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Some physico-mechanical properties of composite biomaterials on the basis of biogenic hydroxyapatite with magnetic additives

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The effect of ferromagnetic additives in the form of magnetite on composite systems based on biogenic hydroxyapatite (BHA) and obtained by physico-mechanical and chemical methods was studied. It was established that the specific density of BHA/Fe_3O_4 powder obtained by physico-mechanical method for introduction of magnetite is close to that of trabecular bone. Herewith, the density of heat-treated pressed samples from this biomaterial is almost equal to that of cortical bone. Experiments in vitro revealed that a decrease in the material bioresorption is bound with a gradual increase in the amount of Ca^{2+} in the filtrates accompanied by a sharp decrease in PO_4^{-3}, Fe^{2+} and Fe^{3+} amounts within 5–7 days. The characteristics of elasticity were established to depend on the porosity and chemical composition of samples, decreasing with introduction of ferromagnetic additives that is probably due to the release of thermally induced carbon dioxide while decompositioning of iron oxalates. The results of this study indicate the expediency of using BHA-based biomaterials in orthopedic practice for restoration of bone tissue functions.

Keywords: biogenic hydroxyapatite, magnetite, bioresorption, mechanical properties.

Исследовано влияние ферромагнитных добавок в виде магнетита на композитные системы, полученные с использованием физико-механического и химического способов. Обнаружено, что удельная плотность порошка БГА/Fe_3O_4, полученного физико-механическим способом внесения магнетита, приближена к удельной плотности трабекулярной кости. При этом термически обработанные образцы этого биоматериала имеют удельную плотность, максимально приближенную к этой величине кортикальной костной ткани. В экспериментах *in vitro* установлено, что уменьшение скорости растворения вызвано постепенным увеличением количества Ca^{2+} в фильтратах при резком уменьшении PO_4^{-3}, Fe^{2+} и Fe^{3+} на протяжении 5–7 суток. Показано, что характеристики упругости зависят от пористости и химического состава образцов, уменьшаясь при добавлении ферромагнитных добавок, вероятно, из-за выхода диоксида углерода при термолизе вследствие реакции разложения оксалатов железа. Результаты исследований показали целесообразность использования указанных биоматериалов на основе БГА в ортопедической практике для восстановления функций костных тканей.

Деякі фізико-механічні властивості композитних біоматеріалів на основі біогенного гідроксиапатиту з магнітними добавками. *О.М.Отиченко, Т.Є.Бабутіна, Д.П.Зяткевич, Ю.Г.Безимянний, А.М.Колесников, О.М.Будиліна, Л.С.Проценко, О.Ю.Коваль, І.В.Уварова.*

Досліджено вплив феромагнітних добавок у формі магнетиту на композитні системи, одержані з використанням фізико-механічного та хімічного способів. Встановлено, що питома густина порошку БГА/Fe_3O_4, отриманого фізико-механічним способом внесення магнетиту, наближена до питомої густини трабекулярної кістки. При цьому термічно оброблені зразки даного біоматеріалу мають питому густину, максимально наближену до даної величини кортикальної кісткової тканини. У дослідах *in vitro* встановлено, що зменшення швидкості розчинності зумовлене поступовим збільшенням кількості Ca^{2+} у фільтратах при різкому зменшенні PO_4^{-3}, Fe^{2+} та Fe^{3+} протягом 5–7 доби. Показано, що характеристики пружності залежать від пористості та хімічного складу зразків, зменшуючись із додаванням феромагнітних добавок, імовірно, через вихід діоксиду вуглецю при термолізі внаслідок реакції розкладу оксалатів заліза. Результати досліджень показали доцільність використання зазначених біоматеріалів на основі БГА у ортопедичній практиці для відновлення функцій кісткових тканин.

1. Introduction

Nowadays, the tendency to increasing the number of patients who require reconstructive interventions on the skeleton remains topical. Therefore, the restoration of bone tissue, damaged or lost through various destructive processes, is a vital problem, along with satisfaction of the need in high-quality and harmless biomaterials for orthopedics [1].

From an engineering point of view, bone demonstrates a wide variation in morphology. However, for the development of osteomaterials only two of its types, cortical and trabecular, are most often considered. These types differ primarily in porosity and, accordingly, in the specific density. As known, the average density for hydrated cortical and trabecular tissues of the femur is 1.86 and 0.30 g/cm^3, respectively. Herein the spongy trabecular bone tissue is very sensitive to the specific density [2]. On a nanometer scale, a compact bone tissue consists of organic (30 wt. %), inorganic (60 wt. %) phases and water (10 wt. %) [2–4]. Its main inorganic component is hydroxyapatite (HA).

For medical purposes, synthetic [5, 6] and biogenic HA (BHA) [7] are used. Both of the types have a high level of biocompatibility (being nontoxic, nonantigenic, and noncarcinogenic), but possess only osteoconductive properties [7, 8]. Therefore, in most cases, in order to achieve the needed property of HA, the latter is taken as a basic component in manufacturing various composite systems via introduction of organic or inorganic additives. Among which iron and iron oxides occupy a marked place [9]. Along with various engineering applications (catalysis, storage of information, optoelectronics), magnetic nanoparticles and suspensions based on them are also used in medicine, in particular for contrasting images in magnetic resonance imaging, diagnosis of various diseases at the initial stage, purification and separation of various biological substances, as well as for treatment of malignant tumors and targeted drug delivery [10–13].

In medical practice, the above composite biomaterials are widely used as components of scaffolds [14], coatings on metal implants [15, 16], materials-fillers (powder or granules) of small bone defects that are not exposed to significant loads, etc. [7]. Between the surface of a ceramic implant and the surrounding tissues, an active interaction takes place, owing to which the implant undergoes biological resorption with the formation of bone tissue [17, 18].

For synthesis the composite systems HA/Fe or HA/Fe_3O_4 a coprecipitation method with the following hydrothermal method [19] can be used. Also known a sol-gel method [20], a biomimetic method [21], neutralization [22], method with using of ultrasonic irradiation [23] or spray-drying technique [24]. Another method is the introduction of the magnetite (powder or suspension) directly into the process of HA synthesis [25]. Less widespread is the use of mechanical and mechano-chemical methods [26, 27].

In accordance with the above, the aim of this work was to produce composite systems based on BHA via doping it with magnetite (obtained from the precipitate of iron oxalate) followed by heat treatment and to study the effect of ferromagnetic additives on the physical and mechanical properties of medicine-aimed materials on the basis of BHA.

Table 1. Physicochemical characteristics of studied powders

Material	Specific surface area, m^2/g	Element contents, wt. %		Specific density, g/cm^3
		Fe_{total}	C_{total}	
BHA (<160 μm)	5.60	0.03	–	0.57
BHA/Fe_3O_4 phys.-mech. method	7.38	1.07	0.51	0.40
BHA/Fe_3O_4 chem. method	8.82	1.01	0.62	0.61

2. Experimental

The present study was carried out using the following materials and samples:

— powder "Osteoapatyt Keramichny" (Ukraine), which is BHA obtained from cattle bone with a particle size of under 160 μm [28];

— powder composite materials BHA/Fe_3O_4 obtained by two methods: 1) *via* including BHA directly in the process of obtaining iron oxalate due to interaction of oxalic acid dehydrate with iron sulfate solution stabilized with a mixture of isopropanol and sucrose (chemical method) and 2) *via* mechanical mixing of BHA microgranules with iron oxalate in the hydrosuspension state (physico-mechanical method), followed by low temperature thermolysis in vacuum (10^{-1} mm Hg) for 2 h at 500°C (below the Curie point for magnetite, 572°C) [29];

— compact samples from these materials in the form of cylinders (diameter 10.0±0.2 mm, mass 1.9±0.3 g) subjected to thermolysis under the above conditions.

Total iron, calcium, and phosphorus contents in the studied powders and filtrates were determined by photocalorimetric method on a "FEK-56M" (Russia) device. The amount of total carbon was determined using an express analyzer "AN-7529" (Belarus). The specific surface area of the biomaterials was measured by the method of thermal desorption of nitrogen on a "MPP2" (Czech Republic) apparatus. The phase composition of the powder materials was controlled by X-ray diffractometer "DRON-3.0" under Co–K_α radiation. Surface morphology of the compact materials was studied using the microscope "JEOL Superprobe 733" with gold (Au) deposition.

Investigation of specific mass lost was conducted *in vitro* through placing powder materials in an inorganic model medium, namely 0.9 % sodium saline ("Arterium", Ukraine), for 2, 5, and 7 days under the thermostatic conditions at a temperature close to that of the human body (36.6–37.0°C).

The dissolution rate (Q_R), as a characteristic of the specific mass lost, was determined by the formula (1):

$$Q_R = \frac{\Delta m}{m_s \cdot t} \cdot 100, \quad (1)$$

where Δm is the change in a sample mass during the stay in the model medium; m_s is the initial mass; t is the time of the sample stay in the model medium [30].

The samples mass was determined on the analytical balance of "OHAUS Pioneer PA214C" firm ("OHAUS Corporation", China) with an accurate within 0.0001 g.

Dynamic characteristics of elasticity were determined on the basis of measuring the propagation rate of longitudinal elastic wave, the excitation parameters of which were chosen by taking into account the structural characteristics of compact samples. The wave velocity was determined from the time of end-to-end passage of the elastic momentum at a frequency of 0.6 MHz using a radio pulse method with a discrete delay under shock excitation of the transducer [31]. For this, a hardware complex for precise acoustic measurements, developed in Frantsevich Institute for Problems of Materials Science of the NAS of Ukraine, was used [32].

The velocity of the longitudinal elastic wave propagation (c), was determined by the formula (2):

$$c = \frac{h}{t - t_0}, \quad (2)$$

where h is the height of pressed sample, cm; t is the measured time, sec; and t_0 is the systematic error of measurement, sec.

The relationship between the elasticity (E), the velocity of elastic waves (c), and the density (ρ) was taken as follows (3):

$$E = \rho \cdot c^2. \quad (3)$$

Minimization of the measurement error caused by variation in the contact layer thickness and penetration of contact lubri-

Table 2. Results of chemical analysis of filtrates

Material	Content elements, mg								
	2 days			5 days			7 days		
	Ca	P	Fe	Ca	P	Fe	Ca	P	Fe
BHA (<160 μm)	0.33	0.02	–	0.40	0.01	–	0.48	0.003	–
BHA/Fe_3O_4 phys.-mech. method	0.37	0.02	0.09	0.48	0.01	0.01	0.55	0.015	0.020
BHA/Fe_3O_4 chem. method	0.30	0.02	0.08	0.35	0.06	0.03	0.44	0.017	0.017

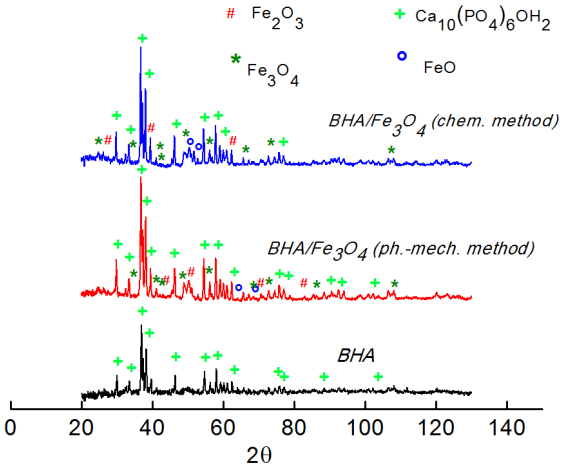

Fig. 1. Diffractograms of studied powder materials.

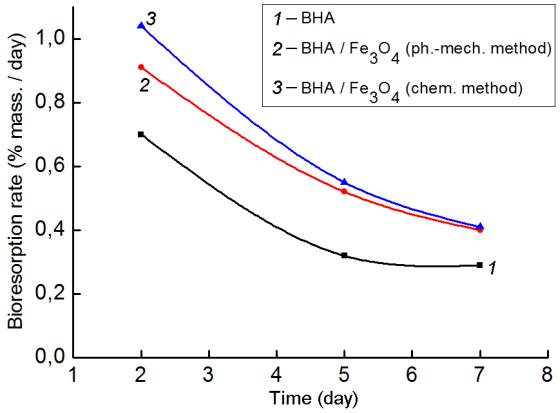

Fig. 2. Results of *in vitro* experiments.

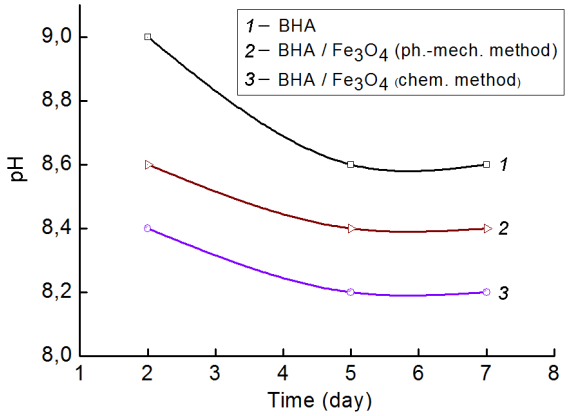

Fig. 3. Changes of pH level for filtrate during the experiment (pH (NaCl) = 7).

cant into the material as well as the preservation of sample integrity were provided with using a specific measurement procedure: in a special acoustic chamber, radiating and receiving transducers were aligned with the sample and pressed to it through a polymeric film using a normalized load. Herewith the measurement error was 1.5 %.

3. Results and discussion

It is known that the chemical composition, specific density, and porosity are the main characteristics which should be taken into account in designing any biomaterial for reconstruction of bone tissue. The corresponding data for used in the work pure and doped BHA powders are given in Table 1. These data correlate with the data of the X-ray phase analysis (Fig. 1).

When comparing the values of the specific density in Table 1 and ones for bone tissue, one can see that the density of the undoped BHA and BHA/Fe_3O_4 obtained by the physico-mechanical method is close to the average specific density of trabecular bone, 0.30 g/cm^3. This fact may be related to the presence in BHA of a predominant number of particles sizing within 20–80 μm. Whereas the sample obtained by mechanical blending of BHA and prepared iron oxalate is characterized by a significant number of particles in the size range <1 μm (12.8 %) and within 6–20 μm (total 41.8 %). For the sample BHA/Fe_3O_4 obtained by chemical method, the presence of particles <2 μm (total 27.1 %) and within 8–20 μm (total 35.9 %) is characteristic [24]. Comparison of the specific density of the powders with that of human bones leads to the conclusion on expediency of using BHA-based biomaterials to restore the functions of trabecular bone tissues.

Table 3. Physical characteristics of studied compact samples

Material	Shrinkage				Specific density, g/cm^3	Porosity, % (total/open)
	$\Delta m/m$	$\Delta h/h$	$\Delta d/d$	$\Delta V/V$		
BHA (<160 μm)	−0.009	−0.001	0.0	−0.001	1.80	42.2/38.8
BHA/Fe_3O_4 phys.-mech. method	−0.055	0.002	0.0	0.002	1.82	41.9/39.6
BHA/Fe_3O_4 chem. method	−0.042	−0.009	0.0	−0.041	1.67	47.0/35.6

In order to forecast the behavior of the studied materials in the human body, a series of experiments *in vitro* were conducted. The results of which for solid-state remains of sample are showed on Fig. 2.

Since during the formation of bone tissue, on the biomaterial surface, mineralization of the surrounding cells takes place due to release of Ca^{2+}, PO_4^{3-}, Fe^{2+}, Fe^{3+}, and other ions from the implanted material. It seemed reasonable to perform a chemical analysis of filtrates for the content of Ca, P, and total Fe (Table 2) and pH change (Fig. 3) after the experiment *in vitro*.

There is a significant amount of negatively charged ions (PO_4^{3-}, Cl^-, OH^-) and alkali compound, in particular $Ca(OH)_2$, were identified in the filtrate of BHA for first 2 days. It can be argued comparing to the data of Table 2 and Fig. 3. Decreasing of pH level with a time (Fig. 2), probably, caused by a decrease of phosphate ion content by forming an insoluble compound $Ca_3(PO_4)_2$ and formation of acidic compounds ($CaCl_2$). An increase of Ca content with a decrease of P and Fe content is characteristic for both materials doped with magnetite (Table 2). Thus, in the filtrate of these materials likely to be a variation in the amount of the alkali compounds: from minimal content of $Ca(OH)_2$ with maximum content of $Fe(OH)_2$ during first 2 days to the conversely situation during 7 days. This can reduce the dependence of pH level on time for materials BHA/Fe_3O_4 (Fig. 3). Furthermore, the acidity of solutions is also influenced by a decrease in the content of phosphate ions and the probable formation of $CaCl_2$.

Current world medicine uses HA-based biomaterials in the form of both powder (or granules of various sizes) and blocks of various shapes and volumes. Therefore in the work, cylindrical blocks (diameter 10.0±0.2 mm, mass 1.9±0.3 g) were formed by dry pressing in a mold under a pressure of 10 MPa and subsequently subjected to heat treatment in vacuum (10^{-1} mm Hg) for 2 h at 500°C. Their physical characteristics are presented in Table 3.

From the data of Table 3, it is evident that the specific density of the pressed samples of undoped BHA and BHA/Fe_3O_4 obtained by physico-mechanical method is very close to the average specific density of cortical bone tissue, 1.86 g/cm^3, which leads to the conclusion on expediency of using thermally treated BHA-based blocks with various shapes and volumes to restore the function of cortical bone tissue. Furthermore, a significant loss of mass (−0.055) with a slight increase in height (0.002), and, accordingly, in volume (0.002), may be a result of releasing a certain amount of carbon dioxide during iron oxalate decomposition. Herewith, almost all porosity of the cylindrical sample is open (39.6 % at total 41.9 %). For the sample BHA/Fe_3O_4, obtained by the chemical method, mass loss after thermolysis (−0.042) occurs as well. However, its height and volume decrease, which is directly reflected in the open porosity (35.6 % at total 47.0 %). The microstructure images of the studied pressed samples (Fig. 4) reveal a sufficient roughness of the surface of all materials, which in the case of implantation will facilitate the adhesion of osteoblasts to the surface. The presence of surface defects and 10–18 μm areas of pore accumulation in unalloyed BHA is presumably a result of pressing. The surface of the sample prepared from material obtained by physico-mechanical method consists of pore chains smaller than 600 nm with a few surface defects in the form of 3.5–7.0 μm pores. It can also be traced to the presence of grain agglomerates sizing within the range of 400 nm–3.5 μm. The surface of the sample prepared from material obtained by the chemical method is characterized by the presence of the significant number of 29–30 μm areas, which contain grain agglomerates of 3–10 μm and pores of 6–17 μm. This may indicate the release of a greater amount of carbon dioxide due to the decomposition of iron oxalate, compared to the previous sample.

Taking into account the mentioned differences of alloyed materials, their dynamic

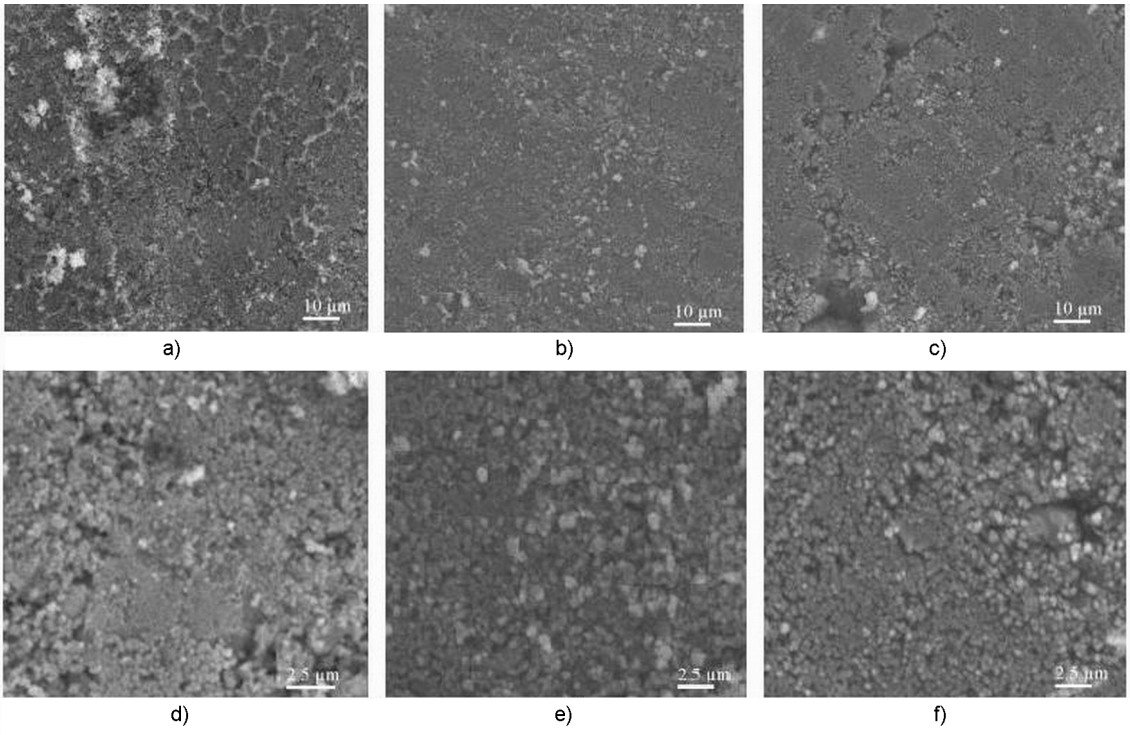

Fig. 4. Microstructure of materials studied: BHA: (a) (×100), (d) (×5400); BHA/Fe_3O_4 obtained by physico-mechanical method: (b) (×100), (e) (×5400); BHA/Fe_3O_4 obtained by chemical method: (c) (×100), (f) (×5400).

characteristics were determinates. The elasticity of BHA and BHA/Fe_3O_4 obtained by physico-mechanical and chemical methods was equal 15, 7.8, and 4.3 GPa, respectively. These values are close to the elastic characteristics of cortical bone tissue [4]. Difference in the elasticity of doped materials can be related to the difference in the particle size spectrum of initial powders. Particles of different sizes form different structures of samples under pressing, which additionally changed under the decomposition of iron oxalate. This is confirmed by the difference in the propagation time of longitudinal elastic wave (8.2 sec for samples obtained by physico-mechanical method and ~ 11 sec for the sample obtained by chemical method), which, according to formula (2), significantly influences the velocity of the elastic wave propagation as well as elastic characteristics, according to formula (3).

4. Conclusions

Composite BHA/Fe_3O_4 systems with almost the same content of iron and carbon, required for normal metabolism in case of implantation, have been obtained via doping BHA with ferromagnetic additives yielded by decomposition of iron oxalate. Herein the specific density of both powdered and compact materials from pure BHA and BHA/Fe_3O_4 produced by physico-mechanical method is almost equal to that of trabecular and cortical bone tissue, respectively. The dissolution rate of doped materials is higher than that of pure BHA, probably owing to the additional release of iron ions. In spite of different dissolution rates, all materials tend to equilibrium at the expense of slight increase in the number of Ca ions and parallel decrease in that of P and Fe ions due to precipitation. However, their elastic characteristics differ by about twice: 7.8 and 15.0 GPa for BHA and BHA/Fe_3O_4. As for the compact sample from the BHA/Fe_3O_4 system produced by chemical method, it is characterized by the lowest elasticity, 4.3 GPa, and the highest porosity (47 %), which may be due to CO_2 release during the thermal decomposition of iron oxalate.

The composite BHA/Fe_3O_4 systems produced by different methods exhibit different characteristics and thus make it possible to select the most suitable material according to the demands of implantation in different regions of the human musculoskeletal system.

References

1. A.Herrera, J.Mateo, J.Gil-Albarova et al., *BioMed. Res. Int.*, **2015**, ID 386461, (2015).
2. T.M.Keaveny, W.C.Hayes, Mechanical Properties of Cortical and Trabecular Bone in Bone: A Treatise, ed. B.K.Hall. CRC Press, New York (1992).
3. T.M.Keaveny, E.F.Morgan, Yeh Bone Mechanics in Yeh Standard Handbook of Biomedical Engineering & Design, ed. M.Kutz. McGraw-Hill, New York (2003).
4. I.V.Uvarova, P.P.Gorbyk, S.V.Gorobets et al., Nanomaterials of Medical Application. Naukova Dumka, Kyiv (2014).
5. N.Kurgan, V.Karbivskyy, V.Kasyanenko, *Nanoscale Res. Lett.*, **10**, 41 (2015).
6. A.Mishra, N.Khobragade, K.Sikdar et al., *Adv. Mater. Sci. Eng.*, **2017** , doi: 10.1155/2017/9814624 (2017).
7. S.Panseri, C.Cunha, T.D.D'Alessandro et al., *PLoS ONE*, **6**, 1 (2010).
8. Ie.V.Pylypchuk, Yu.O.Zubchuk, A.L.Petranovskaya et al., *Chem., Phys. Technol. Surf.*, **3**, 326 (2015).
9. Q.Li, G.Zhou, T.Wang et al., *J. Nanomaterials*, in press, doi: 10.1155/2015/835604 (2015).
10. F.Marquez, G.M.Herrera, T.Campo et al., *Nanoscale Res. Lett.*, **7**, 210 (2012).
11. Y.I.Golovin, *Studies of TSK*, **18**, 1954 (2013).
12. K.J.Tapan, M.K.Reddy, M.A.Morales et al., *Mol. Pharm.*, **5**, 316 (2008).
13. S.Prijic, J.Scancar, R.Romih et al., *Membrane Biol.*, **236**, 167 (2010).
14. D.S.Kuznetsova, P.S.Timashev, V.N.Bagratashvili et al., *Modern Technol. Med.*, **6**, 201 (2014).
15. J.V.Rau, I.Cacciotti, A.De Bonis et al., *App. Surf. Sci.*, **307**, 301 (2014).
16. Ya.Li, C.T.Nam, C.P.Ooi, *J. Physics: Conf. Series.*, **187**, doi: 10.1088/1742-6596/187/1/012024 (2009).
17. X.Lu, Y.Leng, *Biomaterials*, **26**, 1097 (2005).
18. L.Ploux, A.Ponche, K.Anselme, *J. Adh. Sci. Tech.*, **13–14**, 2165 (2010).
19. D.-L. Trandafir, C. Merestean, R.V.F. Turcu et. al., *Ceram. Int.*, **40**, 11071 (2014).
20. O. Kaygili, S.V. Dorozhkin, T. Ates et. al., *Ceram. Int.*, **40**, 9395 (2014).
21. E.M. Muzquiz-Ramos, D.A. Cortes-Hernandez, *J. Escobedo-Bocardo, Mater. Lett.*, **64**, 1117 (2010).
22. A. Tampieri, T. D'Alessandro, M. Sandri et. al., *Acta Biomater.*, **8**, 843 (2012).
23. D. Gopi, M.T. Ansari, E. Shinyjoy et. al., *Spectrochim. Acta Part* A, **87**, 245 (2012).
24. K. Donadel, M.D.V. Felisberto, M.C.M. Laranjeira et. al., *Anais da Academia Brasileira de Ciencias*, **89, 179 (2009).**
25. L. Dong, Z. Zhu, Y. Qui et. al., *Chem. Eng. J.*, **165**, 827 (2010).
26. T. Iwasaki, Mater. Sci. - Adv. Topics, in press, doi: 10.57725/54344 (2017).
27. S. Sprio, S. Panseri, A. Adamiano et. al., Front. in Nanosci. and Nanotech., 3, 1 (2017).
28. UA Patent 61938 (2003).
29. O.M.Otychenko, T.Ye.Babutina, O.R.Parkhomey et al., *Chem., Phys. Technol. Surf.*, **8**, 10 (2017).
30. O.M.Otychenko, N.D.Pinchuk, O.R.Parkhomey et al., *Nanostruct. Mater. Sci.*, **2**, 45 (2014).
31. Yu.G.Bezimyanniy, *Akustychn. Visnyk.* **2**, 3 (2006).
32. Yu.G.Bezimyanniy, E.A.Kozirackiy, A.N.Kolesnikov et al., *Visnyk Natsionalnogo Tehnichn. Universutetu "KhPI"*, **19**, 15 (2014).
33. O.Otychenko, T.Babutina et al., *Nanosistem., Nanomater., Nanotehnol.*, **15**, 185 (2017).