World Journal of Medical Sciences 10 (2): 204-207, 2014
ISSN 1817-3055

DOI: 10.5829/idosi.wjms.2014.10.2.82182

Clotting Co-Factor and Bees Extract in Dentin Stabilization

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Abstract: The aim of this study was to investigate the dentin collagen stabilization property of propolis and vitamin K using tensile bond strength testing. Occlusal reduction was done in 25 extracted human mandibular first molars. Following the surface pre-treatment (propolis, Vit K, 17 % EDTA), dentin bonding agent and composite resin was applied and cured. The specimens were sectioned and subjected to tensile bond strength testing. Propolis showed significantly higher mean bond strength when compared to all other experimental groups.

Key words: Vitamin K · Propolis · Dentin · Stabilization

INTRODUCTION

Collagen in biological tissue is strengthened by the formation of native cross-links which provides fibrillar resistance against enzymatic degradation, thus increasing the tensile strength. The stable and effective bond between composite material and dentin surface is achieved due to primer and adhesive penetration into the demineralised dentin forming the hybrid layer [1]. The structural stability of the collagen fibers with the resin monomers accounts for the better bond strength.

Vital dentin is inherently wet, but collagen collapse occurs on drying demineralized dentin [2]. The concept of moist bonding is preferred as it enhances bond strength as water preserves the porosity of the collagen network for monomer interdiffusion. The further stabilization of collagen fibrils by biological agents to increase the mechanical properties and decrease the enzymatic degradation should be an important application in restorative dentistry. Several synthetic and natural substances have been used to stabilize the dentin collagen with inter and intra-molecular cross-links. The application of two naturally stabilizing agents like proanthocyanidin and genipin to dentin collagen significantly improves the ultimate tensile strength, indicating its potential value in restorative dentistry.

Propolis is a natural resinous material that honey bees collect from various plant species [3]. This material has been extensively used in dentistry due to its ability to stimulate mineralization (for protection against caries), as a storage media for avulsed teeth, as an intracanal medicament in endodontic procedures, as a pulp capping material due to its ability to stimulate reparative dentin formation and for treatment of dentin hypersensitivity. Flavanoids in propolis, being natural anti-oxidants and free radical scavengers, increase collagen synthesis as well as accelerate the conversion of soluble collagen to insoluble collagen during development to enhance collagen stabilization.

Vit K is a fat soluble vitamin, essential for the functioning of several proteins involved in blood coagulation. It has the ability to bind to calcium ions and is required for the activation of coagulation cascade. Vitamin k is an essential factor to hepatic gamma glutamyl carboxylase that adds a carboxyl group to glutamic acid residues on factors II, VII, IX and X. On adding the gamma carboxyl group to glutamate residues on the immature clotting factors, vitamin k is oxidized, which is important for collagen stabilization [4].

Hence based on their collagen stabilizing properties, propolis and vitamin k were used in this study for dentin surface pre-treatment.

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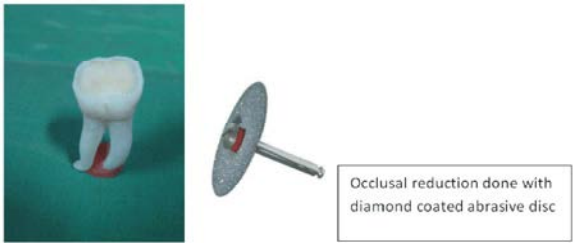
Aim of the Study: The aim of this in-vitro study was to compare the tensile bond strength of dentin surface after pre-treatment with propolis and Vitamin-K.

MATERIALS AND METHODS

Twenty five recently extracted caries free human mandibular molars were selected. They were cleaned with slurry of pumice and immersed in 0.2 % chloramine T solution for disinfection. Occlusal reduction was done to expose the mid coronal dentin with diamond discs.



After 5 min ultrasonication in distilled water to remove debris, the prepared surfaces were etched with 37 % phosphoric acid for 15 sec and rinsed with water spray for 1 min. Excess water was removed from the specimens using absorbent paper.

The specimens were randomly divided into 4 groups. The following solutions were used to pre-treat the prepared dentin surfaces for 5 min. (Table 1). Both vitamin K and propolis available in liquid form, was applied on the prepared dentin surface using applicator brush and kept for 5min.

PROPOLIS VITAMIN K

Table 1: Experimental Groups

Groups	Surface Pretreatment Solutions
I	Propolis (n=5)
II	Vitamin K (n=5)
III	17% EDTA, (n=5)
IV	No pre-treatment, negative control (n=5)

Following surface pre-treatment, bonding agent (Prime and Bond NT, DENTSPLY) was applied and cured for 20 sec. Composite material (Ceram X-nano ceramic restorative, DENTSPLY) was applied in incremental layers to achieve a total thickness of 5 mm and each increment was cured for 40 sec. The restorations were finished and polished with flexible discs (Super snap kit). For the negative control, no surface pretreatment was done, after acid etching, bonding agent and composite resin was applied and cured. All the specimens were then stored in distilled water at 37°C for 24 hrs before the testing.

Tensile Bond Strength Testing: The specimens were sectioned perpendicular to the adhesive interface using a water cooled diamond impregnated saw at 400 rpm to result in resin dentin specimen with a cross sectional area of 2 mm x 6 mm. For testing the tensile bond strength the specimens were embedded on either side in cylinders of self cured acrylic resin (length-15mm, breadth-15mm, height-25mm)

Specimens mounted on self cure acrylic resin blocks for tensile testing.

The test specimens were individually fixed to the tensile strength test device to position the adhesive area perpendicular to the long axis of the tensile force. The tensile strength test was performed with the Instron Universal Testing machine (Dept of Testing, CIPET, Guindy) at a crosshead speed of 2 mm / min. The load value in Newton's was divided by the cross sectional area in sq mm at which fracture occurred at the adhesive interface which was calculated to obtain tensile bond strength value in Mpa. ($N / mm^2 = Mpa$)

The tensile strength bond strength values were recorded in Mpa and statistical analysis was done. Kruskal-Wallis One-Way ANOVA was used to calculate the P-value. Mann-Whitney U-Test followed by Bonferroni correction method was employed to identify the significant groups at 5% level.

RESULTS

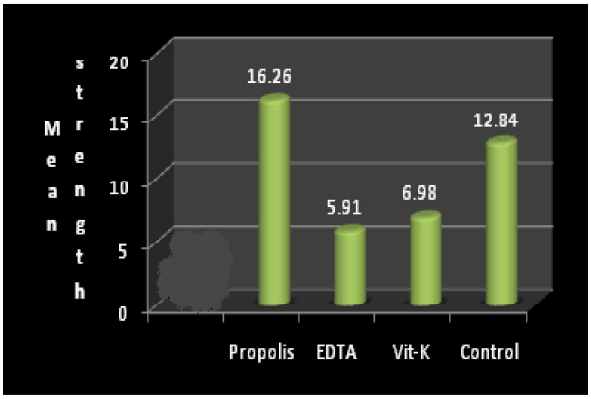
The tensile bond strength values in Mpa for the different experimental groups are shown in Table 2.

The mean tensile bond strength of all the experimental groups differed from that of the control group. Propolis showed significantly higher mean bond strength when compared to all other experimental groups. The Vitamin K group did not show a significant increase in the bond strength. There was no statistically significant difference between EDTA and Vit K groups. The significant probability value was P< 0.05 %.

Table 2: ComparisionOf The Mean Bond Strength Values Among The Different Experimental Groups

Groups	MEAN ± S.D(Mpa)
I-Propolis	16.26 ± 2.83
II-Vitamin K	6.98 ± 1.00
III-EDTA	5.91 ± 0.94
IV-No pretreatment	12.84 ± 0.30

COMPARISION OF THE MEAN BOND STRENGTH VALUES AMONG THE DIFFERENT EXPERIMENTAL GROUPS.



DISCUSSION

Collagen stabilization is necessary to achieve optimum bond strength. The presence of a collagen layer allows establishment of a stress relieving layer at the tooth-adhesive interface. In normal demineralised dentin, the spaces between collagen fibrils are maintained by water during the moist bonding technique. Air drying causes evaporation of this water collapse of collagen fibril network [5]. However in caries affected dentin the water content is more and the Knoop hardness is only half of the normal dentin. Thus inspite of moist bonding, there is less resin infiltration and reduced bond strength. So besides moist bonding, additional methods of collagen stabilization such as dentin surface pre-treatment is needed to obtain optimal bond strength values.

Dentin surface pre-treatment with propolis significantly increased the bond strength 16.26 Mpa when compared to vitamin K group 6.98 Mpa. This was due to the presence of flavonoids in propolis that react with collagen proteins via four mechanisms-covalent interactions, ionic interactions, hydrogen bonding and hydrophobic interactions[6]. This in turn decreases the enzymatic degradation of restoration interfaces, modifying the tooth surface with collagen cross-linking, producing fibrillar and collagen stabilization [7]. Flavonoids and caffeic acid present in propolis are known to play an important role in the stimulation of various enzyme systems, cell metabolism, circulation and collagen formation contributing to dentin stabilization by propolis.

Following propolis, vitamin k (6.98 Mpa) and EDTA (5.91 Mpa) have did not improve the bond strength compared to the control group (12.84 Mpa). Vitamin k was used in this study as it is known for its collagen stabilization potential due to its ability to stimulate the

formation of intermolecular cross-links between the tropocollagenmolecules[8]. Besides this vitamin K restores the disturbances in fibrillogenesis due to its potential to induce collagen and mucopolysaccharide metabolism[9]. But the negative result obtained with Vit K may be due to the reduced pre-treatment time and due to the use of Vit K analogue (menaphthone sodium bisulphate) that contained only 5.2 mg of Vit K.

The drawbacks of this *in vitro* study may be related to the small sample size and the large cross sectional area 2x6 mm (use of tensile bond strength test rather than microtensile testing). According to Sano *et al.*, there is an inverse relationship between bond strength and bond area: the smaller the area, the greater is the bond strength [10]. A small surface improves the specimen in terms of stress distribution in having a reduced number of internal defects, due to the smaller surface area. Larger specimens have a greater number of defects such as air bubbles, water blisters, surface roughness, or regions of resin / solvent phase separations, which can serve as stress concentrations areas during bonding. Hence, specimens with small cross sectional surface area have higher bond strength values [11].

Propolis can be considered as one of the pre-treatment regimes prior to application of bonding agents. This new collagen stabilizing agent seems to have a promising future to achieve a restoration with stable bonding abilities. However further studies are needed to evaluate collagen stabilization potential of vitamin K, thickness of the hybrid layer that will highlight the clinical importance of these pre-treatment systems.

CONCLUSION

Dentin surface pre-treatment with propolis prior to application of bonding agent significantly increased the bond strength. There was no statistically significant difference between Vitamin K and EDTA groups.

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