Evaluation of push-out bond strength of different concentrations of chitosan nanoparticles incorporated composite resin and eighth-generation bonding agent for class II restoration: An *in vitro* study

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**Abstract**

**Aim:** The aim of this study is to evaluate the push-out bonding strength of class II cavities of maxillary molars restored with different concentrations of chitosan nanoparticles (CSN) incorporated in universal composite resin and eighth-generation dentin bonding agent (DBA).

**Materials and Methods:** Seventy extracted human maxillary first molar teeth were cleaned and mounted in acrylic mold up to 2 mm below cementoenamel junction and mesio-occlusal Class II cavities with standard measurements were prepared. CSN 2% and 0.25% powder were added to the DBA and composite resin. The samples then arbitrarily divided into three groups for restoration: Group 1: composite restoration without CSN (control group) (*n* = 10), Group 2: 2% CSN (*n* = 30), and Group 3: 0.25% CSN (*n* = 30). Groups 2 and 3 were further subdivided into three subgroups (*n* = 10 each) subgroup A. CSN incorporated in composite resin. B. CSN incorporated in DBA and C. CSN incorporated in composite and DBA. After restoration, push-out bond strength was evaluated in each sample using a universal testing machine, and data collected were statistically analyzed by one-way analysis of variance and *Post hoc* Tukey tests (*P* ≤ 0.05).

**Results:** Subgroup 3A (0.25% CSN + composite) has shown an increase in bond strength among all experimental groups with no significant difference between group 1 (control group).

**Conclusion:** CSN 0.25% incorporated in composite or DBA exhibits no harmful effect on the bond strength of class II restorations. Hence, it can be used to improve the antibacterial action and longevity of composite resin.

**Keywords:** Chitosan nanoparticles; composite restoration; posterior restorations; push-out bond strength

**INTRODUCTION**

Resin composites have a wide range of applications in restorative dentistry, such as for pit and fissure sealing, as a liner under restorations, temporary and permanent restorations, and extensive restorations such as for inlays, onlays, cosmetic veneers, etc. The success of composite restorations depends on the longevity of resin composite restorations. Over the past two decades, composite restorations are well established in restorative dentistry due to improvements in adhesive properties and the material itself. Composites provide minimal preparation

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and can be easily used to restore the lost tooth structure for long-lasting restorations.[9]

Class II cavity preparations are done in posterior teeth, that are subjected to heavy occlusal forces.[10] Composite restorations in these cavities are difficult to restore and technique sensitive. Despite several advantages of composite restorations, there are some drawbacks such as polymerization shrinkage, postoperative sensitivity, no antibacterial activity, marginal deficiencies, and secondary caries that affect the success of composite restorations, particularly in posterior complex restorations.[5,6] Therefore, further improvement of composite restorative materials is needed.

Neo Spectra ST (Dentsply Sirona, USA), a nanoceramic universal composite resin with novel (Sphere TEC) filler component system that consists of spherical filler granules combined with an optimized resin matrix, has been developed that is designated for use in direct and indirect restorations. It has better handling properties, provides good esthetics in anterior restorations, and provides longevity.[2]

With changing technology, dentin bonding agents (DBAs) had evolved over time from total etching to self-etch adhesives. DBAs of the eighth generation produce higher bond strength, have longer shelf-life, and stress absorption. Previously, it was a challenge to achieve good bond strength to self-etching agents as there is no rinse after etching, therefore causing sensitivity to teeth and the lower acidity of etching and decreases the bond strength.[7,8] GC G-Premio Bond (GC Dental Products Corp, Kasugai, Japan) can be used as a self-etch and total etch DBA. It has a unique combination of three functional monomers: 4-Methacryloxyethyl trimellitate anhydride, methacroyloxyloxydecyl dihydrogen phosphate, and methacryloxyloxydecyl dihydrogen thiophosphate, notably excluding HEMA, ensures excellent stability and bonding.[3]

Chitosan, a naturally occurring polysaccharide, has gained popularity in dentistry including restorative applications. It exhibits good biocompatibility, chelating action, and exhibits antimicrobial efficacy against a wide range of microbes. It promotes the biomimetic reconstruction of enamel and enhances resin infiltration and formation of the hybrid layer under composite restorations.[9] Chitosan nanoparticles (CSNs) incorporated in DBAs increases the antibacterial and anti-inflammatory properties to protect the pulp. It is also used as a cavity liner/preconditioning dentin to prevent inflammatory response in the pulp.[10]

Since there are no studies reported incorporating CSN both in composite resin and bonding agents for class II cavities; hence, this study aims at evaluating the push-out bonding strength of different concentrations of CSN incorporated in composite resins and eighth-generation DBAs in class II restorations of maxillary molars.

**MATERIALS AND METHODS**

Institutional ethical clearance reference no – IEC/2020-2021/S-17 was obtained for this in vitro study and was done accordingly. The materials used in this study were eighth-generation DBA, GC G-Premio Bond (GC Dental Products Corp, Kasugai, Japan), NeoSpectra universal composite restorative material (Dentsply Sirona, USA), and CSN 0.25% and 2% (SRL Pvt. Ltd., Hyderabad, India).

Seventy human extracted maxillary molar teeth recently extracted for periodontal reasons were collected, cleaned with Ultrasonic Scalers (woodpecker piezo scaler UDS-J, China), and stored in distilled water for further use not more than a month.

**Preparation of experimental materials**

Experimental composite resins were prepared according to Mirani et al.[11] different concentrations of 2% and 0.25% CSN in powder form were incorporated into commercial composite resin in a 1:1 ratio by weight. About 1 mg of components were weighed separately using an analytical balance (ACZET Model CZ 310, Ahmedabad, India). For both concentrations, the weighed CSN powder was mixed together with preweighed composite resin in a 50 ml glass beaker with a sterile glass rod in a dark room until the homogeneous mix was obtained.

To prepare the experimental DBA for CSN 2% and CSN 0.25% groups, about 1 mg of chitosan powder was incorporated with 1 ml of bonding agent by vigorous mixing in a vortex, without exposure to the light according to the methodology described by de Carvalho Nunes et al.[7]

**Specimen preparation**

All the teeth specimens were mounted 2 mm below the cementoenamel junction (CEJ) in acrylic blocks followed by the preparation of mesio-occlusal class II cavities on the tooth specimen with standard dimensions of 2 mm pulpal depth using straight bur (Mani, Hyderabad, India, 2 mm of buccolingual width using inverted bur, and the gingival floor on the mesial side was prepared 1 mm below the CEJ with new straight fissured diamond bur (No-010) (Mani, Hyderabad, India) in high-speed air water-cooled handpiece (Drillerz-EM, Hyderabad, India) [Figure 1].

The specimens were arbitrarily allotted into three groups for restoration as follows: Group 1: composite restoration without CSN (control group) \( n = 10 \), Group 2: 2% CSN \( n = 30 \), and Group 3: 0.25% CSN \( n = 30 \).

Groups 2 and 3 were further subdivided into three subgroups \( n = 10 \) each.
In all the specimens of Group 1 (control group), DBA was applied and left undisturbed for 10 s, then air-dried and light-cured for 20 s followed by placement of Bioclear Matrix with retainer (Tacoma, WA, USA) and stabilized for tight contact; followed by the placement of composite restorative material into the cavity by the incremental technique of 1 mm thickness and light-cured for 20 s.

In Groups 2 and 3, specimens were restored with 2% and 0.25% CSN incorporated in experimental composite resin and DBA as described above.

In all the groups, finishing and polishing were done with a composite polishing kit (Shofu Dental India Private Limited, India) and specimens were then stored in humid conditions for 24 h.

**Evaluation of push-out bond strength**

A reference point was marked on all the specimens for the placement of a testing needle at the center of the tooth at the interface between the prepared cavity and restoration for the pointed bur to be inserted. The specimens were placed under a universal test machine (Instron JOEL 3352, USA) for push-out bond strength. A needle consisting of a round cross-sectional area of 0.5 mm gauge, length of 1 mm, and radius curvature of 0.6 mm was inserted in each specimen at the speed of 1 mm/min until the restoration gets dislodged from the cavity, and data were recorded [Figure 2].

**Statistical analysis**

The Microsoft R Open software version 4.0.2 (Microsoft Corporation, Washington, USA) was used for the statistical analysis of the collected data. The mean and standard deviation values were calculated and analyzed using the one-way analysis of variance and post hoc Tukey tests ($P \leq 0.05$).

**RESULTS**

One-way analysis showed the highest push-out bond strength was obtained in Group 3A (0.25% CSN + Composite) 623.2 N followed by Group 1 (control group) 568.8 N, 2A (2% CSN + Composite) 475.4 N, 3B (0.25% CSN + DBA) 368.8 N, 3C (0.25%CSN + DBA + Composite) 263.4N, 2B(2%CSN + DBA) 165.2 N, and least in 2C (2% CSN + DBA + Composite) with 73.2 N [Table 1 and Graph 1]. Based on multiple comparisons by post hoc Tukey test, no statistical significance difference between Groups 1, 3A, and 2A; between 2A and 3B; and between 3C, 2B, and 2C ($P \leq 0.05$) [Table 2].

**DISCUSSIONS**

Composite restorations are widely used for direct and indirect restorations due to esthetics and their ability to conserve the tooth structure; along with long-term success rate, it is an economic restorative material with high strength and bond stability to the tooth structure but composite restorations have certain drawbacks such as secondary caries, polymerization stresses, shrinkage during light curing, postoperative sensitivity, and no antibacterial activity.

Thus, researchers continue to improve the composites with new advancements to restore the lost tooth structure with minimal drawbacks and longevity of the restoration for successful treatment. The technical advantages and evolution of composites resin in restorative dental materials has made it the material of choice for restoring posterior restorations including class II cavities as they are subjected to heavy occlusal stresses, thus the clinicians acknowledged the importance of conserving the tooth structure with minimal preparation to preserve the remaining tooth structure, to strengthen the tooth, and to reduce the fracture of the tooth.

Solomon et al. found that teeth restored with light-cured composite restoration with beta quartz insert showed significantly higher values compared to the group without...
Numerous studies were conducted to improve composite restorations with the reduction in microleakage and demineralization of dentin. Studies have been done evaluating the incorporation of CSN into different restorative materials such as resin-modified cement and glass-ionomer cement. In the present study, improvements in the composite restoration have been attempted by the addition of CSN to DBA and also in composite resin for class II cavities.

The adhesive system has undergone great progress in dentistry for decades in use with a composite restorative material. The quality and success of restoration also depend on the adhesive material used. Kudva et al. evaluated shear bond strength of a bioceramic material added to composite resin using three universal adhesives and two different aging periods and its results showed that the highest shear bond strength was observed with eighth-generation DBA GC G-Premio Bond compared to other experimental groups. This system depends on the effectiveness of enamel and dentin bonding.

Incorporating chitosan into a composite and DBA effectively inhibits the growth of bacteria and attains good bond stability when incorporated in a DBA. Adhesive systems enhance the bond strength of composite resin restorations, de Carvalho Nunes et al. presented different forms of bond strength to dentin by adding 0.2% and 0.5% of CSN to total-etch and self-etch adhesives in class V cavities and concluded that chitosan incorporated composites have improved mechanical properties including bond strength with self and total-etch adhesives. The results of the present study are in accordance with the above studies,

### Table 2: Multiple comparison between groups by post hoc Tukey test

| Comparison group | Difference | P     | Remark     |
|------------------|------------|-------|------------|
| Group 1 (no CSN) control group | Group 2A (2% CSN+composite) | 93.4 | 0.2808 | Not significant |
|                   | Group 2B (2% CSN+DBA) | 403.6 | <0.0001 | Significant |
|                   | Group 2C (2% CSN+DBA+composite) | 495.6 | <0.0001 | Significant |
|                   | Group 3A (0.25%CSN+Composite) | 54.4 | 0.8286 | Not significant |
|                   | Group 3B (0.25%CSN+DBA) | 200.0 | 0.0006 | Significant |
|                   | Group 3C (0.25%CSN+DBA+Composite) | 305.4 | <0.0001 | Significant |
| Group 2A (2% CSN+composite) | Group 2B (2% CSN+DBA) | 310.2 | <0.0001 | Significant |
|                   | Group 2C (2% CSN+DBA+Composite) | 402.2 | <0.0001 | Significant |
|                   | Group 3A (0.25%CSN+Composite) | 147.8 | 0.0169 | Significant |
|                   | Group 3B (0.25%CSN+DBA) | 106.6 | 0.1579 | Not significant |
|                   | Group 3C (0.25%CSN+DBA+Composite) | 212.0 | 0.0003 | Significant |
| Group 2B (2% CSN+DBA) | Group 2C (2%CSN+DBA+composite) | 92.0 | 0.2969 | Not Significant |
|                   | Group 3A (0.25%CSN+Composite) | 458.0 | <0.0001 | Significant |
|                   | Group 3B (0.25%CSN+DBA) | 203.6 | 0.0005 | Significant |
|                   | Group 3C (0.25%CSN+DBA+Composite) | 98.2 | 0.2301 | Not significant |
| Group 2C (2% CSN+DBA+composite) | Group 3A (0.25%CSN+Composite) | 550.0 | <0.0001 | Significant |
|                   | Group 3B (0.25%CSN+DBA) | 295.6 | <0.0001 | Significant |
|                   | Group 3C (0.25%CSN+DBA+Composite) | 190.2 | 0.0012 | Significant |
| Group 3A (0.25% CSN+DBA) | Group 3B (0.25%CSN+DBA) | 254.4 | 0.00002 | Significant |
|                   | Group 3C (0.25%CSN+DBA+Composite) | 359.8 | <0.0001 | Significant |
| Group 3B (0.25% CSN+DBA) | Group 3C (0.25%CSN+DBA+composite) | 105.4 | 0.1669 | Not significant |

CSN: Chitosan nanoparticle, DBA: Dentin-bonding agent
showing that 0.25% CSN added to composite enhanced the bond strength in class II cavities.

In a study, class V cavity restored with 0.2% chitosan added microhybrid composite resins were examined for microleakage and concluded that chitosan incorporation improved the properties when used with total-etch or self-etch adhesives in addition, providing an antibacterial effect.[18] In the present study, a decrease in bond strength was found in both concentrations of chitosan added in DBA compared to chitosan incorporated composite resin, these results might be due to the physicochemical characteristics of the self-etch adhesive system used in this study, and are in accordance to Dacoreggio et al., who evaluated particle size, physicochemical characteristics of DBA with self-etch and total-etch DBA, and analyzed microtensile bond strength and interfere micromorphology. The assessment made after 24 h and 6 months showed an increase in particle size and polydispersity index significantly over time.[19] Chitosan was added to the universal adhesive system and in total etching showed greater gelatinolytic activity and compromising the restorative procedure. There is also a reduction in the formation of dentin tags when chitosan was used in total-etch dentin adhesive strategy due to greater colloidal stability.[20]

Botelho et al. in a study evaluated 2.5% chitosan applied after dentin acid etching or incorporated into an adhesive system interferes with microtensile strength of the interface tooth resin and concluded that the microtensile resistance of an adhesive system modified with the CSN group, where chitosan was manipulated as part of the adhesive system, showed lower and statistically significant values than the other nonaging groups, demonstrating maintenance of the resin/dentin bonding interface.[21]

In this study, results showed that the highest bond strength values were obtained with chitosan 0.25% incorporated in composite resin compared to 2% chitosan incorporated in DBA and composite. These results show that when the concentration of CSN increases the bond strength value decreases. The results of this study are in agreement with Mohamed et al., who evaluated the microtensile bond strength of chitosan incorporated composite resin to dentin using self-etch adhesives after aging. 0.2% chitosan is statistically showed a significant increase in bond strength than chitosan 2.5%.[9] Hence, it can be stated that low concentrations of CSN can be added to the composite resin to achieve good bond strength and reduce microleakage which improves the mechanical properties of composite resin restorations and exhibits an antimicrobial effect preventing recurrent caries with the longevity of the restoration. However, further in vivo and in vitro studies should be evaluated for confident use of this combination of restorative materials for the long-term success of composite restorations.

**CONCLUSION**

Composite resin or DBA incorporated with CSN 0.25% did not affect the push-out bond strength of class II restorations compared to without CSN. Hence, it can be incorporated to improve the antibacterial action and longevity of composite resin restoration.

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**Conflicts of interest**

There are no conflicts of interest.

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