Original Article

MedInform

DOI: 10.18044/MEDINFORM.2024111.1762

ISSUE 1, 2024

A study of the cytotoxicity of resin-modified glass-ionomer cement

Janet Kirilova

Department of Conservative Dentistry, Faculty of Dental Medicine, Medical University, Sofia, Bulgaria

Abstract

Background. It is crucial to maintain the vitality of the dental pulp by using biologically tolerable materials when placed on dentin near the dental pulp. Calcium-hydroxide cement has been established as such a material. Resin-modified Glass-ionomer cement (RMGIC) is another group of materials. The results on the biological tolerance of different types are contradictory.

Aim. Therefore, we conducted a study to compare the cytotoxicity of glass-ionomer cement available in powder and liquid packages with individual capsule doses on cell cultures.

Material and methods. The materials used in this study are zink phosphate cement AdhesorTM Fine (GR.1), resin-modified glass-ionomer cement Fiji LC II (Gr.2) powder-liquid package; Fiji LC II (Gr3) individual capsules package, calcium-hydroxide cement Basic L (Gr.4). The study was conducted on human skin-muscle embryonic fibroblasts (diploid cells) cell cultures.

Results. The study's results indicate that RMGIC Fuji LC II and calcium-hydroxide cement studies have approximately the same degree of relative growth on cell cultures. However, in powder-liquid packaging, the results deteriorate over time, while individual ones retain their qualities.

Conclusions. The present study's limitation is that RMGIC packages influenced the materials' cytotoxicity degree. Individual capsules of RMGIC had a better degree of cytotoxicity close to the calcium-hydroxide cement. Further research is needed along these lines.

Keywords: Cytotoxicity, dental pulp, resin-modified glass-ionomer cement, individual doses

Introduction

It is crucial to maintain the vitality of the dental pulp by using biologically tolerable materials when placed on dentin near the dental pulp (1). Calcium-hydroxide cement has been established as such a material (2). Glass-ionomer cement (GIC) is another group of materials with significant advantages such as chemical adhesion of hard dental tissues, fluorine emission for caries protection, similar coefficient of thermal expansion with hard dental tissues, modulus of elasticity similar to dentin, and good biological tolerance(3). However, our research has shown that some glass-ionomer cements are not always biologically tolerable when placed close to the dental pulp (3). In addition, resin-modified glass-ionomer cement (RMGIC) is available in powder-liquid and capsule options for machine stirring in single doses. According to our previous studies, the cytotoxicity of some RMGIC approaches that of calcium-hydroxide cement. Still, its cytotoxicity deteriorates over time when using the same packaging (3).

Aim

We conducted a study to compare the cytotoxicity of glass-ionomer cement available in powder and liquid packages with individual capsule doses on cell cultures.

Materials And Methods

The materials used in this study are zink phosphate cement AdhesorTM Fine (Pentron), calcium hydroxy cement Basic L (Icoclar, Lichtenstein), resin-modified glass-ionomer cement Fiji LC II (International CG Corp.) powder-liquid package; Fiji LC II (International CG Corp.) individual capsules package.

The study was conducted on human skin-muscle embryonic fibroblasts (diploid cells) cell cultures - about 100,000 cells per millilitre. The culture medium contains diploid EAGLE-90% 25 µg/ml gentamicin, 50 µg/ml penicillin, 50 µg/ml streptomycin, and 1.25 µg/ml amphotericin B and calf serum - 10%. Cells were subcultured for one week, then transferred to a medium containing 0.02% versen, 25 µg/ml crystalline trypsin in sterile phosphate buffer pH 7.2. The thus prepared cell suspension as a stationary cell culture is poured into a suitable laboratory vessel - Petri dishes. The following dental cements were tested:

- Group 1 -Adhesor -zinc phosphate cement (as a negative control);
- Group 2 -Fuji LC II -glass-ionomer cement- powder and liquid;
- Group 3 -Fuji LC II -glass-ionomer cement- single capsule;
- Group 4 –Basic L –calcium-hydroxide cement (as a positive control);

The materials were prepared according to the manufacturer's instructions, and pieces with a size of 0.00200 ± 0.00050 grams were measured using an analytical balance. Thus, prepared samples of 10 pieces of material are placed in a thermostat for 24 hours at 37°C at 50% humidity to complete the curing processes. Each petri dish is then gassed with CO_2. Test specimens are placed in the centre of a petri plate. We put 5 ml of cell suspension in each petri dish. Ten Petri dishes are prepared for each material. With each experiment, control from the cell culture is also left, which, under equal conditions of passage, nutrient medium, temperature, pH, and others, shows the reliability of the changes in the experiment. Cultivation and maintenance of the medium with cell cultures is carried out at 37°C, an atmosphere of 5% CO_2 and 95% relative humidity. Results are reported at the 24th and 72nd hour.

The study is repeated after one year using the same methodology and for the same time intervals. The dental materials and packaging are the same. During this period, pads and stoppers were made from them. They are stored at room temperature.

Changes in the culture medium were observed under an inverted phase-contrast microscope at the same magnification (x 160). Evaluation of the tested materials is done based on the morphological changes. The pH of the medium is also tested using a standard indicator method. The results are calculated according to the following parameters:
1. Relative growth of cells in the culture – calculated in percentages;
2. Cell morphology;

Cell cultures are stained with trypan blue. The dye is permeable only to dead cells; live cells do not stain.

Results

The results of the relative growth rate are shown in Table No. 1.

Table No. 1. Degree of the relative growth of cell cultures under the influence of the studied materials.

Periods / Materials	Period A				Mann-Whitney P<0,05	Period B – after 12 months				Mann-Whitney P<0,05
	24th hour		72nd hours			24th hour		72nd hours		
	N	%	N	%		N	%	N	%	
Gr.1 Adhesor	5	13±3,46	5	14,6±3,57	P=0,451[b]	5	10,8±2,77	5	13,8±2,58	P=0,115[b]
Gr.2 Fuji LC II powder and liquid	5	81,8±2,58	5	96,6±3,13	**P=0,009[a]**	5	74±2,91	5	75,6±2,30	P=0,343[b]
Gr.3 Fuji LC II in single doses - capsule	5	83,7±2,58	5	94,1±3,13	**P=0,009[a]**	5	87,6±3,13	5	95,8±2,71	**P=0,009[a]**
Gr.4 Basic L	5	87,8±2,16	5	97,8±2,77	**P=0,009[a]**	5	87,8±2,16	5	96,6± 3,78	**P=0,009[a]**
	5	100	5	100		5	100	5	100	

Note: the 24th and 72nd hours of the study (marked with A - after opening the packages) and after 12 months - the 24th and 72nd hours (marked with period B - used containers after 12 months). A indicates statistical significance (p< 0.05), and B shows a lack of statistical significance (p> 0.05).

The results for zinc phosphate cement(gr.1) show no statistically significant difference for the individual times in periods A and B and when comparing the results between different periods. This material maintains low relative cell growth (13-14.6%). It is probably related to its composition's longer-lasting emission of toxic substances. Phosphoric acid is emitted up to 6 days after its hardening.
The results for calcium hydroxide cement(gr.4) show a high degree of relative growth (87.8% to 97.8%). 100% was not reached for the periods studied, although calcium hydroxide cements are considered the

gold standard in pulp capping agents. There is a statistically significant difference between the 24th and 72nd hours for periods A and B (p=0.009). No statistically significant difference was found initially and after using the packaging material for one year. The type of packaging that does not allow material contamination is probably essential here since it is in individual packaging.

The results for Fuji-LC II(gr.2) showed good indicators regarding the relative growth rate of cell cultures at the 24th and 72nd hours (81.8% to 96.6%) (p=0.009) of period A. For the second study period B (use of the power-liquid material after one year), the results worsened (74% to 75.6%), with no statistical significance between the 24th and 72nd hours of the experiment. Comparing the results after opening the package and using the material for one year shows a statistically significant decrease in the relative growth rate of the cell cultures ($p<0.05$).

One year after using the material from the package, Fuji LC II cement provoked a statistically significantly lower degree of relative cell growth than the first study. Cells adjacent to the Fuji LC II material have a normal morphology with a regular spindle shape. In single cells, roundings are observed in the protoplasm.

The results for Fuji-LC II(gr.3) showed good indicators regarding the relative growth rate of cell cultures at 24 and 72 hours (83.7% to 94.1%) (p=0.009) of period A. For the second study period B (use of the single capsule after one year), the results are almost the same (87.6% to 95.8%), with no statistical significance between the 24th and 72nd hours of the experiment.

Discussion

Cell culture studies are crucial in establishing the cytotoxic effect of the investigated types of cement. They are also a reliable and accessible method for analysing dental materials (4).

It is characteristic of the group of resin-modified glass-ionomer cement that there is a difference in the degree of relative growth of the cell cultures at the 24th hour compared to the corresponding one at the 72nd hour of incubation (3). It is lower for Fuji-LC II and is close to that of calcium-hydroxide cement. Regardless of the incubation time, the most pronounced cytotoxic effect was exerted by phosphate cements both at the 24th hour and at the 72nd hour. Calcium-hydroxide cement is the "gold standard" of pulp capping agents. The present study confirms these results.

Calcium-hydroxide cement has an alkaline pH and stimulates the formation of protective dentin. When calcium hydroxide interacts with tissue fluid buffers, phase transformations occur. With this, the mechanical resistance of the calcium hydroxide cement is disturbed. As a result, the cement disintegrates over time (5). Gaps are formed at the place of their placement, which is unfavourable in the treatment of dental caries (5). Beneath calcium-hydroxide cement, a dentin bridge is formed with tunnels, and the dentin is not dense (2). Numerous calcifications are included in the pulp, representing a severe difficulty in future endodontic treatment (5).

Calcium-hydroxide cements do not adhere adhesively to dentin and disintegrate after etching. After about two years of placement, they could break the adhesive bond of materials for restoration, thereby increasing microleakage. Today, this leads to the search for alternative materials such as pulp coating agents – mineral trioxide aggregate, propolis, and Biodentine. Evidence shows that a dentine bridge is formed in more teeth than calcium-hydroxide cement (4).

Studies by Muller on the cytotoxicity of calcium-hydroxide cement found different degrees of relative tissue culture growth in Dyract and KERR (6). By the third day of the experiment, it was between 75%-99% (grade 4 according to the index of Nacamura, 1983). According to J. Muller, for the later study periods - the sixth and ninth days of the experiment, the cytotoxicity of Dycal worsened from 25% to 49% (6). Our results for Basic L at 24 hours are 87% and 72 hours at 96%.

Sidhu SK and Schmalz G. summarise the results of various studies on the cytotoxicity of glass-ionomer cement. They conclude that a severe reaction of the revealed dental pulp is observed with direct pulp capping, noting that a thin layer of dentin protects against the components of dental materials (9). Our previous study also confirmed that different cytotoxicity to cell cultures was observed in various types of glass-ionomer cement (3). However, it was proved that RMGIC Fuji LC II and calcium-hydroxide cement Basic L had similar cytotoxicity. Interestingly, when this study was repeated after a year from the same package, the results changed; the same cement showed degraded cytotoxicity. This gave grounds to include a capsule pack in the present study. The present study found no deterioration of the cytotoxicity indicator when a capsule packaging of the glass-ionomer cement Fuji – LC II was used.

The study was repeated using the same methodology after one year with the same materials stirred manually and from the same packaging. For the period between the two studies, the materials were used in clinical settings and stored at room temperature. In the second study, the RMGIC Fuji LC II showed a statistically significantly higher degree of cytotoxicity than the first period (in the initial research, the qualities of the material were close to calcium-hydroxide cement). Probably during the use of materials, the residue in their packaging is contaminated bacteriological, which worsens their medico-biological qualities. The calcium-hydroxide cement used as a positive control in this study did not change its values after one year of use from the same package. It was probably due to its non-contaminating packaging (tubes).

A study by Chumpraman and co-authors proved that including bioactive molecules in the composition of modified glass-ionomer cement leads to the proliferation of cells in stem cells from dental pulp and potentiates calcium deposition. This study discovered possibilities for investigated modifications of glass-ionomer cement and direct pulp capping (7).

Similar studies by Hii and co-authors prove that nano RMGICs have a low degree of cytotoxicity on cell cultures of dental pulp relative to conventional GICs (8).

Induction of dentine regeneration is an essential property of the materials used for direct and indirect pulp capping. A study by Martins MD, Tuygunov N and co-authors found good biotolerance and osteoinductive potential of glass-ionomer cements in bone healing in fractures (9,10).

Conclusion

The present study's limitation is that RMGIC packages influenced the materials' cytotoxicity degree. Individual capsules of RMGIC had a better degree of cytotoxicity close to the calcium-hydroxide cement. Further research is needed along these lines.

References

1. Duncan HF, Present status and future directions- vital pulp treatment and pulp preservation strategies. Int Endod J.2022:55(suppl. 3):497-511.
2. Kitasako Y, Ikeda M, J.Tagami J. Pulpal responses to bacterial contamination following dentin bridging beneath hard-setting calcium hydroxide and self-etching adhesive resin system. Dent. Traumatol. 2008;24: 201-206.
3. Kirilova J. Glass-ionomer cement as a biomaterial for the treatment of proximal dental caries of chewing teeth ("sandwich" restoration). PhD thesis, Sofia, 2013: 230.
4. Sidhu SK, Schmalz G. The biocompatibility of glass-ionomer cement materials. A status report for the American Journal of Dentistry. Am J Dent. 2001 Dec;14(6):387-96. PMID: 11949800.
5. Kuzmanova Y, Dimitrova I. Endodontic Perforations. Modern clinical approaches and tools in treatment. Sofia, 2021, SOFTTRADE, 211.
6. Muller J. et al. An experimental study on the biocompatibility of liting cements based on glass-ionomer as compared with calcium hydroxide. DentMater. 1990;6(1): 35-40.
7. Chumpraman A, Tannukit S, Chotigeat W, Kedjarune-Leggat U. Biocompatibility and mineralization activity of modified glass ionomer cement in human dental pulp stem cells. J Dent Sci. 2023 Jul;18(3):1055-1061. doi: 10.1016/j.jds.2022.11.024. Epub 2022 Dec 5. PMID: 37404606; PMCID: PMC10316452.
8. Hii SC, Luddin N, Kannan TP, Ab Rahman I, Nik Abdul Ghani NR. The Biological Evaluation of Conventional and Nano-Hydroxyapatite-Silica Glass Ionomer Cement on Dental Pulp Stem Cells: A Comparative Study. Contemp Clin Dent. 2019 Apr-Jun;10(2):324-332. Doi: 10.4103/ccd.ccd_581_18. PMID: 32308298; PMCID: PMC7145261
9. Magalhães GAP, Thomson JJ, Smoczer C, Young LA, Matos AO, Pacheco RR, Souza MT, Zanotto ED, Puppin Rontani RM. Effect of Biosilicate® Addition on Physical-Mechanical and Biological Properties of Dental Glass Ionomer Cements. J Funct Biomater. 2023 May 30;14(6):302. doi: 10.3390/jfb14060302. PMID: 37367266; PMCID: PMC10299142.
10. Tuygunov N, Zakaria MN, Yahya NA, Abdul Aziz A, Cahyanto A. Efficacy and bone-contact biocompatibility of glass ionomer cement as a biomaterial for bone regeneration: A systematic review. J Mech Behav Biomed Mater. 2023 Oct;146:106099. doi: 10.1016/j.jmbbm.2023.106099. Epub 2023 Aug 30. PMID: 37660446.

Corresponding author:

Janet Kirilova
Department of Conservative Dentistry, Faculty of Dental Medicine
Medical University, Sofia;
1, St. Georgi Sofiiski blvd., 1431 Sofia, Bulgaria.
e-mail: janetkirilova@gmail.com

Kirilova J. A study of the cytotoxicity of resin-modified glass-ionomer cement. Medinform 2024; 11(1):1762-1767.