



The Effect of Chitosan and Fuji II LC Compared to Activa on Microleakage Characteristics of Dentin

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Article Type	ABSTRACT
Research Paper	Background and Objective: The polymerization of resin component and ionic activity of resin modified glass ionomer cement (Fuji II LC) is reduced due to present of resin that cause shrinkage and microleakage. This study aims to evaluate the effect of chitosan and Fuji II LC compared to Activa on microleakage characteristics of dentin. Methods: In this experimental study, 30 premolars, free of decay, restoration, cracks, and fractures (as assessed by light cure), extracted for orthodontic purposes, were used. The study consists of three separate groups of samples. Group 1: 10 teeth treated with Fuji II LC (negative control), Group 2: 10 teeth treated with Activa (positive control), and Group 3: 10 teeth treated with chitosan added to Fuji II LC. Class V cavities were made on the buccal surfaces and restorations were performed in all groups according to the manufacturer's instructions. Two coats of nail polish were applied to the restoration in a 1 mm area near its margin. The teeth were thermocycled for 30 seconds in a water bath at $5-55 \pm 1-2^\circ\text{C}$ (500 cycles). The samples were then incubated in 2% methylene blue for 24 hours. The teeth were bisected using the buccolingual method and then examined. Findings: There was a significant microleakage in Fuji II LC (0.828) and Chitosan-Fuji II LC (0.259) and between Fuji II LC and Activa (0.207). The highest amount of microleakage was found at the gingival margin. Conclusion: The results of the study showed that Activa and the addition of chitosan to Fuji II LC had a positive effect on microleakage, while Fuji II LC showed the highest microleakage with a significant negative difference. Keywords: Activa, Chitosan, Fuji II LC, Microleakage.

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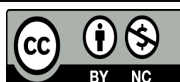
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Introduction

Resin component is added to the original cement to create a hybrid substance known as a resin-modified glass ionomer cement restoration (RMGIC or Fuji II LC) to enhance the physical and mechanical qualities (1). The attachment of Fuji II LC to the tooth structure depends on two linked phenomena: micromechanical interlocking retention and chemical reaction (2). As a result of the polymerization process of the resin component, the Fuji II LC retains some of the drawbacks, such as a tendency to shrink, which may cause microleakage (3). They are usually more prone to syneresis and imbibition due to the setting reaction (4). Activa-Bio-ACTIVE (Pulpdent, Watertown, MA, USA) is a modified restorative RMGIC composed of reactive glass particles and polyacids that undergo an acid-based reaction and light polymerization or chemical curing (5, 6). Additionally, Activa includes bioactive fillers and a shock-absorbing resin component.

Chitosan (CH) is a naturally occurring linear polysaccharide made from chitin molecules. Chitin is typically transformed into its deacetylated derivative. Chitin is a nitrogenous polysaccharide that is white, firm, and rigid and is present in invertebrates and is the main component of the exoskeletons of crustaceans, such as shrimp and crabs, and the cell walls of fungi (7). Chitosan has antioxidant, antimicrobial, and tumor-fighting properties (8, 9). CH has many applications in dentistry, such as the delivery of oral drugs, modification of dentifrices, prevention of caries, repair of enamel, regeneration, remineralization, hemostasis and pulpotomy, modification of glass ionomer cement, 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 (10)), and antibacterial activity. Chitosan had highly antibacterial properties against *Enterococcus faecalis* when mixed with gutta-percha in an in vitro study by AL-Jobory et al (11) when compared to commercial gutta-percha (control) in the treatment of endodontic conditions.

Methods

This is an in vitro experimental, comparative study conducted in the University of Technology in Baghdad city, and College of Dentistry, University of Baghdad from December 20, 2022 until September 8, 2023. The study was performed 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 (project no.683322) with ethics code 683 on November 10, 2022.

The teeth selected in this study included 30 permanent premolars free from caries, restorations, cracks, fractures, or other structural defects (checked by light cure and naked eye), extracted for orthodontic purposes from patients aged between 13 and 18 years. The teeth were obtained from several special clinics and stored in deionized (DI) water containing 0.2% thymol solution to prevent bacterial growth for about four months until the necessary number of samples was obtained (12). To prevent deterioration, the media used for storage were replaced at least every two months (13). Then, the teeth were polished using a traditional, low-speed handpiece and non-fluoridated pumice (14).

The roots of teeth were covered with a layer of wax placed below the CEJ to act as the soft tissue and as a separating medium between the root and the silicon. A custom mold of plastic was utilized, and different colors of nail polish were applied as codes to distinguish each group. The plastic mold was filled with an elastomeric silicon impression substance, and each tooth was placed individually in the silicon to a level lower than the CEJ (15).

Cavity preparation: Under air-water cooling, a class V cavity with the following dimensions was done on each tooth buccal surface with: 4 mm mesiodistally, 2 mm occluso-lingivally, and 2 mm in depth. A modified dental surveyor was utilized to prepare the cavities in all samples uniformly (16). During the preparation, in order to maintain the long axis of the burr perpendicular to that of tooth, a high-speed headpiece was placed on the surveyor's moving arm (17). A disposable band and retainer were used to standardize the cavity width and length; an opening (4×2 mm) was made in the band, and it was placed over the buccal surface of tooth. By using a diamond depth-oriented burr (2 mm), the depth was standardized. After preparing five teeth, the bur was changed. The depth and width of the cavity were measured using a digital caliper and a periodontal probe (16).

Chitosan solution preparation: Glacial acetic acid (1.8 mL) and distilled water (100 mL) were mixed in a 100-mL container. Chitosan nanoparticles weighing 20 mg were dissolved in 0.3 N acetic acid and added to a 100-mL flask, yielding a 0.2 mg/mL chitosan solution (18).

Formulation of modified Fuji II LC liquid with chitosan: To produce a concentration of 10% (v/v) chitosan with glass ionomer liquid, 0.1 mL of the chitosan solution (0.2 mg/mL) was mixed with 0.9 mL of the Fuji II liquid (19). Then 3.2-1.0 g of Fuji II LC with chitosan (Fuji II LC-CH, 3.2:0.9:0.1 Fuji II powder: Fuji II liquid: chitosan liquid) was prepared and applied to the cavity after mixing for 20 seconds (20).

Sample grouping and application of restorative material: In Group 1 (Fuji II LC, 10 teeth), Shad A2 (GC Corp. Japan), all cavity areas were cleaned and gently dried without dehydration for 20 seconds, using 10% polyacrylic acid conditioner (GC Corp. Japan) before being filled with Fuji II LC.

In Group 2 (Activa, 10 teeth), (Pulpdent Corp. USA), 37% phosphoric acid (Any-Etch, Korea) was used for cavity etching. Each of the three steps of etching, washing, and drying, took 20 seconds. The single bond (3M ESPE Germany) adhesive system was applied based on the guidelines provided by the manufacturer. The bio-Activa restorative material was applied and cured for 20 seconds.

In Group 3 (Fuji II LC with Chitosan, 10 teeth), all cavity areas were cleaned and gently dried without dehydration for 20 seconds using 10% polyacrylic acid conditioner and restored with Fuji II LC-Chitosan.

Microleakage measurement: After removing the silicon blocks, each sample was cleaned and polished using a non-fluoridated paste. The samples were prepared by drying the teeth and covering the entire tooth surface with two coatings of nail polish, except for a 1-mm border around the filling margins. The root apices of the teeth were sealed with a sticky wax material before being submerged for 24 hours in 2% methylene blue dye. Next, the specimens were thermocycled by soaking the teeth alternatively into 5-55±1-2°C water bath chambers with 30 seconds immersion in each bath with 10 seconds of transition time for 500 cycles. (21) Then, the samples were cleaned under running water and dried (16). The surface dye and nail polish were removed with a blade and a disk, respectively (16). Blocks were made from the samples by casting them in transparent epoxy resin, utilizing a custom plastic mold that was 3×2×1 cm in size. The teeth were subsequently divided into sections using a SAW process for cutting sections and 0.01 grit disks with water cooling. The tooth was divided into two parts along its long axis. The center of the filling included bucco-lingual sectioning of the teeth (16). Microleakage was calculated according to the formula of Bertrand et al. (22):

It/Lt= The total infiltration ratio in mm.in which:

It= Io+Ic

Io: The.length of dye penetration along the,occlusal margin in mm.

Ic: The length of dye penetration along the,cervical margin in mm.

Lt: The total,length of the interface between the tooth surface and restoration was recorded in millimeters.

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, Levene test for testing homogeneity of variance, One way ANOVA with Dunnett's T3 Post hoc test. At $p < 0.05$, the results appeared to be significant.

Results

The lowest microleakage was observed in the Activa group, followed by the Fuji II LC-Chitosan group. The Activa group had lower microleakage than the Fuji II LC-Chitosan group by an average of 0.05220. The difference between Activa and Fuji.II LC-Chitosan was not statistically significant ($p=0.838$). The highest microleakage was seen in the Fuji.II LC group, which had higher microleakage than the Fuji.II LC-Chitosan group by an average of 0.56909. The difference between the Fuji II,LC-Chitosan and Fuji II.LC groups was statistically significant ($p=0.000$). The Activa group had lower microleakage than the Fuji II LC group by an average of 0.62129; the difference between Activa and Fuji.II,LC was also statistically significant ($p=0.000$). Fuji II.LC was statistically significant when each group was compared with the others, as shown in Figure 1. Table 1 provides the statistical analysis of microleakage of groups and regions. Among the various types of restoration, the gingival margin showed more leakage than the occlusal margin.

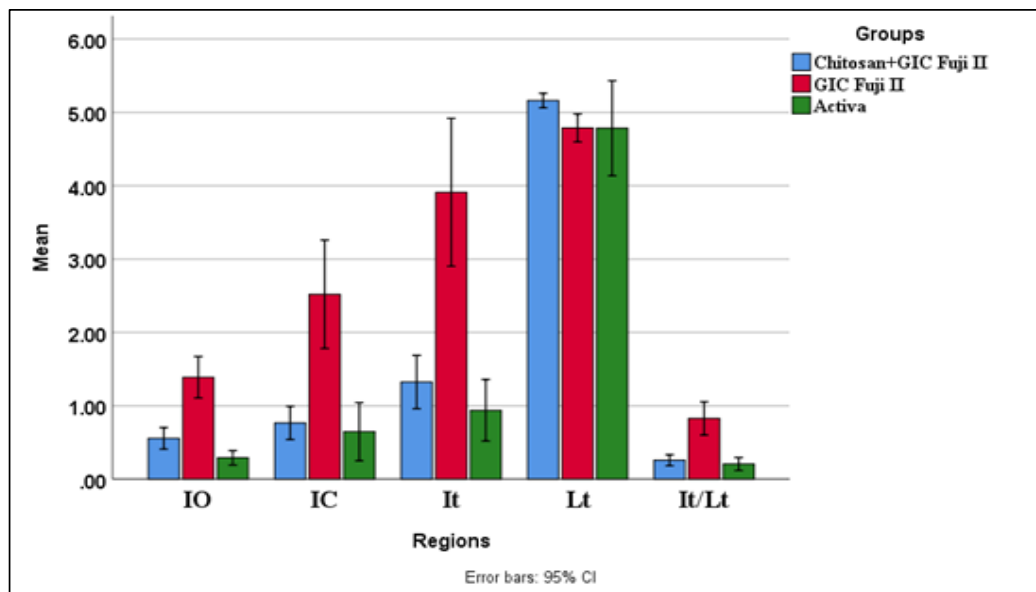


Figure 1. Microleakage in regions of filling materials

Table 1. Descriptive and statistical tests of microleakage in groups and regions

Regions	Chitosan+Fuji II LC	Fuji II LC	Activa	F	p-value
	Mean±SD	Mean±SD	Mean±SD		
Io	0.557±0.205	1.389±0.394	0.291±0.138	45.510	0.000
Ic	0.767±0.315	2.521±1.033	0.648±0.554	22.390	0.000
It	1.324±0.508	3.910±1.409	0.939±0.587	30.258	0.000
Lt	5.162±0.140	4.788±0.266	4.785±0.905	1.549	0.005
It/Lt	0.259±0.104	0.828±0.317	0.207±0.124	28.151	0.000

Discussion

The present study showed that the lowest microleakage occurred in the Activa group, followed by the Fuji II LC-Chitosan group, and the highest microleakage was seen in the Fuji II LC group. The cervical margin demonstrated more leakage than the occlusal margin among all types of restoration. This finding is in agreement with the results reported by previous studies (23-25). These studies demonstrated that regardless of the restorative material, greater microleakage occurred at the gingival borders than at the occlusal or axial walls. In contrast, the work of Alkhudhairy et al. showed a moderate degree of microleakage in Activa class II (box alone) cavities of maxillary premolars. The difference between these findings may be due to variations in the design of the cavities in the teeth (26).

The lowest microleakage in the present study was in Group 2 (Activa), and the highest microleakage was in Group 1 (Fuji II LC), which is in line with the results of Omidi et al. (27). It was determined through comparison that the method by which the material attaches to the tooth structure causes the microleakage of Activa to be less than that of Fuji II LC. An acid-base reaction and polymerization reaction between the 2-hydroxyethyl methacrylate (HEMA) and urethane dimethacrylate (UDMA) monomers of the resin matrix produces the setting of the Fuji II LC. Additionally, the reduced filler in Fuji II LC indicates a higher resin content, increasing microleakage and shrinkage during polymerization (23, 24).

The highest microleakage is seen in the Fuji II LC group, maybe due to the mixing process during preparation, which may have caused bubbles to develop, causing leakage (27). The enhanced leakage may also have been due to the thermocycling process used; this is considered the primary factor in increased microleakage due to the large number of hot and cold water cycles used. This work agrees with the study by Gerdolle et al., who found that the Fuji II LC restorative experienced the most shrinkage compared to the composite and compomer. They concluded that the higher degree of microleakage in Fuji II LC may be caused by multiple factors, including higher polymerization shrinkage (23).

Less microleakage was observed in this study than in other studies of Fuji II LC. In these investigations, Fuji II LC was compared to various GICs and composites, although their sample storage conditions and storage times differed from the current research. It has been claimed that water sorption, which causes the material to hygroscopically expand, gives light cured resin reinforced restorative cements and good sealing capabilities by reducing the gap between the restoration and the tooth (28, 29). In our investigation, varying storage conditions could result in various degrees of water absorption and, as a result, various degrees of microleakage.

Additionally, the microleakage decreased when chitosan was added to Fuji II LC in Group 2. This result agreed with Perchyonok, who stated that microleakage was not significantly increased by adding 10% (v/v) of chitosan nano diamond powder, or a combination of 10% (v/v) to glass ionomer cement (30). It disagreed with Abraham et al., who stated that the microleakage of GIC was not significantly affected by the addition of 10% (v/v) chitosan (31). The contradictions in the results could be related to variations in the type of GIC and tooth.

In the present study, the addition of chitosan improved Fuji II LC properties (Chitosan-Fuji II LC had positive effect on microleakage). Greater microleakage occurred at the gingival margin than at the occlusal or axial walls. Activa had the lowest microleakage, and Fuji II LC had the highest. The chitosan added to Fuji II LC decreased the microleakage compared with pure Fuji II LC. Thus, chitosan had positive effects on microleakage property.

Conflict of interest: The authors have no conflicts of interest to declare.

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