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The Remineralizing Effect of Incorporating Ca-Phosphate and Ca-Fluoride Nanoparticles into the Self-Etch Adhesives Used in Restoring Class I Cavities

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ABSTRACT

Purpose: This study was carried out to investigate the remineralizing effect of amorphous calcium phosphate (ACP) and calcium fluoride (CaF_2) nanoparticles incorporated in two-step self etch- adhesives (in vivo). **Materials and Methods:** The nanoparticles were incorporated into the bond of Clearfil™ SE Bond at a mass fraction of 10%. A total of ten patients in the age range of 17-30 years old, having at least 3 carious lower molars, were enrolled in this study (split mouth design). Class I cavity preparation was performed, leaving the caries affected dentine at the pulpal floor. The 30 molars were divided into 3 groups (n=10) according to the type of adhesive used (A); namely: self-etch adhesive, Clearfil SE Bond (Control)(A_1), Clear Fill SE Bond incorporated with ACP NPs (A_2) and Clearfil SE Bond incorporated with CaF_2 NPs (A_3). After restoration placement, each tooth was radiographically evaluated at baseline (I_0), after 1 month (I_1) and after 3 months (I_2) to assess the remineralizing effect of the adhesive systems used. **Results:** The adhesive containing ACP nanoparticles induced the highest remineralization potential during all the study intervals, followed by the adhesive containing (CaF_2) nanoparticles. **Conclusion:** nanoparticles are capable of remineralizing the caries affected dentin when incorporated into two-step self etch adhesive.

KEYWORDS

Calcium-Fluoride,
Calcium-Phosphate,
Nanoparticles,
Self-etch Adhesives

INTRODUCTION

The long-term clinical success of resin composite restorations depends mainly on the dentine bonding systems. Adhesion enhances sealing of the cavity margin, this is important for the protection of the restoration against development of secondary caries [(1)]. In latest decades,

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bonding to tooth substrates with self-etch adhesive (SEA) systems has gained popularity. Self-etch adhesive systems are characterized by; short time of application and lower technique sensitivity [2].

Dramatic changes of the role of adhesive dentistry have occurred from the end of the 20th century. From the mainly reparative dentistry of the 20th century, contemporary dentistry has undergone a shift towards what is known as minimal intervention (MI) approach[3]. This approach avoids unnecessary tooth sacrifice and leaves caries-affected dentin as the clinical bonding substrate. In case of the caries-affected dentin the collagen fibrils still show intermolecular cross-links and distinct cross-banding patterns, and therefore are physiologically remineralizable[4]. Reminearlization of remaining caries affected dentin may be encouraged by the use of bioactive, ion-releasing base materials [5].

Currently, nanotechnology is experiencing rapid growth, with many potential applications in dentistry [6]. Recently, nanoparticles have been incorporated into the dental adhesives. The filler particles have many advantages when added into dental adhesives. The fillers penetrate into the dentinal tubules leading to fortifying the bond strength of adhesive to dentin. Additionally the fillers tend to decrease polymerization shrinkage, and increase the modulus of elasticity of the adhesive layer [7]. The performance of dental adhesives may be enhanced in other aspects including the bioactivity. A bioactive dental material may induce remineralization of adjacent tooth substance, gaps closure between material and tooth and may improve bond strength over time [8]. In dental applications, the sustained release of calcium and phosphate is essential in providing super saturation conditions needed for the arrest of demineralization and enhancing remineralization [9].

Accordingly, this study aimed to incorporate amorphous calcium phosphate and calcium fluoride nanoparticles into two-step self-etch adhesive, in order to investigate the remineralizing effect of these nanoparticles on caries affected dentin in vivo.

MATERIALS AND METHODS

Preparation of Nanoparticles:

The nanoparticles were prepared using the spray-drying technique [10].

Characterization of Nanoparticles:

1. Fourier Transform Infra-Red Spectroscopy:

Fourier Transform Infra-Red (FTIR) spectra of the ACP, and CaF_2 NPs were obtained using FTIR spectrometer (FT/IR-6100, Spectrometer, Jasco, Deutchland). The powders were blended with potassium bromide at a mass ratio of 1:400, and then the formed mixture was pressed into a pellet 13 mm in diameter in an evacuated die. The FTIR spectrum was recorded in the range 400–4000 cm^{-1} with a resolution of 2 cm^{-1} [10].

2. Z- Potential Measurements:

Surface charge measurement of the nanoparticles was done following their suspension in a phosphate buffer solution pH 7.4. The Z potential values of the NPs were measured using a Zetasizer Nano ZS (Malvern Instruments Ltd., UK) at a temperature of 25 °C and were measured using laser Doppler anemometry [10].

3. Transmission Electron Microscope (TEM) and Selected Area Electron Diffraction:

The particle morphology, size and selected area electron diffraction (SAED) were examined by Transmission Electron Microscope (TEM) operating at 80 kV (model JEM-1230, Jeol, Tokyo, Japan)[10].

Patients' Selection:

Patients enrolled in this study were selected from the outpatient clinic of Operative Department, Faculty of Dental Medicine for Girls, Al-Azhar University. A total of ten patients in the age range of 17-30 years old were selected for this study. Each patient should have at least three carious molars, indicated for simple class I restoration (active and cavitated caries lesions extending to dentin but free of proximal caries, according to bitewing radiography), with a total of 30 molars for the study [11]. The patients were informed about the clinical procedures, experimental rationale and possible risks. Then they were asked to sign a written consent. Approval of the ethics committee at Faculty of Dental Medicine for Girls, Al-Azhar University was obtained.

Patients' Examination:

In the first appointment, a diagnostic sheet was made for every patient enrolled in the study. A detailed medical history was taken. A complete dental history and a thorough clinical examination were conducted on each of the selected cases. A periapical and bitewing radiographs of the selected tooth were taken before treatment to exclude any cases with pulp involvement, or periapical lesion. Impressions from the upper and lower arch are made using alginate impression (Cavex CA37 Dust-Free Alginate Impression Material, Fast Set). The alginate impressions were poured with dental plaster in order to obtain stone models. Jaw relation was used to mount the models onto the semi-adjustable articulator. The biteblock (part of the posterior parallel kit) was individualized for each patient with an acrylic resin (Pattern ResinH, GC America Inc., Alsip, IL). This was done in order to ensure that the radiographic technique is accurately reproduced [12,13].

Allocation of Patients to Treatment Groups:

The patients were subjected to split mouth design, where the side with one carious molar was allocated to control group (A_1), while on the other side, the first molar was allocated to ACP group (A_2), and the second molar was allocated to CaF_2 (A_3) group. Each molar tooth was finally restored with resin composite restoration. Then each tooth was radiographically evaluated at baseline (immediately after restoration) (I_0), after 1 month (I_1) and after 3 months (I_2) to assess the remineralizing effect of the adhesive systems used.

Preparation of Cavities:

Occlusal caries was removed using fissure bur with round end #245 (Komet, Lemgo, Germany) (1 bur/5 cavities) [11]. Caries was removed from everywhere in the cavity except for that on the pulpal floor, where only the overlying soft biological material was removed. Then caries indicating dye (Sable™ Seek® caries indicator, Ultradent products Inc, South Jordan, UT, USA) was used to differentiate between the superficial caries infected dentin and the deep caries affected dentin.

Application of the Self-Etch Adhesive:

According to each group, one of the three adhesives was used. The adhesives were applied into the prepared cavities, after rubber dam isolation. The manufacturer instructions were accurately followed. Primer was applied for 20 sec, mild air dry followed by the application of the bond, then mild air flow and light cured for 10 sec.

Restoring the Cavities with Resin Composite:

Resin composite (**Filtek™ Z250 XT Universal Restorative) (3M, ESPE**) was applied into the cavity using Teflon tipped instrument. Oblique increments (2 mm /each) of resin composite were inserted and light-cured (20 s) [11], using a LED light-curing unit of 470 wavelength (Elipar 3M, ESPE) with light intensity of 1000 mW/cm^2.

Radiographic assessment:

Patients were assessed radiographically; using intra-oral digital radiograph (X MIND, France) with exposure parameters were 70 Kilovoltage (Kv_p) and 0.63 milliampere (mA) using an image plate (size 2) (3x4) (Durr Dental, Germany). Then these images were transferred to the digital scanner. Barrier envelopes were used to wrap the image plate during imaging to prevent cross infection (Image plate protecting bag, No. SDT-XR57, smile dental, NiHEN). A posterior XCP parallel kit (Dentsply International

Inc, U.S.A) was used with special bite block customized for each patient for standardization and reproducibility. After irradiation using the posterior parallel kit the plate was then removed from the barrier envelope, scanned by Durr vista scan and the image was stored to the computer. The radiographic procedures were performed at baseline, 1 and 3 months intervals.

Assessment of Remineralization:

The radiodensity of the samples were measured using standaradized and reproducible method to assure proper calibration. The average values for image density were recorded, which was considered to be the sample's initial radiodensity (baseline) that was compared to other values throughout the follow up periods.

In each sample a line was drawn at the bottom of the cavity parallel to the cement-enamel junction (CEJ). The starting and ending points for this line were standaradized (for each sample) by readings (in radiodenisty) provided directly via the previously mentioned software. Such line was fixed for each sample throughout the assessment intervals **(Figure 1).**

These procedures were done at baseline, and then repeated during the follow up visits (1 and 3 months). Having all the conditions standardized, change in the radiodenisty of the image, indicated change in the remineralizaton of the dentin. The mean values (provided by the software) and percentage change in dentin radiodensity, for each tooth, at different intervals were calculated.

The percentage of change in image density (in vivo study) was calculated by the following formula:

$$\frac{\text{Value after-Value beforeX100}}{\text{Value before}}$$

Data was then tabulated and statistically analyzed.

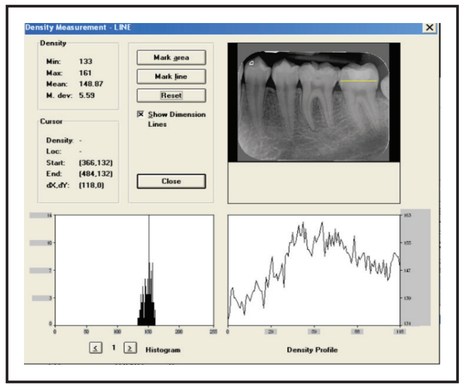

Figure (1): DBSWin software

STATISTICAL ANALYSIS

Statistical analysis was then performed using a commercially available software program (SPSS 19; SPSS, Chicago, IL, USA). As data related to image density were parametric, significance of the difference between different groups was evaluated using one way analysis of variance (ANOVA) test, followed by Tukey's post hoc test when ANOVA indicated a significant difference. The level of significance was set at $P < 0.05$

RESULTS

Table 1, Figure 2, showed that, in the first interval (baseline to one month), the highest mean percent increase was recorded in the calcium phosphate group (A_2) (15.44±5.32), whereas the lowest mean percent increase was recorded in control group (A_1) (3.41±1.28). ANOVA test revealed that the difference between groups was statistically significant (p=0.000). Tukey's post hoc test revealed no significant difference between calcium phosphate and calcium fluoride groups. In the second interval (one month to three months), the highest mean percent increase was recorded in the calcium phosphate (A_2) (11.05±3.05), whereas the lowest mean percent increase was recorded in Calcium fluoride (A_3) (4.13±0.97). ANOVA test revealed that the difference between groups was statistically significant (p=0.00). Tukey's post hoc test revealed a significant difference between each 2 groups.

Overall (baseline to 3 months), the highest mean percent increase was recorded in the calcium phosphate (A_2) (27.1±8.51), whereas the lowest mean percent increase was recorded in control group (A_1) (10.67±3.37). ANOVA test revealed that the difference between groups was statistically significant (p=0.00). Tukey's post hoc test revealed a significant difference between each 2 groups.

Table (1): *Comparison of percent change between study groups within the same interval (ANOVA test)*

	Groups	Mean	SD	P
Baseline to one month	Control (A_1)	3.41	1.28	0.000*
	ACP (A_2)	15.44[a]	5.32	
	CaF_2 (A_3)	14.96[a]	4.48	
One month to 3 months	Control (A_1)	7.03	2.52	0.000*
	ACP (A_2)	11.05	3.05	
	CaF_2 (A_3)	4.13	0.97	
Baseline to 3 months (Overall)	Control (A_1)	10.67	3.37	0.000*
	ACP (A_2)	27.1	8.51	
	CaF_2 (A_3)	19.43	6.83	

*Significance level p<0.05, *significant. Tukey's post hoc test: Mean values sharing the same superscript letter* **within the same comparison** *are not significantly different.*

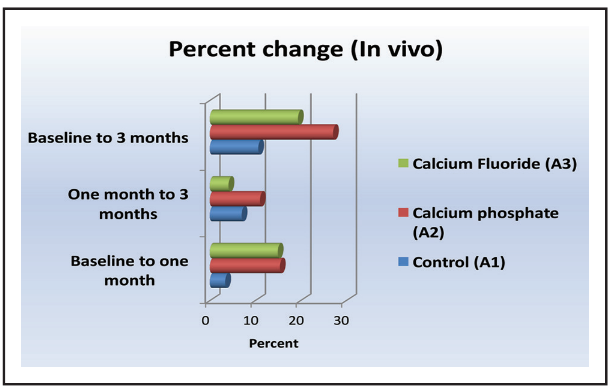

Figure (2): Bar chart showing percent change in different intervals in all groups

DISCUSSION

Dental adhesive technology has evolved towards complex formulations with simplified clinical procedures. Self-etch adhesives became more promising than other adhesive systems sue to the reduced technique sensitivity, shorter clinical application time and less incidence of post-operative sensitivity.

In vivo demineralization occurs with the dissolution of Ca and P ions from the tooth structure into the saliva. On the other hand, remineralization occurs with mineral precipitation into the tooth structure to increase the mineral content [14]. Amorphous calcium phosphate and calcium fluoride nanoparticles could play an important role in providing theses ions needed for remineralization, since they are characterized by much higher surface area than the traditional particles, thus leading to an increased ion release. Incorporation of these nanoparticles into adhesives, will lead to easier penetration into the dentin [14].

An adhesive material that is capable of supplying beneficial ions such as calcium, phosphate and fluoride needed for remineralization, would be useful for clinical applications. Therefore, this study aimed to evaluate the effect of incorporating amorphous calcium phosphate nanoparticles (ACP NPs), and calcium fluoride nanoparticles (CaF_2 NPs) into Clearfil™ SE Bond (two-step self-etch adhesive) on the remineralization of dentin in vivo.

Clearfil™ SE Bond adhesive system was chosen for this study. It is a two-step self-etch dental adhesive that contains methacryloxydecyl phosphate (MDP) as a functional monomer. This monomer chemically bond to Ca of hydroxyapatite (HAP) forming stable calcium-phosphate salts, along with only a limited surface-decalcification effect [15]. Additionally, being mild self-etch adhesive; it tends to keep collagen not only encapsulated and thus protected by HAP, but also provide the potential to chemically interact with HAP [16].

The ACP nanoparticles were used because of their good biocompatibility and bioactivity. Moreover, it is a precursor that can convert to apatite, similar to the minerals in tooth enamel and dentin[17]. Amorphous calcium phosphate nanoparticles release calcium and phosphate ions, thus inhibit demineralization and enhance remineralization[18]. Similary CaF_2 nanoparticles have been chosen because of the effectiveness of fluoride in promoting remineralization and inhibiting demineralization. The fluoride is incorporated into the minerals of the tooth as fluoroapatite or Fluoride-enriched hydroxyapatite, both are characterized by reduced solubility[19].

Clinically, the coronal dentine lesion can be divided into two structurally distinct zones. The outer, superficial zone that has suffered the ravages of the carious attack for the longest period and ultimately consists of irreversibly acid demineralized dentine. The collagen in this zone has been exposed to excessive proteolytic degradation, which prevents any reconstitution of the molecular crosslinks. The inner, deeper zone of the lesion is composed of dentine that has been reversibly attacked by the carious process and thus can be repaired when subjected to favourable conditions. It is thought that this zone should be retained at the cavity floor to allow remineralization to occur after placement of a suitable restoration [20].

A dye was developed, whose active ingredient was propylene glycol combined with a visible dye stain. This was said to bind to collagen cross-links and thus enabled discrimination between the two structural zones in carious dentine, reportedly staining only the outer, irreversibly damaged zone [21]. Sable™ Seek® caries indicator was selected for the in vivo part of this study as it was found to be the closest among caries indicators to neutrality.

Remineralization was assessed using digital imaging. Digital radiographic density measurements were used as it is a non invasive, alternative method to measure density changes which describes mineral gain or loss in dental tissues. Vista Scan-system was used to monitor density changes (pixel gray measurements)[22].

In the oral environment the adhesives are subjected to stresses, humidity, bacteria, thermal and pH fluctuation and many other factors that could affect its performance. Being able to induce remineralization of the caries affected dentin under such circumstances is considered one of the important advantages of the adhesive.

Results of the current study showed that, adhesive system containing ACP nanoparticles, showed the highest mean percent of change in the image density, from baseline to 3 months. This means it was the highest group capable of inducing remineralization to the remaining caries affected dentin, followed by calcium fluoride group then the control group. This may be due to the high release of Ca, P and F ions which are known for their remineralizing capabilities. The Ca, P and F ion release from adhesive may be highly beneficial and can serve as seed crystals to facilitate remineralization in hybrid layer and at the tooth-restoration margins. The higher capability of ACP nanoparticles to induce remineralization compared to the CaF_2 nanoparticles may be attributed to the amorphous nature of the calcium fluoride. This amorphous nature is characterized by more dissolution rate and higher ion release compared to the crystalline structure of CaF_2.

These findings are in accordance with previous studies that showed that adhesive containing ACP nanoparticles when placed in class I cavity in rats showed a tertiary dentin thickness that was five to six-fold that of the control adhesive without nanoparticles [23, 24]. Also, these findings are in agreement with other results that reported that that CaF_2 nanoparticles are effective in increasing the fluoride concentration in the oral fluids, this enhance tooth remineralization[25,26].

CONCLUSION

Under the conditions of the present study the following conclusions could be drawn, nanoparticles are capable of remineralizing the caries affected dentin when incorporated into two-step self etch adhesive. This could be a promising approach for the prevention of secondary caries at the cavity margins. Moreover, nanoparticles still have many aspects and useful applications in the field of dentistry.

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