

Effect of Two Different Concentrations of Chlorhexidine Digluconate on the Shear Bond Strength of Resin Composite to Biodentine using Self-etch and Etch-rinse Bonding Strategies: An In-vitro Study

POOJA BARGHARE¹, SUMANTHINI MARGASAHAYAM², ANURADHA PATIL³, DIVYA NAIK⁴, AMISHA SAOJI⁵



ABSTRACT

Introduction: Chlorhexidine (CHX) has been most commonly used as an antimicrobial component as well as for disinfection before the placement of restorations. CHX has demonstrated successful inhibition of dentin Matrix Metalloproteinase (MMP) collagenolytic activity when used in concentrations of 0.2% and 2%, however this may influence the bond strength of composite resin to Biodentine when applied before adhesive procedures. The present study endeavours to evaluate a bonding strategy that would best suit the preconditioning with CHX.

Aim: To evaluate the effect of two different concentration of CHX on Shear Bond Strength (SBS) between resin composite and Biodentine, using self-etch and etch-rinse bonding strategies.

Materials and Methods: The present in-vitro study was carried out in the Department of Conservative Dentistry and Endodontics in Mahatma Gandhi Mission Dental College and Hospital, Navi Mumbai, Maharashtra, India, from January 2022 to May 2022 on 108 acrylic blocks. A central cavity was prepared in the acrylic block and randomly divided into six main groups. Group 1 (control) Biodentine placement; self-etch adhesive application followed by resin composite. Group 2 (control) Biodentine placement; etch and rinse adhesive application followed by resin composite. Since two different bonding strategies, self-etch and etch-and-rinse were used, two control groups were included. Each of these bonding methods has different application protocols and bonding mechanisms, which could independently affect the SBS between resin composite and Biodentine, therefore two control groups were included. Including both controls allows for a fair and unbiased comparison with the experimental groups where CHX (at 0.2% or 2%) was used. This ensures that any observed differences in bond strength can be accurately attributed to the effect of CHX, rather than differences between the bonding strategies themselves. Group 3 Biodentine placement; application of 0.2% CHX prior to application of self-etch adhesive followed

by resin composite placement. Group 4 Biodentine placement; application of 2% CHX prior to application of self-etch adhesive followed by resin composite placement. Group 5 Biodentine placement; for etch and rinse adhesive system application 0.2% CHX after acid etching prior to application of adhesive followed by resin composite placement. Group 6 Biodentine placement; for etch and rinse adhesive system application 2% CHX after acid etching prior to application of adhesive followed by resin composite placement. Thereafter the samples were subjected to the shear bond testing in a universal testing machine. The obtained data was tabulated and statistically analysed for normality using Shapiro-wilk test. Further statistical analysis was done using One-way Analysis of Variance and Two-way ANOVA followed by Tukey-Kramer Test for pair-wise comparisons. Post-hoc test was done to compare control group compared with CHX treated group irrespective of bonding technique.

Results: Surface treatment of Biodentine with 0.2% or 2% CHX reduced the bond strength of composite resin to Biodentine irrespective of bonding strategies. The One-way ANOVA test showed there was statistically significant difference in SBS of the resin composite bonded to Biodentine when the different bonding strategies and surface treatment are applied ($p < 0.001$). Two-way ANOVA test showed statistically significant difference in SBS between etching technique and different concentrations of CHX with $p < 0.001$. A higher SBS was observed when the Biodentine was pretreated with 0.2% CHX (Group 3 and Group 5) compared to 2% CHX (Group 4 and Group 6) ($p < 0.001$). Post-hoc test statistically significant difference found in SBS when control groups when compared to groups treated with CHX, irrespective of bonding technique ($p < 0.001$).

Conclusion: Application of 2% and 0.2% CHX on Biodentine reduced the SBS with resin composite, irrespective of the bonding strategy. Between the self-etch and etch-rinse adhesive system used in this study, etch-rinse showed higher SBS for 0.2% CHX compared to self-etch adhesive system.

Keywords: Cavity disinfectants, Dentine, Dental bonding agents, Matrix metalloproteinase, Microleakage

INTRODUCTION

Vital Pulp Therapy (VPT) is a strategy for treating significant dental caries, dental trauma, restorative operations, or iatrogenic causes that have weakened but not completely destroyed the pulp tissue [1]. Comparing the VPT to traditional root canal treatments, there are many benefits [2]. The maintenance of the tooth's vitality in situations with immature apices is advantageous

for continuing root development, increasing the strength of the damaged tooth [3].

Theracal, Biodentine (BD), Mineral Trioxide Aggregate (MTA), and other Calcium Hydroxide (CH) and calcium oxide-based compounds are the most widely used agents for direct and indirect pulp capping [4]. A disadvantage of CH is the occurrence of so-called "tunnel defects" in the reparative dentin created underneath CH, which has

no inherent adhesive properties and makes for a weak seal [5]. As a direct pulp capping substance, MTA proved to be more successful and superior to CH [6]. It also proved to be simpler to apply and had better results in preserving long-term tooth vitality [7]. The practitioner is still faced with difficulties due to the material's lengthy setting time, poor handling characteristics, expensive material cost, and discolouration possibility [8].

Resin composites in combination with dental adhesive systems are widely used to restore the cavitated teeth. However, the failure rate of resinous restorations is relatively high [9]. Resin composite to dentin bond is created by impregnating the dentin substrate with blends of resin monomers, and the stability of the bonded interface of dentine and resinous material [10].

The CHX has been most commonly used as an antimicrobial component as well as for disinfection before the placement of restorations [11,12]. CHX is a bis-guanide that acts by adsorbing onto the bacterial cell wall resulting in leakage of intracellular components of bacteria. The use of 2% CHX digluconate has been recommended for cleansing prepared cavity surface, owing to its ability to significantly reduce the levels of oral microorganisms in a short period of time, including *S.mutans* present mostly in dentinal tubules [12].

Moreover, in-vitro and in-vivo studies have suggested that the application of 2% CHX solution, a MMP inhibitor, to acid-etched dentin for 60 s could minimise the degradation of the dentin bond over time [13-15]. Specific host-derived MMPs, responsible for the breakdown of the collagen matrices (MMP-2, MMP-8, and MMP-9), can be inhibited by low concentrations of CHX (0.0001–0.02%) [16]. However, application of CHX may hamper the bond between Biodentine and resin composite [13].

There are very few studies in literature which have evaluated bonding of resin composite and Biodentine under influence of different concentrations of CHX. Hebling J et al., (2005), they reported that CHX arrests subclinical degradation of dentin hybrid layers [14]. Therefore, the purpose of present study was to evaluate effect of two different concentration of CHX on bond strength between resin composite and Biodentine, using self-etch and etch-rinse bonding strategies.

The null hypothesis tested was that there is no difference in the effect of two different concentrations of CHX digluconate on the SBS of resin composite to Biodentine, when bonded using self-etch and etch-rinse bonding strategies. Alternate hypothesis tested was there would be a difference in the effect of two different concentrations of CHX digluconate on the SBS of resin composite to Biodentine, when bonded using self-etch and etch-rinse bonding strategies.

MATERIALS AND METHODS

This in-vitro study was conducted in the Department of Conservative Dentistry and Endodontics at Mahatma Gandhi Mission Dental College and Hospital, Navi Mumbai, Maharashtra, India from January 2022 to May 2022. after obtaining due permission from the Institutional Ethics Committee (no. of IEC approval MGM/DCH/IEC/004/2021).

Sample size calculation: The sample size was calculated based on SBS when pretreated with 2% CHX is 12.16 (2.62 MPa) on Biodentine and without pre-treatment 15.25 (3.84 MPa) from the pilot study. For a power of 90% and alpha value of 0.05 sample size was calculated on F test using a One-way ANOVA analysis by means of Stata software (MedCalc Statistical Software version 18.2.1 (MedCalc Software, Ostend, Belgium; <http://www.medcalc.org>; 2018). Samples size was calculated to be 108 and divided into 6 groups with 18 samples in each group [17]. The mean SBS values (12.16 and 15.25) were sourced from an Institutional study.

Study Procedure

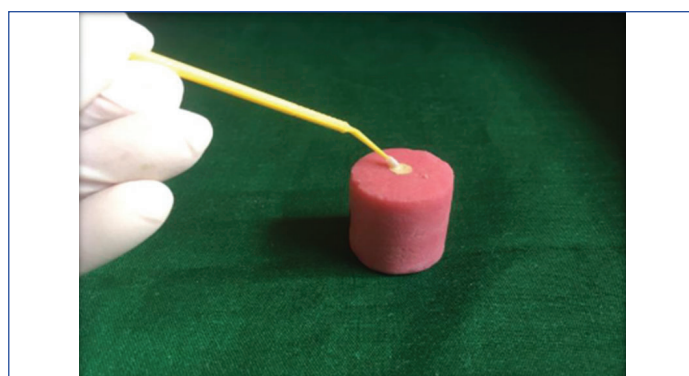
A total of 108 acrylic blocks measuring (20x20x25 mm height) were prepared using cold-cure acrylic resin (DPI RR cold-cure acrylic resin, Dental Product of India, a division of the Bombay Burmah Trading Corporation Ltd., Mumbai, India) and polished using sandpaper. The acrylic blocks were then randomly divided into six main groups, namely Group 1, Group 2, Group 3, Group 4, Group 5 and Group 6. A central cavity was prepared in the acrylic block using a straight tungsten carbide bur in an airtor hand piece using high speed [Table/Fig-1]. The dimensions of the cavity were 5 mm in diameter and 2 mm deep in all the blocks and it was assigned to six groups. Biodentine (Septodont, Headquarters: SaintMaur-des-Fossés, France) was placed inside the cavity for all the group [Table/Fig-2] and a cylinder of resin composite (3M Espe Filtek Z350, headquarters-Maplewood minnesota) was bonded to the Biodentine based [Table/Fig-3,4] on the group allocation as follows:



[Table/Fig-1]: Prepared cavity of 2 mm depth and 5 mm in diameter.

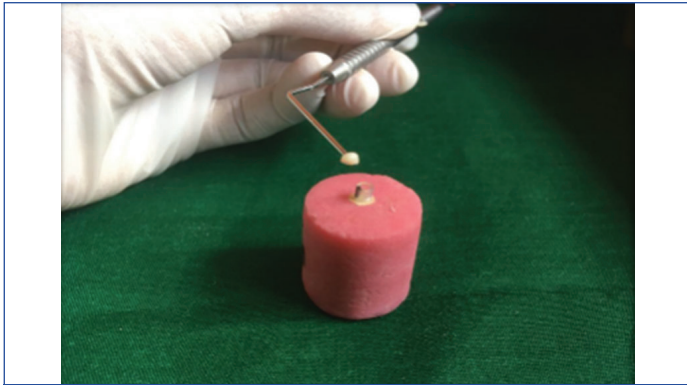


[Table/Fig-2]: Placement of Biodentine.

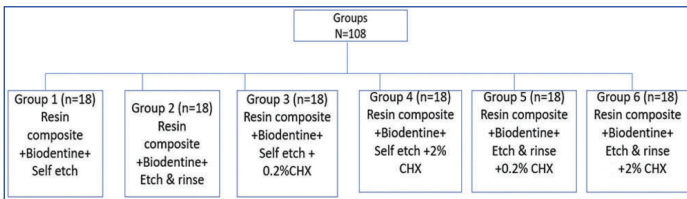


[Table/Fig-3]: Application of adhesive.

A plastic tube was used to aid in the proper condensation of the composite material and to produce a cylinder shape with a uniform height of 2 mm. Additionally, the curing light was passed through the plastic tube to ensure adequate polymerisation of the composite. This setup ensured: Standardisation of specimen shape and size, which is crucial for consistency in SBS testing and controlled light-curing conditions, improving the reliability of the polymerisation process across samples. Sample distribution has been depicted in [Table/Fig-5].



[Table/Fig-4]: Cylinder of composite prepared by condensing composite into plastic tube



[Table/Fig-5]: Sample distribution.

Group 1: (Control) Biodentine placement; self-etch adhesive (3M ESPE single bond adhesive, headquarters-Maplewood minnesota) application [Table/Fig-3] followed by resin composite.

Group 2: (Control) Biodentine placement; etch and rinse adhesive application followed by resin composite.

Group 3: Biodentine placement; application of 0.2% CHX digluconate for one min prior to application of self-etch adhesive followed by resin composite placement.

Group 4: Biodentine placement; application of 2% CHX digluconate for one min prior to application of self-etch adhesive followed by resin composite placement.

Group 5: Biodentine placement; for etch and rinse adhesive system application 0.2% CHX digluconate for one min after acid etching prior to application of adhesive followed by resin composite placement.

Group 6: Biodentine placement; for etch and rinse adhesive system application 2% CHX digluconate for one min after acid etching prior to application of adhesive followed by resin composite placement.

In the present study, Biodentine specimens without composite were stored for 72 h, 37°C at 100% humidity in a laboratory incubator to allow complete hardening of the materials in stimulated intraoral conditions. After 72 hrs, the blocks were removed from incubator followed by resin composite placement. This duration is often used in in-vitro studies for early bond assessment: It allows researchers to measure how well the material initially adheres, before long-term factors like degradation or fatigue set in [18], Standardisation: Many similar studies use 24, 48, or 72-hour time frames to maintain consistency and enable comparison, Practicality: It is a manageable period for laboratory-based experiments and provides quick preliminary results, Material setting and interaction: Some materials (like Biodentine or resin composites) may need time to fully set or interact at the interface, so 72 hrs ensures that early chemical bonding and mechanical interlocking are complete.

Thereafter the samples were subjected to the shear bond testing in a universal testing machine (Instron India) [Table/Fig-6]. Universal testing machine applies a controlled, reproducible forces to bonded surface, quantifies the maximum forces (in MPa or N) the bond can withstand before failure and ensures consistency across all test samples for scientific comparison. A crosshead speed of 1 mm/

min was applied to each sample using a knife-edge device until the bond between the Biodentine and resin composite material failed.



[Table/Fig-6]: Sample subjected to testing.

STATISTICAL ANALYSIS

Data was captured using MS Excel and assessed for errors. Statistical analysis was done using MedCalc Statistical Software version 18.2.1 (MedCalc Software, Ostend, Belgium; <http://www.medcalc.org>; 2018). The data obtained was presented using descriptive statistics such as mean, standard deviation, maximum and minimum values as shown in table followed by graphs and charts. Data was tested for normality distribution using Shapiro-wilk test (skewness and kurtosis). It was found to be normally distributed $p>0.05$. Further analysis was done using One-way ANOVA and Two-way ANOVA. Pair-wise comparison was done with the Tukey–Kramer test to evaluate which group showed a significant difference. The level of significance was set at 5%.

RESULTS

The results of the study showed that surface treatment of Biodentine with 0.2% or 2% CHX reduced the bond strength of composite resin to Biodentine irrespective of bonding strategies [Table/Fig-7]. The highest mean SBS was observed in Group 1 (no CHX pre-treatment) while Group 6 in which 2% CHX with etch and rinse bonding strategy showed the least value.

| Groups | Mean±SD | Minimum MPa | Maximum MPa | Standard error | 95% Confidence Interval (CI) |
|---------|----------------|-------------|-------------|----------------|------------------------------|
| Group 1 | 12.5578±1.7818 | 9.2300 | 16.2400 | 0.4200 | 13.2100-14.8709 |
| Group 2 | 10.0883±1.4114 | 8.1200 | 12.5700 | 0.3327 | 10.1344-12.2331 |
| Group 3 | 8.0441±0.9398 | 6.3600 | 9.3900 | 0.2215 | 8.1889-9.3900 |
| Group 4 | 7.2282±0.8182 | 5.5700 | 8.6600 | 0.1928 | 7.4867-8.4951 |
| Group 5 | 9.1158±1.4585 | 6.4900 | 11.2700 | 0.3438 | 9.6756-11.1123 |
| Group 6 | 5.0628±0.5590 | 4.3600 | 6.2400 | 0.1318 | 5.0555-6.0106 |

[Table/Fig-7]: Descriptive statistics of SBS (MPa) value in all the six groups.

Two-way ANOVA test showed statistically significant difference in SBS between etching technique and different concentrations of CHX with $p<0.001$ [Table/Fig-8]. Intergroup comparison by Tukey-Kramer test showed that SBS of Group 3 (0.2% CHX, Self-etch technique) was statistically significant when compared to groups 1, 2, and 6. Group 4 (2% CHX, self-etch technique) showed statistically significant difference when compared to group 1, 2, 5 and 6. Group 5 (0.2% CHX, etch-rinse technique) showed a statistically significant difference when compared to groups 1, 4, and 6 [Table/Fig-9].

A higher SBS was observed when the BD was pretreated with 0.2% of CHX (Group 3 and Group 5) was used compared to 2% of

| Source* | Sum of squares | DF | Mean Square | F | p |
|-----------------------|----------------|-----|-------------|---------|-----------|
| Intervention | 369.960 | 2 | 184.980 | 121.179 | <1.0E-14 |
| Material | 165.813 | 1 | 165.813 | 108.623 | <1.0E-14 |
| Intervention*Material | 49.453 | 2 | 24.726 | 16.198 | 0.78E-006 |
| Residual | 155.703 | 102 | 1.526 | | |

[Table/Fig-8]: Two-way ANOVA table showed difference in SBS between etching technique and different concentration of CHX.

p is value statistically significant; DF: Degree of freedom Source source of data sets used for calculation (1.0E-14 is scientific notation for, which equals 0.00000000000001 - an extremely small number. The "<" symbol means that the p-value is even smaller than. the "Intervention" factor has a very strong effect on the outcome.)

| Groups | n | Mean±SD | Different (p<0.05) from factor nr |
|--------|----|----------------|-----------------------------------|
| (1) 1 | 18 | 12.5578±1.7818 | (2)(3)(4)(5)(6) |
| (2) 2 | 18 | 10.0883±1.4114 | (1)(3)(4)(6) |
| (3) 3 | 18 | 8.0978±0.9398 | (1)(2)(6) |
| (4) 4 | 18 | 7.2728±0.8182 | (1)(2)(5)(6) |
| (5) 5 | 18 | 9.2311±1.4585 | (1)(4)(6) |
| (6) 6 | 18 | 5.0911±0.5590 | (1)(2)(3)(4)(5) |

[Table/Fig-9]: Intergroup comparison of SBS value between all groups with different bonding strategies and different concentration of CHX used.

Test applied- Tukey-Kramer test ,Statistically significant difference (p <0.05) from the corresponding group

CHX [Table/Fig-10] (Group 4 and Group 6), irrespective of bonding technique (p<0.001) [Table/Fig-11, 12].

| Factors | Mean difference | Std. Error | Pa | 95% Cla |
|---------|-----------------|------------|----------|--------------------|
| 1 - 2 | 2.4781 | 0.2378 | <1.0E-14 | 2.0065 to 2.9498 |
| 2 - 1 | -2.4781 | 0.2378 | <1.0E-14 | -2.9498 to -2.0065 |

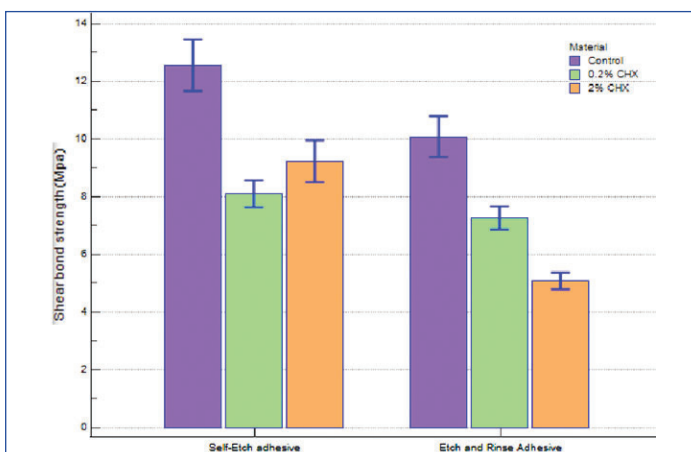
[Table/Fig-10]: Pair-wise comparisons of materials with different concentration of CHX used.

Pa Bonferroni test applied, highly significant -p-value of the statistical test used to compare factor 1 and factor 2. 95% Cla-95% CI for the mean difference. The p-value <0.05 was considered statistically significant. a Bonferroni corrected

| Factor | Mean difference | Std. Error | Pa | 95% Cla |
|--------|-----------------|------------|----------|--------------------|
| 1 - 2 | 3.6378 | 0.2912 | <1.0E-14 | 2.9289 to 4.3466 |
| - 3 | 4.1619 | 0.2912 | <1.0E-14 | 3.4531 to 4.8708 |
| 2 - 1 | -3.6378 | 0.2912 | <1.0E-14 | -4.3466 to -2.9289 |
| - 3 | 0.5242 | 0.2912 | 0.2245 | -0.1847 to 1.2330 |
| 3 - 1 | -4.1619 | 0.2912 | <1.0E-14 | -4.8708 to -3.4531 |
| 2 | -0.5242 | 0.2912 | 0.2245 | -1.2330 to 0.1847 |

[Table/Fig-11]: Pair-wise comparisons using Post-hoc test when compared to groups treated with CHX irrespective of bonding technique.

Std error- standard error, p-values -(Pa), 95% Cla. Statistically significant differences are indicated by p < 0.05.



[Table/Fig-12]: Comparison between self-etch and etch-rinse technique in presence of CHX.

The Two-way ANOVA analysis showed that etching technique and CHX concentration had a very strong and statistically significant

effect on SBS. The extremely small p-value (< 1.0E-14) confirms that the observed differences are not due to chance, meaning the "intervention" (etching + CHX) had a real and powerful impact on the outcome.

The One-way ANOVA test showed there was statistically significant difference in SBS of the resin composite bonded to Biodentine when the different bonding strategies and surface treatment are applied (F (5,102)=76.676 p<0.001) [Table/Fig-13]. The self-etch system had a higher bond strength when the BD was pretreated with 0.2% CHX compared with 2% CHX used. The etch and rinse group showed better bond strength when 0.2% CHX was applied.

| Source of variation | Sum of squares | DF | Mean square |
|------------------------------------|----------------|-----|-------------|
| Between groups (influence factor) | 585.2249 | 5 | 117.0450 |
| Within groups (other fluctuations) | 155.7026 | 102 | 1.5265 |
| Total | 740.9275 | 107 | |

[Table/Fig-13]: Differences in SBS of the resin composite bonded to Biodentine when the different bonding strategies and surface treatment are applied.

Test applied - One-way ANOVA*p is value statistically significant P <0.001; DF: Degree of freedom

DISCUSSION

In the present study, the bonding of composite resin to Biodentine after pre-treatment with 2% and 0.2% CHX were evaluated. After comparative analysis of findings showed both concentrations of CHX (0.2% and 2%) reduced the SBS between resin composite and Biodentine, the highest SBS was observed in the control group with self-etch adhesive and no CHX pre-treatment, Among CHX-treated groups, 0.2% CHX showed comparatively higher SBS than 2% CHX, suggesting that higher concentration may be more detrimental, the etch-rinse adhesive system showed superior SBS values compared to self-etch adhesives. The results of the study found that the use of 2% and 0.2% of CHX and different bonding strategies reduced the SBS between resin composite and Biodentine. Therefore, the null hypothesis stands rejected.

These findings are consistent with previous studies by Loguercio AD et al., and Sabatini C, they reported that the CHX pre-treatment can improve the longevity of resin-dentin bonds yet higher concentrations might lead to bond degradation over time [13,19]. Alzraikat H et al., observed the different adhesive strategies yielding varying bond strengths with calcium silicate materials, aligning with the present result showing self-etch adhesives outperform etch-rinse systems [20]. Therefore, present study supports existing literature while providing specific evidence for the interaction between CHX concentration, adhesive strategy, and bonding efficacy to Biodentine.

The CHX, a cationic antimicrobial agent, inhibits dentin MMP collagenolytic activity at concentrations of 0.2% and 2%, thus improving the stability of the adhesive interface over time [19]. Applying CHX to acid-etch dentin prior to the use of etch-and-rinse adhesives may prevent the degradation of collagen fibrils, besides its antimicrobial property [21]. Study conducted using inhibition assay technique on MMP-2 and MMP-9 activity. At a concentration of 0.03%, CHX produced a complete inhibition of MMP-2 and -9 gelatinase activities. The inhibitory effect of CHX was concentration dependent. The minimal concentration of CHX needed to inhibit the MMP-9 is 0.002%, while MMP-2 activity is more sensitive, being inhibited at a CHX concentration as low as 0.0001%, and MMP-8 is inhibited by 0.02% CHX [21].

The CH, MTA and BD can be used as a pulp capping agent among many others [22]. Biodentine is a bioactive, biocompatible material suitable for use in furcal perforations, retrograde filling, pulp capping and can be applied directly in the restorative cavity as a bulk dentin substitute [23]. It has been shown to stimulate tertiary dentine formation and is well tolerated by the pulp tissue when it is in direct contact (in cases of direct pulp capping) forming reparative dentine [24,25]. Biodentine sets faster when compared to MTA. In a study

conducted by Nowicka A et al., compared the pulpal response to MTA and BD, they observed that placement of MTA was more time consuming and difficult in handling in comparison to Biodentine [23]. Major drawback of MTA is its prolong initial setting time (2 hrs 45 min). Conversely, for Biodentine, as initial setting time is 12 mins, shorter than MTA thus making it possible for immediate placement of resin composite within single visit [26,27].

In clinical conditions, after the placement of the pulp capping agent, the subjacent residual dentine surface may be treated with CHX in order to achieve stable dentin resin bonds [28]. In such conditions, the biodentin also may be layered with CHX. This may have an effect on the bonding of resin composite to the BD surface.

In the present study, single bond universal when applied in self etch mode showed better SBS value without pretreated with 2% CHX and 0.2% CHX. This may be due to the chemical interaction achieved through specific functional monomers, such as 10-Methacryloyloxydecyl dihydrogen phosphate (10-MDP), 4 MET (4-methacryloxyethyl trimellitic acid) and phenyl-P. The ionic bond formation of the carboxylic/phosphate groups of these functional monomers to Calcium of Hydroxyapatite was first proven by Yoshida et al using X-ray photo-electron spectroscopy [29]. Self-etch adhesive systems also contain 2-hydroxyethyl methacrylate monomer because most of the acidic monomers are low water-soluble and to increase the wettability of dentin surface. Bi- or multi-functional monomers are added to provide strength to the cross-linking formed from monomeric matrix [30]. Furthermore, self-etch adhesive systems do not require a separate acid conditioning step and moist post-rinse control they are considered simplified adhesive materials. They offer some advantages over conventional etch-and-rinse systems, such as reduction of postoperative sensibility and less sensitive technique [31].

The reduction in bond strength due to CHX seems to be concentration dependent, attributed to the mechanism of calcium ions, released from the dentin during the self-etch process, that can essentially inhibit the effect of CHX on MMPs through their chelation property [21,32,33]. Therefore, CHX percentage in the primer or resin may not have an immediate effect, however can significantly affect the resin- dentin bond stability over a period of time as observed by Kazemi-Yazdi H et al., [34]. Campos EA et al., reported that dentin pre-treatment with concentrations of CHX above 0.12% CHX negatively affected μ TBS of dentin substrates [35].

The findings of present study have direct implications for clinical practice in restorative dentistry and VPT. Avoiding CHX Pre-treatment on Biodentine: As both 0.2% and 2% CHX reduced SBS, it is advisable to avoid CHX application on Biodentine when planning composite restorations. Preference for Self-Etch Systems: Clinicians may prefer self-etch adhesive systems when working with Biodentine, especially when no CHX is used, due to their superior bond strength and simplified technique. Selective use of low-concentration CHX: If CHX disinfection is deemed necessary using lower concentrations (e.g., 0.2%) may be less harmful to bond integrity than higher concentrations. The present study contributes to evidence-based decision-making by highlighting how material interactions influence long-term restoration success.

Future research should focus on clinical trials replicating intraoral conditions such as saliva contamination, pH fluctuations, and mechanical loading are essential to confirm these findings. Extended evaluation of bond strength over time (e.g., 6-12 months) will help assess the aging effect and degradation of the adhesive interface. Testing two-step or multi-step adhesives might yield better results than single-step systems and should be explored. Evaluating the bonding performance of CHX with materials like MTA, TheraCal, or newer bioactive cements can offer broader insight. Future studies should also measure micro-tensile bond strength, flexural strength, and marginal integrity to comprehensively assess clinical performance.

Limitation(s)

Limitations of the study include the in-vitro study design where, intraoral conditions like pH, temperature and moist conditions could not be recreated during study procedure. This study as preliminary one, evaluated the resin composite bonded to bio dentine after 72 hrs, studies with longer duration of bond of resin composite to bio dentine need to be carried out in along mechanical properties like micro tensile strength to evaluate durability of the bond. Currently there is a number of calcium silicate-based cement that has therapeutic effect on pulp and should be evaluated for adhesion to composite resin.

CONCLUSION(S)

Within the limitation of current in-vitro study the following conclusion were arrived at application of 2% and 0.2% CHX on biodentine reduced the SBS with resin composite irrespective of the bonding strategy. The reduction in bond strength was dependent on the concentration of CHX. Between self-etch and etch and rinse adhesive system used in this study, etch &rinse showed higher bond strength values compared to self-etch adhesive system at 0.2% of CHX and self-etch performed better at 2%.

REFERENCES

- [1] Asgary S, Parhizkar A. Importance of 'time' on 'haemostasis' in vital pulp therapy - letter to the editor. *Eur Endod J.* 2021;6(1):128-29.
- [2] Ward J. Vital pulp therapy in cariously exposed permanent teeth and its limitations. *Aust Endod J.* 2002;28(1):29-37.
- [3] Caplan DJ, Cai J, Yin G, White BA. Root canal filled versus non-root canal filled teeth: A retrospective comparison of survival times *J Public Health Dent.* 2005;65(2):90-96.
- [4] Jain B, Tiku A. A comparative evaluation of shear bond strength of three different restorative materials to biodentine and TheraCal Ic: An in-vitro study. *Int J Appl Dent.* 2019;5(2):426-29.
- [5] Hilton TJ. Keys to clinical success with pulp capping: A review of the literature. *Oper Dent.* 2009;34(5):615-25.
- [6] Zhu C, Ju B, Ni R. Clinical outcome of direct pulp capping with MTA or calcium hydroxide: A systematic review and meta-analysis. *Int J Clin Exp Med.* 2015;8(10):17055-60.
- [7] Tran XV, Salehi H, Truong MT, Sandra M, Sadoine J, Jacquot B, et al. Reparative mineralized tissue characterization after direct pulp capping with calcium-silicate-based cements. *Materials (Basel).* 2019;12(13):2102. Published 2019 Jun 29.
- [8] Camilleri J. Investigation of Biodentine as dentine replacement material. *J Dent.* 2013;41(7):600-10.
- [9] Drummond JL. Degradation, fatigue, and failure of resin dental composite materials. *J Dent Res.* 2008;87(8):710-19.
- [10] Nakabayashi N, Kojima K, Masuhara E. The promotion of adhesion by the infiltration of monomers into tooth substrates. *J Biomed Mater Res.* 1982;16(3):265-73.
- [11] Carrilho MR, Carvalho RM, de Goes MF, di Hipólito V, Geraldelli S, Tay FR, et al. Chlorhexidine preserves dentin bond in-vitro. *J Dent Res.* 2007;86(1):90-94.
- [12] Matthijs S, Adriaens PA. Chlorhexidine varnishes: A review. *J Clin Periodontol.* 2002;29(1):01-08.
- [13] Loguerico AD, Stanislawczuk R, Polli LG, Costa JA, Michel MD, Reis A. Influence of chlorhexidine digluconate concentration and application time on resin-dentin bond strength durability. *Eur J Oral Sci.* 2009;117(5):587-96.
- [14] Hebling J, Pashley DH, Tjäderhane L, Tay FR. Chlorhexidine arrests subclinical degradation of dentin hybrid layers in-vivo. *J Dent Res.* 2005;84(8):741-46.
- [15] Brackett MG, Tay FR, Brackett WW, et al. In-vivo chlorhexidine stabilization of hybrid layers of an acetone-based dentin adhesive. *Oper Dent.* 2009;34(4):379-83.
- [16] de Souza LB, de Aquino SG, de Souza PP, Hebling J, Costa CA. Cytotoxic effects of different concentrations of chlorhexidine. *Am J Dent.* 2007;20(6):400-04.
- [17] MedCalc Software bvba. MedCalc Statistical Software version 18.2.1. Ostend, Belgium; 2018.
- [18] Nowicka A, Lipski M, Parafiniuk M, Sporniak-Tutak K, Lichota D, Kosierkiewicz A, et al. Response of human dental pulp capped with biodentine and mineral trioxide aggregate. *J Endod.* 2013;39(6):743-47.
- [19] Sabatini C. Effect of a chlorhexidine-containing adhesive on dentin bond strength stability. *Oper Dent.* 2013;38(6):609-17.
- [20] Alzraikat H, Taha NA, Qasrawi D, Burrow MF. Shear bond strength of a novel light cured calcium silicate based-cement to resin composite using different adhesive systems. *Dent Mater J.* 2016;35(6):881-87.
- [21] Gendron R, Grenier D, Sorsa T, Mayrand D. Inhibition of the activities of matrix metalloproteinases 2, 8, and 9 by chlorhexidine. *Clin Diagn Lab Immunol.* 1999;6(3):437-39.
- [22] Acharya S, Gurunathan D, Assiry AA, Luke AM, Shetty KP, Karobari MI. Comparison of Modified NeoPutty MTA®, Biodentine, and Calcium Hydroxide in indirect pulp therapy in deciduous teeth: An *in-vivo* clinical study. *Int J Clin Pediatr Dent.* 2024;17(9):1025-29.

- [23] Malkondu Ö, Karapinar Kazandağ M, Kazazoğlu E. A review on biodentine, a contemporary dentine replacement and repair material. *Biomed Res Int*. 2014;2014:160951.
- [24] Shayegan A, Jurysta C, Atash R, Petein M, Abbeele AV. Biodentine used as a pulp-capping agent in primary pig teeth. *Pediatr Dent*. 2012;34(7):e202-e208.
- [25] Laurent P, Camps J, About I. Biodentine(TM) induces TGF-β1 release from human pulp cells and early dental pulp mineralization. *Int Endod J*. 2012;45(5):439-48.
- [26] Parirokh M, Torabinejad M. Mineral trioxide aggregate: A comprehensive literature review--Part I: Chemical, physical, and antibacterial properties. *J Endod*. 2010;36(1):16-27.
- [27] Rajasekharan S, Martens LC, Cauwels RG, Verbeeck RM. Biodentine™ material characteristics and clinical applications: A review of the literature. *Eur Arch Paediatr Dent*. 2014;15(3):147-58.
- [28] Breschi L, Mazzoni A, Nato F, Carrilho M, Visintini E, Tjäderhane L, et al. Chlorhexidine stabilizes the adhesive interface: A 2-year in-vitro study. *Dent Mater*. 2010;26(4):320-25.
- [29] Yoshida Y, Nagakane K, Fukuda R, Nakayama Y, Okazaki M, Shintani H, et al. Comparative study on adhesive performance of functional monomers. *J Dent Res*. 2004;83(6):454-58.
- [30] Van Landuyt KL, Snauwaert J, De Munck J, Peumans M, Yoshida Y, Poitevin A, et al. Systematic review of the chemical composition of contemporary dental adhesives. *Biomaterials*. 2007;28(26):3757-85.
- [31] Van Meerbeek B, Peumans M, Poitevin A, Mine A, Van Ende A, Neves A, et al. Relationship between bond-strength tests and clinical outcomes. *Dent Mater*. 2010;26(2):e100-e121.
- [32] Frassetto A, Breschi L, Turco G, Marchesi G, Di Lenarda R, Tay FR, et al. Mechanisms of degradation of the hybrid layer in adhesive dentistry and therapeutic agents to improve bond durability-- A literature review. *Dent Mater*. 2016;32(2):e41-e53.
- [33] Maravić T, Comba A, Cunha SR, Angeloni V, Cadenaro M, Visintini E, et al. Long-term bond strength and endogenous enzymatic activity of a chlorhexidine-containing commercially available adhesive. *J Dent*. 2019;84:60-66.
- [34] Kazemi-Yazdi H, Saeed-Nezhad M, Rezaei S. Effect of Chlorhexidine on durability of two self-etch adhesive systems. *J Clin Exp Dent*. 2020;12(7):e663-e669.
- [35] Campos EA, Correr GM, Leonardi DP, Pizzatto E, Morais EC. Influence of chlorhexidine concentration on microtensile bond strength of contemporary adhesive systems. *Braz Oral Res*. 2009;23(3):340-45.

PARTICULARS OF CONTRIBUTORS:

1. Postgraduate Student, Department of Conservative Dentistry and Endodontics, MGM Dental College and Hospital, Navi Mumbai, Maharashtra, India.
2. Head, Department of Conservative Dentistry and Endodontics, MGM Dental College and Hospital, Navi Mumbai, Maharashtra, India.
3. Professor, Department of Conservative Dentistry and Endodontics, MGM Dental College and Hospital, Navi Mumbai, Maharashtra, India.
4. Professor, Department of Conservative Dentistry and Endodontics, MGM Dental College and Hospital, Navi Mumbai, Maharashtra, India.
5. Postgraduate Student, Department of Conservative Dentistry and Endodontics, MGM Dental College and Hospital, Navi Mumbai, Maharashtra, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Sumanthini Margasahayam,
Head, Department of Conservative Dentistry and Endodontics, MGM Dental
College and Hospital, Navi Mumbai, Maharashtra, India.
E-mail: margsuman@gmail.com

PLAGIARISM CHECKING METHODS: [\(Jain H et al.\)](#)

- Plagiarism X-checker: Mar 07, 2025
- Manual Googling: Sep 13, 2025
- iThenticate Software: Sep 15, 2025 (2%)

ETYMOLOGY: Author Origin

EMENDATIONS: 7

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? NA
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Mar 03, 2025**

Date of Peer Review: **May 18, 2025**

Date of Acceptance: **Sep 17, 2025**

Date of Publishing: **May 01, 2026**