Original Article

Effect of Incorporating Chitosan to Resin Modified Glass Ionomer Cement on Shear Bond Strength to Dentin (An *In vitro* Comparative Study)

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ABSTRACT

Aim: Resin-modified glass ionomer cement tends to shrink due to polymerization of the resin component. Additionally, they are more prone to syneresis and imbibition during the setting process. This *in vitro* study evaluates the impact of chitosan, a biopolymer that is, both biomaterial and biocompatible, on the strength of dentin bonding and compares it with ACTIVA Bio-ACTIVE Restorative. The present study was aimed to assess the impact of including chitosan into Fuji II on the shear bond strength between. the restoration material and tooth dentin, in contrast to Bioactiva in permanent teeth. **Materials and Methods:** A total of 30 premolar teeth were recently extracted. The study involved three distinct sample groups. Group 1 (10 teeth) is the negative control (Fuji II), Group 2 (10 teeth) is the positive control (ACTIVA Bio-ACTIVE Restorative), and Group 3 (10 teeth) is treated with a mixture of Chitosan and Fuji II (CH-Fuji II). Each tooth's buccal and palatal cusps were eliminated to achieve a horizontal surface. Using a periodontal probe, 1.5 mm from the mesial pit to the mesial marginal ridge were removed. Restoration was implemented in all groups following manufacturer directions. Thermocycling the teeth by immersing them in a water bath with temperatures ranging from 5°C to 55°C (± 1–2°C) for 30 s (500 cycles). Each sample was attached to the universal testing machine's jig at a cross-head speed of 1 mm/min. Shear force was used until breakage, and the bond's adhesive strength was then calculated. Statistical analysis using ANOVA with Dunnett's T3 *post hoc* test. Results were significant at $P < 0.05$. **Results:** Statistically significant difference was present between Chitosan and Fuji II and between Chitosan and Activa by reducing the shear bond strength. **Conclusions:** Addition of chitosan to Fuji II had a negative effect on the shear bond with a significant difference while Activa and Fuji II exhibited favorable shear bond strength.

Keywords: *ACTIVA Bio-ACTIVE Restorative, chitosan, Fuji II LC, RMGIC, SBS*

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Introduction

In order to enhance the physical and mechanical characteristics and address the limitations of traditional GICs, a resin element has been included in the standard cement to create a hybrid material known as RMGICs. Including RMGICs eliminates the need

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for a follow-up appointment as dentists have complete control over the working time. This is because they can finalize and refine the restoration immediately after light-curing. Compared to traditional GICs, RMGICs exhibit superior flexural and tensile strength and reduced moisture sensitivity.[1] The attachment of RMGIC to the tooth structure depends on two linked phenomena: First, micromechanical interlocking retention; second, the self-adhesion process by chemical reaction.[2] However, the RMGIC (Fuji II) still had some drawbacks, such as the presence of resin decreases ionic activity, which hurts the bonding tendency, and as a result of the polymerization process of the resin component, it has a certain tendency to shrink.[3]

ACTIVA-BioACTIVE (Pulpdent Corp., USA) is a modified restorative RMGIC that includes bioactive fillers as well as a shock-absorbing resin component, all of which increase flexural strength, resilience to fractures, and wear resistance.[4] According to the manufacturer, ACTIVA is recommended for all direct anterior and posterior fillings and can be used as a replacement basis.[5]

In nature, chitin molecules that have undergone partial or total deacetylation can be converted into Chitosan (CH). This linear polysaccharide is derived from marine shellfish like crab, lobster, krill, cuttlefish, shrimp, squid pens, insect species, yeast, mushrooms, fungus, and their cell walls. CH is a biopolymer, biomaterial, and biocompatible and has antioxidant, antimicrobial, and tumor-fighting properties used in medical treatments.[6]

No published studies indicate the toxic effects of chitosan-based compositions on humans. Further, it has received safety certifications from the US FDA (Food and Drug Administration) and the EU (European Union) for wound healing and nutritional use. CH has many applications in dentistry, such as oral drug delivery, dentifrice modification, caries prevention, enamel repair, regeneration, adhesion and dentin bonding, hemostasis and pulpotomy, modification of glass ionomer cement, and remineralization. According to Al-Ward and Radhi,[7] a chitosan/NANO-HA compound slurry remineralizes artificial white spot lesions.[7] Guided tissue regeneration: when a bone defect is treated with CH/beta-tricalcium phosphate (TCP), either alone or in combination with a scaffold, the process of bone regeneration is accelerated via increased osteoblast proliferation, decreased osteoclast activity, and the mineralization of the bone matrix.[8] Antibacterial activity: chitosan had highly antibacterial properties when mixed with gutta-percha in an *in vitro* study by AL-Jobory and AL-Hashimi,[9] against Enterococcus faecalis when compared to commercial (control) gutta-percha in the treatment of endodontic conditions.[9]

The RMGIC had polymerization shrinkage because of the presence of resin that decreases ionic activity and may affect bond strength. This study was performed with the addition of chitosan to RMGIC to improve this property.

Materials and Methods

This is an *in vitro* experimental, comparative study done at the University of Technology in Baghdad City, and the College of Dentistry/University of Baghdad began from December 8, 2022 to September 20, 2023 where 30 freshly extracted sound permanent premolar teeth were used as study sample. The size of the samples for testing bond strength was measured by using G power 3.0.10 (Program written by Franz-Faul, Universitatit Kiel, Germany) with a power of study = 80% and alpha error of probability = 0.05, two sided. Teeth were collected from multiple special health centers with patient consent. Teeth were stored in deionized (Dl) water containing 0.2% thymol solution for about 4 months till the sample size was completed to prevent bacterial growth.[10] To minimize deterioration, the storage medium should be replaced at least once every 2 months.[11] Then, nonfluoridated pumice was used to polish the teeth using a traditional low-speed handpiece.[12]

Manufacture of acrylic blocks using a specifically developed cubic silicone mold with interior dimensions of 1.5 × 1.5 × 1.5 cm. The cement–enamel junction was marked, and to represent the plane of seating the teeth within the acrylic, a second marking was created 2 mm apical to the first marking. After pouring a cold-cure acrylic resin into the silicon mold per the manufacturer's directions, the tooth was placed inside until complete polymerization.[13]

Selection criteria

The inclusion criteria were teeth selected in this study were free from caries, restoration, cracks, fractures, or other structural defects extracted for orthodontic purposes with age ranging between 13 and 18 years. Exclusion criteria: Caries, restoration, cracks, fractures of teeth.

Teeth preparation

Samples were poured into an acrylic block to analyze the SBS of the exposed dentin surface. Using a diamond bur (NTI, Germany), the buccal and palatal cusps were cut into sections by utilizing a periodontal probe to measure the distance from the mesial pit

to the mesial marginal ridge which was 1.5 mm. To create a smooth horizontal surface, the saw was used to cut under running water.[13] Each dentin surface was polished with 600-grit silicon carbide paper[14] with running water for 20 s in one direction and then for another 20 s in the other direction to establish a standardized smear layer.[15] Using a 10-cm-long piece of sandpaper (Al-Ugaily Trading Company, China), scratch the dentin surface 4X before washing to get rid of any dirt; you can achieve a flat, polished dentin surface.[16]

Preparation of chitosan solution

In a container with a 100-mL volume containing glacial acetic acid (1.8 mL) and distilled water (100 mL), Chitosan nanoparticles (CDH, India) weighing 20 mg were dissolved in 0.3 N acetic acid and added to a flask with a 100-mL capacity, yielding a 0.2 mg/mL chitosan solution.[17]

Formulation of modified glass ionomer cement liquid with chitosan

To produce a concentration of 10 v/v% of glass ionomer liquid, including chitosan, 0.1 mL of the chitosan solution (0.2 mg/mL) was mixed with 0.9 mL of the glass ionomer liquid.[18] Then 3.2–1.0 g (3.2 Fuji II powder + 0.9 Fuji II liquid + 0.1 chitosan liquid). Afterwards, it was applied to the cavity after mixing for 20 s.[19]

Sample grouping and application of restorative material

A specific Teflon-made device was developed for standardizing the process of putting restorations on the dentin surface. This device comprises a cylinder containing the acrylic block. This translucent Teflon cover can be removed and is secured to the cylinder by four screws and a removable metal bar with a screw in the middle to secure the acrylic block to the Teflon cover. Four screws are positioned opposite each other around the cylinder to secure the acrylic block and guide the dentin surface. Teflon cover for insert restoration with a 4 mm diameter opening.[13]

In Group 1 (Fuji II) shad A2 (GC Corp. Japan) and Group 3 (CH + Fuji II), 10% polyacrylic acid conditioner (GC Corp. Japan), all of the dentin surfaces were treated for 20 s before being rinsed and gently dried without desiccation. The hole was filled with restorative materials.

In Group 2 (Activa) (Pulpdent Corp., USA), 37% phosphoric acid (Any-Etch, Korea) was utilized for etching. Each of the three steps—etching, washing, and drying—took 20 s. As an adhesive system, Single Bond Universal (3M ESPE, Germany) was used according to the instructions provided by the manufacturer. The bioactive restorative material was applied and cured for 20 s.

A plastic tool was used to apply restoration in a single increment through the previously mentioned hole in the cover, and a celluloid strip was then applied to cover it. The celluloid strip was covered with a glass slide containing a 200-g load for 1 min. After removing the load and glass slide, any more restoration was wiped off before light curing; the celluloid strip was near the light-curing apparatus.[13]

SBS measurement

By thermocycling, the specimens are soaked alternately in water bath chambers that range from 5 to 55 ± 1–2°C for 30 s at a time, with 10 s between each bathing cycle. This process is repeated 500X.[20] Then, the samples were cleaned under running water and dried.[19] Each sample was attached to the universal testing machine's jig (Instron machine, WDW-50, LARYEE, China) using an acrylic mold in such a way that the chisel rod. was held vertically to the dentin-restoration contact from the buccal side. At a cross-head speed of 1 mm/min, shear force was used until breakage, and the bond's adhesive strength was then calculated.[13] The SBS was estimated in Newton and converted to MPa (N/mm^2) by dividing it by the dentin-restoration interface's surface area (12.56 mm^2).[21]

$$\text{SBS} = \frac{\text{Force (Newton)}}{\text{Surface area (mm2)}}$$

Surface area. = πr 2, π = 3.14 3.14*2*2 = 12.56 mm^2; r = radius = 2 mm

Statistical analysis

The statistical package for social science (SPSS version 22, Chicago, Illinois, USA) was used for data description, analysis, and presentation. This included the use of the chart bar, mean, and standard deviation (SD), as well as the Shapiro Wilk test for normality, the Levene test for testing homogeneity of variance, one-way ANOVA with Dunnett's T3 posthoc test, and Pearson correlation. At $P < 0.05$, the results appeared to be significant.

Results

A normalcy test was carried out according in Table 1. This table indicated that shear bond strength (SBS) was normally distributed among the groups using the Shapiro–Wilk test at $P > 0.05$ ranged (0.055–0.126).

The results are shown in Table 2. The decrease in the SBS of F2LC after the addition of CH resulted in the mean SBS of CH-Fuji II (Group 3), Fuji II (Group 1), and ACTIVA (Group2) being 3.1051 (1.33754), 11.4251 (6.58165), and 7.6035 (4.01810) Mpa, respectively, with significant differences between the 3 groups ($P = 0.001$).

Table 1: Findings below show that SBS is normally distributed among groups using the Shapiro Wilk test at $P > 0.05$ range (0.055–0.126)

Tests of Normality			
Groups	Shapiro–Wilk		
	Statistic	df	*P* value
Chitosan + GIC Fuji II	0.848	10	0.055
GIC Fuji II	0.858	10	0.072
Activa	0.879	10	0.126

Table 2: Descriptive and statistical test of SBS among groups using one-way ANOVA

Groups	Mean	± SD	Df	F	*P* value
Chitosan + GIC Fuji II	3.1051	1.33754	2	8.495	0.001 Sig.
GIC Fuji II	11.4251	6.58165			
Activa	7.6035	4.01810			

Levene *P* value = 0.026 significant

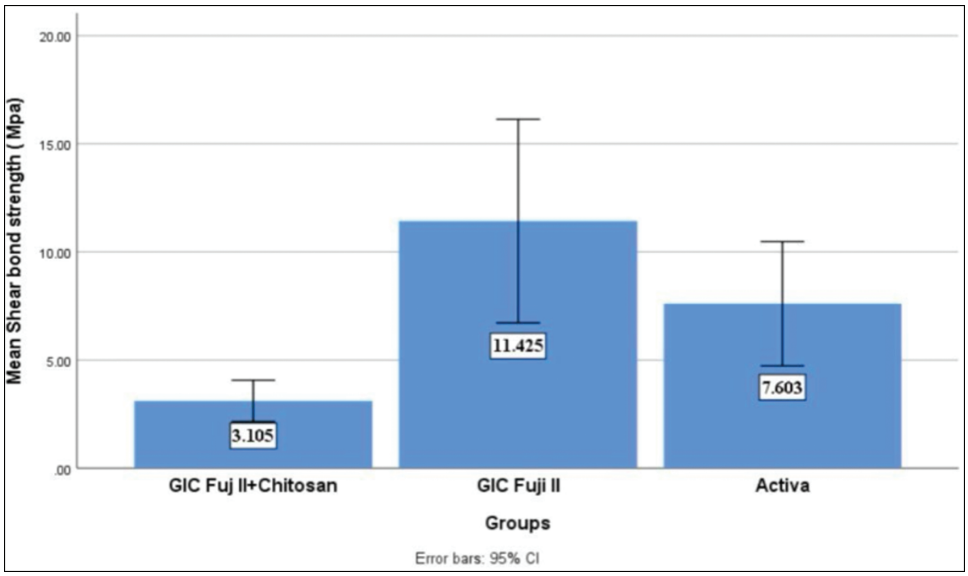

Figure 1: Bar chart showing the comparison between groups (Chitosan II, Activa) of adhesive strength

As shown in Figure 1, Fuji II had the highest SBS, followed by ACTIVA, whereas CH-Fuji II had the lowest SBS.

Following multiple pairwise comparisons using one-way ANOVA with Dunnett's T3 posthoc test, results showed that Group 3 had the lowest SBS with a statistically significant difference when comparing each group with the others, as shown in Table 3.

Mode of failure

The results of the failure modes of CH-Fuji II (Group 3) showed that 50% (5 specimens) had an adhesive failure and 50% (5 specimens) had a mixed (adhesive–cohesive) failure. In contrast, Fuji II (Group 1) showed that 70% (7 specimens) had adhesive failure and 30% (3 specimens) had mixed (adhesive-cohesive) failure; the mode of failure of ACTIVA (Group 2) was only adhesive failure 100% (10 specimens), as shown in Table 4, Figure 2.

Scanning electron microscopy (SEM) evaluation

More dentinal tubules were closed in the Fuji II and Activa groups, and many resinous tags were shown, which explained the higher bond strength. In the CH-Fuji II group, many dentinal tubules remained open, which may be due to CH altering the original properties of Fuji II and affecting bond strength, as shown in Figure 3.

Discussion

The simplest method for evaluating the effectiveness of adhesive systems is bond strength testing, since it is quick, simple, and accurate.[22] The present study showed that the

Table 3: Multiple pairwise comparisons of SBS among groups using Dunnett's T3

Groups		Mean Difference	*P* value	Lower 95% CI	Upper 95% CI
Chitosan+GIC FUJI II	GIC Fuji II	−8.32005	0.009	−14.356	−2.284
	Activa	−4.49841	0.018	−8.228	−0.769
GIC Fuji II	Activa	3.82164	0.346	−2.692	10.336

Table 4: Distribution of failure mode among groups

Groups	Adhesive		Mixed	
	N	%	N	%
GICFuji II + Chitosan	5	50.00	5	50.00
GIC Fuji II	7	70.00	3	30.00
Activa	10	100.00	0	.00
Total	28	70.00	12	30.00

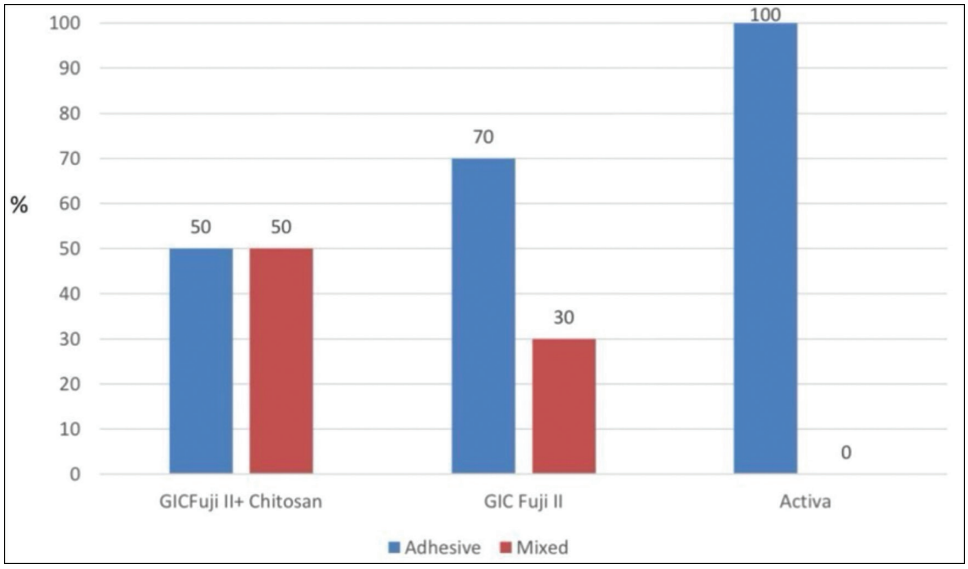

Figure 2: Bar chart showing the failure mode among groups

highest mean value of SBS was noted in the Fuji II control group and the Activa group. The lowest SBS was noted in the CH-Fuji II group, which may be due to chitosan interacting with the polyacrylic acid in Fuji II and forming a polymer complex that may affect the chemical properties and setting reaction of Fuji II. The difference between CH and Fuji II was statistically substantial and was lower than the Activa group. The difference between chitosan and Activa was statistically significant ($P = 0.035$).

All the samples were subjected to thermocycling, an *in vitro* method in which the restoration and the teeth were exposed to temperatures and conditions comparable to those in the oral cavity. Thermocycling between 5°C and 55°C. with a dwell time of 30 for 500 cycles as a method of universal standardization.[11,20] This approach was chosen for this investigation because it was consistent with previous research on various restorations. Thermocyling stresses the bonding between the resin and the teeth, as well as depending on the adhesive technique used, the bond strength might be impaired.[23] May be this was the reason CH added to Fuji II decreased the SBS.

One sample was randomly selected from each group. The failure surfaces were sputter-coated with gold using Sputter Coater, and specimens were analyzed with SEM. SEM results agreed with each group's SBS findings; more resin tags, resin impregnations beyond the hybrid layer, and more micromechanical interlocking were present in the Fuji II group and Activa group, influencing and increasing the bond strength. In the CH-Fuji II group, observe the tubules' partial exposure and mineral clearance. Consequently, we noted a decrease in resin impregnations, and these results verified SBS data.

This result disagreed with Perchyonok[24] who showed that the use of a small amount of CH might significantly

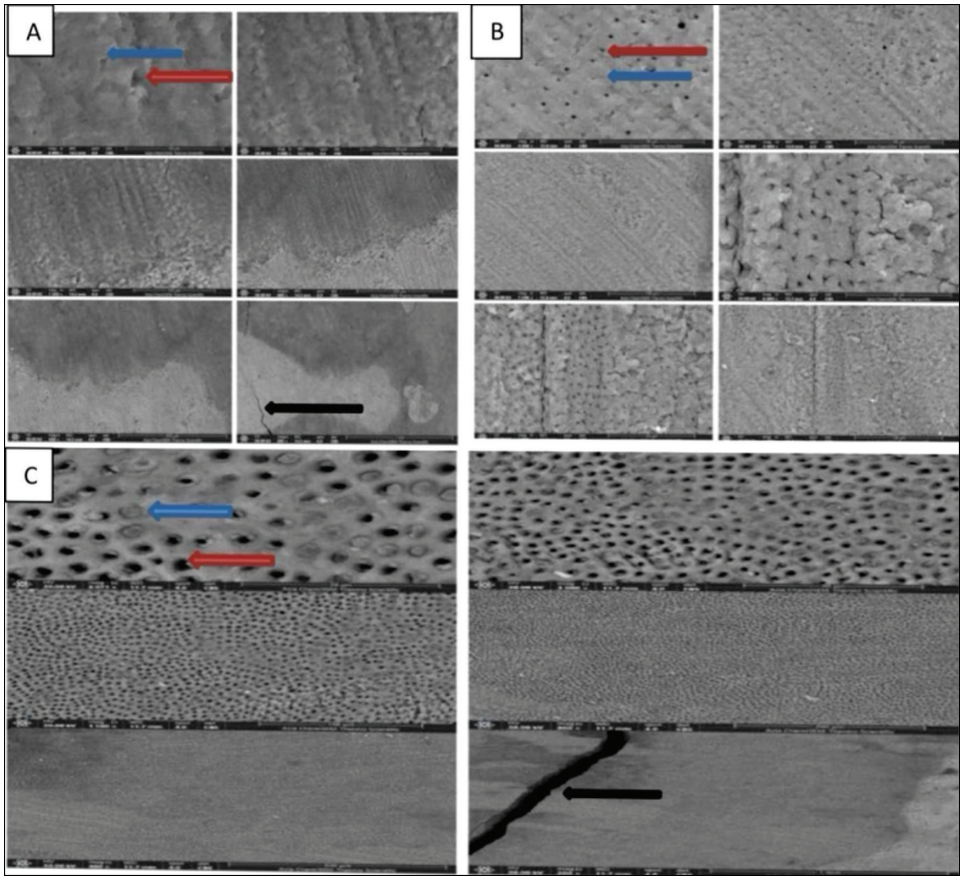

Figure 3: Scanning electron microscopy photographs of dentin (A) Fuji II failures are more adhesive and slightly mixed, showed more closed dentinal tubules (blue arrow) and slight cracks in dentin surface (black arrow); (B) Activa failures are adhesive and only showed some of the dentinal tubules closed (blue arrow) and some opened (red arrow); (C) CH. Failures are adhesive and mixed (adhesive-cohesive), showing some obliterated dentinal tubules by resinous tags (blue arrows), while numerous tubules are still opened (red arrows) and there are cracks in dentin surface (black arrow)

improve the strength of a commercial GIC. The difference in results may be due to thermocycling, the difference in study design, the amount of chitosan added, and the type of GIC in the present study. Fuji II (RMGIC) in the powder–liquid ratio was used instead of conventional GIC, and the presence of resin in Fuji II may have led to an increase in the shrinkage and a decrease in the bond strength.

In disagreement with Mohamad *et al.,*[25] who stated CH had increased SBS than Fuji II since they used CH as pretreatment and Fuji II in capsule form and mixed in an amalgamator, not manually. Al-Taee *et al.*[26] concluded that the RMGIC's physical characteristics were statistically significantly influenced by the mixing method and the addition of reactive glass additives to cement. But in this current study, CH was mixed with the liquid part of Fuji II (in powder–liquid form) in a manual way that may lead to the incorporation of air during the mixture that may affect the results.

Sodagar *et al.* [27] showed that blending CH with composites did not affect bond strength. Ibrahim *et al.*[28] and Hodhod *et al.*[29] state that adding CH did not affect the clinical performance of GIC cement, but the conditions of those studies differ from the present study.

Regarding the failure mode, CH-Fuji II showed a mixture of adhesive and mixed (adhesive-cohesive) failure modes. This result may be due to CH and manual mixing that may lead to air incorporation. Fuji II showed a mixture of adhesive and mixed (adhesive-cohesive) modes of failure, in agreement with Al-Hasan and Al-Taee.[30] Although Fuji II was capsulated (no manual mixing), the cause may be due to the bonding properties of the material. An increase in the proportion of mixed failures denotes strengthening the bond due to improved adhesive contact.[31] The percentage of mixed materials in this study was low, denoting a weaker bond strength. Whereas ACTIVA showed that only an adhesive mode of failure might be because no mixing was required (mixing by a particular gun), which resulted in an agreement with Al-Hasan and Al-Taee, 2022.[30]

Since, in the present study, the addition of chitosan does not improve Fuji II properties (CH- Fuji II had a negative effect), other studies need to be performed with different proportions of chitosan added to improve the restoration of Fuji II. Limitations of this study were the difficulty in sample collection criteria (sound premolar teeth without caries, restoration, cracks, fractures). Fuji II is not readily available as a powder or liquid but is more available as capsule.

Conclusion

The addition of chitosan to Fuji II had a negative effect on the shear bond, with a significant difference between chitosan, Fuji II, and ACTIVA. However, Activa and Fuji II Light Cure exhibited satisfactory SBS. Additional study investigations utilizing varying ratios of chitosan are necessary to enhance the regenerative properties of the material.

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Conflicts of interest

There are no conflicts of interest to disclose.

Author contributions

ATK and MSK jointly contributed for completing this study.

Ethical policy and institutional review board statement

This research was an *in vitro* comparative study and had been accomplished at the University of Technology in Baghdad and the College of Dentistry/University of Baghdad after obtaining approval from the Scientific Committee of the Department of Pedodontics and Preventive Department and the Ethics Committee of the College of Dentistry/University of Baghdad on November 10, 2022 (Project no. 683322).

Patient declaration of consent

Not applicable.

Data availability statement

Information related to the paper is available.

List of Abbreviations

F2LC Fuji II Light Cure
CH Chitosan
SD Standard Deviation
SBS Shear bond strength
RMGIC resin-modified glass ionomer cement
°C degrees Celsius

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