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EFFECTIVENESS OF ORGANIC VERSUS INORGANIC ANALOGUES FOR TREATMENT OF WHITE SPOT LESIONS: AN IN VITRO STUDY

Moshera Hamed Yaseen Abd-Elbar[1*], Enas Mahmoud El-Ddamony[2], Rania El-Saady Badawy[3]

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- E-mail address: mosherahamdi@gmail.com

1. Demonstrator in Dental materials Department, Faculty of Dentistry, Hours University
2. Lecturer of Dental Materials, Faculty of Dentistry, Suez Canal University
3. Associate Professor of Dental Materials, Faculty of Dentistry, Suez Canal University

ABSTRACT

Introduction: Demineralization denotes the dissolution of the calcium and phosphate ions from the hydroxyl-apatite/tooth structure into the plaque and saliva caused by the acidic attack. White spot lesions start at the outermost layer of the teeth, damaging the dental hard tissues through the loss of mineral ions from the hydroxyapatite lattice at the surface. An ideal remineralizing agent should mimic the organization and micro-architecture of natural tooth structure mineral crystals to the greatest extent possible. **Aim: S**tudy aimed to evaluate and compare the effect of two remineralizing agents on remineralization of initial enamel carious lesions. Furthermore, the impact of both remineralizing agents and one infiltrating resin material on the color of demineralized enamel and their ability to mask white spot lesions was investigated. **Subjects and Methods:** 50 intact, freshly extracted permanent human anterior central incisors (extracted from diabetic patients) were selected and stored in distilled water at room temperature (22^{0}C). Selected teeth were classified into two main groups (Control and Demineralized) according to the type of treatment. **Results:** Significant differences resulted between treatments for ΔE at p<0.001. Demineralized enamel showed the highest significant color change values (6.03±0.4). All treatments of demineralized enamel significantly restored the tooth color. **Conclusion:** Curodont Repair and MI Paste Plus can fill up defects and micropores on demineralized tooth structure, significantly restoring the tooth color.

INTRODUCTION

Tooth decay, or dental caries, is an widespread, microbiological infectious teeth disease resulting in localized dissolution and deterioration of the calcified dental structure[(1)]. It is a multifactorial disease that occurs because of interactions within the plaque community, host physiology, oral flora, diet, fluoride, pH, and the nature of the tooth enamel.

Moreover, dental caries is a dynamic process produced by the imbalance between the demineralization and remineralization of the hard tooth structure[(2)].

Demineralization is the release of calcium and phosphate ions from the hydroxyl-apatite/tooth structure into the plaque and saliva as a result of an acidic assault. Dietary acid absorbed in food or beverages microbiological assault by intraoral bacteria(3). On the other hand, the process of remineralization involves the deposition of calcium and phosphate, and other ions from the saliva into the previously demineralized tooth structure(4).

While both processes may occur concurrently in the oral cavity, a lesion is formed only when the rate of demineralization surpasses the rate of remineralization(3). Though this balance is not restored by early intervention measures, including remineralization treatment and dental plaque control, caries gradually propagate from enamel to dentine, leading to tooth cavitation and further loss of tooth structure(5).

The null hypothesis (H_0) tested; was that no variation exists between the organic and inorganic analogous treatment of demineralized enamel lesions regarding their remineralization ability and color restoration.

MATERIALS AND METHODS

I- Materials:

Materials used in this study are listed in Table 1.

Table (1) *Brand name, Composition, and Manufacturer of the Materials*

Brand name	Composition	Presentation	Manufacturer
1. CURODONT ™ REPAIR	Each Curodont cylinder contains Smart self-assembling peptide (P11-4) molecules water for activation. The applicators are coated with the active substance (FMOC-peptide synthesis	Cylinders containing 0.05 ml solution	Credentis AG, Windisch, Switzerland
2. MI Paste Plus	Pure water, glycerol, CPP-ACP, d-sorbitol, CMC-Na, propylene glycol, silicon dioxide, titanium dioxide, xylitol, phosphoric acid, sodium fluoride, butyl p-hydroxybenzoate (available in 5 flavors: melon, mint, strawberry, tutti-frutti, and vanilla).	The tube containing 40g (35ml) paste	GC America Inc.
3. ICON ®	Methacrylate-based resin matrix, TEGDMA, initiators, additives	(0.30 ml) syringe etch, (0.45 ml) syringe dry, (0.45 ml) syringe infiltrant, and six smooth surface tips	(DMG, Hamburg, Germany)
4. Demineralizing solution	1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, 50 mM CH3COOH, and 3 mM NaN_3.	Liquid	Pharmacology Department, Faculty of Pharmacy, Mansoura University

II -Methods:

II.1 Teeth selection and Sample Grouping (Randomized Regimen):

A total of 50 intact, freshly extracted permanent human anterior central incisors (extracted from diabetic patients) were selected for this study. Immediately after extraction, teeth were thoroughly washed under running water to remove blood and mucous. The teeth were scaled to remove calculus and remnants of periodontal ligaments.

II.2 Creation of White-Spot Lesions (WSLs):

To designate the test area, the enamel surface of each tooth was partly covered with two successive layers of acid-resistant nail varnish (Amanda Long Lasting, Milano, Italy), leaving an experimental window of sound enamel (3x3 mm). A rectangular piece of modeling wax temporarily covered the window of sound enamel until drying of the nail varnish[6].

For demineralization, the piece of wax was removed. The specimens were held by their roots and placed individually into plastic tubes filled with demineralizing solution (1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, 50 mM CH_3COOH, and 3 mM NaN_3.) at pH 4.8 (30 mL) for 72 hours, which was changed daily (every 24 h). After3 days of immersion (**figures 1**), the superficial lesion of specimens was subjected to surface analysis by (SEM)[7]. For treatment, all samples were taken out of the solution, rinsed with running tap water for 5 min, then rinsed again with distilled water for 30 s and dried with oil-free air spray to be visualized for their chalky white appearance. After detecting the opaque white area, an acid etchant of 35% phosphoric acid solution (Ultradent Products Inc., South Jordan, Utah, USA) was applied to the chalky area of the tooth for 30 seconds, which was followed by rinsing with distilled water, and then air-dried. This treatment effectively removed the white opaque layer, exposing the WSLs [8].

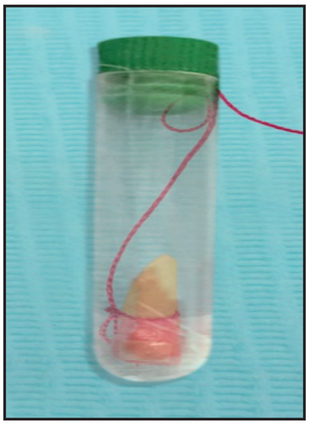

Fig. (1) Incisors placed in demineralizing solution, in plastic tubes.

II.3 Remineralization of Teeth

Demineralized teeth were further classified into three subgroups, according to the type of repair material used (either being remineralized or resin-infiltrated) in a single application method[9]. All teeth were stored in distilled water at 37°C until tested after 1 week. After rinsing the surface of the demineralized tooth with water and drying, the treatment material was applied according to the manufacturer's instructions, followed by storage in distilled water until characterization.

III. Materials Characterization

III.1. SEM Examination and Elemental Analysis (EDXA) Testing

A SEM (Model Quanta 250 FEG (Field Emission Gun) attached to an EDX unit (Energy Dispersive X-ray Analysis), with accelerating voltage 30 KV and a magnification of 14x up to 1000000 resolutions for Gun.1n. (FEI Company, Netherlands), was used to examine the enamel surfaces for each group (control, demineralized, treated).

All teeth were dried before characterization, and if the exposed enamel area was not visible and measurable, the tooth was discarded.

RESULTS

Characterization of the Materials

SEM Examination

SEM examination of the untreated enamel (control group) revealed a smooth, regular, and intact surface of the flat polished enamel with fine scratches. The structural arrangement was characteristic of the normal enamel (keyhole pattern) with no morphological irregularities, with an observed uniformity of the prismatic surface layers, as shown in Figure (2A).

Scanning electron microscopy (SEM) of the demineralized enamel showed an irregular surface and dissolved prism cores, denoting surface dissolution. Also, the surface revealed a honeycomb pattern with a marked increase in surface porosities figure 2B.

SEM Examination and EDAX of White spot lesion treated with Curodont Repair (DT_1): SEM micrographs showed the honeycomb pattern of enamel. Enamel prisms showed very high deposition of minerals that were limited to scanty aggregated particles near the interprismatic enamel, leaving the prism cores nearly vacant, as shown in figure 2C.

SEM Examination and EDAX of White spot lesion treated with MI Paste Plus: SEM micrographs showed that the honeycomb pattern of demineralization was visible in most of the irregular surfaces of enamel. However, Cotton-like globular particles filled almost the entire enamel cores with partial coverage of the opened prisms, as shown in figure 2D.

SEM Examination and EDAX of White spot lesion treated with Icon resin infiltrant: SEM micrographs showed deposition of sponge-like aggregates within the enamel prism cores (Figure 2E).

Results of Elemental analysis

Mean values of the elements for control and differently treated enamel surfaces are graphically presented in figure (3). The results revealed that demineralized enamel surfaces treated with Curodont Repair (DT_1) showed the highest calcium (Ca) and phosphorous (P) values ($27.6{\pm}0.3^{a}$, $14.1{\pm}0.5^{a}$), followed by MI Paste Plus (DT_2) ($21{\pm}2.2^{b}$, $10.4{\pm}1^{b}$) respectively. Icon (DT_3), on the other hand, showed Ca and P values ($17.8{\pm}0.4^{c}$, $9.1{\pm}0.1^{bc}$), which are like the Ca and P values for demineralized enamel ($17.1{\pm}\ 0.2^{c}$, $8.2{\pm}0.3^{c}$), but lower than those of the control group (19.2 ± 0.2^{bc}, 9 ± 0.5^{bc}) (Figure 3).

Accordingly, control enamel revealed the highest Ca:P ratio $2.2{\pm}0.1^{a}$, followed by demineralized enamel surfaces ($2.1{\pm}0.1^{a}$) and finally demineralized enamel surfaces treated with remineralizing or resin infiltrant materials (2 ± 0^{a}).

The highest mean carbon (C) content was detected in demineralized enamel surfaces ($29.2{\pm}1.5^{a}$), followed by the control enamel ($27{\pm}3.2^{a}$).

The results also revealed that oxygen content in demineralized enamel surfaces treated by Curodont Repair (DT_1) possessed the highest mean value ($45.7{\pm}0.8^{a}$), followed by those treated with MI Paste Plus™ ($34.6{\pm}7.1^{a}$) compared to the rest of the surfaces.

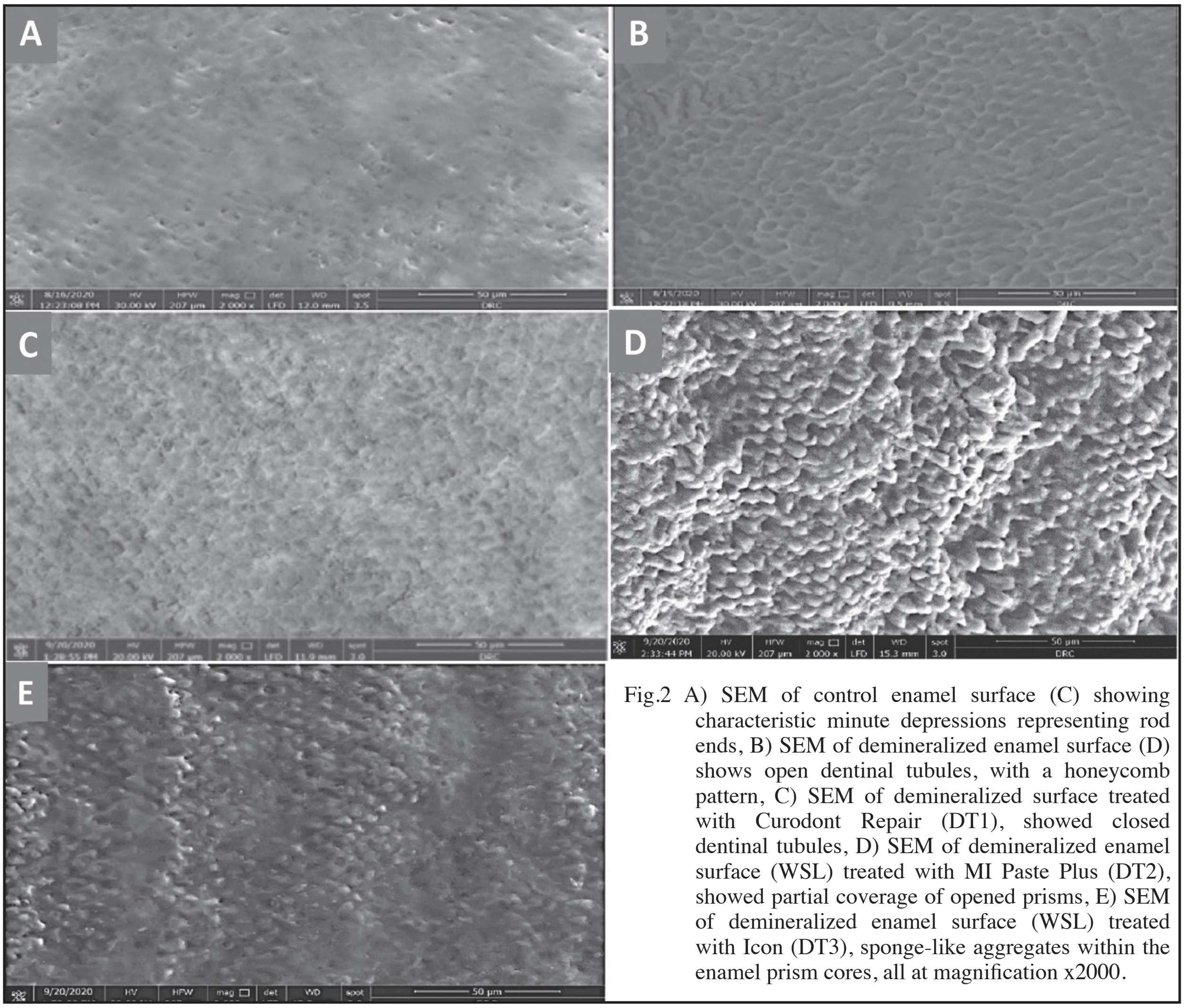

Fig.2 A) SEM of control enamel surface (C) showing characteristic minute depressions representing rod ends, B) SEM of demineralized enamel surface (D) shows open dentinal tubules, with a honeycomb pattern, C) SEM of demineralized surface treated with Curodont Repair (DT1), showed closed dentinal tubules, D) SEM of demineralized enamel surface (WSL) treated with MI Paste Plus (DT2), showed partial coverage of opened prisms, E) SEM of demineralized enamel surface (WSL) treated with Icon (DT3), sponge-like aggregates within the enamel prism cores, all at magnification x2000.

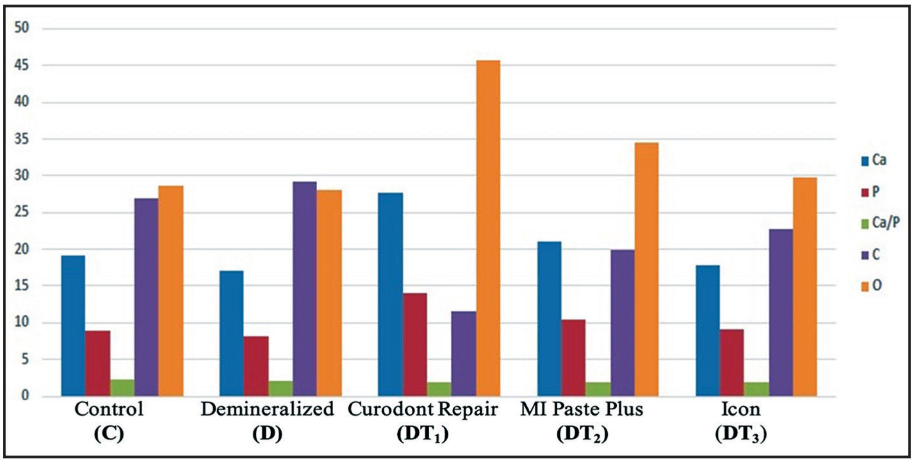

Fig. (3) Concentrations (at %) of elements on differently treated enamel surfaces. **Abbreviations: D:** Demineralized enamel surface, **DT_1:** Demineralized enamel treated with Curodont Repair, **DT_2:** Demineralized enamel treated with MI Paste Plus, **DT_3:** Demineralized enamel treated with Icon. Different lowercase letters denote significantly different values.

DISCUSSION

Dental caries are a major public health problem influenced by several pathological factors, mainly acidogenic bacteria, salivary dysfunction, and dietary carbohydrates. These factors interact together to finally result in 'demineralization' of the tooth structure due to dissolving its carbonated hydroxyapatite minerals[10–12]. The process of dental caries covers a continuum starting from the first molecular changes in apatite crystals of the tooth to a visible White spot lesion (WSL), passing through the dentin, to finally result in cavitation[11]. The continual imbalance between pathological and protective factors results in the dissolution of apatite crystals and a net loss of Ca, P, and other ions from the tooth, known as 'demineralization'.

WSL is considered the earliest evidence of dental caries, which can be caused by an imbalance between demineralization and remineralization processes[13]. White spot lesions can appear on any tooth surface where the microbial biofilm can develop and remain for a prolonged period[14,15].

The decrease in the mineral content beneath the intact enamel surface is due to the obvious dissolution of apatite crystals, accompanied by the final loss of ions, including calcium and phosphate, from the tooth structure (demineralization). As demineralization progresses, the inter-crystalline spaces enlarge, and the lesion becomes more visible without air drying[16].

Before cavitation of the enamel occurs, therapeutic agents can be used to remineralize and reverse the caries process[17].

Therefore, modern dentistry mainly plans to manage early carious lesions prior to cavitation. This can be achieved through remineralization to stop disease progression and thus improve aesthetics, strength, and function. However, even after remineralization and arrest of the caries process, the unsightly white spots often persist.

This study aimed to evaluate the effect of two different commercially available remineralizing agents and a resin infiltrant on the surface composition and color change of artificially induced white spot enamel lesions. A demineralizing solution was applied to the tooth surfaces to simulate early enamel lesions to create caries-like subsurface enamel lesions with an intact surface.

An SEM assessment of differently treated enamel surfaces was performed in this study. The results revealed a smooth enamel surface with few cracks before demineralization. However, after demineralization, irregular surface and dissolved prism cores denoting surface dissolution were detected on the surfaces of artificial carious lesions. Both remineralizing agents, Curodont Repair and MI Paste Plus, filled up defects and micropores on demineralized tooth structure. This finding may be explained by precipitation of a new HA layer, sealing micropores, and defects, as previously declared in several studies conducted on the effect of remineralizing agents and CPP-ACP products[18–20].

These results agreed with **Kirkham et al.,**[21] showing that monomeric self-assembling peptide Curodont Repair (P11-4) diffuses into the subsurface of early caries lesions and self-assembles into 3-D fibrillar scaffolds in response to local conditions of high ionic strength and acidic pH within the lesion body. As shown in earlier studies by X-ray diffraction and energy-dispersive X-ray spectroscopy, the assembled P11-4 scaffold can promote de novo hydroxyapatite crystal nucleation and supports mineral crystal growth in the process of biomimetic mineralization. It can regenerate minerals for enamel repair using a method analogous to the enamel matrix during enamel formation.

Furthermore, **Brunton et al**.[22], and **Jablonski-Momeni et al**.[23], and **Schlee et al**.[24] have declared that P11-4 promotes subsurface remineralization in the presence of saliva, both in vitro and in vivo.

Takahashi et al.[25] revealed by SEM (SEM) that the treatment of demineralized enamel surfaces with P11-4 could inhibit demineralization and facilitate hydroxyapatite crystal formation. P11-4 is now the basis of a product, Curodont Repair, which is approved and available for clinical use in treating early enamel lesions.

Results also revealed an increase in Ca (27.6±0.3) and P (2.0±0.0) percentage for Demineralized enamel treated with Curodont Repair. Due to the combination impact of enhanced mineral acquisition and prevention of mineral loss, peptide therapy greatly boosted net mineral gain. Additionally, P11-4 in its assembled state triggered de novo hydroxyapatite nucleation. Self-assembling peptides, based on these results, may be effective for modulating mineral behaviour during in situ dental tissue engineering[21].

These results agreed with **Wierichs et al.**[26], who assessed the effectiveness of approved and new treatment approaches to inhibit demineralization and, as a result, hide simulated enamel caries lesions. The application of a self-assembling peptide to nonactivated caries lesions is intended to enhance remineralization and conceal their unpleasant look. **Kobeissi et al.**[27] compared the effectiveness of the SAP11-4 vs. tricalcium phosphate fluoride (TCPF) in remineralization of WSLs in young permanent teeth. SAP11-4 was effective in treating WSLs. However, the success of guided enamel regeneration by the SAP11-4 through biomineralization has proven the superiority of this material.

Higher carbon percentage was recorded in Control and Demineralized enamel surfaces. The enamel in question has a high carbon content but 20% lower calcium and phosphate concentrations[28].

Prasada et al.[6] reported that inner enamel surfaces displayed higher carbonate content compared to the outer enamel. Comparison based on the intensity ratio of carbonate to phosphate peaks. Results also showed decreased C percentage for remineralized groups. This may be due to their ability to deliver mineral ions to rebuild the outer mineral-rich enamel layer covering the inner carbon-rich layer.

It is worth mentioning that enamel surfaces treated by Icon and MI Paste Plus revealed a higher carbon percentage (22.8±5.2and 19.9±6.8 respectively) compared to Curodont Repair (11.6±1.4). This finding may be due to the carbon-rich organic compounds present in Icon and MI Paste Plus.

CONCLUSIONS

1. Both remineralizing agents, Curodont Repair and MI Paste Plus, are approved and available for clinical use in the treatment of early enamel lesions, as they fill- up defects and micropores on demineralized tooth structure.
2. Curodont Repair showed best values in remineralization of demineralized teeth, as P11-4 facilitate remineralization and mask their unfavorable appearance.

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