Evaluation of The Micromorphological Patterns and Chemical Changes of Enamel surface Following Application of Three Remineralizing Agents

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Abstract:
Aim: To 1) Quantitatively evaluate the remineralizing potential of Icon infiltrant, CPP-ACP containing paste and fluoride gel on artificial enamel subsurface lesions using scanning electron microscopy with energy dispersive x-ray analysis (SEM-EDX), and 2) Evaluate the micro-morphological patterns of enamel subsurface lesions.
Materials and Methods: 80 freshly sound extracted human incisors were collected for this study.Ten teeth (C1) were left intact (positive control). 70 teeth used to create artificial enamel lesion by using 37% phosphoric acid gel. Another ten teeth (C2) left after decalcification (negative control). The remaining sixty teeth were randomly divided into three experimental equal groups (20 teeth each): Group 1: resin infiltrant, Group 2: CPP-ACP tooth mousse and Group 3: Sodium fluoride gel. Each group was further subdivided into two sub-groups (10 teeth each), subgroup A: examined after 24 hours and subgroup B: examined after six months storage in artificial saliva. Each material was applied following manufacture instructions. Each sample was sectioned into two halves longitudinally in the facio-palatal plane. were observed under an enviromental scanning electron microscopy (SEM) after 24 hours following the treatment procedures, as well as, after six months aging in artificial saliva. The other half will be subjected to elemental analysis under high-vacuum using energy dispersive x-ray system (EDX).
Results: The results of this study showed that after remineralization, there was a significant difference between the groups (P>0.001) when calcium and phosphorous ratios (Ca:P) were compared as seen in one-way ANOVA test, showing greater potential of remineralization for CPP-ACP followed by fluoride and Icon group. CPP-ACP was better in immediate and delayed groups to be followed by fluoride and finally infiltrant.
Conclusions: Under the limitations of this study, the outcomes reveled that CPP-ACP performed better than fluoride and Icon in remineralizing the demineralized enamel.
Keywords: Remineralization, demineralization, incipient lesion, resin infiltration, SEM, EDX.

Introduction

In dentistry, a white spot lesion (WSL) can be described as a localized area of enamel porosity that is caused by tooth minerals loss [1] from the deep layers of enamel while the surface enamel is somehow sound.[2] The clinical evidence of such WSL can be noted as early as 2 weeks from the formation of the initial biofilm.[3]
Recently, caries diagnosis and management have been massively improved due to the advanced knowledge of caries formation. Caries formation is an active process of sequential stages of demineralization/remineralization.[4] A carious lesion may progress, revert or stay unchanged based on a stability between demineralization and remineralization phases.[4] Advancement of sciences helped us to further develop the management of dental caries; moving it in the direction of biological therapeutic application rather than surgical restorative application. This is what we call "minimal intervention dentistry".[4]
Minimal intervention dentistry (MID) means using the least invasive techniques to save most of the tooth structure and patient's pain.[5, 6] MID is depends mainly on potential remineralization of early carious lesions by using different remineralizing agents.[5]
Casein phospho-peptide amorphous calcium phosphate complex (CPP-ACP) is used in some remineralizing agents and its application has reflected advantageous results in limiting the progression of early carious lesions. However, the applications of CPP-ACP in a paste form, have shown controversial outcomes[7-10] and it is crucial to determine the benefits of different forms of remineralizing agents before their clinical application recommendations.[11]
Fluoride has an essential cario-static effect on initial dental lesions, when it creates calcium fluoride structures that is not soluble in the oral conditions. However, it is still crucial to evaluate the amount of fluoride absorption into demineralized enamel lesions after its topical application and to further determine its effect on enhancing enamel resistance to future decay.[12]
The use of these effective therapeutic approaches is still questionable in esthetic areas due to the limitation of remineralization taking place only at the lesion surface.[13-15] In addition, significant time is needed to achieve the required remineralization results which happen only when these therapeutic approaches are applied at the initial stages of lesion formation and the patient's compliance is another important factor to consider.[14]
Recently, resin infiltration technique has been developed. This low-viscosity resin can stop the initial enamel lesion by infiltrating into enamel, arresting further diffusion of demineralizing agents into enamel.[16, 17] Moreover, this resin mechanically supports the enamel form and prevents enamel cavitation.[18-20] Many *in vitro* reports [21-23] evaluated resin penetration into artificially produced carious lesions,

using different adhesives and sealants have found that these materials act as diffusion barriers at the lesion surface with very superficial penetration.[24] Therefore, a "low viscosity resin with a high penetration coefficient" was needed to address the limitation of these materials. Additionally, this low viscosity resin has the ability to lose its white non-transparent color and restore the healthy enamel shade and translucency.[15, 25-27]

The main goal of modern dentistry is to "non-invasively" handle the non-cavitated dental lesions by enhancing remineralization to control lesion disease progression, restore tooth form, esthetics, strength and function.[28] Therefore, this research project was carried out to investigate the efficacy of three remineralizing agents; Icon resin infiltrant, CPP-ACP paste and Fluoride gel.

Materials And Methods

This in-vitro study was carried out on three types of incipient caries treating agents with different chemical compositions: A resin infiltrating material; Icon (Dental material company, DMG, Hamburg, Germany), CPP-ACP-containing paste; GC Tooth Mousse (Recaldent GC Corp., Tokyo, Japan) and 1.1% sodium fluoride gel; Flor-Opal (Ultradent Products, Inc., USA).

A total of 80 freshly extracted human incisors free of caries, defects or restorations were collected from patients scheduled for complete denture construction. The teeth were collected after obtaining patient's informed consent to use their extracted teeth for research purposes under a protocol approved by the Institutional Review Board of the Mansoura University. The collected teeth were stored in 2% sodium azide solution for 3 days, then pumiced and kept for one day in distilled water before use.[19, 29]

Twenty teeth were left as control groups which are divided into two equal groups (10 per each): C1, intact teeth without decalcification or treatment (positive control) and C2 (negative control) that were just undergone decalcification with the use of 37% phosphoric acid (Super Etch, SDI, Victoria, Australia). The remaining sixty teeth were randomly divided into three experimental groups (20 teeth each) based on the three remineralizing materials used, Group 1: resin infiltrant, Group 2: CPP-ACP tooth mousse and Group 3: Sodium fluoride gel. Each group was further subdivided into two sub-groups (10 teeth each), subgroup A: examined after 24 hours and subgroup B: examined after six months storage in artificial saliva.

Preparation of artificial carious lesion

For each tooth in the negative control group and experimental groups, phosphoric acid gel was applied on the labial surface of each tooth and left for 20 minutes to demineralize the sandblasted enamel surface sufficiently to simulate early enamel carious lesions.[30-32] Teeth were then washed under distilled deionized water then, air dried and stored till treatment.

Application of treating agents

Each material was applied according to manufacture instructions as follow:

Resin infiltrant (Group 1):[20]

Each carious lesion produced was treated with resin infiltration. The application of resin was done as follows: 15% HCl was applied for 2 minutes on the lesion, and then etchant was rinsed for 30 seconds and air dried. About 99% ethanol was applied for 30 seconds and air dried followed by resin infiltrant application, which was left on the lesion for 3 minutes before curing. This was light cured for 40 seconds. The application of resin was repeated for 1 minute and again light cured for 40 seconds.

CPP-ACP tooth mousse(Group 2):

CPP-ACP tooth mousse was applied onto the artificial carious lesions, on enamel surfaces, and left for 3 minutes.[8, 33] Enamel surfaces were rinsed with distilled water and dried with water/oil free air stream, and then the specimens were stored in artificial saliva at 37±1°C. This step was repeated every 12 hours for 1 day in case of immediate specimens and 6 months in case of delayed specimens.[34]

Sodium fluoride gel (Group 3):

Approximately 2 mm of sodium fluoride gel was applied to the artificial carious lesions, on enamel surfaces, using a special mini brush. The gel was left in contact with tooth surface for 4 hours then washed with distilled water till the surface become clean, and dried with water/oil free air stream. This step was repeated every 24 hours according to manufacturer's instructions [6], so that it was applied for a single time in case of immediate specimens and for 6 months in case of delayed specimens. It should be recalled that artificial saliva was changed every 24 hours.

Specimens preparation for SEM and EDX

Each sample was sectioned into two halves longitudinally in the facio-palatal plane using a low-speed diamond saw under running water (Band Saw; ExaktApparatebau, Norderstedt, Germany). Then polished to a smooth surface by using silicon carbide paper with different grades of roughness. Then washed under distilled water for 30 s, dried with oil free air syringe. All specimens were observed under an enviromental scanning electron microscopy (SEM) after 24 hours following the treatment procedures, as well as, after six months aging in artificial saliva. The other half will be subjected to elemental analysis under high-vacuum using energy dispersive x-ray system (EDX).

Figure (1): labial surface after application of icon-infiltrant

Figure (2): CPP-ACP paste applied on the labial surface of the tooth

Results

The collected data were analyzed using the Statistical Package of Social Science (SPSS) program for Windows (Standard version 21). The normality of data was first tested with one-Shapiro test. Continuous variables were presented as mean±standard deviation (SD). Data were subjected to statistical analysis by one-way analysis of variance (ANOVA) to determine the progression of caries like lesions in terms of change in enamel mineral content (calcium and phosphate) using EDX method.

After remineralization, the results showed significant difference between the groups with p-value less than 0.05, when Ca:P were compared, showing greater potential of remineralizationfor CPP-ACP followed by fluoride and then Icon group regarding all immediate and delayed groups.

Table 1: One-way ANOVA test results for immediate and delayed samples regarding calcium level among the studied groups.

	Groups	ANOVA test (F value)	p-value
Immediate	Control (Normal) vs. tested groups	851.82	<0.001*
	Control (Demineralization) vs. tested groups	435.15	<0.001*
Delayed	Control (Normal) vs. tested groups	801.27	<0.001*
	Control (Demineralization) vs. tested groups	855.83	<0.001*

Table 2: One-way ANOVA test results for immediate and delayed samples regarding phosphorus level among the studied groups.

	Groups	ANOVA test (F-value)	p-value
Immediate	Control (Normal) vs. tested groups	89.15	<0.001*
	Control (Demineralization) vs. tested groups	18.32	<0.001*
Delayed	Control (Normal) vs. tested groups	93.10	<0.001*
	Control (Demineralization) vs. tested groups	68.71	<0.001*

Scanning electron microscopy evaluations and EDX reports

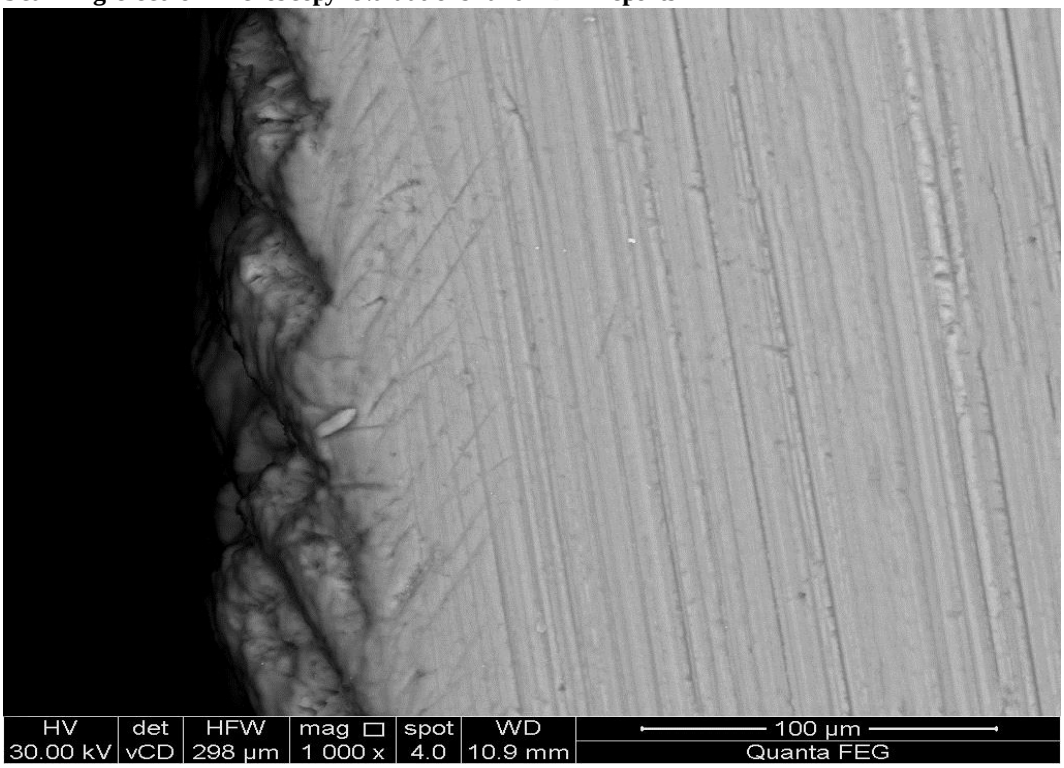

Figure (4): Structural analysis of demineralized enamel sample by SEM.

Graph 1: Elemental analysis of demineralized enamel sample by SEM- EDX analysis

Discussion

The naturally achieved remineralization of carious lesions, by salivary ions, can be enhanced by external factors or elements such as fluoride which is the most commonly used remineralizing agent. Consequently, developing new strategies for caries management and improvements in the remineralization process has become the prime demand in the last two decades.When the acid attacks enamel surface, the pH begins to go down, in presence of topical fluoride, new and larger crystals that contain more fluoride begin to form, thereby, reducing the enamel demineralization by forming fluoro-hydroxyapatite crystals and enhancing remineralization.[35] CPP-ACP creating stable systems capable of supplying bio-available calcium and phosphate directly to the lesion or to the surrounding biofilm.[36]Arresting enamel lesions by infiltration with resins is a promising approach for the medical model of incipient enamel carious lesions treatment.[20]The concept of resin infiltration aims at arresting the incipient enamel carious lesions by obstructing the diffusion pathways for acids and dissolved minerals in enamel.[16]

37 % phosphoric acid gel was selected for this study due to its easy availability.[31]Backscattered electron mode was used in this study, as it gives better mineral density images compared to the secondary electron method.[37]In this laboratory study,the CPP-ACP group provides the greatest remineralizing effect either immediately or after storage in artificial saliva after six months and there is a significant difference in comparison with the other tested materials. This achieved by continued remineralization patterns of CPP-ACP over time throughout the whole body of the lesion.[38]The decreased remineralization capacity of fluoride by time could be explained by that the topical application of a high concentration of fluoride gel presented higher initial rates of minerals deposition, which is lowered gradually with time.[6]In this study, the Icon group showed the least remineralizing efficacy among the tested groups in both immediate and delayed evaluations. This can be explained by the concept of resin infiltration itself. Paris et al.[39]compared the penetration depth following conditioning with 37% phosphoric acid or 15% HCl and concluded that etching with 15% HCl gel is more suitable than 37% phosphoric acid gel as a pretreatment for carious lesions intended to be infiltrated. About 99% ethanol used for dehydration before infiltrant application and the infiltrant

resin was applied twice over the lesion in order to occlude the space generated by the shrinkage. The application time was kept at 3 minutes according to manufacturer's instructions.

Limitations

As the study is limited to in vitro conditions, further clinical trials are necessary to assess the remineralization potential and longevity of Icon. As in vitro conditions may be different when compared to the in vivo with dynamic complex biological system.

Conclusion

The outcomes of this study revealed that CPP-ACP has the best remineralizing efficacy among all tested materials. Topical fluoride perform well as a superficial remineralizing agent. Icon showed the least remineralizing efficacy among all tested materials.

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