Chimica Techno Acta
published by Ural Federal University
eISSN 2411-1414
chimicatechnoacta.ru

LETTER
2023, vol. 10(4), No. 202310402
DOI: 10.15826/chimtech.2023.10.4.02

Ceramic materials based on lanthanum zirconate for the bone augmentation purposes: cytocompatibility in a cell culture model

Maria Ulitko [ab], Yulia Antonets [a], Irina Antropova [cd]*, Albert Mullabaev [d], Elena Volokitina [cd], Anna Kasyanova [ad], Ekaterina Loginova [e], Nataliia Tarasova [d]

a: Ural Federal University, Ekaterinburg 620002, Russia
b: Institute of Medical Cell Technologies, Ekaterinburg 620026, Russia
c: Ural State Medical University, Ekaterinburg 620028, Russia
d: Institute of High-Temperature Electrochemistry, Ural Branch of the Russian Academy of Sciences, Ekaterinburg 620137, Russia
e: Prior-M, Ekaterinburg 620027, Russia
* Corresponding author: aip.hemolab@mail.ru

This paper belongs to a Regular Issue.

Abstract

Creation of new ceramic materials for the bone augmentation purposes that combine the absence of cytotoxicity, high strength and osseointegration characteristics is an urgent modern task. In this work, the cytocompatibility of ceramic materials based on lanthanum zirconate ($La_2Zr_2O_7$) was determined to assess the prospects for their use as implants and components of human joint endoprostheses. The effect of ceramic materials based on undoped and alkali-earth (Ca, Sr) doped $La_2Zr_2O_7$ on the viability and proliferative activity of human cells was evaluated. The release of elements into the culture medium was also evaluated.

Keywords

lanthanum zirconate
bioceramics
osteoreplacement material
cytocompatibility
cell culture

Received: 03.08.23
Revised: 11.08.23
Accepted: 14.08.23
Available online: 16.08.23

Key findings

- During the interaction with the studied materials, the human cell viability is sufficient to maintain their regenerative potential.
- Doping of $La_2Zr_2O_7$ with Ca or Sr slowing down the adaptation of human fibroblasts to the ceramic material.
- Sr, Zr and La were found in the culture medium, which did not affect cytocompatibility during the cultivation period.

1. Introduction

Bone defects caused by injuries or diseases are becoming more common and have a huge impact on the patient life quality [1]. Bone grafting and repair with autologous material is the gold standard, because it has a number of advantages: osteoconduction, osteoinduction and stimulation of osteogenesis. But possible complications should be taken into consideration: persistent pain syndrome may occur at the bone collection site (in situ), an infectious and inflammatory process may develop, an aesthetic defect may also occur [2, 3]. In this regard, the search for a new material for bone replacement is actively underway. Such materials should be biocompatible, promote cell adhesion, proliferation and differentiation without adverse effects on host tissue [4, 5]. Due to good biocompatibility, ceramic osseosubstituting materials have become widely used in practical medicine [6]. The presence of zirconium in ceramics significantly improves mechanical properties of the material [7, 8], without having toxic effect on preosteoblasts and improving the reaction of osteoblasts [9, 10]. Addition of lanthanum has a significant effect on corrosion resistance of the material, moreover, it has an inhibitory effect on the formation of osteoclasts [11, 12]. Doping ceramic materials can contribute to the process of osteogenesis and rapid bone healing [13, 14]. The doping quality depends on the type of ion used.

Doping with calcium promotes the synthesis of osteopontin, which is involved in bone formation and promotes cell attachment and proliferation on the implant surface [15]. The calcium-doped lanthanum zirconate demonstrates positive results in the process of bone remodeling and repair [16, 17]. The introduction of strontium into osteogenic materials is also considered promising [18]. It has been

shown that the introduction of strontium into calcium phosphate ceramics leads to an improvement in biocompatibility, osteoconductivity and strength [19]. Doping ions can contribute to osteogenesis and angiogenesis, induce effective regeneration of bone tissue. On the other hand, excessive accumulation of doping ions can provoke cytotoxicity and inhibition of biological activity [20]. Creation of new ceramic materials for the bone augmentation purposes that combine the absence of cytotoxicity, high strength and osseointegration characteristics is an urgent modern task.

In this research lanthanum zirconate was used, it was chosen was mainly due to the fact that its crystal structure is resistant to various substitutions, including calcium and strontium ions. Earlier basic physical and chemical properties of lanthanum zirconate doped with alkaline earth (Ca, Sr) were studied, the influence of the synthesis method and modification of doping impurities on the target characteristics of materials was determined [21].

In this work, the cytocompatibility of ceramic materials based on lanthanum zirconate was determined to assess the prospects for their use as implants and components of human joint endoprostheses. The effect of three samples of complex oxides based on lanthanum zirconate: undoped $La_2Zr_2O_7$, calcium-doped $La_{0.9}Ca_{0.1}Zr_2O_{6.95}$, and strontium-doped $La_{0.9}Sr_{0.1}Zr_2O_{6.95}$ on the viability and proliferative activity of human cells was evaluated. The release of elements into the culture medium was also evaluated.

2. Experimental

Samples of bioceramics $La_2Zr_2O_7$, $La_{0.9}Ca_{0.1}Zr_2O_{6.95}$ and $La_{0.9}Sr_{0.1}Zr_2O_{6.95}$ were obtained as described earlier [21].

To study the cytocompatibility of these materials, a culture of human dermal fibroblasts was used. Dermal fibroblasts are a good cell model, since they represent a heterogeneous cellular population of connective tissue cells that play an important role in regeneration and the regulation of homeostasis processes. The use of dermal fibroblasts as test cultures to study the cytotoxicity of various biologically active materials (including bone replacement materials) is a standard research practice [22, 23]. Cells were provided by the laboratory of cell cultures of the Institute of Medical Cell Technologies in Yekaterinburg. The cells were cultured in Eagle DMEM ("Biolot") medium with glutamine (1%), in the presence of 10% embryonic calf serum ("Biolot") and gentamicin (50 mg/l) at 37 °C, in a humidified atmosphere of 5% CO2.

Samples of bioceramics: $La_2Zr_2O_7$ (1), $La_{0.9}Ca_{0.1}Zr_2O_{6.95}$ (2) and $La_{0.9}Sr_{0.1}Zr_2O_{6.95}$ (3) in the form of round plates with a diameter of 5 mm and a thickness of 2 mm were sterilized for 30 minutes with ultraviolet radiation, washed with saline solution, dried in a sterile laminar flow box and placed into the wells of a 24-well tablet. A suspension of human fibroblasts in the volume of 500 µl was applied to ceramic plates. Wells without samples of ceramics served as a control. The cells were cultured without changing the medium for 5 days. The study of cytocompatibility of ceramic materials included the determination of viability and proliferative activity of cells after 24, 72 and 120 hours of cultivation respectively.

Cell viability was assessed using a hemocytometer for the absorption of trypan blue by dead cells according to the international standard ISO 10993-5. The counting of living and dead cells was carried out after their disaggregation with a mixture of trypsin and versene in a ratio of 1:3 and staining with a 0.4% solution of trypan blue. Only dead cells were stained. Based on the data obtained, the viability index (VI) and the proliferation index of the culture were calculated. The viability index was determined by the Equation 1.

$$VI = (\text{viable (living)cells number})/ /(\text{total number of cells}) \cdot 100\% \quad (1)$$

The proliferation index was defined as the ratio of the number of grown cells to the initial number of cells. To assess the statistical significance of the differences between the control group and each of the experimental groups, the non-parametric Mann-Whitney criterion was used. At $p \leq 0.05$, the differences were considered statistically significant.

In order to determine the potential release of Ca, Sr, Zr and La from the ceramic materials $La_2Zr_2O_7$, $La_{0.9}Ca_{0.1}Zr_2O_{6.95}$ and $La_{0.9}Sr_{0.1}Zr_2O_{6.95}$, samples of the medium were taken after 24 and 72 hours of human fibroblast cultivation. The control was the culture medium from wells without ceramic samples. The concentration of Ca and Sr doping elements in DMEM medium samples was determined by inductively coupled plasma mass spectrometry on a NexIon 2000 device (Perkin Elmer, USA). The sample of the DMEM medium of about 0.5 g was weighed in PTFE beakers on analytical scales with the accuracy of 0.0001 g. 2 cm^3 of nitric acid purified by distillation without boiling was added to a beaker with a sample attachment, the beaker was covered with a PTFE lid and heated at a temperature of 150 °C until the release of nitrogen oxides ceased. After that, the contents of the beaker were quantitatively transferred into polypropylene measuring flasks with a capacity of 50 cm^3 and brought to the mark with deionized water with the specific electrical resistance of 18.2 MΩ cm. The initial solution was diluted with 1 wt.% nitric acid solution and analyzed on a mass spectrometer. Calibration characteristic of the spectrometer, which expresses the dependence of analytical signal value of ^{43}Ca and ^{88}Sr isotopes on the mass concentration of elements, was established using three standard calibration samples with concentrations of Ca, Sr, 10, 50 and 100 ppb and three series of measurements. Calibration solutions were prepared from high-purity single-element samples of PerkinElmer Pure grade Aquatic Standards. Spectral overlays on the ^{43}Ca isotope were eliminated by ammonia gas in the mass spectrometer reaction cell. Control sample of the DMEM medium was used as a blank sample. The arithmetic mean of three parallel measurements was taken as the result of the analysis.

 DOI: 10.15826/chimtech.2023.10.4.02

3. Results and Discussions

In the course of the experiment a change in the viability and proliferative activity of cells was observed when they were cultured on ceramic samples. After 24 hours of cultivation on samples (2) and (3), there is a decrease in the viability of fibroblasts relative to the control by 11.4% and 16.5%, respectively. At the same time, the fibroblast viability index during cultivation on sample (1) did not differ significantly from the control (Figure 1). There was no proliferative activity of fibroblasts after 24 hours of cultivation, both in the control and on experimental samples, which corresponds to the lag phase – the period when cells adapt to the new environment (Figure 2).

After 72 hours of cultivation, a lower level of cell viability is observed relative to the control for all samples, including sample (1). The proliferative activity of fibroblasts on all experimental samples lags behind the control, however, it increases relative to a 1-day period, which indicates the development of compensatory reactions in cell culture in response to the action of ceramic materials. After 120 hours of cultivation, the viability of fibroblasts increases to the control level in wells containing samples (1) and exceeds 60% of the control in wells with samples (2) and (3). The proliferative activity of cells on all ceramic materials is significantly lower than the control level, but increases relative to the previous period. At the same time, it was noted that cell proliferation occurs much more actively when grown on ceramic samples (1). The continued gradual increase in proliferative activity and cell viability index during cultivation on ceramic samples may indicate successful adaptation of human fibroblast cells to the effects of ceramic materials due to extracellular regeneration mechanisms.

Thus, when human fibroblasts interact with ceramic materials $La_2Zr_2O_7$ (1), $La_{0.9}Ca_{0.1}Zr_2O_{6.95}$ (2) and $La_{0.9}Sr_{0.1}Zr_2O_{6.95}$ (3), cell viability varies within acceptable values and is sufficient to maintain their recovery potential. Doping of lanthanum zirconate with calcium or strontium slows down the adaptation of human fibroblasts during cultivation on the studied ceramic materials without significant differences between $La_{0.9}Ca_{0.1}Zr_2O_{6.95}$ and $La_{0.9}Sr_{0.1}Zr_2O_{6.95}$. It can be assumed that the introduction of additional ions (calcium or strontium) into the structure of the lanthanum zirconate leads to the need for additional time for the adaptation of cells growing in culture on the studied materials. Additional studies are needed to elucidate the nature of this phenomenon.

The results of determining the concentrations of Ca, Sr, Zr and La in DMEM culture medium samples in the dynamics of human fibroblast cultivation are shown in Figure 3. The concentration of Ca ions in the culture medium with ceramic samples did not exceed the control level, which indicates the absence of this element release from all studied ceramic samples during the study period (Figure 3a).

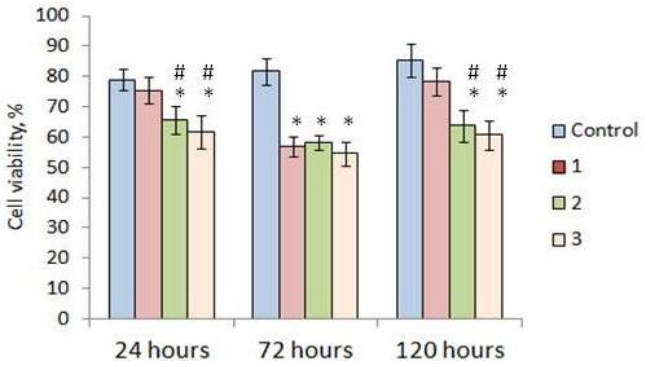

Figure 1 Influence of bioceramic samples on the viability of human fibroblasts. $La_2Zr_2O_7$ (1), $La_{0.9}Ca_{0.1}Zr_2O_{6.95}$ (2) and $La_{0.9}Sr_{0.1}Zr_2O_{6.95}$ (3); * – differences with control are statistically significant ($p<0.05$); # – differences in doped samples with undoped lanthanum zircon are statistically significant ($p<0.05$).

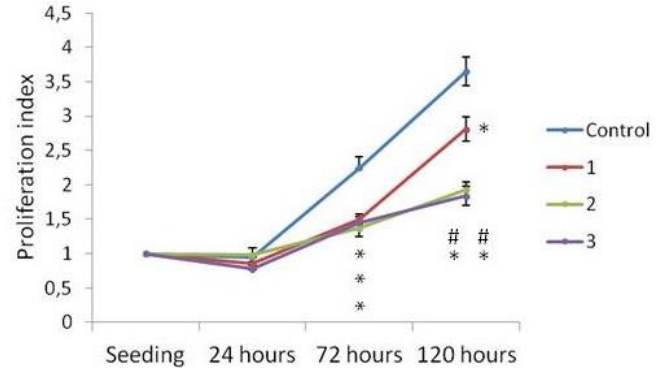

Figure 2 Human fibroblast culture proliferation index when exposed to ceramic samples: $La_2Zr_2O_7$ (1), $La_{0.9}Ca_{0.1}Zr_2O_{6.95}$ (2) and $La_{0.9}Sr_{0.1}Zr_2O_{6.95}$ (3); *– differences with control are statistically significant ($p<0.05$); # – differences in doped samples with undoped lanthanum zircon statistically significant ($p<0.05$).

The analysis revealed the presence of Sr ions in the medium with the samples of the material (3), while it was noted that the concentration of these ions increased strating from the first to the third day of cultivation (Figure 3b).

The level of Zr ions in the medium during cell culture on samples (1) did not differ from the control. Cultivation on samples (2) resulted in a slight increase in the concentration of Zr in the medium by the end of the third day. When using samples (3), an increase in the concentration of Zr ions in the medium was detected on the 1st day of cultivation with a slight further increase by the 3rd day (Figure 3c).

When culturing fibroblasts on samples (1), a slight release of La ions into the medium was observed without a significant increase in the dynamics of the studied period. For samples (2), there was also no increase in the release of La ions from the 1st to the 3rd day, although their release was 2 times higher than on samples (1). The release of La ions from samples (3) into the medium was more active compared to samples (1) and (2), and a significant increase in the concentration of this ion was noted the 1st to the 3rd day (Figure 3d).

 DOI: 10.15826/chimtech.2023.10.4.02

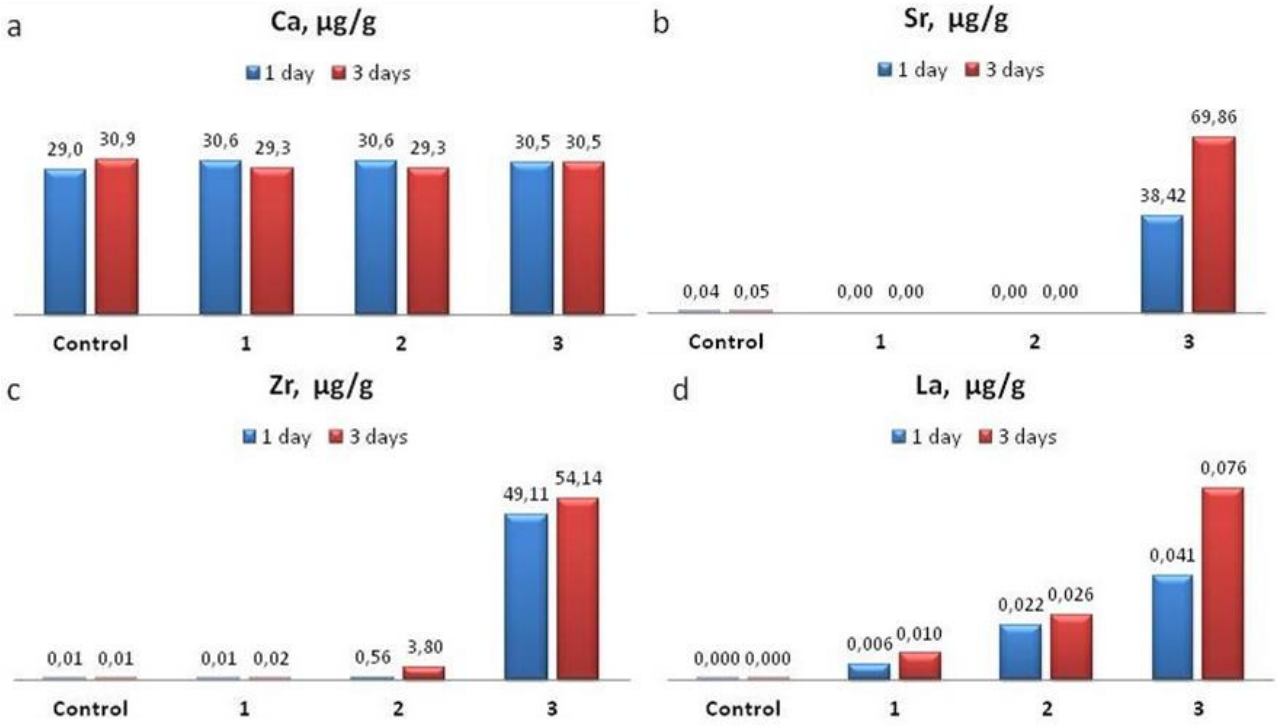

Figure 3 The concentration of Ca (a), Sr (b), Zr (c) and La (d) in the medium after 1 and 3 days of cultivation of human fibroblasts on ceramic samples: $La_2Zr_2O_7$ (1), $La_{0.9}Ca_{0.1}Zr_2O_{6.95}$ (2) and $La_{0.9}Sr_{0.1}Zr_2O_{6.95}$ (3).

The study showed that Ca is resistant and is not released from the studied samples into the culture medium. Sr and Zr ions are less stable, they are released into the medium mainly from the material (3). La is the least stable, and these ions are also released into the medium most actively from the material (3). It should be noted that despite the obvious differences in the release of Sr, Zr and La from the samples of materials (2) and (3), no significant differences in the viability and proliferative activity of fibroblasts during cultivation on these samples within the cultivation period were found.

4. Limitations

The limitation of this work is the inability to establish the effect of new ceramic materials on the body as a whole in the dynamics of the regenerative process after bone injury, which is the driving impulse for the next stage of preclinical studies using laboratory animals.

5. Conclusion

This paper presents the results of the first stage of preclinical studies of new complex oxides based on lanthanum zirconate, created for bone augmentation. Cytocompatibility of ceramics based on undoped and doped lanthanum zirconate ($La_2Zr_2O_7$, $La_{0.9}Ca_{0.1}Zr_2O_{6.95}$ and $La_{0.9}Sr_{0.1}Zr_2O_{6.95}$) was determined. It was established that during the interaction of human fibroblasts with the studied ceramic materials, the viability of cells varies within acceptable values and is sufficient to maintain their regenerative potential. Doping of lanthanum zirconate with calcium or strontium affects cytocompatibility by slowing down the adaptation of human fibroblasts to the ceramic material. Additional studies are needed to elucidate the nature of this effect. It should also be taken into account that some growth retardation at the initial stages of regeneration may not affect the regeneration process as a whole. To test this assumption, it is necessary to conduct animal experiments.

It was found that Ca is resistant and is not released from the studied samples into the culture medium. The release of Sr, Zr and La ions into the culture medium was detected, but this did not significantly affect the proliferative activity and viability of human fibroblasts during the cultivation period. Cytocompatibility of new ceramic materials was determined on the culture of human fibroblast cells. However, this work is the first stage of preclinical studies of new complex oxides based on lanthanum zirconate.

• Supplementary materials

No supplementary materials are available.

• Funding

This work was supported by the Russian Science Foundation (grant no. 22-25-20037), https://www.rscf.ru/en.

• Acknowledgments

None.

 DOI: 10.15826/chimtech.2023.10.4.02

• Author contributions

Conceptualization: M.U., I.A., N.T.
Data curation: M.U, I.A.
Funding acquisition: E.V.
Investigation: Y.A., E.L., A.K., A.M.
Methodology: M.U., A.M.
Validation: M.U., I.A, A.M.,
Visualization: I.A.
Writing – original draft: M.U., I.A.
Writing – review & editing: I.A., N.T.

• Conflict of interest

The authors declare no conflict of interest.

• Additional information

Author IDs:
Maria Ulitko, Scopus ID 56766009100;
Irina Antropova, Scopus ID 18338795600;
Albert Mullabaev, Scopus ID 57156662300;
Elena Volokitina, Scopus ID 57194755505;
Anna Kasyanova, Scopus ID 57192873241;
Natalia Tarasova, Scopus ID 37047923700.

Websites:
Ural Federal University, https://urfu.ru/en/;
Ural State Medical University, https://usma.ru/en/main/;
Institute of Medical Cell Technologies, https://celltechnologies.ru/;
Institute of High Temperature Electrochemistry UB RAS, http://www.ihte.uran.ru/;
Prior-M, https://www.prior-m.ru/.

References

1. Valtanen RS, Yang YP, Gurtner GC, Maloney WJ, Lowenberg DW. Synthetic and Bone tissue engineering graft substitutes: What is the future? Injury. 2021;52:S72–S77. doi:10.1016/j.injury.2020.07.040
2. Schlundt C, Bucher CH, Tsitsilonis S, Schell H, Duda GN, Schmidt-Bleek K. Clinical and research approaches to treat non-union fracture. Curr Osteoporos Rep. 2018;16(2):155–168. doi:10.1007/s11914-018-0432-1
3. Le BQ, Nurcombe V, Cool SM, van Blitterswijk CA, de Boer J, LaPointe VLS. The Components of bone and what they can teach us about regeneration. Mater (Basel). 2017;11(1):14. doi:10.3390/ma11010014
4. Carotenuto F, Politi S, Ul Haq A, De Matteis F, Tamburri E, Terranova ML, Teodori L, Pasquo A, Di Nardo P. From soft to hard biomimetic materials: tuning micro/nano-architecture of scaffolds for tissue regeneration. Micromach (Basel). 2022;16;13(5):780. doi:10.3390/mi13050780
5. Tang G, Liu Z, Liu Y, Yu J, Wang X, Tan Z, Ye X. Recent trends in the development of bone regenerative biomaterials. Front Cell Dev Biol. 2021;9:665813. doi:10.3389/fcell.2021.665813
6. Pina S, Rebelo R, Correlo VM, Oliveira JM, Reis RL. Bioceramics for osteochondral tissue engineering and regeneration. Adv Exp Med Biol. 2018;1058:53–75. doi:10.1007/978-3-319-76711-6_3
7. Depprich R, Naujoks C, Ommerborn M, Schwarz F, Kübler NR, Handschel J. Current findings regarding zirconia implants. Clin Implant Dent Relat Res. 2014;16(1):124–137. doi:10.1111/j.1708-8208.2012.00454.x
8. Skjöldebrand C, Tipper JL, Hatto P, Bryant M, Hall RM, Persson C. Current status and future potential of wear-resistant coatings and articulating surfaces for hip and knee implants. Mater Today Bio. 2022;15:100270. doi:10.1016/j.mtbio.2022.100270
9. Chen Y, Roohani-Esfahani SI, Lu Z, Zreiqat H, Dunstan CR. Zirconium Ions Up-Regulate the BMP/SMAD Signaling Pathway and Promote the Proliferation and Differentiation of Human Osteoblasts. Plos One. 2015;10(1):e0113426. doi:10.1371/journal.pone.0113426
10. Bhowmick A, Pramanik N, Jana P, Mitra T, Gnanamani A, Das M, Kundu PP. Development of bonelike zirconium oxide nanoceramic modified chitosan based porous nanocomposites for biomedical application. Int J Biol Macromol. 2017;95:348–356. doi:10.1016/j.ijbiomac.2016.11.052
11. Pantulap U, Arango-Ospina M, Boccaccini AR. Bioactive glasses incorporating less-common ions to improve biological and physical properties. J Mater Sci Mater Med. 2021;33(1):3. doi:10.1007/s10856-021-06626-3
12. Jiang C, Shang J, Li Z, Qin A, Ouyang Z, Qu X, Li H, Tian B, Wang W, Wu C, Wang J, Dai M. Lanthanum Chloride Attenuates Osteoclast Formation and Function Via the Downregulation of Rankl-Induced Nf-κb and Nfatc1 Activities. J Cell Physiol. 2016;231(1):142–151. doi:10.1002/jcp.25065
13. Mabrouk M, Ibrahim Fouad G, Beherei HH, Das DB. Barium oxide doped magnesium silicate nanopowders for bone fracture healing: preparation, characterization, antibacterial and *in vivo* animal studies. Pharmaceut. 2022;14(8):1582. doi:10.3390/pharmaceutics14081582
14. Zhu DY, Lu B, Yin JH, Ke QF, Xu H, Zhang CQ, Guo YP, Gao YS. Gadolinium-doped bioglass scaffolds promote osteogenic differentiation of hBMSC via the Akt/GSK3β pathway and facilitate bone repair in vivo. Int J Nanomed. 2019;14:1085–1100. doi:10.2147/IJN.S193576
15. Jung GY, Park YJ, Han JS. Effects of HA released calcium ion on osteoblast differentiation. J Mater Sci Mater Med. 2010;21(5):1649–1654. doi:10.1007/s10856-010-4011-y
16. Izmodenova MYu, Gilev MV, Ananyev MV, Zaytsev DV, Antropova IP, Farlenkov AS, Tropin ES, Volokitina EA, Kutepov SM, Yushkov BG [Bone Tissue Properties after Lanthanum Zirconate Ceramics Implantation: Experimental Study]. Travmatologiya i ortopediya Rossii [Traumatology and Orthopedics of Russia]. 2020;26(3):130-140. (In Russian). doi:10.21823/2311-2905-2020-26-3-130-140
17. Antropova IP, Volokitina EA, Udintseva MYu, Yushkov BG, Tyumentseva NV, Kutepov SM. [Effect of Lanthanum Zirconate Ceramic on the Dynamics of Hematological Parameters and the Bone Remodeling Markers: Experimental Study]. Travmatologiya i ortopediya Rossii [Traumatology and Orthopedics of Russia]. 2022;28(1):79–88. (In Russian). doi:10.17816/2311-2905-1704
18. Chen Y, Liu Z, Jiang T, Zou X, Lei L, Yan W, Yang J, Li B. Strontium-substituted biphasic calcium phosphate microspheres promoted degradation performance and enhanced bone regeneration. J Biomed Mater Res Part A. 2020;108:895–905. doi:10.1002/jbm.a.36867
19. Silva ADR, Pallone EMJA, Lobo AO. Modification of surfaces of alumina-zirconia porous ceramics with Sr^{2+} after SBF. J Aust Ceram Soc. 2020;56:517–524. doi:10.1007/s41779-019-00360-4
20. Pereira P, Neto AS, Rodrigues AS, Barros I, Miranda C, Ramalho-Santos J, Pereira de Almeida L, Ferreira JMF, Coelho JFJ, Fonseca AC. *In vitro* evaluation of biphasic calcium phosphate scaffolds derived from cuttlefish bone coated with poly(ester urea) for bone tissue regeneration. Polymers (Basel). 2023;15(10):2256. doi:10.3390/polym15102256
21. Tarasova N, Galisheva A, Belova K, Mushnikova A, Volokitina E. Ceramic materials based on lanthanum zirconate for the

 DOI: 10.15826/chimtech.2023.10.4.02

bone augmentation purposes: materials science approach Chimica Techno Acta. 2022;2:9. doi:10.15826/chimtech.2022.9.2.09

22. Bokov AE, Bulkin AA, Davydenko DV, Orlinskaya NY, Egorikhina MN, Rubtsova YP, Charykova IN, Kovylin RS, Yudin VV, Chesnokov SA, Morozov AG, Mlyavykh SG, Aleynik DY. Biological Response to a Novel Hybrid Polyoligomer: *in vitro* and *in vivo* models. Sovrem Tekhnologii Med. 2021;12(6):36–44. doi:10.17691/stm2020.12.6.05
23. Hadzik J, Jurczyszyn K, Gębarowski T, Trytek A, Gedrange T, Kozakiewicz M, Dominiak M, Kubasiewicz-Ross P, Trzcionka-Szajna A, Szajna E, Simka W. An experimental anodized and low-pressure oxygen plasma-treated titanium dental implant surface-preliminary report. Int J Mol Sci. 2023;24(4):3603. doi:10.3390/ijms24043603