Antibacterial activity and adhesive properties of a chitosan-containing dental adhesive

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Objective: To evaluate the antibacterial activity of adhesive resin incorporating chitosan as well as the adhesive characteristics. Method and Materials: An experimental adhesive was prepared by adding 0.12%, 0.25%, 0.5%, and 1% (w/w) chitosan solution to Single Bond adhesive resin. The solution of chitosan was prepared by dissolving 2 g of chitosan powder in 1 liter of 1% (v/v) acetic acid. Single Bond without chitosan was used as a control. The antibacterial activity was evaluated using a direct contact test against Streptococcus mutans. The viscosity, degree of conversion, pH, and microtensile bond strength (μTBS) values of the experimental adhesives to dentin were evaluated. Data were analyzed using the ANOVA and Tukey tests. Statistical significance was set at the .05 probability level. Results: The antibacterial properties of freshly prepared and aged experimental adhesives incorporating chitosan were found to exhibit an inhibitory effect on the growth of Streptococcus mutans compared with the unmodified adhesive resin (P < .05). The viscosity of the experimental adhesives increased with increasing the concentrations of chitosan incorporation into the adhesive. However, the degree of conversion and pH values decreased with increasing the concentrations of chitosan incorporation into the adhesive. Experimental adhesives incorporating 0.12% and 0.25% (w/w) chitosan showed no significant differences in the μTBS values compared with the control (P > .05). However, the incorporation of 0.5% and 1% (w/w) chitosan into the dental adhesive significantly decreased the μTBS (P < .05). Conclusion: Adhesive resin that contains 0.12% (w/w) chitosan is a promising antibacterial adhesive that does not adversely affect adhesive properties. (Quintessence Int 2012;43:603–613)

Key words: adhesive properties, antibacterial activity, chitosan, dental caries, microtensile bond strength, viscosity

Dental caries is an infectious disease of bacterial etiology.1 The treatment of caries in clinical situations is a challenge due to the lack of a comprehensive and precise diagnosis of the extent of dentinal caries.2,3 Active bacteria may inadvertently be left behind by incomplete caries removal. Accordingly, restorative materials that reveal antibacterial activity are helpful for eliminating the harmful effects caused by either residual bacteria or bacterial microleakage.2

Significant trials of incorporating antibacterial components into dental adhesive systems have been investigated to improve the biologic sealing ability of the restorative materials, including methacryloyloxydodecyl pyridinium bromide (MDPB),3,4 inorganic agents,5 methacryloxyethyl cetyl dimethyl ammonium chloride (DMAE-CB),6,7 and chlorhexidine8,9 with varying degrees of success. Therapeutic enhancement may consequently be achieved when combining antibacterial agents with dental adhesive systems. The incorporation of chlorhexidine into a self-etching primer improved the antibacterial activity against cariogenic microorganisms. However, incorporation of chlorhexidine with higher concentrations decreased the bond strength of adhesive to dentin.8 Hence, the specific antibacterial agent chosen and its quantity are significant for incorporation into the dental adhesive system. The additive should not adversely affect the basic physical properties of dental adhesive systems, and further improvements are needed to enhance their antibacterial activities.

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Chitosan is a natural polysaccharide biopolymer produced by alkaline partial deacetylation of chitin (Fig 1). Chitin is a straight homopolymer consisting of (1,4)-linked N-acetyl-glucosamine units found in the exoskeleton of crustaceans such as crabs and shrimp. Chitosan is composed of copolymers of glucosamine and N-acetyl-glucosamine with two free hydroxyl groups and one primary amino group for each C6 structure unit. These free amino groups provide chitosan a positive charge that permits its reaction with negatively charged surfaces. Chitosan is generally regarded as nontoxic, biocompatible, and biodegradable and is inherently antibacterial in nature. One of the suggested mechanisms of chitosan’s antibacterial characteristics is that the interaction between positively charged chitosan and a negatively charged bacterial cell could change the bacterial cell permeability, leading to the leakage of intercellular components and cell death.10–13 In recent years, the use of chitosan has become a significant area of research in dentistry.12–14 Chitosan may act as a barrier against acid penetration, since it interferes with demineralization of the tooth enamel.12

The objective of this study was to investigate the effect of chitosan incorporation on the antibacterial activity and adhesive properties of a commercial dental adhesive. The null hypothesis tested was that the incorporation of increasing concentrations of chitosan had no effect on the antibacterial activity or the adhesive characteristics of the dental adhesive.

**METHOD AND MATERIALS**

Adper Single Bond (lot no. 5FE, 3M ESPE), a commercially available adhesive resin that has no effective antibacterial constituent,15 was used in this study. An experimental adhesive was prepared by adding chitosan (85% deacetylated, Sigma-Aldrich) solution at 0.12%, 0.25%, 0.5%, and 1% (w/w) to Single Bond. The chitosan solution was prepared by dissolving 2 g of chitosan powder in 1 liter of 1% (v/v) acetic acid.13 Single Bond without chitosan was used as the control (Table 1).

**Antibacterial activity—Test microorganism and growth conditions**

*Streptococcus mutans* (*S* *mutans*) (ATCC 27351) was obtained from the Department of Microbiology, Faculty of Medicine, Mansoura University, Mansoura, Egypt. *S* *mutans* was grown aerobically from frozen stock cultures.
in brain heart infusion (BHI) (Oxoid) broth containing 8 μg/mL of bacitracin (Sigma-Aldrich) for 48 hours at 37°C before use. *S. mutans* is naturally resistant to bacitracin; consequently, this antibiotic was added to the growth media to prevent microbial contamination throughout the experiments.\(^16\)

**Direct contact test**
The direct contact test (DCT)\(^17\)–\(^19\) is based on turbidometric determination of bacterial growth in 96-well microtiter plates by using a microplate reader to measure the absorbance of transmitted light through the specimens. Smaller amounts of transmitted light indicate greater specimen absorbance and greater density of bacterial cells.\(^20\) A sterile 96-microtiter plate (Becton Dickinson) was held vertically, and the sidewalls of 8 wells were coated with 15 μL of each tested experimental adhesive without it flowing and wetting the bottom of the well (this would interfere with the light path through the microplate well and lead to false readings). The experimental adhesive was light cured for 10 seconds using a quartz-tungsten halogen curing device (XL2500, 3M ESPE). The output of the curing light (670 mW/cm\(^2\)) was verified with a radiometer (Optilux radiometer, Kerr-Sybron). The experimental adhesive in each well was light cured at the same distance from the light-curing device and without exposing the adjacent well to the light by fitting an opaque plastic jig onto the end of the light-curing tip.\(^21\)

Then, a 10-μL bacteria suspension (1 × 10\(^6\) bacteria) was placed on the tested material, and the plate was incubated in a vertical position for 1 hour at 37°C. After that, the plate was placed horizontally. BHI broth supplemented with 25 μg/mL bacitracin (235 μL) was added to each well and gently mixed (DELFIA Plateshake, PerkinElmer) for 2 minutes. The positive control consisted of one set of eight uncoated wells in the same microtiter plate, containing bacterial inoculum and processed as described before. However, the negative control consisted of one set of eight wells coated with the test materials, containing an equal volume of uninoculated fresh medium. The antibacterial properties of the experimental tested adhesives were examined 1 hour after polymerization (fresh preparations). Similar experiments were conducted where the experimental tested adhesives were aged in 280 μL of phosphate-buffered saline (PBS) (Sigma-Aldrich) containing 25 μg/mL of bacitracin for 7 days at 37°C before assaying. During the aging period, the PBS was replaced every 24 hours. Absorbance at 650 nm (A\(_{650}\)) was determined in each well every 20 minutes for 16 hours by using a temperature-controlled spectrophotometer set (VICTOR X Multilabel Plate Readers, PerkinElmer) at 37°C to determine the maximum change in A\(_{650}\). Automixing was done before each reading to establish a homogenous bacterial cell suspension. Data were recorded in optical density units. The baseline represented the values obtained from the negative control wells and was then subtracted from the respective experimental data.

**Viscosity measurement**
Viscosity of the experimental adhesive was measured using a rotational rheometer (AR2000, TA Instruments) in the controlled-rate mode. The measurements were made at 25°C with 40-mm diameter and 2-degree cone angle. The shear rate range tested was 10 to 100/second. At each shear rate, shear was applied for 60 seconds before the viscosity measurement. Five measurements over the shear rate range were averaged for each experimental adhesive.

**Degree of conversion**
The degree of conversion was measured using Fourier transform infrared spectroscopy (FTIR, Spectrum One; Perkin-Elmer). One drop (10 μL) of each experimental adhesive was placed between celluloid strips (GC) to achieve a thin film. Before covering the experimental adhesives with the upper celluloid strip, they were gently air dried for 5 seconds to allow solvent evaporation. An FTIR spectrum of the uncured material was recorded, and the specimens were then photoactivated for 10 seconds with the light-curing unit. Each specimen was carefully removed with a narrow surgical knife and stored for 24 hours in a dark, dry environment until the FTIR analysis of the degree of conversion. All the spectra were obtained in a transmission
mode with 32 scans in the range of 4,000 to 800 cm\(^{-1}\) and at a resolution of 4 cm\(^{-1}\). Five specimens were performed for each experimental adhesive. The degree of conversion of each specimen was determined from the ratio of absorbance intensities of the aliphatic bond (C=C) (peak height at 1,638 cm\(^{-1}\) ) against the internal standard before and after curing of the specimen. The aromatic carbon–carbon bond (peak height, 1,608 cm\(^{-1}\) ) absorbance was used as an internal standard. The degree of conversion was determined using the following equation:

\[
DC(\%) = \left( 1 - \frac{A_{\text{aliphatic}}}{A_{\text{aromatic}}} \right) \times 100
\]

where \(A_{\text{aliphatic}}\) is the absorbance peak at 1,638 cm\(^{-1}\) of the cured specimen, \(A_{\text{aromatic}}\) is the absorbance peak at 1,608 cm\(^{-1}\) of the cured specimen, \(A_{\text{aliphatic}}\) is the absorbance peak at 1,638 cm\(^{-1}\) of the uncured specimen, and \(A_{\text{aromatic}}\) is the absorbance peak at 1,608 cm\(^{-1}\) of the uncured specimen.

**pH measurement**

The pH values of the experimental adhesives were determined by a pH meter (Accumet Research AR25, Fisher Scientific). The pH meter was calibrated with 2.0 and 1.0 standard buffer solution. To measure the pH value, the probe was rinsed with distilled water, shaken, blotted of excess water, and put into the test tube containing 2 mL of the experimental adhesive. The pH measurement was recorded after 2 minutes. After each measurement, the probe was rinsed with ethyl alcohol and distilled water, shaken, and blotted of excess water to remove any traits of previously measured adhesive. Calibration was then performed. Five readings were taken for each group, and the mean pH value was calculated and recorded.

**Microtensile bond strength (μTBS)—Tooth preparation**

The teeth used in this study were obtained by protocols that were reviewed and approved by the appropriate institutional review board of the Faculty of Medicine and Dentistry, Mansoura University, Mansoura, Egypt. Fifty human caries-free third molars extracted for orthodontic reasons or as a result of periodontal disease were collected and stored in PBS at 4°C. Only teeth that were free of caries and cracks when examined under a stereomicroscope (Olympus SZX-ILLB100, Olympus Optical) at 10× magnification were used.

The teeth were randomly divided into five groups of 10 teeth for each group of control/experimental adhesive materials. The occlusal enamel was removed perpendicularly to the long axis of the tooth using a low-speed diamond saw (Isomet 1000, Buehler) under water cooling to expose a flat dentin surface. The surface was polished on wet 600-grit silicon carbide paper for 1 minute to produce a standard smear layer. The dentin surfaces were thoroughly rinsed with water spray and immediately air dried with moisture-free air.

**Bonding and restorative procedures**

The dentin surfaces were etched with 35% phosphoric acid gel (Etchant, lot no. 1ER, 3M ESPE) for 15 seconds. The etched surfaces were then rinsed with distilled water for 10 seconds and blotted dry. Two consecutive coats of the adhesive resin (control/experimental) were then applied using a saturated disposable brush, gently air dried with oil-free compressed air for 5 seconds, and then light cured for 10 seconds.

Following adhesive application, a composite resin, Filtek Z-250 (shade A2, lot no. 4AN; 3M ESPE) was built up in increments to a height of 5 mm. Each increment was light cured for 40 seconds. After that, the specimens were stored in distilled water at 37°C, 100% humidity for 24 hours before sectioning.

**Microtensile bond strength testing**

After 24 hours storage in distilled water at 37°C, the bonded teeth were vertically sectioned into serial slabs and further into beams with cross-sectional areas of approximately 1 mm\(^2\) using a low-speed diamond saw under water cooling. Each tooth yielded between 4 and 6 beams for bond testing. The specimens were then attached to a Bencor Multi-T testing device (Danville Engineering) using a cyanocrylate adhesive
(Zapit, Dental Ventures of America) and stressed to failure in tension using a universal testing machine (model TT-B, Instron) at a crosshead speed of 1 mm/min. The exact dimension of each fractured beam was then individually measured using a digital caliper (Mitutoyo), from which the μTBS was calculated in MPa. Beams that failed prematurely while being cut or glued were included as 0 MPa in the calculation of the mean μTBS.

Failure analysis
Debonded specimens were examined under a stereomicroscope at 50× magnification to evaluate the fracture pattern. The modes of failure were classified according to one of four types: type 1, cohesive failure within the composite resin; type 2, adhesive failure (failure across the bonding interface); type 3, cohesive failure in dentin; and type 4, mixed failure. Representative debonded specimens from each group were selected, sputter-coated with gold (Sputter Coater S150A), and observed using a scanning electron microscope (SEM) (JXA-840A, JEOL) at magnifications of 200× and 2,000×.

Statistical analysis
All data were statistically analyzed (SPSS 13.0, IBM) using one-way analysis of variance (ANOVA) and Tukey multiple comparison tests. Statistical significance was set at .05.

RESULTS
Freshly tested specimens showed antibacterial activity of experimental adhesive incorporating 0.12%, 0.25%, 0.5%, and 1% (w/w) chitosan. Bacterial growth of experimental adhesives incorporating chitosan was significantly reduced when compared with the control ($P < .05$). After aging the materials for 7 days, experimental adhesive incorporating 0.12%, 0.25%, 0.5%, and 1% (w/w) chitosan maintained their antibacterial property (Table 2).

The viscosity (Pa s), degree of conversion, and pH values of all groups are presented in Table 3. The viscosity increased with increasing concentrations of chitosan incorporation into the Single Bond dental adhesive. There was a significant difference in the viscosity of Single Bond + 0.5% and Single Bond + 1% (w/w) chitosan compared with the control ($P < .05$). On the other hand, the degree of conversion decreased with increasing concentrations of chitosan incorporation into the Single Bond dental adhesive. There was a significant difference in the degree of conversion of Single Bond + 0.5% and Single Bond + 1% (w/w) chitosan compared with the control ($P < .05$). The pH values significantly decreased with increasing concentrations of chitosan incorporation into the Single Bond dental adhesive for all experimental adhesive groups compared with the control group ($P < .05$).

<table>
<thead>
<tr>
<th>Group</th>
<th>Fresh material (1 h)</th>
<th>Aged material (7 d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>0.123 ± 0.010×</td>
<td>0.503 ± 0.034×</td>
</tr>
<tr>
<td>Single Bond + 0% chitosan</td>
<td>0.153 ± 0.022×</td>
<td>0.468 ± 0.031×</td>
</tr>
<tr>
<td>Single Bond + 0.12% chitosan</td>
<td>0.066 ± 0.019×</td>
<td>0.157 ± 0.025×</td>
</tr>
<tr>
<td>Single Bond + 0.25% chitosan</td>
<td>0.057 ± 0.018×</td>
<td>0.136 ± 0.020×</td>
</tr>
<tr>
<td>Single Bond + 0.5% chitosan</td>
<td>0.045 ± 0.006×</td>
<td>0.126 ± 0.019×</td>
</tr>
<tr>
<td>Single Bond + 1% chitosan</td>
<td>0.044 ± 0.005×</td>
<td>0.122 ± 0.19×</td>
</tr>
</tbody>
</table>

SD, standard deviation. Mean values with the same superscript lowercase letter (column) are not significantly different ($P > .05$), while the mean values with different superscript lowercase letters (column) are significantly different ($P < .05$).
The mean μTBS (MPa), standard deviation (SD), the number of pretesting failures (ptf), the number of tested specimens (n), and the percentage of failure modes of all groups are summarized in Table 4. There was no significant difference in the bond strength values between the experimental adhesives incorporating 0.12% and 0.25% (w/w) chitosan compared with the control group (P > .05). However, the incorporation of 0.5% and 1% (w/w) chitosan into the dental adhesive significantly decreased the μTBS (P < .05). When the μTBS was plotted against the viscosity, the μTBS started to decrease significantly as the values of the viscosity began to increase starting from experimental adhesives incorporating 0.5% and 1% (w/w) chitosan (Fig 2a). On the other hand, when the μTBS was plotted against the degree of conversion and pH, the μTBS started to decrease as the values of the degree of conversion and pH began to decrease starting from experimental adhesives incorporating 0.5% and 1% (w/w) chitosan (Figs 2b and 2c). Most failure modes were mixed failures (type 4) and cohesive failures in the composite resin (type 1) in both the control and experimental adhesives, followed by cohesive failures in dentin (type 3). There were few adhesive failures (type 2) (Table 4 and Fig 3).

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Mean ± SD of viscosity (Pa s), degree of conversion, and pH of each tested experimental adhesive and Tukey analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Viscosity</td>
</tr>
<tr>
<td>Single Bond + 0% chitosan</td>
<td>0.027 ± 0.004a</td>
</tr>
<tr>
<td>Single Bond + 0.12% chitosan</td>
<td>0.029 ± 0.005ab</td>
</tr>
<tr>
<td>Single Bond + 0.25% chitosan</td>
<td>0.031 ± 0.004bc</td>
</tr>
<tr>
<td>Single Bond + 0.5% chitosan</td>
<td>0.034 ± 0.005bc</td>
</tr>
<tr>
<td>Single Bond + 1% chitosan</td>
<td>0.036 ± 0.004c</td>
</tr>
</tbody>
</table>

SD, standard deviation. Mean values with the same or common superscript lowercase letters (column) are not significantly different (P > .05), while the mean values with different superscript lowercase letters (column) are significantly different (P < .05).

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Mean ± SD of the microtensile bond strength (μTBS) (MPa) and percentage distribution of failure modes of debonded specimens, in each experimental group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>μTBS (MPa) Mean ± SD [ptf/n]</td>
</tr>
<tr>
<td>Single Bond + 0% chitosan</td>
<td>30.77 ± 2.86 [5/47]</td>
</tr>
<tr>
<td>Single Bond + 0.12% chitosan</td>
<td>32.34 ± 3.01 [6/50]</td>
</tr>
<tr>
<td>Single Bond + 0.25% chitosan</td>
<td>29.34 ± 2.04 [8/44]</td>
</tr>
<tr>
<td>Single Bond + 0.5% chitosan</td>
<td>22.36 ± 2.18 [7/49]</td>
</tr>
<tr>
<td>Single Bond + 1% chitosan</td>
<td>20.23 ± 1.14 [10/45]</td>
</tr>
</tbody>
</table>

SD, standard deviation; ptf, pretesting failure; n, total number of tested specimens. Mean values with the same superscript lowercase letter are not significantly different (P > .05), while the mean values with different superscript lowercase letters are significantly different (P < .05).

Type 1, cohesive failure within the composite resin; type 2, adhesive failure (failure across the bonding interface); type 3, cohesive failure in dentin; and type 4, mixed failure.
Fig 2 μTBS vs (a) viscosity, (b) degrees of conversion, and (c) pH of the experimental adhesives incorporating chitosan.
DISCUSSION

The results of this study reject the null hypothesis since the incorporation of increasing concentration of chitosan enhanced the antibacterial activity of the adhesive resin; however, the higher concentrations of chitosan solution (0.5% and 1% [w/w]) incorporated into the adhesive resin affected the adhesive properties.

Bacteria invariably remain trapped in the dental tissue during removal of carious substrate, since neither the clinical parameters of dentin hardness and color nor the caries-detector dyes are capable to ensure complete removal of microorganisms.\(^{24,25}\) Differentiation between affected and infected dentin is still a problem that frequently occurs in daily clinical practice. A conventional surgical approach to remove caries will not certainly render caries-free restorations; however, it may result in additional unwarranted removal of sound tooth structure and potential exposure of the pulp.\(^{3,26,27}\)

Additionally, there has been significant attention in the treatment and management of caries with less surgical intervention—the minimally invasive approach—\(^{27,28}\) However, it is possible that some active bacteria remain while awareness is focused on the removal of less tooth structure.\(^{4}\) With some pioneer bacteria in the remineralizable, affected layer after minimally invasive caries removal, the subsequent use of materials that have an antibacterial or bactericidal effects provide an adjunct treatment contributing to suppression of residual infection and increasing the survival of the restored tooth.\(^{3}\) Accordingly, adhesive systems with antibacterial effects would be helpful, which was the purpose of the present study.

**Fig 3** Representative SEM photomicrographs of the fractured surface along the dentin side. (a) A mixed failure of Single Bond + 0.12% chitosan group (remaining scratches from the surface preparation may be observed) and (b) at a higher magnification, tubules are occluded by resin tags. (c) A mixed failure of Single Bond + 0.25% chitosan group and (d) at a higher magnification, the dentinal surface covered by the resin with no exposure of dentinal tubules (Ar, adhesive resin; C, composite; D, dentin; Hy, hybrid layer).
The DCT was introduced\textsuperscript{17} to determine the effect of direct contact between the tested materials and the test microorganism. The DCT is a quantitative method and provides information on the viability and bacterial growth rate. Single Bond is a weak acidic adhesive system (pH, $\approx$ 4.3) (see Table 3). Theoretically, this adhesive system should display some antibacterial activity prior to curing since the acidity of adhesives has been recognized as an inhibiting factor to bacteria growth.\textsuperscript{29,30} Nevertheless, its limited antibacterial activity may disappear after curing.\textsuperscript{30} According to the results of the current study, for the unmodified adhesive (Single Bond + 0% chitosan), the set specimens did not produce bacterial growth inhibition (see Table 2). This finding is in accordance with Prati et al.\textsuperscript{31} who found that most cured dentin bonding resins are not antibacterial. On the other hand, experimental adhesives incorporating chitosan exhibited a bacteriostatic effect, demonstrating inhibition of bacterial growth compared with control adhesive (Single Bond + 0% chitosan), both fresh and aged specimens. It could be explained by the polycationic properties of chitosan, presented by the positively charged $\text{NH}_3^+$ groups of glucosamine, which could be the major factor contributing to its interaction with negatively charged surface components of bacteria, resulting in extensive cell surface alterations, leakage of intracellular substances, and damage to vital bacterial activities.\textsuperscript{11,32,33} Consequently, dental adhesives containing chitosan could prevent the invasion and growth of bacteria and will offer a new pattern of caries management.

Even though dental adhesives have new biologic characteristics, they would not be clinically helpful if their basic adhesive properties are hindered by the new additive.\textsuperscript{4} Accordingly, evaluation of viscosity, degree of conversion, pH, and $\mu$TBS of the experimental adhesive incorporating chitosan were considered. When the concentrations of chitosan were altered, the properties of the experimental adhesives, such as the viscosity, degree of conversion, pH, and $\mu$TBS were also changed (see Tables 3 and 4).

In this study, the $\mu$TBS to dentin of experimental adhesive incorporating 0.12% or 0.25% (w/w) chitosan was not significantly different from that of the control ($P > .05$). However, the $\mu$TBS of the former slightly increased, although not significantly, compared with the control group (see Table 4). On the other hand, the $\mu$TBS decreased significantly with increasing the concentration of chitosan (0.5% and 1% [w/w]) in the adhesive resin compared with the control group ($P < .05$). This could be explained by the fact that the measurements of the viscosity of experimental adhesive indicated that the viscosity of Single Bond adhesive increased with increasing concentrations of chitosan incorporation (see Table 3 and Fig 2a). This reduction in bond strength might be due to the inferior infiltration of the adhesive resin into the demineralized dentin due to a significant increase in the viscosity of experimental adhesives (Single Bond + 0.5% [w/w] chitosan and Single Bond + 1% [w/w] chitosan) ($P < .05$). This explanation is in accordance with Imazato et al.\textsuperscript{4} Furthermore, the fracture mode analysis revealed that the adhesive failures across the bonding interface (type 2) increased as the concentration of chitosan solution increased in the experimental adhesive (see Table 4). This result showed that the adhesion of the experimental adhesive to dentin decreased with increasing concentrations of chitosan incorporation.

From the results of the present study, the decrease in the $\mu$TBS of the experimental adhesives containing more than 0.25% (w/w) chitosan were in accordance with that of degree of conversion and pH values of those adhesives (see Figs 2b and 2c). The activity of the amine accelerator commonly used in the dental polymeric materials was known to be retarded in an acidic condition.\textsuperscript{34} Similar to the connection between the increments in the incremental filling of composite resin,\textsuperscript{35} the copolymerization between the top of the hybrid layer and the adhesive layer or between the adhesive layer and the composite resin will be mediated by the oxygen-inhibited layer of unpolymerized matrix monomers.\textsuperscript{34} The acidity of the adhesive resin may impede the acceleration of the polymerization at the interface between the top of the adhesive layer and the composite resin.\textsuperscript{34} This could explain in part the decrease in $\mu$TBS for the experimental adhesives incorporating 0.5% and 1% (w/w)
chitosan. In addition, from the viewpoint of polymerization kinetics, the increase in the viscosity of adhesive due to higher concentration of chitosan incorporation might cause the decrease of μTBS by lowering the degree of conversion of thick adhesive as the reactivity of major monomer was restricted.

This study has highlighted that chitosan could be considered a significant constituent in the adhesive resin to maintain long-lasting success of the restoration, suggesting a promising usage of chitosan in resisting bacterial growth at the dentin-resin interface. A limitation of this study was that only one type of dental adhesive and one type of bacteria were tested; these findings may not extend to other adhesives due to the variations in the chemistry. The effect of dental adhesives incorporating chitosan on the other common cariogenic bacteria and the influence on the bond strength to dentin and enamel after aging for a long period should be considered in future studies to determine the most efficient concentration of chitosan.

CONCLUSION

Based on the results presented and within the limitations of this study, the following conclusions can be made:

- Experimental adhesive resin containing chitosan exhibited an inhibitory effect on the growth of S. mutans compared with the unmodified adhesive.
- Adhesive resin incorporating 0.12% (w/w) chitosan is a promising antibacterial adhesive that does not adversely affect adhesive properties.

REFERENCES