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Original Article

Dentistry Section

Comparison of Two Remineralising Agents on the Microshear Bond Strength of Composite Resin to Artificially Demineralised Dentin: An In-vitro Study

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ABSTRACT

Introduction: Bonding efficacy depends on the structural integrity of dentin, which is compromised by demineralisation. Remineralising agents like Nanobioactive Glass (NBG) and Theobromine (TH) can enhance dentin hardness and bonding performance. NBG promotes Hydroxyapatite (Hap) formation, while TH, a cocoa-derived compound, increases microhardness and tubule occlusion. However, their comparative effects on bond strength are not well established.

Aim: To evaluate and compare the effect of TH and NBG on the microshear Bond Strength (μSBS) of nanohybrid composite resin bonded to artificially demineralised dentin after one and seven days of application testing.

Materials and Methods: This was an in-vitro study conducted in Department of Conservative Dentistry and Endodontics of MIDSR Dental College, Latur Maharashtra, India in the month of February 2024. A total of 32 non carious extracted human molars were used for this in-vitro study and randomly allotted in four groups (n=8). Group-1 sound dentin, Group-2 demineralised dentin. Group-3 consisted of demineralised dentin with NBG application and Group-4 of demineralised dentin with TH application, which were further divided into two subgroups (A and B) each for one day and seven days application, respectively (n=4 for subgroups). Remineralising agents were applied for one day and seven days according to groups allocated with samples stored in artificial saliva between applications. All specimens were prepared by bonding using a self-etch adhesive and nanohybrid composite resin. The μSBS was tested with the help of a universal testing machine at day 1 and day 7. Statistical analysis was done with Statistical Package for Social Sciences (IBM SPSS Statistic for window, version 21.0. Armonk, NY: IBM Corp.) at 95% Confidence Interval (CI) and 80% power to the study. Analysis of Variance followed by Tukey's Honestly Significant Difference (HSD) test was applied to check statistically significant difference between groups ($p<0.05$).

Results: Demineralisation significantly reduced dentin strength compared to sound dentin ($p<0.001$). At seven days, NBG significantly increased stress at maximum load (20.34±1.03 MPa) compared to demineralised dentin and TH groups ($p<0.001$), with a significant time-dependent improvement ($p<0.0001$). TH showed significantly higher values than demineralised dentin ($p<0.001$) but no significant change between one and seven days ($p>0.05$). NBG demonstrated strength comparable to sound dentin at seven days ($p>0.05$).

Conclusion: The TH and NBG agents enhanced bond strength of composite resin to demineralised dentin. TH performed better at Day 1, while NBG exhibited greater efficacy over time at Day 7.

Keywords: Nanobioactive glass, Nanohybrid, Remineralisation, Theobromine

INTRODUCTION

Bonding efficacy in restorative dentistry depends on the structural and chemical characteristics of the underlying dentin. Carious or acid-challenged dentin exhibits reduced mineral content and altered collagen fibrils, which hinder the development of a hybrid layer and resin infiltration [1,2].

The process of remineralisation is one of the bases of minimally invasive dentistry. In the oral environment, teeth are exposed to continuous cycles of demineralisation and remineralisation [3]. Dentin and enamel become structurally weaker when the balance shifts toward mineral loss. When such compromised dentin is treated, restoration procedures frequently result in increased microleakage and decreased bond strength [4,5].

The NBG, a material composed of silica that reacts with tissue fluids to produce hydroxyapatite (HAp). Bioactive glasses tend to improve bond strength, increase dentin hardness and reduce hypersensitivity [3]. It also contains calcium and phosphate. Improved remineralisation and tubule occlusion is attributed to its increased surface area and improved reactivity due to its particle size [6].

As a bioactive remineralising agent, a naturally occurring methyl-xanthine from cocoa, has been acknowledged by many researchers named TH. It reduces the adhesion of Streptococcus mutans to the dentin, thus increasing the microhardness of the dentin and encouraging the growth of crystallites [7,8]. TH has ability to alter surface morphology and increase tubule occlusion which leads to improved dentin bonding [9].

Although TH and nanobioactive glass have been studied independently, there are no direct comparisons of their remineralising effects on bond strength of composite resin to dentin [1,2,9].

Therefore, the present study was planned with an aim to evaluate and compare the μSBS of composite resin with artificially demineralised dentin treated with NBG and TH after one and seven days, respectively.

MATERIALS AND METHODS

The present was an in-vitro study which was conducted in the Department of Conservative Dentistry and Endodontics of MIDSR Dental College, Latur, Maharashtra, India in the month of February 2024. Ethical clearance was obtained from Ethical committee of college (Ref No.: MIDSR/IEC-177/837/169/2024).

Sample size calculation: Sample size was calculated using the following formula with [4] as reference:

N = (σ1 2 + σ2 2 /κ) (Z1 - α /2 + Z1 - β)2

Δ2

The notation for the formulae are:

N = sample size

σ1 = standard deviation of Group-1

σ2 = standard deviation of Group-2

Δ = difference in group means

κ = ratio = 1

Z1 − α/2 = two-sided Z value (e.g., Z=1.96 for 95% confidence interval).

Z1 − β = power

N= (2.74 x 2.74) + (2.1 x 2.1) (1.96 +0 .84)2/(-3.94 x -3.94) = 7

The minimum sample size calculated was seven samples per group.

The authors had selected a sample size of eight per group.

A total of 32 freshly extracted non carious human permanent molars, without cracks, restoration or hypoplasia extracted for periodontal reasons were used for this study. They were kept at 4°C in a 0.1% thymol solution until use [9]. The crown of each tooth was sectioned apical to the Cemento-Enamel Junction (CEJ) using a diamond saw to obtain flat mid-coronal dentin surfaces.

These teeth were divided into four groups (n=8) Group-1: sound dentin (positive control), Group-2: demineralised dentin (negative control). Group-3 consisted of demineralised dentin with NBG application and Group-4 of demineralised dentin with TH application. All the groups were further divided into two subgroups each for one day (A) and 7days (B) application.

Group-1: Positive control: The samples were kept in artificial saliva without demineralisation.

Group-2: Negative control: The samples were demineralised and kept in artificial saliva.

Group-3: The samples were treated with NBG particles mixed with artificial saliva by applying it on demineralised dentin for one minute for one day and seven days at 24-hour interval.

Group-4: The samples were treated with TH mixed with artificial saliva by applying it on demineralised dentin for five minutes for one day and seven days at 24-hour interval.

They were further divided into subgroups of one-day and seven-days (n=4).

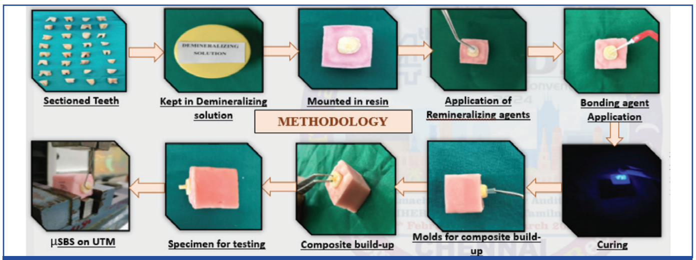
Demineralisation protocol [1] Except for Group-1 (positive control), samples were immersed in demineralising solution (2.2 mM $CaCl_2$, 0.05 M lactic acid and 2.2 mM NaH_2PO_4 adjusted to pH 4.5) for 72 hours to produce artificial demineralisation.

Remineralising protocol: A total of 20 milligrams (0.02 g) of Nanobioactive glass powder composed of 45 wt% Silica, 25 wt% Calcium Oxide, 25 wt% Sodium Oxide and 5 wt% Phosphorus Pentaoxide, was mixed with one millilitre of the artificial saliva then the mixture applied on the demineralised surface of each specimen, rubbed with sterilised cotton bud for one minute. For the one-day group the procedure was done once, while for the one week group the procedure was repeated for seven days [1].

For TH group, 20 gm TH containing toothpaste (Theodent classic, Manufacturer: Theodent, Country of Origin: USA) was mixed with 100 ml of artificial saliva and applied same as for NBG group for one-day group and seven days group, respectively [7].

The specimens were kept in artificial saliva (pH 7.2) between the applications. The artificial Saliva was prepared using Trisodium Phosphate - 3.90 mM, Sodium Chloride - 4.29 mM, Potassium Chloride- 17.98 mM, Calcium Chloride - 1.10 mM, Magnesium Chloride - 0.08 mM, Sulfuric acid - 0.50 mM, Sodium Bicarbonate - 3.27mM, distilled water. The artificial saliva was changed every 24 hours [1].

After remineralisation treatment, samples were bonded using G-Premio Bond using manufacturer's instructions (applying to the tooth for 10 seconds, drying for five seconds under maximum air pressure and light-curing for 10 seconds). Nanohybrid Composite microcylinders (Filtek Z350 XT, 3M ESPE) of 3 mm height and 1 mm diameter were placed with the help of paediatric i.v. tubing as molds and light-cured using an LED curing unit (I Led Plus, Woodpecker, India). The step-wise methodology is shown in [Table/Fig-1].



[Table/Fig-1]: Step-wise methodology.

Before testing, specimens were kept at 37°C in artificial saliva between applications.

Testing for Microshear Bond Strength (µSBS):

Each specimen was horizontally secured with tightening screws to the lower fixed compartment of a universal testing machine (Company: FSM) and data recorded using computer software (Bluehill Universal). A loop prepared from an orthodontic wire was wrapped around the bonded micro cylinder assembly as close as possible to the base of micro cylinder and aligned the loading axis of the upper movable compartment of the testing machine. A shearing load with tensile mode of force was applied through universal testing machine at acrosshead speed of 0.5mm/min.

STATISTICAL ANALYSIS

A structured proforma was used to collect the data. Microsoft Excel (v.2013) was used to enter and sort the data that was obtained. With Statistical Package for Social Sciences (IBM SPSS Statistic for window, version 21.0. Armonk, NY: IBM Corp.) at 95% CI and 80% power to the study statistical analysis was done. The Shapiro-Wilk and Kolmogorov-Smirnov tests were used to determine normal distribution of the data and found to be normally distributed. Mean, standard deviation was measured for descriptive statistics. To evaluate a statistically significant difference in the groups ($p<0.05$), the ANOVA and Tukey's HSD tests were used.

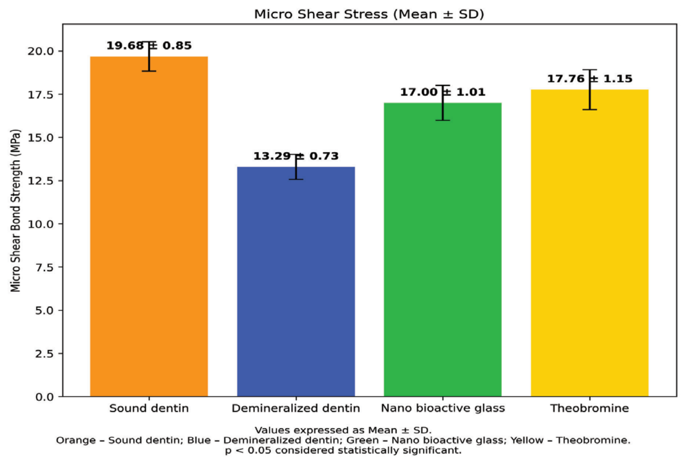
RESULTS

The µSBS values of the different experimental groups expressed as mean±standard deviation is illustrated in [Table/Fig-2,3]. The sound dentin group (Group-1) demonstrated the highest bond strength with a mean value of 19.68±0.85 MPa, indicating optimal bonding performance under normal dentin conditions.

The demineralised dentin group (Group-2) showed the lowest µSBS values (13.29±0.73 MPa), suggesting that demineralisation adversely affects the bonding capability of dentin.

Groups	Treatment	Stress at Maximum Load (MPa) Mean (μSBS)±SD
Group-1	Sound dentin (positive control)	19.68±0.85
Group-2	Demineralised dentin (negative control)	13.29±0.73
Group-3	Demineralised dentin + nanobioactive glass	17.00±1.01
Group-4	Demineralised dentin + Theobromine (TH)	17.76±1.15

[Table/Fig-2]: Mean μSBS and standard deviation values (MPa).



[Table/Fig-3]: Graphical representation of Mean μSBS and standard deviation values of the different experimental groups.

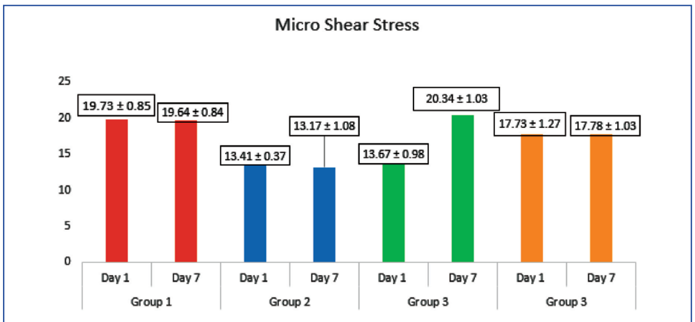
Treatment with nanobioactive glass (Group-3) resulted in an increase in bond strength (17.00±1.01 MPa) compared to untreated demineralised dentin, indicating the remineralising potential of nanobioactive glass in improving dentin bonding.

Similarly, the Theobromine (TH)-treated group (Group-4) showed a mean μSBS of 17.76±1.15 MPa, which was higher than the demineralised dentin group and slightly higher than the nanobioactive glass group, suggesting that TH may enhance the bonding properties of demineralised dentin.

The μSBS values of all experimental subgroups expressed as mean±standard deviation is shown in [Table/Fig-4,5].

Group	Treatment	Stress at Maximum Load (MPa) Mean(μSBS)±SD
1A	Sound dentin	19.73±0.85
1B	Sound dentin	19.64±0.84
2A	Demineralised dentin	13.41±0.37
2B	Demineralised dentin	13.17±1.08
3A	Demineralised dentin + nanobioactive glass	13.67±0.98
3B	Demineralised dentin + nanobioactive glass	20.34±1.03
4A	Demineralised dentin + Theobromine (TH)	17.73±1.27
4B	Demineralised dentin + Theobromine (TH)	17.78±1.03

[Table/Fig-4]: Mean μSBS and standard deviation values (MPa) within the sub-groups.
Subgroup A and B denote time interval at Day 1 and Day 7, respectively.



[Table/Fig-5]: Graphical representation of mean μSBS and standard deviation values (MPa) of all experimental subgroups.

Among all subgroups, Group-3B (Demineralised dentin treated with nanobioactive glass on Day 7) demonstrated the highest bond strength (20.34±1.03 MPa). The sound dentin subgroups (1A and 1B) also showed high bond strength values of 19.73±0.85 MPa and 19.64±0.84 MPa, respectively.

The demineralised dentin subgroups (2A and 2B) exhibited the lowest μSBS values, with mean values of 13.41±0.37 MPa and 13.17±1.08 MPa, indicating that demineralisation significantly reduced dentin bond strength.

In the nanobioactive glass treated group, the Day 1 subgroup (3A) showed a bond strength of 13.67±0.98 MPa, whereas the Day 7 subgroup (3B) demonstrated a marked increase in μSBS (20.34±1.03 MPa), suggesting an improvement in bonding performance over time.

Similarly, the TH treated subgroups (4A and 4B) exhibited mean μSBS values of 17.73±1.27 MPa and 17.78±1.03 MPa, respectively, which were higher than the untreated demineralised dentin groups but lower than the sound dentin and nanobioactive glass Day 7 subgroup.

Statistical analysis was performed using One-way ANOVA to compare the mean μSBS values among the eight experimental subgroups. Differences were considered statistically significant with p<0.001 [Table/Fig-6].

Source of variation	SS	df	MS	F value	p-value
Between groups	261.32	7	37.33	40.21	<0.001
Within groups	22.28	24	0.93		
Total	283.60	31			

[Table/Fig-6]: One-way ANOVA.

Post-hoc Tukey's multiple comparison analysis revealed that the demineralised dentin groups (2A and 2B) showed significantly lower μSBS values compared to sound dentin groups (1A and 1B) and the nanobioactive glass day 7 subgroup (3B) (p<0.001).

The nanobioactive glass day 7 subgroup (3B) demonstrated significantly higher bond strength compared to 3A, 2A, 2B, 4A and 4B (p<0.05). However, no statistically significant difference was observed between the sound dentin subgroups (1A and 1B) and subGroup-3B.

The TH subgroups (4A and 4B) showed significantly higher μSBS values compared to the demineralised dentin groups, but were not significantly different from the sound dentin groups.

At Day 1, TH (4A) 17.73±1.27 showed higher μSBS values compared to the NBG group (3A) 13.67±0.98 showing statistically significant result (p<0.05) [Table/Fig-7].

Comparison	Mean difference	p value	Significance
1A vs 1B	-0.09	1.000	NS
1A vs 2A	-6.32	<0.001	Significant
1A vs 2B	-6.56	<0.001	Significant
1A vs 3A	-6.06	<0.001	Significant
1A vs 3B	0.61	0.983	NS
1A vs 4A	-2.00	0.108	NS
1A vs 4B	-1.95	0.125	NS
1B vs 2A	-6.23	<0.001	Significant
1B vs 2B	-6.47	<0.001	Significant
1B vs 3A	-5.97	<0.001	Significant
1B vs 3B	0.70	0.965	NS
1B vs 4A	-1.91	0.140	NS
1B vs 4B	-1.86	0.161	NS
2A vs 2B	-0.24	1.000	NS
2A vs 3A	0.26	0.999	NS

2A vs 3B	6.93	<0.001	Significant
2A vs 4A	4.32	<0.001	Significant
2A vs 4B	4.37	<0.001	Significant
2B vs 3A	0.50	0.995	NS
2B vs 3B	7.17	<0.001	Significant
2B vs 4A	4.56	<0.001	Significant
2B vs 4B	4.61	<0.001	Significant
3A vs 3B	6.67	<0.001	Significant
3A vs 4A	4.06	<0.001	Significant
3A vs 4B	4.11	<0.001	Significant
3B vs 4A	-2.61	0.015	Significant
3B vs 4B	-2.56	0.018	Significant
4A vs 4B	0.05	1.000	NS

[Table/Fig-7]: Tukey's post-hoc multiple comparison.
NS = Not significant

DISCUSSION

The present study analysed the effect of remineralising agents like NBG and TH on µSBS of nanohybrid composite resin bonded to artificially demineralised dentin for one and seven days. The results indicated that TH improved bond strength significantly at Day 1, whereas NBG showed greater improvement by D
ay 7 ($p<0.001$). These findings can be explained by the distinct mechanisms of action of the two agents.

Elmalawany LM et al., assessed the impact of TH and Casein Phospho-Peptides/Amorphous Calcium Phosphate with Fluoride (CPP-ACPF) on the resin-dentine bond strength, microhardness and dentine morphology and found that µTBS was comparable to sound dentine (46.38±12.18) and the demineralised group demonstrated statistically the lowest µTBS ($p<0.001$) [2].

Demineralisation decreases the availability of calcium and phosphate ions, disturbs collagen fibrils network and impairs the micromechanical and chemical bond of resin adhesives [3]. Consequently, the negative control group showed the lowest µSBS values, consistent with results of earlier studies highlighting poor bonding to demineralised substrates [2,4,5,7].

The TH facilitates the growth of hydroxyapatite crystallites rapidly and also facilitates the occlusion of dentinal tubules. In addition, TH has been reported to exhibit antimicrobial activity against cariogenic microorganisms, which may further contribute to its protective effect on dentin [10]. TH enhances hybrid layer formation in the short period by stiffening the dentin matrix and decreasing collagen collapse during adhesive infiltration [7,11]. This interprets the significantly higher Day 1 µSBS in the TH group compared to NBG. However, the constant value observed at Day 7 might suggest that its effect is only till mineralisation of surface, with little enhancement of subsurface dentin over time.

The NBG exhibited improvement at Day 7. This can be explained by its unique property of sustained release of calcium, phosphate and silica ions, which induce Hydroxycarbonate Apatite (HCA) formation in the demineralised collagen matrix of demineralised dentin. The particle size being nanoscale make it possible to penetrate deeper into dentinal tubules and react much longer, thus resulting in continuous strengthening and improved adhesive interaction. The progressive build-up of an HCA layer facilitates both micromechanical interlocking and chemical bonding via 10-Methacryloyloxydecyl Dihydrogen Phosphate (10-MDP)-calcium salts in self-etch adhesives [12-15].

The universal adhesive used in this study (G-Premio Bond) has 10-MDP monomer, which interacts with calcium ions in dentin thus forming chemically bond with those ions [16]. In demineralised dentin, decreased calcium negatively affects this interaction. Pretreatment with remineralising agents replenishes calcium and phosphate, thus providing stable MDP-Ca nanolayering. TH provides immediate surface calcium for bonding, while NBG progressively enriches calcium availability, explaining the crossover effect observed in Day 1 and Day 7 [17,18] Results agree with Elmalawany LM et al., who found TH enhanced bond strength after short-term use [2] and with Mohamed AD and Abielhassan M who reported increasing µSBS with longer application of NBG [1].

From a clinical perspective, TH may be suitable for situations requiring immediate bonding to demineralised dentin, whereas NBG offers a more durable, long-term strengthening effect [19,20]. Incorporating these agents as a pretreatment step could improve restoration longevity, especially in cases of caries-affected dentin or minimally invasive cavity preparations.

Limitation(s)

The present was an in-vitro study conducted over short durations of time intervals (only 1 day and 7 days). Oral factors such as saliva composition, biofilm and occlusal loading were not simulated. Future studies with long-term aging, thermocycling and nanoleakage analysis are needed.

CONCLUSION(S)

The NBG and TH significantly improved the µSBS of demineralised dentin. TH exhibited a rapid remineralising effect by stimulating calcium and phosphate ions, producing larger crystals and showing superior µSBS at 1 day. However, after seven days, NBG demonstrated greater improvement due to increased mineral matrix formation. Overall, both NBG and TH effectively enhanced the bond strength of demineralised dentin.

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