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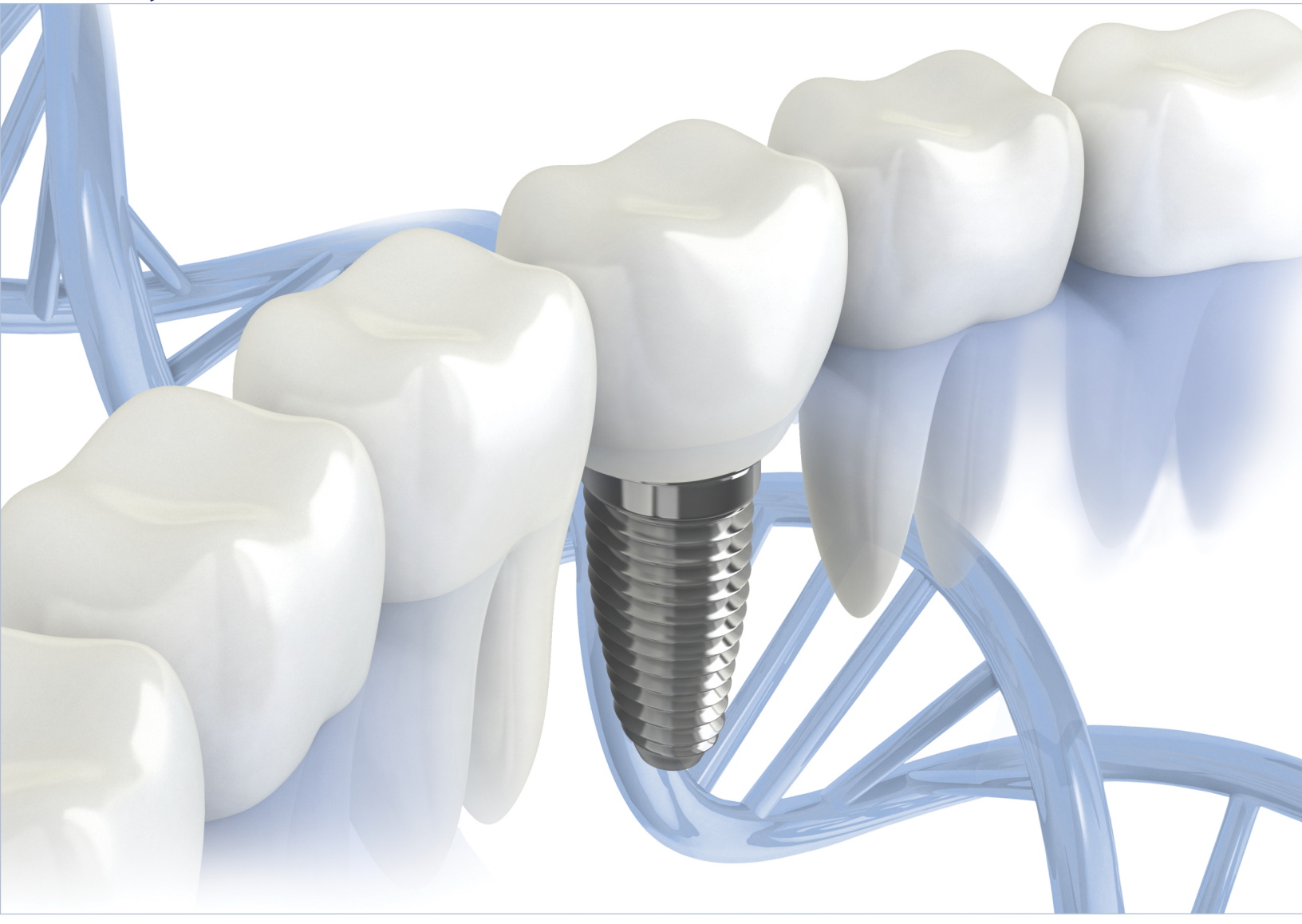

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Original Research

A Comparison of Antibacterial Inhibitory Effect on *Streptococcus mutans* and Tensile Strength between Chitosan-Based Bonding Adhesives and Commercial Products

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Abstract

Background: Adhesive bonding is the material used to attach a bracket to the enamel surface of the tooth. *Streptococcus mutans* contributes to enamel demineralization during orthodontic treatment. **Objectives:** To analyze the antimicrobial inhibitory effect of *Streptococcus mutans* bacteria and tensile strength of chitosan and $CaCO_3$-based adhesive bonding material. **Materials and Methods:** The investigation constituted laboratory experimental research featuring analytical observation and a random sampling method. The antibacterial inhibitory effect of chitosan and $CaCO_3$-based adhesive bonding against *Streptococcus mutans* involved six groups: two control groups using commercial light cure and self-cure adhesive bonding products and four groups using adhesive bonding consisting of 75% $CaCO_3$ + 17.6% Bis-GMA + 22.4% MMA with various percentages of chitosan composition (A1: 25%, A2: 50%, A3: 75%, and A4: 100%) each group consisting of two samples (n = 12). A diametric test was conducted consisting of three samples (n = 15) to measure the tensile strength of each group. Data were analyzed by a combination of one-way analysis of variance and least significant difference tests. **Result:** The antibacterial inhibitory effect showed significant differences between groups (A1: 2.9467 ± 0.4163, A2: 3.6500 ± 0.6245, A3: 5.1267 ± 0.2517, A4: 4.7267 ± 0.9238; P = 0.0000; $P < 0.05$). A diametric tensile strength test confirmed significant differences between groups (A1: 7.2733 ± 5.0046, A2: 6.7667 ± 4.4346, A3: 6.4533 ± 2.9994, A4: 1.0058 ± 1.0058, K1: 15.6167 ± 3.1250; P = 0.009; $P < 0.05$). **Conclusion:** Chitosan-based adhesive bonding with good tensile strength has an antibacterial inhibitory effect against *Streptococcus mutans*.

Keywords: *Adhesive bonding, chitosan, diametric tensile strength, enamel demineralization, orthodontics, Streptococcus mutans*

Introduction

Adhesive bonding has, since its first use in 1980, become an important material for bracket placement on the enamel surfaces of teeth during orthodontic treatment. Several varieties of adhesive bonding such as composite resins, glass ionomer cement, resin-modified composite (compomers), and polyacid modified composites (compomers) have been developed.[1,2]

Three weeks after bracket placement on the enamel surface, cariogenic plaque was visible around the resin and gingival margin. On average, two of every three teeth to which adhesive bonding was applied experienced postorthodontic treatment enamel opacity. During orthodontic treatment, the enamel temporarily demineralized. Previous studies have reported the colonization of a variety of microorganisms and the accumulation of dental plaque on the orthodontic adhesive bonding surface. Orthodontic adhesive bonding material should contain an antibacterial agent to minimalize enamel demineralization during and after orthodontic treatment.[3,4]

Several ingredients are used to inhibit biofilm colonization that contributes to the development of dental caries. One such ingredient is chitosan which possesses antimicrobial properties and constitutes a natural, unbranched homopolymer obtained from chitin, an abundant by-product of seafood processing through a deacetylation reaction with alkali. Chitosan is a biopolymer derived from crustaceans such as shrimp, shellfish, and crabs and represents a biomaterial with the ability to reduce the attachment of dental plaque *in vitro*. It also induces antimicrobial activity against

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certain pathogenic bacteria present in the oral cavity involved in the formation of dental plaque and periodontitis disease such as *Actinobacillus actinomycetemcomitans, Streptococcus mutans*, and *Porphyromonas gingivalis*.[5-7]

The aims of this study were to analyze and compare the antimicrobial inhibitory effect against *Streptococcus mutans* and tensile strength between adhesive chitosan and $CaCO_3$-based material.

Materials and Methods

The study was approved by the Health Research Ethical Clearance Commission, Universitas Airlangga, and represented an experimental laboratory with posttest-only control group design. The research was conducted at the Microbiology Laboratory, Airlangga University, and the Materials and Metallurgy Laboratory, Faculty of Industrial Engineering, Institute of Sepuluh November, Surabaya, East Java, Indonesia. The randomly selected samples, two of which were contained in each group, were adhesive bonding $CaCO_3$, Bis-GMA, and MMA, each containing a different percentage composition of chitosan.

The samples were divided into five groups: control group 1: commercial adhesive bonding material products – light cure (Xeno Ortho: Bis-GMA) and self-cure (Monologue: Bis-GMA resin); treatment group A1: adhesive bonding with 25% chitosan + 75% $CaCO_3$ + 17.6% Bis-GMA + 22.4% MMA; treatment group A2: adhesive bonding with 50% chitosan + 50% $CaCO_3$ + 17.6% Bis-GMA + 22.4% MMA; treatment group A3: adhesive bonding with 75% chitosan + 25% $CaCO_3$ + 17.6% Bis-GMA + 22.4% MMA; and treatment group A4: adhesive bonding with 100% chitosan + 17.6% Bis-GMA + 22.4% MMA.

The brain heart infusion (BHI) broth media used consisted of calf brain solids 12.5 g, beef heart infusion solids 5 g, procteose peptonei 50 g, glucose 2 g, sodium chloride 5 g, and disodium phosphate 2.5 g. A total of 37 g was dissolved in 1 L of distilled water, sterilized in an autoclave at a temperature of 121°C for 15 min and, subsequently, stored at 10–30°C; pH 7.4 ± 0.2.

The tryptone yeast cystine (TYC) media used consisted of tryptone 15 g, yeast extract 5 g, L. sistin 0.2 g, sodium sulfite 0.1 g, sodium chloride 1 g, disodium phosphate anhydroxy No. 2120 0.8 g, sodium bicarbonate 2 g, sucrose 50 g, and sodium acetate anhydroxy 12 g. About 98 g was dissolved in 1 L of aquadest and agitated for 10 min, heated for 15 min at 121°C, and, finally, cooled to 47°C. The medium was then ready for mixing with *Streptococcus mutans*.

Streptococcus mutans (ATCC® 25175) in TYC agar was taken. *Streptococcus mutans* was immersed in BHI broth suspension with a standard 0.5 McFarland and incubated at 37°C for 24 h under anaerobic conditions. About 0.1 mL of the BHI media was transferred to a Petri dish containing TYC media and spread on its surface. The Petri dish was divided into three sections with a well in each section. Each platinum well with 2-mm diameter was inserted into an anaerobic jar and incubated for 24 h at 37°C. Measurement of the inhibition zone of each sample was taken using sliding term 0.05 mm accuracy.

Streptococcus mutans inhibition zone was analyzed after 48 h of incubation on a TYC agar medium with chitosan adhesive bonding materials, $CaCO_3$, Bis-GMA, MMA, and adhesive bonding commercial products light cure (Xeno Ortho: Bis-GMA) and self-cure (Monologue: Bis-GMA resin system with a quartz filler and benzoyl peroxide initiator).

Scanning electron microscopy (SEM) was performed to analyze the morphology of chitosan-based surface adhesive bonding and commercial product adhesive bonding. A spectroscopy type Nicolet iS10 Fourier transform infrared (FTIR) test was performed to analyze the chemical composition of the chitosan-based adhesive bonding material and compare it with adhesive bonding commercial product. Chitosan-based adhesive bonding was divided into six groups. Energy-dispersive X-ray (EDX) detector system Inspect S50 was used to analyze the constituent elements of chitosan-based adhesive bonding. A tensile strength diametral test was performed to determine tensile strength. Each sample was placed in a position perpendicular to the surface of the Shimatzu Autograph AG-10TE. The cross head speed was set on the autograph controller, and load numbers were recorded on this compressive strength test monitor in Newton units.

Results

The antibacterial inhibitory effect against *Streptococcus mutans* showed a difference in each sample (A1, A2, A3, A4) as well as commercial product light cure and self-cure adhesive bonding (Xeno Ortho) [Figure 1].

Group A3 demonstrated the optimum antibacterial inhibitory effect against *Streptococcus mutans*, whereas group A1 possessed the greatest tensile strength [Table 1]. Antibacterial inhibitory effect and diametric tensile strength test results were normally distributed in all groups [Table 2] with homogeneous variants [Table 3]. One-way analysis of variance (ANOVA) test results confirmed significant differences in antibacterial inhibitory effect in each group [Table 4], but no significant difference in diametric tensile strength [Table 5]. A least significant difference test result showed a significant difference in antibacterial inhibitory effect in each group [Table 6].

SEM confirmed group A1 as having a rough honeycombed surface with wide, deep pores that were spherical at several surface points. Group A2 appeared homogeneous with small, fine, spherical sand-like particles. Its many pores have smaller spacing. Group A3 has heterogeneous, spherical, rectangle, trapexohedral trihedrons with larger particle size when compared with A2. Group A4 has a rough surface, a heterogeneous form with an arrangement that resembles a high-level building, spherical trihedrons

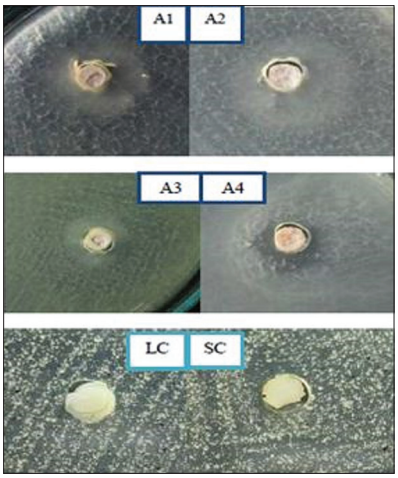

Figure 1: Antibacterial inhibitory zone in each group

Table 1: Mean result of antimicrobial test and diametral tensile strength test

Treatment	Antimicrobial test		Diametral tensile strength	
	Mean	SD	Mean	SD
A1	2.9467	0.04163	7.2733	5.0046
A2	3.6500	0.06245	6.7667	4.4346
A3	5.1267	0.02517	6.4533	2.9994
A4	4.7267	0.09238	1.3533	1.0058
Light cure	-	-	15.6167	3.1250
Self-cure	-	-	-	-

SD: Standard deviation

Table 2: Result of one-sample Kolmogorov–Smirnov test

No.	Sample type	Sample	Sig (2-tailed)
1	Samples of bonding material subjected to an antimicrobial test	12	0.648
2	Samples of bonding material subjected to a diametral tensile strength test	15	0.904

with pores at several points on its surface. The control group has both large and small, uniform, spherical particles similar to those in groups A2 and A3 with large, irregular particles, and rectangle shape [Figure 2].

EDX confirmed that chitosan-based adhesive bonding materials consist of carbon (C), nitrogen (N), oxygen (O), sodium (Na), magnesium (Mg), aurum (Au), and calcium (Ca) [Figure 3] [Table 7]. FTIR test results can be seen in Figure 4.

Discussion

Adhesive bonding material is important in orthodontic treatment whose impressive progress has been indicated by research since 1980. Composite resin was widely used as an adhesive bonding material due to its suitable mechanical properties, adequate bonding strength, and ease of use.[1,8]

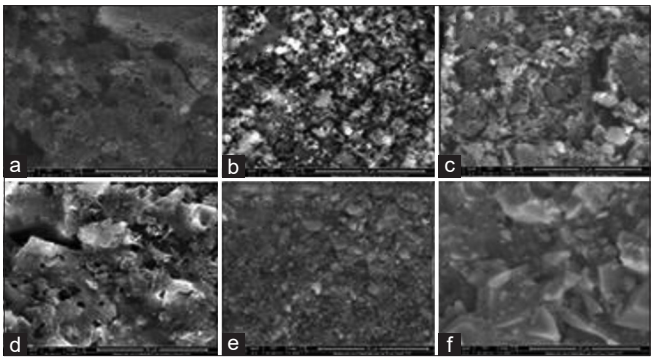

Figure 2: (a) SEM diagonal cut on the surface of bonding material of group A1, (b) group A2, (c) group A3, (d) group A4, (e) control group 1, and (f) control group 2

Table 3: Antimicrobial homogeneity test result of all bonding materials' sample

No.	Sample type	Sample	Levene statistic	Sig.
1	Samples of bonding material subjected to a antimicrobial test	12	3.114	0.088
2	Samples of bonding material subjected to a diametral tensile strength test	15	1.624	0.243

Table 4: One-way ANOVA test results of antimicrobial tests

	Sig.
Between groups	0.0000
Within groups	

ANOVA: Analysis of variance

Chitosan-based adhesive bonding possesses the highest antimicrobial inhibition zone against *Streptococcus mutans* with a significant antibacterial inhibitory effect between groups due to its cationic nature, whereas commercial adhesive bonding product demonstrates no such effect. The electrostatic interaction between positively charged RN (CH3) 3+ sites and negatively charged microbial cell membranes is regarded as responsible for cellular lysis and assumed to be the main antimicrobial mechanism. Charged chitosan can also interact with essential nutrients thereby interfering with microbial growth. Consequently, it is expected that polymers with higher charge densities result in improved antimicrobial activity. The antimicrobial effectiveness of chitosan and its derivative against Gram-positive and Gram-negative bacteria is somewhat controversial. Unmodified chitosan generally acts more effectively against Gram-negative than on Gram-positive strains. Such superior antimicrobial efficiency has been attributed to bacterium wall characteristics, considering that the Gram-negative cell wall is thinner and, consequently, more susceptible than that of the Gram-positive.[5,7]

SEM was used to view the surface of adhesive bonding materials based on chitosan and adhesive bonding

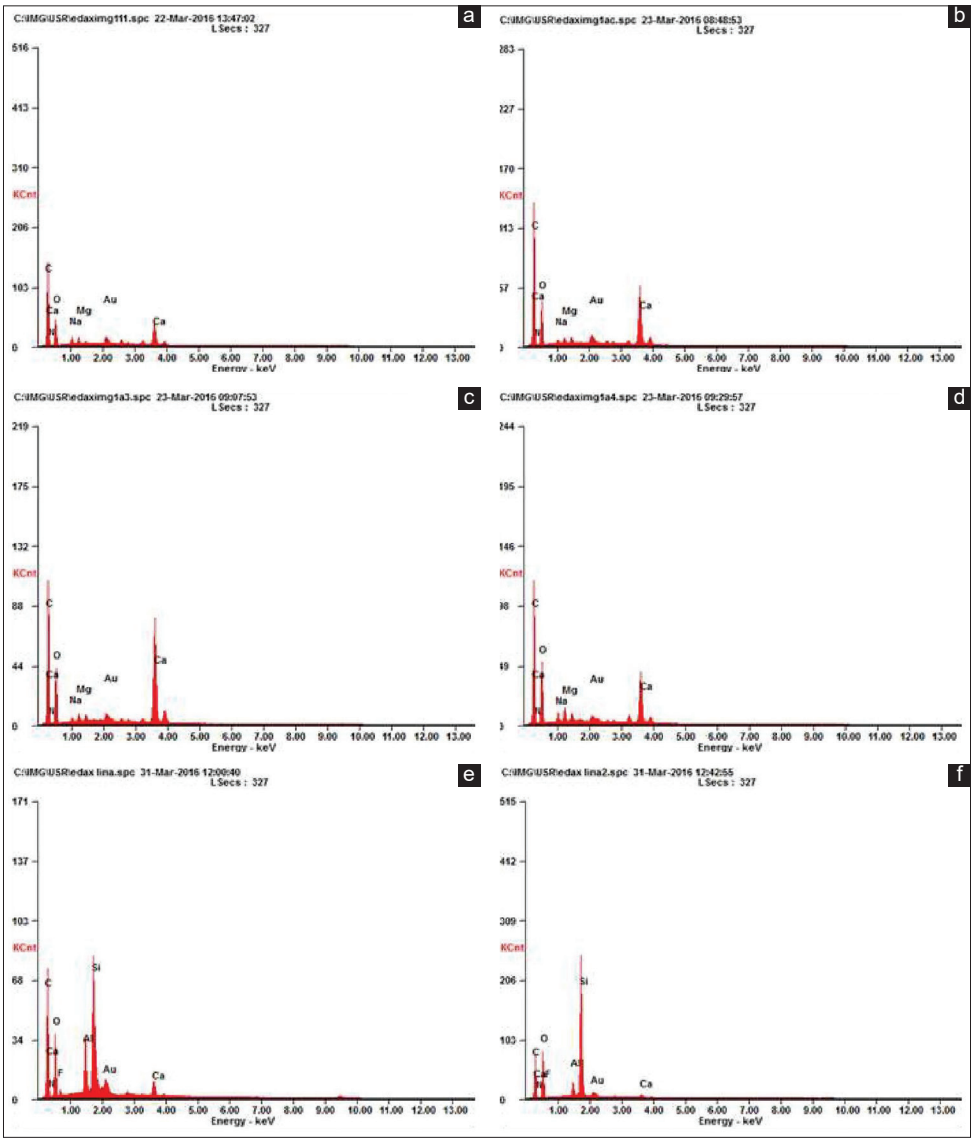

Figure 3: EDX test result on chitosan-based adhesive bonding materials: (a) A1, (b) A2, (c) A3, (d) A4, (e) light cure, (f) self-cure

Table 5: One-way ANOVA test results of diametric tensile strength tests

	Sig.
Between groups	0.009
Within groups	

ANOVA: Analysis of variance

Table 6: Results of least significant difference test

Treatment group	A1	A2	A3	A4
A1	-	0.000	0.000	0.000
A2	-	-	0.000	0.000
A3	-	-	-	0.000
A4	-	-	-	-

commercial products. Chitosan-based adhesive bonding at 100% concentration has the roughest surface. Previous studies showed the attachment area between brackets–adhesive bonding material–enamel junction became an area for biofilm layer formation and bacterial attachment. The rough surface in the attachment area is difficult to clean. The rough area was the most conductive for the formation of biofilm and adhesion layers of bacteria in orthodontic patients.[9-11]

The composition of the constituent elements, observed using an EDX detector system, showed chitosan-based adhesive bonding material to consist of carbon (C), nitrogen (N), oxygen (O), sodium (Na), magnesium (Mg), aurum (Au), and calcium (Ca). Commercial adhesive bonding product consisted of carbon (C), nitrogen (N), oxygen (O), fluorine (F), aluminum (Al), silica (Si), aurum (Au), and calcium (Ca). FTIR analyzes the constituent structure of a chitosan-based adhesive bonding material. FITR results found bond between OH and NH. C6–OH bond derived from chitosan structure, C = O, C = C, CO, CN bond from the monomers used such as *bisphenol-A-glycidyl-dimethacrylate* and *methyl methacrylate*. In addition, CH and COS bond was derived from the *calcium carbonate* structure.[12,13]

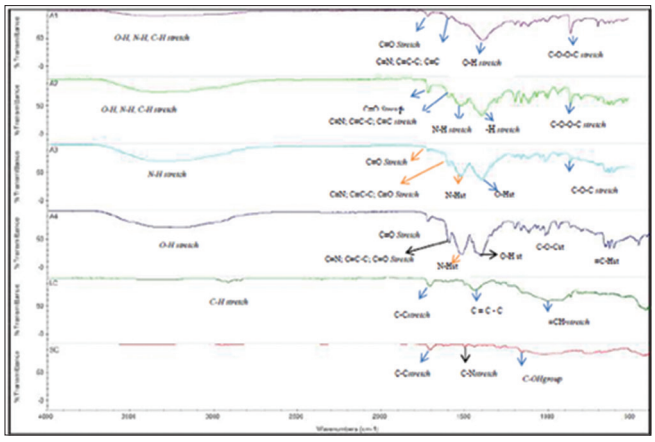

Figure 4: Fourier transform infrared spectroscopy chitosan-based adhesive bonding materials

Table 7: Comparison of the element constituent value of chitosan bonding material with commercial product bonding material

Element constituent (wt%)	Chitosan-based bonding materials				Commercial product bonding materials	
	A1	A2	A3	A4	Light cure	Self-cure
C	44.63	35.52	23.61	42.51	27.86	28.34
N	01.26	01.74	02.15	00.97	01.23	01.76
O	27.34	35.16	39.45	38.19	29.31	34.54
Na	01.76	01.90	02.21	02.44	-	-
Mg	02.15	02.05	02.33	03.26	-	-
F	-	-	-	-	02.44	00.15
Al	-	-	-	-	06.04	02.97

The attachment of brackets to the teeth plays a key role in orthodontic treatment. Formerly, this was achieved by bonding the teeth, but the introduction of acid etching of enamel and direct bonding of brackets have led to changes in orthodontic practice. Chitosan adhesive bonding material possesses good tensile strength. The difference in test results was due to the variety of adhesive bonding materials contained in each product based on the manufacturing process, including the composite matrix resin, the size of the fillers, the bond between the fillers, and the matrix resins.[1,14,15]

Conclusion

Chitosan-based adhesive bonding at 75% concentration has the highest antibacterial inhibitory zone against *Streptococcus mutans*, whereas chitosan-based adhesive bonding with 25% concentration has the highest diametric tensile strength. The composition of chitosan-based adhesive bonding was *natrium* (Na) and *magnesium* (Mg), whereas adhesive bonding commercial product was *fluorine* (F) and *aluminum* (Al).

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Conflicts of interest

There are no conflicts of interest.

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