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ENAMEL PRISM-LIKE TISSUE REGENERATION USING DIFFERENT AGAROSE-BASED HYDROGELS AS PREVENTIVE TREATMENT APPROACHES FOR DENTIN EROSION

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ABSTRACT

Objectives: The purpose of the study was to compare the efficiency of different agarose-based hydrogels in the treatment of dentin erosion. **Methodology:** Eighteen human molars were collected. Radicular part of each molar was removed, while coronal part was sectioned mesiodistally. Two dentin slabs were obtained from each molar. Dentin specimens were immersed in Coca Cola beverage (Coca-Cola® Co., Egypt) for 25 hours. Specimens in group I were stored in artificial saliva (control), in group II they were treated with agarose hydrogel system, in group III chitosan was added, and in group IV agarose hydrogel system was loaded with Emdogain. Hydrogels were applied to specimens for 5 hours. This procedure was repeated daily for 15 days. Morphology of all specimens was investigated by Scanning Electron Microscope. **Results:** A smear layer occluding some dentinal tubules was visible in images of sound dentin, while after immersion in Coca Cola images showed exposure of tubules' orifices. In group I, images revealed partially obliterated and opened tubules, while in group II they revealed partial obliteration of all tubules. Additionally, images of group III exhibited mild sclerosed dentinal tubules with enamel prism-like grown crystals, and in group IV presented regenerated crystals assembled into parallel bundles. **Conclusion:** Dentin erosion cannot be treated with artificial saliva. Agarose hydrogel group resulted in formation of enamel prism-like structures, Chitosan group showed densely packed crystal layer covering dentin surface, and Emdogain group revealed the formation of regenerated crystals and parallel bundles masking dentinal tubules openings.

KEYWORDS: Agarose- chitosan- Emdogain- biomimetic remineralization.

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INTRODUCTION

Human teeth are a fascinating biological material, a constant concern regarding public health, and an economically relevant factor of global importance for human society[1,2]. Structurally, an enamel layer covers the dentin core in the crown portion of the tooth, while a cementum layer covers the dentin core in the root.

By weight, dentin is made up of around 70% inorganic matrix, 20% organic matrix, and 10% water[3]. Apatite crystals (Ca10(PO4)6(OH)2)4 make up the inorganic portion, whilst 10% non-collagenous proteins (NCPs) and 90% type I collagen make the organic one[5]. The dentin macroscopic architecture is defined by the presence of numerous 1μm parallel dentinal tubules that are oriented radially from the pulp to the dentin–enamel junction. These tubules are surrounded by a highly mineralized cuff called the peritubular dentin containing apatite crystals and a small amount of collagen, while the interstitial space between these cuffs is termed the intertubular dentin[6,7].

Dental erosion is the process by which non-bacterial acids chemically dissolve the minerals that make up tooth structure. It can have an intrinsic (gastro-esophageal) or extrinsic (diet) origin. Recent major lifestyle changes have resulted in increased consumption of foods and beverages rich in acids, making erosion a key therapeutic concern[8,9].

In vitro, tooth minerals already dissolve at pH values below 5.5[10]. The presence of Ca2+ and PO4 3– ions in saliva and plaque fluid, which can function as a buffer system, might cause variations in this essential pH value in the oral cavity[10]. Furthermore, the time of exposure, quality and quantity of the pellicle layer, and the subsequent activities taken after consuming acidic food, all affect the actual effectiveness and extent of damage caused by an acid attack[11]. The dentin of healthy teeth is not subjected to the harsh circumstances found in the oral cavity, such as sharp variations in pH and temperature. On the other hand, under specific clinical circumstances such severe gingival recession or localized enamel layer abrasion, this might be the case[12].

The dramatic effect of erosion on dental tissues presents as modifications to the surface shape, variations in surface roughness, and even mineral loss in teeth[13-15]. In dentin erosion, denaturation of dentin organic proteins and proteoglycan molecules exposes the collagen fibrils. The exposed fibrils could be further susceptible to degradation by bacteria and matrix metalloproteinases (MMPs), which progressively weaken the dentin matrix[16,17]. An attempt to stabilize this collagen ultrastructure can significantly enhance the mechanical integrity of dentin, and preventing further degradation causing desensitization.

The creation of new minerals is facilitated by the supersaturation of saliva with Ca2+ and PO4 3– ions under physiological conditions. However, this natural remineralization process is slow and insufficient to counteract the extrinsic erosive impacts of teeth that must endure throughout their lifetime.[18]. Therefore, using non-restorative preventive techniques to preserve the integrity and health of demineralized, non-cavitated dentin is the recommended course of action[19]. Biomimetic remineralization is a prominent and well-established concept on the scientific research network.

Ionic and non-ionic agarose hydrogel is applied over the lost non-carious tooth enamel to create a favorable biomimetic milieu for human enamel and dentin remineralization. Agarose, a naturally occurring polysaccharide derived from red algae, has been shown to generate thermo-reversible hydrogels that resemble gel-like organic matrices and facilitate the growth of prism-shaped hydroxyapatite crystals[20]. It is one of the non-collagenous biological materials that is employed as a template in tissue engineering to regulate the self-assembly of the hydroxyapatite nanocrystals into nanorod crystals because of its repeating anionic -OH groups[21,22]. The adjustable qualities of agarose, in addition to its great biocompatibility and inexpensive cost, make it

ideal for a range of medical applications, including drug delivery systems and the regeneration of bone and cartilage[23-25].

The enamel matrix derivatives (EMDs) are marketed under the brand name Emdogain. Emdogain is a product primarily employed in the remineralization of dentin and enamel as well as directed tissue regeneration[26,27]. In addition, chitosan and its derivatives have emerged as a new class of novel biomaterials[28]. One of the chitosan derivatives, phosphorylated chitosan, has metal-chelating, osteo-inductive, antibacterial, and biocompatible qualities. It functions as a non-collagenous protein analog that imitate the natural protein functional domains which control the biomimetic remineralization process. Furthermore, incorporation of biopolymers such as chitosan (β 1→4 N-acetyl glucosamine) to the inorganic component has been attempted to crosslink and/or stabilize the collagen fibrils. It is structurally similar to that of glycosaminoglycans, a ground substance of dentin[29].

The null hypothesis of this study was that no significant differences between all treatment modalities in the management of dentin erosion.

MATERIALS AND METHODS

Study design and eligibility criteria

The experiment is a descriptive, in-vitro study. It was submitted for consideration and approval by the Scientific Research Ethics Committee, Faculty of Dentistry, Suez Canal University, (711/2023) 30-10-2023. All the materials obtained were in their analytical grade.

Specimens' collection & preparation

A total of eighteen anonymously human molars were collected for use in the current study[30]. All molars were taken out of patients in the age group of (25-40) for therapeutic purposes. The study omitted any teeth with cracks, fractures, white spot lesions, fillings, hypoplastic diseases, or obvious cavities[31]. Any soft tissue that was still present was manually scaled, then the molars were immersed in 1% chloramine-T solution for 72 hours for disinfection[32]. After examination, molars were kept in distilled water with 0.1% thymol in a refrigerator at 4°C to prevent the growth of bacteria or fungi till the study start (they were utilized within 1 month of their extraction)[31,33].

The radicular portion of each molar was removed, while the coronal portion was sectioned mesiodistally by a diamond coated disc (Buehler, IL, USA) under water coolant. Two dentin slabs of 3 mm thickness each were obtained from each molar by using hard tissue microtome (Yushuoda Hard Tissue Microtome, Liaoning, China). A total of thirty-six dentin specimens were obtained and mounted on resin blocks. The dentin specimens were then polished using 600 grit silicon-carbide papers. Only six reference dentin specimens from total were selected randomly, and coated with gold sputtering for baseline evaluation of dentinal tubule using Scanning Electron Microscope (SEM) (Hitachi S 3400 N SEM, Tokyo, Japan)[34].

Beverage exposure

The dentin specimens were then coated with waterproof nail varnish leaving a workable window exposed of approximately 3 × 3 mm at the center by using sticky tape[34]. In order to expose the dentinal tubules, all dentin specimens were immersed in Coca Cola beverage (Coca-Cola® Co., Egypt) (10 specimens/250 ml) for a total of 25 hours (Coca Cola was replaced every 5 hours). Following Coca exposure, specimens were rinsed with distilled water and dried[35]. Only six specimens were subjected to SEM examination to confirm the eroded and open dentinal tubules. According to the experimental hydrogels, remaining dentin specimens (n=24) were divided randomly into 4 groups (n = 6) and allocated as follows: group I: received no treatment (control); group II: treated with agarose hydrogel; group III: treated with agarose hydrogel+ chitosan; group IV: treated with agarose hydrogel+ Emdogain.

Preparation of experimental hydrogels

Calcium chloride ($CaCl_2$) agarose hydrogel was prepared by dissolving 0.5g agarose powder (Genetic Analysis Grade, Fisher Bio-Reagents, UK) into 100 ml of 0.13M (1.91g) of $CaCl_2$ solution. The latter was prepared by dissolving $CaCl_2.2H_2O$ (Analytical Reagent Grade, Fisher Chemical, UK) in deionized water (Sigma-Aldrich, St. Louis, MO, USA). On the other hand, Na_2HPO_4 agarose hydrogel containing 500 ppm fluoride was prepared by dissolving 0.5g agarose powder into 100 ml of 0.26M (4.63g) of Na_2HPO_4 solution containing 500 ppm (0.3g) fluoride. The latter was prepared by dissolving $Na_2HPO_4.2H_2O$ (EMSURE-Merck KGaA, Germany) and NaF (DHARMA, USA) in deionized water[36]. Both mixtures were left to soak for 30 minutes then heated at 150°C till completely dissolved and left at room temperature till gelation and then stored in the refrigerator till using[37].

In case of "chitosan hydrogel", Calcium chloride (Ca Cl_2) hydrogel was prepared by dissolving 0.13M (1.91g) of $CaCl_2$ into 1% (v/v) acetic acid (Laboratory Reagent Grade, Fisher chemical, UK). One gram of chitosan (ACROS ORGANICS. New Jersy, USA. Geel, Belgium) was added, stirred and heated at 150°C till completely dissolved. Finally, 0.5g agarose was added to the previous solution[38]. On the other hand, in case of "Emdogain hydrogel" (Straumann, Basel, Switzerland.), 4 ml of the previously prepared Ca Cl_2 hydrogel was added to 0.2ml of Emdogain (30mg/ml) to achieve a final concentration of 1.5mg/ml Emdogain + $CaCl_2$ agarose hydrogel[37].

Application of hydrogels

At the time of application, the prepared hydrogels were preheated to 55°C in water bath. A layer of 1mm thickness of Calcium chloride (Ca Cl_2) hydrogel was applied first on the surfaces of dentin specimens using plastic syringe, and kept around two hours till gelation. A second layer of Na_2HPO_4 hydrogel was then added carefully[36]. The combined hydrogels were kept on the dentin surfaces for 5 hours, rinsed in distilled water (Sigma-Aldrich, St. Louis, MO, USA), and then stored in artificial saliva which was prepared by using [Na3PO4 (3.90mM), KCl (17.98mM), NaCl (4.29mM), MgCl2 (0.08mM)] (Sigma-Aldrich, St. Louis, MO, USA), CaCl2 (1.10mM) (Analytical Reagent Grade, Fisher Chemical, UK), [NaHCO3 (3.27mM), H2SO4 (0.50mM)][37,39]. This process was repeated daily for 30 days. The specimens of control group were stored in artificial saliva for a period of 30 days which was replenished daily[40]. Finally, the specimens were examined under SEM for evaluation of tubules occlusion after 5, 10, and 15 days[34]. Qualitative assessment of the photomicrographs was done based on the surface characteristics and patency of the dentinal tubules at 2000× and 9000 × magnifications.

RESULTS

Top view SEM images of sound dentin specimens in transverse (T.S) & longitudinal (L.S) sections showed smear layer covering the dentinal tubules. The tubules were formed by smooth peritubular dentin (red arrow) surrounding the spaces of odontoblastic process (blue arrows). The asterisks refer to smooth intertubular dentin structure (figure 1). After immersing the dentin specimens in Coca Cola beverage, SEM images in T.S. & L.S. revealed the removal of smear layer and enlarged dental tubules openings (blue arrows) surrounded by less regular intertubular dentin in some areas (asterisks). (figures 2).

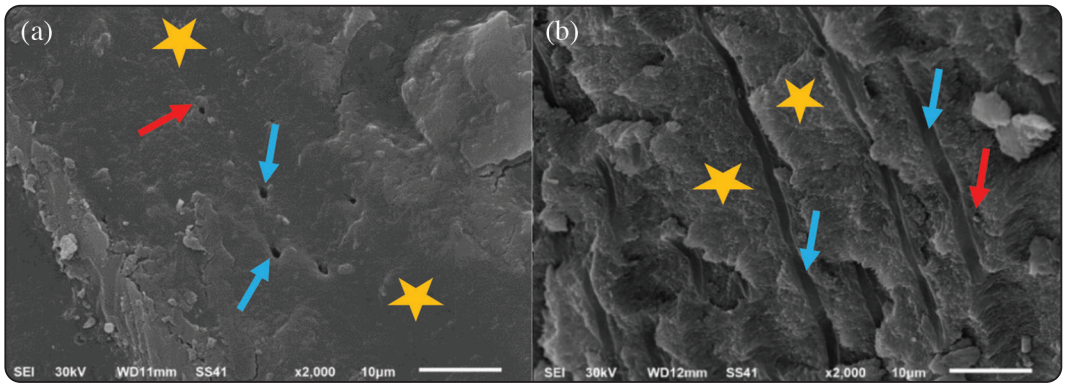

Fig. (1) T.S. and L.S of dentinal tubules before immersion in Coca Cola beverage.

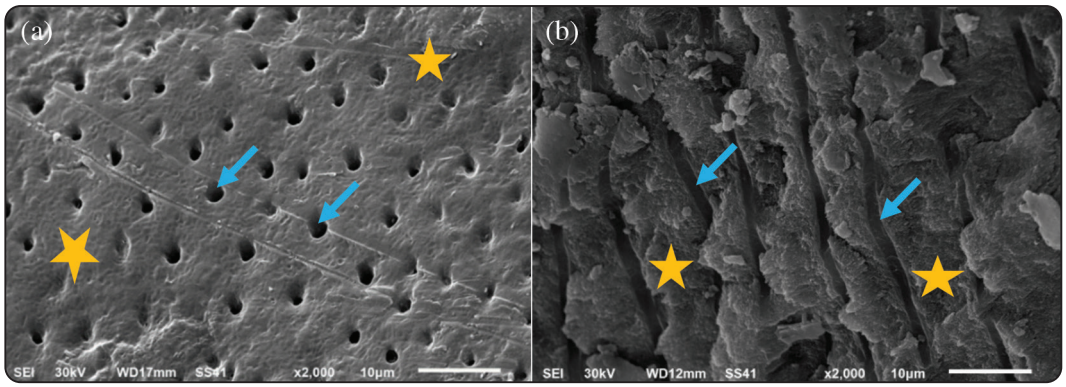

Fig. (2) T.S. and L.S of dentinal tubules after immersion in Coca Cola beverage.

- Group I (control group):

In group I after 5 days of storage of dentin specimens in artificial saliva, top view SEM image showed opened and partially occluded dentinal tubules (blue arrows) surrounded with thin peritubular dentin (red arrows) (figure 3-A), while the high power of the green inset (figure 3-B) showed less regular inter tubular dentin (asterisks) with small calcified particles deposited and scattered on dentin surface (yellow arrows).

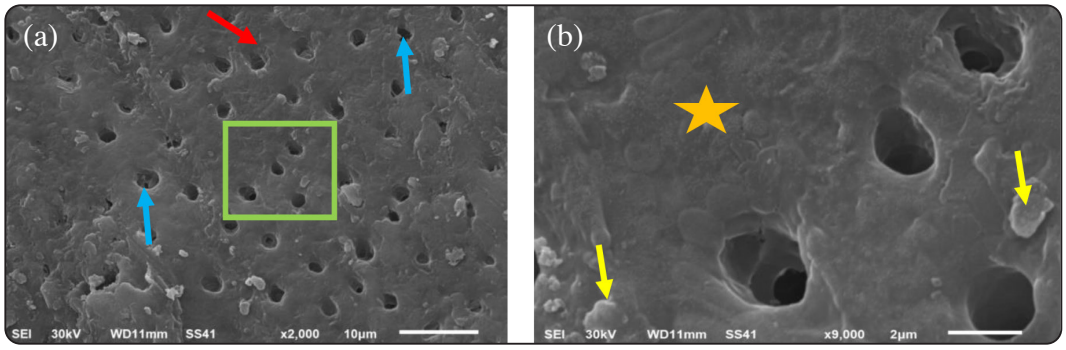

Fig. (3) T.S. of dentinal tubules after 5 days of storage of dentin specimens in artificial saliva

Figure 4-A presented SEM image in T.S after 10 days in artificial saliva which showed less regular inter tubular dentin (asterisks) with partially open dentinal tubules (blue arrows), while under high magnification (figure 4-B) revealed calcified like materials attached to the dentin surface& on the walls of the open tubules (yellow arrows).

After 15 days in artificial saliva, SEM image in T.S revealed open dentinal tubules (blue arrows) with the deposition of external hard like masses scattered on the dentin surface (green arrows) (figure 5-A). Under high magnification, the image showed fully opened tubules (blue arrows) surrounded with rough inter tubular (asterisks) & peritubular dentin (red arrows) (figure 5-B), while SEM image in L.S. showed opened dentinal tubules under low magnification (blue arrows) (figure 6).

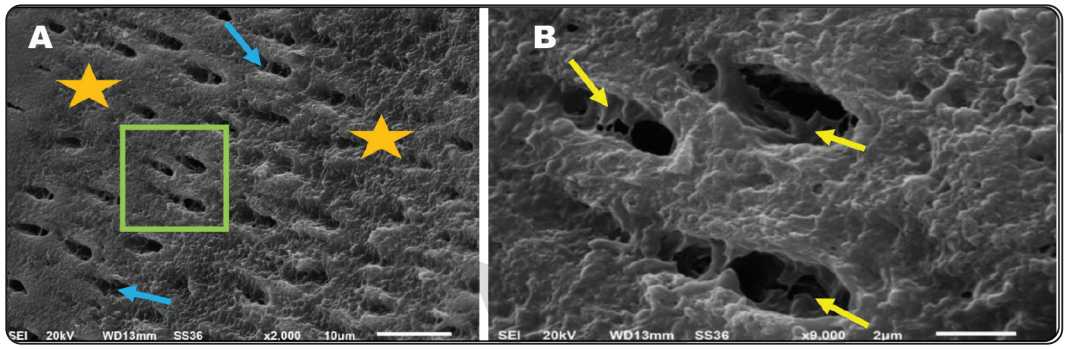

Fig. (4) T.S. of dentinal tubules after 10 days of storage of dentin specimens in artificial saliva

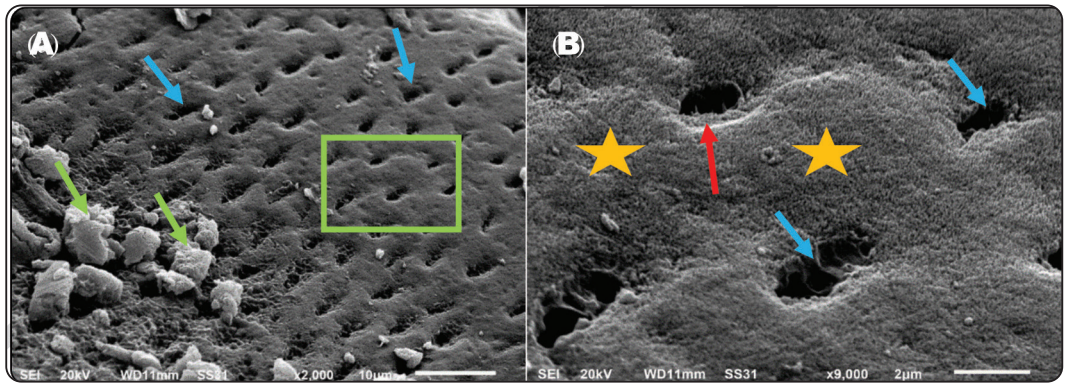

Fig. (5) T.S. of dentinal tubules after 15 days of storage of dentin specimens in artificial saliva

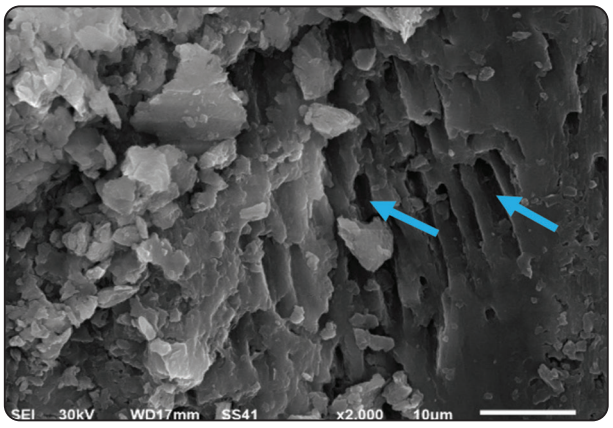

Fig. (6) L.S. of dentinal tubules after 15 days of storage of dentin specimens in artificial saliva

- Group II (treated with agarose hydrogel):

Regarding to group II, the image in T.S after 5 days treatment with agarose hydrogel showed regular inter tubular dentin, and thin peritubular dentin (red arrows) surrounding the space of odontoblastic process (blue arrows) (figure 7-A), while figure 7-B at high magnification revealed that not all the tubules were fully opened. Some minerals were deposited blocking some dentinal tubules on their walls (blue arrows), in addition to small calcified nodules appeared on the surface (yellow arrows).

After 10 days of treatment, SEM image in figure 8-A presented partially opened dentinal tubules (blue arrows), and rough dentin surface with scattered minerals clusters deposited on it. In addition, under high magnification figure 8-B revealed calcified like material attached to both inter tubular (asterisks) & peritubular dentin (red arrows). Furthermore, after 15 days SEM image in T.S. and under low magnification showed homogenous dentin surface (asterisks) (figure 9-A), while under high magnification revealed that almost the tubules were partially obliterated with enamel prism-like structures (blue arrows) (figure 9-B). In addition, it revealed the deposition of calcified masses on the dentin surface & on the walls of dentinal tubules (yellow arrows). In L.S., the SEM image also demonstrated the partial obliteration of tubules and the presence of calcified like materials on their walls (figure 10).

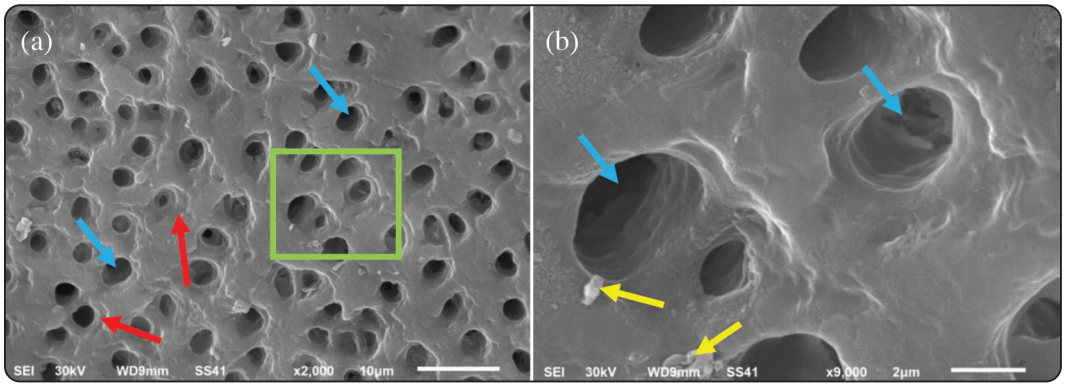

Fig. (7) T.S. of dentinal tubules after 5 days of treatment with agarose hydrogel

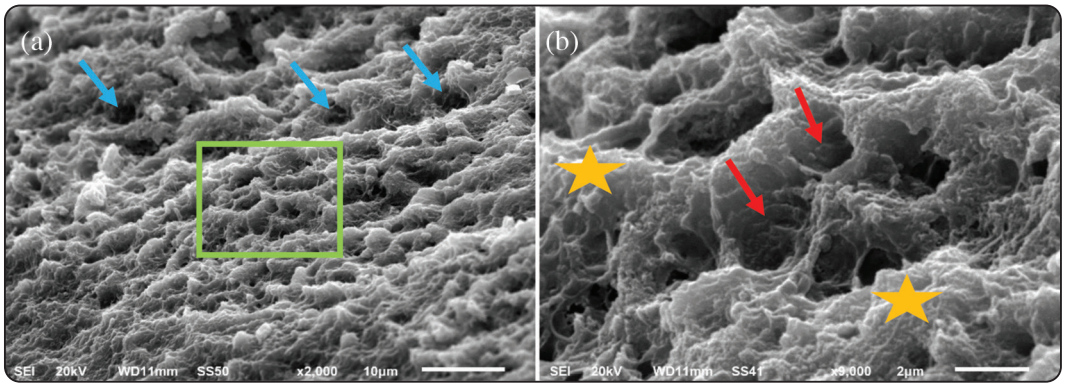

Fig. (8) T.S. of dentinal tubules after 10 days of treatment with agarose hydrogel

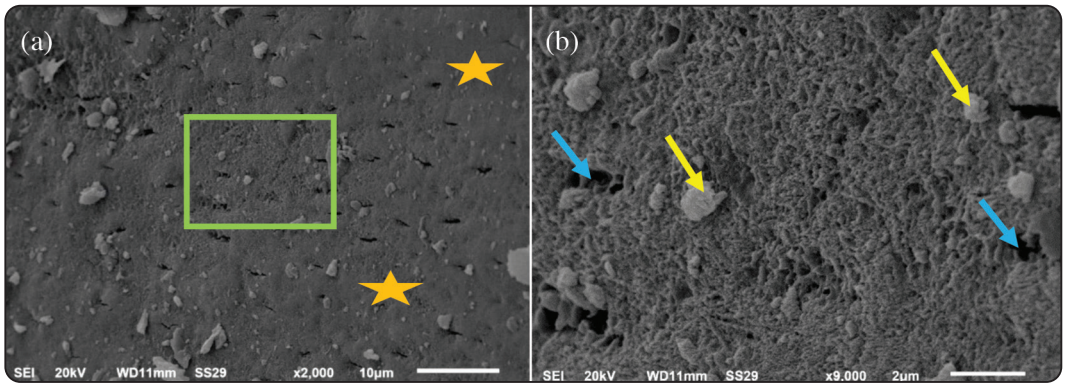

Fig. (9) T.S. of dentinal tubules after 15 days of treatment with agarose hydrogel

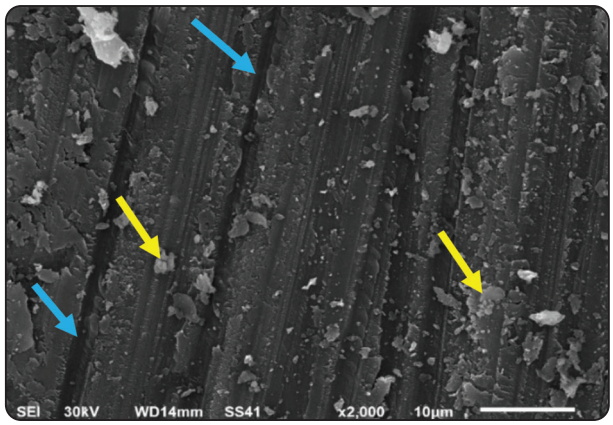

Fig. (10) L.S. of dentinal tubules after 15 days of treatment with agarose hydrogel

- Group III (treated with agarose hydrogel + chitosan):

The SEM image in T.S. after 5 days treatment of dentin specimens with chitosan showed rough dentin surface (asterisks) with clearly opened dentinal tubules (blue arrows) (Figure 11-A), while the high power of green inset of figure 11-A revealed small calcified nodules (yellow arrows) attached to dentin surface and on the walls of dentinal tubules (figure 11-B). After 10 days of treatment, low magnified SEM image (figure 12-A) presented mild sclerosed dentinal tubules (blue arrows), and rough dentin surface with deposition of prism-like structures (yellow arrows). In addition, SEM image under high magnification (figure 12-B) exhibited prism-like structures scattered on the dentin surfaces (yellow arrows).

Figure 13-A presented SEM image in T.S after 15 days treatment and showed irregular dentin surface. Nearly almost the dentinal tubules were obliterated with enamel prism-like grown crystals which were closely clusters to form a uniform layer on the underlying dentin surface (yellow arrows). The image under high magnification (figure 13-B) exhibited calcified prism-like structures on the dentin surface & on the walls of tubules (yellow arrows). Furthermore, in L.S., SEM image revealed the obliteration of dentinal tubules (blue arrows) with calcified materials (yellow arrows) (figure 14).

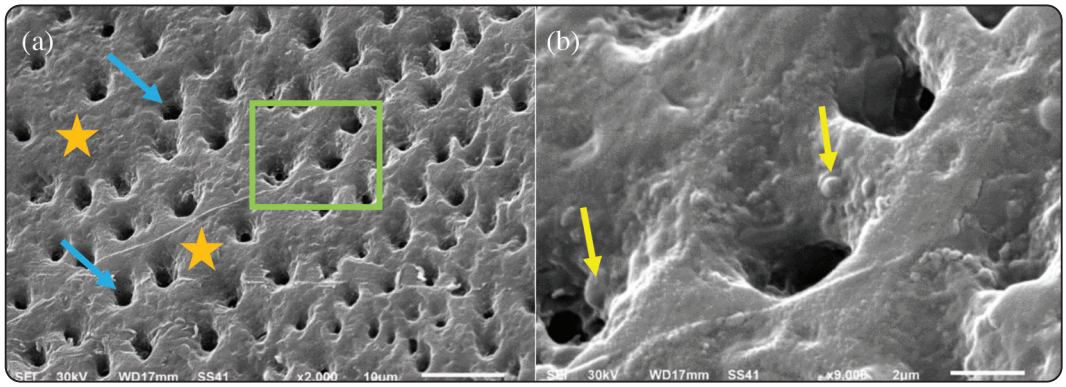

Fig. (11) T.S. of dentinal tubules after 5 days of treatment with chitosan

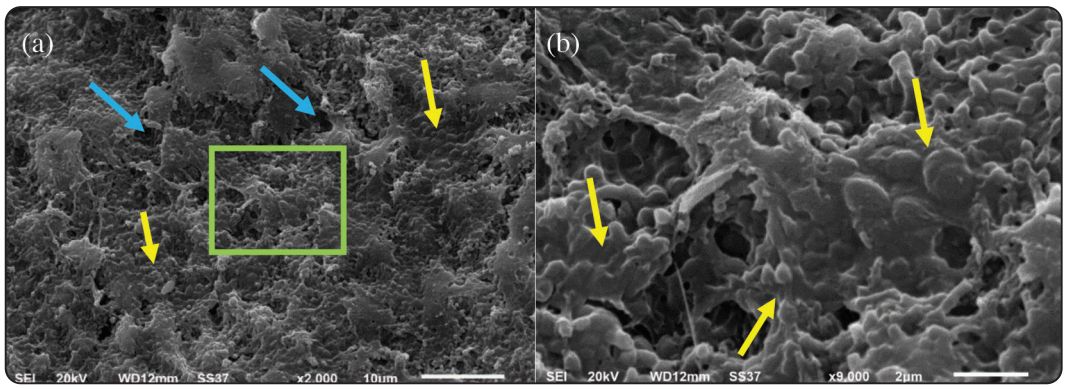

Fig. (12) T.S. of dentinal tubules after 10 days of treatment with chitosan

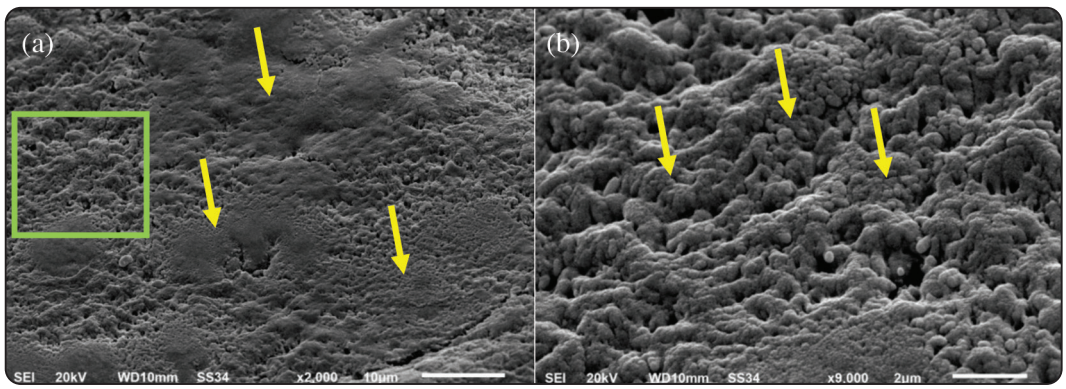

Fig. (13) T.S. of dentinal tubules after 15 days of treatment with chitosan

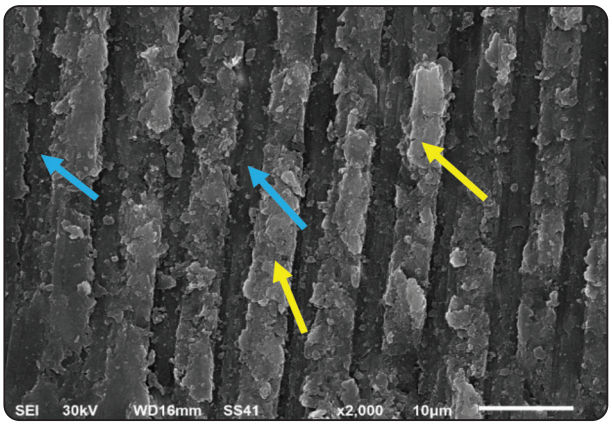

Fig. (14) L.S. of dentinal tubules after 15 days of treatment with chitosan

- Group IV (treated with agarose hydrogel + Emdogain):

Viewing from T.S., the SEM image of dentin specimens after 5 days treatment with Emdogain showed clear spaces of odontoblastic process (blue arrows) (figure 15-A), while figure 15-B under high magnification exhibited sclerotic dentinal tubules (blue arrows) surrounded with smooth peritubular dentin (red arrows), and less regular inter tubular dentin (asterisks).

Figure 16-A presented the image of dentin specimens after 10 days and revealed less regular dentin surface with partially occluded tubules (blue arrows). In addition, prism like structures deposited on the main bulk of the dentin forming a honeycomb-like morphology. Furthermore, high power of great inset in figure 16-A showed less regular inter tubular dentin (asterisks) and the prism like structures partially obliterated the dentinal tubules (blue arrows) (figure 16-B).

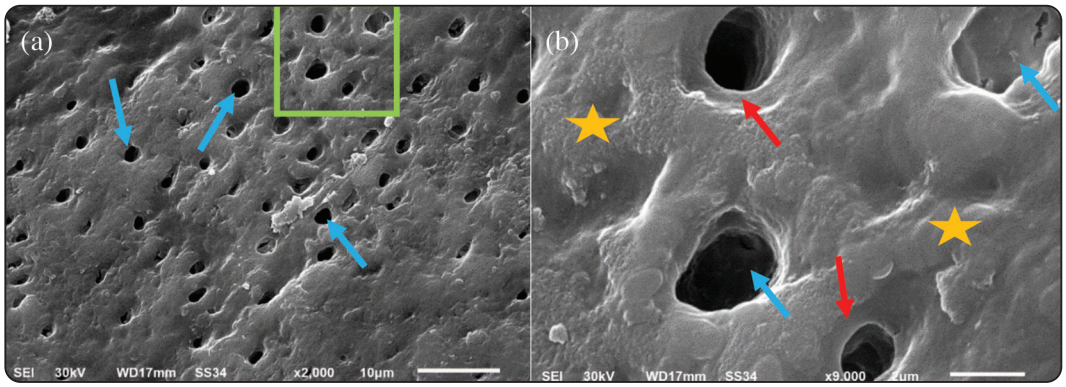

Fig. (15) T.S. of dentinal tubules after 5 days of treatment with Emdogain

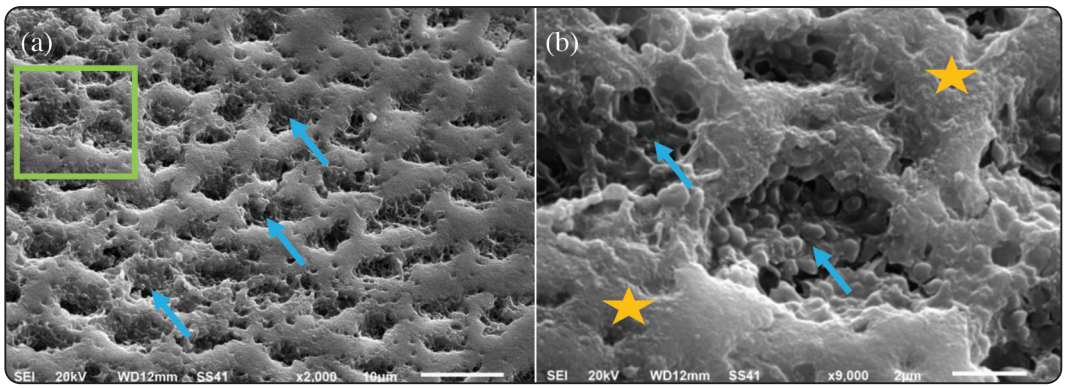

Fig. (16) T.S. of dentinal tubules after 10 days of treatment with Emdogain

Top view of SEM image under low and high magnifications after 15 days treatment exhibited regenerated crystals (yellow arrows) with incomplete developed hexagonal structure which are quite like natural enamel crystals. In addition, the crystals were found fused together forming densely packed crystal-like layer covering the main bulk of the dentin (figure 17 A&B). Furthermore, figure 18 A&B revealed that the crystals (yellow arrows) assembled into parallel bundles (orange arrows) forming enamel prism-like structures covering the dentin surface and masking the openings of dentinal tubules. Also, SEM image in L.S. showed the occlusion of the tubules (blue arrows) and the presence of calcified masses on the dentin surface (yellow arrows) (figure 19).

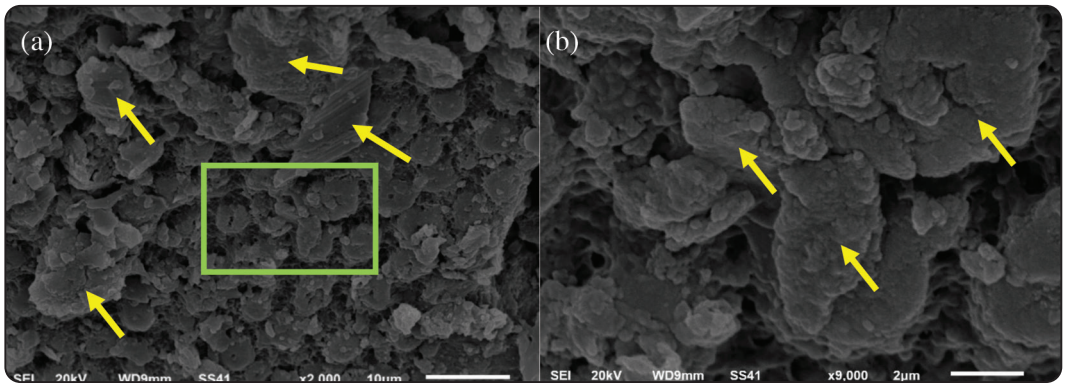

Fig. (17) T. S. of dentinal tubules after 15 days of treatment with Emdogain

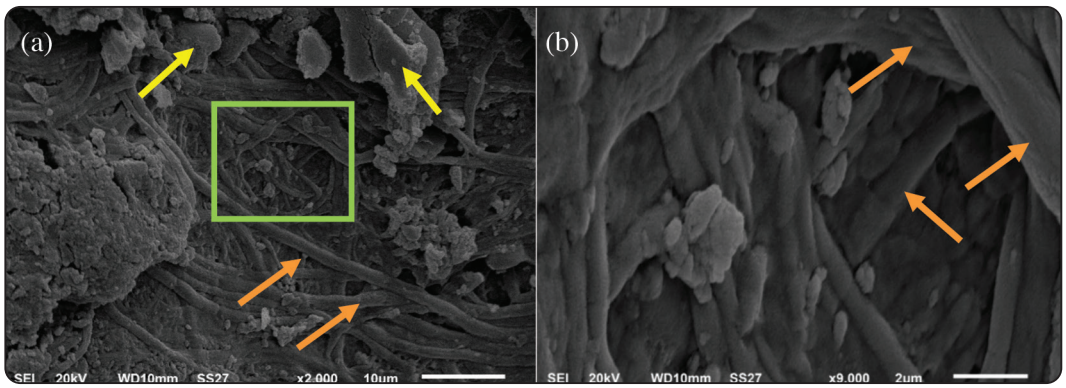

Fig. (18) T. S. of dentinal tubules after 15 days of treatment with Emdogain

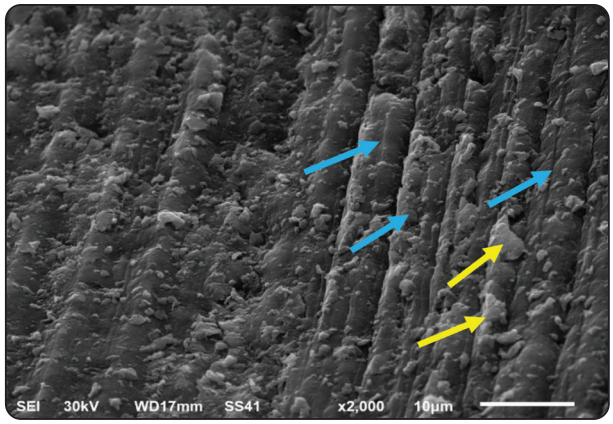

Fig. (19) L. S. of dentinal tubules after 15 days of treatment with Emdogain

DISCUSSION

Dental erosion is a condition of increasing concern, as if it is not early diagnosed and treated it may cause irreversible damage to the dentition in subjects of all ages.[41] There is common awareness within the dental professions that dental erosion is on the increase.[42]

Park et al., 2017 found that nutritional factors also contribute significantly to tooth erosion, as occupation accounted for 37.7% of the erosion and diet for 23.1%[43]. Consequently, consumption of acidic foods and beverages that may erode teeth should be carefully considered. Moreover, the hydroxyapatite dissolves at pH 3.0, so beverages with a pH value of less than 3.0 have a higher risk of producing tooth erosion. This pH threshold determines the potential of erosion due to a beverage.[44] Accordingly, Coca Cola beverage with a PH value of 2.76 was selected as an erosive agent in the current study.

The non-invasive therapeutic technique of remineralization of the superficial dental tissue has garnered more attention in recent times, and its therapeutic significance has been widely acknowledged[45]. Solutions or gels that are supersaturated with calcium, phosphate, and fluoride are the common agents used for enamel and dentin remineralization.[46] The aim to remineralize dentin is to regenerate mineralized collagen matrix and form hydroxyapatite crystals to block the open dentinal tubules in order to manage sensitivity, erosion, and other conditions in a therapeutic setting.[47] Thus, the study objective was to evaluate the effectiveness of different agarose-based hydrogels in the treatment of dentin erosion.

The agarose which is considered as a biomimetic mineralization system was chosen due to its potential effect for repairing exposed dentin. The hydrogel form was found to be the most versatile growth media for crystals and clinically easier to handle than a solution system.[48] Chitosan was added to the agarose hydrogel model to enhance the agarose molecules ability to cross-link with collagen fibers[49,50], and to function as a reservoir for calcium and phosphate ions [51,52]. Furthermore, Emodogain was added to the agarose hydrogel to mimic the biomineralization process that organic matrix proteins trigger in the formation of tooth enamel.[37,53] The application of the hydrogels was conducted and lasted for 5 hours/daily which corresponds with the least amount of human sleep time, since people could apply the hydrogels in their mouths for an entire night when used clinically.

Sound dentin specimens were first examined under SEM which revealed smear layer covering the dentinal tubules and occluding some of them (figure1), while after immersion of specimens in Coca Cola beverage for 25 hours the images showed the absence of that layer and the exposure of tubules orifices (figure 2). Our results came in agreement with the study of **Han et al., 2017** denoting the removal of the inorganic components from the surface of dentin exposing the organic matrix (mainly type I collagen).[36]

Regarding to group I (control group), it was important to record SEM findings after 5 days of storage of specimens in artificial saliva. Examination exhibited the obliteration of dentinal tubules with small calcified particles scattered on dentin surface (figure 3 A&B). The previous finding was still seen even after 10 days, where SEM images showed calcified like materials attached to the walls of the open tubules (figures 4 A&B). Our findings were confirmed by **Fateema et al., 2023** who found in their study that remineralization may be achieved without remineralizing agent. This may be due to the readily available mineral components found in artificial saliva.[54]

Additionally, after storing the dentin specimens in artificial saliva for 15 days, SEM images presented fully opened dentinal tubules (figures 5 A&B). This was confirmed by **Han et al., 2017** who reported little mineralization in their study and explained the absence of prism like structures formation by the absence of fluoride, as it proven to be essential

for the creation of the ordered needle-like apatite crystals.[36] Furthermore, the absence of crystals formation in the control group was also observed by **Zaharia et al., 2017**. They explained these results by the inability of the eroded dentin surface consisting of only type I collagen matrix to promote the mineral crystal deposition due to the absence of NCPs and/or lack of seed mineral crystals.[55]

Concerning the different experimental hydrogels, SEM images of group II (treated for 5 days with agarose hydrogel) revealed the occlusion of opened dentinal tubules with the deposition of minerals on their walls (figure 7 A&B). The current results might be due to the biomineralization process in which the organic matrix acted as a template, and utilized a sequestering mechanism to manage the molecular interaction between the polymer and minerals to create the mineral crystallites. It was assumed that agarose hydrogel mimics the gel-like organic matrix environment of natural tooth formation.[56,57]

The same SEM findings were observed after 10 days presenting partially occluded tubules with scattered minerals clusters on the dentin surface (figures 8 A&B). This came in agreement with **Han et al., 2017** reporting that agarose hydrogel mineralization system occluded the dentinal tubules after 24 hours of application on rabbits' incisors.[36] They assumed that agarose hydrogel served as a reservoir for the replenishment of mineral precursors. In addition, the fiber-nanoscale-amorphous calcium phosphate complex may have a consistent and regulated size due to the limited space in the gel network. Dentinal tubule blockage and the calcification of demineralized collagen fibrils were the outcomes of the amorphous initial particles' aggregation and assembly.[58,59]

However, this occlusion was assumed to take place as a layering technique as reported by **Ning et al., 2012** concluding that this occlusion was crystal deposition starting layer by layer forming very dense crystals and covering almost the entire dentin surface.[60,61] They confirmed the current assumption by L.S SEM image after material application for 15 days (figure 10) showing the partial obliteration of dentinal tubules and the presence of calcified materials on their walls.

Regarding the current findings after 15 days of treatment, SEM images showed that almost the dentinal tubules were partially obliterated with enamel prism-like structures (figures 9 A&B). Similar findings were reported by **Ning et al., 2012** using agarose gel loaded with calcium and phosphate for biomimetic mineralization of acid etched dentin for 10 days (240 hours) revealing large number of nanocrystals deposited on the dentin surface. They claimed that this was due to agarose being a polyanionic polysaccharide containing repetitive -COOH and -HSO3 groups in its monomeric units, while the collagen molecule carries a positive charge in the physiological environment. While the agarose gel created a hydrogel milieu where mineralization could take place, agarose molecules bound to the positively charged collagen molecules on the dentin surface. So, agarose hydrogel loaded with calcium and phosphate ions acts as a template to induce nucleation and growth. The hydroxyapatite crystals are then deposited and arranged densely, thus bonding to the dentin substrate.[60]

The formation of newly grown crystals was also observed by **Zaharia et al., 2017** under SEM examination.[55] These were randomly self-assembled onto the eroded dentin surface, some of which were deposited around the dentinal tubules and an initiation of the tubule's occlusion had been detected after 7 days (168 hours) of treatment with agarose hydrogel. They explained their findings by the presence of agarose gel which facilitated the formation of nanorod-like hydroxyapatite crystals during immersion into artificial saliva by attaching to the collagen matrix and inducing the formation of transient amorphous phosphates.[62]

Concerning the results of group III, the SEM images (figures 11A&B) after 5 days of treatment represented the same results showed in group II

after the same period. Nearly all SEM images after 10 days of treatment exhibited mild sclerosed dentinal tubules with deposition of prism-like structures on dentin surface forming a honeycomb-like morphology in some areas (figures 12 A&B). The present results were related to chitosan which serves as a scaffold for the formation of stable calcium phosphate-based layer onto the surface of the demineralized dentin by increasing the cross-linking between the dentin collagen fibers and agarose molecules.[50] Our results came in agreement with **Musat et al., 2021** who found in their study that, in addition to acting as a moderating agent in the creation of hydroxyapatite nanorods, natural polysaccharide chitosan biopolymers supplied an array for self-assembling hydroxyapatite nano-building units. Therefore, in order to recreate an enamel hierarchical structure biomedically, chitosan can operate as a bioactive macromolecule[63].

After 15 days of treatment, SEM image in L.S (figure 14) showed the occlusion of dentinal tubules with calcified materials, and in T.S (figure 13 A&B) revealed the obliteration of dentinal tubules with prism-like grown crystals forming a uniform layer on the underlying dentin surface. These findings were confirmed with **Zaharia et al., 2017** since they observed the formation of homogeneous surface of a continuous compact layer with the shrinkage of dentinal tubules' diameter in the group treated with agarose hydrogel mineralization system loaded with chitosan after 4 days (96 hours).[55] Their results were explained by the act of chitosan molecules as a chelating agent, serving as a scaffold for the formation of a complex composite calcium phosphate-based layer onto the surface of the demineralized dentine.[47] Besides, the amino group (-NH2) in chitosan makes it extremely reactive with food and cariogenic acids in the mouth. Furthermore, hydrogen ions of acid are captured resulting in an overall positive charge that gives a bio-adhesive property to negatively charged erosive surfaces preventing further acid penetration with preservation of enamel crystal orientation.[64]

When agarose hydrogel was loaded with Emdogain as presented in group IV. The SEM images after 5 days of treatment revealed some clear spaces of odontoblastic process, also sclerotic dentinal tubules were observed in high magnification image (figure 15 A&B). This might be due to the significant increase in (Ca) and (P) contents. These minerals depositions were explained by the formation of calcium-based biominerals at a template via stable pre-nucleation clusters, with aggregation into an amorphous precursor phase and transformation of this phase into a crystal.[65]

Furthermore, SEM images of specimens from this group treated for 10 days presented the deposition of prism like structures which partially obliterated the dentinal tubules and forming a honeycomb-like morphology (figure16 A&B). This could be due to the amelogenin enamel protein which representing about 90% of Emdogain composition, as it has a direct role in initiating nucleation, controlling crystal growth, and affecting the spacing of crystallites[37].

The sequence of amelogenin is typically divided into three prominent amino acid domains: a hydrophobic N-terminal domain, the central hydrophobic proline-rich region, and the hydrophilic C-terminal domain.[66] The N-terminus has been associated with matrix self-assembly, as it contains phosphorylated serine which plays an important role in stabilizing amorphous calcium phosphate and preventing unwanted mineralization, while the central hydrophobic region has been speculated to control crystal spacing.[67,68] Moreover, the hydrophilic C-terminal has been demonstrated to facilitate amelogenin protein solubility and its adhesion to the crystal surface, besides it is essential for the arrangement of the crystals into parallel arrays as shown in (figure 17) resembling a SEM image of a case after treatment for 15 days.[66]

Moreover, SEM images after 15 days showed regenerated crystals which were found fused together forming crystal-like layer covering the dentin surface (figure17A&B), while the assembling

of these crystals into parallel bundles was observed in other images (figure 18 A&B). In addition, SEM image in L.S showed the occlusion of dentinal tubules (figure 19).These findings were explained by the aggregation of Emdogain with calcium and phosphate ions followed by development of oriented apatite crystals.[53] The presence of amelogenin in Emdogain can guide the formation of highly ordered apatite crystals, besides it has a role with the presence of fluoride in promoting the oriented bundle formation of needle-like fluoridated hydroxyapatite forming an enamel-like layer.[69,70]

The novel biomimetic mineralization system (agarose hydrogel mineralization system) was selected to serve the hypothesis of this research. Our study reported that agarose hydrogel mineralization system loaded with Emdogain was the best treatment modality in the management of dentin erosion. This was to rebuild enamel-like tissue covering the remineralized dentin surface, enhance growth and nucleation of enamel crystals, and occlude the open dentinal tubules.[36] This system design was informed by the processes of enamel development in hydrogel microenvironments and organic matrix-mediated biomineralization .[60]

LIMITATIONS

- The limitations of the study were mainly related to time constraints where the follow-up of remineralization ability after a long time would have given more clear results regarding the remineralization potential of each protocol.
- The application of remineralizing agents for more than 5 hours daily would lead to more pronounced effect.
- Being dependent on collecting human teeth, limitations could be attributed to the insufficient sample size for statistical measurements which led to compensating this through teeth sectioning.

CONCLUSION

- Within the limitations of this study, the following conclusions could be drawn:
- Artificial saliva alone was not effective to manage erosive dentin.
- The agarose hydrogel system alone or loaded with chitosan seems as acceptable treatment modality.
- The addition of Emdogain to agarose system represented the best treatment modality in the management of dentin erosion.

RECOMMENDATIONS

- The present biomimetic synthesis is a step towards the creation and design of innovative biomaterials for use in restorative and reparative dentistry in the future.
- The in situ remineralization of enamel in the presence of amelogenin, enamelin, and related enzymes would be a potentially fruitful clinical use of biomimetic synthesis of enamel-like material in dentistry. Clinical research is therefore extremely important.
- The findings suggested a possible clinical application for treating disorders like erosion, wear, and dentin hypersensitivity that are related to the dentin.

Authors Contribution

All authors have read and agreed to the published version of the manuscript.

Conflict Of Interest

The authors declare no conflict of interest.

REFERENCES

1. Teaford MF, Smith MM and Ferguson MWJ Development, Function and Evolution of Teeth. Cambridge University Press (2000).
2. Van Loveren C .Toothpastes. Monographs in Oral Science. S. Karger AG Basel, 2013, vol. 23.

3. He, Z.; Chen, L.; Hu, X.; Shimada, Y.; Otsuki, M.; Tagami, J.; Ruan, S. Mechanical properties and molecular structure analysis of subsurface dentin after Er:YAG laser irradiation. J. Mech. Behav. Biomed. Mater. 2017, 74, 274–282. [CrossRef] [PubMed]
4. Abou Neel, E.A.; Aljabo, A.; Strange, A.; Ibrahim, S.; Coathup, M.; Young, A.M.; Bozec, L.; Mudera, V. Demineralization-remineralization dynamics in teeth and bone. Int. J. Nanomed. 2016, 11, 4743–4763. [CrossRef] [PubMed]
5. Mazzoni, A.; Tjäderhane, L.; Checchi, V.; Di Lenarda, R.; Salo, T.; Tay, F.R.; Pashley, D.H.; Breschi, L. Role of dentin MMPs in caries progression and bond stability. J. Dent. Res. 2015, 94, 241–251. [CrossRef] [PubMed]
6. Arola, D.D.; Reprogel, R.K. Tubule orientation and the fatigue strength of human dentin. Biomaterials 2006, 27, 2131–2140. [CrossRef] [PubMed]
7. Weiner, S.; Veis, A.; Beniash, E.; Arad, T.; Dillon, J.W.; Sabsay, B.; Siddiqui, F. Peritubular dentin formation: crystal organization and the macromolecular constituents in human teeth. J. Struct. Biol. 1999, 126, 27–41. [CrossRef] [PubMed]
8. Leslie A. Ehlen, Teresa A. Marshall, Fang Qian, James S. Wefel, and John J. Warren. Acidic beverages increase the risk of in vitro tooth erosion. Nutr Res. 2008; 28(5): 299–303.
9. Curtis DA, Jayanetti J, Chu R, Staninec M. Managing dental erosion. Today's FDA. 2012; 24(44–5):47
10. Dawes C. What is the critical pH and why does a tooth dissolve in acid? Journal of the Canadian Dental Association. 2003; 69: 722–724.
11. Lussi A and Ganss C (eds). Erosive Tooth Wear: From Diagnosis to Therapy. 2014; vol. 20. S. Karger, Basel, Swtizerland.
12. Gillam DG. Dentine Hypersensitivity: Advances in Diagnosis, Management, and Treatment. Springer International Publishing (2015).
13. Hara A, Livengood S, Lippert F, Eckert G, Ungar P. Dental surface texture characterization based on erosive tooth wear processes. J Dent Res 2016; 95:537–542. https:// doi. org/ 10. 1177/ 00220 34516 629941
14. Passos VF, Melo MA, Vasconcellos AA, Rodrigues LK, Santiago SL. Comparison of methods for quantifying dental wear caused by erosion and abrasion. Microsc Res Tech 2013; 76:178–183. https:// doi. org/ 10. 1002/ jemt. 22150
15. Braga SRM, De Faria DLa, De Oliveira E, Sobral MAP. Morphological and mineral analysis of dental enamel after erosive challenge in gastric juice and orange juice. Microsc Res Tech 2011; 74:1083–1087. https:// doi. org/ 10. 1002/ jemt. 20998.
16. Amaral SFD, Scaffa PMC, Rodrigues RDS, Nesadal D, Marques MM, Nogueira FN, et al. Dynamic influence of pH on metalloproteinase activity in human coronal and radicular dentin.Caries Res 2018;52:113-8.
17. Klont B, Damen JJ, ten Cate JM. Degradation of bovine incisor root collagen in an in vitro caries model. Arch Oral Biol 1991;36:299-304.
18. Cochrane NJ, Cai F, Huq NL, Burrow MF and Reynolds EC. New approaches to enhanced remineralization of tooth enamel. Journal of Dental Research 2010; 89: 1187–1197.
19. Giacaman RA, Muñoz-Sandoval C, Neuhaus KW, Fontana M, Chałas R. Evidence-based strategies for the minimally invasive treatment of carious lesions: Review of the literature. Adv Clin Exp Med. 2018;27:1009-16.
20. Zarrintaj, P.; Manouchehri, S.; Ahmadi, Z.; Saeb, M.R.; Urbanska, A.M.; Kaplan, D.L.; Mozafari, M. Agarose-based Biomaterials for Tissue Engineering. Carbohydr. Polym. 2018, 187, 66–84, doi:10.1016/j.carbpol.2018.01.060.
21. Fowler, C.E.; Li, M.; Mann, S.; Margolis, H.C. Influence of surfactant assembly on the formation of calcium phosphate materials— A model for dental enamel formation. J. Mater. Chem. 2005, 15, 3317–3325, doi:10.1039/B503312H.
22. Cao, Y.; Mei, M.L.; Li, Q.L.; Lo, E.C.M.; Chu, C.H. Agarose Hydrogel Biomimetic Mineralization Model for the Regeneration of Enamel Prism like Tissue. ACS Appl. Mater. Interfaces 2014, 6, 410–420, doi:10.1021/am4044823.
23. Luo, Y.; Sinkeviciute, D.; He, Y.; Karsdal, M.; Henrotin, Y.; Mobasheri, A.; Önnerfjord, P.; Bay-Jensen, A. The minor collagens in articular cartilage. Protein Cell 2017, 8, 1–13, doi:10.1007/s13238-017-0377-7.
24. Hu, J.-X.; Ran, J.-B.; Chen, S.; Jiang, P.; Shen, X.-Y.; Tong, H. Carboxylated Agarose (CA)-Silk Fibroin (SF) Dual Confluent Matrices Containing Oriented Hydroxyapatite (HA) Crystals: Biomimetic Organic/Inorganic Composites for Tibia Repair. Biomacromolecules 2016, 17, 2437–2447, doi:10.1021/acs.biomac.6b00587.
25. Campos, F.; Bonhame-Espinosa, A.B.; Vizcaino, G.; Rodriguez, I.A.; Durand-Herrera, D.; Lopez-Lopez, M.T.; Sánchez-Montesinos, I.; Alaminos, M.; Sánchez-Quevedo, M.C.; Carriel, V. Generation of genipin cross-linked fibrin-agarose hydrogels tissue like models for tissue engineering applications. Biomed. Mater. 2018, 13, 025021, doi:10.1088/1748-605X/aa9ad2.

26. Grandin, H.M.; Gemperli, A.C.; Dard, M. Enamel matrix derivative: a review of cellular effects in vitro and a model of molecular arrangement and functioning. Tissue Eng. Part B Rev. 2012, 18, 181–202. [CrossRef] [PubMed]
27. Schmidlin, P.; Zobrist, K.; Attin, T.; Wegehaupt, F. In vitro re-hardening of artificial enamel caries lesions using enamel matrix proteins or self-assembling peptides. J. Appl. Oral Sci. 2016, 24, 31–36. [CrossRef] [PubMed]
28. Noaman KM, Al-Samoly WM, Al-Hariri AA-K. Evaluation of the degree of remineralization of subclinical carious lesions using chitosan and conventional remineralizing agents (An in vitro study). Egypt J Hosp Med. 2020;78:182–9.
29. Rinaudo M. Chitin and chitosan: Properties and applications. Prog Polym Sci 2006;31:603-32. http://dx.doi.org/10.1016/j. progpolymsci.2006.06.001.
30. Mona M. El Sayed, Maha A. Niazy, Hadeel F. Mohamed. Evaluation of Remineralizing Potential of Cranberry and Chitosan on Demineralized Dentin (An in Vitro Study). ADJ-for Girls, Vol. 9, No. 1, January (2022) — PP. 83:93
31. Fatima Saeed J. Al-Ward1 , Nada Jafer MH. Radhi. Combined Effect of Nanohydroxyapatite and Chitosan on Remineralization of Initial Enamel Lesion in Vitro. The Egyptian Journal of Hospital Medicine (January 2023) Vol. 90, Page 107- 112.
32. Coelho, A.; Amaro, I.; Apolónio, A.; Paula, A.; Saraiva, J.; Ferreira, M.M.; Marto, C.M.; Carrilho, E. Effect of Cavity Disinfectants on Adhesion to Primary Teeth—A Systematic Review. Int. J. Mol. Sci. 2021, 22, 4398, doi:10.3390/ijms22094398.
33. J. C. Favaro, E. Ribeiro, R. D. Guiraldo, M. B. Lopes, A. M. F. Aranha, and S. B. Berger, "Effect of mouth rinses on tooth enamel surface," Journal of Oral Science, vol. 62, no. 1, pp. 103–106, 2020.
34. Gurucharan I, Derick Isaac DR, Madhubala MM, Vijay Amirtharaj L, Mahalaxmi S, Jayasree R, et al. Effect of chitosan and hydroxyapatite nanocomposite on dentin erosion: An in-vitro study. J Int Oral Health 2022;14:509-17.
35. Ehlen LA, Marshall TA, Qian F, Wefel JS, Warren JJ: Acidic beverages increase the risk of in vitro tooth erosion. Nutr Res 28: 299-303, 2008.
36. Han, M., et al., In vivo remineralization of dentin using an agarose hydrogel biomimetic mineralization system. Scientific reports, 2017. 7: p. 41955.
37. Cao, Y., et al., Enamel prism-like tissue regeneration using enamel matrix derivative. Journal of dentistry, 2014. 42(12): p. 1535-1542.
38. Zaharia, A., et al., Human Dentine Remineralization Under Non-colagen Materials Action. REVISTA DE CHIMIE, 2017. 68(5): p. 928-932.
39. Jose P, Sanjeev K, Sekar M. Effect of green and white tea pretreatment on remineralization of demineralized dentin by CPP-ACFP-An invitro microhardness analysis. J Clin Diagn Res. 2016;10:85–9.
40. Abdelaziz RH, Mohamed AA, Talaat DM. Effect of two remineralizing agents on mirohardness of initial enamel caries like lesions in young permanent teeth. Alex Dent J. 2019; 44: 45-49.
41. Joshi M, joshi N, Kathariya R, Angadi P, Raikar S. Techniques to Evaluate Dental Erosion: A Systematic Review of Literature. J Clin Diagn Res. 2016 Oct;10(10):ZE01-ZE07.
42. Paryag A, Rafeek R. Dental erosion and Medical Conditions: An Overview of Aetiology, Diagnosis and Manengement. West Indian Med J.2014 Sep;63(5):499-502.
43. Park SM, Shin MS, Kong MS, Kin HD: Distribution of dental erosion and its related factors among workers in factories that use acids. J Korean Acad Oral Health 40: 222-230, 2016. https://doi.org/10.11149/jkaoh.2016.40.4.222
44. Reddy A, Norris DF, Momeni SS, Waldo B, Ruby JD: The pH of beverages in the United States. J Am Dent Assoc 147: 255-263, 2016. https://doi.org/10.1016/j.adaj.2015.10.019.
45. Arifa MK, Ephraim R, Rajamani T. Recent Advances in Dental Hard Tissue Remineralization: A Review of Literature. Int J Clin Pediatr Dent. 2019 Mar-Apr;12(2):139-144.
46. Li,Y.; Liu,M.; Xue,M.; Kang, Y.; Liu, D.; Wen,Y.; Zhao,D.; Guan,B. Engineered Biomaterials Trigger Remineralization and Antimicrobial Effects For Dental Caries Restoration. Molecule 2023,28,6373.
47. Nimbeni,S.B.; Nimbeni,B.S.; Divakar, D.D. Role of Chitosan in remineralization of enamel and dentin. A Systematic Review. Int.J.Clin. Pediatr. Dent. 2021,14,562-568.
48. Li, H., et al., Gel incorporation inside of organic single crystals grown in agarose hydrogels. CrystEngComm, 2011. 13(4): p. 1060-1062.
49. Daood, U., et al., Effect of chitosan/riboflavin modification on resin/dentin interface: spectroscopic and microscopic investigations. Journal of Biomedical Materials Research Part A, 2013. 101(7): p. 1846-1856.
50. Persadmehr, A., et al., Bioactive chitosan nanoparticles and photodynamic therapy inhibit collagen degradation in vitro. Journal of endodontics, 2014. 40(5): p. 703-709.

51. Ruan Q, Liberman D, Bapat R et al. Efficacy of amelogenin-chitosan hydrogel in biomimetic repair of human enamel in pH-cycling systems. Journal of biomedical engineering and informatics, (2016);2(1): 119.
52. Simeonov M, Gussiyska A, Mironova J et al. Novel hybrid chitosan/calcium phosphates microgels for remineralization of demineralized enamel–a model study. European Polymer Journal, (2019);119: 14-21.
53. Cao, Y., et al., A novel oligopeptide simulating dentine matrix protein 1 for biomimetic mineralization of dentine. Clinical oral investigations, 2014. 18(3): p. 873-881.
54. Fatima Saeed J. Al-Ward1 *, Nada Jafer MH. Radhi. Combined Effect of Nanohydroxyapatite and Chitosan on Remineralization of Initial Enamel Lesion in Vitro. The Egyptian Journal of Hospital Medicine (January 2023) Vol. 90, Page 107- 112.
55. Zaharia, A., et al., Human Dentine Remineralization Under Non-colagen Materials Action. REVISTA DE CHIMIE, 2017. 68(5): p. 928-932.
56. Wu, X.-T., et al., An electrophoresis-aided biomineralization system for regenerating dentin-and enamel-like microstructures for the self-healing of tooth defects. Crystal Growth & Design, 2014. 14(11): p. 5537-5548.
57. Jiang,F.; Xu,X-W.; Chen,F-Q.; Weng, H-F.; Chen,J.; Ru,Y.; Xiao, Q.; Xiao, A-F. Extraction, Modification and Biomedical Application of Agarose Hydrogels: A Review. Mar. Drugs 2023,21,299.
58. M. Edén. Structure and formation of amorphous calcium phosphate and its role as surface layer of nanocrystalline apatite: Implications for bone mineralization. Materialia 17 (2021) 101-107. https://doi.org/10.1016/j.mtla.2021.101107.
59. Kim, J., et al., Functional biomimetic analogs help remineralize apatite-depleted demineralized resin-infiltrated dentin via a bottom–up approach. Acta biomaterialia, 2010. 6(7): p. 2740-2750.
60. Ning, T.Y., et al., Biomimetic mineralization of dentin induced by agarose gel loaded with calcium phosphate. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 2012. 100(1): p. 138-144.
61. Denis T. Yucesoy, Hanson Fong, John Hamann, Eric Hall, Sami Dogan, and Mehmet Sarikaya. Biomimetic Dentin Repair: Amelogenin-Derived Peptide Guides Occlusion and Peritubular Mineralization of Human Teeth. ACS Biomater.Sci.Eng.2023,9,1486-1495.
62. Cao, C.Y., et al., Methods for biomimetic remineralization of human dentine: a systematic review. International journal of molecular sciences, 2015. 16(3): p. 4615-4627.
63. Muşat, V.; Anghel, E.M.; Zaharia, A.; Atkinson, I.; Mocioiu, O.C.; Buşilă, M.; Alexandru, P. A Chitosan-Agarose Polysaccharide-Based Hydrogel for Biomimetic Remineralization of Dental Enamel. Biomolecules 2021, 11, 1137. https://doi.org/10.3390/ biom11081137.
64. Husain, S., et al., Chitosan biomaterials for current and potential dental applications. Materials, 2017. 10(6): p. 602.
65. Gopinathan G, Jin T, Liu M, Li S, Atsawasuwan P, Galang M-T, Allen M, Luan X and Diekwisch TGH .The expanded amelogenin polyproline region preferentially binds to apatite versus carbonate and promote apatite crystal elongation. Front. Physiol. (2014); 5:430.
66. Lu, J.-x., Y.S. Xu, and W.J. Shaw, Phosphorylation and ionic strength alter the LRAP–HAP interface in the N-terminus. Biochemistry, 2013. 52(13): p. 2196-2205.
67. Le Norcy, E., et al., Leucine-rich amelogenin peptides regulate mineralization in vitro. Journal of dental research, 2011. 90(9): p. 1091-1097.
68. Margolis, H.C., S.-Y. Kwak, and H. Yamazaki, Role of mineralization inhibitors in the regulation of hard tissue biomineralization: relevance to initial enamel formation and maturation. Frontiers in physiology, 2014. 5: p. 339.
69. Fan, Y., Z. Sun, and J. Moradian-Oldak, Controlled remineralization of enamel in the presence of amelogenin and fluoride. Biomaterials, 2009. 30(4): p. 478-483.
70. Ruan, Q., et al., Amelogenin–chitosan matrix for human enamel regrowth: effects of viscosity and supersaturation degree. Connective tissue research, 2014. 55(sup1): p. 150-154.