/P ratio over the 10-day interval from 2.17 to 2.27, but less than group I at the 20-day interval.

Although saliva has some remineralization potential, it is insufficient on its own to raise the level of calcium and phosphate needed for effective remineralization [25]. The remineralization process involves forming an acid-resistant, hyper-mineralized layer on the surface of the tooth, which serves as a nucleus for further mineral deposition [26]. Typically, this layer is composed of a fluorapatite-like mineral, formed through seeding calcium, and phosphate ions from saliva and another source. However, sufficient calcium and phosphate ions must be present for the surface layer to be penetrated and for mineral deposition to be promoted within the lesion's body for remineralization to occur [27].

Previous studies confirmed the remineralization ability of artificial saliva by deposition of some minerals at the affected dentin surface, but they didn't regain the normal composition of dentin [25–27].

In a study [28], dentin samples from extracted, caries-free human third molars were demineralized using a 0.5 M (EDTA) solution. SEM revealed a few tiny minerals deposited in the dentinal tubules for the demineralized control group, all treated dentin samples were kept in an artificial saliva solution with a pH of 7.0. These results were consistent with our investigation.

Group III (MI paste) was treated using the GC MI paste plus after demineralization with the active ingredient 'Recaldent'. Top-view SEM, Fig. 4e at 10-day intervals showed the partially occluded dentinal tubules due to the deposition of inorganic material. The ability of MI paste plus to release calcium, phosphate, and fluoride that is bioavailable to the tooth surface showed layers of granular deposits that were like cobblestones appearance, and a few peritubular dentin layers began to appear around dentinal tubules orifices as an opaque area. While the side-view scanning, Fig. 4f showed slight material extension inside dentinal tubules, some irregularities, and un-remineralized areas of the dentin surface.

The main mineral needed for remineralization is HAP. The amount of HAP in the tooth structure increases during the remineralization process, making the calcium-to-phosphorus ratio an important indicator of the process' effectiveness. A higher ratio is typically linked to better mineralization and increased resistance to decay [29].

Elemental analysis of group III treated specimens' surfaces showed that the Ca/P ratio is 2.277 which increased from 2.17 (G II), so there was a statistically significant difference ($P < 0.05$). The EDX results after a 20-day interval, the Ca/P ratio increased from 2.27 to 2.4, that difference in means of the Ca/P ratio indicates more minerals regain over time. These results confirmed the results of SEM images of the top-view, Fig. 5e which showed a more homogeneous dentin surface which explains the more deposition of inorganic material by time and increased remineralization process than the 10-day interval and noted narrowing of dentinal tubules orifices as the deposited material distributes around the orifices to regain the peritubular layers so the distance between orifices increased (intertubular dentin). The side-view, Fig. 5f proved a smoother dentin surface than the 10-day interval and more extension of material inside dentinal tubules.

A previous study [30] compared Clinpro White Varnish, Duraphat Varnish, and Colgate Sensitive Pro-Relief dentifrice to assess the effectiveness of MI Paste Plus as a remineralizing agent on dentinal permeability. SEM analysis of acid-etched dentin samples treated with GC Tooth Plus revealed layers of granular deposits with a cobblestone appearance. Only a few orifices of dentinal tubules were visible, and the surface looked to be uniformly coated, occluding the dentinal tubular orifice. These results support our findings.

In contrast to our findings, a study [31] compared GC MI paste plus to NaF and Clinpro tooth crème and found that it had a weaker ability to occlude dentinal tubules.

Several studies evaluated MI Paste Plus as a remineralizing agent and compared it with other agents, the results showed the ability of MI Paste to remineralize the demineralized dentin surface [30,31].

Remineralization using MI paste or pastes containing HAP can deposit HAP on the surface and interior of certain tubules, both between and on the collagen bundles. However, the HAP that was deposited failed to integrate with the dentin's structure but only slightly increased hardness [32].

Group IV (Chitosan group) was treated using a chitosan hydrogel after demineralization. The top-view SEM, Fig. 4g at 10-day intervals showed multiple opaque areas appeared around dentinal tubules (peritubular dentin) due to the more deposition of minerals, leading to narrowing of dentinal tubules and showing partially occluded dentinal tubules while other orifices are completely occluded by chitosan hydrogel material, While the side-view scanning, Fig. 4h showed a coated layer of minerals deposition on the dentin surface and show the extension of material inside tubules and filling them.

EDX values of the Ca/P ratio indicate a highly statistically significant difference between the demineralized group (group II) and the chitosan-treated group (group IV). The EDX values of the Ca/P ratio of this group at a 20-day interval increased from 2.38 to 2.47. When comparing the ratio of this group with the Ca/P ratio of G III (MI Paste) in the two-time intervals, Table 2 showed that chitosan has a great ability to remineralize the demineralized dentin. The elemental analysis of this group's samples showed and confirmed the maximum value of the Ca/P ratio between the four groups. Table 3 represents the mean Ca/P and standard deviation values with highly statistically significant differences between all the groups.

After 20-days of immersion in artificial saliva, the top-view SEM, Fig. 5g showed masking of the dentin surface with chitosan hydrogel deposited material, showed multiple completely occluded dentinal orifices with other partially blocked, with increasing intertubular dentin distances between orifices. While the side-view scanning, Fig. 5h showed a thick coated layer of remineralized material deposition on the dentin surface and showed the extension of material inside dentinal tubules by filling them, this group showed the maximal remineralization among the three groups.

In this case, chitosan molecules acting as a chelating agent served as a scaffold for the formation of a complex composite calcium phosphate-based layer onto the surface of the demineralized dentine. Compared with the peritubular dentin area of the demineralized sample, the surface of this chitosan-remineralized dentine sample shows a shrinking of tubule diameter promoted by the development of the new composite layer [33].

In a previous study [34] the use of phosphorylated chitosan for biomimetic surface remineralization of partially demineralized dentin was examined. According to these findings, phosphorylated chitosan significantly increased the amount of calcium and phosphate ions that were deposited on the partially demineralized dentin's surface. This promoted the growth of crystals and the creation of a layer of HAP, a substance that is an essential part of both dental enamel and dentin, which confirms our study.

Moreover, another research [35] using carboxymethyl chitosan for dentin remineralization found that carboxymethyl chitosan/amorphous calcium phosphate greatly accelerated the remineralization process and strengthened dentin.

On the other hand, the demineralized dentin surface receives calcium and phosphate ions through the gel diffusion method, such as chitosan hydrogel, which can produce HAP. Due to the affinity of calcium ions to the dentin phosphoproteins, it has been discovered that the gel diffusion approach preferentially deposits HAP on the collagen bundles rather than on the spaces between them where it already exists. The development of HAP on the collagen bundles could facilitate the incorporation of the deposited HAP into the dentin structure [36].

In summary, the gel diffusion method using chitosan hydrogel appears to be a promising approach for promoting the remineralization of demineralized dentin, as it can deposit HAP on the collagen bundles and potentially integrate the deposited HAP into the structure of dentin. Further studies are needed to optimize the formulation and delivery of chitosan hydrogel and to evaluate its long-term efficacy and safety. So, according to previous studies, it was proved that chitosan is the material of the future. As it increases and enhances the remineralization action of defective dentin lesions.

FTIR results of sound premolars (GI) were used as a reference to monitor the different changes in de and re of dentin. In the demineralization process (GII), decreased the intensity of the band po_4^{-3}. After demineralization of the dentin slices, the peak of PO_4^{-3} was where the P–O bond's antisymmetrical stretching peak could be found. The peak appeared at 1045 cm^{-1} in the 10-day interval and at 1040 cm^{-1} in the 20-day interval, Fig. 6. These peaks indicated that the dentin had been demineralized.

After the remineralization of demineralized dentin samples by recaldent MI Paste and chitosan hydrogel for the two-time intervals, increased the intensity of the po_4^{-3} band, these characteristic absorption peaks of PO_4^{-3}, demonstrating that the dentin's remineralization process was complete.

The roughly increasing peak positions and peak intensities of the infrared spectrum of the essential samples in the natural dentin slices proved that many HAP crystals were deposited on the surface.

Our results agreed with a previous study [37] that used the FTIR test to evaluate the remineralization process of dentin slices using the combination of casein phosphopeptide-amorphous calcium phosphate with sodium tripolyphosphate.

4.1. Conclusion

Overall, chitosan shows promise as a natural alternative to fluoride for remineralizing teeth and regenerating dental tissues. However, more study is necessary to fully understand its possibility and maximize its use in dental applications.

4.2. Recommendations

(a) Further studies should be carried out for a prolonged period to prove these re-mineralizing agents' best results and action.
(b) Additional *in vivo* studies, clinical trials, and research are required to move chitosan-based remineralizing gels from research to clinical usage.

Ethical statement

The study was carried out according to the recommendations of the Faculty of Dental Medicine for Girls at Al-Azhar University's Research Ethics Committee (REC-BI-23-01).

Funding

No funders.

Conflict of interest

There are no conflict of interest.

References

[1] Mathew MG, Soni AJ, Khan MM, Kauser A, Charan VS, Akula SK. Efficacy of remineralizing agents to occlude dentinal tubules in primary teeth subjected to dentin hypersensitivity in vitro: SEM study. Fam Med Prim Care Rev 2020;9:354–8.
[2] Marrelli M, Codispoti B, Shelton RM, Scheven BA, Cooper PR, Tatullo M, et al. Dental pulp stem cell mechanoresponsiveness: effects of mechanical stimuli on dental pulp stem cell behavior. Front Physiol 2018;9:1685.
[3] Kharouf N, Reitzer F, Ashi T, Daras N, Haikel Y, Mancino D. Effectiveness of etching with phosphoric acid when associated with rubbing technique. J Stomatol 2021;74:16–21.
[4] Velasco-Hogan A, Huang W, Serrano C, Kisailus D, Meyers MA. Tooth structure, mechanical properties, and diet specialization of Piranha and Pacu (Serrasalmidae): a comparative study. Acta Biomater 2021;134:531–45.
[5] Fleck C, Burke M, Ganzosch G, Mueller C, Currey JD, Zaslansky P. Breaking crown dentine in whole teeth: 3D observations of prevalent fracture patterns following overload. Bone 2020;132:115178.
[6] Noaman KM, Al-Samoly WM, Al-Hariri AA. Evaluation of the degree of remineralization of subclinical carious lesions using chitosan and conventional remineralizing agents (An in vitro study). EJH 2020;78:182–9.
[7] Weerakoon AT, Cooper C, Meyers IA, Condon N, Sexton C, Thomson D, et al. Does dentine mineral change with anatomical location, microscopic site, and patient age? J Struct Biol X 2022;6:100060–8.
[8] Nakano Y, DenBesten P, Goldberg M. Structure of collagen-derived mineralized tissues (dentin, cementum, and bone) and non-collagenous extra cellular matrix of enamel. ECM Biominera Dent Tissue Struct 2021;10:3–4.
[9] Yucesoy DT, Fong H, Hamann J, Hall E, Dogan S, Sarikaya M. Biomimetic dentin repair: amelogenin-derived peptide guides occlusion and peritubular mineralization of human teeth. ACS Biomater Sci Eng 2023;9:1486–95.
[10] Roberts WE, Mangum JE, Schneider PM. Pathophysiology of demineralization, part I: attrition, erosion, abfraction, and noncarious cervical lesions. Curr Osteoporos Rep 2022;20:90–105.
[11] Carrouel F, Viennot S, Ottolenghi L, Gaillard C, Bourgeois D. Nanoparticles as anti-microbial, anti-inflammatory, and remineralizing agents in oral care cosmetics: a review of the current situation. Nanomaterials 2020;10:140–71.
[12] Arifa MK, Ephraim R, Rajamani T. Recent advances in dental hard tissue remineralization: a review of literature. Int J Clin Pediatr Dent 2019;12:139–44.
[13] Philip N. State of the art enamel remineralization systems: the next frontier in caries management. Caries Res 2019;53:284–95.
[14] Abdelaziz RH, Mohamed AA, Talaat DM. Effect of two remineralizing agents on microhardness of initial enamel carieslike lesions in young permanent teeth. Alex Dent J 2019;44:45–9.
[15] Simeonov M, Gussiyska A, Mironova J, Nikolova D, Apostolov A, Sezanova K, et al. Novel hybrid chitosan/calcium phosphates microgels for remineralization of demineralized enamel–a model study. Eur Polym J 2019;119:14–21.
[16] Nimbeni SB, Nimbeni BS, Divakar DD. Role of chitosan in remineralization of enamel and dentin: a systematic review. Int J Clin Pediatr Dent 2021;14:562–8.
[17] Belmar da Costa M, Delgado AH, Pinheiro de Melo T, Amorim T, Mano Azul A. Analysis of laboratory adhesion studies in eroded enamel and dentin: a scoping review. Biomater Investig Dent 2021;8:24–38.
[18] Yassen AA, Safy RK. Grape seed extract and dentin remineralization. Egypt Dent J 2018;64:1719–26.
[19] Thilander B, Pena L, Infante C, Parada SS, de Mayorga C. Prevalence of malocclusion and orthodontic treatment need in children and adolescents in Bogota, Colombia. An epidemiological study related to different stages of dental development. Eur J Orthod 2001;23:153–68.
[20] Thomas CS, Sharma DS, Sheet D, Mukhopadhyay A, Sharma S. Cross-sectional visual comparison of remineralization efficacy of various agents on early smooth surface caries of primary teeth with swept source optical coherence tomography. J Oral Biol Craniofac Res 2021;11:628–37.
[21] Huang C, Cao P. Tuning Ca: P ratio by NaOH from monocalcium phosphate monohydrate (MCPM). Mater Chem Phys 2016;181:159–66.
[22] Abdel Samie BM, Al-Yasaki MA, Gad NA. The effect of mesoporous silica nanoparticles desensitizing slurry alone or combined with calcium phosphate on dentin permeability (An in-scanning electron microscope study). ADJ for Girls 2021;8:19–26.
[23] Li Y, Hu X, Ruan J, Arola DD, Ji C, Weir MD, et al. Bonding durability, antibacterial activity and biofilm pH of novel adhesive containing antibacterial monomer and nanoparticles of amorphous calcium phosphate. J Dent 2019;81:91–101.
[24] Rouhani A, Ghoddusi J, Naghavi N, Al-Lawati G. Scanning electron microscopic evaluation of dentinal tubule penetration of Epiphany in severely curved root canals. Eur J Dermatol 2013;7:423–8.

[25] Yadav RK, Bharti D, Tikku AP, Verma P, Shakya VK, Pandey P. Comparative evaluation of remineralizing effect of fluoride and nonfluoride agents on artificially induced caries using different advanced imaging techniques. J Conserv Dent 2022;25:26–31.
[26] Farooq I, Bugshan A. The role of salivary contents and modern technologies in the remineralization of dental enamel: a narrative review. F1000Research 2021;9:171–84.
[27] Zhang J, Lynch RJ, Watson TF, Banerjee A. Chitosan-bioglass complexes promote subsurface remineralisation of incipient human carious enamel lesions. J Dent 2019;84:67–75.
[28] Tao S, Fan M, Xu HH, Li J, He L, Zhou X, et al. The remineralization effectiveness of PAMAM dendrimer with different terminal groups on demineralized dentin in vitro. RSC Adv 2017;7:54947–55.
[29] Huang Y, Song B, Zhou X, Chen H, Wang H, Cheng L. Dental restorative materials for elderly populations. Polymers 2021;13:828–40.
[30] Berkathullah M, Farook MS, Mahmoud O. The effectiveness of remineralizing agents on dentinal permeability. BioMed Res Int 2018;2018(5):1–12.
[31] Zafar MS, Amin F, Fareed MA, Ghabbani H, Riaz S, Khurshid Z, et al. Biomimetic aspects of restorative dentistry biomaterials. Biomimetics 2020;5:34–75.
[32] Eissa MM, Ashour DG, Ahmed S. "Chapter 4 - Nanotechnology in non-cavitated lesions and tooth hypersensitivity", Nanotechnology in conservative dentistry. Woodhead Publishing; 2021. p. 83–103.
[33] El Gezawi M, Wolfle UC, Haridy R, Fliefel R, Kaisarly D. Remineralization, regeneration, and repair of natural tooth structure: influences on the future of restorative dentistry practice. ACS Biomater Sci Eng 2019;5:4899–919. https://doi.org/10.1021/acsbiomaterials.9b00591.
[34] Xu Z, Neoh KG, Lin CC, Kishen A. Biomimetic deposition of calcium phosphate minerals on the surface of partially demineralized dentine modified with phosphorylated chitosan. J Biomed Mater Res 2011;98:150–9.
[35] Santoso T, Djauharie NK, Ahdi W, Latief FD, Suprastiwi E. Carboxymethyl chitosan/amorphous calcium phosphate and dentin remineralization. J Int Dent Medical Res 2019;12:84–7.
[36] Ibrahim IE, Karam SS, Aly HM. Biomimetic enamel remineralization using chitosan hydrogel (an in vitro study). Alex Dent J 2018;43:116–21.
[37] Zhou Z, Ge X, Bian M, Xu T, Li N, Lu J, et al. Remineralization of dentin slices using casein phosphopeptide–amorphous calcium phosphate combined with sodium tripolyphosphate. Biomed Eng Online 2020;19:1–3.