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Cytotoxicity investigation of a new hydroxyapatite scaffold with improved structural design

Nikola Sjerobabin[1], Božana Čolović[2], Milan Petrović[3], Dejan Marković[3], Slavoljub Živković[3], Vukoman Jokanović[2]

[1]Clinical Hospital Center "Dr Dragiša Mišović – Dedinje", Belgrade, Serbia;
[2]University of Belgrade, Institute for Nuclear Sciences "Vinča", Belgrade, Serbia;
[3]University of Belgrade, Faculty of Dental Medicine, Belgrade, Serbia

SUMMARY
Introduction Biodegradable porous scaffolds are found to be very promising bone substitutes, acting as a temporary physical support to guide new tissue regeneration, until the entire scaffold is totally degraded and replaced by the new tissue.
Objective The aim of this study was to investigate cytotoxicity of a synthesized calcium hydroxyapatite-based scaffold, named ALBO-OS, with high porosity and optimal topology.
Methods The ALBO-OS scaffold was synthesized by the method of polymer foam template. The analysis of pore geometry and scaffold walls' topography was made by scanning electron microscope (SEM). The biological investigations assumed the examinations of ALBO-OS cytotoxicity to mouse L929 fibroblasts, using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromidefor (MTT) and lactate dehydrogenase (LDH) tests and inverse phase microscopy.
Results The SEM analysis showed high porosity with fair pore distribution and interesting morphology from the biological standpoint. The biological investigations showed that the material is not cytotoxic to L929 cells. Comparison of ALBO-OS with Bio-Oss, as the global gold standard as a bone substitute, showed similar results in MTT test, while LDH test showed significantly higher rate of cell multiplication with ALBO-OS.
Conclusion The scaffold design from the aspect of pore size, distribution, and topology seems to be very convenient for cell adhesion and occupation, which makes it a promising material as a bone substitute. The results of biological assays proved that ALBO-OS is not cytotoxic for L929 fibroblasts. In comparison with Bio-Oss, similar or even better results were obtained.
Keywords: hydroxyapatite; cytotoxicity; MTT; LDH

INTRODUCTION

Bone is a dynamic and highly vascularized tissue which has significant self-healing capacity for the repair of small fractures. However, bone grafts are needed to provide support, fill bone defect, and enhance biological repair in larger ones. Due to source limitation of autogenic bone grafts and immunologic rejection problem of natural bone grafts, bone tissue engineering technique has become a crucial strategy for permanent repair of bone defects in current tissue transplantation methodologies and grafting [1, 2, 3].

Hence, 3D biodegradable porous structures well-known as scaffolds are found to be a promising solution because they act as a temporary physical support to guide new tissue regeneration until the entire scaffold is totally degraded and replaced by the new tissue. Ideally, these temporary scaffolds should be porous in order to accommodate cell growth and facilitate both tissue regeneration and vascularization [4, 5, 6]. Furthermore, they should also be biocompatible, mechanically strong, and have a biodegradation rate similar to the cell/tissue growth rates. Three more properties should be fulfilled: (i) the material should establish a stable direct connection with patient's bone, both structural and functional, (ii) the material should be osteoinductive, and (iii) it should be osteoconductive, for recruitment of mesenchymal progenitor cells and guided bone deposition on the scaffolds surface.

Scaffold has to favor cell attachment, proliferation and differentiation, bone growth, and *in vivo* revascularization. It should also possess mechanical properties matching those of target tissue, designed geometrically and functionally to mimic native extracellular matrix environment as much as possible [7–10]. A ceramic carrier designed to mimic bone structure hierarchy covered by polymer thin films with appropriate properties is probably one of the best solutions for the above requested scaffold construction.

ALBO-OS scaffold was developed as innovative product following the aforementioned requirements. It is a composite of calcium hydroxyapatite (CHA) and poly-lactide-co-glycolide (PLGA) with very high porosity and pronounced topography of the inner walls. PLGA (coating CHA in ALBO-OS) belongs to the family of synthetic biodegradable polyesters commonly used in tissue engineering, as it satisfies the requirements of mechanical

Correspondence to:
Vukoman JOKANOVIĆ
Institute for Nuclear Sciences "Vinča"
Mike Petrovića Alasa 12–14
11001 Belgrade
Serbia
vukoman@vinca.rs

properties and convenient processing [11, 12]. It has favorable degradation rate that matches the rate of healing of damaged bone tissue [13]. This composite was made in an attempt to enhance the scaffold surface for various cell activities, including cell adhesion, proliferation and release of cytoplasmic metabolites, changes of the cell shape, density of their occupations, etc.

Presented *in vitro* study was aimed at investigating the biological response (cytotoxicity and cell adhesion) of fibre-like cells to ALBO-OS. The potential of such scaffold was compared with Bio-Oss through 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromidefor (MTT) and lactate dehydrogenase (LDH) tests; cell line of mouse fibroblasts L929 was used for investigation of biological response to the implanted material.

OBJECTIVE

The aim of this study was to investigate cytotoxicity of the synthesized scaffold on the base of calcium hydroxyapatite and poly(lactic-co-glycolic acid) (PLGA), with improved structural design, on the cell line of mouse fibroblasts L929 using MTT and LDH assays and inverse phase microscopy.

METHODS

Scaffold preparation

The synthesis of scaffold named ALBO-OS consisted of three stages: synthesis of CHA powder, production of CHA granules, and deposition of a thin film of PLGA on the surface of the granules.

CHA powder was synthesized hydrothermally from approximately stoichiometric mixture of $(NH_4)_2HPO_4$ and $Ca(OH)_2$, by procedure given in our previous investigations [14, 15]. The procedure, in brief, consisted of the following: 500 ml of a 2.32 cmol aqueous solution of $(NH_4)_2HPO_4$ was poured into 500 ml of a 3.02 cmol aqueous solution of $Ca(OH)_2$, under vigorous stirring. The mixture's pH value was adjusted to 7.4, and it was autoclaved at 150°C under a pressure of 5×10^5 Pa for 8 h. After the hydrothermal treatment, the precipitate was decanted, dried at 80°C for 48 h, washed with deionized water, and ultracentrifuged. In the next step, a mixture of 5 g of hydrothermally synthesized CHA and 1.5 g of poly(ethylenevinyl acetate)/poly(ethylene vinyl versatate) (PEVA/PEVV) was made and further processed in the autoclave at 120°C for 2 h. The obtained CHA powder was further used for production of CHA granules.

The CHA powder was mixed with water to form ceramic slurry. To enhance the wetness of the slurry and its rheological properties, 2% of PVA was added. Polyurethane (PU) foam with adequate pore size distribution was then dipped into the slurry followed by gently squeezing the foam several times to allow the slurry to penetrate the foam and the excess slurry to be squeezed out. Compressed air through an air gun was used to avoid a blockage of pores. The ceramic slurry-coated PU foam was left to dry at room temperature and was then heated in an oven at 600°C to burn out the PU foam, and then sintered at 1,200°C for 4 h. The obtained porous CHA compact was further disintegrated into granules of suitable size.

The final step in ALBO-OS synthesis was the deposition of PLGA thin film on the surface of CHA granules. PLGA pellets (50:50, M = 45,000–70,000; Durect Corporation, Cupertino, CA, USA) were dissolved in chloroform to obtain 1% w/w solution which was further poured over CHA granules. After the solvent evaporation, thin PLGA film was formed at the granules' surface [16].

Scaffold characterization

For structural analysis of CHA granules, as the main component of ALBO-OS, the methods of X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) were used. For XRD analysis, Philips PW 1050 diffractometer (Koninklijke Philips N. V., Amsterdam, the Netherlands) with Cu-Kα1-2 lamp was used, and the data were collected in the 2 θ range from 9° to 67°, in steps of 0.055°, and with exposure time of 2 seconds per step. For FTIR analysis, spectrometer 380 Nicollet FTIR (Thermo Electron Corporation, Waltham, MA, USA) was used. FTIR spectra were taken in the spectral range 4,000–400 cm^{-1}.

Scanning electron microscopy (SEM) was used to analyze the microstructure of pore walls of CHA granules. Sample preparation for SEM was performed by steaming with gold in BALZERS, and the samples were observed under a JSM-5300 microscope (JEOL USA, Inc., Peabody, MA, USA).

Total porosity of the synthesized CHA scaffolds was determined by using the following equations: bulk density (ρB) = weight of the sample divided by volume of the sample; theoretical density of the CHA (ρ_0) = 3.16 g/cm^2; relative density (R.D.) = ($\rho B/\rho_0$) × 100%; and total porosity = 100% - R.D. The dimensions and the weight of each sample were measured and recorded using the weight of displaced liquid (water) with measured quantity of the sample. Three identical specimens were used to determine the total porosity.

Nano-porosity of the CHA scaffold was estimated by high-pressure mercury intrusion porosimeter (Carlo Erba Porosimeter 2000 with Milestone 100 Software System). The porosimeter operates in the pressure interval 0.1–200 MPa, enabling estimation of pores in interval 7.5–15,000 nm.

Biological examination

Biological samples

Study included three different materials (ALBO-OS, Bio-Oss, and control) and three independent experiments (three biological replicates). In each experiment, five wells were analyzed for each material, which makes 15 samples in total, for each material.

Cell culture

The mouse fibroblasts L929 were grown in appropriate containers, in a culture medium (DMEM) with addition of 10% fetal calf serum (FCS), L-glutamine (3 mM), penicillin (100 IU/ml) and streptomycin (100 mg/ml). After supplementation, pH of the medium solution was adjusted to 7.2 using a bicarbonate buffer. Thereafter, the solution was filtered through a filter with a pore size of 0.22 µm. Cells were grown at the temperature of 37°C in air with 5% CO_2.

To optimize the growing conditions of cells, experimental curves of cell growth were defined, depending on the growth time. The cells were monitored using alamarBlue (Thermo Fisher Scientific, Waltham, MA, USA) assay (at 570 nm) after two, 24, 48, 72, and 96 hours. The growth curves after seeding 500, 1,000, 2,000, 4,000, and 8,000 cells were investigated. Based on the growth curves, the time required to double the cell number was determined and on its basis the optimal number of cells for experiments was determined. That number was 6,000 cells/well in the 96-well plate, for indirect toxicity testing, and 100,000 cells/well in the six-well plate for direct toxicity testing [17, 18, 19].

MTT test

For the test of indirect cytotoxicity (MTT test) the extracts were obtained by suspending the granules of ALBO-OS in culture medium (DMEM with10% FCS) at a concentration of 0.02 g/ml and incubated at 37°C for one, 24, or 72 hours with constant agitation. After incubation, extract solutions were centrifuged for 10 minutes at 2,000 rpm, decanted and filtered through a 0.22 µm filter (to eliminate any solid particles of the material). pH values of the obtained solutions were determined and they were used for MTT test.

For MTT test, L929 cells were seeded in 96-well cell culture plates in 100 µl growth medium at concentration of 6,000 cells/well. After 24 h of culture in a humidified atmosphere with 5% CO_2 at 37.1°C, medium was removed and replaced with 100 µl of previously prepared extract dilutions (obtained after one-, 24-, or 72-hour contact of medium and ALBO-OS). Extract of Geistlich BioOss (as a global gold standard) obtained only after 72-hour contact of BioOss with culture medium was used for comparison of cell viability on these two materials. Cells cultured in growth medium served as controls. After 72 h of incubation with each extract, the cell culture was treated with 20 µl/well of MTT (5 mg/ml in PBS) and incubated for further 4 h in humidified atmosphere with 5% CO_2 at 37.1°C. Then, MTT was removed and 100 µl 10% SDS in 0.01 M HCl was added in order to dissolve the formazan crystals. The next day, the optical density (OD) was read on ELISA multiwell microplate reader at 570 nm [17, 18, 19].

LDH test

To determine direct cytotoxicity, a thin (300 µm – 1 mm) ALBO-OS coating of the granules was deposited on round polypropylene flakes, which were further fixed to 6-well plates and sterilized with γ-rays (25 kGy, 48 h). BioOss granules prepared in the same way were used for comparison with ALBO-OS. Then, the optimal number of L929 cells (100,000 per well) was added. Further, plates were incubated for one, two and seven days in humidified atmosphere with 5% CO_2 at 37.1°C. The number of viable cells was estimated after physical lysis. The cells were lysed by alternate freezing and defrosting in three cycles, then centrifuged and LDH level was determined from the obtained supernatant. Supernatant aliquots of each well were transferred to a new 96-well plate and the LDH quantified using an enzymatic kit (Sigma Aldrich, St. Louis, MO, USA). Number of cells was determined by the level of LDH using previously constructed calibration curve. The optical density was read on microplate reader at 490 nM.

Statistical analysis

The results are presented as mean ± standard deviation (SD). The differences between groups (independent samples) were analyzed using One-way Analysis of Variance (ANOVA) and Student's t-test. Statistical analysis was performed using SPSS statistical software version 15.0 (SPSS, Chicago, Illinois). All p-values less than 0.05 were considered to be significant.

Phase contrast microscopy

Cells were observed under phase contrast microscope (Leica Microsystems, Wetzlar, Germany) after contact with the extracts of ALBO-OS for 24, 48, and 72 h. Their morphology and the difference in cell density compared to controls (cells cultivated in culture medium, for 24, 48, and 72 h also) were evaluated [17, 18, 19]. The percentage of survived cells in ALBO-OS extracts was obtained by analysis of contrast phase microscopy images for each well in each experiment (15 images). The results are presented as mean percentage ± standard deviation of percentage for each group.

RESULTS

Scaffold granules characterization

The obtained XRD spectrum (Graph 1) showed that the phase composition of CHA scaffold granules corresponds to carbonate calcium hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ (JCPDS 9–432). All characteristic diffraction peaks were present: (002) at 2θ = 25.98°, (120) at 2θ = 29.03°, (121) at 2θ = 31.87°, (300) at 2θ = 33.07°, (310) at 2θ = 39.94°, (222) at 2θ = 46.82° and (123) at 2θ = 49.54°.

FTIR spectrum of CHA granules (Graph 2) showed the bands characteristic for CHA. The bands around 1,092, 1,046, 603, and 568 cm^{-1} correspond to PO_4^{3-} group. Vibrations of OH^- groups were detected at about 632 cm^{-1}. Vi-

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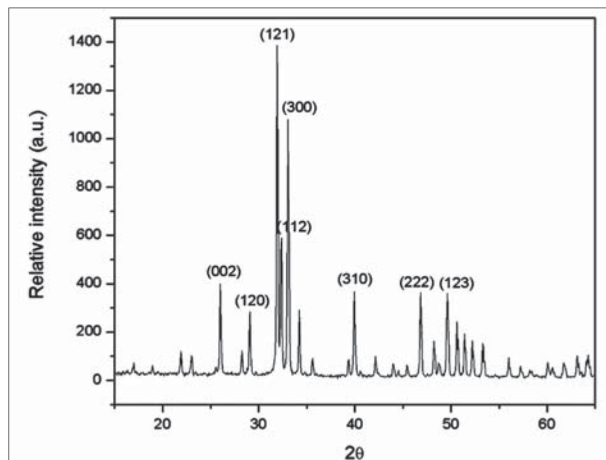
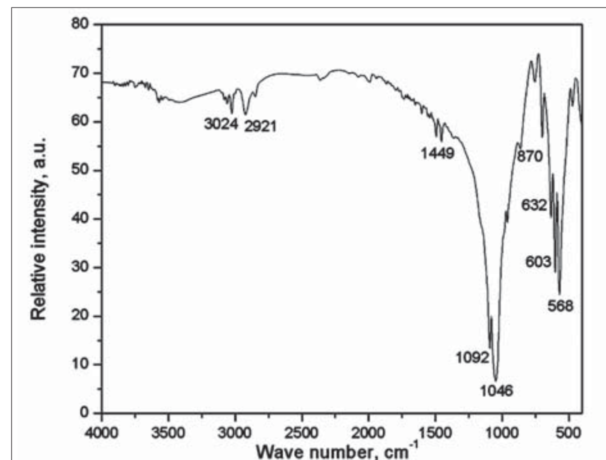


Graph 1. XRD pattern of CHA scaffold granules



Graph 2. FTIR spectra of CHA scaffold granules

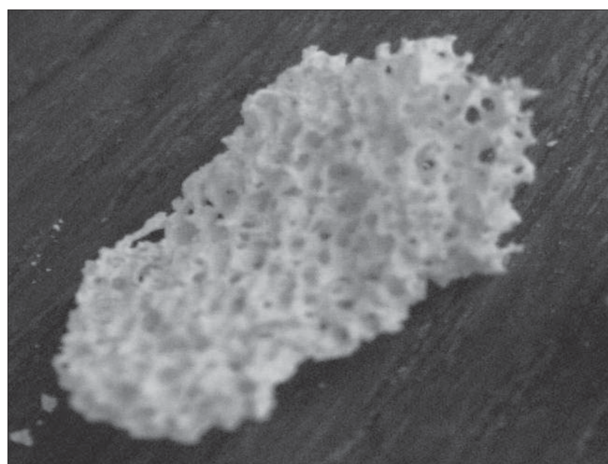


Figure 1. Enlarged photograph of the CHA compact

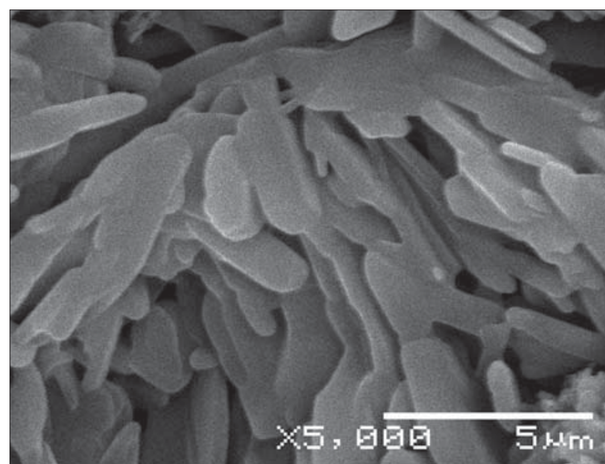


Figure 2. SEM: Typical appearance of the inner walls of CHA scaffold granules at 150,000× magnification

brations of CO_3^{2-} group at 1,449 and 870 cm^{-1} indicate the presence of carbonate groups in the apatite structure that partially replace OH^- ions. Based on this, it was identified that the carbonate apatite type B had been obtained, which is the most active form of carbonated apatite (type B predominates in the bones of young people, while the A type predominates in the bones of elderly people). The bands at 2,921 cm^{-1} and 3,024 cm^{-1} correspond to the ν(C-H) symmetric and asymmetric (respectively) stretching vibrations of intercalated PEVA/PEVV at the surface of CHA particles. A broad band between 3,150 cm^{-1} and 3,500 cm^{-1} can be attributed to the OH stretching vibrations, which belong to various hydroxyl groups present in the sample.

Porous CHA compacts had very porous 3D macrostructure (Figure 1). The pores were cylindrical and interconnected, with a diameter of 0.1–1 mm, while most of them have a diameter of 0.2–0.3 mm. The average scaffold porosity was 87 ± 8%. Scaffold granules obtained by scaffold disintegration were from 300 µm to 1 mm.

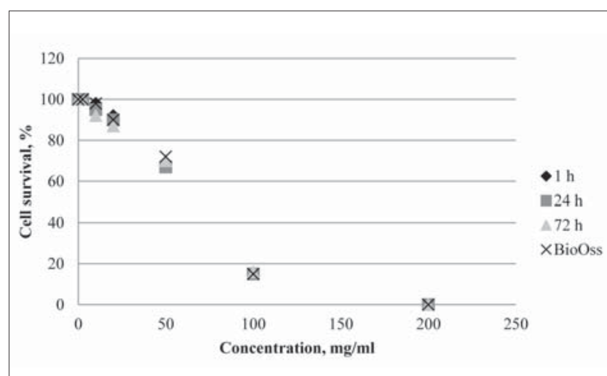
The structure of the inner walls of CHA scaffold granules is shown on SEM micrograph (Figure 2). A well-defined internal geometry could be observed with the elongated grains of a mean diameter 0.5–1.5 µm, and length 1.7–4 µm.

Investigations of the nano-structure of the scaffold walls, by mercury porosimetry, showed the presence of nano-pores with diameters of 30–400 nm, with prevailing pores in the range of 100–200 nm.

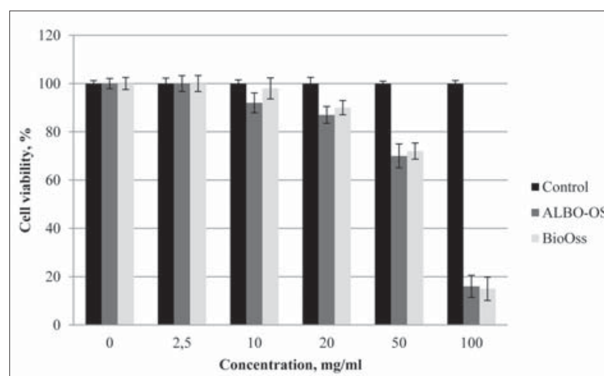
Indirect cytotoxicity

MTT test

The results of MTT test are presented as the percentage of viable L929 fibroblasts in the presence of ALBO-OS extracts at different concentrations (obtained after ALBO-OS degradation in the culture medium after one, 24, and 72 hours) compared to the controls, which represent a pure cell culture of L929 cells without ALBO-OS (Graph 3). Concerning cell survival relative to the concentration of the extract, we noticed the following: for concentration of 200 mg/ml (100% extract), the cytotoxic effect was total (the number of survived cells was negligible); for concentration of 100 mg/ml (two times diluted extract) survival rate was about 15%; for concentration of 50 mg/ml (four times diluted 100% extract) survival was 60–70%, and for concentrations up to 20 mg/ml, the percentage of survival was 80–100%, regardless of the contact time with the cell extract. Similar results were obtained for BioOss. BioOss extract was obtained after a 72-hour degradation in culture



Graph 3. Survival of cell culture of L929 mouse fibroblasts in the presence of ALBO-OS extract at different concentrations, for the extracts obtained after exposure of culture medium to ALBO-OS for one, 24, and 72 hours, and in the presence of the extract of BioOss; survival in the control group was 100%. Results are given as mean values of three independent experiments with five wells in each (n = 15). Standard deviations (SD) were between ± 5 (for higher concentrations) and ± 15 (for lower concentrations).



Graph 4. MTT viability assay of L929 mouse fibroblasts incubated with extracts of ALBO-OS and BioOss (obtained after exposure of culture medium to ALBO-OS/BioOss for 72 hours). Data are expressed as percentage over the control cells, considering the control group as 100% (cells without exposure to materials). Results are given as mean values of cell viability of three independent experiments with five wells in each ± SD (n = 15).

medium and the same concentrations were used as in the case of ALBO-OS.

As it is evident from Graph 3, cell survival was only slightly dependent on the contact time between the growth medium and the material, while it was highly dependent on the extract concentration.

In order to define the difference between ALBO-OS and BioOss more precisely, only results obtained in the presence of ALBO-OS and BioOss extracts obtained after 72 h were compared (Graph 4). Statistically significant difference between them was noticed only for concentrations of 10 and 20 mg/ml ($p < 0.05$).

Phase contrast microscopy

The morphology of cells that were in contact with extracts of ALBO-OS for 24, 48, or 72 h, were observed by phase contrast microscopy (Figures 3a, 3b, and 3c). An increase of cytotoxicity with the increase of time the medium and ALBO-OS are in contact could be observed (Figure 3a). Observing the contact of cells with the ALBO-OS extract obtained after one hour of contact with the medium, it can be seen that the cell morphology was similar while their density was lower than that in the control group (that was the case only in contact with the culture medium). When the extract obtained after a 24-hour contact was used for cell cultivation, cell density was also lower, compared to the control, and morphology was changing from elongated forms, typical for fibroblasts, to spheroidal form. In the case of extract obtained after 72 hours, cell number was significantly lower than in the control group and cells were spherical in shape.

From Figure 3b it is obvious that the cytotoxicity increased with the increase of contact time of the culture medium with ALBO-OS. Observing the cells cultured in the ALBO-OS extract obtained after one hour of contact with the medium, cell morphology was similar, while their density was lower than in the control group. In the case of the extracts obtained after 24 and 72 hours, cell density was significantly lower than in the control group, and the spherical morphology was observed. There was no significant difference between the cells that were in contact with the extract of ALBO-OS obtained after 24 and 72 h.

The cytotoxicity continued to grow with the increase of contact time between the culture medium and ALBO-OS (Figure 3c). In contact of the cells with the extract of ALBO-OS obtained after one hour, the cell morphology was similar, while their density was lower than in the control group. When the extract obtained after 24 and 72 hours were used, cell density was significantly lower than in the control group and the morphology was spherical. A significant difference was observed in the density of cells that were seeded in the extract of ALBO-OS obtained after 24 and 72 hours of contact with the culture medium.

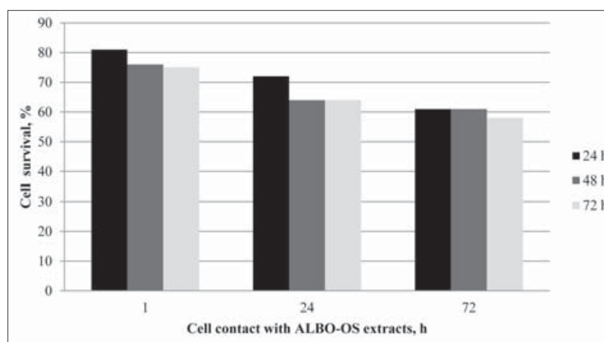
It is evident from Graph 5 that the number of viable L929 cells slightly decreased when they were exposed to the products of ALBO-OS degradation over a period of 24, 48, and 72 hours, i.e. cytotoxicity slightly increased with the increase of contact time between the cells and the ALBO-OS extracts. The ratio between the density of survived cells in three different ALBO-OS extracts (obtained after one-, 24-, and 72-hour contact of ALBO-OS and medium) and control (expressed as percentage) showed mild decrease with the increase of contact time between ALBO-OS and medium (Graph 5).

Comparatively observing the presented results, the test material ALBO-OS showed quite similar results from the standpoint of cytotoxicity on L929 fibroblast culture as the Geistlich BioOss (Geistlich Pharma North America Inc., Princeton, NJ, USA) gold standard (Graph 3).

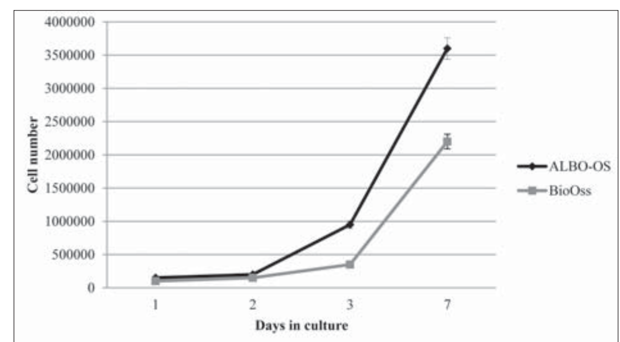
Direct Cytotoxicity

Cell proliferation, shown in Graph 6 as the number of cells adhered to the surface as a function of time, showed clearly that the number of cells grows during the entire period of ALBO-OS contact with the cells. The initial cell number in the well was 100,000. On the first day of the experiment

Figure 3. Phase contrast microscopy images of mouse fibroblasts L929 treated for: a) 24 h, b) 48 h, and c) 72 h, with extracts obtained after contact of culture media with ALBO-OS for one, 24 and 72 hours (magnification 100×)



Graph 5. Percentage of survived cells in ALBO-OS extracts (obtained after contact of medium and ALBO-OS for one, 24, and 72 hours) compared with survived cells in control, attained from contrast phase microscopy images obtained after 24-, 48-, and 72-hour contact with cells. For each sample 15 images were analyzed. SDs were between ± 5 and ± 10.



Graph 6. The number of L929 cells adhered on ALBO-OS and BioOss. Data are expressed as mean values ± SD of three independent experiments with five wells in each (n = 15).

the number of cells in the presence of ALBO-OS did not significantly increase, but from the second day of the experiment the cells were extensively multiplied and their number was growing exponentially, so that it was 3.6×10^6 cells per well on the seventh day. Obviously, ALBO-OS did not show cytotoxicity in direct contact with the L929 fibroblast cell culture because the number of cells grew steadily over the entire period of contact. Statistically significant difference between ALBO-OS and BioOss was noticed after two days ($p < 0.001$).

DISCUSSION

High average ALBO-OS scaffold porosity (nearly 87%) and its macrostructure, as it is shown in Figure 3a, mainly built from cylindrical and interconnected pores with a diameter of 200–300 μm, is very suitable for convenient adhesion of bone cells. The other very important element for cell adhesion and proliferation is very specific nanotopology of scaffold pore walls, with properly distributed hills and valleys, which enables the attachment of cell filopodia, as it is shown in Figure 3b.

Considering the results of MTT assay (Graph 3), it is evident that cell survival was highly dependent on the extract concentration, while it was just slightly dependent on the extraction time. The results obtained for ALBO-OS and BioOss for extraction time of 72 hours were very similar, with statistically insignificant difference. Similar results, for systems with PLGA alone or a component of a composite with CHA, were obtained in other investigations, showing the cytotoxicity increase with the extract concentration, caused mainly by the decrease in pH after PLGA degradation to PGA and PLA [20–23].

Phase contrast microscopy showed that the cytotoxicity of ALBO-OS slightly increased with the increase of contact time between cells and ALBO-OS extracts, while the increase of its cytotoxicity was more pronounced for longer extraction time when cell morphology became slightly changed (Figure 3 and Graph 5).

LDH assay showed that after the second day of the experiment the cells were already significantly multiplied, growing exponentially over the entire period of contact. This was also confirmed in previous investigations of the same material (ALBO-OS) on dental pulp stem cells, where LDH test showed favorable effect of the material on the cell proliferation [21]. The comparison of the number of cells grown on ALBO-OS and BioOss showed that in first two days, when cells were in the adaptation phase, the difference was not so significant although the number of cells grown on ALBO-OS was about 1.5 times higher. After three days, significant difference could be noticed (the number of cells on ALBO-OS was about 2.7 times higher than on BioOss), while after seven days of cultivation, the cell number on ALBO-OS was only 1.6 times higher. The difference in cell number on ALBO-OS and BioOss was particularly expressed after three days of cultivation. This can be assigned to shorter adaptation period of cells to ALBO-OS, due to its synthetic origin, while BioOss is of natural origin, containing remained collagen fibres, which induces corresponding immune response. The results for hydroxyapatite-PLGA composite scaffolds obtained in investigations of other researchers also showed the increase of cell number with contact time, but the data are not completely comparable because the increase of cell number on ALBO-OS was significantly faster [24, 25].

CONCLUSION

The paper describes synthesis of hydroxyapatite scaffold with surface modified using PLGA thin film, signed as ALBO-OS. The phase analysis of ALBO-OS was made by XRD and FTIR, while its geometrical structure and appearance of thin walls of porous body were investigated by SEM. The macroscopic structure showed a high degree of interconnected pores with a diameter of 0.1–1 mm. Specific morphology, shown by SEM, indicated that ALBO-OS probably has great potential as a synthetic bone substitute.

Biological investigations, using MTT and LDH tests and inverse phase microscopy, confirmed that this material is not cytotoxic for fibroblast cells L929. The MTT test showed similar results for ALBO-OS and Bio-Oss, while LDH test of cell viability showed significantly better results for ALBO-OS, probably due to its synthetic origin.

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Испитивање цитотоксичности носача на бази хидроксиапатита с унапређеним структурним дизајном

Никола Сјеробабин[1], Божана Чоловић[2], Милан Петровић[3], Дејан Марковић[3], Славољуб Живковић[3], Вукоман Јокановић[2]
[1]Клиничко-болнички центар „Др Драгиша Мишовић – Дедиње“, Београд, Србија;
[2]Универзитет у Београду, Институт за нуклеарне науке „Винча“, Београд, Србија;
[3]Универзитет у Београду, Стоматолошки факултет, Београд, Србија

КРАТАК САДРЖАЈ
Увод Порозни биодеградабилни носачи су се показали као добри заменици кости јер делују као привремена физичка потпора за усмерену регенерацију ткива док се читав носач потпуно не разгради и замени новим ткивом.
Циљ рада Циљ овог рада је био да се испита цитотоксичност носача ткива на бази калцијум-хидроксиапатита, високе порозности и оптималне топологије, названог *ALBO-OS*.
Методе рада Заменик кости *ALBO-OS* је синтетисан методом матрице направљене од полимерне пене. Геометрија пора и зидова носача анализиране су помоћу скенирајуће електронске микроскопије (СЕМ). Биолошка истраживања изведена су испитивањем цитотоксичности *ALBO-OS*-а на мишјим фибробластима *L*929 помоћу *MTT* и *LDH* тестова и фазно контрастне микроскопије.
Резултати СЕМ анализа је показала велику и равномерну порозност и занимљиву морфологију са биолошког становишта. Биолошка истраживања показала су да материјал није цитотоксичан. Поређењем материјала *ALBO-OS* и *Bio-Oss*, који је, глобално, златни стандард међу заменицима кости, добијени су слични резултати на *MTT* тесту, док су резулати *LDH* теста показали значајно већи број ћелијских деоба у контакту са *ALBO-OS*-ом.
Закључак Дизајн носача са становишта расподеле величине пора и топологије је веома погодан за адхезију и насељавање ћелија, због чега има велики потенцијал као заменик кости. Резултати биолошких тестова су показали да *ALBO-OS* није цитотоксичан за *L*929 фибробласте. У поређењу са материјалом *Bio-Oss*, добијени су слични или бољи резултати.
Кључне речи: хидроксиапатит; цитотоксичност; *MTT*; *LDH*

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