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Comparison between Chitosan Hydrogel and Recaldent Paste in Enamel Re-mineralization of Induced Enamel Demineralized Lesions

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ABSTRACT

Purpose: This study aimed to compare between the effect of chitosan and GC MI paste plus on re-mineralization of demineralized enamel in-vitro. **Subjects and methods:** Extracted upper premolars from orthodontic patients. Sample premolars were examined one by scanning electron microscope with energy dispersive X-ray spectroscopy to determine the Ca/P ratio before demineralization and the other by Fourier transform Infrared spectroscopy to determine the degree of conversion of bonds. After demineralization, two premolars samples were selected; one was examined by scanning electron microscope with energy dispersive X-ray spectroscope and the other was examined by FTIR to determine the degree of conversion of bonds. The remaining premolars were divided equally into 3 groups: control group (GI) premolars were re-mineralized using artificial saliva only for 14 days, (GII) premolars were re-mineralized by chitosan hydrogel for 14 days and (GIII) premolars were re-mineralized by GC MI paste plus for 14 days. **Results:** They were 46 premolars and after both negative and positive control, they were divided into 3 groups 14 premolar each where, GI showed least re-mineralization occurred, where the peripheries of prisms were lost. GII chitosan group showed the most re-mineralized results as it was not only increase in minerals but also thickness of enamel by addition of minerals' layer on the surface and GIII GC MI paste plus showed average re-mineralization process. **Statistical results:** GII showed the highest mean % of re-mineralization followed by GIII and then GI. Statistical difference was significant. **Conclusion:** Chitosan is better re-mineralizing agent than GC MI paste plus.

KEYWORDS

Enamel,
Re-mineralization,
Chitosan.

- Paper extracted from Master thesis titled" Comparison between Chitosan Hydrogel and Recaldent Paste in Enamel Re-mineralization of Induced Enamel Demineralized Lesions".

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INTRODUCTION

Enamel is a unique teeth hard tissue, forms the outermost covering of teeth[1]. Its hardness is due to the highly organized crystalline calcium phosphate of 96% mineral with the remaining 4% consisting of organic components and water. The organic content is mainly with 90% amelogenin protein. Enamel consists of organized array of hydroxyapatite crystals that grew along the C-axis[2].

The main component of enamel is rods, which are bundles of stratified crystallites that are woven into complex architecture that are 3-5 μm in diameter[3], where the most abundant protein amelogenin acts as a key factor in controlling the orientation and elongated growth of enamel rods during the mineralization process[4]. Secondly is interrod enamel which surrounds and packs between the rods[5]. The third structure is aprismatic enamel, which refers to the structures containing hydroxyapatite crystals that show no mesoscale or macroscale alignment[6].

Dental caries is not only continuous damage of teeth leading to their loss, but also it is a process of alternative demineralization and re-mineralization[7]. The equilibrium between both the pathological factors and the preventive methods is the only engine behind the re-mineralization process[8].

The best way to treat demineralized, non-cavitated enamel is to protect its integrity and health by non-restorative preventive methods[9]. Early caries lesions can be quickly stopped by re-mineralization and good oral hygiene in adults, but it is much more difficult in children and adolescents[10,11].

On the scientific research network, biomimetic re-mineralization has a strong and visible presence. These methods rely on the formation of well-organized hydroxyl apatite crystals that mimic enamel, as well as the control of crystal growth and bio-mineralization[12].

Re-mineralization of enamel is a well-accepted preventive concept for maintaining enamel integrity. Fluoride varnishes and amorphous calcium phosphate were among the commercial products introduced to the dental sector[13,14]. Several studies have shown that fluoride varnish and other topical applications have the least impact on the re-mineralization process[15,16].

Furthermore, after treatment with these agents and amorphous calcium phosphate, the crystals produced were poorly organized and morphologically irregular[17-19] . Other studies were conducted to build up nano crystals; including the sol–gel method, mechano-chemical process, electrochemical deposition, crystallization under magnetic field, hydrothermal crystallization. But these approaches need severe reaction conditions like high pressure, high temperature and also may contain very harmful reagents[20,21] .

Recently, a re-mineralizing water-based cream containing hydroxyapatite, fluoride and xylitol has been introduced. It has been claimed that hydroxyapatite fills eroded enamel, fluoride seals dentinal tubules and xylitol acts as an antibacterial agent[22].

Different systems of re-mineralization have been investigated to achieve the goal of building up enamel like micro-structure; calcium phosphate nanoparticles, amyloid like peptides, enamel matrix derivatives and other organic materials[23].

Chitosan hydrogel is derived from chitin. Chitin is a naturally abundant polysaccharide, and the supporting material of crustaceans and insects. It was lately found on wide range in medical field as it is non-toxic and antibacterial[24,26]. Also 'Recaldent' paste is trade mark in enamel re-mineralization depending on mixing casein phosphopeptide amorphous calcium phosphate with fluoride to give synergistic effect of re-mineralization[26].

The null hypothesis of this study is that chitosan not a better re-mineralizing material for the biomimetic regeneration of Enamel than Recaldent paste.

MATERIAL AND METHODS

1. Extracted teeth:

These extracted upper 46 premolars were collected from orthodontic patients after extraction as their age ranged from 15 to 20 years. They were selected for the study excluding any teeth with deformities or cracks. The study was performed according to the guidelines of the Research ethical committee, Faculty of Dental Medicine for Girls Al-Azhar University (REC-BI-19-03).

2. Chitosan powder:

Molecular weight = 100:300KDa, DD= 85% Supplied by Loba-chemie and produced as Hydrogel, by Nanogate co.

Figure (1) Prepared hydrogel of chitosan powder

3. **Recaldent (GC MI paste plus calcium and fluoride):** this product is for professional use containing the active ingredient RECALDENT™ (CPP-ACP), a special milk-derived protein that has a unique ability to release bio-available calcium and phosphate (and fluoride in MI Paste Plus) to tooth surfaces. From dental store supplied by Dentacart.com from USA.

4. Artificial saliva:

Supplied by Nanogate Company.

The selected upper 46 premolars were thoroughly cleaned ultrasonically of debris and any organic material on their surfaces, then immersed in ethanol for 3 days.

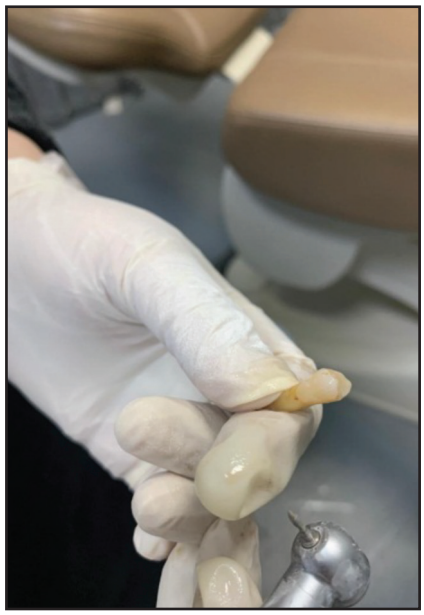

Figure (2) Cleaning teeth ultrasonically

- Two reference premolars were selected randomly; scanned by electron microscope with EDX to determine the Ca/P ratio, and by FTIR for –percentage of bond conversion.
- **Demineralization process:**

  The upper premolars were demineralized using EDTA 5% for 14 days as the solution was changed every 2 days without washing the teeth[27].

Demineralized upper premolars were selected; one was scanned by electron microscope with EDX to determine the amount of minerals lost. And the other was examined by FTIR to calculate the Degree of conversion Percentage and, this acted as baseline uncured sample for further statistical analysis.

Re-mineralization process:

The rest of premolars were randomly assigned to 3 groups (14 premolars each), as follow: **Group I:** demineralized group which was left in artificial saliva, where saliva was changed every 2 days for 14 days.

Group II: demineralized group was re-mineralized using chitosan hydrogel, as a layer of chitosan was painted on the crown of the teeth, left to dry for 2 hours and then immersed in artificial saliva. This process was repeated every 2 days for 14 days[28].

Group III: demineralized group was re-mineralized using GC MI paste plus as a layer of GC MI paste was painted on the crown of the teeth, left to dry for 2 hours and then immersed in artificial saliva. This process was repeated every 2 days for 14 days[29].

- After re-mineralization process one premolar from each group was examined using SEM with EDX, and the rest were examined by FTIR.

RESULTS

Scanning electron microscope

The SEM examination was focused on evaluating the topographic changes of the surfaces of all study groups.

Reference sample

Showed smooth surface without any minerals loss (fig.3A,4A).

Artificial enamel lesions

Scanning image of the demineralized premolar showed loss of minerals and crystals in the core of the prisms and also showed surface irregularities. Demineralization increased the enamel porosities size (fig.3B,4B).

Group I (control group)

This group was treated by artificial saliva for natural re-mineralization simulating the oral cavity. The scanning images showed partial re-mineralization for the hydroxyapatite crystals in the core of the prism but the crystals at the peripheries were lost (fig.5A, 6A).

Group II (chitosan hydrogel group)

This group of demineralized enamel was treated using chitosan hydrogel as re-mineralizing agent. The scanned picture showed the maximal re-mineralization among the three groups, where the crystals were not only regenerated in the core and peripheries but also there was a dense layer of minerals deposited on the surface of enamel (fig.5B, 6B).

Group III (Recaldent paste)

This group was treated after demineralization using the GC MI paste plus with the active ingredient 'Recaldent'. The scanned image showed regeneration of the crystals in the core and the peripheries (fig.5C, 6C).

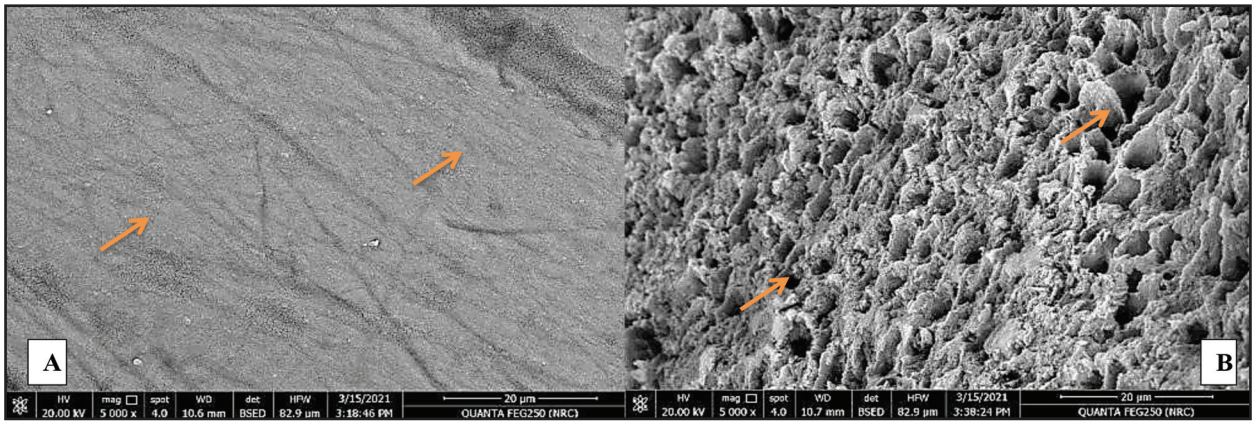

Figure (3): A) SEM reference premolar showing smooth surface of enamel and no rod ends and porosities(X5,000). B) SEM of demineralized premolar showing rough surface irregularities and lost crystals (X5,000).

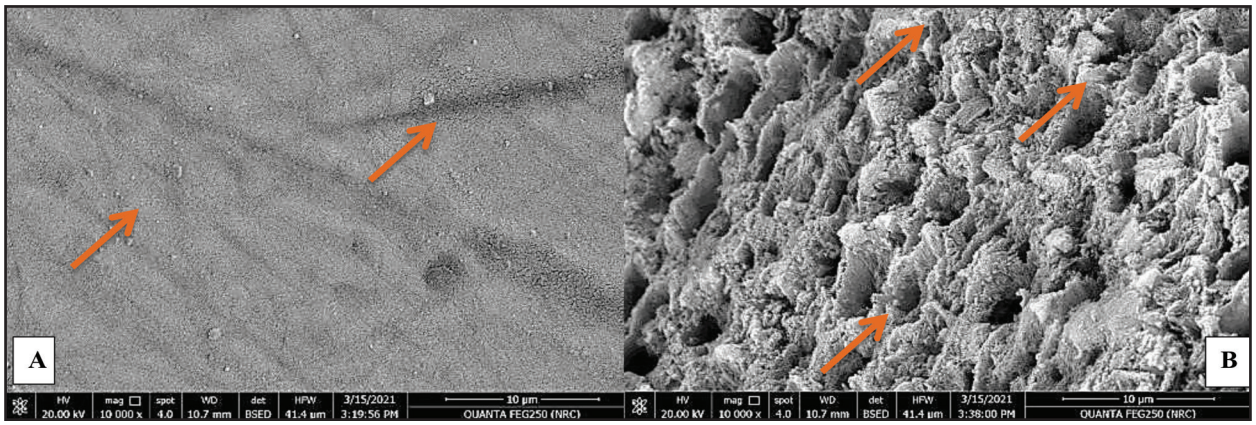

Figure (4): A) Reference premolar at magnification (X10,000) showing the surface topography of the reference premolar. B) SEM of demineralized premolar at magnification (X10,000) confirming the demineralization

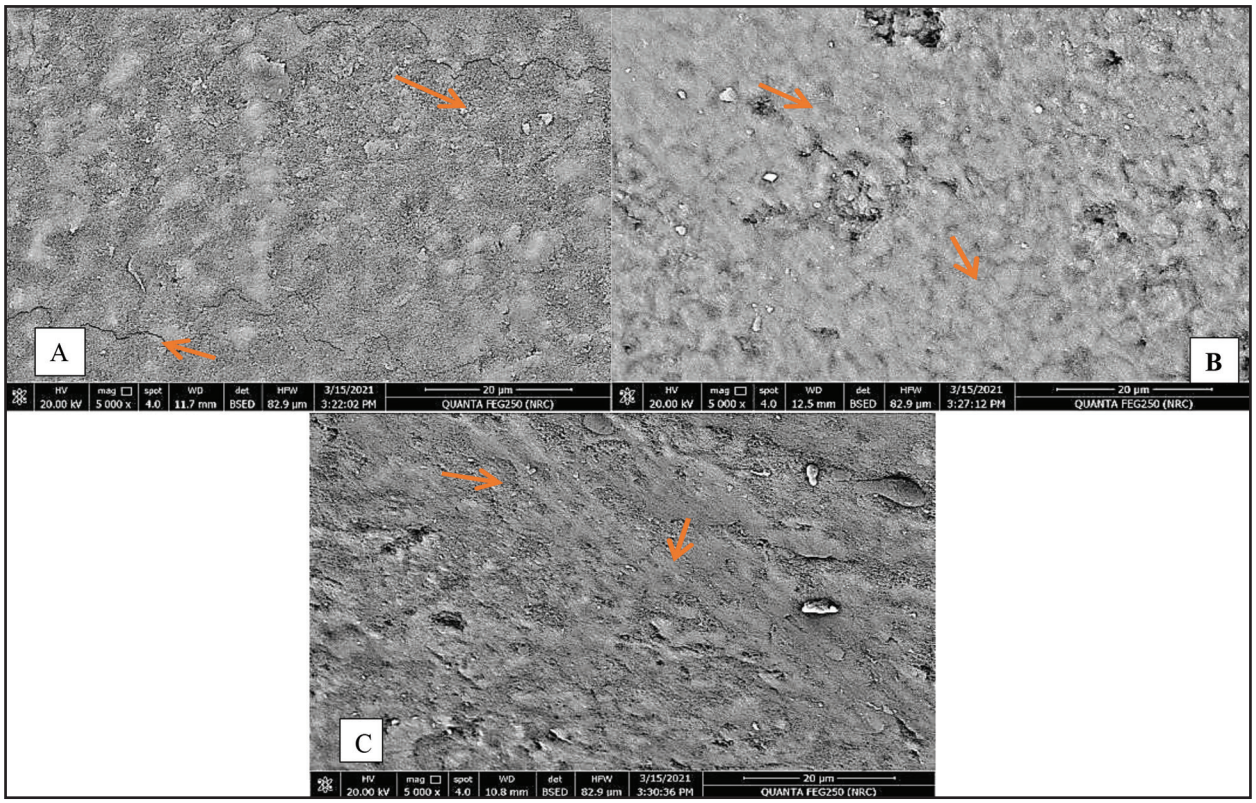

Figure (5): A) SEM of group I (control group) showing enamel rods which are relatively intact with a preferential loss of rod peripheries(X5,000). B) SEM of group II (chitosan hydrogel) showing the elimination of almost all of the inter-rod spaces, bundles of crystals forms enamel rod-like structures (X5,000). C) SEM of group III (GC MI paste plus) showing reduced inter-rod spaces (X5,000).

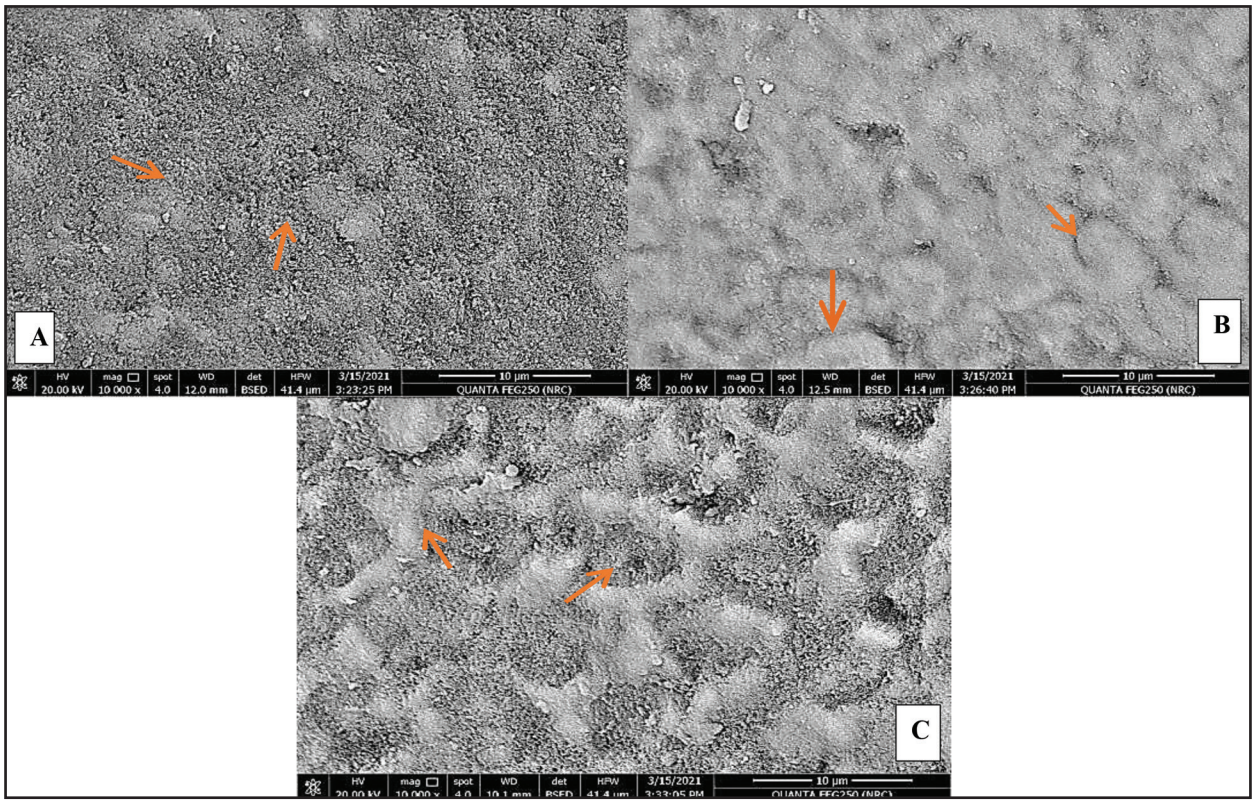

Figure (6): A) SEM of group I (control group) showing loss of rod core and spacing between crystal bundles (X10,000). B) SEM of group II (chitosan hydrogel) showing dense layer of nano-rod crystals on the enamel surface (X10,000). C) SEM of group III (GC MI paste plus) showing that regenerated crystals eliminating the spaces caused by etching (X10,000).

Energy Dispersive Analysis X-Ray Results

Table (1): *The Ca and P content (weight %) of demineralized and re-mineralized enamel was measured and converted into Ca/P ratios for each group.*

	Ca%	P%	Ca/P
Reference	50.52	31.76	1.59
Demineralized	35.51	33.73	1.05
GI (Control group)	41.32	34.65	1.19
GII (Chitosan group)	41.24	18.44	2.23
GIII (GC MI paste plus)	42.78	30.90	1.38

EDX Analysis showed that group II (chitosan group) have the greatest Ca/P ratio, followed by group III (Recaldent) and at last group I the control group.

FTIR analysis

Degree of conversion (DC%) was determined by estimating the changes in the peak height ratio of the absorbance intensities of aliphatic C=C peak at 1638 cm^{-1} and that of an internal standard peak of aromatic C=C at 1608 cm^{-1} during polymerization, in relation to the un cured material (demineralized premolar), where the reference sample = 0.84, while demineralized sample= 3.89. The ratio was calculated using the following formula:

$$DC\ (\%) = \left(\frac{R\ (cured)}{R\ (uncured)} \right) \times 100$$

- *DC (%)*= (1-3.89/0.84*) X 100= -3.83,* the negative results confirm the breakage occurred in the bonds and loss of minerals.

Statistical results

Table (2)*: GII showed the highest mean % of degree of conversion, comparing to GII and GIII. The difference was highly significant* **(Fig. 4,5)**.

Groups	Group I	Group II	Group III	ANOVA	p-value
Mean±SD	59.323±2.03	73.631± 2.03	65.577±2.03	156.455	≤0.00001**
Range	57.293- 61.353	71.601-75.661	63.547- 67.607		

*** highly significant at p≤0.00001*

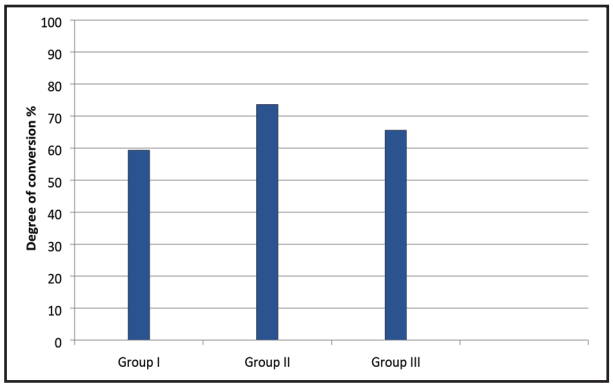

Figure (7) Bar chart representing mean and SD values of degree of conversion% in studied groups.

DISCUSSION

The present study aimed to discover the most effective re-mineralizing agent of artificial carious lesions of enamel, between Chitosan and Recaldent paste.

By using EDTA 5% to produce artificial carious lesions, as it took average from 8 to 16 days for PH 5.0 to achieve the needed artificial lesion as the loss of minerals was not only at the surface but also at the depth. According to previous study, EDTA changed every 2 days to prevent the increase in concentration of un dissociated acid as it decreases the mineral out-flux[27]. The premolars were divided into 3 groups. Where GI, we used only artificial saliva for re-mineralization, GII chitosan was used as re-mineralizing agent beside artificial saliva[25], then GIII GC MI paste plus was used as re-mineralizing agent beside artificial saliva[26].

Chitosan was introduced in several medical fields, but in dentistry it doesn't overtake the in vitro studies[24]. Chitosan macromolecules act as a template for the Ca/P deposition and thus amorphous to poorly crystalline non stoichiometric hydroxyapatite was in situ formed[29] . Chitosan is the deacetylated form of the natural polysaccharide – chitin. It is soluble if exposed to acidic conditions (pKb NH 2 = 6.5) and is a great antimicrobial agent[30,31]. Chitosan is biocompatible and biodegradable. Below its pKb, the amino groups of chitosan become protonated, which makes its macro molecules positively charged in acidic media. This overall positive charge makes chitosan adhesive to negatively charged surfaces such as tooth enamel[30].

But Recaldent paste is widely used as re-mineralizing agent in dental field after laser and bleaching processes and even after scaling and polishing[26,27] . GC MI paste plus is a commercial product that combines CPP-ACP and 900 ppm fluoride (CPP-ACPF), aiming to provide more therapeutic effects than MI Paste, which contains CPP-ACP alone[32].

By Scanning of GI by electron microscope revealed the natural minimal re-mineralization of enamel by salivary secretion without any synthetic intervention.

By examination of both groups GII, and GIII by scanning electron microscope and Fourier transform infrared spectroscopy and statistical analysis. GII (chitosan) showed more significant results of re-mineralization than GIII (Recaldent paste).

In current research chitosan showed the great ability of re-mineralizing the artificial lesions of enamel. Where these results also agreed with a study which reported that chitosan hydrogel in an attempt to prove that it is a promising material in biomimetic regeneration of dental hard tissues, and concluded that chitosan properties qualify it for straight forward clinical use[24]. By using several studies as template researchers proved the efficacy of chitosan in inhibiting the demineralization process, and he reported that chitosan not only inhibit the demineralization process but also increase PH in acidic medium and prevent damage of enamel surface[25].

Other researchers reported that the hybrid type of chitosan micro-gels for re-mineralizing of enamel, and reported that chitosan is effective agent that enhance growth and nucleation of enamel crystals[29]. Another study conducted to induce bone like apatite depending on the chitosan hydrogel cross, which is used as template for ion assembly[30]. Other researchers evaluated and confirmed that chitosan has bactericidal effect on both gram negative and gram positive E.coli[31]. And finally, paper reported the properties of chitosan in order to be a critical additive for dental materials, and reported that it will be the future starting from restorative dentistry passing by engineered scaffolds and reaching periodontal complex healing[22]. High Ca content in chitosan may be the reason behind its high ability of re-mineralizing the demineralized enamel.

On the other hand studies showed the ability of Recaldent paste in re-mineralizing the demineralized enamel by evidences. A study evaluated the effect of MI paste plus on demineralized enamel by both acid and laser etching and proved its ability in re-mineralization[26]. Researchers compared between MI paste plus and nano-hydroxy paste and reported the efficacy of both pastes[28]. A study showed the effect of MI paste plus on white lesions after fixed appliance and reported the improvement of the white lesions after its application[33]. Other studies were conducted and reported MI paste plus re-mineralizing properties, and concluded significant decrease in white spot lesions found after orthodontic treatment[32]. And finally, a comparison between the effect of MI paste plus and plain MI paste on enamel softened buy cola drink and reported that MI paste plus showed higher re-mineralization potential [34].

CONCLUSION

From the previously mentioned results, it could be concluded that chitosan has great ability in re-mineralization of demineralized enamel than Recaldent (GC MI paste plus).

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RECOMMENDATIONS

1. Evaluating multiple types of casein derivatives and natural elements in order to re-mineralize enamel for preventive dentistry
2. Further studies should be done over a longer period in order to prove the positive action of these re-mineralizing agents.
3. Similar studies should be done on other types of re-mineralizing agents.

DECLARATION STATEMENT

Authors declare no conflict of interest.

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