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Evaluation of grape seed extract (*Vitis vinifera*) as a crosslinker on the stability of dentine collagen in total-etch adhesive systems: An in-vitro study

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ABSTRACT

The bond strength between resin-dentine is determined by the integrity of collagen, dentine and resin monomers. The susceptibility of the dentine bond results from the instability of the bonding structure that adheres to the bonding material. This can be achieved by using collagen cross-linker, synthetically and naturally in dentine substrates which are effective in protecting collagen fibrils from degradation, as proanthocyanidine. The aim of this study was to see the effect of grape seed extract as a crosslinker on the stability of dentine collagen and see the differences between groups. This study used 27 fresh premolars or third molars which were divided into 3 groups, namely group I giving grape seed extract 6.5%, group II giving chlorhexidine 2%, and group III only giving bonding ingredients. Samples were analyzed using SEM with 1000x magnification. Data was analyzed using the kappa statistic, Kruskal-Wallis, dan Mann-Whitney. The result of the study significant differences between treatment groups (P <0.05), the 2% chlorhexidine group showed the highest value compared to the grape seed extract group and without treatment. Grape seed extract can be used as an alternative to chlorhexidine as a crosslinker to maintain bone strength of the composite restoration

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INTRODUCTION

The use of composite resins for dental restorations in the past decade to restore carious teeth has greatly increased. The need for aesthetics and low toxicity makes popular composite resins. The initial problem in using composite resins is low durability due to the use and loss of anatomical shape after use (Irawan, 2008). Composite resins contain bonds that are not stable, so they are easily degraded by acid or low pH. Acid causes polymer degradation and filler components, which can affect the hardness and roughness of the composite resin surface (Basri *et al.*, 2017). Various studies have shown that there is a decrease in composite resin bonds in dentine which in this case can be attributed to the complexity of dentine structures such as increasing tubular numbers and diameter with intertubular dentine matrix which is much less than superficial dentine (Srinivasulu *et al.*, 2012). Successful adhesion to enamel with a strong adhesive value height cannot be achieved equivalent to dentine. Dentine has higher water and organic content than

enamel, which causes dentine to be moist so that the adhesion system must be hydrophilic (Puspitasari *et al.*, 2014). Dentine has a composition of 70% minerals (apatite crystals), 18% is in the form of organic components namely collagen type 1 and non-collagen proteins while 12% is water (Puspitasari *et al.*, 2014). Dentin collagen is needed to form a cross bond that can provide strength and stabilization, so it is needed as a biomaterial (Castellan *et al.*, 2013). Infiltration of resin monomers into dentinal tubular tissue and inter-tubule will form micromechanical retention areas called hybrid layers. Micromechanical retention between resin and dentin is the main mechanism of the resin-dentine bond. The bond strength between resin-dentine is determined by the integrity of collagen, dentine and resin monomers (Generosa *et al.*, 2017; Heymann *et al.*, 2011). The resistance of the hybrid layer depends on the stability of each component, such as collagen fibrils and polymer chains. However, collagen fibrils which are not fully infiltrated by resin monomers when exposed to acid etching, inhibit optimal protection against denaturation. Unprotected collagen is more susceptible to creep, and cyclic fatigue ruptures after functioning for a long time. In the fibril, collagen resins are filled and surrounded by water, which participates in the matrix hydrolysis of resin by esterases and collagen by collagenolytic enzymes (Hass *et al.*, 2016). The susceptibility of the dentine bond results from the instability of the bonding structure that adheres to the bonding material. This is due to physical and chemical factors including hydrolysis and enzymatic degradation by Matrix Metaloproteinases (MMPs) which can increase the risk of degradation of collagen dentine in hybrid layers (Hass *et al.*, 2016; Green *et al.*, 2010). In the degradation of hybrid layers by MMP's, MMPs inhibitors act to protect collagen in the hybrid layer. Chlorhexidine 2% can inhibit MMP-2, -8, -9, and even at low concentrations can maintain the integrity of the hybrid layer. The role of chlorhexidine is to delay dentine demineralization but does not stop the degradation of the bonds permanently. However, chlorhexidine also has deficiencies in the form of discoloration and bitter taste (Dennis, 2013; Mangundjaja *et al.*, 2000). The mechanical properties of collagen and its resistance to enzymatic degradation can be increased by increasing intra-cross and molecular formation. This can be achieved by using synthetic and natural collagen cross-linker in the dentine substrate before bonding procedures (Vidhya *et al.*, 2013). The use of collagen cross-linking agent is proposed to improve the mechanical properties of the dentine matrix, reduce the level of biodegradation of collagen, increase the nature of dentine resin bonding, and extend the life of adhesive restoration (Castellan *et al.*, 2013). One natural collagen cross-linker is proanthocyanidin. Proanthocyanidin, called condensed tannins, is a structure of flavan-3-ol (Aguiar *et al.*, 2014). Proanthocyanidin is found in fruits, vegetables, nuts, seeds, and flowers (Al-Ammar *et al.*, 2009). Grape seed compounds include flavonoids, polyphenols, anthocyanins, proanthocyanidins, procyanidines and the stilbene derivative resveratrol (Ahmed *et al.*, 2015; Sayed *et al.*, 2016). Grape seeds extract has been reported to have a broad spectrum of pharmacological and therapeutic effects such as antioxidative, anti-inflammatory, and antimicrobial activities, as well as cardioprotective, hepatoprotective, and neuroprotective potentials (Ahmed *et al.*, 2015) There has been a lot of research testing about proanthocyanidin in increasing shear bond strength and tensile bond strength but examining the part of dentin collagen directly does not yet exist, so researchers are interested in knowing the effects of proanthocyanidin on grape seed extract on the stability of dentine collagen.

MATERIALS AND METHODS

Sample

Type of this study was the experimental in-vitro laboratory study. The design was a post-test group design with a control group. The study sample consisted of 27 premolars and third molars.

Methods

50 gr of fresh grape seeds were used to make grape seed extract with an additional 70% ethanol. Teeth that had been removed then planted in a cast. Cavity preparation was performed on the occlusal surface. The first group was applied by grape seed extract, the second group was applied by chlorhexidine, and the third group was only applied with the bonding agent. After that, teeth were cut into two parts vertically, then remove the roots and the only remaining dentin and composite resin Samples were analyzed using SEM with 1000 magnification. After that, the results obtained were tested with Kruskal Wallis.

RESULTS AND DISCUSSION

From the results of Table 1, it shows that there is an effect of extract of grape seeds as a crosslinker on the stability of dentine collagen. It is known that there are significant differences between the treatment groups ($P < 0.05$). In Chart 1, the group applied chlorhexidine 2% showed the highest value compared to the grape seed extract group and without

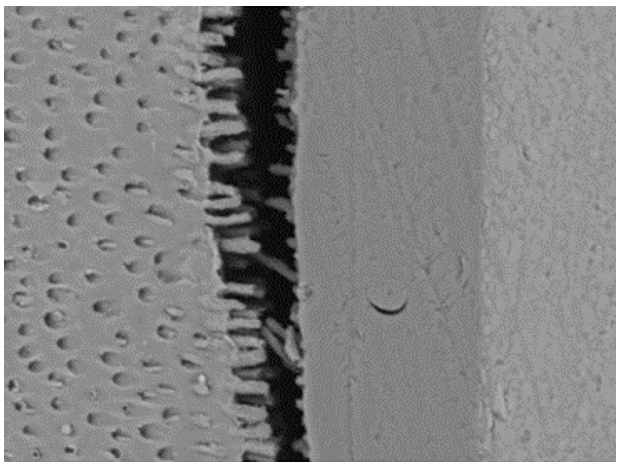

Figure 1: Group of grape seed extract

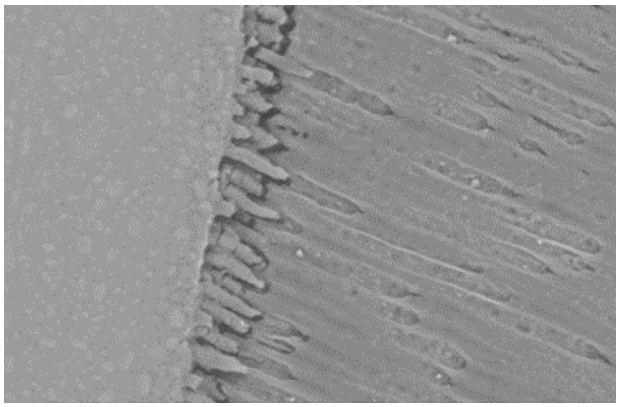

Figure 2: Group of chlorehexidine(CHX)

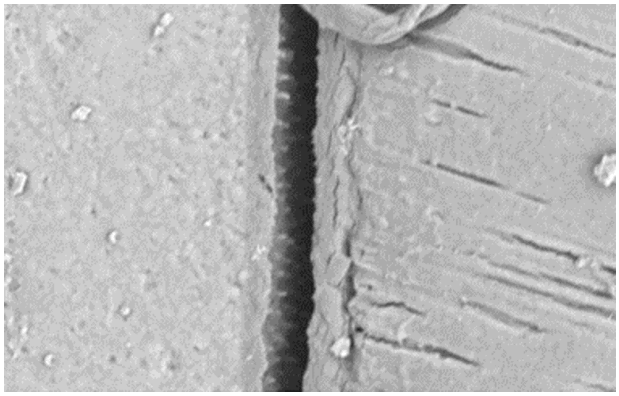

Figure 3: Group of negative control (only bonding agent)

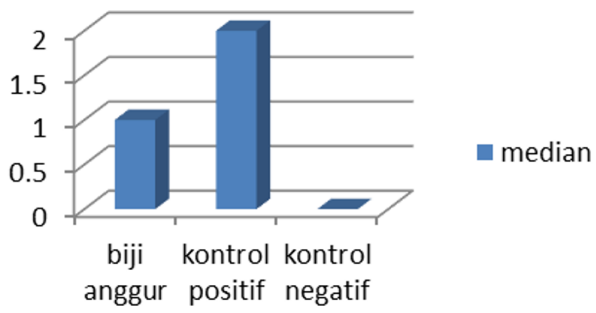

Chart 1: Median of each group

treatment

Table 1: Result of Kruskal Wallis test

Group	N	X±SD	Median	P
Grapeseed	9	1.11±0.601	1	0.002
CHX	9	1.89±0.782	2	
Negative Control	9	0.44±0.864	0	

Biomodification of the demineralization of the collagen matrix with crosslinking agents plays an important role in increasing dentin biomechanics. Strengthening collagen fibrils by crosslinker agents can reduce enzymatic degradation, which is important for enhancing hybrid layer stability and endurance of restoration adhesions (Epasinghe *et al.*, 2012). Collagen crosslinker is an agent that forms inter and intramolecular crosslinks with type 1 collagen in dentine and helps stabilize the matrix (Khan *et al.*, 2017). The process of bonding to dentine occurs through a micromechanical mechanism by forming a hybrid layer. Bonding occurs by impregnating the dentine substrate through a mixture of resin monomers to obtain a homogenous hybrid layer that is highly dependent on the stability of the interfacial resin-dentine bond. At the same time, the hybrid layer is very important for dentine bonding, which is also the weakest and most vulnerable component of the resin-dentine surface (Al-Ammar *et al.*, 2009). Proanthocyanidine shows significant results in the strength of the bond in deep dentin (Srinivasulu *et al.*, 2012). This can be attributed to the specificity of proanthocyanidine to facilitate the proline hydroxylase enzyme, which catalyzes proline hydroxylation, which is a step in collagen biosynthesis. In collagen fibrils through exogenous crosslinker agents, not only can they increase the modulus of elasticity of the collagen matrix and its efficacy, but it can also ensure long-term stability of the hybrid layer by increasing the resistance of the collagen matrix. The use of crosslinker agents in the study can increase resistance to collagenase through the closure of open parts of the collagen matrix. In addition, crosslinkers have the ability to inhibit collagen molecules from gliding past each other under mechanical pressure (Balalaie *et al.*, 2018). Proanthocyanidine or condensed tannins consist of thick flavon-3-ol subunits, catechins, epicatechin and epicatechin-3-O-gallate and are bound through C4-C8. This component is responsible for biological properties such as free radical cleansing and antioxidant activity. Proanthocyanidine contains several electron donor sites (hydroxyl sites) which allow binding

of unstable molecules or free radicals by donating hydrogen atoms.Proanthocyanidin consists of a high hydroxylation structure that is capable of forming non-soluble complexes with carbohydrates and proteins (Epasinghe *et al.*, 2012). Proanthocyanidine and proteins interact in four ways: covalent interactions, ionic interactions, hydrogen bond interactions, and hydrophobic interactions (Srinivasulu *et al.*, 2012). Crosslinker mechanism between proanthocyanidine and collagen mainly formed by hydrogen bonds between amine carbonyl proteins and phenolic hydroxyl groups in addition to covalent and hydrophobic bonds which are the main forces to stabilize collagen given proanthocyanidin and stabilize mechanical strength (Nagpal *et al.*, 2016). (Figure 1) Proanthocyanidin can form hydrogen bonds by forming bridges with hydroxyl ions, carboxyl, amines and collagen dentin amides (Generosa *et al.*, 2017). The hydrogen bond between the phenolic hydroxyl group and the amide carbonyl group can increase the stability of collagen fibers and improve the mechanical properties of collagen (Ahmed *et al.*, 2015). In the process of forming hydrogen bonds, Proanthocyanidin molecules can replace water molecules in the extrafibrillary collagen spacev (Generosa *et al.*, 2017). Application of 2% chlorhexidine, which is the most significant group among grape seed groups and negative controls. This is due to the inhibiting properties of MMP, which can prevent the binding of metal ions, such as zinc and calcium to MMP, thus inhibiting its catalytic activity. Chlorhexidine can only electrostatically bind to demineralize dentine collagen, which slowly diffuses out of the collagen matrix through a competitive desorption mechanism in the presence of other cations. The interplay between chorheksidine and the dentine matrix is based on the electrostatic force between the exhibited NH3 in the chlorhexidine molecule and COOH- or OH- in the negatively charged dentine. When applied in high concentrations, chlorhexidine can cause oversaturation of the binding part of the enzyme, and remain bound to collagen fibrils for later release (Sabatini and Pashley, 2014). (Figure 2) Application of 2% chlorhexidine for one minute to acidic dentine, as a clinical protocol both for wetting back collagen tissue, and reducing degradation of resin-dentine bonds (Sabatini and Pashley, 2014). From result and discussion, the use of chlorhexidine 2% is better to form resin tags when compared with 6.5% grape seed extract and the negative control shows the lowest results in resin tag formation (Figure 3).

CONCLUSION

In conclusion, grape seed extract can be considered to be used as an alternative crosslinker in composite resin restoration to maintain its adhesion to dentin.

Conflict of interest

The authors declare that they have no known competing for financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors Contribution

All authors conceived the original idea and designed the experiment, performed the experiment, collected and analyzed data. All authors contributed to the interpretation of the results, wrote the manuscript, read and approved the final manuscript.

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