RESEARCH ARTICLE

Evaluation of Immediate and Delayed Microleakage of Class V Cavities Restored with Chitosan-incorporated Composite Resins: An *In Vitro* Study

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Abstract

Aim and objective: To evaluate and compare the microleakage of unmodified microhybrid composite and 0.2% chitosan-incorporated composite in class V cavities restored immediately and after 3 months of storage in artificial saliva.

Materials and methods: Sixty human permanent maxillary premolars were collected and standardized class V cavity prepared on the buccal surface of each tooth with dimensions: mesiodistally 3 mm, occluso cervically 2 mm, and depth of 1.5 mm and restored with microhybrid composite and chitosan-incorporated composite resins respectively and randomly divided: Group I: control-microhybrid composite ($n = 30$): (a) 15 teeth tested immediately (b) 15 teeth tested after 3 months. Group II–restored with chitosan + composite ($n = 30$): (a) 15 teeth tested immediately (b) 15 teeth tested after 3 months. Specimens were stored in artificial saliva following which a dye extraction test was carried out using a spectrophotometer.

Results: There was no statistically significant difference in microleakage score between the chitosan-composite group and unmodified composite group when evaluated immediately after placing the restoration. Microleakage values of the unmodified composite group increased significantly after 3 months of storage in artificial saliva and values of the chitosan-composite group did not differ significantly even after 3 months of storage. Microleakage was seen significantly less in the chitosan-composite group compared to the unmodified composite group after 3 months of storage in artificial saliva.

Conclusion: It can be concluded that chitosan-incorporated composite seems to have improved mechanical properties and forms a more stable bond when compared with unmodified microhybrid composite in addition to being antibacterial.

Clinical significance: Considering the advantageous properties of this material, it may be clinically useful in restoring class V cavities in patients with high caries risk. However, further *in vitro* and *in vivo* studies need to be carried out.

Keywords: Dye extraction, Hybrid composite, Microhybrid composite, Microleakage, Resin-based composites.

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Introduction

Incidents of dental caries, in general, have declined over a period owing to effective preventive protocols and enhanced dental care.[1] However, in children[2] gross dietary inadequacy, poor oral hygiene, high sugar diet, or systemic illness have led to increased prevalence of several cervical carious defects and, at times, gingival recession, which initiates caries on the root surfaces. Restoration of these defects is challenging and technically demanding, whether the etiology is early childhood caries, rampant caries, adult caries, erosion, or tooth wear.

The etiology and progression of these lesions being multifactorial[3] coupled with limitations in implementing preventive protocols, difficulties in isolation and bonding to root dentin, selecting a restorative material for such lesions has become a hard task.[3] Therefore, one prefers to select a material that is less technique sensitive, has a low modulus of elasticity to allow the restoration to flex with the tooth on masticatory load,[3] and has less propensity for plaque accumulation.

To date, the preferred material of choice is composite due to its properties like aesthetics, adhesion, and conservation of tooth structure.[3] However, in class V composite restoration, the primary challenge is to deal with the marginal quality of the restoration.

The low modulus of elasticity flowable resins is recommended as the material of choice for class V lesions.[3] Still, their lower filler content often leads to polymerization shrinkage, which leads to poor mechanical properties. The stress generated at the tooth-restoration interface often exceeds the bond strength of the restorative material, which leads to microscopic gap formation that eventually causes microleakage.[3]

A microleakage eventually progresses to a discolored margin on the tooth-restoration interphase, sensitivity postoperatively, and finally pulpal damage due to the formation of secondary caries.[4]

Numerous *in vitro* and *in vivo* studies have revealed a more incredible amount of bacteria and plaque accumulating on the

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surface of composite resin[5] than other restorative materials, such as amalgam[6] and glass ionomer[6] or dental hard tissues such as enamel.[5,7] *In vitro* enamel demineralization because of plaque accumulation has been observed around different RBCs.[5] During *in vivo* conditions, the growth of plaque adjacent to the restoration margins may lead to secondary caries, which may limit the longevity of RBCs.[5,7] So recurrent caries can be prevented by the effect of the antibacterial property of any restorative material.

Although mechanical properties of RBCs have been improved substantially since their development,[4] their antibacterial properties are still considered unsatisfactory by dentists as well as investigators.[7] Hence, attempts have been made to incorporate antibacterial agents into resin components to provide antibacterial activity and decrease plaque accumulation.[5] Various agents like chlorhexidine,[5] antibiotics,[7] magnesium, zinc oxide,[5] silver ions,[7] iodine,[7] calcium,[6] and quaternary ammonium compounds[7] are added to the resin-based composites.

Antibacterial agents have been incorporated by two means—adding them into the bonding material or the resin composite. In the first technique, Imazato et al. extracted favorable output after MDPB (methacryloyloxydodecyl pyridinium bromide) was assimilated with adhesive resin and self-etching primer.[8]

A second technique[8] has been considered by incorporating several bioactive materials: MDPB, CHX (chlorhexidine diacetate), triclosan, material containing silver (Navarone, Amenitop), cetylperidium chloride, etc., into the resin composites.[8] Various antibacterial agents such as antibiotics, iodine, and quaternary ammonium compounds have also been tried.[7]

A simple way to release any antibacterial agent into a wet environment is to add these agents directly into the resin matrix.[9] Nevertheless, it was observed that a significant proportion of the antibacterial agents filter out within a few days, resulting in short-term effectiveness.[8] On the other hand, if the antibacterial agent is incorporated into the monomer, it leads to an unfavorable impact on their mechanical properties.[8] Hence, there is always a quest for newer natural biomaterials.

Recently, a biomaterial chitosan (2-amino-2-deoxy-β-D-glucan) has surfaced as a remedy for potential bio dental applications. It is considered non-toxic, biocompatible, biodegradable, and antibacterial.[10] Moreover, when used as a bioadhesive polymer, it has been demonstrated to have extended retention inside the oral cavity. Several studies have proved that chitosan, due to its bactericidal and/or bacteriostatic characteristics, can be used to prevent dental caries. It has already been proved that chitosan inhibits the growth of *S. mutans*, which is most frequently found caries causing bacteria. When the bacteria's negatively charged microbial cell surface comes in contact with the amino groups in chitosan (positively charged), it leads to leakage of all the intracellular content and loss of barrier function of the microbial cell wall.[11]

Chitosan, when incorporated in resin composite, is said to increase the biocompatibility, decrease the adsorption capacity of bacteria without altering the flexural strength and mechanical properties.[8]

To provide a good seal, the restorative material should adhere well to the tooth structure so that the integrity is maintained in static mode and during function over some time as degradation of seal causes collapse of the restoration due to microleakage.[4]

Therefore, the stability and retention of new materials in the oral cavity are more valued over a period of time as leaching of these agents might alter the bonding and compromise the properties of the restorative material. Since no data have been reported so far on the extended durability of the seal of this experimental resin, we have undertaken the *in vitro* study to assess the microleakage of chitosan + composite resin to dentin in class V cavities over 3 months duration.

Materials and Methods

Source of Data

Sixty non-carious extracted human permanent maxillary premolars (extracted for various reasons) were collected from the Oral Surgery Department at Dayananda Sagar College of Dental Sciences.

Inclusion Criteria

Unbroken, non-carious, permanent maxillary premolars with complete root formation.

Exclusion Criteria

- Carious teeth.
- Previous restoration.
- Pre-existing fractures or cracks.
- Previous endodontic treatment.
- Non-carious lesions (attrition, fluorosis).

Infection control protocols for extracted teeth collected for educational purposes:

OSHA and CDC recommendations and guidelines were followed for collection, sterilization, storage, and handling of extracted teeth used in the study.

Materials and Armamentarium

- High-speed aeroter handpiece (NSK Japan).
- Straight handpiece (NSK Japan).
- Diamond and carbide bur (Komet, Gebr. Brasseler).
- 35% phosphoric acid (3M ESPE).
- Bonding agent (3M ESPE).
- Microhybrid composite resin (Brilliant NG coltene whaledent).
- Light curing unit (Bisco Inc., Schaumburg, IL, USA).
- Nail varnish.
- Artificial saliva.
- Thermocycling unit 12–2% methylene blue.
- UV photo spectrometer.
- 0.2% chitosan gel (Everest Biotech).
- Centrifuge apparatus.

Methods of Collection of Data

Sixty freshly extracted maxillary premolars were collected and cleaned of debris with a rubber cup and pumice slurry. 0.5% chloramine was used to disinfect the samples, and artificial saliva was used as a storage medium. The samples were rested at 5°C for <1 month before the restorative procedures were carried out. Then, standardized class V cavities were prepared using an aerator and round diamond bur under air-water cooling. A new bur was used for every fifth preparation. On the buccal surface of each tooth, a cavity was prepared with a guideline of 1 mm above CEJ, which resulted in the gingival margin in dentin whereas the occlusal margin in enamel. This was done in accordance with a study done by Tavangar et al.[12]

The dimensions of the cavities were: depth of 1.5 mm, width of 3 mm, and length of 2 mm. To gauge the cavity dimensions, William's graduated periodontal probe was used. Subsequently, all teeth were arbitrarily assigned into two experimental groups of 30 teeth in each group. They were next divided into two subgroups ($n = 15$): Group I—Control-microhybrid composite ($n = 30$):

- 15 teeth tested immediately.
- 15 teeth tested after 3 months.

Group II—Restored with chitosan + composite

- 15 teeth tested immediately.
- 15 teeth tested after 3 months.

The teeth were prevented from dehydration by immersing in artificial saliva at room temperature before preparing for restoration. 37% phosphoric acid was used for etching, followed by bonding agents were applied on all walls of the cavity. Subsequently, composite resins (microhybrid) and chitosan-incorporated composite resins were used for restoration, respectively, and light-cured for 40 seconds. The polishing of the restorations was done using Sof-Lex abrasive disks (3M ESPE) followed by storing the specimens in artificial saliva. The samples were placed in an incubator (37 ± 2°C) at 100% humidity for 24 hours for group I immediate and group II immediate samples, whereas group I (b) and group II (b) samples were stored for 3 months duration. The samples were thermocycled using a thermocycling apparatus at 5 and 55°C in the water bath with a dwell time of 60 seconds each for 10,000 cycles, just before subjecting the test specimens for dye extraction (Figs 1 to 4).

Preparation of chitosan: 0.012 g of deacetylated chitosan with a particle size of 0.001 g was mixed homogeneously with 4 g of microhybrid composite (Brilliant NG coltene whaledent) in a 50 mL glass beaker in a dark room to achieve the desired concentration of 0.2% chitosan-based composite.

Methodology for Dye Extraction Test

After the restoration, the apical portion of all the teeth was sealed with sticky wax.

Dye extraction method: The samples were first immersed in 2% methylene blue solution for 24 hours, including 1 mm of restorative margins covered with two layers of nail varnish. Twenty-four hours later, the nail varnish from the samples was removed by the use of polishing disks, followed by rinsing it under tap water for 30 minutes. Next, vials containing 65 wt% nitric acids were used to immerse the samples. It was rested for 3 days so that the nitric

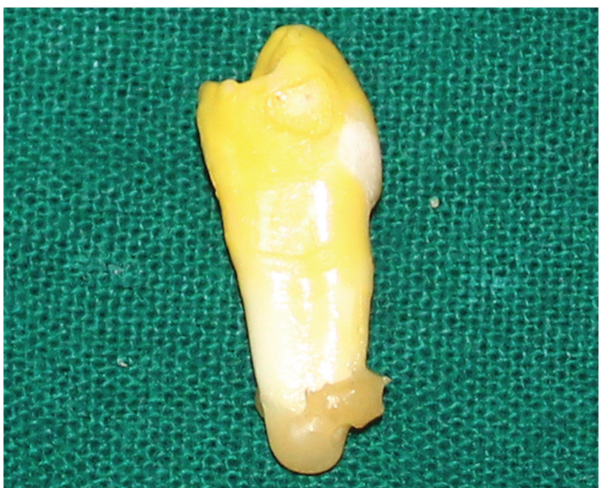

Fig. 1: Class V cavity prepared with dimensions verified with a periodontal probe

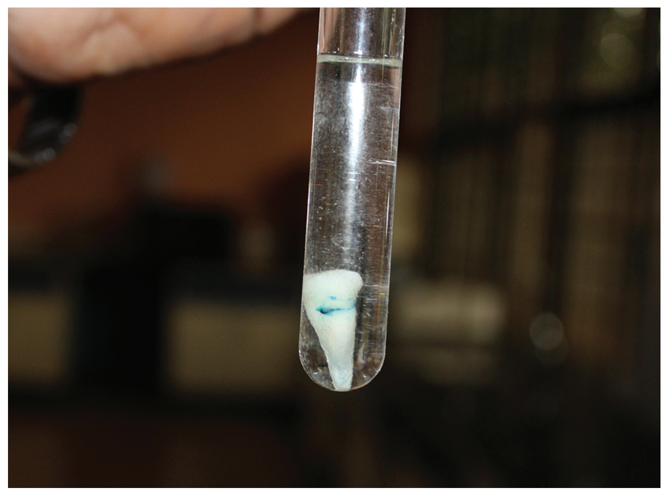

Fig. 2: After removal of nail varnish restored tooth is immersed in 65 wt% nitric acid

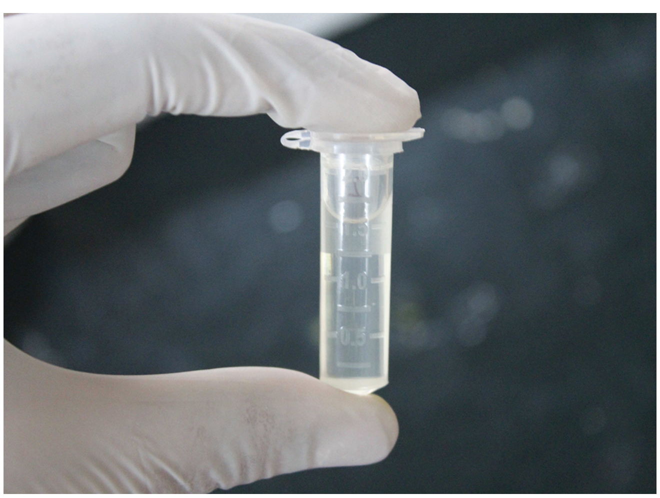

Fig. 3: After centrifugation, supernatant is collected from the tooth solution

Fig. 4: Dimension of the cavity were verified with a perio probe

acid dilutes methylene blue present within the restoration-dentin interface. In each vial, there was 1,000 µL of 65 wt% nitric acid present. Centrifugation of the contents within the vials was carried out at 14,000 rpm for 5 minutes, and then a plate with 100 µL of the supernatant was transferred. Using concentrated nitric acid as the blank, an automated spectrophotometer at 550 nm was used to measure the quantity of dye absorption. Therefore, the light absorption of methylene blue by the spectrophotometer indicated the microleakage of the restoration.[13]

Results

Statistical Analysis

Statistical Technique Used

The comparison of the mean dye penetration (in nm) between group I and group II at immediate and after 3 months interval was made using the independent student's *t*-test.

The comparison of the mean dye penetration (in nm) between immediate and 3 months samples within group I and group II was done using the Student's paired *t*-test.

Computations

The following tables depict the results from the independent student's *t*-test and Student's paired *t*-test and the *p* value.

Note:

Group I: Composite; group II: chitosan + composite

The immediate test results demonstrated that group I exhibited a mean microleakage score of 0.015 nm and group II a mean microleakage of 0.0096 nm. There was no statistically significant difference between the mean microleakage scores when the *p* value was set at $p < 0.05$.

The samples tested after 3 months demonstrated that the mean leakage of group I was 0.0158 nm and group II of 0.0117 nm. Here, a statistically significant difference between the mean microleakage scores was found when the *p* value was set at $p < 0.05$.

Comparison of mean dye penetration (in nm) between immediate and 3 months samples in group I and group II.

Note:

Group I: Composite; group II: chitosan + composite

Comparing within the groups: group I (control group) demonstrated a mean microleakage of 0.0105 nm on immediate testing and 0.0158 nm after 3 months. After comparison, a statistically significant difference was evident between the two groups with a *p* value: $p < 0.05$.

Within group II (chitosan + composite group): the immediate mean microleakage score was 0.0096 nm, and 3 months later, the mean microleakage score was 0.0117 nm. After comparison, it was found that there was no evidence of any statistically significant difference between the two groups with a *p* value: $p < 0.05$.

Discussion

Placing the cervical restorative margins with a complete seal has always been challenging and technically demanding due to the difficulty in isolation, insertion, contouring, finishing, and polishing which often results in secondary caries. It is commonly experienced that the least durable type of restoration in the oral cavity is class V restorations.[3] Hence, one is always on a constant outlook for a suitable component that possesses good adhesive and antibacterial properties for such lesions. Similarly, here in our study, we aimed to evaluate the seal-ability of the newly formulated chitosan-modified composite restoration when placed in class V lesions.

The success of any restoration depends on the size and position of the cavity margins,[14] depth of the lesion.[15] From the literature review, it is summarized that most cervical lesions are sensitive to sweet, cold, or air as they are moderately deep.[16] In our study to mimic a relatively deep lesion, the cavity dimension was standardized based on the study design suggested by Tavangar et al.[12]

The location of these lesions makes a selection of the restorative material an arduous task, as there is always a constant application of masticatory load, which has a destructive effect on tooth flexure in the cervical region. So, this demands a material with a low modulus of elasticity which allows the restoration to flex with the tooth.[3]

Microhybrid composite is considered the material of choice in class V lesions.[17] They possess a low modulus of elasticity, allowing the restoration to flex with the tooth rather than de-bond on load compared to more rigid macro-filled Composite. Moreover, they have substantially improved wear resistance compared with older composites. Additionally, the polymerization shrinkage forces are sufficiently less in microhybrid composite than the conventional composite restoration in class V situations resulting in less microleakage.[18] Hence, in our study, the material of choice for restoration was microhybrid composite.

A series of studies have proven the fact that there is a greater number of bacteria and plaque accumulation on the surface of composite resins[5] and enamel demineralization owing to plaque accumulation compared with other restorative materials,[6] which may lead to secondary caries thereby limiting the longevity of RBCs.[5,7]

Although mechanical properties may have improved substantially since their development,[4] the antibacterial properties of RBCs are still considered unsatisfactory by dentists and investigators.[7] Several bioactive materials and antibiotics such as MDPB,[8] triclosan, antibiotics,[7] iodine,[7] quaternary ammonium compounds, etc.,[7] have been added, but these agents' durability was durable not found to be satisfactory. It was seen that within a few days, a large proportion of these agents released out, resulting in a decrease in their concentration. Furthermore, they may be toxic and disrupt microbial homeostasis.[8]

So the prerequisite for the addition of any new component to resin is that it should neither adversely affect the physical and mechanical property nor affect the long-term adhesion to the tooth structure and it should also have sustained release. Thus, at the current date, an active area of research in our field is to improve the currently available resin-based material. In this context, special attention should be driven towards the area of bioadhesive polymers and chitosan-based dentine replacement materials.

An extensive amount of research has been carried out on chitosan-based materials[11] for various dental applications[10] such as modification of dentifrices, enamel repair and mineralization, oral drug delivery, incorporation in adhesives and dentin bonding agents, improvement in various restorative materials such as GIC,[19] RBC,[19] MTA,[20] etc., as it has versatile physicochemical and biological characteristics. It is said to be highly compatible and can blend with any restorative material.

Incorporation of chitosan in resin-based composite was done based on research work carried out by Mirani et al.[7] He has evaluated the antibacterial efficacy of chitosan-based composite and found it to be superior to that of commercial RBCs. In a study done by

Kim et al.,[8] the antibacterial effect and mechanical properties of composite resin were evaluated using three different (low, medium, and high) molecular weights of chitosan. They concluded that the addition of chitosan with low and high molecular weight showed suitable antimicrobial properties without altering the mechanical properties of the resin.

Based on the above findings, in our study, 0.012 g of deacetylated chitosan with the particle size of 0.001 g was mixed homogeneously with 4 g of microhybrid composite (Brilliant NG coltene whaledent) in a darkroom to achieve the desired concentration of 0.2% chitosan-based composite.

The bonding potential of chitosan-based composite has been evaluated immediately after restoration in almost all the studies in literature,[4,7] however, the stability and retention of any modified restorative material are valued over a period of time as leaching of the additive agents might change the bonding and compromise the properties of restorative material. Literature shows that a significant change in hardness takes place between 9 weeks and 12 weeks[21,22] of storage when compared to immediate samples. Hence, in our study, we incubated the samples for 3 months in artificial saliva.

Several storage media such as artificial saliva, acids, or ethanol solutions have been used in various studies.[22] The basic assumption in creating artificial saliva is its similarity to natural saliva in terms of physical characteristics and chemical composition.[23] It has been reported that when compared with distilled water,[24] the leaching of fillers was significantly more after storage in artificial saliva. Storing in artificial saliva simulates our oral cavity better than distilled water which justifies storage of samples in the artificial saliva in an incubator at 37°C at 100% humidity as an aging protocol in the current study.

The physical properties such as flexural strength, bond strength, and structural durability of RBCs are degraded by the action of water absorption within a resin matrix. In the present study, we utilized a method to simulate the intraoral aging conditions,[25–28] as the late physical and chemical degradation phenomena occur as a function of time. Hence, in our study, we used thermocycling where a water bath was set at 5 and 55°C with one minute dwell time for each 10,000 cycles.

There are a plethora of studies undertaken over the years on class V composite restorations[13] to explore various methods to evaluate microleakage beneath a restoration. Most of these studies have used various dyes as leakage tracers (dye penetration: considered as the gold standard). The technique has several limitations.[29,30]

As a result, two quantitative methods (dye-extraction and fluid filtration technique) have surfaced, which provides a better predictive value than the qualitative methods. Although the fluid filtration technique gives a similar result as dye extraction, the water present penetrates into all the irregularities. As a result, the filtration value reduces over time.[13] Hence, dye extraction method was used in our study to evaluate microleakage.

On analyzing the results of our study, it was found that the unmodified composite group showed a mean microleakage score of 0.0105 nm when tested immediately, which increased to 0.0158 nm at the end of 3 months. These values were in accordance with the results obtained by many researchers previously.[13,31]

Studies have shown that long-term chemical and mechanical degradation occurs under *in vivo* conditions due to the presence of water at the interface leading to hydrolysis within the collagen fibrils or resin bonded interface. Hydrolysis also occurs due to break-in covalent bonds between the polymers; when water contacts the ester bonds, it wears away the resin mass. This phenomenon leads to resin matrix degradation within the hybrid layer over a period of time.[32,33] These factors may have contributed to the increased microleakage score seen in the unmodified microhybrid composite group after 3 months of storage in artificial saliva.

On comparing the samples tested immediately after restoration, the chitosan + composite group showed a low mean microleakage value (0.096 nm) compared with the unmodified composite group (0.0105 nm), although a statistically significant difference was not evident between the two groups. This indicated that incorporation of chitosan had not adversely affected the bonding property of composite to dentin.

Comparing the mean microleakage values of chitosan-incorporated composite-immediate test group and 3 months interval group, there was no significant increase in the microleakage score (from 0.0096 to 0.0117 nm), indicating that there was hardly any bond degradation occurring even after 3 months of storage in artificial saliva.

On comparing the microleakage after 3 months, the mean microleakage score of the unmodified composite group (0.0117 nm) was significantly higher than the chitosan + composite group (0.0105 nm).

Conventional microhybrid composite has low elastic modulus, which indicates the greater ability for the restoration to flex with the tooth to accommodate inherent modulus of the tooth[17] but compared to the adhesive or cohesive strength of the substrates if the stress proportion exceeds, it leads to separation of the restoration from the tooth surface. Despite significant improvements to date, the weakest area for composite restoration is the bond interface. The durability and stability of resin-bonded restoration on dentin surfaces are still questionable.[32]

The reduction in the microleakage value evident in the chitosan modified composite group may have attributed to the following: chitosan-incorporated in the composite may act as a space occupier since the amine groups make it very reactive along with –OH group as revealed in the Fourier transform infrared spectroscopy (FTIR) analysis. Further, chitosan acted as an inert filler having –NH chain and high nitrogen content (6.89%) resulted in improved adhesion between the constituents of composite resin, less leaching of resin monomer into the liquid prevented hydrolytic bond degradation, decreasing the volumetric shrinkage compared to regular composites thereby reducing microleakage.[34]

It has also been speculated by Satheesh et al. in his study that we can access a uniform dispersion of the additive when <2.5 wt% chitosan loading is used. When the mechanical and tensile strength of the material is stable, the thermal stability of the system also increases. They are of the opinion that an improvement in the adhesion between components is due to excess free amine groups through chitosan chains and incorporation of HMDA hardener.[34]

Several authors have proved that chitosan, when used in 0.12 and 0.25% (w/w), maintains the stability of the material, and does not hamper the adhesive properties of the bonding system.[35] A similar mechanism could have been responsible for the reduction of microleakage, as evident in our study.

It appears that the chitosan-modified composite is an exciting combination, beneficial especially in class V cavities. It may be considered a replacement to traditional microhybrid composites in class V cavities as it combines the benefits of antibacterial properties and better marginal seal even after 3 months.

However, further *in vitro* and long-term *in vivo* studies regarding its antibacterial effectiveness, water sorption, solubility, and long-term stability and the refinement in formulation technique are necessary before it can be recommended for routine clinical usage.

Conclusion

The present *in vitro* study draws the following conclusions (within its limitation).

A statistically significant difference in mean microleakage scores was not evident between chitosan containing composite group and the unmodified composite group when evaluated immediately after placing the restoration, which indicated that the addition of chitosan had not interfered with the bonding of composite resin to the tooth.

Microleakage values of the unmodified composite group increased significantly after 3 months of storage in artificial saliva, suggesting considerable bond degradation.

The microleakage value of the chitosan-incorporated composite group did not differ significantly even after 3 months of storage in artificial saliva, suggesting that there was no bond degradation.

There was significantly less microleakage seen in the chitosan-containing composite group compared to the unmodified composite group after 3 months of storage in artificial saliva suggesting more excellent stability of bond of chitosan containing composite resin over unmodified composite resin.

Clinical Significance

Based on the results of our study and that found in literature, it is evident that chitosan-incorporated composite in addition to being antibacterial also seems to have improved mechanical properties and more stable bonding when compared with the unmodified microhybrid composite. Considering the above advantageous property of this material, their use may be clinically valuable in restoring class V cavities in patients with high caries risk. However, further *in vitro* and *in vivo* studies have to be done to evaluate this novel restorative material for its long-term durability, bonding ability, color stability, solubility, and most importantly, retention of its antibacterial property over a more extended period of time.

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