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Push-Out Bond Strength and Dentinal Penetration of a Novel Herbal-Based Pulp Capping Agent: An *In vitro* Study

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Abstract

BACKGROUND: Pulp capping agents should have proper sealing effect to enhance dental pulp tissue healing.

AIM: The purpose of this study was to compare the effectiveness of grape seed extract (GSE) and mineral trioxide aggregate (MTA) to penetrate to dentin and their push-out bond strength at two time intervals (1 and 3 months) when used as pulp capping agents either singly or combined to each other.

MATERIALS AND METHODS: This study was conducted on 120 human single-rooted anterior teeth. Sixty dentin disks were randomly divided into three groups (n = 20) based on the material used; MTA, GSE, and a combination of MTA and GSE. A universal testing machine was used to determine the push-out bond strength for 1 and 3 months. At the same time intervals, extra 60 teeth with the same groups were utilized to quantify the degree of capping material penetration within the dentinal tubules using scanning electron microscopy. Analysis of variance with multiple comparison *post hoc* test was used to evaluate the data where $p < 0.05$.

RESULTS: MTA had the highest push-out bond strength and penetration depth measurement into dentinal tubules at 1 month, followed by MTA combined with GSE, while GSE had the lowest push-out bond strength and penetration depth measurement. Nevertheless, GSE had the greatest values in both tests at 3 months, followed by MTA, while MTA coupled with GSE had the lowest value in both tests.

CONCLUSIONS: Push-out bond strength and dentinal penetration depth were improved with time except for the MTA group testing its dentinal penetration depth. GSE shows good push-out bond strength and dentinal penetration depth.

Introduction

Direct pulp capping is a vital pulp therapy (VPT) method in which the exposed vital pulp is covered by a pulp capping material to encourage reparative dentin development [1]. Dental pulp stem cells are synchronized by overlapping stages of propagation, multiplication, and mineralization of pulp cells produced by odontoblast-like cells [2]. To boost this regeneration property, a variety of materials have been used, such as calcium hydroxide, adhesive systems, and mineral trioxide aggregate (MTA). While some have been found to be cytotoxic, as calcium hydroxide-based compounds, others are not [3], [4].

MTA has been identified as having excellent sealing capabilities among the available materials. The colloidal gel formed by hydration of hydrophilic particles has several unique properties, the most notable of which is its ability to seal and to be set in a moist environment, where humidity aids in setting [5], [6]. MTA offers improved biocompatibility, but it has a long setting time, leads to tooth discoloration, is difficult to manipulate, and is pricey [7], [8].

Natural compounds derived from plants have been the subject of recent studies for use as medicinal agents. Oligomeric proanthocyanidin (PA) complexes and PA grape seed extract (GSE) are primarily approved for their antioxidant activities [9]. Moreover, these chemicals have antibacterial, antiviral, anticarcinogenic, anti-inflammatory, anti-allergic, and vasodilatory properties [9]. Abraham *et al.* used the 5th and 7th generations of bonding agents to test the influence of GSE on the bonding strength of composite resin to bleached enamel. Due to its antioxidant properties, GSE considerably increased the bond strength of composite resin to bleached enamel following bleaching [10]. Recent research used GSE as an endodontic irrigant due to its antimicrobial action and it was comparable to 2% chlorhexidine gel [11]. Interestingly, GSE maintained the dentin microhardness [12].

Several factors influence successful direct pulp capping, the most important of which is the dislodgment of the capping material due to either the condensation forces applied on the final restoration or the occlusal loads. Consequently, adequate bond strength eradicates routes of leakage and inters remaining bacteria [13]. It was shown that penetration

of an endodontic material into the dentinal tubules will enhance marginal adaptation, increase mechanical retention, and entomb residual bacteria [13].

To the best of our knowledge, there have been no previous studies that tested GSE as a pulp capping material, either alone or in combination with MTA regarding sealing ability. Thus, the aim of this research was to assess how the combination of GSE, and MTA might affect dentinal penetration depth and push-out bond strength over time. The null hypothesis was that there was no significant difference between either MTA or GSE or their combination.

Materials and Methods

Sample size calculation

The study was approved by the Research Ethics Committee (REC) at the College of Dentistry of Suez Canal University (registered No. 327/2021). All steps were accomplished in accordance with the relevant guidelines and regulations. The sample size was obtained using the G* power software statistical analysis (Latest ver. 3.1.9.7; Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) according to D'Aviz *et al.* [11]. One hundred and twenty teeth were sufficient to detect the effect size of 0.4 and a power (1-β) of 80% at a significant level of (α) level of 0.05.

Teeth selection

The present study was conducted on 120 single-rooted extracted human anterior teeth. Teeth were selected from the oral surgery clinic at the College of Dentistry, Suez Canal University. The teeth were collected from unknown patients irrelevant to the study that had extraction due to periodontal problems. The teeth were radiographed to exclude cracks and caries. The selected teeth were soaked in 2.5% sodium hypochlorite for 2 h to enable disinfection.

Preparation for the pulp exposure

One hundred and twenty teeth were subjected to a Class V preparation on their labial surface coronal to the gingival margin. Inverted cone bur size 1 (Dentsply Maillefer, Tulsa, Oklahoma, USA) at high speed (30,000 rpm) contra-angle handpiece (NSK, Tokyo, Japan) was used under a water coolant until an exposure was noticed at the floor of the cavity. The tested materials were applied on the exposure site and then final restoration was placed using inter mediate restorative material (Dentsply, Charlotte, USA).

Sample randomization and grouping

Randomization was utilized using Microsoft Excel. Later, blind allocation was performed where the samples were coded and placed in opaque envelopes. The samples were divided into three main groups utilizing the three materials: White MTA (WMTA) Angelus (Angelus, Londrina, Brazil); GSE (Nu Sci., HerbStore, USA); MTA + GSE with 40 samples for each group. Samples were then divided into two subgroups, each with 20 samples, based on time intervals (1 month and 3 months). Subsequently, 10 samples for each subgroup were examined independently for the push out bond strength and dentinal penetration.

Preparation of the GSE

The GSE powder used in the present study was purchased from the company as a readymade powder. GSE powder was mixed with distilled water with a 3:1 ratio on a glass slab to reach a creamy consistency that was maintained during the application to the exposure site.

Preparation of the mixture of MTA and GSE

On the glass slab, the powder was divided into four equal amounts; GSE was blended 25% by weight into MTA where the powder constituents were vibrated in a mixer for an hour. The initial quantity of the powder was inserted into the bath to begin the mixing process. The distilled water was added to the resulting mixtures. To obtain the required consistency, the powder was blended into the water to achieve a uniform mix. As a result, the water/powder ratio was 1:3:3 where the weight of GSE was equal to MTA. The resultant mix was in creamy consistency that was maintained during the application to the exposure site.

Push-out bond strength

A total of 60 dentin disks with a thickness of 1.50.2 mm and a lumen of 1.3 mm were employed using an IsoMet diamond saw (Buehler, Lake Bluff, NY, USA). The disk was embedded in a polyvinyl ring with self-curing acrylic resin (Prothyl repair EVO, Zhermack, Badia Polesine, Italy). The dentin surface was polished with 600 grit silicon carbide paper and later cleaned in an ultrasonic cleanser for 10 min. The samples were randomly divided into three groups, 20 of each, according to tested capping materials (MTA, GSE, and mixture of MTA and GSE). Twenty samples of each examined material were further divided into two subgroups (n = 10) for push-out bond strength testing at 1 and 3 months. For the MTA group, the materials were mixed according to the manufacturers' instructions. MTA powder was mixed with distilled water with a 3:1 ratio on a glass slab to reach a creamy consistency that was

maintained during the application to the exposure site. For the GSE and the mixture of MTA and GSE groups, the steps were as mentioned earlier. Each sample was compressed after being mounted in a loading fixture using a computer-controlled materials testing machine (Model LRX-plus; Lloyd Instruments Ltd., Fareham, UK) with a load cell of 5 kN. Using computer software, the data were captured and loaded at a crosshead speed of 0.5 mm/min (Nexygen-MT; Lloyd Instruments). Plungers apply a load of diameter size (1 mm). Only the filling is contacted to move it downward, according to the plunger diameter chosen. The bond intensity was considered from the observed peak load divided by the measured surface area as defined by the following formulation: Bond = F/A = π h 2 (r). Where, π is the 3.14 constant, the sample thickness in millimeters is the radius of r1 and h. Extrusion of the filling material indicated failure, which was confirmed by a dramatic drop in the load-deflection curve of the computer program.

Penetration depth measurements

Scanning electron microscope (SEM) was accustomed to assess the amount of capping material diffusion into the dentinal tubules. A #2 diamond disk was used to cut the crowns of the teeth at the cementoenamel junction. The samples were sliced with a diamond disk under sprayed water after the capping materials were applied. The bottom 3 mm of the cervical region is left. The sample was then dehydrated for 10 min in a succession of solutions with ethanol concentrations ranging from 50% to 100% in 10% increments, before being dried for 24 h at room temperature in a closed jar containing silica gel. In the upper notch, a chisel and hammer were used to cut cross-sections, which were then coated by ion sputtering with Pt (E-1030, Hitachi High Technologies, Tokyo, Japan). Photographs of the coronal part were taken using a SEM at ×500, ×1000, and ×1500, depending on the degree of material diffusion (SU-8220, Hitachi High Technologies, Tokyo, Japan). The general penetration of the substance in the images was tested using Adobe Photoshop 7.0.0.

Statistical analysis

The Statistical Package for the Social Sciences version 26 was used to code and enter the data (IBM Corp., Armonk, NY, USA). The mean and standard deviation were used to summarize the data. Data were investigated for normality using Kolmogorov–Smirnov and Shapiro–Wilk tests. Data displayed normal distribution. The analysis of variance with multiple comparisons *post hoc* test was used to compare the groups. The paired t-test was used to compare the two timings in each group. Correlations between quantitative variables were done using Pearson correlation coefficient. Statistical significance was defined as $p < 0.05$.

Results

Regarding the push-out bond strength, at 1 month, there was a significant difference between the three groups, with the MTA group having the greatest value, followed by the MTA + GSE group, and the GSE group having the lowest. No significant difference was found between MTA and MTA + GSE groups. In addition, no significant difference was found between the MTA + GSE and GSE groups, Table 1.

Table 1: Mean values of push-out bond strength of the different tested pulp capping agents at different time intervals 1 and 3 months

	MTA group	Grape seed group	MTA + Grape seed group	p value
Push-out bond strength at 1 month (megapascal)	^{A}4.02 ± 0.21	^{B}2.7 ± 0.3 *	^{AB}3.82 ± 0.12	<0.001
Push-out bond strength at 3 months (megapascal)	^{B}5.59 ± 0.33	^{A}6.86 ± 0.06 *	^{C}5.16 ± 0.18	<0.001
	< 0.001	< 0.001	< 0.001	

Mean values with the same superscript letters are not statistically significant at $p \leq 0.05$. Mean values with different superscript letters are statistically significant at $p \leq 0.05$.

At 3 months, there was a significant difference between the three groups, with the GSE group having the greatest value, followed by the MTA group, and the MTA + GSE group having the lowest, Table 1.

Push-out bond strength increased significantly in each group from 1 to 3 months, Table 1.

Regarding the penetration depth, at 1 month, there was a significant difference between the three groups, with the MTA group having the greatest value, followed by the GSE group, and the MTA + GSE group having the lowest value. No significant difference was found between the GSE and MTA + GSE groups (Table 2 and Figure 1).

Table 2: Mean values of dentinal penetration depth of the different tested pulp capping agents at different time intervals 1 and 3 months

	MTA group	Grape seed group	MTA + Grape seed group	p value
Dentinal penetration depth at 1 month (micrometer)	^{A}211.2 ± 46.87	^{B}41.7 ± 4.21	^{B}32.96 ± 3.02	<0.001
Dentinal penetration depth at 3 months (micrometer)	^{B}60.12 ± 4.18	^{A}90.94 ± 8.75	^{C}44.18 ± 5.32	<0.001
	0.001	<0.001	0.001	

Mean values with the same superscript letters are not statistically significant at $p \leq 0.05$. Mean values with different superscript letters are statistically significant at $p \leq 0.05$.

At 3 months, there was a significant difference between the three groups, the GSE group had the highest value, followed by the MTA group, while the MTA + GSE group had the lowest, Table 2 and Figure 2.

Tubular penetration depth increased significantly in each group from 1 to 3 months, with the exception of the MTA group, which showed a significant drop in penetration depth from 1 to 3 months, Table 2.

Correlation between push-out bond strength and dentinal penetration depth at both time intervals showed strong positive correlation, Table 3.

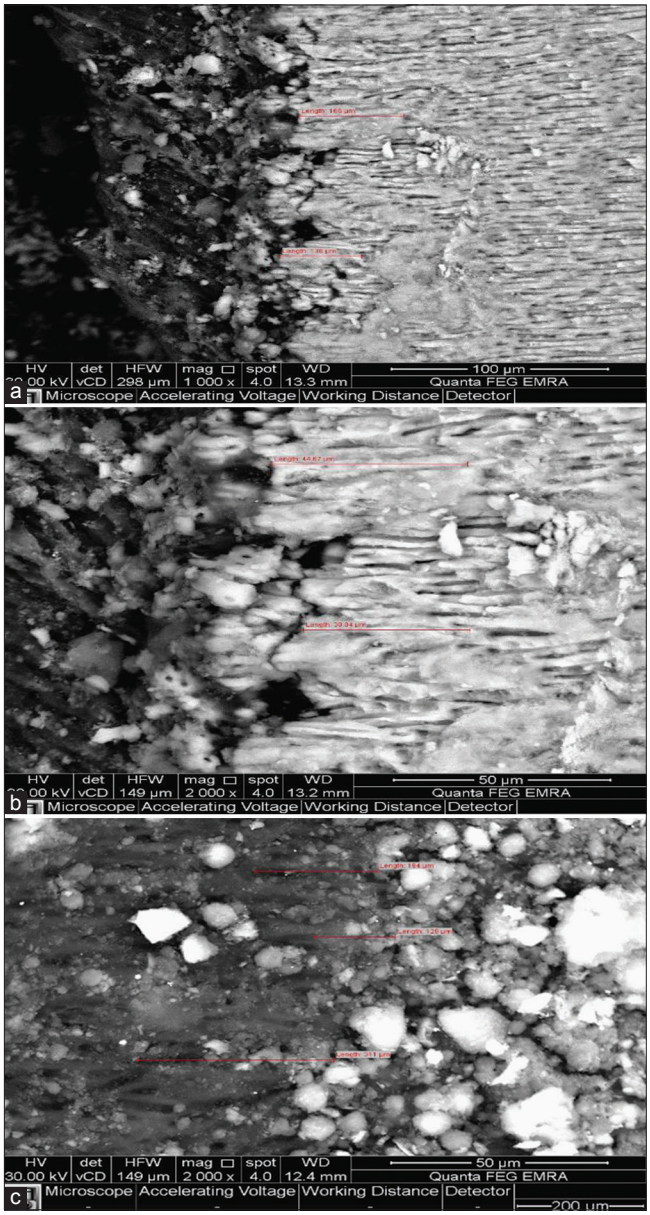

Figure 1: Representative SEM micrographs of dentinal penetration depth of the tested capping materials. (a) MTA, (b) GSE, and (c) MTA + GSE at 1 month

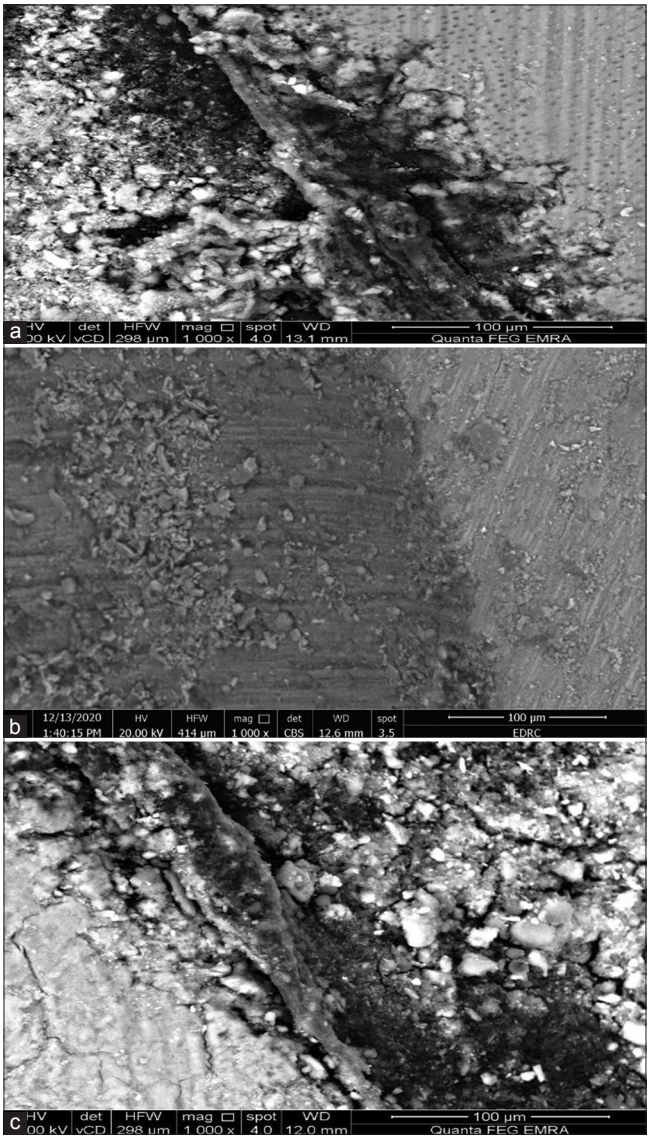

Figure 2: Representative SEM micrographs of dentinal penetration depth of the tested capping materials. (a) MTA, (b) GSE, and (c) MTA + GSE at 3 months

Discussion

To achieve a successful VPT, the bond quality of capping biomaterials to dentin is crucial [14]. The adherence of a material to the surrounding dentin should be unaffected by any dislocation pressures utilized during functional procedures [15]. In the present study, the material sealing to the surrounding dentin was evaluated using a push-out bond strength test to assess the resistance of the material to dislodgment [16], [17].

The material employed in this study was WMTA, which is made up of 80% Portland cement and 20% calcium sulfate-free bismuth oxide to reduce setting time [7]. GSE was also used as it showed promising results when used as an endodontic irrigant and before the application of composite [9], [10], [11], [12]. Subsequently, the present study investigated a new dental material (combination of WMTA and GSE) that combines WMTA biocompatibility with acceptable setting time, handling characteristics, chemical properties, and antioxidant capabilities of GSE, and compared it to each of its components independently.

Table 3: Correlation between bond strength and penetration depth at 1 and 3 months

Pearson correlation coefficient	Penetration depth at 1 month (micrometer)
Push-out bond strength at 1 month (megapascal)	
r	0.542
p value	0.037
N	15
Pearson correlation coefficient	Penetration depth at 3 months (micrometer)
Push-out bond strength at 3 months (megapascal)	
r	0.904
p value	<0.001
N	15

$p < 0.05$

Results showed that the tested pulp capping materials showed statistical significance in push-out bond strength values as time progressed. MTA and MTA + GSE had the highest push-out bond strength values at 1 month, while GSE had the lowest. In spite of the absence chemical link between MTA and root dentin, interfacial deposits have been recorded as a result of the reaction between phosphate in body fluid and the calcium and hydroxyl ions generated by MTA [18]. These deposits were deposited in the crevices between MTA and root dentin, increasing the frictional resistance of MTA [19], which grew over time. As previously stated, PA is found abundantly in GSE, which has enormous remineralization potential by time [20], stabilizing the collagen matrix [21], preventing caries and remineralization occurs at the enamel [22] which explained why GSE showed after 3 months the maximum push-out bond strength among the tested materials. This was in accordance with a study done by Atabek and Özden [23] which has shown that when compared to the use of 10% sodium ascorbate, the use of 6.5% Pa-rich GSE to deep dentin considerably improved shear bonding strength values of composite to dentin. The highest bond strength of the GSE in comparison to the MTA could be clarified by the higher number of collagen cross-links that have strengthened the stability of collagen [24]. As a result of its cross-linking effect, GSE improved the mechanical properties of dentinal collagen and preserved the collagen matrix from degradation by exogenous collagenase [25]. The matrix metalloproteinases 3 (MMPs 3) are inhibited by PA [26]. GSE also actively regulates apatite formation during mineralization by charging interaction with amorphous calcium phosphate which favors dental remineralization [26]. On the other hand, combining MTA + GSE did not show any privilege compared to MTA or GSE groups. It seemed that the combination might affect the mechanical and chemical bond of both materials to the dentin.

Furthermore, this study evaluated depth of penetration of the tested materials into dentinal tubules by means of the SEM at the same time intervals. The most important advantages of this technique are producing highly detailed images of dentinal tubules and their content and allowing observing the material within the dentinal tubules at distant region from the root canal wall [27], [28]. The main disadvantage is the difficulty of making systemic analysis at low magnifications. Another drawback is the possibility of manufacturing artifacts during the preparation [29].

The capacity of an endodontic material to penetrate dentinal tubules can be correlated with the number and size of dentinal tubules, the particle size of the material, and the material setting reaction. Dentinal tubules are structures in the wall of the pulp that ranges from 2 to 3.2 μm in diameter [30]. In cervical dentin, the number of tubules is the highest, with a substantial decrease in the mean tubule compactness in radicular dentin [31]. For material to permeate the tubules, the particle size must be smaller than the tubule diameter. For WMTA, the mean particle size is 2–3 μm [32]. GSE, by comparison, has an average particle size of 1.3 μm. This may be explained results of this study that GSE group has highest penetration depth at 3 months.

Results of the present study displayed that MTA group demonstrated lower penetration depth in 3 months. The previous MTA studies investigated the apatite layer that formed on top of it, as well as the interfacial gaps and dentinal tubules. As a result, this physiochemical reaction aids in the synthesis of HA between MTA and dentin, which improves sealing capacity and biocompatibility [30], [33]. Apatite formation is mainly driven by the liberation of calcium into biological fluids. When used as a capping material, MTA has been shown to facilitate calcium release into the hard tissues that develop underneath it [34], [35]. Combining MTA + GSE did not favor the penetration of the material into dentin compared to MTA or GSE groups. It could be justified that the combination might affect the mechanical and chemical bond of the materials to the dentin.

Interestingly, there was a strong positive correlation between the push-out bond strength and dentinal depth penetration at both time intervals. This result was contradictory to a previous study done on sealers, where there was no correlation [36]. The correlation in this study was also inconsistent to another as the effect of push-out bond strength was weak on the penetration depth [37]. Different methodologies using different sealers rather than pulp capping materials might justify the variation.

The null hypothesis was partly rejected. The result of the present study confirmed that there is a positive correlation at different time intervals between push-out bond strength and the penetration depth of GSE into the dentinal tubules.

Conclusions

According to the limitation of this study, we can conclude that GSE has favorable properties regarding its push-out bond strength and penetration depth into dentinal tubules making it an effective and promising natural pulp capping material. Further study is required to confirm the remineralizing potential effect of GSE checking the quality of dentin bridge formed using a histopathological study.

Authors' Contributions

M.R and H.E proposed the ideas; H.E and M.R collected data; M.R and M.S analyzed and interpreted

data; M.R, H.E, and M.S critically reviewed the contents and drafted and critically revised the article. The author(s) read and approved the final manuscript.

The datasets acquired during and/or analyzed during the present study are available from the corresponding author on reasonable request.

Data Availability

The datasets used and/or analyzed during the present study available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study was approved by the REC at the College of Dentistry of Suez Canal University (registered No. 327/2021) and was conducted in accordance with the relevant guidelines and ethical regulations.

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