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# Effect of dimethyl sulfoxide pretreatment on bonding of universal adhesives to pulp chamber dentin

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ABSTRACT

*Aim and objective:* To evaluate the effect of 50 % (v/v) dimethyl sulfoxide (DMSO) pretreatment on immediate (24 h) and delayed (6 months) microtensile bond strength (μTBS) of an etch-and-rinse adhesive and two universal adhesives to dentin.

*Materials and methods:* A total of 144 extracted human molars were decoronated, pulp chambers exposed, and roots sectioned 2 mm apical to the CEJ. Teeth were randomly divided into six experimental groups (n = 24), based on surface pretreatment and adhesive system used: Group 1A: Adper Single Bond 2 (ASB) Group 2A: Tetric N-Bond Universal (TNBU) Group 3A: Prime & Bond Universal (PNBU) Groups 1B, 2B, and 3B received the same adhesives, respectively, following a 50 % (v/v) DMSO wet bonding pretreatment. All adhesives were applied per manufacturer's instructions, followed by composite resin build-up. Ten samples per group were tested for μTBS at 24 h and ten after 6 months of storage. Failure mode was analyzed. Four additional samples per group underwent SEM evaluation to observe the resin–dentin interface.

*Statistical analysis:* Data were analyzed using one-way ANOVA and Tukey's post hoc test for intergroup comparisons. Unpaired t-tests were applied for two-group comparisons. Significance was set at $p < 0.05$.

*Results:* DMSO pretreatment significantly improved both immediate and long-term μTBS ($p < 0.05$) across all adhesives. PNBU exhibited the highest bond strength at both time intervals.

*Conclusion:* Dentin pretreatment with 50 % DMSO prior to adhesive application enhances immediate μTBS and preserves bond durability at 6 months, regardless of adhesive system used.

## 1. Introduction

Achieving an effective coronal seal may have equal or even greater impact than the quality of root canal treatment in predicting the clinical success of endodontically treated teeth.[1] Due to their ability to bond micromechanically, composite resins are being more often used for post-endodontic restorations. Endodontically treated teeth have compromised structural integrity and are prone to fracture, making adhesive resin restorations essential for reinforcement.[2] However, pulp chamber dentin poses greater challenges to resin adhesion than coronal dentin.[3] Root canal irrigants reduce dentin microhardness and flexural strength.[4] Additionally, residual sealer or pulp tissue, high C-factor cavity, limited light polymerization, and irregular secondary dentin further complicate resin–dentin bonding.[5] Although adhesive dentistry has evolved enormously in past few years, the durability of dentin bonding with universal adhesives still remains questionable especially when complexities involved in post-endodontic restorations are considered.[6]

Bonding to dentin is considered to be much more challenging as compared to enamel as it is a vital, hydrated, biologically active substrate with substantial amounts of organic constituent, complex tubular arrangement with dentinal fluid, differences in permeability of superficial and deep dentin and presence of endogenous proteases.[7] Adhesion to this dynamic structure involves resin infiltration into the demineralized collagen mesh to form hybrid layer, which is the weakest link in the resin-dentin interface.

To prevent accelerated disintegration of exposed dentin collagen fibrils by endogenous dentinal enzymes like cysteine cathepsins and

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matrix metalloproteinases, various approaches have been assessed like ethanol wet-bonding technique, application of collagen cross-linking agents, protease inhibitors or chelators on dentin surface and agitation for deeper adhesive penetration.[8,9]

The ethanol wet bonding technique is regarded as clinically impractical because of the amount of time required for multiple ethanol applications to achieve sufficient dentin dehydration.[10] Moreover, application of dentin-bio modifiers also has its own limitations in enhancing the longevity of adhesive-dentin bonds because most techniques do not sufficiently deal with both hydrolytic deterioration of adhesive resin and collagen degradation.

Pretreatment with Dimethyl sulfoxide or DMSO- wet bonding technique has come to light as novel approach in adhesive dentistry and various studies have examined the role of DMSO in improving resin-dentin bond durability.[11–15] The effect of DMSO pretreatment on dentin bonding remains controversial, as improved long-term bond strength has been reported with 2-step etch-and-rinse adhesives, while other studies found no significant benefit for either etch-and-rinse or universal adhesive systems.[11,16,17] These contradictory findings suggest that the effect of DMSO on dentin bonding may be highly dependent on adhesive composition and substrate characteristics. No studies have evaluated DMSO pretreatment across multiple universal adhesive systems with time-dependent aging on pulp chamber dentin. Our protocol fills that translational gap with the aim to evaluate the effect of pulp chamber dentin pre-treatment with DMSO on immediate and delayed μTBS of an etch and rinse adhesive and two different universal adhesives. The null hypothesis tested was that DMSO pretreatment of pulp chamber dentin had no effect on bond strength and bond durability of universal adhesives and etch and rinse adhesive.

## 2. Material and method

One hundred and forty-four freshly extracted molars were collected and examined under a stereomicroscope to exclude teeth with caries, cracks, restorations and defects. The collected teeth were completely cleaned using ultrasonic scaler followed by thorough cleaning with pumice slurry and rubber prophylactic cups rotating at slow speed in a contra-angled micromotor handpiece and stored in 0.1 % thymol solution and kept in a refrigerator at an approximate temperature of 4 °C for not more than 6 months till commencing the experiment. Teeth were collected after obtaining the patient's consent under a protocol approved by the institutional ethics and review board under protocol number KDCRC/IERB/22/2022/22.

A power analysis was established by G*Power version 3.0.1(Franz Faul universitat, Kiel, Germany). Total minimum calculated sample size of 120 samples for μTBS (20 samples per experimental group; total 6 groups) yielded the significance level ($\alpha = 0.05$) and power of test (1-β err prob = 0.95).

### 2.1. Specimen preparation

The pulp chamber was exposed by using a gypsum model trimmer under water coolant, and the roots were sliced at a distance of 2 mm below the CEJ using a slow-speed diamond disk (Microdont) under vigorous water irrigation. The pulp was extracted using endodontic tools followed by rinsing with distilled water for 60 s and pulp chamber was completely air-dried. Using wax, specimens were mounted vertically on an acrylic plate at the level of CEJ, to facilitate restorative procedures. Samples were divided into six groups ($n = 24$) according to different dentin pretreatment and different adhesives used for μTBS test.

**Group 1A**. (ASB) Adper Single Bond 2 adhesive (3M ESPE): Acid etching with 36 % phosphoric acid (Detrey conditioner 36, Dentsply Sirona) for 15 s was done followed by rinsing with water for 10 s and then blot dried. After this, adhesive was applied according to manufacturer's instructions.

**Group 1B**. (ASB + DMSO) Adper Single Bond 2 adhesive with DMSO pre-treatment: Acid etching for 15 s was done followed by rinsing with water for 10s and blot drying with cotton pellet. Leaving dentin surface slightly moist, active application of 50 % DMSO for 60s using micro brush was done, followed by blot drying with filter paper. After that, the adhesive was applied and light cured.

**Group 2A**. (TNBU) Tetric N-Bond Universal (Ivoclar Vivadent): Application of universal adhesive in self-etch mode was done according to manufacturer's instructions.

**Group 2B**. (TNBU + DMSO)) Tetric N-Bond Universal with DMSO pre-treatment: Dentin surface was kept slightly moist, active application of 50 % DMSO for 60 s using micro brush was done, followed by blot drying with filter paper. After that the adhesive was applied in selfetch mode.

**Group 3A**. (PNBU) Prime &Bond Universal (Dentsply Sirona): Application of universal adhesive in self-etch mode was done according to manufacturer's instructions.

**Group 3B**. (PNBU + DMSO) Prime & Bond Universal with DMSO pre-treatment: Dentin surface was kept slightly moist, active application of 50 % DMSO for 60 s using micro brush was done, followed by blot drying with filter paper. After that, universal adhesive was applied in the self-etch mode.

After the adhesive application, composite rein (3M™ Filtek™ Z350 XT Universal Restorative, Shade A2; 3M ESPE, St. Paul, MN, USA) was filled into the pulp chamber incrementally in 2 mm layers and each increment was light cured for 20 s using Blue Phase light curing unit (Ivoclar) at a light intensity of 600 mW/cm$^2$ until the cavity was filled.

Ten samples from each group were stored in distilled water at 37 °C for 24 h before μTBS (immediate testing). The other ten samples from each group were stored in artificial saliva (Wet Mouth, ICPA Heath Products Ltd.) at 37 °C in an incubator for 6 months before the μTBS test (delayed testing). Storage solution was changed every 2 weeks.

### 2.2. μTBS test and fracture mode analysis

The restored samples were sectioned using a hard-tissue low speed microtome (IsoMet 1000 Precision Saw). Each sample underwent horizontal sectioning followed by vertical sectioning to obtain three beams of resin dentin interface with a cross-sectional area of 1 ± 0.05 mm$^2$ (Fig. 3). The beams were fixed to a custom-made jig with cyanoacrylate glue and a tensile load administered at 1 mm/min crosshead speed until restoration failure. Universal testing Machine (Instron®, Instron Corp.) was used to estimate μTBS. The force applied for each specimen was recorded in Newtons and divided by cross-sectional area to calculate bond strength in Mega Pascals (MPa). Each tooth's μTBS was represented by the mean bond strength of three beams obtained from that tooth. All debonded samples were analyzed under a stereomicroscope (ALCO® Stereo Zoom Microscope, ALCO Instruments, India) at 45× magnification to define the location of the bond failure, categorized as Adhesive (a), Cohesive (c) and Mixed (m).

### 2.3. Scanning electron microscope study

Four teeth per group were bonded in the same way as described for the μTBS test, two were used for immediate testing and the other two was stored in artificial saliva for delayed testing. The specimens were sectioned to have slabs with resin-dentin interface involving pulp chamber lateral wall. The slabs were polished with SiC papers (#600, #800, and #1000) under running water followed by polishing with diamond pastes (Direct Dia Diamond Polishing Paste; Shofu). The specimens were fixed in 10 % formalin for 24 h and subjected to acid-base treatment. Specimens were immersed in 6N HCl for 30 s, rinsed in distilled water followed by 10-min immersion in 1 % NaOCl, and final rinse with distilled water. After dehydration in ascending grade of ethanol up to 100 %, the specimens were sputter-coated with platinum sputter coater (JFC 1600, Auto Fine Coater) and analyzed in scanning electron microscope (JEOL JSM 6490). Images were acquired at 1000 × magnification to observe hybrid layer formation, resin tag penetration, and interfacial adaptation.

## 3. Statistical analysis

Data analysis was performed using SPSS software (version 21.0, IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) was employed to assess overall intergroup differences, followed by Tukey's post hoc test for pairwise comparisons. A p-value of <0.05 was considered indicative of statistical significance.

## 4. Results

In our study statistically significant increase in immediate and delayed dentin μTBS after DMSO pretreatment was observed (Tables 1 and 2). PNBU depicted highest shear bond strength values regardless of the DMSO pretreatment at 24 h and at 6 months. At 24hrs, PNBU (Group 3A) depicted highest bond strength while TNBU (Group 2A) showed the lowest bond strength when applied according to manufacturer's instructions (without pretreatment). However, the difference was not statistically significant. At 24 h, PNBU + DMSO (Group 3B) depicted a significantly higher bond strength than TNBU + DMSO (Group 2B) (p = 0.048) and ASB + DMSO (Group 1B) (p = 0.012) while ASB + DMSO (Group 1B) showed the lowest bond strength when DMSO pretreatment was done.

After 6 months of storage in artificial saliva, a significant fall in bond strength was observed for all adhesives when applied according to manufacturer's instructions. Therefore, the mean difference between immediate and delayed bond strength was found to be significant for groups without pretreatment that is ASB (Group 1A) (p = 0.008), TNBU (Group 2A) (p = 0.049) and PNBU (Group 3A) (P = 0.007) (Tables 1 and 2). When samples of DMSO pretreated groups were stored for 6 months in artificial saliva, only a non -significant fall in bond strength was observed for all adhesives where PNBU + DMSO (Group 3B) showed highest bond strength followed by TNBU + DMSO (Group 2B) and ASB + DMSO (Group 1B), with statistically significant difference between ASB + DMSO (Group 1B) and PNBU + DMSO (Group 3B) (p = 0.040). There was no significant difference seen between immediate and delayed bond strength for groups with DMSO pretreatment. Application of DMSO preserved the resin dentin bond strength at 6 months for all adhesives.

Scanning electron microscopic observation revealed good adaptation at resin-dentin interface for all adhesives regardless of DMSO treatment when evaluated at 24 h. However, after six months of ageing only DMSO treated groups exhibited satisfactory interfacial seal (Fig. 1).

Adhesive failures were most common, especially in delayed groups.

**Table 1**
Inter-group comparison of bond strength among immediate and delayed groups with and without DMSO pre-treatment (unpaired *t*-test; p < 0.05).

| Groups | Immediate Mean (SD) | Delayed Mean (SD) | Unpaired *t*-test | p -Value |
|---|---|---|---|---|
| Group 1A **(ASB)** | 28.13(4.21) | 22.05(4.88) | t = 2.975 | p = 0.008* |
| Group 1B **(ASB + DMSO)** | 32.28(4.45) | 30.65(5.04) | t = 0.766 | p = 0.453 |
| Group 2A **(TBU)** | 27.39(4.24) | 24.07(5.52) | t = 1.507 | p = 0.049* |
| Group 2B **(TBU + DMSO)** | 34.74(4.47) | 32.8(7.07) | t = 0.932 | p = 0.364 |
| Group 3A **(PBU)** | 31.63(4.47) | 25.09(5.14) | t = 3.034 | p = 007* |

p > 0.05 – no significant difference.
*p < 0.05 – significant difference.

**Table 2**
Comparison of bond strength between groups with and without DMSO pre-treatment at immediate (24 h) and delayed (6 months) testing periods (unpaired *t*-test; p < 0.05).

| Adhesive system | Comparison | Immediate testing (p value) | Delayed testing (p value) |
|---|---|---|---|
| ASB | Group 1A vs Group 1B | 0.046* | 0.001* |
| TBU | Group 2A vs Group 2B | 0.001* | 0.010* |
| PBU | Group 3A vs Group 3B | 0.005* | <0.001* |

p > 0.05 – no significant difference.
*p < 0.05 – significant differenceTable legends-.

Cohesive failures were rare across all groups (Fig. 2).

## 5. Discussion

This study uniquely investigated the effect of 50 % DMSO wet-bonding on pulp chamber dentin using contemporary universal adhesives, evaluating both immediate and six-month microtensile bond strength and interfacial integrity. DMSO pretreatment significantly enhanced immediate and delayed bond strength for both universal adhesives and the etch-and-rinse adhesive, resulting in rejection of the null hypothesis.

DMSO pretreatment before Adper Single Bond 2 adhesive application significantly improved the immediate bond strength and maintained the strength of the resin-dentin bond after six months of storage in artificial saliva. This may be attributed to enhanced interaction between DMSO biomodified dentin surface and adhesive monomers.

DMSO increases collagen inter-fibrillar spacing, which may be due to removal of water by DMSO or by direct modification in collagen structure by DMSO.[18] This improves infiltration of monomers into dentin substrate and improves the immediate bond strength.[19,20] Similarly Guo et al. and Li et al. also reported improved bond durability of Adper Single Bond 2 adhesive after DMSO treatment of acid-etched dentin.[12,16] DMSO pre-treatments have been reported to enhance bonding to air-dried dentin also, thereby challenging the current concept of conventional water-wet bonding with etch-and-rinse adhesives.[21,22]

Also, in our study pretreatment with 50 % DMSO before universal adhesive application showed statistically significant increase in immediate bond strength than both of the universal adhesives and preserved the resin dentin bond strength at 6 months for both the universal adhesives. Wendlinger et al. also reported that DMSO pre-treatment resulted in improved bonding of universal adhesives to eroded dentin over a four-year evaluation period in both etch-and-rinse and self-etch mode.[23]

DMSO can bind to hydrophobic component of an enzyme, leading to unfolding of the protein and protein denaturation. Various previous studies have reported reduction in metalloproteinase activity by DMSO which contributed to enhanced durability of the hybrid layer.[11,12,22] Zabeu et al. demonstrated that DMSO pretreatment could reduce gelatinolytic activity for a universal adhesive under etch-and-rinse mode or stabilize the gelatinolytic reactions for self-etching strategies.[24] Similarly, Sismanoglu et al., concluded that DMSO-wet bonding was effective to improve the quality of resin-dentin bonds of the tested universal adhesive.[25] They suggested that 50 % DMSO could be a feasible alternative to improve resin-dentin bond quality. A 50 % concentration of DMSO is widely regarded as optimal because lower concentrations may not sufficiently reduce the contact angle or effectively modify dentin surface energy, resulting in limited enhancement of resin wettability and penetration.[12,13,17,21,22] In contrast, higher concentrations have the potential to excessively disturb collagen ultrastructure, adversely affect dentin mechanical properties, or interfere with polymerization processes, which may ultimately compromise the durability

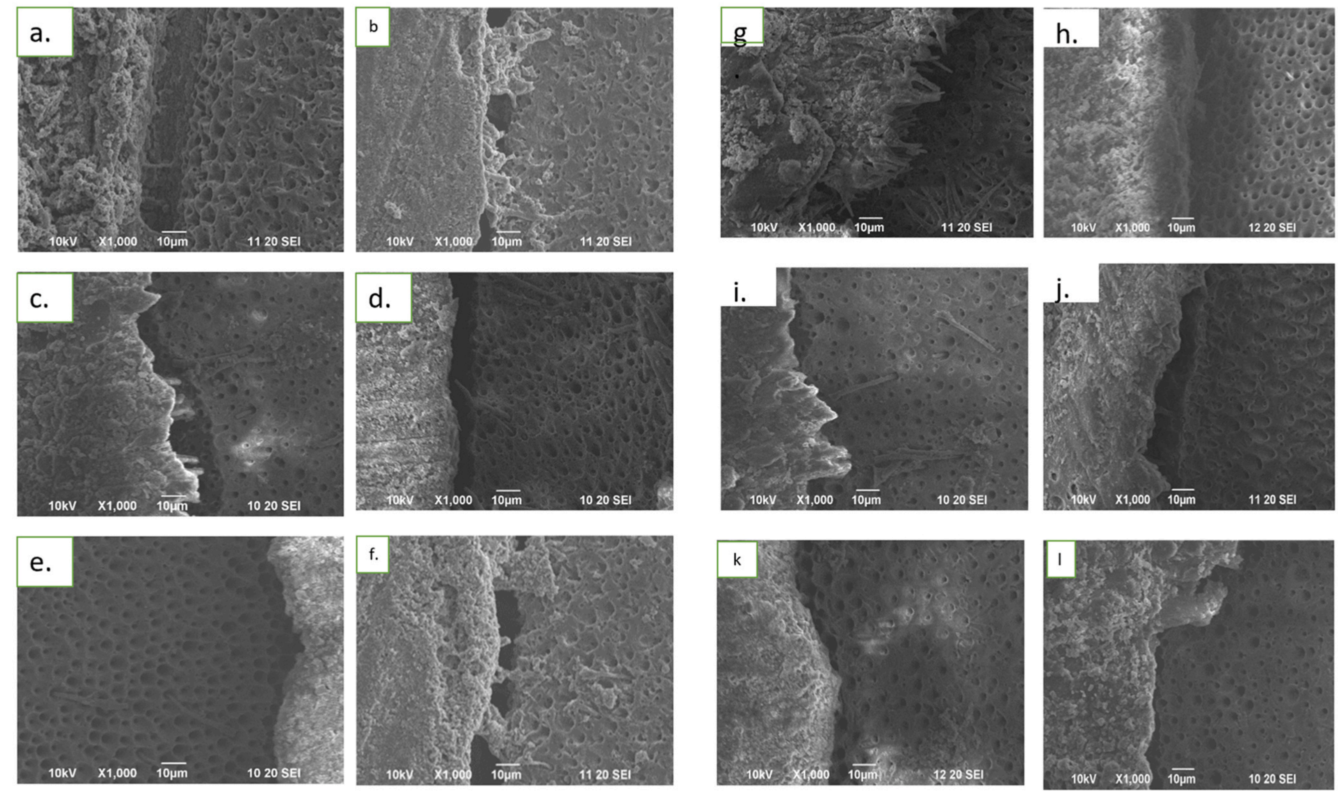


**Fig. 1.** SEM ( × 1000) photomicrograph of resin-dentin interface of Group 1A (Adper Single Bond 2 adhesive without DMSO Pretreatment): (a) Immediate; (b) Delayed – poor interfacial seal is evident at six months; Group 2A (Tetric N-Bond Universal Adhesive without DMSO Pretreatment): (c) Immediate; (d) Delayed - poor interfacial adaptation at six months. 3A (Prime & Bond Universal without DMSO Pretreatment): (e) Immediate; (f) Delayed - interfacial gap is evident at six months. Group 1B (Adper Single Bond 2 adhesive with DMSO Pretreatment): (g) Immediate; (h) Delayed - interfacial seal is seen at both time periods. Group 2B (Tetric N-Bond Universal Adhesive with DMSO Pretreatment): (i) Immediate; (j) Delayed - good interfacial adaptation can be seen even at six months. Group 3B (Prime & Bond Universal Adhesive with DMSO Pretreatment): (a) Immediate; (b) Delayed - good interfacial seal is present even after six months.

of the adhesive interface. Consequently, a 50 % DMSO concentration offers a balanced approach, providing effective improvement in dentin wettability and resin infiltration while avoiding undesirable structural or bonding alterations.[25] Supporting these findings, Balakrishnan et al. and Cardenas et al. demonstrated enhanced bonding performance of universal adhesives following DMSO pretreatment.[26,27] When used according to manufacturer's instructions Prime & Bond Universal exhibited higher immediate and delayed bond strength than Tetric N-Bond Universal but the difference was not statistically significant. Prime & Bond Universal adhesive showed highest shear bond strength values which may be due to the exceptional chemical constituents of Prime N Bond universal adhesive. It incorporates a proprietary “Active-Guard Technology,” characterized by a balanced combination of hydrophilic and hydrophobic monomers that enhances moisture tolerance and ensures dependable adhesion. The formulation is designed to perform consistently under both wet and dry conditions, with the primary objective of achieving high and stable bond strength.[28]

While the comparable bond strength of Tetric N-Bond Universal adhesive may be due to compositional matrix, which is composed of a blend of hydrophilic (HEMA), hydrophobic (D3MA) and intermediate (BIS-GMA) monomers along with 10-MDP.[29]

Universal adhesives undergo same pattern of hydrolytic breakdown observed with other simplified adhesives, despite the advantages of the simplified clinical procedure. In our study universal adhesives were applied in self-etch strategy. Self-etch strategy was chosen over etch and rinse because universal adhesives have the capability to bind chemically to the inorganic hydroxyapatite in dentin.[30] With the etch and rinse technique, there is deeper demineralization of peritubular and intertubular dentin, resulting in a demineralized dentin zone without resin impregnation at the bottom of the hybrid layer, which is more vulnerable to hydrolytic and enzymatic degradation.[30] Thus, self-etching is preferred because the depth of the hybrid layer which is formed in dentin is less but is better in quality when compared to that of etch and rinse technique. Besides, self-etch technique has lesser application time, fewer steps and is less technique sensitive. Both the universal adhesives used in our study contain 10-MDP monomer which binds ionically to calcium that is still connected to collagen fibrils, generating a stable and water-resistant ca-monomer salt.[29]

Contrary to our study, Dadvar et al. stated that when universal adhesives were used in the self-etch technique, there was no significant difference between DMSO applications and non-application modes in terms of adhesive bond strength.[31] However, use of DMSO in an etch and-rinse technique could dramatically enhance the universal adhesive-dentin μTBS. Contradictory to our results, Mirzaei et al. reported that dentin pretreatment with DMSO before universal adhesive (G-Premio Bond) application, significantly reduced the μTBS of the adhesive in both self-etch and total-etch mode.[32] Probably, the high acidity of G-Premio Bond (pH-1.5) adhesive creates etch patterns comparable to etching with 37 % phosphoric acid and therefore, it was not benefitted with DMSO treatment. On the other hand, Prime & Bond.

Universal and Tetric N-Bond Universal adhesives are considered as “mild-etching” adhesives. These adhesive systems cause incomplete demineralization of the dentin leading to a hybrid layer formation that has a thickness of 1 μm or less.[33] Karadas et al. also reported that DMSO increased μTBS to wet and dry dentin of some universal adhesives but not all when used in etch and rinse mode.[34] Mello et al. reported that the presence of DMSO along with other adhesive solvents hindered proper evaporation which neutralized any potential benefit of DMSO

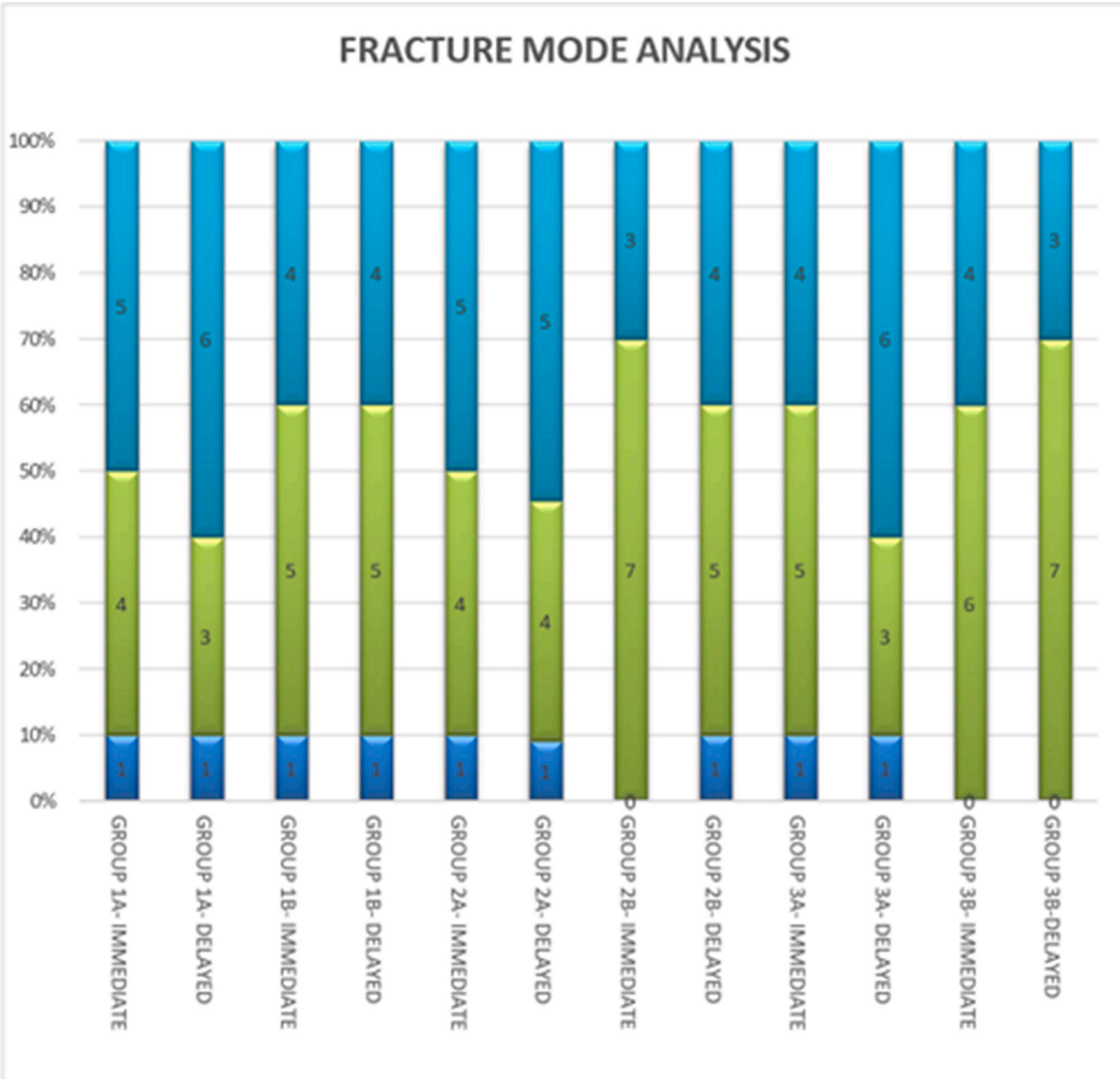


**Fig. 2.** Fractographic Analysis (Group 1A (ASB), Group 1B (ASB + DMSO), Group 2A (TNB), Group 2B (TNB + DMSO), Group 3A (PBU), Group 3B (PBU + DMSO), Top segment- Cohesive failure, Middle segment- Adhesive failure, Bottom segment- Mixed failure).

pretreatment and compromised bonding for universal adhesive in etch and rinse or self-etch mode.[35]

DMSO is not contained in adhesives but it is completely soluble with water, resin monomers of adhesives, and a number of organic solvents. Studies have demonstrated that DMSO is a useful solvent for specific adhesive components, such as HEMA, BISGMA, UDMA, TEGDMA and photo initiator, camphorquinone.[20,36] By adding DMSO to the adhesive formulation, less water, HEMA, and other solvents may be required to wet and penetrate the etched dentin surface, thereby enabling the use of hydrophilic monomers in smaller amounts. Additionally, DMSO as an adhesive solvent need not be necessarily evaporated, which should lessen the technique sensitivity of the etch and rinse dentin bonding, even under drastic air-drying conditions.[22] DMSO has been approved by the FDA for use as a carrier in several medical products and has a long history of application in biomedicine as a pharmaceutical solvent and penetration enhancer. Existing evidence suggests that DMSO alone does not adversely affect odontoblastic cell viability, adhesion, or survival.[37] However, due to its strong penetrative capacity, there is a potential for enhanced diffusion of resin components toward the pulp.[38] This dual characteristic highlights both the potential benefits of DMSO in improving resin–dentin interactions and the need for cautious application. Consequently, further well-designed laboratory and clinical studies are necessary to clearly establish its biological safety and to develop reliable guidelines for its use in adhesive dentistry. If proved beneficial in future investigations, DMSO-bonding protocols could be seen as practical and cost-effective substitutes to increase the quality and, consequently, durability of resin-dentin bonds. DMSO- bonding methods have fewer steps than ethanol-wet bonding, they are less technique sensitive and more user friendly to administer clinically.

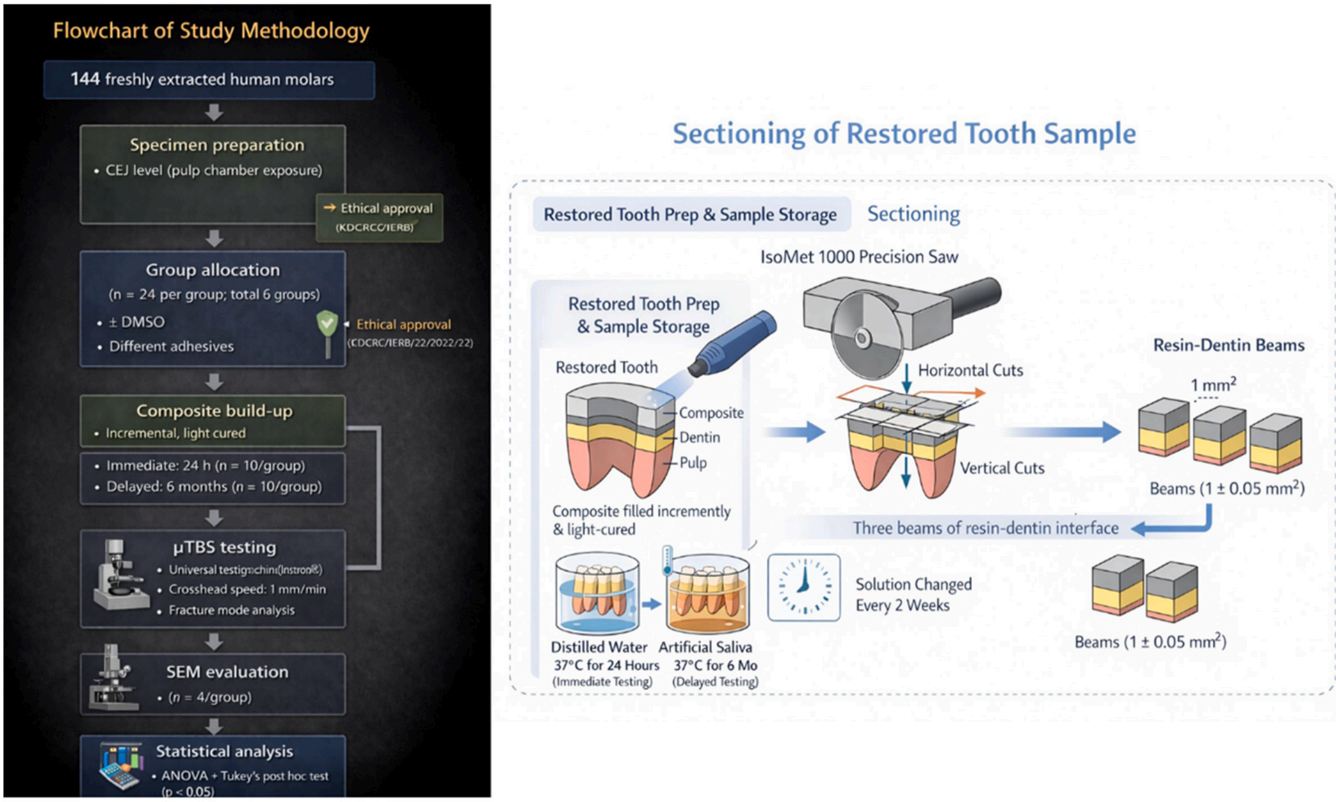


**Fig. 3.** Schematic diagram of Methodology.

## 6. Limitations

Even though the current study produced encouraging results, there are some drawbacks. Limited number of samples were evaluated under SEM providing only a qualitative analysis. As the study was done in vitro, the behaviour of agents under clinical conditions cannot be predicted. The bonding procedures and storage of samples were not done under simulated pulpal pressure. Bonding to caries affected dentin may yield different results. These factors and the specific concentration of DMSO used in this study may have an impact on the outcomes. Further studies are needed to assess the DMSO effect on different adhesives, at different concentration and time.

## 7. Conclusion

Pretreatment of pulp chamber dentin surface with 50 %(v/v) DMSO before adhesive application improved the immediate bond strength of etch-and-rinse adhesive and universal adhesives when applied in self-etch mode. Furthermore, DMSO pretreatment was able to prevent the fall in bond strength over six months and preserved the bond strength of all the adhesives tested regardless of their etching mode. The suggested DMSO-based biomodification of the dentin substrate seems to be an appropriate method for creating hybrid layers that are more resilient to hydrolytic deterioration over time. Further research is required to understand the role of DMSO in preserving the longevity of post-endodontic resin restorations under clinical conditions.

## Patient consent

This study does not involve human participants, identifiable patient data, or images, and hence, patient or guardian consent was not required.

Procedure approved by institutional ethics and review board (reference no: KDCRC/IERB/22/2022/22.

## Ethical approval

The study protocol was reviewed and approved by the Institutional Ethics Committee of [Kothiwal Dental College and Research Centre, Moradabad, Uttar Pradesh, India], with approval number (reference no: KDCRC/IERB/22/2022/22.).

The extracted teeth used in this in vitro study were obtained from the department with prior consent and stored per institutional bioethical guidelines.

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The study was self-funded by the authors.

## Declaration of competing interest

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

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