Original scientific papers

UDK 615.277:77.026.341
DOI 10.7251/COMEN2002141J

CYTOTOXIC EFFECT OF NEWLY SYNTHESIZED NANOMATERIALS FOR POTENTIAL DENTAL APPLICATION

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Abstract: Introduction: Biocompatibility is an essential feature of any dental material. Few materials can be said to be biologically inert since most contain potentially harmful or irritating ingredients. This study aimed to determine the cytocompatibility of newly synthesized nanomaterials based on calcium aluminates and calcium silicates for potential dental applications.

Material and methods: The cytotoxicity of calcium aluminate-based nanomaterials (*ALBO-CA*), calcium silicate (*ALBO-CS*) and calcium silicate hydroxyapatite (*ALBO-CSHA*) was examined using the MTT test on the human line of human fibroblasts (MRC-5) according to ISO standard (ISO 10993- 5: 2009) in comparison with the calcium aluminate cements EndoBinder (Binder was, São Carlos, SP, Brazil). For the analysis, the eluates of the investigated materials in the growth medium were diluted to a concentration of 4.7, 9.4, 18.8, 37.5 and 75, 0 mg. Qualitative verification of results was performed by a light microscope (Carl Zeiss). The mean values and standard deviations of the MTT test results were done in Microsoft Excel.

Results: All tested concentrations of *ALBO-CA*, *ALBO-CS*, and EndoBinder resulted in a high survival of cells in culture. The strongest cytotoxic effect was *ALBO-CSHA* with IC_{50} = 46.44 after the first cycle of testing; IC_{50} = 55.52 after the second cycle; or IC_{50} = 55.42 after the third repetition of the MTT test.

Conclusion: *ALBO-CA* and *ALBO-CS* nanomaterials have shown a cytocompatible effect comparable to EndoBinder. The obtained results are certainly encouraged to continue researching these materials in the future and other experimental and clinical studies.

Keywords: nanomaterials, calcium aluminates, calcium silicates, cytotoxicity.

1. INTRODUCTION

The fact that there is no ideal dental material on the market with ideal biocompatibility characteristics encourages the development of a large number of new materials to create a cement that causes mild and short-term inflammation of the surrounding tissue [1,2].

Any new synthetic material intended for medical use must undergo numerous tests to ensure the reliability of the application and the quality of the material for everyday clinical practice.

Biocompatibility is defined as the ability of the material to perform a certain function within the host organism after application, without causing a negative response [3,4].

The biocompatibility of dental materials is very important because they are in intimate contact with human oral tissues over a longer period. The characteristic of the material to interact with the biological tissue and thereby creating a stable bond is crucial for biocompatibility. Biocompatibility of the material is reflected in several parameters: (a) cytotoxicity (systemic and local), (b) genotoxicity, (c) mutagenicity, (d) carcinogenicity and (e) immunogenicity [5].

Initially, the biocompatibility of dental materials is most often performed through cytotoxicity tests, as cytotoxic materials cause an inflammatory reaction when in contact with the surrounding tissue, which can affect the outcome of the treatment [6,7].

The MTT test is one of the most commonly used methods for assessing the potential cytotoxicity of dental materials, thanks to simple and fast performance, precision and reproducibility [8].

In the last few years, the great influence of nanotechnology in all areas, including in the field of

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biomedical science, has been noted. The size of the nanomaterial particles (<100nm) is similar to the size of biological molecules and structures (proteins 5nm, organelles 100-200nm) [9]. Nanomaterials can mimic the surface properties of natural tissues and are highly cytocompatible and biocompatible, and show excellent properties for use in tissue engineering and regenerative medicine. These materials can overcome the problems of solubility and stability and ensure the release of the drug into the desired tissue. Extremely tiny nanoparticles representing drug carriers show a very rapid release of the drug substance and the ability to penetrate the target tissue. Nanopartner drugs of less than 200 nm can undergo lung and spleen filtering and facilitate drug penetration from the blood vessel into the tumor tissue or through various physiological membranes [10].

Nanomaterials, in addition to being very similar to biological tissues, also have a very favorable surface for the regeneration of bone and cartilage tissue due to the good adherence of proteins and their important role in cell regeneration. This is their main advantage over conventional or micron materials [11].

At the end of ten years, a slightly higher emphasis was placed on the research of calcium aluminate and calcium silicate elements. Calcium aluminate types of cement have several different advantages over calcium-silicate cement, including flexible adhesion time (depending on the additive) resistance to high temperature, better resistance to abrasion and improved resistance to aggressive environments [12,13]. Also, they possess greater mechanical strength than calcium silicate or calcium phosphate cement, the ability to add different filers and more flexible handling properties [13-15]. CAC, as opposed to other dental materials, shows significantly more resistance to caries due to nanostructured deposition on tissue walls, which further induces their increased corrosion resistance [13]. CACs also confirmed biocompatibility, exhibited bacteriostatic and antibacterial properties that are not primarily related to the pH or specific type of ion and their concentration or reducing agents [16, 17]. Instead, they mainly refer to the hydration process and the microstructure obtained during the hydration of the cement.

This study aimed to determine the cytotoxic potential of nanoparticles based on calcium aluminates and calcium silicates, synthesized by nanotechnology, an innovative combination of the hydrothermal sol-gel method and the methods of self-combating waves.

2. MATERIAL AND METHODS

The cytotoxicity testing of the material was performed on cell culture under in vitro conditions using the MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) at the Oncology Institute in Belgrade, following recommendations of International ISO Standard (ISO 10993-5: 2009, Part 5: Test for cytotoxicity: in vitro method).

2.1. Tested materials

The cytotoxicity of calcium aluminate nanomaterials (ALBO-CA), calcium silicate (ALBO-CS) and calcium silicate hydroxyapatite ALBO-CSHA were investigated. Calcium aluminate cement EndoBinder (Binderware, Sao Carlos, SP, Brazil) was used as controls.

For the synthesis of the active calcium aluminate system, it was necessary to first synthesize individual components: calcium aluminate ($CaOAl_2O_3$), calcium carbonate ($CaCO_3$) and monocyclic Bi_2O_3 or $BaSO_4$ (as an X-ray contrast).

The calcium aluminate phase was synthesized using $CaCl_2$ * $5H_2O$ and the aluminate salt (AlOOH) was obtained by hydrothermal treatment. Aluminum-2-butoxide is dissolved in a mixture of ethanol and water (in a ratio of 1: 4). This mixture was then heated to 85 [deg.] C., with vigorous stirring. After 2 hours of heating, the solution was cooled to room temperature, H_2SO_4 was added. The resulting solution was mixed with a stoichiometric amount of $CaCl_2x5H_2O$ (Merck, Germany) and transferred to an autoclave, where it remained for the next 5 hours at a temperature of 150 ° C and a pressure of 5 bar. The resulting gel was dried at 150 [deg.] C. for evaporation of water.

Calcium chloride tetrahydrate ($CaCl_2x4H_2O$) (Sigma-Aldrich, St. Louis, MO) was used as a precursor in the synthesis of calcium carbonate. The amount of 5 mmol $CaCl_2x4H_2O$ is dissolved in 50 ml of ethylene glycol (Sigma-Aldrich) by ultrasound at 40 ° C (Elmasonic S30H). Then, 10 mmol of $NaHCO_3$ was dispersed in 50 ml of ethylene glycol dropwise over 30 minutes with mechanical stirring. The resulting dispersion was then heated at 30 [deg.] C. for 30 min. Calcium carbonate was then separated from the murky solution by centrifugation (3.4 g, 30 min), washed several times in a mixture of water and ethanol (1: 4) and finally, only in water. Sulphonyl dodecyl sulfate (0.5%) was added as an agglomeration agent. The resulting nanoparticles

were then exposed to ultrasound for 30 min, with strong mechanical stirring for 5 hours. The resulting powder, after drying at 120 [deg.] C for 5 hours, was heated to 500 [deg.] C for one hour to give a calcium carbonate phase.

Monocyclic Bi_2O_3 was produced by calcination Bi $(NO_3)_3$ (Chemical, Croatia) at 450 ° C for 20 hours. This procedure was performed to obtain a stable tetragonal Bi_2O_3 phase, saturated with oxygen. In addition to Bi_2O_3, $BaSO_4$ is also used as a contrast agent.

The new calcium aluminate called ALBO-CA was obtained by mixing $CaCO_3$ and Bi_2O_3 or $BaSO_4$ with a calcium aluminate phase in a ratio of 2: 2: 1. This mixture was mixed with water (water: powder 1: 2) to obtain the consistency of the paste.

For the synthesis of the active calcium silicate system, the individual components were first synthesized: calcium silicate phase 2β-$CaSiO_4$ (β-C2S) and Ca_3SiO_5 (C3S) and calcium carbonate ($CaCO_3$). The synthesis of calcium carbonate proceeded in the manner described above.

The calcium silicate phase was synthesized using $CaCl_2$ x $5H_2O$ (Merck, Germany), and the silica salt was obtained by hydrothermal treatment. Stoichiometric amounts of $CaCl_2$ x $5H_2O$ (35.59 g), silica salt (15 g 30% salt solution) and C2S: C3S in a ratio of 2: 1 were used to obtain the silicate active phase (40% contained in the mixture). 4.55 g of Al ($C_2H_3O_2$) was added to this mixture to provide small amounts of the active C_3A phase (3.01%). As an oxidizing agent, to initiate the combustion reaction, 71.3 g of ammonium nitrate (NH_4NO_3) and 53.51 g of citric acid ($C_6H_8O_7CH_2O$) were added to the mixture, which was used as fuel during the combustion reaction. After drying at 80 [deg.] C to obtain the gel, all the samples were subsequently dried at a temperature of 150 [deg.] C to remove all the water between silica particles. In the next phase, the increase in temperature to 180°C caused the gel to burn. The gel gradually turned into a foam, in the end, there was strong self-sufficiency of the reaction of combustion with the release of a large number of gases. The rapid release of large quantities of gas products during combustion led to the dissipation of the heat of combustion and the temperature growth threshold, which reduced the possibility of early, partial synthesis of the primary particles, which is important for maintaining the final activity of the mixture. After such a high temperature and self-expansion of the fraction, the samples were quickly dried using copper plates to minimize the crystallization phase and obtained high reactivity of the obtained β-C2S and C3S phases. The freed black powder contained carbon residues and its calcification was continued at 650°C for 4 hours to finally produce a product with a small crystallite size. After this thermal treatment, the powder is further ground to obtain the silicate phases to be used in the final cement mixtures.

In addition to the β-C2S and C3S phases, which comprised 60% of the total amount of the mixture, additional components were used, such as calcium carbonate ($CaCO_3$) or dehydrated gypsum in the amount of 20% and $BaSO_4$ (Merck, Germany) also in an amount of 20% due to the X-ray contrast of the mixture.

The composition of ALBO-CSHA was: 40% hydroxyapatite 20% mixture of C2S and C3S in a ratio of 2:1, 20% calcium carbonate ($CaCO_3$) and 20% $BaSO_4$ (Merck, Germany). Both of these mixtures, after grinding, and better homogenization are mixed with water (water: powder in a ratio of 1: 2) to obtain cement paste.

2.2. Preparation of examined types of cement for MTT

Stock extracts of the examined ingredients were prepared in a complete feeding medium by incubating 100 mg of each cement in a 1 ml feeding medium for 24 hours at 37°C. The extracts were then centrifuged and filtered (0.20 μm), and diluted with a nutrient medium to the respective working concentrations. The feeding medium used is RPMI 1640 medium in which 3 mmol / L of l-glutamine, 100 mg / mL of streptomycin, 100 IU / mL penicillin, 10% FCS and 25 mM Hepes are added to pH 7.2 with a bicarbonate solution.

2.3. Nutrient base and reagents

PMI 1640 medium with and without phenol, L-glutamine, penicillin/streptomycin, HEPES and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were purchased from "Sigma Chemicals Co". Fetal chestnut (FCS) was obtained from the Veterinary Institute (Novi Sad). Immediately before use, MTT was dissolved in PBS at a pH of 7.2 at a concentration of 5 mg/ml and filtered through a Millipore filter (0.20 μm).

2.4. Cell line

In this work, a normal cell line purchased from the American Type Culture Collection, MRC-5 (normal human fibroblasts of the lungs), was used. The cells were grown in a single-layer culture, in a complete nutrient medium, at a temperature of 37 ° C

in air, enriched with 5% CO2 and saturated aqueous steam.

2.5. Determination of the intensity of the cytotoxic effect of the investigated cement

The cytotoxic effect of the tested tetraoxanes was evaluated indirectly by determining the survival of the target cells after their growth in the presence of these agents.

Cell survival (S-Engl. Survival) was estimated as the ratio of the number of surviving cells in the sample treated with the examined agent (NL) and the number of cells in the control sample in which the cells grew only in the presence of a nutrient medium (NK).

$$S\ (\%) = (N_L/N_K)\cdot 100$$

From these values, a diagram is presented which represents the percentage of survival of the cells in the function of the concentration of the test compounds. From this diagram, the IC50 value was determined (a concentration that reduces the survival of the target cells to 50% relative to the control sample).

2.6. Cell treatment (determination of cell survival)

The MRC-5 cells were piled into microtiter plates with 96 open-bottomed openings (5000 cells per aperture in 50 μl of the substrate), and 20 hours later, after cell adherence, five different concentrations of examined extracts were added to the platelets in order to make the final concentrations in the range of 4.7-75 mg / mL. After the addition of cell extracts, the cells were incubated for 72 hours at 37°C in air, saturated aqueous steam and 5% CO_2. After 72 hours, the cells were photographed on an inverted microscope, and 10 μl of MTT solution (5 mg/ml in PBS) was added to the plates. Cell incubation lasted for 4 h followed by reduction of MTT in colored formats by interruption by adding 100 μl of 10% SDS.

The absorbance was measured at 570 nm, 24 h later. Since the absorbance of the newly formed formazan is directly proportional to the number of living cells, in order to obtain cell survival (S%), A sample with cells treated with different concentrations of the tested tetraoxane (AL) was divided by the absorbance of the untreated cells (AK) and then this value multiplied by 100. The absorbance of blanc is always taken from the absorbance of the appropriate sample.

$$S(\%)=(A_L/A_K)\cdot 100$$

What is it:

AL - Absorbance of the sample in which the cells grew in the presence of the investigated extracts

AK - Absorbance of control cells.

The mean values and standard deviations of the MTT test results were done in Microsoft Excel.

3. RESULTS

The results of cytotoxicity testing of the material are shown in Tables 1 and 2 and Figure 1.

Cytotoxicity of ALBO-CA calcium aluminate, ALBO-CS calcium silicate,ALBO-CSHA calcium silicate hydroxyapatite, and EndoBindera was tested in five decreasing concentrations of 75.0, mg /ml, 37.5 mg/ml, 18.8 mg/ml, 9.4 mg/ml and 4.7 mg/ml.

Table 1. Concentrations of investigated factors that induce survival decline (IC50) MRC-5 cells

	experiment			mean value		standard deviation
				IC_{50} [mg/mL]		
	I	II	III	Av	±	Sd
ALBO/CSHA	46,44	55,52	55,42	52,5	±	5,2
ALBO/CA	75	>75	>75	>75	±	
ALBO/CS	>75	>75	>75	>75	±	
EndoBinder	>75	>75	>75	>75	±	

All ALBO-CA calcium aluminate concentrations tested, ALBO-CS calcium silicate and EndoBinder resulted in high survival of cells in culture. The strongest cytotoxic effect was ALBO-CSHA with IC50 = 46.44 after the first cycle of testing; IC50 = 55.52 after the second cycle; or IC50 = 55.42 after the third repetition of the MTT test.

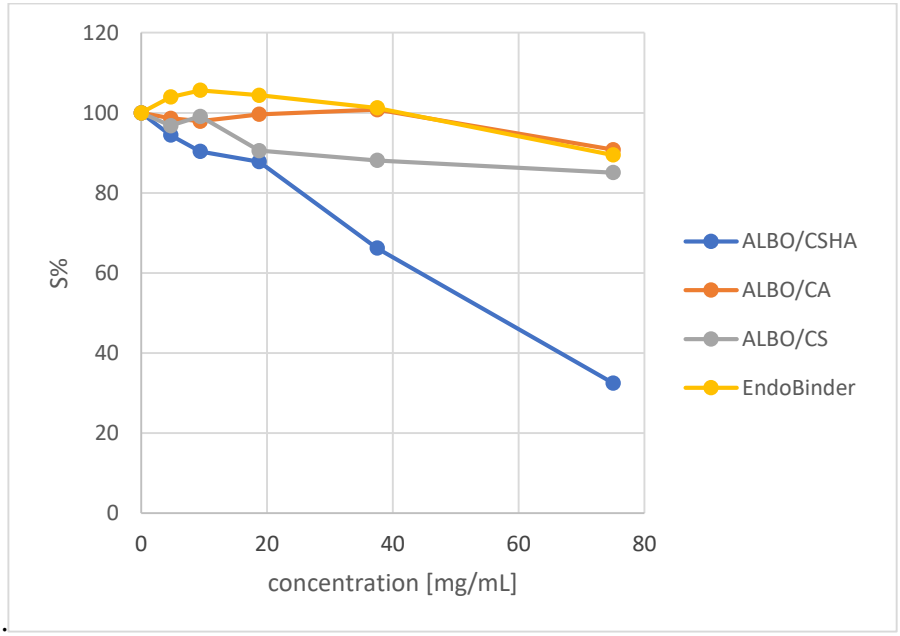

Figure 1. Survival of MRC-5 cells after 72 hours of continuous action of the eluate of the examined cells.

Table 2. Survival of MRC-5 cells after 72 hours of continuous action of the eluate of the investigated elements.

c [mg/mL]	ALBO/CSHA	ALBO/CA	ALBO/CS	EndoBinder
0,0	100,0	100,0	100,0	100,0
4,7	94,4	98,6	96,8	103,9
9,4	90,3	97,9	99,1	105,6
18,8	87,8	99,6	90,6	104,4
37,5	66,2	100,8	88,1	101,2
75,0	32,5	90,8	85,1	89,5

Calcium aluminate ALBO-CA, calcium silicate ALBO-CS and EndoBinder did not exhibit cytotoxic effects, and a very high percentage of survival of cells was also observed at the highest applied concentration of these materials (75.0, mg/mL): for ALBO-CA 90.8%, for ALBO-CS 85.1% and for EndoBinder 89.5% (Table 2, Figure 1)

Following expectations, the dependence of the cytotoxic effect of ALBO-CSHA on the applied concentration of this material was determined. The highest survival rate of the cells (94.4%) was observed at the lowest applied concentration of the material (4.7 mg/mL), while the lowest survival rate (32.5%) was observed at the highest applied concentration of this cement (75.0 mg/mL) (Table 2, Figure 1)

4. DISCUSSION

In this study, the cytotoxic activity of the investigated calcium aluminate cement (ALBO-CA), calcium phosphate cement (ALBO-CS), calcium silicate hydroxyapatite (ALBO-CSHA) and EndoBindera (control) was determined under in vitro, MTT assay conditions. The MTT test was selected for easy performance and precision. The MTT test is based on the principle that the number of survivors after the incubation of the culture of cells with cytotoxic matter and after staining with MTT reagent, is proportional to the colored formazan content, which can be determined spectrophotometrically. Viability is expressed as the absorption of treated cells relative to absorbance of control [18,19].

The MRC-5 cell line was derived from normal fetal lung tissue of the 14-week old fetus. This cell line is used to test the cytotoxicity of substances, because of its high sensitivity. MRC-5 cells are cultured adhered, and according to morphological characterization, they are similar to fibroblasts. They can pass through 42 to 46 duplications of the population before the onset of senescence [20].

Permanent cell lines have advantages over primary ones because they are easier and faster to reproduce and have an unlimited lifetime [21,22].

According to the results of this study, calcium aluminate (ALBO-CA), pure calcium silicate (ALBO-CS) and EndoBunder did not exhibit

cytotoxic effects, and a very high percentage of survival of the cells was observed at the highest applied concentration of these materials.

These results are consistent with the results of the research carried out by Silva et al. (2012), in which MTT test on the cell line 3T3 fibroblasts, calcium aluminate cement EndoBinder (Binder were, Sao Carlos, SP, Brazil) and calcium silicate cement MTA did not exhibit a cytotoxic effect in direct contact with fibroblasts. In their study, only calcium hydroxide exhibited the effects of cytotoxicity ($P < 0.05$), which is explained by the lower solubility of EndoBinder and MTA, and therefore the poor dissociation of calcium and hydroxyl ions compared to calcium hydroxide. A more pronounced dissociation of calcium and hydroxyl ions contributes to toxicity, as it leads to the formation of an alkaline medium. In conditions with an elevated pH, the denaturation of adjacent cells and proteins is triggered by the trigger mechanism for cell death [23].

The results of the study by Chinese scientists Kai-Chun Chang et al.(2014) are also in line with these findings because they confirmed the cytocompatibility of tricalcium aluminate products produced by the sol-gel method (designated as PSC-91, PSC-73, and PSC-55), with the exception of PSC-55 which showed mild cytotoxicity compared to other groups, after 1 day and after 3 days of incubation. This is explained by the increase of C3A content from 30% to 50% in the composition of this cement, which significantly improved binding time and mechanical strength, but also deteriorated its biological properties [24].

Gutiérrez et al. (2017) have come up with a result that matches the findings of this study confirming that calcium aluminate cement (CAC) and calcium aluminate cement with the addition of an aqueous solution of LiCl (CAC1) are bioactive materials that do not cause hemolysis and in vitro conditions do not exhibit cytotoxic effects on the cell line of fibroblast L929. According to their results, the number of living cells after direct contact with cement CAC1 is greater than the number of cells after direct contact with cement CAC, since CAC1 cement is more hydrated than CAC due to Li [25].

In the study of Soares et al. (2017), the experimental calcium aluminate chitosan collagen scaffold proved to be a bioactive and cytocompatible material capable of increasing the odontogenic potential of human pulping cells (HDPCs). This result is explained by the synergy of the bioactive effects of the organic matrix of chitosan-collagen and calcium-aluminate microparticles [26].

In some studies, as in this study, calcium aluminate cement has shown better results concerning calcium silicates, an increase in the number of cells, higher cellular viability, higher alkaline phosphatase activity, and greater expression of osteoblastic markers. The authors explain this by the stoichiometric relationship between calcium and aluminates, which are the base of the CAC + system, which further resulted in lower solubility and poor dissociation of Ca and OH ions compared to calcium silicate MTA [27,28].

On the other hand, cases have been reported where calcium aluminate cement Quick-Set (Primus Consulting, Bradenton, FL) and calcium silicate cement (Dentsply Tulsa Dental Specialties, Tulsa, OK) showed initially greater cytotoxicity than control, and after 15 days' biocompatibility, both types of cement were comparable to control [29]. These Chinese scientists have concluded that both calcium aluminate cement Quick-Set and WMTA possess a negligible in vitro toxicological effect time-dependent from the elimination of toxic components. The 1:1 dilution cement showed significantly higher cytotoxicity compared to dilution of 1:10 or 1: 100 ($P < 0.05$).)

According to the results of this study, the strongest cytotoxic effect was expressed by CS-HA. This result is probably due to somewhat inferior physical and physiological properties of CS-HA, which in comparison with experimental CS showed greater water absorption, higher porosity, and solubility. The consequence of these properties could be the release and accumulation of a higher amount of ion that further resulted in greater reductions in the survival of the cells.

These results are somewhat comparable with the results of the study Jingzbi Ma et al. (2011) confirming the biocompatibility of calcium silicate and calcium phosphate nanocomposites on biocompatibility, but it was noticed by the authors that shorter bonding of materials, with longer leaching, could lead to the release of a higher amount of ion with a negative impact on biocompatibility [30].

In a study by Gomes Filha et al. (2009). HA, which is part of the nanostructured CS-HA examined here, showed a marked cytotoxic effect on cell NIH-3T3 (mouse fibroblasts) [31].

Contrary to our results, Petrović et al. (2014) in his research CS-HA exhibited minor cytotoxicity concerning CS, and especially about MTA, which these authors explain with a different chemical composition of the material and lower pH values [32].

5. CONCLUSION

The ALBO-CA and ALBO-CS nanomaterials showed a cytocompatible effect comparable to EndoBinder while ALBO-CSHA showed a cytotoxic effect. This experiment with experimental nanostructured calcium aluminate and calcium silicate cement was done in laboratory conditions where the samples of the test materials were contacted with a large amount of liquid, and consequently, the very osmotic effect of the cement was more pronounced. It is recommended the results of the test to be obtained in the future and further tests to be conducted in clinical conditions, where a smaller amount of material is brought into contact with moisture surrounding tissue.

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ЦИТОТОКСИЧНО ДЕЈСТВО НОВОСИНТЕТИСАНИХ НАНОМАТЕРИЈАЛА ЗА ПОТЕНЦИЈАЛНУ СТОМАТОЛОШКУ ПРИМЈЕНУ

Сажетак: Увод: Биокомпатибилност је есенцијално својство сваког материјала за стоматолошку примјену. Малобројни су материјали за које се може рећи како су биолошки инертни, јер већина садржи потенцијално штетне или надражујуће састојке. Циљ ове студије је био да се утврди цитокомпатибилност новосинтетисаних наноматеријала базираних на калцијум-алуминатима и калцијум-силикатима, за потенцијалну стоматолошку примјену.

Материјал и методе: Цитотоксичност наноматеријала на бази калцијум алумината (*ALBO-CA*), калцијум силиката (*ALBO-CS)* и калцијум-силикат хидроксиапатита (*ALBO-CSHA*) испитивана је примјеном МТТ теста на ћелијској линији хуманих фибробласта (MRC-5) према ISO стандарду (ISO 10993-5: 2009) у компарацији са калцијум алуминатним цементом EndoBinder (Binderware, São Carlos, SP, Brazil). За анализу су коришћени елуати истраживаних материјала у медију за раст, разријеђени до концентрација од 4,7, 9,4, 18,8, 37,5 і 75,0 mg. Квалитативна верификација резултата изведена је свјетлосним микроскопом (Carl Zeiss). Средње вриједности и стандардне девијације резултата МТТ теста су рађене у Microsoft Excel-u.

Резултати: Све тестиране концентрације *ALBO-CA, ALBO-CS* и EndoBinderay резултирале високим преживљавањем ћелија у култури. Најснажније цитотоксично дејство испољио је *ALBO-CSHA* sa IC_{50}=46,44 након првог циклуса тестирања; IC_{50} = 55,52 након другог циклуса; односно IC_{50} = 55,42 након трећег понављања МТТ теста.

Закључак: Наноматеријали *ALBO-CA* і *ALBO-CS* показали су цитокомпатибилан ефекат упоредив са EndoBinderom док је *ALBO-CSHA* испољио цитотоксично дејство. Препорука је да се наставе истраживања ових материјала у будућности и другим експерименталним и клиничким студијама.

Кључне ријечи: наноматеријали, калцијум-алуминати, калцијум-силикати, цитотоксичност.

Paper received: 9 September 2019
Paper accepted: 15 October 2019