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ORIGINAL ARTICLE

Cytotoxicity Comparison of a Calcium Silicate-Based Resin Cement *versus* Conventional Self-Adhesive Resin Cement and a Resin-Modified Glass Ionomer: Cell Viability Analysis

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ABSTRACT

Objective: To compare the cytotoxicity level of a new calcium silicate-based resin cement (TheraCem) with two commonly used cements, including a conventional self-adhesive resin cement (Panavia SA) and a resin-modified glass ionomer cement (FujiCem2), on the human gingival fibroblast cells after 24 and 48 hours. **Material and Methods:** Twelve discs of each cement type were fabricated. The extract of cement disks was made by incubating them in the cell medium. Human gingival fibroblast cells were cultured and exposed to cement extracts for 24 h and 48 h. MTT assay was performed on extracts and optical density and cell viability rates were calculated by the spectrophotometer device at 570 nm. Data were analyzed using ANOVA and Tukey HSD tests. **Results:** The cell viability rates after 24 hours and 48 hours were as follows: TheraCem: 89.24% and 85.46%, Panavia SA: 49.51% and 46.57% and FujiCem2: 50.63% and 47.36%. TheraCem represented the highest cell viability rate. However, no significant difference was noted between Panavia SA and FujiCem2. Time had no significant effect on cell viability. **Conclusion:** TheraCem exhibited the best results among three tested cements and was considered non-toxic. Panavia SA and FujiCem2 were not significantly different regarding the cell viability rate. Time had no significant effect on the cytotoxicity level of cements.

Keywords: Glass Ionomer Cements; Fibroblasts; Resin Cements.

Introduction

Cementation procedure plays a crucial role in the long-term clinical success of prosthetic restorations [1]. Nowadays, a wide variety of dental materials are used in the cementation process [2], such as resin-modified glass ionomers and self-adhesive resin cements, which are among the most commonly used cements in clinic [3]. The broad application of these materials has drawn attention to their possible side effects, most importantly, their cytotoxicity. Since cements and luting agents are in direct contact with gingiva, they might cause adverse reactions in adjacent gingival tissues and fibroblast cells. Thus, their biocompatibility, cytotoxicity, and subsequent side effects are a matter of concern for clinical application. It is believed that released monomers such as Bis-GMA, UDMA, Bis-EMA, TEGDMA, and DEGDMA in cement composition are responsible for cement cytotoxicity [4].

Resin-modified glass ionomers such as FujiCem2 possess various outstanding properties such as chemical bonding to the tooth structure, fluoride release, and enhancing remineralization of carious lesions [5-7]. They are also less water-soluble and have higher flexural and compressive strengths compared to conventional glass ionomers. However, according to the studies, their cytotoxicity is questionable and, in some cases, has been higher compared to the other cement types [5,8].

In addition to resin-modified glass ionomers, self-adhesive resin cements, such as Panavia SA, have been used generally for cementation since they decrease the cementation clinical steps compared to conventional resin cements. However, many studies have reported higher cytotoxicity levels of these cements [9-12].

Recently, calcium and phosphate ions have been added to some resin cements such as TheraCem to compensate for the demineralization that happens during bonding procedure of resin cements. TheraCem enhances remineralization by providing an alkaline environment; however, its cytotoxicity has been raising concerns and needs further research [13,14].

Many laboratory methods are used to evaluate materials cytotoxicity, MTT assay is one of the most commonly used methods [15]. In this method, NADPH-dependent oxidoreductase enzymes reduce the solution of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) to insoluble formazan, and this reaction leads to the color change of the solution from yellow to purple [16]. The amount of NADPH-dependent oxidoreductase enzymes is an indicative of the number of living cells.

In the present study, we aimed to compare the cytotoxicity level of three commonly used cements, namely a self-adhesive resin cement (Panavia SA), self-adhesive resin cement reinforced by calcium (TheraCem), and resin-modified glass ionomer cement (FujiCem2) on the human gingival fibroblast cells after 24 and 48 h.

Material and Methods

Sample Size

The sample size was calculated with regard to a similar study [17] using SPSS software (IBM SPSS Statistics for Windows, Version 25.0, IBM Corp., Armonk, NY, USA). The sample size was calculated to be six specimens in each experimental group.

Preparation of the Specimens

Before the preparation of the specimens, standardized molds (4 mm in diameter and 1.1 mm in height) were made using a flexible glass. Then, cement specimens were prepared according to the manufacturer's

instructions. For each type of cement, twelve cement disks were made; six for cytotoxicity evaluation after 24 h and six for cytotoxicity evaluation after 48 h. The detailed information and lot number of the cements used in the present study are listed in Table 1.

Table 1. Material, composition, manufacturer, and lot number of three used cements.

Material	Type	Component	Manufacturer	LOT Number
Panavia SA Luting Plus	Self-Adhesive Resin Cement	MDP, bis-GMA, hydrophobic Dimethyl methacrylate, HEMA, silanated barium glass filler, silanated colloidal silica	Kuraray Noritake Dental Inc., Tokyo, Japan	A30145
FujiCem 2	Resin-Modified Glass Ionomer Cement	30-40% polyacrylic acid, polybasic carboxylic acid, poly (nbutylmethacrylate), 30-40% distilled water, 2% silica powder, 20% silicone dioxide, 2-3% benzenesulfonic, acid sodium salt	GC Dental, Tokyo, Japan	1307041
TheraCem	Self-Adhesive Resin Cement	Base: <50% Portland cement, <50% Ytterbium, w/Barium Glass, <5% ytterbium fluoride, <5% Bis GMA Catalyst Paste: <30% 10-MDP, <5% HEMA	Bisco Inc., Schaumberg, Illinois USA	1800004067

For Panavia SA luting plus, the equal amount of A and B pastes was placed on the sterile glass slab and mixed with a spatula for 10 s. Then, the cement was transferred to the standard mold and irradiated with a light cure device with 800-1400 mW/cm² power intensity for 10 s. For TheraCem, cement was placed in the standard mold using the manufacturer's automix tip and irradiated by the light cure device for 20 s. For FujiCem2, cement pastes were mixed using the manufacturer's automix tip and were placed in the standard mold. Specimens were irradiated by the light cure device for 10 s.

Finally, all set cement specimens were placed in sterile plastic tubes; each sample was placed in one tube. The specimens underwent sterilization by exposing tubes to UV light for 20 min.

Extraction Process

Concerning the surface of each specimen (0.39 cm²) and ISO 10993-5 guidelines [18], the extract ratio of 3 cm²/ml was considered. A mixture of PRMI 1640 medium (HyClone, Logan, UT, USA) supplemented with 10% fetal bovine serum, 1% penicillin, and 1% streptomycin, with a total volume of 130 µm was added to each of the tubes.

Tubes were placed in a 5% CO_2 incubator (MCO-19A1C, SANYO Electric Co., Osaka, Japan) at 37 °C and 95% humidity for 24 h. At the end of the incubation period, extracts were retained for further MTT assay.

Cell Culture and MTT Assay

HGF2 PL2 cells were seeded in a 96-well cell culture dish (Microplate, 96 Well, Greiner Bio-One Co., Frickenhausen, Germany) at a density of 5×10^3-10×10^3 cells/well. 100µL of cell suspension was added to each well. The culture dish was incubated for 24 h at 37 °C and 100% humidity to ensure the attachment of the cells to the culture dish. After the incubation, each cement extract was added to each of the wells. For positive control, DMSO 50% was added to one well, and one well-containing cell suspension was left empty to be used as a negative control. After that, 10 µL of the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) was added to each well. The culture dish was covered with aluminum foil and placed in the incubator at 37 °C and 5% CO_2 for 4 h. At the end of the incubation period, the culture dish was examined by an inverted

microscope (CKX41, OLYMPUS Corp., Tokyo, Japan) to observe the presence of purple deposits. The solution in the wells that contained purple deposits was drained and replaced with 100 µL of DMSO, which was poured in each well. The wells were left in the dark for 4 h. Then, an optical assessment was performed using an ELISA reader (ELx808, BioTek Instrument Inc., Vermont, USA) at 570 nm to measure optical density (OD) of the specimens. Cell viability rate was calculated using the following formula: Viability = ($OD_{Test}/OD_{Control}$) ×100. The parameters for cytotoxicity were those adopted according to the ISO 10993 guidelines [19].

Statistical Analyses

The effects of cement type and time on cytotoxicity levels were evaluated using two-way ANOVA. In addition, the one-way ANOVA and Tukey HSD test were performed to compare the cytotoxicity levels in different experimental cement groups. The analyses were performed using SPSS software (IBM SPSS Statistics for Windows, Version 25.0, IBM Corp., Armonk, NY, USA) and the significance level was considered to be 0.05.

Results

The mean cell viability rates of three experimental cement groups are shown in Table 2. Two-way ANOVA analysis showed no significant difference between cytotoxicity levels after 24h and 48h (p=0.55) and time had no significant effect on the cement cytotoxicity level. However, pairwise analysis of cell viability rates revealed a significant difference among different experimental cement groups.

Table 2. Cell viability rates of experimental cement and control groups after 24h and 48h.

Group	24h Mean ± SD	48h Mean ± SD
TheraCem	89.24 ± 2.80	85.46 ± 9.52
Panavia SA	49.51 ± 6.74	46.57 ± 3.68
FujiCem2	50.63 ± 12.24	47.36 ± 8.96
Negative Control	99.59 ± 13.62	99.95 ± 15.34
Positive Control	38.92 ± 5.79	25.15 ± 2.35

SD: Standard Deviation.

Concerning the cement type, the positive control showed the lowest rate of cell viability (Figure 1).

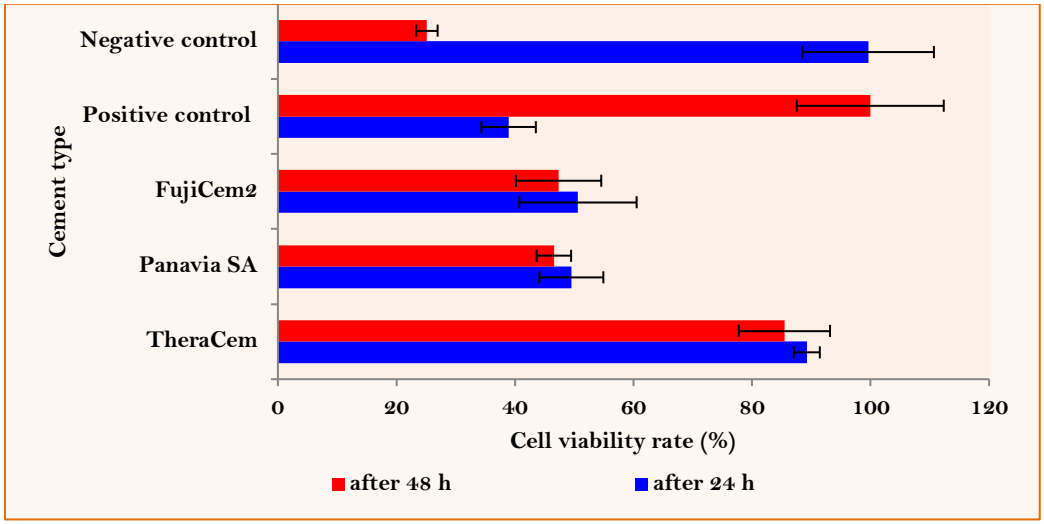

Figure 1. Means and 95% confidence interval (CI) of cell viability rates of the experimental groups (T: TheraCem, P: Panavia SA, G: FujiCem2) after 24 hours and 48 hours.

FujiCem2 and Panavia SA exhibited cell viability rates slightly higher than that of positive control. However, no significant difference was noted between FujiCem2 and Panavia SA in this regard. TheraCem and negative control showed the highest rates of cell viability, respectively. Among three cement types, only TheraCem showed a cell viability rate higher than 70%. FujiCem2 and Panavia SA were cytotoxic since their cell viability rates were less than 70%.

Discussion

This study aimed to compare the cytotoxicity level of the three commonly used cements during cementation procedures, namely a self-adhesive resin cement (Panavia SA), a self-adhesive resin cement reinforced by calcium (TheraCem), and a resin-modified glass ionomer cement (FujiCem2) on the human gingival fibroblast cells after 24 and 48 h of exposure. In the present study, we used human gingival fibroblast cells since in the oral cavity, cements are in direct contact with the gingiva and their possible cytotoxicity might affect the gingival fibroblast cells.

According to ISO 7405 [20], cements exhibit the highest level of cytotoxicity in the first 24 h. Therefore, we evaluated the cytotoxicity of cements after 24 h and 48 h to assess the correlation between the time and cytotoxicity level. Our results show that TheraCem exhibited less cytotoxicity than Panavia SA and FujiCem2. No significant difference was noted between Panavia SA and FujiCem2. It should be noted that higher levels of cytotoxicity do not necessarily lead to a higher rate of apoptosis and cell necrosis. Rather, it could indicate reduced metabolic activities in a higher number of cells [10].

Regarding the cement type, our findings are inconsistent with the results of a study conducted by Sismanoglu et al. [21]. They assessed the cytotoxicity level of four self-adhesive cements, including TheraCem, Panavia SA, Beauticem SA, and RelyX U200. Accordingly, Panavia SA showed the highest cell viability rate and TheraCem showed the least after 24 h. Differences between our results and their study might be due to the different sample preparation, extraction methods and curing times. Moreover, they transferred the cements to the extraction media after the completion of the chemical setting of the Panavia and TheraCem. This could have decreased the amount of monomer released in the extraction media since the chemical setting was completed before transferring into the extraction media.

Considering the relation between the time and the cytotoxicity level, we found no association between the time and cytotoxicity level after 48 h. However, some previous studies have reported the opposite; the cytotoxicity level of different cements has increased significantly after 72 h [21,22]. Therefore, one possible explanation might be that those studies have used longer periods for the extraction process.

According to the literature, 3 types of interactions happen between the monomers in the cement formulation: synergic, additive, and antagonist interaction. In other words, these interactions might increase or decrease the overall cytotoxicity of cement compared to the cytotoxicity of its individual components. In the first 24 h to 48 h, the antagonist interaction primarily occurs [23]. On the other hand, synergic and additive reactions mostly happen after 48 h. Thus, the cements possibly require longer periods of exposure time to exhibit their cytotoxicity, which was a limitation of our study.

Three factors are associated with the cytotoxicity level of cements; first, cement acidity induces cell apoptosis by increasing the activity of caspase. Second, the time duration which cement remains acidic, and third, the amount of monomers and unreacted components in the cement [21]. Accordingly, TheraCem PH increases in the first 24 h due to the formation of calcium hydroxide, which decreases the cement acidity in the

first 24 h while Panavia SA maintains its acidity for a longer time. This might explain why Panavia SA has exhibited higher cytotoxicity levels than TheraCem.

Moreover, Panavia SA contains various monomers such as Bis-GMA, TEGDEMA, UDMA, and HEMA in its formulation. Resin-modified glass ionomer cements such as FujiCem2 also contain HEMA and TEGDEMA and their cytotoxicity has been established in a previous study [10]. Despite the Panavia SA and FujiCem2, the amounts of HEMA and Bis-GMA monomers in TheraCem formulation are significantly low (<5%) [24]. The cytotoxicity of these monomers have been shown in the previous studies [25,26]. Lower concentrations of these monomers in TheraCem composition might contribute to the less cytotoxicity level of this cement compared to Panavia SA and FujiCem2.

It is noteworthy to mention that both FujiCem2 and TheraCem contain fluoride. High doses of fluoride can cause damage to the antioxidant system and induce inflammation and cell apoptosis [27]. However, the fluoride amount in TheraCem is low and insufficient to cause such effects.

Sufficient polymerization of methacrylate-based materials is necessary to improve their biocompatibility [10]. If the curing energy of the cement is inadequate, high amounts of unreacted monomers remain in cement mass and might increase the cement cytotoxicity. In the present study, we used the curing times suggested by the manufacturers; 20 s for TheraCem and 10 s for Panavia SA and FujiCem2. It is possible that 10 s of curing have not been sufficient for complete polymerization of Panavia and FujiCem2 and thus, a higher amount of unreacted monomers have remained in these two cements, which has increased the cytotoxicity levels of these two cements.

Finally, there is another speculation to explain the underlying cause of the higher cytotoxicity level of FujiCem2 cement; according to the literature, resin-modified glass ionomer cements such as FujiCem2 undergo more marginal destruction in the oral cavity compared to conventional resin cements. Consequently, higher amounts of monomers might release from resin-modified glass ionomer cements during marginal destruction, which is attributed to the higher levels of cytotoxicity [28,29].

The main limitation of the present study is that the study setting does not completely simulate the clinical conditions. For instance, the cytotoxicity level of the cements was evaluated using MTT assay. However, in MTT assay, the cells are directly exposed to the tested materials, while in the oral cavity, harmful materials are constantly washed out by the help of circulating saliva. Thus, MTT assay might exhibit exaggerated results compared to the clinical setting. Moreover, in the oral cavity, restorations act as barriers between the cement and light cure device and interfere with the polymerization process while we directly cured the specimens using a light cure device without any interference.

Further studies are recommended to evaluate the cytotoxicity level of cements by using other cytotoxicity evaluation methods rather than the MTT assay. Moreover, investigating the association between the cement concentration and cytotoxic effect on human gingival fibroblast cells is recommended.

Conclusion

TheraCem showed the least cytotoxicity level, and Panavia SA and FujiCem2 had no significant difference in cytotoxicity level. Additionally, Time had no significant effect on the cytotoxicity level of each cement type. Only TheraCem showed a cell viability rate higher than 70% and was considered non-toxic.

Authors' Contributions

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All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support the findings of this study can be made available upon request to the corresponding author.

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