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Remineralization Potential of Grape Seed Versus Amorphous Calciumphosphate-Nanoparticles on Sound and Caries Affected Dentin

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ABSTRACT

Objectives: This study was conducted to evaluate the remineralization potential of grape seed extract versus amorphous calcium phosphate nanoparticles on sound and caries affected dentin.

Materials and methods: Grape seed extract powder (GSE) was added to Clearfil SE primer with a concentration of 0.5 wt% to obtain primer + GSE while nanoparticles of amorphous calcium phosphate (NACP) were incorporated into the Clearfil SE adhesive at mass fractions of 20% to obtain adhesive + NACP. Class V cavity preparation was prepared on the anterior teeth of fifteen white rabbits. Rabbits were divided into three main groups according to the type of material used **Group A** (primer + GSE) **Group B** (adhesive + NACP), **Group C** (sodium fluoride NAF as positive control). Each group was subdivided into two subgroups according to type of substrate in which right side was act as sound dentin while left side was act as artificial caries affected dentin. After 10 days the dentin was examined using EDX analysis to determine amount of calcium and phosphorus in each sample. **Results:** There was no statistically significant difference between mineral content of different materials compared with positive control group for both sound and caries affected dentin. **Conclusion: -** Grape seed extract and nano amorphous calcium phosphate can produce remineralization of sound and caries affected dentin as sodium fluoride.

KEYWORDS

Grape seed extract, Calcium Phosphate Nanoparticles, Remineralization

INTRODUCTION

Dental caries is considered a highly prevalent disease that targets a large public [1]. It results from a dynamic imbalance present in the oral

A paper extracted from Doctor's Thesis entitled "Remineralization Potential of Grape Seed Versus Amorphous Calciumphosphate-Nanoparticles and their Effect on Bond Strength of Resin Composite to Sound and Caries Affected Dentin"

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environment in alternating periods of dissolution and mineral replacement leading to mineral loss[2]. Prevention of extension by remineralization of caries is highly desirable and is one of the cornerstones of minimal invasive dentistry [3].

Apart from caries, resin–dentin bonding is another major reason for dentin demineralization. The formation of resin–dentin bonds is accomplished predominantly by micromechanical retention via resin penetration and entanglement of exposed collagen fibrils in the partially or completely demineralized dentin. This is achieved by etching dentin with acids or acidic resin monomers derived from self-etching primers/adhesives to expose the collagen fibrils [4].

In recent years, much attention has been focused on research and education related to the identification of food components and development of food products seeking prevention and health promotion [5]. Proanthocyanidins (PA) are substances that have been the target of recent studies aiming to control or treat carious lesions[6]. In addition, PA increases the synthesis of collagen, accelerates the conversion of insoluble collagen to soluble collagen during development and decreases the rate of enzymatic degradation of the collagen matrix[7].

Grape seed extract (GSE) is a rich source of proanthocyanidin (PA), mainly composed of monomeric catechin and epicatechin, gallic acid and polymeric and oli-gomericprocyanidins [8]. Another approach is to incorporate calcium phosphate (CaP) particles into dental resins to promote remineralization and avoid demineralization [9]. Adhesives containing CaP particles could remineralize the remnants of tooth lesions in the cavity as well as the acid-etched dentin, and hence are promising to improve the longevity of the restorations [10].

Recently, bonding agents containing nanoparticles of amorphous calcium phosphate (NACP) were developed. These bonding agents could release high levels of Ca and P ions to induce remineralization and combat caries. The NACP adhesive was “smart” because it could substantially increase the Ca and P ion release at a low cariogenic pH when these ions would be most needed to combat caries. [11]

Even with the emergence of new preventive measures, fluoride is still considered one of the most prominent elements because it not only has chemical qualities but physiological properties as well, and is of great interest and importance to the dentist [12]. The effect of proanthocyanidin (PA) in combination with tri-calciumphosphate (TCP) and fluoride (F) on resistance to collagen degradation and remineralization of artificial caries lesions was evaluated. One hundred and twenty five dentine blocks, approximately 5 mm × 5 mm × 5 mm in dimension, were prepared from the middle third of root of non carious single rooted teeth and randomly divided into five groups based on treatments: (i) 6.5% PA, (ii) TCP + F, (iii) TCP + F + 6.5% PA, (iv) 1000 ppm fluoride (Positive control) and (v) deionized water (control). Each specimen was subjected to pH cycling at 37 C for 8 days. Lesion depth and mineral loss was evaluated using microradiography and confocal laser scanning microscopy. The type of crystal formation was determined by XRD spectra. The lowest lesion depth and mineral loss were observed in the TCP + F + PA group. The XRD patterns showed hydroxyapatite formation on TCP + F-treated artificial caries lesions, which were not altered by the addition of PA. The addition of PA to TCP+F significantly reduced collagen degradation depth, when compared to TCP only group. Lesion depth was the lowest in the PA and TCP+F+PA groups following collagenase degradation [13]. Therefore the aim of the present study was to evaluated remineralization potential of grape seed extract versus amorphous calcium phosphate nanoparticles on sound and caries affected dentin.

MATERIALS AND METHODS

Preparation of bonding agent containing GSE:

Grape seed extract powder (Myoprotein, PO Box 612, Northwich, CW9 9hx, UK) was added to Clearfil SE primer (Kuraray Noritake dental Inc. Japan) with a concentration of 0.5 wt% to obtain primer + GSE [14].

Preparation of bonding agent containing NACP:

The nanoparticles of amorphous calcium phosphate (Nanotech Laboratory prepared) were incorporated into the Clearfil SE adhesive at mass fractions of 20% to obtain adhesive + NACP [11].

Selection of rabbits:

Fifteen healthy adult female NewZealand white rabbits weighing between 3.5 and 4 kg with age of three month were obtained from the Medical Experimental Practice and Research Centre, in accordance with local ethical committee. Rabbits were kept in individual metal cages at room temperature under veterinary supervision. They were fed a standard diet and water [15].

Rabbits grouping:

Rabbits enrolled in this study were divided equally into three main groups of five rabbits each according to type of material used, **Group A** (primer + GSE), **Group B** (adhesive + NACP), **Group C** (sodium fluoride NAF as positive control). Each rabbit contain four teeth (two upper and two lower). A total of sixty teeth were subdivided into two subgroups according to the type of substrate in which right side of each rabbit used as sound dentin whereas the left side used as artificial caries affected dentin.

Cavity preparation:

Initially, all rabbits were sedated with 3cc propofol I.V as induction followed by 1cc propofol as maintainace. Standardized Class V cavity preparation was prepared on the buccal surfaces of both upper and lower permanent anterior teeth using small carbide round bur size (#009) which were changed after every four teeth [16]. Low speed hand piece was used to prepare the cavities by cutting tooth structure until the entire head of round bur disappeared. An endodontic file stopper was placed at the termination of the bur head to control the depth. After application of rubber dam, the left side of upper and lower teeth was etched using 37% phosphoric acid for 30sec followed by rinsing for 30 sec to obtain artificial caries affected dentin.

Bonding procedure and restoration of cavities:

The bonding agent was applied according to the manufacturer's instructions as follows:

For **GSE group**, the primer + GSE was applied by micro brush with rubbing motion and left for 20 sec followed by gentle air drying for 10 sec. The adhesive was applied, thinned by gentle air flow for 5 sec and light cured for 20 sec with a 420- 480 nm LED curing device. For **NACP group**, the primer was applied as group A, followed by the adhesive + NACP.

For **NAF group**, NAF varnish was applied by micro brush, then the primer was applied followed by adhesive as group A. The cavities were restored with resin composite and light cured for 20 sec with LED curing device.

Extraction of teeth:

All rabbits were sacrificed after 10 days following the restoration, and teeth were extracted. The teeth were cut cervico-occlusal through the center of class V restoration into two halves using diamond disk and copious amount of water. The dentin around composite was examined using EDX analysis to determine amount of calcium and phosphorus in each sample.

RESULTS

(Table 1 and 2) and (Figure 1a-f)

Two-way ANOVA results showed that material, substrate and the interaction between the two variables had no statistically significant effect on mean Ca weight % or P weight %. Since the interaction between the two variables is non-significant, so the variables are independent from each other.

Either with sound or caries affected dentin, there was no statistically significant difference of Ca weight % and P weight % between GSE, NACP or NAF.

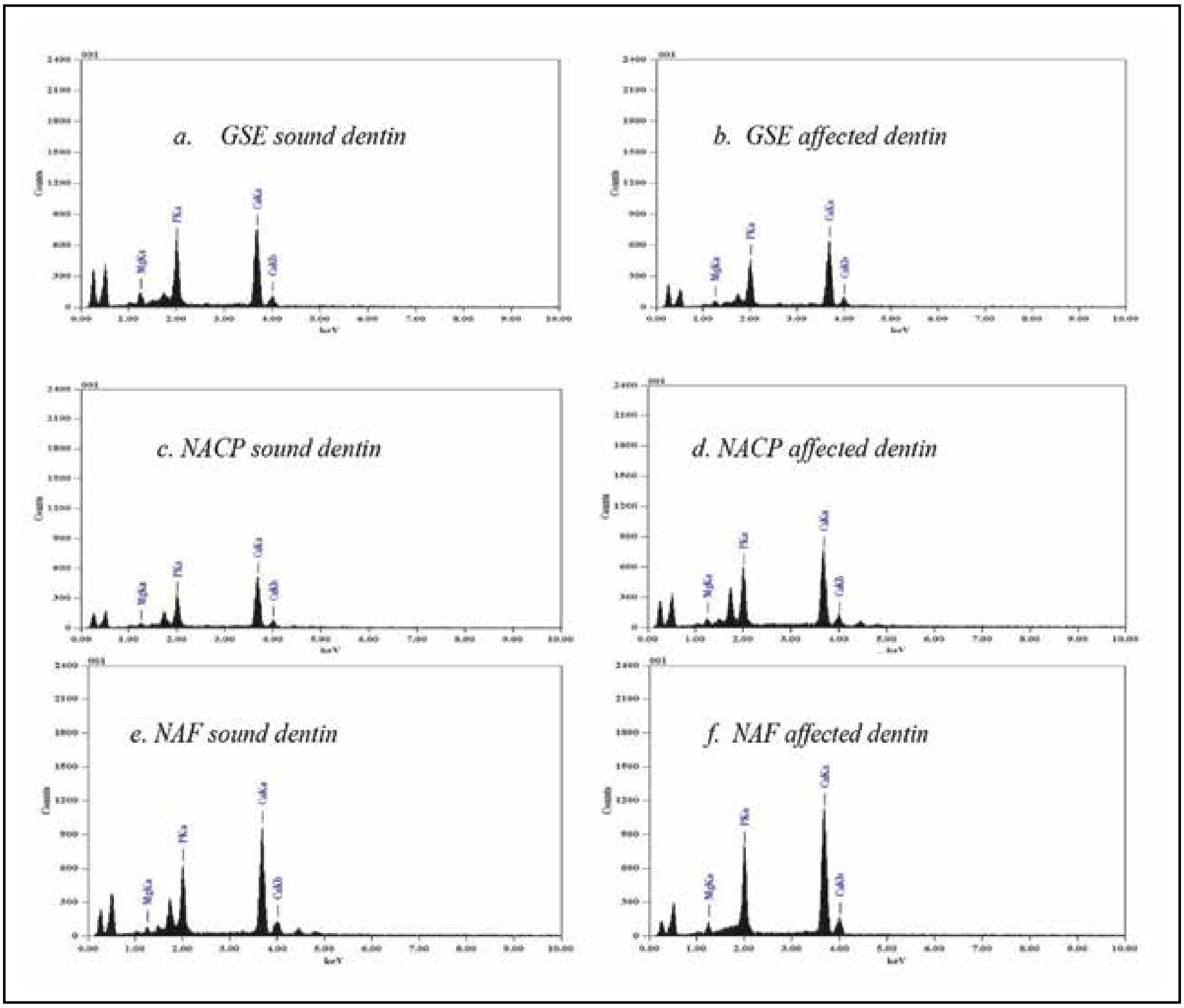

Fig. (1) EDX analysis of different materials a) GSE sound dentin, b) GSE caries affected dentin, c)NACP sound dentin, d) NACP caries affected dentin, e)NAF sound dentin, F) caries affected dentin

Table (1) *The mean, standard deviation (SD) values and results of two-way ANOVA test for comparison between Ca weight % in the three materials*

Substrate	Grape seed extract		CaP		NaF		*P*-value
	Mean	SD	Mean	SD	Mean	SD	
Sound dentin	57.23	2.51	57.47	4.74	56.18	1.93	0.739
Affected dentin	57.19	2.48	55.37	2.29	55.18	1.52	0.462

**: Significant at $P \leq 0.05$*

Table (2) *The mean, standard deviation (SD) values and results of two-way ANOVA test for comparison between P weight % in the three materials*

Substrate	Grape seed extract		CaP		NaF		*P*-value
	Mean	SD	Mean	SD	Mean	SD	
Sound dentin	37.44	3.01	35.99	2.81	36.33	1.03	0.454
Affected dentin	35.55	1.23	37.23	0.93	36.18	0.80	0.374

**: Significant at $P \leq 0.05$*

DISCUSSION

Resin–dentin bonding is a major reason for dentin demineralization [17]. The formation of resin dentin bonds is accomplished predominantly by micromechanical retention via resin penetration and entanglement of exposed collagen fibrils in the partially or completely demineralized dentin. This is achieved by etching dentin with acids or acidic resin monomers derived from self-etching primers/adhesives to expose the collagen fibrils [4]. Under the combined challenges of enzymes, temperature and functional stresses, regions of incomplete resin infiltration within the dentin hybrid layer is susceptible to degradation, resulting in damage of interfacial integrity, reduction in bond strength and ultimately, the failure of resin–dentin bonds. Thus, remineralization of demineralized dentin has important consequences for control of dentinal caries as well as improvement of dentin bonding stability[17,18].

The limited remineralization action of fluoride and the inadequate acid resistance of fluorapatite to carbonated drinks are still an issue among dental experts [19]. This, besides the known side effects of fluoride, highlights the need for alternative approaches to preserve this important structure. Hence, the present study evaluated the remineralization potential of grape seed extract and amorphous calcium phosphate nanoparticles on sound and caries affected dentin.

The remineralization potential of GSE and NACP when comparing by NAF as positive control described by EDX as measuring Ca and P percentage, show no statistically significant difference. For GSE it could be attributed to the newly induced collagen crosslinks by PA. Collagen cross-linking has recently been found to enhance extra-fibrillar and intra-fibrillar mineralization processes in densified reconstituted collagen films [20]. It was suggested that collagen is not a passive scaffold as has been previously thought. Rather, it actively controls and templates apatite formation during mineralization through charge interaction with Amorphous Calcium Phosphate (directing ACP infiltration) and mediating its nucleation into the crystalline phase[21].

Furthermore, the stabilized collagen matrix acts as a mechanical barrier, which prevents ingress of acid and further loss of calcium and phosphate ions out of the lesions. This was in accordance with previous studies which showed that GSE has the ability to modify dentin collagen and to enhance remineralization of the substrate. [6,22-24].

For NACP it may be attributed to release of amorphous calcium phosphate, the precursor form of hydroxyapatite, which subsequently initiates the hydroxyapatite formation [25]. Also NACP with a high surface area possessed a high ion releasing capability, which leads to release higher concentration of Ca and P ions [26].

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. Although saliva contains Ca and P ions, the remineralization of tooth lesions can be significantly promoted by increasing the solution concentrations of Ca and P ions to levels higher than those in natural oral fluids. Furthermore, when marginal gaps occur at the tooth-restoration interface, a NACP adhesive could greatly increase the local Ca and P ion concentration to promote remineralization and inhibit demineralization at the margins, where secondary caries usually occurs. Therefore, an important approach to the inhibition of demineralization and the promotion of remineralization was to develop CaP-containing restorations [27].

Indeed, CaP-filled resins released Ca and P ions to supersaturating levels with respect to tooth mineral, which were shown to protect the teeth from demineralization, or even regenerate lost tooth mineral [9, 27].

CONCLUSION

Grape seed extract and nano amorphous calcium phosphate can produce remineralization of sound and caries affected dentin

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