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Comparison of Antibacterial Activity, Cytotoxicity, and Fluoride Release of Glass Ionomer Restorative Dental Cements in Dentistry

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Statistical Analysis C
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Background: This study aimed to compare the antibacterial activity, cytotoxicity, and fluoride release of 4 different glass ionomer cements (GIC).

Material/Methods: A total of 200 samples were prepared: Riva Silver, a silver-reinforced GIC; Equia Forte HT, a glass hybrid GIC; ChemFil Rock, a zinc-added GIC; and Ketac™ Molar Easymix. Using the agar diffusion test for antibacterial activity, 30 samples from each group were analyzed for *Streptococcus mutans*, *Lactobacillus acidophilus*, and *Actinomyces naeslundii*. The areas around the samples where no bacterial growth occurred were digitally measured and recorded. For cytotoxicity analysis, the WST-1 test was performed on 10 samples from each group using the L929 mouse fibroblast cell line. The fluoride release property was evaluated using an ion-selective electrode method on 10 samples from each group.

Results: The group that used Ketac™ Molar Easymix showed the lowest antibacterial activity against *S. mutans*, *L. acidophilus*, and *A. naeslundii*. In all 3 days of cytotoxicity testing, the group that used Riva Silver was found to be the least toxic material, while the group that used ChemFil Rock did not have viable cells after the 1st day. In all 4 materials, fluoride release values gradually increased since the first day, with Ketac™ Molar Easymix having the highest fluoride release.

Conclusions: Of all the GICs tested, Ketac™ Molar Easymix demonstrated the least antibacterial activity despite having the highest fluoride release, while Riva Silver was found to be the least cytotoxic material.

Keywords: Fluorides • Glass Ionomer Cement

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Background

Dental caries is one of the most common chronic diseases observed in people of all ages worldwide [1]. As a result of advancements in dentistry, new minimally invasive techniques and materials have been developed to protect remaining tooth structures and to minimize pulp damage [2-4]. After removing the caries layer using this conservative approach, infected tissues and microorganisms may remain in the dentin cavity [1,5,6]. This situation necessitated the development of materials that increase remineralization and antibacterial activity via their fluoride-releasing properties. GICs, one of the most commonly used materials for this purpose, were introduced to dentistry by Wilson and Kent [7] in the 1970s.

Conventional GICs (CGICs) are made by combining water-soluble polymeric acids with calcium- or strontium-based alumina-silicate glass powders and adding fluoride [8]. Their advantages include remineralization of dental hard tissues, such as enamel and dentin, as a result of fluoride release; a dentin-like thermal expansion coefficient and modulus of elasticity; chemical bonding to mineralized tooth tissues; good biocompatibility; and an inhibitory effect on the growth of cariogenic bacteria after incomplete caries removal procedures [9]. Despite these advantages, CGICs have some disadvantages, including a long curing time, high sensitivity and brittleness to moisture during the initial curing reaction, microleakage, low wear resistance, and a short working time [10-12]. To minimize these disadvantages and improve mechanical properties, different materials have been added to GIC, and different categories such as resin-modified GICs (RMGICs), metal-reinforced GICs (MRGICs), high-viscosity glass ionomers, and giomers have been developed [9,13].

In minimally invasive approaches, the possibility of microorganisms remaining in the cavity increases the risk of restoration failure due to secondary caries [14]. Secondary and initial caries have similar microbiology. Streptococci, Lactobacilli, and Actinomyces are involved in the early stages of bacterial invasion in caries development. *Streptococcus mutans* is the primary bacterium responsible for caries formation. *Lactobacillus acidophilus* is responsible for the progression of caries and the formation of secondary caries. *Actinomyces naselundii* is associated with root caries and can invade dentinal tubules [15,16]. The use of materials with antibacterial or bactericidal effects, especially in minimally invasive approaches, will provide additional treatment by suppressing residual infection and increasing the life of the restored tooth [17]. Because of their low pH and fluoride-releasing properties, GICs exhibit antibacterial properties immediately after placement in the cavity [18].

GICs are also known for their ability to remineralize dental hard tissues, such as enamel and dentin, as a result of fluoride release into the oral environment [19]. The release of fluoride from the restorative material and the continuous supply of low levels of fluoride ions in the biofilm–saliva–tooth interaction play important roles in caries prevention [20].

Although restoration materials help restore tooth health, the products released by these materials can directly or indirectly affect the surrounding tissues as they pass through the dentin canals to the pulp during and after the polymerization process [21,22]. Many of these materials come into contact with or interact with body tissues and fluids; therefore, not only their mechanical and physical properties, but also their biological compatibility should be considered when selecting materials [23]. Biocompatibility is defined as the ability of a material to function in living organisms and induce an appropriate tissue response [24]. The biocompatibility of GICs depends on the components that decompose during the curing process. Given that both components of GICs (glass powder and polyacid liquid) consist of a spectrum of chemical formulas, the risk of these materials causing toxic effects *in vivo* has been partially evaluated by *in vitro* tests [25].

Therefore, this study aimed to compare the antibacterial activities of 4 different GICs against *S. mutans*, *L. acidophilus*, and *A. naselundii*; their cytotoxicity on a mouse fibroblast cell line; and their fluoride release properties.

Material and Methods

Approval was obtained from the Dicle University, Faculty of Dentistry Local Ethical Committee (Protocol number 2021-61).

In this *in vitro* study, 4 GICs were used: Riva Silver, a silver-reinforced GIC (SDI, Australia); Equia Forte HT, a glass hybrid GIC (GC, Tokyo, Japan); ChemFil Rock, a zinc-added GIC (Dentsply De Trey, Konstanz, Germany); and Ketac™ Molar Easymix, a CGIC (3M-ESPE, Germany) (**Table 1**).

Preparation of Samples

For the analyses, a total of 200 disk-shaped samples (30 for antibacterial activity, 10 for cytotoxicity, and 10 for fluoride release) with a thickness of 2 mm and a diameter of 5 mm were prepared from each material in accordance with the manufacturers' recommendations, as in the studies of Ranjani et al [26].

The Riva Silver, Equia Forte HT, and ChemFil Rock GICs in capsule form were mixed in an amalgamator for the time periods specified by the manufacturers. The samples were pressed between a celluloid strip and a glass coverslip and placed in plastic rings using the applicator to prevent air bubble formation, remove excess material, and obtain a flat, smooth surface.

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Table 1. Glass ionomer cements used in the study.

Materials	Type	Manufacturer	Composition
Riva Silver	Silver-alloy reinforced GIC	SDI, Australia	Liquid: acrylic acid homopolymer; tartaric acid. Powder: glass powder; acrylic acid homopolymer; alloy powder
Equia Forte HT	Bulk fill glass hybrid restorative system	GC, Tokyo, Japan	Powder: fluoroaluminosilicate glass, polyacrylic acid, iron oxide Liquid: polybasic carboxylic acid, water
ChemFil Rock	Zinc-reinforced glass ionomer	Dentsply, Konstanz, Germany	Calcium-aluminium-zinc-fluoro-phosphor-silicate glass, polycarboxylic acid, iron oxide pigments, titanium dioxide pigments, tartaric acid, water
Ketac™ Molar Easymix	Conventional glass-ionomer cement	3M-ESPE, Germany	Powder: Al-Ca-La fluorosilicate glass, 5% copolymer acid (acrylic and maleic acid) Liquid: Polyalkenoic acid, tartaric acid, water

After curing at room temperature, the samples removed from the plastic rings were polished with polishing disks.

Ketac™ Molar Easymix was prepared manually by mixing powder and liquid with a spatula. Similarly to other materials, it was pressed between a glass and a celluloid strip and placed in plastic rings. After curing at room temperature, the samples removed from the rings were polished with polishing disks.

Evaluation of Antibacterial Efficacy

The agar diffusion test was performed on 30 samples from each group using standard bacterial isolates of *S. mutans* ATCC 25175, *L. acidophilus* ATCC 11975, and *A. naeslundii* 12104. Immediately after dilution with 0.5 mL of trypticase soy broth liquid medium and 4 h of incubation at 37°C, the lyophilized *S. mutans* isolate was planted in 5% sheep blood agar (SBA) (ThermoScientific Oxoid, England) medium using the dilution method. The cultured medium was incubated for 48 h in a 5-10% CO_2 environment (in a wax desiccator) before being sub-cultured to CCA medium for the second time. The lyophilized *L. acidophylus* isolate was reconstituted with 0.5 mL of Man, Ragosa, and Sharpe (MRS) broth and seeded on MRS agar solid medium immediately after 4 h of incubation at 37°C. The lyophilized *A. naeslundii* isolate was diluted in thioglycolate medium and seeded on Actinomyces agar solid medium. The inoculated MRS agar and Actinomyces agar media were incubated at 37°C for 48 h in a Ziplock bag with anaerobic medium (Anaerocult P [Merck, Germany]). After the second subculture, the bacteria were prepared for the study by repeating the cultivation procedures.

The agar diffusion test was performed using Mueller-Hinton fastidious (MHF) agar medium, which is recommended for use in antibiotic susceptibility tests of *S. mutans* and anaerobic bacteria according to EUCAST standards. After incubation, 4-5 pure bacterial colonies grown in the medium were picked with a sterile cotton swab and kept in brain–heart infusion broth, and bacterial suspensions were prepared at 0.5 McFarland turbidity (1×10^8 CFU/mL). The prepared bacterial suspensions were diluted at a rate of 1/100 in sterile 0.9% NaCl, and the final bacterial concentration was determined as 10^6 CFU/mL. Three media were used to test each glass ionomer; a sterile swab stick was used to spread suspensions of *S. mutans*, *L. acidophilus*, and *A. naeslundii* on the entire surface of the medium. After inoculation on MHF agar medium, wells with a diameter of 5 mm were made in the media using the wide end of sterile glass Pasteur pipettes. The prepared samples were placed in the wells using sterile forceps. The MHF agar media inoculated with *S. mutans* were incubated in a 5-10% CO_2 environment, while the MHF agar media inoculated with *L. acidophylus* and *A. naeslundii* were incubated in an anaerobic environment at 37°C. Two percent chlorhexidine was used as the control group. On the 2nd, 4th, and 6th days, the areas around the samples where no bacterial growth occurred were digitally measured and recorded. The measurements were taken from the 2 outermost points of the area around the samples, where there was no reproduction.

Evaluation of Cytotoxicity

Using the L929 mouse fibroblast cell line, WST-1 analysis was performed on 10 samples from each group. The cells were placed in 24-well culture plates at 1×10^4 cells/cm^2 and incubated for 1 day in an incubator at 37°C and 5% CO_2. After subjecting the disk samples to UV sterilization, they were placed in the prepared experimental medium and incubated in a low glucose Dulbecco's Modified Eagle's Medium containing 10% fetal bovine serum and 1% penicillin/streptomycin. The control group consisted of L929 cells that had not been treated with any material. After our samples were placed in the experimental environment, WST-1 tests were performed at the end of the 1st, 3rd, and 7th days.

After the samples were removed from the experimental medium on the specified days, WST-1 reagent (WST-1 Cell Proliferation Assay Reagent (Roche)) was added in a 1: 10 ratio. After 2 h

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of incubation under appropriate conditions, the absorbances were measured at 450 nm using a monochromatic microplate reader (Microplate Reader, VersaMax, Molecular Devices, USA).

Evaluation of Fluoride Release

The fluoride releases of 10 samples from each group were evaluated using the ion-selective electrode method.

The ion-selective electrode method was employed for the analyses using the ionometer device (Thermo Orion 720 A+), with a fluoride electrode (Orion 9609 BNWP), a 0.1 MF standard (Orion ionplus-application solution-940906), and a special filling liquid (Orion ionplus-filling solution-900061) placed in the fluoride electrode. The TISAB II solution (Orion ionplus-application solution-940909) was prepared in a 1: 1 ratio and used as an anti-interference buffer. Depending on the concentration range to be studied, 5 calibration standards (0.1, 1, 10, 50, and 100 ppm) were used to calibrate the electrode.

After weighing and recording the weight of each sample with a precision balance, 5 mL of distilled water was added to the sample, and the sample was placed in an oven at 37°C.

On the 1st, 2nd, 3rd, 7th, 14th, 21st, and 28th days, the TISAB II solution was mixed in a 1: 1 ratio with the distilled water taken from the tubes containing the groups, and the reading was taken by immersing the electrode. Before and after each measurement, the electrode tip was washed and lightly dried with distilled water to remove any residual fluoride ions.

Statistical Analysis

SPSS version 22 package program and descriptive statistical methods were used in the statistical evaluation of the data in this study, and a value of $p<0.05$ was considered statistically significant.

The Kruskal-Wallis test was used for the multiple comparison of the groups, and the Mann-Whitney U test was used for the pairwise comparison of the groups.

The Friedman test was used for multiple comparisons of groups on repetitive days, and the Wilcoxon test was used for pairwise comparisons.

Results

Antibacterial Efficacy Findings

When we evaluated the antibacterial activity of the 4 different GICs against *S. mutans* in our study, we found that chlorhexidine had the highest values on all 3 days. On the 2nd and 6th days, ChemFil Rock, Equia Forte HT, Riva Silver, and Ketac™ Molar Easymix followed, respectively. On the 4th day, Equia Forte HT, ChemFil Rock, Riva Silver, and Ketac™ Molar Easymix followed, respectively. While there was a statistically significant difference in pairwise comparisons between all groups on each measurement day, there was no statistically significant difference between ChemFil Rock and Equia Forte HT ($p>0.05$).

When the antibacterial activities against *L. acidophylus* were evaluated, chlorhexidine yielded the highest values on all 3 days, followed by Equia Forte HT, ChemFil Rock, Riva Silver, and Ketac™ Molar Easymix on the 2nd day and ChemFil Rock, Equia Forte HT, Riva Silver, and Ketac™ Molar Easymix on the 4th and 6th days. In pairwise comparisons between the groups on the measurement days, there was no statistically significant difference between ChemFil Rock and Equia Forte HT on the 2nd and 4th days ($p>0.05$).

In the evaluation of antibacterial efficacy against *A. naeslundii*, chlorhexidine yielded the highest values on all 3 days, followed by Equia Forte HT, ChemFil Rock, Riva Silver, and Ketac™ Molar Easymix.

Table 2 shows inhibition zone mean values and standard deviations in the evaluation of antibacterial activity against *S. mutans*, *L. acidophilus*, and *A. naeslundii*.

Cytotoxicity Findings

When the number of viable cells in the L929 mouse fibroblast cell line was examined using WST-1 analysis of the 4 different GICs used in our study, the majority of cells were observed in the control group on all 3 days, while there were no viable cells in the ChemFil Rock group after the 1st day. The control group was followed by Riva Silver and Equia Forte HT, respectively, while the least number of cells were detected in the group that used Ketac™ Molar Easymix on the 1st day and in the group that used ChemFil Rock on the 3rd and 7th days.

The mean and standard deviations of viable cell numbers as a result of cytotoxicity analysis are shown in **Table 3**.

Fluoride Release Findings

When the fluoride releases of the 4 GICs were examined, we found that the values increased gradually in each group, starting from the 1st day. While ChemFil Rock yielded the lowest fluoride release values on all measurement days, Ketac™ Molar Easymix yielded the highest fluoride release values. The mean fluoride release values and standard deviations are shown in **Table 4**.

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Table 2. The mean and standard deviation values of the antibacterial activities of the GICs used.

	Groups	Antibacterial activity (mm)			
		Mean±SD (2nd day)	Mean±SD (4th day)	Mean±SD (6th day)	Friedman p*
S. mutans	Riva Silver	11.00±1.19^{cde}	9.38±0.69^{cde}	7.47±0.34^{cde}	<0.001
	Equia Forte HT	13.53±1.17^{ad}	11.93±1.18^{ad}	8.69±0.44^{ad}	
	ChemFil Rock	13.72±0.97^{be}	10.60±0.88^{be}	8.78±0.44^{be}	
	Ketac™ Molar Easymix	8.52±0.48^{abc}	7.58±0.48^{abc}	7.10±0.12^{abc}	
	Chlorhexidene	20.3	18.1	17	
L. acidophylus	Riva Silver	11.00±1.16^{cde}	9.69±1.10^{cd}	7.67±0.33^{ce}	<0.001
	Equia Forte HT	12.50±0.74^{ad}	10.89±0.75^{a}	7.49±0.24^{ad}	
	ChemFil Rock	12.33±0.81^{be}	11.04±0.67^{bd}	8.00±0.13^{bde}	
	Ketac™ Molar Easymix	8.53±0.48^{abc}	7.51±0.36^{abc}	7.01±0.02^{abc}	
	Chlorhexidene	17.9	16.3	15.9	
A. naeslundii	Riva Silver	12.15±0.47^{cd}	11.03±0.95^{cd}	7.10±0.08^{cde}	<0.001
	Equia Forte HT	13.37±0.57^{ad}	12.66±0.67^{ad}	8.19±0.11^{ad}	
	ChemFil Rock	12.79±0.59^{b}	11.94±0.63^{b}	8.01±0.40^{be}	
	Ketac™ Molar Easymix	8.04±0.26^{abc}	7.23±0.20^{abc}	7.01±0.02^{abc}	
	Chlorhexidene	19.5	17.2	15	

* Friedman test was performed, and $p<0.05$ was considered statistically significant. In paired comparisons, the Wilcoxon test was performed, and $p<0.0167$ was considered statistically significant. a, b, c, d, e: There is a statistically significant difference between the same letter symbols in the same column for each bacterium.

Table 3. Mean and standard deviations of viable cell values.

Groups	Cell viability			
	Mean±SD (1st day)	Mean±SD (3th day)	Mean±SD (7th day)	Friedman p*
Riva Silver	32538±1335^{ce}	28247±780^{bc}	43510±4232^{b}	<0.001
Equia Forte HT	31439±464^{ab}	18454±331^{ab}	39135±6514^{a}	
ChemFil Rock	27347±2577^{bde}	–	–	
Ketac™ Molar Easymix	11964±926^{acd}	262.5±43.7^{ac}	21±2.6^{ab}	
Control Group	60377±1559	37710±631	90557±4512	

* Friedman test was performed, and $p<0.05$ was considered statistically significant. In paired comparisons, the Wilcoxon test was performed, and $p<0.0167$ was considered statistically significant. a, b, c, d, e: There is a statistically significant difference between the same letter symbols in the same column for each bacterium.

Discussion

The dentist's understanding of the physical, mechanical, and chemical properties of restorative materials used in pediatric dentistry is critical for the longevity of the restorations and patient satisfaction. The closest restorative material to the ideal is the one that has physical properties similar to the dental tissue, bonds well to enamel and dentin, and does not undergo structural changes in the oral environment [27]. Because of their antimicrobial properties, ease of application,

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Table 4. Mean and standard deviations of fluoride release values.

Groups	Fluoride release values (mg/l)							
	Mean±SD (1st day)	Mean±SD (2nd day)	Mean±SD (3th day)	Mean±SD (7th day)	Mean±SD (14th day)	Mean±SD (21st day)	Mean±SD (28th day)	Friedman p*
Riva Silver	3.21±1.96^{b}	5.47±1.75^{ad}	5.81±1.99^{ad}	9.66±2.53^{ad}	13.34±3.08^{ad}	16.44±4.09^{ad}	18.00±4.09^{ad}	<0.001
Equia Forte HT	3.14±1.63^{a}	5.39±2.77^{be}	6.79±2.82^{be}	10.62±4.96^{be}	12.97±3.39^{be}	17.81±3.68^{be}	20.21±7.41^{be}	
ChemFil Rock	2.16±0.97^{c}	2.64±1.36^{abc}	2.16±0.77^{abc}	4.14±0.97^{abc}	4.99±1.58^{abc}	6.35±1.82^{abc}	6.73±1.77^{abc}	
Ketac™ Molar Easymix	13.39±3.03^{abc}	15.61±3.48^{cde}	17.18±2.83^{cde}	16.42±2.96^{cde}	21.72±5.46^{cde}	25.58±5.14^{cde}	29.85±5.21^{cde}	

* Friedman test was performed, and $p<0.05$ was considered statistically significant. The Wilcoxon test was performed for pairwise comparisons. a, b, c, d, e: There is a statistically significant difference between the same letter symbols in the same column for each bacterium.

and fluoride-releasing properties, GICs are frequently used in dentistry [28]. One of the biggest advantages of GICs is the ability to modify their biological and physical properties by adjusting the powder-to-liquid ratio or changing the chemical formulation to minimize the risk of restoration failure [29].

The antibacterial activities, cytotoxicity, and fluoride releases of Riva Silver, a silver-reinforced GIC; Equia Forte HT, a glass hybrid GIC; ChemFil Rock, a zinc-added GIC; and Ketac™ Molar Easymix, a CGIC derived from glass ionomer-based materials used in dentistry, were evaluated in this *in vitro* study.

Because of their fluoride release properties, CGICs promote the formation of fluorapatite on the tooth surface. This substance is less soluble than hydroxyapatite [30].

Arısu et al [31] reported that CGICs released the highest amount of fluoride as well as more fluoride compared to MRGICs in their study in which they evaluated fluoride releases of 7 different restorative materials.

In their studies comparing fluoride releases of GICs in different environments, Hattab and Amin [32] found that CGICs released more fluoride compared to MRGICs. In the study by Bahammam et al [33] comparing fluoride releases of 4 different GICs, the group using ChemFil Rock showed the least fluoride release on all days.

In our study, the highest fluoride release was observed in the Ketac™ Molar Easymix group, which is the CGIC group, and the least fluoride release was observed in the ChemFil Rock group on all measurement days. This difference between CGICs and MRGICs could be attributed to the faster release of fluoride in the form of NaF and SiF in conventional glass ionomers than fluoride that is bound to silver particles [31].

Although the amount of fluoride released in the materials indicates the antimicrobial capacity of the material, some researchers reported that the low pH value during the curing reactions of GICs was more effective in terms of the antimicrobial effect than the release of fluoride and did not show any antimicrobial activity after curing was completed [34,35]. Coşgun et al [36] reported all GICs had low antimicrobial effects. All of the GICs in our study had a low antibacterial effect.

Saxena and Tiwari [37] evaluated the fluoride-releasing properties and antimicrobial effects of Zirconomer and Fuji IX against *S. mutans*, *Lactobacillus casei*, and *Candida albicans*. In that study, antifungal effects of the GICs used were observed, but it was determined that Zirconomer had a higher antibacterial effect than Fuji IX, a CGIC.

El-Baky and Hussien [38] showed that Fuji IX inhibited the growth of *S. mutans* and *L. acidophilus*, and Shashibhushan et al [39] demonstrated that Fuji IX inhibited the growth of *S. mutans*.

In our study, it was found that Ketac™ Molar Easymix inhibited the growth of *S. mutans*, *L. acidophilus*, and *A. naeslundi*, but had the lowest antibacterial effect compared to other GICs.

Many new-generation GICs have been developed and used to improve the mechanical properties of GICs. However, there have been very few comparative studies demonstrating the cytotoxic effects of these materials [24,40]. Therefore, this study aimed to evaluate GICs in terms of cytotoxicity as well as antibacterial and fluoride-releasing properties.

Cell culture, agar diffusion test, filter diffusion test, and dentin barrier test are common cytotoxicity tests [22]. In our study, we used the standard, easy-to-apply, and less time-consuming cell culture method with the L929 (mouse gingival fibroblast) cell line.

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In the literature, it is emphasized that low pH during curing and various released components cause cytotoxicity. The fluoride ion released from glass ionomers is the most well-known [41,42].

In their study on the cytotoxicity of CGICs and MRGICs, Selimovic-Dragaš et al [25] reported that CGICs and silver-reinforced GICs had fewer toxic effects on osteoblast-like cells compared to other materials. In our study, Riva Silver, a silver-reinforced GIC, was found to be the least toxic material. As a result, we believe that the addition of nano-sized silver particles will be a more promising GIC in terms of biosecurity.

Kanjevac et al [43] investigated the fluoride release of GICs and their cytotoxic effects on human pulp stem cells and concluded that the material that released the most fluoride was the most toxic. In our study, Ketac™ Molar Easymix, which released the most fluoride, was determined to be the most toxic material on the 1st day. On the 3rd and 7th days, it was the most toxic material, followed by ChemFil Rock, which resulted in zero viable cells.

Cell culture data provide insight into the release of undesirable components from this material and possible reactions. These studies should also be supported by *in vivo* evaluation tests.

This study had some limitations. Only 4 different CCIS were compared with each other. Furthermore, it was tested for a limited period of time, as the analyzes were performed *in vitro* under laboratory conditions. Given the highly variable conditions of the oral environment, more precise data can be obtained with different types of CIS or other restorative materials and with longer *in vitro* or *in vivo* analyzes.

Conclusions

Based on the findings of this *in vitro* study, all GICs showed varying degrees of antibacterial activity against *S. mutans*, *L. acidophilus*, and *A. naeslundii* but Ketac™ Molar Easymix showed the least. Although the amount of fluoride required to prevent caries has not yet been determined, all materials used in our study released fluoride at different rates. Therefore, long-term fluoride-releasing dental materials should be preferred until the ideal fluoride concentration is determined in patients with high caries risk because the active ingredients in the structure of GICs have a cytotoxic potential and can alter the metabolism of the pulp cell. In our study, Riva Silver was determined to be the least cytotoxic material. This cytotoxic effect should be considered when using the GICs employed in our study.

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