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Potential Effect of Mangosteen Extract Incorporation into Self-Etch Adhesive on Dentin Bonding

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Abstract

The aim of this study was to investigate the effect of incorporation of mangosteen natural extract into self-etching primer on dentine bond strength. A total of sixty human molars were utilized for the purposes of this investigation. These molars were sectioned at the cement-enamel junction in order to create flat dentine surfaces. Three experimental primers were produced by including 0.5% Mangosteen peel extract (MPE), 0.5% Chlorhexidine (CHX), or 0.5% Grape seed extract into Kerr Optibond Versa (Kerr, USA). The initial Kerr primer was utilized as the control in the experiment. Subsequently, all specimens were subjected to bonding using Kerr Optibond Versa adhesive. The resin composite was then sequentially applied in four layers to create the build-up. Following this, the specimens were immersed in water and housed in a 37 ºC incubator for a duration of 24 hours. The specimens that were bonded together were subsequently divided into beams of 2x2 mm in size and were then subjected to micro tensile bond testing (µTBS). The debonded samples were examined using a scanning electron microscope (SEM) in order to analyze the fracture mode. The results of the study indicate that there were statistically significant differences in the microtensile bond strength (µTBS) between the experimental and control groups, as determined by a Two-way Analysis of Variance (ANOVA) test ($p<0.05$). The post-hoc comparison test revealed a substantial increase in microtensile bond strength (µTBS) with the integration of MPA, as compared to the other two groups ($p<0.001$). In conclusion, the inclusion of MPE in the Kerr Optibond Versa primer has a positive impact on the instant bond strength of a dental restoration.

Keywords: Resin-dentin interface, Dentine bond strength, Mangosteen extract, Self-etching adhesive.

INTRODUCTION

The establishment of a durable and reliable bond between tooth substrate and resin materials continues to pose a significant issue within the field of adhesive dentistry. The main purpose of dental adhesives is to provide adherence to composite materials, allowing them to counter occlusal forces and shrinkage stress that arise from the lining composite. A proficient adhesive should demonstrate the capacity to efficiently minimise any potential seepage that can arise at the perimeters of the restoration. Within the clinical setting, it has been seen that the principal factors contributing to restorative failure include insuficient sealing and hydrolytic degradation of both resin material and dentin collagen. These factors ultimately lead to the loss of retention [1,2].

Despite recent developments in dental adhesive systems, the bonded interface continues to be identified as the most susceptible region of tooth-colored restorations. The breakdown of the hybrid layer, which results in impaired resin-dentine bonding, has been identified as a potential factor contributing to the failure of adhesive restorations [3]. Degradation transpired in both the resin and collagen fibrils [4], resulting in certain regions of collagen fibrils being left exposed and vulnerable to attack by host-derived dentinal matrix metalloproteinase (MMPs) enzymes [5,6].

The utilization of MMP inhibitors was first proposed by Carrilho et al. as a means to mitigate collagen degradation and enhance bond strength [7]. Scheffel et al. found that Chlorhexidine (CHX), a non-specific MMP-inhibitor, has the capacity to prevent the breakdown of collagen and the hybrid layer [8]. An alternative strategy for mitigating collagen breakdown involves the utilization of cross-linking agents. Previous studies have demonstrated that cross-linkers, such as tannic acid and proanthocyanidins, has the

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ability to not only reduce the enzymatic degradation of collagen but also enhance the mechanical properties of dentine [9]. The adhesive systems possess acidic qualities that can activate the intrinsic matrix metalloproteinases (MMPs) present in dentine. It has been observed that mild acids have the ability to stimulate the activation of endogenous matrix metalloproteinases (MMPs) in dentine [10,11]. In addition,self-etch adhesives have the potential to induce the activation of latent matrix metalloproteinases (MMPs) to levels that are close to their maximal capacity, hence leading to the breakdown of resin-dentine bonds.

Additional cross-linkers that have been employed to enhance stabilization of collagen and bonding performance include glutaraldehyde (GA) and grape seed extract (GSE) [11]. Epigallocatechin gallate (EGCG), which is the predominant catechin found in green tea, has demonstrated potential in mitigating dentine erosive demineralization. This effect may be attributed to its ability to inhibit matrix metalloproteinases (MMPs) [12].

Garcinia mangostana L., a member of the Clusiaceae family, is a tropical evergreen tree characterized by its sluggish growth and possession of leathery, glabrous leaves. It is commonly referred to as mangosteen. The height of the tree can range from 6 to 25 meters, and it is primarily found in India, Myanmar, Sri Lanka, and Thailand. The milky white edible section of the mangosteen fruit is in contrast to the dark red pericarp, which constitutes approximately two-thirds of the total fruit weight and is typically discarded as agricultural waste [13,14].

The secondary metabolites present in mangosteen encompass xanthones, anthocyanins, phenolic acids, and condensed tannins [15-17]. Among these compounds, xanthones, anthocyanins, and phenolic acids have been the subject of substantial research. Condensed tannins, referred to as proanthocyanidins (PA), are intricate oligomers or polymers of flavan-3-ol that exhibit a high degree of structural complexity. These compounds are interconnected through B-type and A-type links [18]. Proanthocyanidins exhibit potent antioxidant activity due to their intricate structural variety and associated physiochemical properties. Consequently, study on proanthocyanidins is regarded as the ultimate frontier in the field of flavonoid exploration [19,20]. In their study, Caili Fu et al. examined the purified fraction of oligomeric proanthocyanidins derived from mangosteen pericarps [13]. The investigated fraction had a mean degree of polymerization (DP) of 6.6. In a recent study, researchers developed a modified matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) technique to investigate the condensed tannins found in mangosteen pericarps. This method yielded more comprehensive insights into the monomeric units, interflavan linkages, and substituents present in these tannins [21]. The available literature on the antioxidant activity of condensed tannins from mangosteen pericarps is limited due to the diverse nature and structural complexity of these compounds [13]. The aims of this investigation were to assess the impact of incorporating MPE into a self-etching primer on the strength of dentin bonding and the mechanical characteristics of the bonded interface and compare these effects with those resulting from the incorporation of CHX and GSE. The null hypotheses was that the introduction of MPE, CHX, or GSE does not have any impact on the initial bond strength to dentine.

METHODOLGY

Tooth preparation

The teeth used in this study were collected with an informed consent from the patients. This investigation was reviewed and approved by the Human Research Ethics Committee of International Islamic University of Malaysia (ID Number: 2021-060). In order to assess the bond strength, sixteen non-carious human molars that were recently extracted were utilized. Occlusal dentine was removed ,and a flat dentine surface was formed perpendicular to the longitudinal axis of the tooth through the cutting process of a slow-speed diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) operating under water chilling. A smear layer was generated on every surface by employing #600 SiC paper while being irrigated with water. Four teeth were designated for the subsequent self-etching primers in each group. For the formulation of the experimental primer groups (Table 1), MPE (Mangosteen Peel Extracts, Bionutricia, MY), CHX (Chlorhexidine di-acetate, Sigma-Aldrich, Saint Louis, MO, USA), or GSE (Proanthocyanidins, Kikkoman Biochemifa, Chiba, Japan) was added to Kerr Optibond Versa primer (Kerr, USA). In all categories, the concentration was 0.5 weight percent (%). Every experimental primer's pH value was determined from a digital pH metre (Twin pH B-211, HORIBA, Ltd. Kyoto, Japan). The original SE primer was marked as a control. The primers were used to condition the dentine surfaces in accordance with the manufacturer's instructions. Subsequently, Kerr Optibond Versa adhesive (Kerr, USA) was surfaced and light-cured for a duration of 10 seconds using a MiniLed, Satelec-Acteon, which supplied a power of 600mW/cm^2. A composite resin (Diafil, DiaDent) was progressively applied to the dentine surfaces until it reached a thickness of 5 mm. Each increment underwent 30 seconds of light curing.

Micro tensile bond strength testing

Following a 24-hour period of storage in de-ionized water at a temperature of 37°C, the bonded teeth underwent longitudinal sectioning into consecutive slabs. These slabs were subsequently further sectioned in order to generate composite-dentine beams measuring 0.9mm x 0.9mm. The precise measurements of each beam's dimensions were obtained by employing a set of digital calipers. Any specimen that was in proximity to the pulp horns was excluded from the study because the size of the dentine was insufficient for conducting tensile testing. The quantity of beams utilized for the bond testing varied between 12 and 16 per tooth. The attachment of each beam to the test apparatus was accomplished using an adhesive known as MODEL REPAIR II BLUE, manufactured by DENSPLY-Sankin in Ohtawara, Japan. Subsequently, the beams were subjected to tension until failure occurred, employing a universal testing machine (Instron E3000, located in Canton, MA). The rate of displacement of the machine's crosshead was set at 1 mm per minute. The provided diagram (Figure 1) is presented for reference.

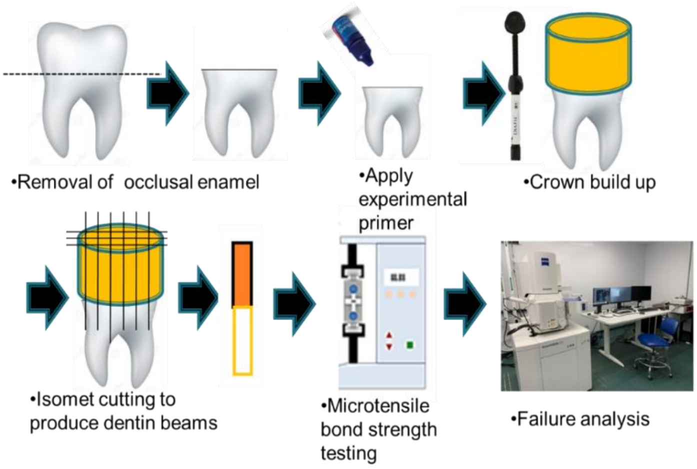

Figure 1: Micro tensile bond strength testing representation

Failure mode analysis

After air-drying and sputter-coating of the fractured dentine surface with gold/palladium, surfaces were investigated using a ZEISS Model-EVO50 scanning electron microscope (SEM). Based on the type and location, the failure modes were classified into four groups: (A) cohesive failure in the dentine; (B) mixed type of failure in the adhesive layer and resin composite; (C) mixed type of failure in the adhesive and retention interface; and (D) cohesive failure in the adhesive layer.

Statistical analysis

The present study used statistical analysis techniques to examine the data. The impact of experimental primers on microtensile bond strength (μTBS) data was assessed through the utilisation of a One-way Analysis of Variance (ANOVA) conducted using Sigma Stat Version 16.0, a statistical software package developed by SPSS in Chicago, IL, USA. Additional statistical analyses were conducted using the Tukey multiple comparisons test to address any significant findings. The level of statistical significance was established at a significance level of 5%.

RESULTS

Micro tensile bond strength

Figure 2 illustrates the microtensile bond strength (μTBS) values obtained from both the experimental and control groups. The results of the one-way analysis of variance (ANOVA) indicated statistically significant differences among the groups that were examined ($p<0.001$). The results of the Tukey post-hoc test indicated a significant difference in the mean microtensile bond strength (μTBS) between the MPE-incorporated group (67.5 ± 8.9 MPa; n=48) and both the control group (53.1 ± 8.8 MPa; n=55) and the experimental groups ($p<0.006$). The CHX-group (49.0 ± 8.7 MPa: n=40) exhibited a slightly reduced bond strength in comparison to the control group. However, this difference was not found to be statistically significant ($p>0.05$). The GSE-group mean ± standard deviation: 44.8 ± 13.8 MPa; n=45) had a considerably reduced bond strength in comparison to the control group ($p<0.001$).

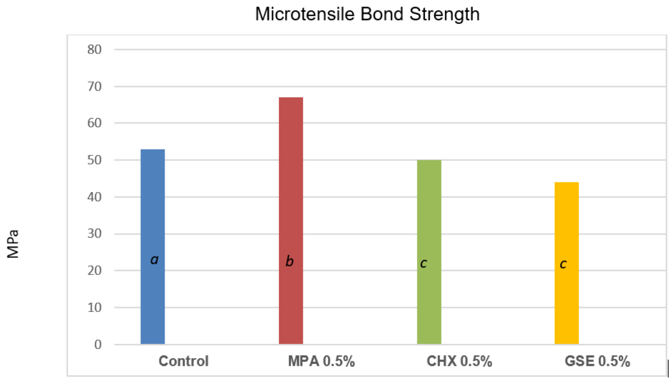

Figure 2: The bar graph illustrates the bond strength following a 24-hour period of water storage. There is no statistically significant difference ($p>0.05$) seen across groups that are identified with the same letters

Failure mode analysis

Figure 3 illustrates the fracture patterns seen on the dentine sides, together with the corresponding percentages of failure modes. The primary cause of failures found in the control group was identified as cohesive failure occurring inside the dentine and adhesive materials. The presence of type (iii) failure, which is defined as adhesive failure at the bonded interface, demonstrated an inverse relationship with increased microtensile bond strength (μTBS) in the MPE incorporated group. Conversely, in the groups incorporating CHX and GSE, a positive correlation was observed between the occurrence of type (iii) failure and lower μTBS. Figure 4 displays representative scanning electron microscopy (SEM) images from each group.

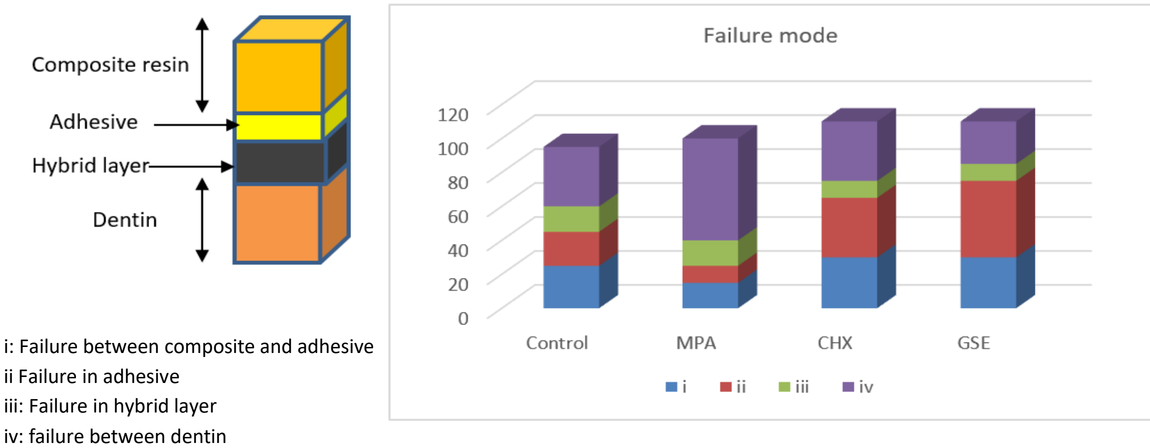

Figure 3: Spatial distribution and areas of fractures in specimens subjected to bond strength testing

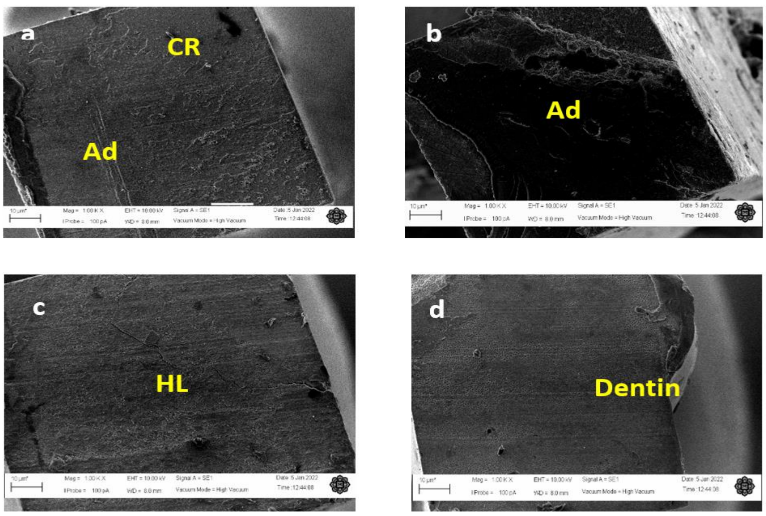

Figure 4: The provided SEM pictures depict frcatured dentine surfaces of experimental groups: a) the control group, b) the group with incorporated MPE, c) the group with incorporated CHX, and d) the group with incorporated GSE. (Composite resin as CR, adhesive as Ad, and the hybrid layer as HL).

DISCUSSION

The objective of our research was to examine the effects of incorporating a natural extract into a self-etching primer on the immediate bond strength and mechanical characteristics of the bonded interface. The findings of the study indicated that the inclusion of MPE led to a notable enhancement in the initial binding strength to dentine. Conversely, the incorporation of GSE was found to drastically decrease this bond strength. The addition of CHX did not have a significant impact on the bond strength. Therefore, the null hypothesis was rejected.

The current investigation observed that the inclusion of MPE resulted in an enhancement of immediate strength. Based on the findings presented by Miguez et al, it is postulated that the mechanical characteristics of the collagen matrix play a crucial role in determining the tensile strength of dentine. In light of this, it is hypothesized that the application of MPE (specific substance) may serve to stabilize the collagen matrix and enhance the mechanical properties of the hybrid layer. Consequently, this improvement in mechanical properties is believed to contribute to the achievement of a high bond strength.

Grape seed extract, previously employed in dentine substrate to investigate their impact on demineralized lesions, with the aim of stabilizing the collagen matrix [23,24]. GSE primarily consists of oligomeric proanthocyanidins, which have gained recognition as a naturally occurring collagen cross-linking agent. The observed cross-linking phenomenon of proanthocyanidins is ascribed to their interaction with proline-rich proteins, such as collagen. The chemical demonstrated an ability to enhance the mechanical characteristics of dentine by means of its cross-linking mechanism [25]. The precise mechanism by which MPA induces cross-linking is not fully understood. However, the basic building block of MPA bears a strong resemblance to complex oligomers or polymers of flavan-3-ol, which are interconnected through B-type and A-type linkages. This unique structural similarity allows MPA to employ a biomimetic approach, along with interactions with non-collagenous components, to enhance the stability of the dentin-resin interface and prolong the lifespan of dental restorations [26]. Numerous research have been conducted to examine the impact of grape seed extract (GSE) on the strength of dentine bonding. These studies have focused on the application of GSE on etched dentine before bonding using etch-and-rinse adhesive systems. The findings of these studies consistently indicate that pre-treatment with GSE leads to enhanced bond strength [27,28]. The researchers hypothesized that the observed increment in the mechanical characteristics of dentine treated with grape seed extract (GSE) is responsible for the better bond strength of etch-and-rinse adhesives. In contrast, a self-etching adhesive system was employed in order to conduct the initial experiment aimed at integrating GSE into a self-etching primer. Inconsistencies in bond strength were observed in our study, contrasting with findings from previous research that employed etch-and-rinse methods. One potential rationale for the disparate outcomes could be attributed to the utilization of distinct adhesive methods.

The CHX group was added as this choice was motivated by past research efforts [29,30] that have explored the usage of CHX in self-etching system. In the present investigation, the inclusion of chlorhexidine (CHX) within the self-etching primer formulation did not yield statistically significant variations in the initial bonding efficacy and mechanical characteristics of the resin-dentine interface, as compared to the control cohort. The utilization of CHX, which has been demonstrated to possess an MMP-inhibitory impact due to its ability to chelate zinc cations ,resulted in a notable enhancement in the initial bond strength of both etch-and-rinse adhesive and self-etch systems. The addition of CHX in a self-etching primer or its application on etched dentin prior to the bonding process resulted in a notable enhancement in the endurance of long-term bonding, as observed over a 12-month period [31]. According to our current investigation, the integration of MPE into self-etching primer is promising,in enhancing the initial bonding performance and mechanical properties of the resin-dentine interface. The combination of MPE-dental adhesives is considered a potential strategy to enhance the longevity of adhesive bonds and ultimately achieve favourable outcomes in clinical adhesive restorations. Additional research is required to assess the extended-term efficacy of MPE and GSE in relation to the resin-dentine bond, as well as to explore other potential applications of MPE within the field of dentistry.

CONCLUSION

To conclude, it can be inferred that the inclusion of MPE in the self-etching primer led to an increased in immediate bond strength. Conversely, the incorporation of GSE in the primer resulted in a notable decrease in bond strength. However, the group that incorporated CHX did not demonstrate any notable impact on this particular characteristic. The capacity of MPE to protect the bonded interface suggests a potential improvement in the long-term bond endurance.

Conflicts of Interest

The author reports no conflicts of interest.

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