Evaluation of the cytotoxicity of selected conventional glass ionomer cements on human gingival fibroblasts

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Abstract

Background. Dentistry materials are the most frequently used substitutes of human tissues. Therefore, an assessment of dental filling materials should cover not only their chemical, physical, and mechanical characteristics, but also their cytotoxicity.

Objectives. To compare the cytotoxic effects of 13 conventional glass ionomer cements on human gingival fibroblasts.

Material and methods. The assessment was conducted using the MTT test. Six samples were prepared for each material. Culture plates with cells and inserts with the materials were incubated at 37°C, 5% CO_2, and 95% humidity for 24 h. Then the inserts were removed, 1 mL of MTT was added in the amount of 0.5 mg/1 mL of the medium, and the samples were incubated in the described conditions without light for 2 h. The optical density was measured with an absorption spectrophotometer at a wavelength of 560 nm.

Results. The cytotoxic effects of the Argion Molar was significantly stronger than the Fuji Triage ($p = 0.007$), Chemfil Molar ($p < 0.0001$), and Ionofil Molar AC Quick ($p < 0.001$). The Fuji IX GP and Fuji IX Extra had a significantly stronger adverse effect than the Chemfil Molar ($p = 0.014$, $p = 0.029$, respectively) and Ionofil Molar AC Quick ($p = 0.017$, $p = 0.034$, respectively). The cements from the low cytotoxicity group were significantly more toxic vs materials whose presence resulted in fibroblast growth ($p < 0.001$).

Conclusions. The research conducted indicates that, although the materials studied may belong to the same group, they are characterized by low, yet not uniform, cytotoxicity on human gingival fibroblasts. The toxic effects should not be assigned to a relevant group of materials, but each dentistry product should be evaluated individually.

Key words: dentistry, fibroblasts, glass ionomer cements

Due to developments in science and technology, there are numerous products for the reconstruction of tooth tissues currently available on the dentistry market. According to Tilberg et al., dentistry materials are the most frequently used substitutes of human tissues.[1] They can affect surrounding structures either directly or indirectly when substances released by the fillings migrate through dentin channels into the pulp during the curing process and/or after it is completed. Therefore, the assessment of dental filling materials should cover not only their chemical, physical and mechanical characteristics, but also their biocompatibility, which is understood as a material's ability to function in live organisms and induce the appropriate tissue response.[2,3] Its measures include cytotoxicity, that is, the effect of a studied material on cell viability.[2] Cytotoxicity is a complex process, as there are numerous mechanisms causing functional and structural changes in cells and tissues.[2,4]

Glass ionomer cements were launched in the dentistry market in the 1970s.[5–8] Their composition was based on fluoroaluminosilicate glass and a liquid part, usually a water solution of polyalkenoic acid.[5–8] Unfortunately, because of their disadvantages, including low mechanical strength, long setting time and high sensitivity to moisture at the beginning of curing, the first products from this group were criticized by clinicians.[5–8] However, these materials had some indisputable advantages: chemical adhesion to mineralized tooth tissues, remineralization and antibacterial properties.[5–8] For these reasons, conventional glass ionomer cements (GIC) have been modernized. They are widely used in dentistry, particularly as materials for reconstructing missing hard tissues in deciduous teeth.

Literature data on the biocompatibility of glass ionomer cements is inconsistent.[9] Many authors suggest their high biocompatibility.[10–13] However, there are reports noting an adverse effect of these materials on live cells.[14–16] Differences in the results obtained by researchers may result from variability in research protocols.[17,18] For this reason, the aim of our study was to compare the cytotoxicity of currently available conventional glass ionomer cements in identical conditions and to verify contradictory reports on their biocompatibility.

Material and methods

Material sample preparation

The test was conducted for 13 conventional glass ionomer materials (GIC), including 2 reinforced with silver (MGIC) in color A3 (Table 1).

The cements studied were packed in capsules. They were prepared immediately before the test, in sterile conditions, using a crusher and shaker as specified by the manufacturer. The prepared materials were applied into plastic rings

Table 1. Materials tested in the study

Material	Manufacturer	Lot
Argion Molar	VOCO GmbH, Cuxhaven, Germany	0936172
Fuji IX GP	GC Corp., Tokyo, Japan	0904201
Fuji IX Extra	GC Corp., Tokyo, Japan	0904211
Riva Silver	SDI Ltd., Bayswater, Australia	G1005031
Fuji IX Fast	GC Corp., Tokyo, Japan	0812174
Fuji Triage	GC Corp., Tokyo Japan	0806051
Chemfil Molar	DENTSPLY De Trey GmbH, Kostanz, Germany	0817100
Ionofil Molar AC Quick	VOCO GmbH, Cuxhaven, Germany	0912304
Ketac Silver	3M ESPE AG, Seefeld, Germany	328538
Ketac Molar Aplicap	3M ESPE AG, Seefeld, Germany	366335
Riva SC	SDI Ltd., Bayswater, Australia	A1004292
Ketac Molar Quick	3M ESPE AG, Seefeld, Germany	387995
Ketac Fil Plus Aplicap	3M ESPE AG, Seefeld, Germany	365422

of 5 mm (inner diameter) × 5 mm (height). The rings with the materials were placed in inserts (Nunc GmbH&Co KG, Wiesbaden, Germany), of a surface area of 0.47 cm^2 and pore diameter of 0.4 µm, which were located in 24-well culture plates (Nunc GmbH&Co KG, Wiesbaden, Germany) containing human gingival fibroblasts. Six samples were prepared for each material. Six wells with inserts without any material constituted the control.

Cell culture preparation

Human gingival fibroblasts from the adherent permanent cell line (ATCC® CRL-2014HGF-1 (LGC Promochem, Warszawa, Poland) were grown in Falcon containers (growth area of 75 cm^2) on DMEM (Dulbecco's Modified Eagle's Medium) (Gibco, Warszawa, Poland) with 10% fetal bovine serum (FBS) (Gibco, Warszawa, Poland) added, at 37°C, 5% CO$_2$, and 95% humidity. When the confluent growth was obtained, the cells were incubated with 0.25% trypsin solution with 0.53 mM EDTA added. Then, a medium with 10% FBS was added to inhibit enzyme activity. The cell suspension diluted in a fresh medium was inoculated in 24-well plates and incubated for 24 h.

Cytotoxicity evaluation

The cytotoxicity of the materials studied was evaluated using the MTT test. It is an indirect method determining cell viability and proliferation on the basis of mitochondrial succinate dehydrogenase activity. In live cells, this enzyme reduces yellow tetrazole salt,

3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide, to formazan precipitating as insoluble grey-purple crystals. The intensity of the solution color after dissolving the crystals, measured by a spectrophotometer, is a measure of cell viability. For low cell survival, low enzymatic activity is found resulting in a low content of purple formazan and lower optical density values.

The culture plates with the cells and applied materials were incubated at 37°C, 5% CO_2, and 95% humidity for 24 h. Then, the inserts with the materials were removed, and 1 mL of medium containing 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) was added to each well at a level of 0.5 mg/mL, and the plates were incubated without light in the conditions described above for 3 h. Afterwards, the fluid was aspirated from the culture, and 1 mL of isopropanol acidified with hydrochloric acid was added. To dissolve the formazan crystals, the solution obtained was stirred for a short time. The optical density (OD) was measured with a double-beam absorption spectrophotometer Lambda EZ 2001 (Perkin Elmer, Waltham, USA) at a wavelength of 560 nm. Cell viability was calculated using the following formula: [mean OD of test group/ mean OD of control group] × 100%.

Cell viability was scored according to the method by da Silva et al.[19] If cell viability exceeded 90%, the material was deemed non-cytotoxic. For cell viability at the 60–90% range, the material was regarded as slightly cytotoxic. For cell viability at the 30–59% range, the material was regarded as moderately cytotoxic. For cell viability below 30%, the material was considered severely cytotoxic.

Statistical analysis

The statistical analysis was performed using the STATISTICA v. 8.0 (StatSoft Sp. z o.o., Kraków, Poland) software package. For comparisons between the groups, the one-way analysis of variance ANOVA and post hoc Tukey's HSD (honestly significant difference) tests were used. The hierarchical cluster analysis with a dendrogram, using the average linkage between groups, was used as the classification method. The level of significance was set at $p < 0.05$.

Results

None of the 13 materials exhibited high or medium cytotoxicity. In all cases, fibroblast survival exceeded 60%. The group with low cytotoxicity consisted of 4 glass ionomer cements: conventional – Fuji IX GP and Fuji IX Extra; and silver-reinforced – Argion Molar and Riva Silver. Other materials studied had no adverse effects. More than 90% of fibroblasts survived in the presence of Fuji IX Fast, Fuji Triage, Chemfil Molar, and Ionofil Molar AC Quick; while in the vicinity of Ketac Silver, Ketac Molar

Fig. 1. Mean cell viability ratios and standard deviations of the materials tested
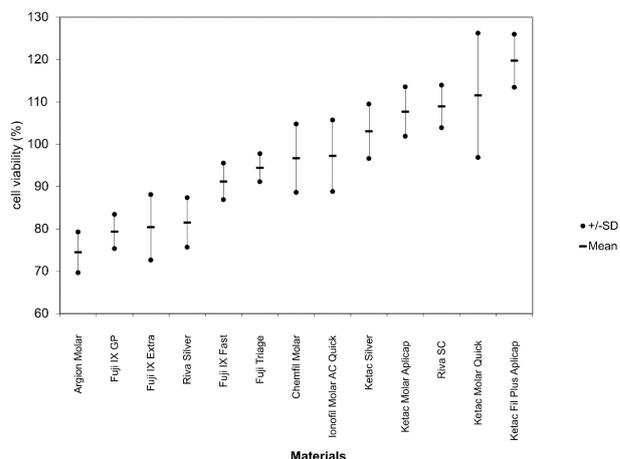
Aplicap, Riva SC, Ketac Molar Quick, and Ketac Fil Plus Aplicap, they multiplied.

One-way variance analysis indicated significant differences in cell survival with different materials ($p < 0.001$). Assessment with Tukey's post hoc test showed that the effect of materials with low cytotoxicity did not differ significantly. A similar relationship applied to the group of materials without an adverse effect on fibroblasts.

Significant differences were found between materials from different groups (Fig. 1).

The cytotoxic effect of Argion Molar was significantly stronger than Fuji Triage ($p = 0.007$), Chemfil Molar ($p < 0.0001$) and Ionofil Molar AC Quick ($p < 0.001$). Fuji IX GP and Fuji IX Extra had a significantly stronger adverse effect than Chemfil Molar ($p = 0.014$ and $p = 0.029$, respectively) and Ionofil Molar AC Quick ($p = 0.017$ and $p = 0.034$, respectively). Cements from the low cytotoxicity group were significantly more toxic vs materials whose presence resulted in fibroblast growth ($p < 0.001$).

The dendrogram (Fig. 2) presents three separate clusters of materials that are most similar to each other in terms of cell survival ratio in the culture.

The tested materials with the most similar cytotoxicity are connected by vertical lines and form a cluster. The position of the lines on the scale (at the top of the figure) indicates the distances between clusters: the closer to the scale center, the greater similarity in cytotox-

Fig. 2. Similarities in the toxic effect of the materials examined
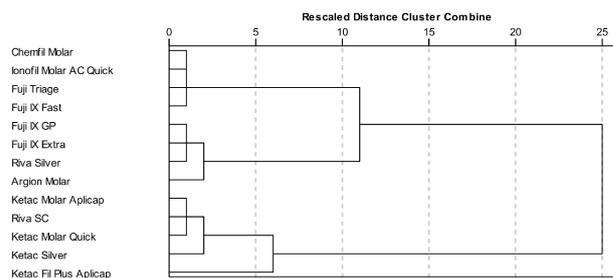
icity. We found the greatest similarity in effects on the viability of fibroblasts in the following groups: Chemfil Molar (96.7%), Ionofil Molar AC Quick (97.2%), Fuji Triage (94.4%) and Fuji IX Fast (91.2%) in the first cluster; the second cluster – Fuji IX GP (79.4%), Fuji IX Extra (80.4%), Riva Silver (81.5%); and the third cluster consisted of Ketac Molar Aplicap (107.7%), Riva SC (108.9%), Ketac Molar Quick (111.5%), and Ketac Silver (103.0%).

Discussion

Studies evaluating the biological characteristics of dentistry materials have been conducted for many years. They have used various methods, including in vitro tests on cell cultures, pre-clinical studies on laboratory animals and clinical trials in patients.[2–4] The use of animals for biocompatibility assessments of dentistry materials represents an ethical problem and has been widely debated. For this reason, the International Organization for Standardization (ISO) recommends in vitro studies with cell cultures and limiting tests conducted on animal models.

Cytotoxic activity can be determined using various laboratory tests. The MTT test is recommended by ISO as a reference, and was used in our experiment. It determines the activity of succinate dehydrogenase, a mitochondrial enzyme present in live cells.[19]

Selection of an appropriate cell line is a very important part of the study during in vitro cytotoxicity assessments. Researchers' opinions on that issue vary. The use of permanent, standard cell lines or primary cells collected from gingiva, periodontium or the pulp is recommended. Permanent cell lines are morphologically and physiologically uniform. Primary cells represent clinical conditions better, but are diversified and have lower viability.[20] The ISO 10993 standard, standardizing in vitro studies, supports the use of permanent cell lines.[2,4,12] In our study, we used a standard human gingiva fibroblast line; and to reproduce conditions similar to clinical, we placed samples of materials on a semi-permeable membrane of inserts.

The study conducted indicates that the evaluated materials were characterized by low or lack of cytotoxicity. Schedle et al. obtained completely different results.[16] Using a flow cytometry method, the authors compared the cytotoxicity of various dentistry materials, including 2 conventional glass ionomer cements. On the basis of the study results, they suggested that for the evaluated cements, the adverse effects on fibroblasts were comparable to the effect of composite materials considered to be very cytotoxic. An unfavorable opinion on the biological characteristics of glass ionomer cements was presented in the study by Milhem et al.[14] They conducted an experiment using Artemia Salina larvae exposed to alcohol eluates of the assessed materials. The results indicated that Ketac Fil cement was more toxic than the composite materials.

In a majority of the published studies, the authors evaluating the biological effects of conventional glass ionomer cements showed that they were characterized by low cytotoxicity.[10–13] The results of our study are consistent with that finding. The group with low toxicity contained 4 materials: Argion Molar and Riva Silver (conventional glass ionomer cements reinforced with silver), and Fuji IX GP and Fuji IX Extra, differing significantly from the remaining 9 products.

The reasons for the cytotoxicity of the evaluated preparations have not been sufficiently explained. The literature contains various interpretations of this phenomenon. The authors emphasize the effect of low pH during setting and the effects of various released components.[17,21–23] Migration of certain ions is most often mentioned. The release of fluoride ions from glass-ionomer cements is commonly known.

Kan et al. investigated the cytotoxicity and fluoride release of 2 resin-modified GICs, a conventional glass-ionomer cement, and a resin composite.[24] Fluoride release and cytotoxicity were correlated, although the fluoride release did not account for the cytotoxicity observed. In their opinion, there were factors other than fluoride responsible for the cytotoxicity. According to Wilson et al., the following ions are released from conventional glass-ionomer cements: F, Na and Si.[25] Recent studies by Nicholson et al. have also demonstrated the release of Al, P, and Ca, depending on the pH solution.[26] The effect of different ions on the cytotoxicity of these materials is still unclear. In the authors' opinion, ions, apart from aluminum, are acceptable in the body and useful for a variety of physiological processes.[26] However, the total amount of aluminum released from glass-ionomer cements is so low that this is not a significant problem.[27] As indicated by Forss,[28] the ion amount leached to the environment is associated with the material's composition. In Tyas' opinion, contemporary glass-ionomer cements consist of fluoroaluminosilicate glass, usually a strontium or calcium salt, and polyalkenoic acid liquid, for example polyacrylic, maleic, itaconic, and tricarballylic acids.[6] The exact chemical compositions of the materials are not provided by the manufacturers.

Stanisławski et al. studied the cytotoxic effects of several ions released from different materials.[23] That study indicates that the F^-, Al^{3+} and Sr^{2+} levels were too low to damage cultured cells. In these authors' opinion, the main factors responsible for cytotoxicity were the presence of copper and silver ions in the case of HiDense – a conventional glass ionomer cement reinforced with silver. In our experiment, we did not study ion release, but the cytotoxicity of Argion Molar and Riva Silver may be related to a similar mechanism.

The studies by Stanisławski et al. and Soheili Majd et al. expanded our knowledge on biochemical mechanisms underlying the cytotoxicity of glass ionomer cements.[15,29] The above-mentioned authors proved that Fuji II and

Ketac Fil Plus Aplicap caused a reduction in cellular glutathione (GSH), one of the most important antioxidants in living organisms. The process of cellular GSH reduction is not fully known yet. Oxidative stress can be a possible cause underlying that phenomenon, possibly dependent on the presence of even small quantities of aluminum and/or iron ions.[23,29]

The results of laboratory experiments should not be directly extrapolated to clinical conditions. According to many authors, during tooth tissue reconstruction, the thickness and permeability of dentine remaining in the cavity should also be considered.[30] Forming a partial barrier, it can reduce the cytotoxic potential of the materials by limiting the availability of water required for hydrolysis of the released components (reduced diffusion) and buffering capacity of hydroxyapatites.

Our research indicates that although the studied materials may belong to the same group, they are characterized by low, yet not uniform, cytotoxicity. This is probably related to differences in their chemical composition, which remains a trade secret of the manufacturers.

Conclusions

It seems fair to conclude that a toxic effect should not be assigned to a relevant group of materials, but each dentistry product should be evaluated individually.

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