Investigation of the radiopacity and cytotoxicity of ALBO-DENT – novel strontium carbonate incorporated calcium silicate based dental cement

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SUMMARY
Introduction Calcium silicate (CS) dental cements have numerous clinical indications in dentistry including pulp capping, root end surgery, perforation repair and apexification/apexogenesis treatment.

Materials and methods Novel CS based dental cement with incorporation of SrCO₃ radiopacifier named ALBO-DENT was used as an experimental cement material while Portland cement (Aalborg, Denmark) and ProRoot MTA (Tulsa Dental, USA) were used as controls. The radiopacity evaluation was performed using digital Trophy Radiographic system with an intention to precisely determine the minimum of radiopaque agent needed to confer to ISO radiopacity requirement. Thereafter, biocompatibility of material was tested in in vitro conditions in mouse fibrosarcoma L929 cell culture treated with materials’ extracts. Cell morphology was observed using phase-contrast microscopy, while cell viability was measured using crystal violet (CV) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assays.

Results Radiopacity evaluation revealed that 30%wt addition of SrCO₃ was necessary to achieve satisfactory radiopacity (3.45 mm Al). Cytotoxicity analysis using CV and MTT assays revealed that pure extracts of ALBO-DENT presented superior biocompatibility when compared to PC and MTA controls while serial dilutions of experimental cements’ extracts as well as that of PC and MTA did not influence L929 cell viability.

Conclusions Novel formulation of CS cement – ALBO-DENT presented satisfactory radiopacity and adequate biocompatibility.

Keywords: calcium silicate; strontium carbonate; radiopacity; dental materials biocompatibility; endodontics

INTRODUCTION
Calcium silicate (CS) dental cements have revolutionized many regenerative endodontic procedures such as root end surgery, apexification/apexogenesis, perforation repair and direct pulp capping [1]. The very first commercial CS-based dental cement - ProRoot MTA (Tulsa Dental, OK, US) has shown significant clinical outcomes [2] and it is composed of type 1 ordinary Portland cement (Aalborg, Denmark) and ProRoot MTA (Tulsa Dental, USA) were used as controls. The radiopacity evaluation was performed using digital Trophy Radiographic system with an intention to precisely determine the minimum of radiopaque agent needed to confer to ISO radiopacity requirement. Thereafter, biocompatibility of material was tested in in vitro conditions in mouse fibrosarcoma L929 cell culture treated with materials’ extracts. Cell morphology was observed using phase-contrast microscopy, while cell viability was measured using crystal violet (CV) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assays.

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Conclusions Novel formulation of CS cement – ALBO-DENT presented satisfactory radiopacity and adequate biocompatibility.

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Our research group has demonstrated the satisfactory properties of two novel CS formulations: one consisting of CS, nano-particulated hydroxyapatite (nano-hydroxyapatite, nHA) and BaSO₄ – ALBO MPCA 1 and another composed of CS, calcium carbonate (CaCO₃) and Bi₂O₃ – ALBO MPCA 2. Their mechanical properties and in vivo safety, after both acute and sub-chronic administration, are documented previously [13–19]. These materials have shown satisfactory setting time, increased pH value, adequate biocompatibility and enhanced neutralization of the bacterial biofilm [20, 21]. It was confirmed that CS enriched with nHA was associated with YbF₃ as radiopacifiers leading to adequate physicochemical and biological characteristics [9, 19].

This study generally served to further improve the quality of ALBO MPCA cements by incorporating the potentially bioactive radiopacifier – strontium carbonate.

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Synthesis of inorganic phases

The novel experimental cement – ALBO-DENT was composed of the following components: calcium silicate, zirconium oxide, strontium carbonate, magnesium silicate, mesosilica and hexaphosphate. Silicate active phase was synthesized from calcium chloride pentahydrate (CaCl₂·5H₂O) (Merck, Germany) and silica sol obtained by hydrothermal treatment. Aluminium acetate (Al(CH₃COO)₃) was added to the mixture to provide the production of a small amount (3.01 %) of active tricalcium aluminate (C₃A) phase. Detailed procedure of used synthesis is given in investigations of Jokanović et al. [14, 15]. SrCO₃ (Sigma-Aldrich, St. Louis, Missouri, USA) was added into the mixture at 10%, 20% and 30% wt. ratio. PC (Alborg, Denmark) and MTA+ (thereafter referred to as MTA) (Cerkamed, StalowaWola, Poland) served as control.

Specimen preparation

All experimental cements and PC were hand-mixed at a powder/liquid ratio of 1 g cement/0.3 ml distilled water, while MTA preparation was performed in accordance with manufacturer’s instructions, using glass mixing pad and stainless steel spatula for cement mixing. The specimens were made using polytetrafluoroethylene (PTFE) ring molds incorporating a cavity of various internal diameter and height depending on the used test. Molds were filled to a level surface with mixed cement.

Radiopacity assessments

Radiopacity was determined in accordance with ISO 6876 [25]. Specimens (n=5) measuring 8 mm in diameter and 1 mm thickness were placed alongside an aluminum step-wedge (99.6 % pure) varying in thickness from 1 to 10 mm in increments of 1 mm each and radiographed by CCD sensor and X-ray unit (Trophy Radiology, Cedex, France) operating at 65 kV, 7 mA, for 0.07 s and at the focus to target distance of 35 cm. Image J for Windows software (National Institutes of Health (NIH), Bethesda, MD, USA) was used to calculate the gray scale values of each specimen and of each aluminium step-wedge thickness. The mean grey scale values were plotted against the number of aluminum steps, the plots were linearly regressed and regressions were used to convert mean grey scale values into millimeters of aluminum.

Cell viability analysis

Preparation of the materials extracts

Cell viability was carried out in accordance with the ISO Standard 10993-5/2005 [26]. Cements were manipulated under sterile conditions. Immediately after mixing, materials were placed into pre-sterilized PTFE molds (12 mm in diameter and 2 mm thick) to set for 24 h in a humidified atmosphere. Thereafter, discs were sterilized by ultraviolet irradiation for 2 h, then immersed in 1 ml complete medium – Dulbecco’s modified Eagle medium (DMEM; Gibco, Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 5 % fetal bovine serum (FBS), 2 mM L-glutamine and penicillin/streptomycin (all from Capricorn Scientific, Ebsdorfergrund, Germany) and incubated for 24 h at 37°C. To prepare eluents for treatment, extracts were diluted with complete culture medium that was used for cultivation of control/non-treated cells.

Cell culture and treatment

The mouse fibrosarcoma L929 cell line (European Collection of Animal Cell Cultures, Salisbury, UK) was cultivated in complete medium and maintained at 37°C, in a humidified atmosphere with 5% CO₂. Cells were prepared for experiments using the conventional trypsinization procedure with trypsin/EDTA and seeded in 96-well flat-bottom plates (5×10⁴ cells/well) for the cell viability assessment. Cells were treated 24 h post-seeding with pure extract (1) and serial dilutions (1:2, 1:4, 1:8, 1:16 and 1:32 (v:v)). Cell viability was assessed after 24, 48 and 72 h treatment.

Cell viability assessment

The number of adherent cells was determined using crystal violet (CV) while mitochondrial dehydrogenase activity was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) test. The CV assay was based on the inability of dead cells to remain adherent. After treatment, the adherent, viable cells were fixed with methanol and stained with 10 % CV solution for 15 min at room temperature. CV dye was dissolved in 33 % acetic acid after rigorous washing with water. MTT test measures mitochondrial-dependent reduction of MTT to formazan by metabolically viable cells. MTT solution was added to the cell cultures in the final concentration of 0.5 mg/ml and cells were incubated for an additional hour. Subsequently, the solution was removed and cells were lysed by dimethyl sulfoxide. The absorbance of dissolved CV dye, corresponding to the number of adherent (viable) cells and the conversion of MTT to formazan, corresponding to the number of cells with an active mitochondria were measured in automated micro-plate reader at 570 nm (Sunrise; Tecan, Dorset, UK). The results were
presented as percentage of viability relative to untreated, control cultures, considered as 100 % viable. The experiments were performed in triplicates.

**Phase contrast microscopy analysis**

Morphological changes in mouse fibrosarcoma L929 cell line were observed and cells photographed under Leica DCF320 phase contrast microscope (Leica Microsystems DMIL, Wetzlar, Germany) equipped with Leica Microsystems DFC320 camera and Leica Application Suite software (version 2.8.1), with 20× magnification.

**Statistical analysis**

The SPSS software program (ver. 20, IBM Corp., Armonk, NY, USA) was employed for statistical analysis. The Shapiro-Wilk test was used to check the normality of data distribution. Afterwards, one-way ANOVA with Bonferroni post-hoc tests was employed to compare obtained radiopacity and cytotoxicity outcomes (p<0.05).

**RESULTS**

The Shapiro-Wilk test for normality found that data were normally distributed and thus they were subjected to one-way ANOVA analysis followed by Bonferroni test. The results of the radiopacity evaluations are presented in Figure 1. One-way ANOVA revealed that the addition of different percentage of radiopacifiers statistically influenced the obtained values of radiopacity. The lowest value of radiopacity was found in PC that was not statistically different when compared with CS+10%SrCO₃ addition, while it was statistically different that all other investigated cements. On the other hand, MTA presented the greatest radiopacity value, statistically higher than in all other cements. Results revealed that 30% wt addition of SrCO₃ conferred the ALBO-DENT radio-density of 3.45±0.09 mm Al that was in accordance with ISO 6876 requirement, while 10 % addition and 20 % addition of SrCO₃ did not conform with ISO standard for 3 mm Al (1.79±0.06 mmAl, 2.3±0.07 mmAl, for 10 % and 20 %, respectively).

Cytotoxicity data are given in Figure 2 and Figure 3, while representative phase-contrast images of the cells treated with extracts of investigated materials are presented in Figure 4. For CV assay (Figure 2), one-way ANOVA showed the statistical difference among tested cements after 24 h (pure extracts, 1:2 and 1:4), 48 h (pure extracts, 1:2 and 1:4) and 72 h (pure extracts and 1:2) (p<0.05). For MTT assay (Figure 3), one-way ANOVA showed the statistical difference for all time points for pure, 1:2 and 1:4 dilutions (p<0.05), while significance was not found for 1:8, 1:16 and 1:32 dilutions (p>0.05). The results obtained for CV and MTT assays are highly complementary. Pure extract of ALBO-DENT presented lower cytotoxicity than PC and MTA for all time points, showed by both CV and MTT assays. For 1:2 dilution, MTA presented significant proliferative potential after 24h. Similarly, treatment with 1:2 and 1:4 dilutions of PC extract exerted statistically higher proliferative potential after 48 h. The rest of dilutions (1:8, 1:16 and 1:32) had no effect on cell viability. Consistent with results obtained using cell viability assays, treatment of L929 cells with ALBO-DENT pure extract for 24 h had no effect on cell morphology, but slightly decreased cell proliferation. Contrary, MTA and PC pure extracts triggered morphological changes typical for cell death, cell shrinkage and rounding and detachment of cells from bottom well (Figure 4).

**DISCUSSION**

This study showed that SrCO₃ might be a radiopacifying agent in CS-based dental cement. It has been shown that 30 % wt addition of SrCO₃ has met ISO requirement for radiopacity and at the same time cement mixture enriched with 30 % wt SrCO₃ showed satisfactory biocompatibility properties.

The idea and reason behind adding SrCO₃ into CS-based cement formulation originate from two reasons.
Figure 2. Cell viability (%) evaluated by the crystal violet (CV) assay after 24 h, 48 h and 72 h exposure of L929 cells to the cements’ eluents – pure extract (1) and different serial dilutions (1:2, 1:4, 1:8, 1:16, 1:32 (v:v)). The data are presented as mean ± standard deviation (SD) values of triplicates from one representative of three independent experiments. Columns with * are statistically different in comparison to control (p < 0.05). SrCO₃ – strontium carbonate; PC – Portland cement; MTA – mineral trioxide aggregate

Figure 3. Cell viability (%) evaluated by the MTT test after 24 h, 48 h and 72 h exposure of L929 cells to the cements’ eluents – pure extract (1) and different serial dilutions (1:2, 1:4, 1:8, 1:16, 1:32 (v:v)). The data are presented as mean ± standard deviation (SD) values of triplicates from one representative of three independent experiments. Columns with * are statistically different in comparison to control (p < 0.05). SrCO₃ – strontium carbonate; PC – Portland cement; MTA – mineral trioxide aggregate
Firstly, Sr is nowadays accepted as a bioactive constituent of many dental materials and biomaterials used in orthopaedic surgery. In addition, modern strategies for bio-activation of the surfaces of titanium implants include their coating with Sr incorporated layers. Secondly, Sr is intentionally used in the form of carbonates since the addition of calcium carbonate (CaCO₃) into CS cements decreases setting time, as it was achieved in Biodentine (Septodont, France) [27].

The results of radiopacity have shown that 30% wt addition of SrCO₃ was necessary to satisfy the radiopacity of ALBO-DENT. The radiopacity of ALBO-DENT was lower than previously found for CS+30%Bi₂O₃ (~11 mm Al) and CS+25%Bi₂O₃ (6.9 mm Al) [28]. The results demonstrated for radiopacity of MTA (6.9 mm Al) corroborate findings of previous studies: 4.86, 6.74, 7.0, 7.5 and 8.0 mm Al [28–31]. The PC did not meet the ISO radiopacity requirement that is in line with previous studies (~0.9 mm Al) [28, 29]. The influence of SrCO₃ on the radiopacity of endodontic ceramics has not been previously mentioned in the literature. The variations in radiopacity come as a consequence of the difference in the atomic number between the constituents [32]. Namely, atomic number of the compounds is directly proportional to the absorption of x-rays. The atomic number of Sr (Z=40) is lower than that in Bi (Z=83) and therefore higher percentage of SrCO₃ is needed to meet ISO radiopacity standard. This is not playing a negative role in the case of SrCO₃ such as with other radiopacifiers addition (i.e. Bi) because Sr may be considered not only as biologically safe, but also biologically active constituent.

Biological safety of dental materials is of paramount importance. Therefore, in vitro and in vivo tests are routinely performed to evaluate material’s biocompatibility before it can be used in clinical practice. The cytotoxicity assessment was performed for the mixture with adequate radiopacity value (30% wt addition of SrCO₃). ISO 10993-5 stipulates that material can be considered as not cytotoxic if it causes less than 30% cells to die in in vitro assays. In our study, two cytotoxicity tests were used: MTT that measures mitochondrial activity of the metabolically active cells and CV that determines the number of the adherent, viable cells. It was demonstrated that novel experimental cement ALBO-DENT performed satisfactory behavior in cell culture, comparable to that of PC and MTA. Presented results showed significantly lower percentage of viable cells in MTA/PC treatments than those found in some studies (80-150%) [7, 33, 34], but they are in agreement with other studies [35, 36]. For PC/MTA 1:2 and 1:4 eluents, the CV and MTT tests showed similar outcomes and are in rough agreement with data documented in the literature [7, 37]. The differences of cell viability results in different studies could arise from variations in specimens’ size (5×3 mm [37], 5×2 mm [12] and 5×1 mm [29]). The toxic potential of PC and MTA pure elute may be a matter of debate, but it is presumably the consequence of its high alkalinity in the closed in vitro cell viability assessment system. It may be speculated that in vivo, where the constant fluid uptake is ensured, these materials may not present negative effect on the surrounding tissue. In any case, novel cement mixture ALBO-DENT presented superior characteristics than widely commercially used ProRoot MTA.

From the clinical point of view, new CS-based experimental material has certain advantages since Sr incorporation may enhance the bone healing during root end canal surgery by activating osteoblasts for improved bone synthesis [22, 23, 24]. In addition, if used for pulp capping procedures, it may stimulate odontoblasts for faster formation of tertiary dentine. These assumptions should be confirmed in the future state of the art researches.

**CONCLUSION**

Newly synthesized CS-based dental cement with 30% wt addition of SrCO₃ as radiopacifying agent meets ISO standard for radiopacity. Biocompatibility of newly synthesized cement, assessed by analysis of cell viability via measurement of the number of adherent, viable cells and viable cells with active mitochondria, is satisfactory and indicates its biological safety.
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Ispitivanje rendgenkontrastnosti i citotoksičnosti ALBO-DENTA – novog kalcijum-silikatnog cementa sa dodatkom stronicjum-karbonata

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KRATAK SADRŽAJ

Uvod Kalcijum-silikatni (KS) dentalni cementi se koriste u brojnim kliničkim indikacijama u stomatologiji koje uključuju direktno prekrivanje pulpe, retrogradnu hiruršku korenu zuba, lećenje perforacija i apektogenezu/apektifikaciju.

Materijali i metode U istraživanju je korišćen novosintetisani cement na bazi KS sa dodatkom SrCO3 kao kontrastnog agensa ALBO-DENTA, dok su kao kontrola korišćeni cement Portland (PC, Aalborg, Denmark) i ProRoot MTA (MTA, Tulsa Dental, USA). Rendgenkontrastnost je ispitivana digitalnom radiografijom primenom aparat TRIPOD, sa namerom da se precizno odredi minimum rendgenkontrastnog agensa koji zadovoljava zahteve standarda ISO za rendgenkontrastnost. Biokompatibilnost materijala je ispitana in vitro, u kulturi celija mišjeg fibrosarkoma L929 tretiranoj ekstraktima ispitivanih materijala.

Rezultati Ispitivanje rendgenkontrastnosti je pokazalo da dodatak 30% SrCO3 dovodi do zadovoljavajućeg kontrasta materijala (3,45 mm Al). Analiza citotoksičnosti KV i MTT metodom je pokazala da čisti ekstrakt ALBO-DENTA pokazuje bolju biokompatibilnost u poređenju sa PC i MTA, dok serijska razblaznjenja ekstraktaka ispitivanih cementa, kao i PC i MTA, nisu uticala na vijabilitet celija L929.

Zaključci Novi cement na bazi KS – ALBO-DENTA pokazuje suzavodnovoljavajuću rendgenkontrastnost i odgovarajuću biokompatibilnost.

Ključne reči: kalcijum-silikat; stronicjum-karbonat; rendgenkontrast; citotoksičnost; endodoncija

UVOD

Kalcijum-silikatni (KS) dentalni cementi doveli su do revolucije u mnogim endodontskim regenerativnim zahvatima kao što su endodontska apektka, apektifikacija/apektogenija. Prvobitno sintetisani komercijalni KS cement, ProRoot MTA (Tulsa Dental, OK, SAD) pokazao je značajne kliničke rezultate, uključujući, endodontska apektka, apektifikacija/apektogeniju, kao i direktno prekrivanje pulpe [1]. Promocija njihove odontogene diferencijacije, proliferacije i aktivnosti, odnosno indukcija humanih stem celija zubne pulpe (SR) na kost i zubna tkiva, među kojima su osteoproliferativni i odontoproliferativni efekti, stimulacija formiranja kosti i angiogeneza, inhibicija čelijske diferencijacije i osteoklastne aktivnosti, odnosno indukcija humanih stem celija zubne pulpe promocijom njihove odontogene diferencijacije, proliferacije i mineralizacije [22, 23, 24]. Cilj ovog istraživanja je bio da utvrdi minimalni udeo SrCO3 kao rendgenkontrastnog sredstva koji zadovoljava zahteve ISO u cementima i da proveri biokompatibilnost ovog materijala.

MATERIJAL I METODE

Sinteza neorganskih faza

Novi eksperimentalni cement ALBO-DENT pokazao je značajne kliničke rezultate, uključujući, endodontska apektka, apektifikacija/apektogeniju, kao i direktno prekrivanje pulpe [1]. Prvobitno sintetisani komercijalni KS cement, ProRoot MTA (Tulsa Dental, OK, SAD) pokazao je značajne kliničke rezultate, uključujući, endodontska apektka, apektifikacija/apektogeniju, kao i direktno prekrivanje pulpe [1].

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UYOD

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Ključne reči: kalcijum-silikat; stronicjum-karbonat; rendgenkontrast; citotoksičnost; endodoncija
količine (3,01%) aktivne trikalcijumaluminatne fazе (C₃A). Detaljnа procedura sintеze korišćеног KS je opisana u istraživanjima Jokanovićа i saradnika [14, 15]. SrCO₃, (Sigma-Aldrich, St. Louis, Missouri, SAD) dodat je u mešavinu kao 10%, 20% i 30% težinski postotak. PC (Aalborg, Danska) i MTA+ (kasnije navоden kao MTA) (Cerkamed, StalowaWola, Polska) služili su kao kontrolа.

**Pripreма uzoraka**

Svi eksperimentalni cementi i PC su ručno zamešani; odnos praha i tečnosti je bio 1 g cеmentа i 0,3 ml destilovane vode, dok je MTA pripremljen prema uputstvu proizvođačа, metalnom špatulom za mešanje cemenata na staklenоj pločici. Uzorci su napravljeni korišćenjem politetrafluoroetilenskih (PTFE) kalupe čemu je izabrano uveličаne od 20×. cementi su pripremani u sterilnim uslovima. Neposredno po Balkanu je odstranjene odlepljene, mrtve ćelije. Adherentne ćelije su fiksirane metanolom u trajanju od 15 minuta na sobnoj temperaturi, a zatim su bojene 1% rastvorom kristal violeta u PBS-u, takođе 15 minuta na sobnoj temperaturi. Boja koja se nije vezala ispirana je vodom, a boja ugrađena u ćelije je rastvorena 33% rastvorom sirćetne kiseline u vodi.

**Rendgenkontrastnost**

Rendgenkontrastnost je utvrđivаnа u skladu sa standardom ISO 6876 [25]. Uzorci (n = 5) dijамetа 8 mm аnd debljine 1 mm postavljeni su zajedno sa aluminijumskim etalonom (99,6% čistoće), čija je debljina varirala od 1 mm do 10 mm sa postepenom povećаnjem od 1 mm, i radiografисани su uz pomoć CCD sensora i izvora x-zraka (Trophy Radiology, Cedex, Француса) radići pri sledеćим параметрима: 65 kV, 7 mA, 0,07 s i rastojаju između izvora zračenja i objekta radiografисаnja od 35 cm. Image J za program Windows (Nаtional Institutes of Health (NIH), Bethesda, MD, SAD) primеnjen je za izраčunavanje stepena sivo-bele skale svakog uzorkа i svake debljine aluminijumskог eta- lona. Srednje vrednosti sivo-bele skale су da се stepen sivo-bele skаle pretvori u milimetre aluminijuma. Eksperimenti су ураđeni u skladu са ISO standardom 10993-5/2005 [26].

**Analiza čelijskог vijabiliteta**

**Priprema ekstrakata materijala.** Anaлиза čelijskог vijabiliteta je urаđena u skladu sa ISO standardom 10993-5/2005 [26]. Cementi су припреманi u stеrilnim uslovima. Neposredno pо sle mašаnja materijала су стављањi u стеrilisane PTFE kalupe (12 mm širine и 2 mm debljine) da ovlаčenu tokom 24 sата u atmosferi zasićenoj vodenом parom. Dobijeni diskovi su zatim sterilisani ultravioletnim zračenjem u trajanju od 2 h, nakon čega су unорjeno u 1 ml kompletног medijuma – Dulbecco modifikovani Eagle medijum (DMEM; Gibco, Thermo Fisher Scientific, Inc., Waltham, MA, SAD), obogaćenog 5% fetalног telечког serumа (FBS), 2 mM L-glutaminom и penici- linom/streptomycinом (Capricorn Scientific, Ebsdorfergund, Nemačka) и inkubирани 24 sата на 37 o C. Kako bi se pripreмили linom/streptomycinom (Capricorn Scientific, Inc., Waltham, MA, SAD), обогаćen 5% fetalnог serumа (FBS), 2 mM L-glutaminom i penцилином/streptomycinom (Capricorn Scientific, Ebsdorfergund, Немачка) и инкубирани 24 сата на 37°C. Kako bi se pripremili, ćelije su rastvoreni pomoću DMSO. Intenzitet dobijenih boja je proporcionalan broju živih ćelija. Nakon izvučаnja, ćelije su rastvorene u 33% rastvorom sirćetne kiseline u vodi.

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**Fazno-kontrastна mikroskopija**

Mорфолоšке promene mišje fibросаркомске ćelijske linije L929 су анализирane и ćelije су фотографисане на talasnoj dužини od 570 nm. Vijabilitet ćelija je procenjen posle 24, 48 и 72 сата.

**Statistička analiza**

Softverski program SPSS (ver. 20, IBM Corp., Armonk, NY, SAD) primеnjen je za statističku analizу. Šapiro-Vilkov test je primеnjen za proveru normalnosti raspodele podataka. Nakon primеnjenje Šapiro-Vilkovog testа normalnosti, normalnost radiokontrastnosti i citotoksičnosti (p < 0,05).

**REZULTATI**

Primena Šapiro-Vilkovog testа normalnosti je pokazala da су добijeni podаци normalno distribuirани te су подвrgnuti jed- nosmernој анализi varijanse (ANOVA) sa Bonferronijevim post-hok testom za poređenje dobijenih vred-nosti radiokontrastnosti и citotokсичности (p < 0,05).
Rezultati procene rendgenkontrastnosti su predstavljeni na Slici 1. Primena ANOVA testa pokazala je da je dodavanje različitog procenata rendgenkontrastnog sredstva uticalo na stepen rendgenkontrastnosti. Najniža vrednost rendgenkontrastnosti izmerena je u PC i nije se statistički razlikovala od cementa sa dodatkom CS + 10% SrCO₃, ali se statistički razlikovala od svih ostalih ispitivanih cementa. S druge strane, vrednost rendgenkontrastnosti MTA 1:2 je pokazala proliferativni potencijal nakon 24 sata. ALBO-DENT je pokazao značajno manju citotoksičnost u postestovima izuzetno komplementarni. Čisti ekstrakt cementa DISKUSIJA izgubile volumen, zaokruglile se i odvojile od podloge. karakteristične za ćelijsku smrt – ćelije su u njihovom prisustvu čisti ekstrakti MTA i PC izazvali su morfološke promene koje su mentalna mitohondrijalnu aktivnost ćelija i KV, koji određuje broj meri mitohondrijalnu aktivnost ćelija i KV, koji određuje broj adherentnih živih ćelija. Pokazano je da novi eksperimentalni cement ALBO-DENT pokazuje zadovoljavajuće ponašanje u ćelijskom vijabilitetu. U ovom slučaju novosintetisani cement ALBO-DENT je poštovao ISO standard od 3 mm Al, što je u skladu sa zahtevima ISO 6876 standarda, dok dodavanje 10%, odnosno 20% SrCO₃ nije bilo u skladu sa zahtevima ISO standarda od 3 mm Al, 2.3 ± 0.07 mm Al, 10%, odnosno 20%). Rezultati citotoksičnosti prikazani su na Slici 2 (KV) i Slici 3 (MTT), a reprezentativne fotografije ćelija tretiranih čistim ekstraktima ispitivanih materijala posmatranih fazno-kontrastnom mikroskopijom prikazane su na Slici 4. Jednomorna analiza varijanse je pokazala da je dodavanje TK testom postoji statistička razlika između ispitivanih materijala nakon 24 sata (čisti ekstrakti, 1 : 2 i 1 : 4), 48 sata (čisti ekstrakti, 1 : 2 i 1 : 4) i 72 sata (čisti ekstrakti i 1 : 2) (p < 0.05). Za podatke dobijene MTT testom ista analiza je pokazala statističku razliku u svim vremenskim tačkama za čist ekstrakt, kao I 1 : 2 i 1 : 4 razblaženja (p < 0.05), dok značajnost nije uočena za 1 : 8, 1 : 16 i 1 : 32 razblaženja (p > 0.05). Rezultati dobijeni KV i MTT testovima su izuzetno komplementarni. Čisti ekstrakt cementa ALBO-DENT je pokazao značajno manju citotoksičnost u podređenju sa PC i MTA, u svim praćenim vremenima. Raz blaženja MTA 1 : 2 je pokazalo proliferativni potencijal nakon 24 sata. Raz blaženja PC 1 : 2 i 1 : 4 je potenciralo proliferaciju ćelija nakon 48 sata, dok ostala raz blaženja (1 : 8, 1 : 16 i 1 : 32) nisu pokazale statistički značajan uticaj na ćelijsku vijabilnost. U skladu sa rezultatima dobijenih testova za ispitivanje ćelijskog vijabiliteta, tretman ćelija L929 sa čistim ekstraktom cementa ALBO-DENT tokom 24 sata nije uticao na morfologiju ćelija, ali je blago inhibirao ćelijsku proliferaciju. S druge strane, čisti ekstrakt MTA i PC izazvali su morfološke promene koje su karakteristične za ćelijsku smrt – ćelije su u njihovom prisustvu izgubile volumen, zaokruglile se i odvojile od podloge.

DISKUSIJA

Ova studija je pokazala da se SrCO₃ može koristiti kao rendgenkontrastno sredstvo kod KS dentalnih cemenata. Pokazano je da 30% težinskog dodatka SrCO₃, zavoljava ISO istraživanja: 4,86, 6,74, 0,7, 5 i 8 mm Al [28–31]. PC nije ispunio ISO standard rendgenkontrastnosti, što je u saglasnosti sa prethodnim studijama (0,9 mm Al) [28, 29]. U ovoj studiji izmerena je u PC i nije se statistički razlikovala od cementa sa dodatkom CS + 10% SrCO₃, ali se statistički razlikovala od svih ostalih ispitivanih cementa. S druge strane, vrednost rendgenkontrastnosti MTA 1:2 je pokazala proliferativni potencijal nakon 24 sata. ALBO-DENT je pokazao značajno manju citotoksičnost u postestovima izuzetno komplementarni. Čisti ekstrakt cementa DISKUSIJA izgubile volumen, zaokruglile se i odvojile od podloge. karakteristične za ćelijsku smrt – ćelije su u njihovom prisustvu čisti ekstrakti MTA i PC izazvali su morfološke promene koje su karakteristične za ćelijsku smrt – ćelije su u njihovom prisustvu izgubile volumen, zaokruglile se i odvojile od podloge. karakteristične za ćelijsku smrt – ćelije su u njihovom prisustvu čisti ekstrakti MTA i PC izazvali su morfološke promene koje su karakteristične za ćelijsku smrt – ćelije su u njihovom prisustvu izgubile volumen, zaokruglile se i odvojile od podloge. karakteristične za ćelijsku smrt – ćelije su u njihovom prisustvu čisti ekstrakti MTA i PC izazvali su morfološke promene koje su karakteristične za ćelijsku smrt – ćelije su u njihovom prisustvu izgubile volumen, zaokruglile se i odvojile od podloge. karakteristične za ćelijsku smrt – ćelije su u njihovom prisustvu čisti ekstrakti MTA i PC izazvali su morfološke promene koje su karakteristične za ćelijsku smrt – ćelije su u njihovom prisustvu izgubile volumen, zaokruglile se i odvojile od podloge. karakteristične za ćelijsku smrt – ćelije su u njihovom prisustvu čisti ekstrakti MTA i PC izazvali su morfološke promene koje su karakteristične za ćelijsku smrt – ćelije su u njihovom prisustvu izgubile volumen, zaokruglile se i odvojile od podloge. karakteristične za ćelijsku smrt – ćelije su u njihovom prisustvu čisti ekstrakti MTA i PC izazvali su morfološke promene koje su karakteristične za ćelijsku smrt – ćelije su u njihovom prisustvu izgubile volumen, zaokruglile se i odvojile od podloge. karakteristične za ćelijsku smrt – ćelije su u njihovom prisustvu čisti ekstrakti MTA i PC izazvali su morfološke promene koje su karakteristične za ćelijsku smrt – ćelije su u njihovom prisustvu izgubile volumen, zaokruglile se i odvojile od podloge. karakteristične za ćelijsku smrt – ćelije su u njihovom prisustvu čisti ekstrakti MTA i PC izazvali su morfološke promene koje su karakteristične za ćelijsku smrt – ćelije su u njihovom prisustvu izgubile volumen, zaokruglile se i odvojile od podloge. karakteristične za ćelijsku smrt – ćelije su u njihovom prisustvu čisti ekstrakti MTA i PC izazvali su morfološke promene koje su karakteristične za ćelijsku smrt – ćelije su u njihovom prisustvu izgubile volumen, zaokruglile se i odvojile od podloge. karakteristične za ćelijsku smrt – ćelije su u njihovom prisustvu čisti ekstrakti MTA i PC izazvali su morfološke promene koje su karakteristične za ćelijsku smrt – ćelije su u njihovom prisustvu izgubile volumen, zaokruglile se i odvojile od podloge. karakteristične za ćelijsku smrt – ćelije su u njihovom prisustvu izgubile volumen, zaokruglile se i odvojile od podloge. karakteristične za ćelijsku smrt – ćelije su u njihovom prisustvu izgubile volumen, zaokruglile se i odvojile od podloge.
ZAKLJUČAK

Novosintetisani cement na bazi KS sa 30% težinskim dodatkom SrCO₃ kao rendgenkontrastnog sredstva zadovoljava zahteve standarda ISO za kontrastnost. Biokompatibilnost novosintetisanog cementa na osnovu analize čelijskog vijabiliteta merenjem broja adherentnih čelija i aktivnosti mitohondrijalne dehidrogenaze je zadovoljavajuća i ukazuje na njegovu biološku bezbednost.

ZAHVALNICA

Ova studija je podržana od strane Ministarstva prosvete, nauke i tehnološkog razvoja Republike Srbije (broj projekata: 451-03-68/2020-14/200017 i 451-03-9/2021-14/200007) i kroz projekat bilateralne saradnje između Ministarstva prosvete, nauke i tehnološkog razvoja Republike Srbije i Ministarstva nauke i tehnologije Narodne Republike Kine (broj projekta: 451-02-818/2021-09/20).