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Analysis of the Microleakage of Fluoride-Loaded Chitosan Nanoparticle-Modified Glass-Ionomer Cement

Florür Yüklü Kitosan Nanopartikülleri ile Modifiye Cam İyonomer Simanın Mikrosızıntı Analizi

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ABSTRACT

Objective: The aim of this study was to evaluate the microleakage of glass ionomer cement modified with fluoride-loaded chitosan nanoparticles.
Methods: 36 human premolars, with Class V cavities prepared on the buccal surface, were randomly divided into 3 groups: Glass ionomer cement (GIC), chitosan-modified glass ionomer cement (CH-GIC), and fluoride-loaded chitosan nanoparticle-modified glass ionomer cement (NCH-GIC). Restorations were polished with aluminum oxide abrasive discs. Then a range of 5 - 55C thermocycling was performed 1500 times. The specimens were immersed in 1% methylene blue dye solution for 24 hours. After the teeth were divided buccolingually into two halves in an occlusoapical direction, the sections were viewed under a light microscope (40X) and the degree of microleakage was evaluated using specific scoring criteria. The Kruskal–Wallis and Wilcoxon tests were used for statistical analyses (α=.05).
Results: There were no significant differences between the groups at the enamel and dentin margins (*P*>.05).
Conclusions: The modification of glass ionomer cement with chitosan or fluoride-loaded chitosan nanoparticles did not affect microleakage.
Keywords: Chitosan, Glass Ionomer Cement, Microleakage, Thermocycling.

ÖZ

Amaç: Bu çalışmanın amacı, florür yüklü kitosan nanopartikülleri ile modifiye cam iyonomer simanın mikrosızıntısının değerlendirilmesidir.
Yöntem: Bukkal yüzeylerine Sınıf V kavite açılan 36 insan küçük azı dişi rastgele 3 gruba ayrıldı.: Cam iyonomer siman (GIC), kitosanla modifiye edilmiş cam iyonomer siman (CH-GIC), ve florür yüklü kitosan nanopartikülle modifiye edilmiş cam iyonomer siman (NCH-GIC). Restorasyonlar alüminyum oksit aşındırıcı disklerle cilalandı. Daha sonra 5 - 55C aralığında 1500 kez termosiklus gerçekleştirildi. Örnekler 24 saat boyunca %1 metilen mavisi boya solüsyonuna daldırıldı. Dişler oklüzoapikal yönde bukkolingual olarak iki yarıya ayrıldıktan sonra, kesitler ışık mikroskobu altında (40X) görüntülendi ve mikrosızıntı skorları spesifik puanlama kriterleri kullanılarak değerlendirildi. İstatistiksel analizler için Kruskal-Wallis ve Wilcoxon testleri kullanıldı (α=,05).
Bulgular: Mine ve dentin kenarlarında gruplar arasında anlamlı bir farklılık bulunmadı (*P*>,05).
Sonuç: Cam iyonomer simanın kitosan veya florür yüklü kitosan nanopartikülleri ile modifikasyonu mikrosızıntıyı etkilememiştir.
Anahtar Kelimeler: Kitosan, Cam Iyonomer Siman, Mikrosızıntı, Termal Siklus

INTRODUCTION

Glass ionomer cement (GIC) is a highly popular material in restorative dentistry for its many advantages, including biocompatibility, fluoride release/uptake, dentin-like elasticity, and adhesion to dental structures.[1-3] However, failures have been reported after treatment due to deficiencies in both mechanical function and bacteriostatic properties.[4] Additionally, due to its relatively low fracture resistance compared to other restorative materials, it can lead to fractures, failure of the bonding, and microleakage between the tooth and GIC.[3,5] To overcome these drawbacks, recent research has investigated biomaterial modifications like chitosan to improve the physical properties of GIC while maintaining its clinical advantages.[6-8]

Chitosan is a natural biopolymer obtained by alkaline N-deacetylation of chitin, which is the main component of the shells of crustaceans such as crabs, shrimp, lobster, and the cell walls of some fungi.[7,8] It is a potential option for GIC modifications with superior properties such as antifungal, antibacterial, anti-plaque, anti-tartar, and hemostatic effects.[6,9] The addition of chitosan to GIC positively affects both

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microhardness and surface roughness results.[10] Modifying the liquid phase of a conventional GIC with 10% v/v chitosan resulted in a notable enhancement the antibacterial property of GIC against S. mutans, while also improving its adhesion to enamel and dentin surfaces.[6,8] Petri et al.[11] reported that the flexural strength of GIC could be significantly enhanced by the addition of chitosan, and the release of fluoride ions from the GIC in the presence of chitosan was catalyzed. Chitosan particles form a better interaction among the different components in the GIC as they reduce the interfacial surface tension.[11] Therefore, it is possible that nano-sized chitosan particles may have an additional benefit, such as greater surface area and charge density, which may allow for better interaction with the surrounding environment.[9] Chitosan nanoparticle formulations have been widely used as drug delivery agents in the field of pharmacology.[12] Nguyen et al.[13] reported that chitosan nanoparticles can also be used in dentistry for the controlled release of fluoride. Furthermore, they showed that fluoride release from fluoride-loaded chitosan nanoparticles is low concentration but continuous. It has been reported that GIC restorations modified with these nanoparticles are more resistant to cariogenic biofilm challenge compared to conventional GICs, thereby preventing secondary caries formation.[14]

However, adding any substance to enhance the properties of the material should not compromise any other properties of the original material. An important determinant in the clinical success of restorative materials in the oral cavity is a good adaptation to the cavity walls, in other words, minimal microleakage.[15] Microleakage is the passage of bacteria, fluid, molecules, or ions between the cavity wall and the restoration. Postoperative pain, marginal discoloration, and secondary caries may occur as a result of microleakage.[16] For these reasons, polymeric biomaterials added to the GIC to enhance physical properties should not affect the adhesive properties and increase the risk of microleakage at the margins of the restoration. It has been reported that the microleakage results of chitosan modified GIC are similar to or better than those of GIC.[15] However, there is a lack of research in the field of dentistry regarding the investigation of microleakage in GIC that has been enhanced with fluoride-containing chitosan nanoparticles. Therefore, the aim of this study was to evaluate the effect of adding chitosan and fluoride-loaded chitosan nanoparticles to GIC on the microleakage of restoration. The null hypotheses tested were: (1) Modification of GIC with both chitosan and fluoride-loaded chitosan nanoparticles would have no effect on enamel leakage scores. (2) Modification of GIC with both chitosan and fluoride-loaded chitosan nanoparticles would have no effect on dentin leakage scores.

METHODS

Thirty-six human premolars, extracted for periodontal and orthodontic reasons were stored in 0.1% thymol solution until the experiment. The research was approved by the Gazi University Faculty of Dentistry Clinical Research Ethics Committee (Date: September 2, 2022, ethical protocol no. 2022.16/1). All teeth had no caries, hypoplastic defect, crack, or restoration. The surfaces were cleaned by hand-scaling from calculus and remaining connective tissue. The materials used in this study and their contents are shown in Table 1.

Preparation of Chitosan Solution

Chitosan solution was prepared by completely dissolving chitosan in 0.5% acetic acid solution. The chitosan was accurately weighed to be 0.5% and 0.25% (w/v) concentrations and added to the 0.5% acetic acid solution. Chitosan solutions were obtained by stirring overnight at room temperature at 500 rpm.[17]

Table 1. The content of the material used and the manufacturers.

Material	Manufacturer	Composition	Lot No
GC Fuji IX GP	GC Corporation, Tokyo, Japan	Fluoroaluminum silicate, glass, polyacrylicacid, polybasic carboxylic acid	2011021
Low molecular weight Chitosan	Sigma-Aldrich, St. Louis, USA	Deacetylated chitin, Poly(D-glucosamine)	STBH2614
Cavity Conditioner	GC Corporation, Tokyo, Japan	77% distilled water, 20% polyacrylic acid, 3% aluminum chloride hydrate	2102091
Riva coat	SDI, Bayswater, Australia	Triethyleneglycoldimethacrylate 20%-30% Diurethanedimethacrylate 60%-70%	19159

Preparation of Fluoride Loaded Chitosan Nanoparticles

Chitosan nanoparticles were prepared via the ionic gelations technique by using sodium tripolyphosphate (TPP) as an anionic linker.[18] Briefly, nanoparticles were obtained by the dropwise addition of TPP solution to the chitosan solution under continuous stirring on a magnetic stirrer at 900 rpm at room temperature for 30 minutes. Based on our previous study, the chitosan:TPP ratio was determined as 2:1.[17]

Formulation optimization studies were carried out to evaluate whether the chitosan concentration, NaF and TPP addition order, and NaF amount had an effect on the formulation characteristics. The prepared nanoparticle formulations during optimization studies were summarized in Table 2. For the preparation of N1 and N2 nanoparticles, NaF was added to the chitosan solution and stirred for 10 minutes before the addition of TPP. At the end of the 10 minutes stirring, TPP solution was added dropwisely and stirred for 30 minutes. The stirring rate was kept constant at 900 rpm during nanoparticle preparation. On the other hand, NaF and TPP were simultaneously added to the chitosan solution dropwisely to obtain nanoparticles with the formulation codes N3 and N4, then stirred for 30 minutes. After preparation, the suspensions were centrifuged at 10,000 rpm for 10 minutes to isolate nanoparticles. During nanoparticle preparation steps, only polypropylene labware was used instead of glass to avoid fluoride adsorption on glass surfaces.[19] Fluroide-loaded nanoparticles were dried in an oven at room temperature and grinded for further use.

Table 2. Fluoride-loaded chitosan nanoparticle formulation codes and optimization parameters

Nanoparticle code	Chitosan concentration (%)	Order of NaF addition	NaF amount
N1	0.5	NaF 10 minutes before TPP	5 mg
N2	0.25	NaF 10 minutes before TPP	3 mg
N3	0.5	Simultaneous addition of NaF and TPP	5 mg
N4	0.25	Simultaneous addition of NaF and TPP	3 mg

Fluoride Release and Characterization of Fluoride Loaded Chitosan Nanoparticles

Particle size distribution (PS), zeta potential (ζP), polydispersity index (PDI), and encapsulation efficiency (EE%) are important parameters used to characterize nanoparticles. 20 µL of prepared nanoparticles were diluted to 2 ml with distilled water and PS, PDI, and ζP were measured with a laser particle size analyzer (Malvern Nano-ZS90, Malvern Instruments LTD, UK).

The encapsulation efficiency was assessed using the centrifugation technique. After preparation, the nanoparticles were centrifuged at 10000 rpm for 10 minutes to precipitate the nanoparticles, the supernatant was completely removed and used to quantify the fluoride after dilutions. %EE is calculated using the following equation:

$$EE\ \% = \frac{W_{FTotal} - W_{FS}}{W_{FTotal}}\ x\ 100$$

W_{FTotal}: the amount of total fluoride added to the nanoparticles,

$W_{FS:}$ the amount of fluoride detected in the supernatant after centrifugation.

To evaluate fluoride release, fluoride-loaded chitosan nanoparticles were prepared and suspended in 50 ml of distilled water following centrifugation. Nanoparticle suspension was kept at 37° C for 14 days in tightly closed polypropylene beakers.[9,20] 1 ml of samples were withdrawn at 2h, 6 h, 1d, 2d, 5d, 7d, 10, and 14 d, equal volume of fresh water was added to the beakers to keep the volume constant at each time point. The samples were kept at 4° C until analysis. In vitro release studies were conducted in triplicate. The release kinetics were also evaluated using DDSolver, for mathematical modeling.

Formulation of glass ionomer powder modified with chitosan and fluoride-loaded chitosan nanoparticles

Petri et al.[11] showed that adding 10% wt chitosan to GIC resulted in better flexural strength. Therefore, in this study, chitosan and fluoride-loaded chitosan nanoparticles were added to the GIC powder at a concentration of 10% wt. The GIC powder was mixed with a vibratory mixer for 10 minutes to obtain a homogeneous distribution of chitosan and/or fluoride-loaded nano-chitosan as possible. The prepared powders were then mixed with the liquid component with a plastic spatula at the recommended powder/liquid ratio (3.6/1.0 g/ml) according to the manufacturer's instructions.

Cavity Preparation and Restoration

The teeth were marked for the borders (cervico-occlusal width 2 mm, mesiodistal width 3 mm) of the total of 36 cavities with a water-resistant pen before the cavity preparations were made. Standard class V cavities (cervico-occlusal width 2 mm, mesiodistal width 3 mm, and depth 1.5 mm) were prepared to all teeth with a cylindrical diamond bur (ISO806314, 014, Hager & Meisinger GmbH, Neuss, Germany) under water cooling by a single researcher. The gingival margins of the cavities were at the dentin surface and 1 mm below the cemento-enamel junction (CEJ), while the occlusal margins were at the enamel surface and 1 mm above the CEJ. The beveling was not applied to the cavity margins. The diamond bur was renewed for each cavity. A periodontal probe (Hu-Friedy Co., Chicago, IL, USA) was used for verifying the dimensions of the cavities. Then the teeth were randomly divided into 3 groups (n=12): GIC, chitosan-modified GIC (CH-GIC), fluoride-loaded chitosan nanoparticle-modified GIC (NCH-GIC).

After applying Cavity Conditioner (GC Corporation, Tokyo, Japan) for 10 seconds with a soft brushing motion to the cavity walls, it was rinsed and dried by directing air flow from the sides to prevent the dentin from drying out. After the cavities were restored, polished using a multi-step finishing/polishing system (Sof-Lex; 3M ESPE, St. Paul, MN, USA). The medium, fine and superfine discs were applied to the resin composite surfaces without water spray and with light intermittent pressure for 10 s with a handpiece speed of 30,000 rpm, rinsed, and dried for 10 s.[21] Then varnish (Riva coat, SDI, Bayswater, Australia) was applied to the surface of the restorations and polymerized with an LED light device (D-Light Pro; GC Corporation, Tokyo, Japan) for 20 seconds. All experimental procedures were performed by a single operator.

Thermocycling

After restoration, the specimens were stored in distilled water at 37°C for 24h and were subjected to 1500 thermo-cycles (Thermocycler; SD Mechatronik, Feldkirchen, Almanya) (5–55°C, dwell time: 25 s, transfer time: 5 s).

Preparation for Assessment of Microleakage

Following thermocycling, all surfaces 1 mm away from the restoration margins were covered with two coats of nail varnish (Golden Rose; Erkul Cosmetics, Istanbul, Turkey) to prevent unacceptable dye penetration. Root apexes were sealed with sticky wax to prevent leakage. The samples were immersed in a 1% methylene blue dye solution for 24 hours at 37°C in an incubator. After the dye protocol, the samples were rinsed under running water and sectioned into two-part buccolingually in an occlusoapical direction through the center of restorations in the mesiodistal direction using a diamond cutting disc. The microleakage degrees of each section were evaluated and scored blindly under light microscopy (Olympus SZ-PT; Olympus Optical CO Ltd, Tokyo, Japan) at 40x magnification. Microleakage scores were determined according to the ordinal scale shown in Table 5.

For statistical analysis, the SPSS Statistics program (v. 22.0; IBM, Chicago, IL) was used. The Kolmogorov-Smirnov and Shapiro-Wilk tests revealed that the distribution of none of the parameters was normal. The Kruskal-Wallis test was used to compare quantitative data among groups while the Wilcoxon test was used to compare within groups. Significance was evaluated at the P<.05 level.

RESULTS

Preparation and Characterization of Fluoride Loaded Chitosan Nanoparticles

Formulation optimization studies evaluated the effects of chitosan concentration, NaF and TPP addition order, and NaF amount on formulation characteristics (Table 2). According to the results presented in Table 3, simultaneous addition of NaF and TPP resulted in higher PDI for N3 and N4 nanoparticles, 0.548 and 0.600 respectively. Since PDI values lower than 0.5 are highly desirable, indicating a narrow particle size distribution, N3 and N4 formulations were deemed inappropriate. On the other hand, addition of NaF 10 min before the TPP addition significantly reduced PDI. As expected, increasing chitosan concentration resulted in larger particle sizes. N3 nanoparticles, were the nanoparticles with the highest particle size. N2 nanoparticles exhibited the lowest zeta potential, and this data was considered as negative fluoride ions were mainly located in the surface of the cationic chitosan nanoparticles. N1 nanoparticles were chosen as the optimum formulation for further studies due to their high encapsulation efficiency, low PDI and higher zeta potential.

Table 3. Characteristics of fluoride-loaded nanoparticles

Nanoparticle code	Particle size (nm)	Polydispersity index (PDI)	Zeta potential (mV)	Encapsulation efficiency (%)
N1	347.8 ± 62.5	0.456 ± 0.084	20.8 ± 0.29	36.5
N2	321.5 ± 28.6	0.451 ± 0.006	13.8 ± 0.40	17.8
N3	510.7 ± 22.8	0.548 ± 0.022	19.8 ± 0.21	18.9
N4	358.8 ± 26.1	0.600 ± 0.032	22.1 ± 1.10	18.0

In vitro fluoride release studies were carried out with N1 nanoparticles. The release profile obtained by the 14 day release study was given in Figure 1. A controlled fluoride release was obtained by chitosan nanoparticles. The release obtained in the first two hours (14.1%) could be related to the burst-release. 26.3% fluoride release was observed in the first six hours and 39% release was completed in the first

24 hours. To understand the mechanism and kinetics of fluoride release from nanoparticles, the *in vitro* release results were fitted to kinetic models via the DDSolver®, the results were presented in Table 4. The release of cargo from chitosan nanoparticles generally starts with a rapid initial release followed by a slower and continuous release (controlled release).[22] To predict drug release from chitosan nanoparticles, frequently used kinetic models include the zero-order model, Hixson-Crowell model, Higuchi model, Weibull model, and Korsmeyer-Peppas model. There are three different ways that chitosan nanoparticles release drugs. The *in vitro* release results were compared with several kinetic models and the three most important criteria, the adjusted coefficient of determination (r^2adjusted), the Akaike Information Criterion (AIC), and the model selection criterion (MSC) were considered for the selection of the fitted model.[23,24] For this purpose, the model with highest r^2 (0.996) and MSC (4.81) on the other hand lowest AIC (37.12), assumed as the best fit model for the *in vitro* release, which is Korsmeyer-Peppas. This model indicates that, the release rate of fluoride through the chitosan nanoparticles was significantly depended on the molecular diffusion rate of the nanoparticulate system.[25]

Table 4. Release kinetics evaluation results

Mathematical Models	*Compliance Criteria*		
	r^2_{adj}	AIC	MSC
Zero Order	0.5823	77.4044	0.3336
First Order	0.9118	63.4035	1.8893
Higuchi	0.9569	56.9541	2.6059
Korsmeyer-Peppas	0.9956	37.1275	4.8089
Hixson-Crowell	0.8822	66.0148	1.5992
Weibull	0.9605	57.5901	2.5352

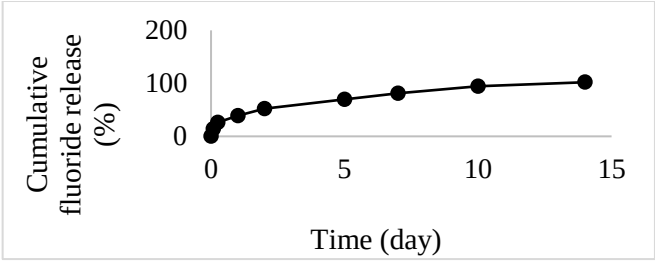

Figure 1. In vitro fluoride release profile N1 nanoparticles (n=3)

Assessment of Microleakage

The microleakage scores at the occlusal and gingival margins of the groups are shown in Table 6. The Kruskal-Wallis test results revealed no significant difference in microleakage scores at the occlusal or gingival margins of groups ($P_{occlusal}$ =.649, $P_{gingival}$ =.365).

The Wilcoxon test revealed significant differences between microleakage scores at occlusal and gingival margins in groups (P_{GIC}=.007, $P_{CH\text{-}GIC}$=.008, $P_{NCH\text{-}GIC}$=.013). Microleakage at the gingival margins was observed to be greater than at the occlusal margins in all three groups. Figure 2 shows the distribution of microleakage scores at the occlusal and gingival margins for all materials and Figure 3 shows light microscopy images of dye penetrations.

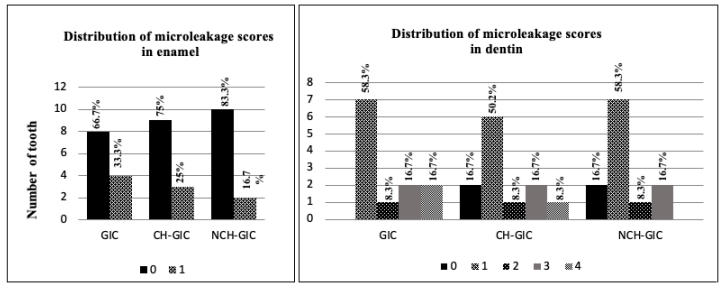

Figure 2. Distribution of microleakage scores at the enamel and dentin margins for all materials

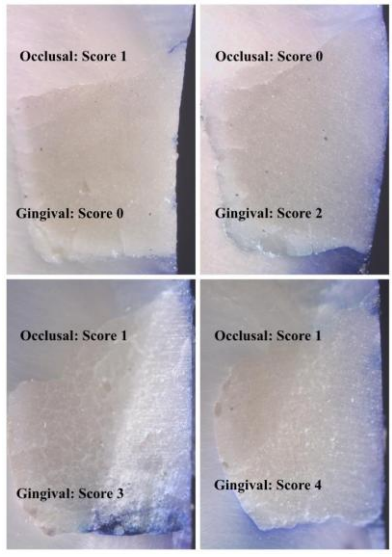

Figure 3. Light microscopy images of dye penetration at occlusal and gingival margins at ×40 magnification

Table 5. Dye penetration score criteria used in this study

Score	Leakage
0	No dye penetration
1	dye penetration up to 1/3 cavity depth
2	dye penetration between 1/3 and 2/3 cavity depth
3	dye penetration more than 2/3 cavity depth without involving the axial wall
4	dye penetration involving the axial wall

Table 6. Frequency and mean ± SD of microleakage scores for enamel and dentin

	Enamel							Dentin					
	0	**1**	**2**	**3**	**4**	**Mean±SD**		**0**	**1**	**2**	**3**	**4**	**Mean±SD**
GIC	8	4	0	0	0	0.33±0.49 ^{Aa}		0	7	1	2	2	1.92±1.24^{Ab}
CH-GIC	9	3	0	0	0	0.25±0.45 ^{Aa}		2	6	1	2	1	1.50±1.24^{Ab}
NCH-GIC	10	2	0	0	0	0.17±0.39 ^{Aa}		2	7	1	2	0	1.25±0.96^{Ab}
p						**0.649**							**0.365**

Note: Capital letters in the columns indicate the difference among groups, and lowercase letters in the rows indicate the difference between enamel and dentin microleakage scores (P < .05). SD: standard deviation; GIC: glass ionomer cement; CH-GIC: chitosan-modified glass ionomer cement; NCH-GIC: fluoride-loaded chitosan nanoparticle-modified glass ionomer cement.

DISCUSSION

The results of this study reveal that the microleakage findings at the occlusal and gingival margins of modified GIC containing 10% (v/v) chitosan or 10% (v/v) fluoride-loaded chitosan nanoparticles were similar to those of conventional GIC. Therefore, the first and second null hypotheses were not rejected.

The adhesion of GIC to dental tissues can be explained by two interconnected mechanisms. Chemical interaction formed between carboxylate groups in the polyacrylic acid and phosphate ions in dental tissue and micromechanical locking caused by self-etching glass ionomers thanks to its polyacid content.[1] Despite their ability to adhere to enamel and dentin, even GIC shows some microleakage at the margins of restorations.[26]

Microleakage is among the major causes of dental restorative failure and may be evaluated by utilizing dyes, chemical tracers, radioactive tracers, SEM analysis, neutron activation testing, and fluid infiltration.[27] This study used dye leakage, which is the commonly used method to assess microleakage.[28] This method is simple, economical, fast, and does not need complicated laboratory instruments.[5,26,27] For dye leakage, dyeing solutions such as 0.5-2 basic fuchsin, 50% silver nitrate, and 0.2-2% methylene blue are used.[5] In this study, 1% methylene blue was used, which is one of the commonly used dyeing solutions. To determine microleakage in restorations, thermocycling is used to simulate temperature changes in the oral environment. In this study, 1500 thermocycles were used, which is equivalent to approximately 6 months of clinical service.[29]

The bond strength of GIC to dentin is lower than that of enamel due to dentin having a higher percentage of water and organic material.[30] In this study, occlusal microleakage scores were found to be more successful than gingival microleakage scores in all three groups. This finding is consistent with other studies that concluded that cervical lesions restored with different types of GICs had more leakage from the gingival margins at the dentin-cementum junction than from the occlusal margins at the enamel. This may be explained to the polar and ionic attraction between the carboxylic groups of polyacrylic acid and the tooth hard surfaces. Due to its strong chelating properties, chitosan allows more polyacrylic acid chains would diffuse into the enamel to displace the phosphate and calcium ions from hydroxyapatite crystals.[5,31]

Recent studies found that 10%(v/v) chitosan-modified GIC has increased bond strength, flexural strength, and antibacterial properties compared to conventional GIC.[6,32] Petri et al.[11] observed that polyacrylic acid networks formed around the inorganic particles when 10%(v/v) chitosan was added to the GIC. In this way, they reported that chitosan improves its mechanical properties by reducing the interfacial tension between the GIC components. A recent study reported that the clinical performance of 10% chitosan-modified glass ionomer in primary molars was comparable to that of unmodified glass ionomer, and its antibacterial effect gives it an advantage.[33] Debnath et al.[6] reported that 10% (v/v) chitosan-modified GIC bonded to enamel 84% more than conventional GIC. Abraham et al.[15] observed that the microleakage findings of 10% (v/v) chitosan-modified GIC were similar to GIC, consistent with the findings of this study.

Kumar et al.[9] reported that 10%(v/v) nano chitosan-modified GIC has better compression and flexural strength compared to conventional GIC. They revealed that because nano chitosan has a larger surface area and a higher positive charge, it further decreases interfacial tension by interacting more with negatively charged polyacrylic acid. Nishanthine et al.[34] reported that nano chitosan-modified GICs released more fluoride than conventional GICs at all time points. Furthermore, chitosan nanoparticles have the ability to control the release of active agents.[35] Altınışık et al.[14] reported that GIC modified with chitosan or fluoride-loaded chitosan nanoparticles had fewer voids (empty spaces within the material) and gaps (spaces between tooth and restorations) compared to conventional GIC.

Given that in vitro studies do not reflect all the variables found in the oral cavity, the findings of the current *in vitro* study cannot be generalized to clinical situation unless adequate clinical trials are conducted to assess the effectiveness of the material *in vivo.* Additional assessments need to be completed to analyze and contrast other mechanical properties such as flexural strength, bond strength, as well as characteristics like hardness, setting time, and working time of the test cement. Moreover, investigations should be carried out to assess the durability and stability of chitosan or fluoride-loaded chitosan nanoparticles modified glass ionomer cement over time.

CONCLUSION

1-The experimental cement containing 10% (v/v) chitosan or 10% (v/v) fluoride-loaded chitosan nanoparticles exhibited microleakage comparable to the unmodified cement.

2- The addition of 10% (v/v) chitosan or 10% (v/v) fluoride-loaded chitosan nanoparticles had no deleterious effect on the microleakage of glass ionomer cement. Therefore, chitosan or fluoride-loaded chitosan nanoparticles modified glass ionomer cement stands out as a promising restorative material.

Ethics Committee Approval: The research was approved by the Scientific Research Ethics Committee of Gazi University Faculty of Dentistry (Date: September 2, 2022, ethical protocol no. 2022.16/1).
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Peer-review: Externally peer-reviewed.
Author Contributions: Concept- H.A., NB. MA., A.B.; Supervision-H.A, NB. MA., A.B.; Resources- H.A., NB. MA., A.B.; Data Collection and/or Processing- H.A., NB. MA., A.B.; Analysis and/or Interpretation- H.A., NB. MA., A.B.; Literature Search- H.A.; Writing Manuscript- H.A., NB. MA., A.B.; Critical Review- H.A., NB. MA., A.B.
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Hakem Değerlendirmesi: Dış bağımsız.
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