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Physico-chemical properties of ZnO doped nanostructured hydroxyapatite

The modification of synthetic biomaterials based on bioapatite by various additives becomes urgent, which would have an antibacterial and antiseptic effect, in order to reduce the risk of transformation of aseptic inflammation to bacterial (osteomyelitis). The solution of this issue can be the inclusion of trace metal ions, such as Zn, Ag and Mg hydroxyapatite (HAP), which present in the biological bone mineral. The addition of zinc ions improves its biological characteristics, since recent studies show its antibacterial properties. This work is devoted to the synthesis and complex study of bioadaptive material based on ZnO doped nanostructured hydroxyapatite, which has properties close to bone tissue. The paper presents new results on the preparation and investigation of a composite material of HAP powder with the addition of ZnO. The investigation was carried out by scanning electron microscopy with the possibility of energy-dispersive analysis, transmission electron microscopy with the possibility of mapping the elemental composition. The morphology and the phase state of the particles have been studied. The ratio of Ca/P corresponds to the generally accepted parameters. In the study, it was found that HAP molecules are embedded in the crystal structure of ZnO and form a chemical bond with the substitution of the Ca atom.

Keywords: nanostructured biomaterials, hydroxyapatite with the addition of zinc oxide, biocomposite materials, calcium phosphates.

1. Introduction

Hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$,HAP], which has similarieties with the inorganic part of human bone by the chemical composition, is widely used in medicine due to its positive properties of biocompatibility, osteoinductive and osteoconductive properties. HAP can form a direct chemical bond with adjacent bone tissue and facilitates the rapid osteointegration of implants. Acceleration of the osteosynthesis process in orthopedics is a key factor, because it leads to a reduction in the duration of patient's treatment, and consequently a reduction in the economic costs of medical discussion [1-9].

In this regard, the modification of synthetic biomaterials based on bioapatite by various additives becomes urgent, which would have an antibacterial and antiseptic effect, in order to reduce the risk of aseptic inflammation transformation to bacterial (osteomyelitis).

A solution to this problem may be the inclusion of trace metal ions, such as zinc, silver and manganese in HAP, which are present in the biological bone mineral. The addition of zinc ions makes it possible to improve its biological characteristics, since recent studies [3] show its antibacterial properties.

Despite the fact that the exact mechanism of ZnO antimicrobial activity is unknown, there are three main assumptions: membrane damage, cellular cell intake of ZnO or Zn^{2+} ions and ion reactivity. These mechanisms and the structure of ZnO are described in [1-3].

Based on the foregoing, the present work is devoted to the complex study andsynthesis of bioadaptive material based on nanostructured hydroxyapatite doped with ZnO, which has properties close to bone tissue.The presented results are the first one in the series of investigation work, which devoted to synthesis and coatings of biocompatible nanostructured biocomposite materials based on calcium apatite.

The purpose of this work is to investigate the influence of ZnO doping on physical properties of hydroxyapatite. The objectives are understand the mechanism of ZnO microcrystals' growing into the HAP, and its' bonding with HAP molecules.

2. Materials and methods of the experiment

The HAP study was carried out by the methods of analysis as scanning electron microscopy (SEM) using the JSM-6390LV microscope with the energy-dispersive microanalysis system INCA Energy Penta FET X3, elemental contrast analysis was carried out on selected aries of samples. High-resolution transmission electron microscopy (HR-TEM)was carried out using an electron microscope JEM-2100, an additional EDAX analysis was performed using the INCA Energy TEM 350 with an X-MAX 80 crystal. All this analytical investigations were conducted at university laboratories of East Kazakhstan State Technical University named after D. Serikbayev.

To 200 ml of 0.2 M solution of zinc nitrate hexahydrate was added 4 ml of a 3 % solution of sodium chloride. To begin the chemical reaction for the formation of the ZnO compound, 15 ml of 25 % ammonia solution was added. In this variant of synthesis, a titanium plate was dropped into the resulting solution, through which an alternating current (2A) was passed for 30 minutes. Further, the entire volume of the solution was heated on an electric plate to a temperature of 80 °C with stirring using a magnetic stirrer. After cooling, the sample was repeatedly rinsed with distilled water until neutral reaction. The precipitated ZnO fraction was separated by centrifugation. As a result, a suspension of ZnO with a moisture content of about 85 % was obtained, which was dried at 37 °C in the next processing step. The dried product was crushed and sieved through a sieve. Thus, ZnO powder was obtained with a dispersion of ≤ 63 μm.

To 6.0 g of a suspension of HAP (85 % moisture) obtained by the above described method, 0.3 g of ZnO powder (≤ 63 μm) was added, which is 33 % of ZnO with respect to dry HA and kneaded with a spatula.

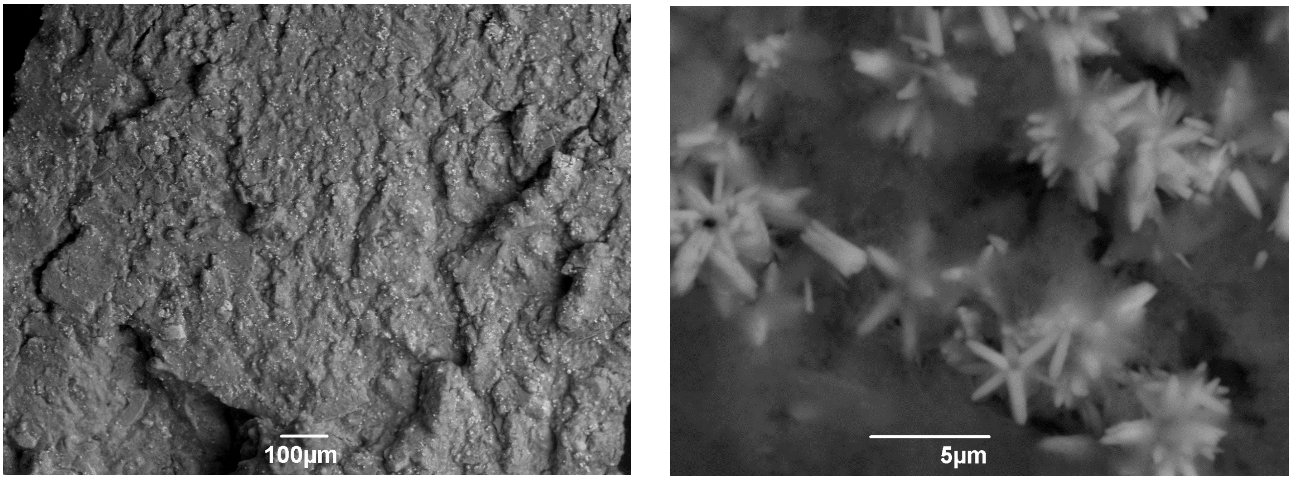
3 Results and discussion

Figure 1a shows a photograph of the microstructure of a HAP doped with ZnO. As you can see, the ZnO crystallites are distributed evenly and are pronounced (white dots). At higher magnification (x3000), it is found in Figure 1b that the ZnO crystallites consist of single crystals crystallized in the form of a 6-star with a size of ≤5 microns. The chemical composition of the synthesized material is shown in Table.

Table

Chemical composition of the synthesized material, in % by weight

Number	O	Al	P	Ca	Zn	Sum
1	34.34	0.35	8.29	13.74	43.28	100.00
2	39.74	0.61	9.27	15.37	35.02	100.00
3	45.23	0.23	14.78	29.41	10.35	100.00
4	44.78	0.27	13.39	27.70	13.86	100.00
Average	41.02	0.36	11.43	21.55	25.63	100.00

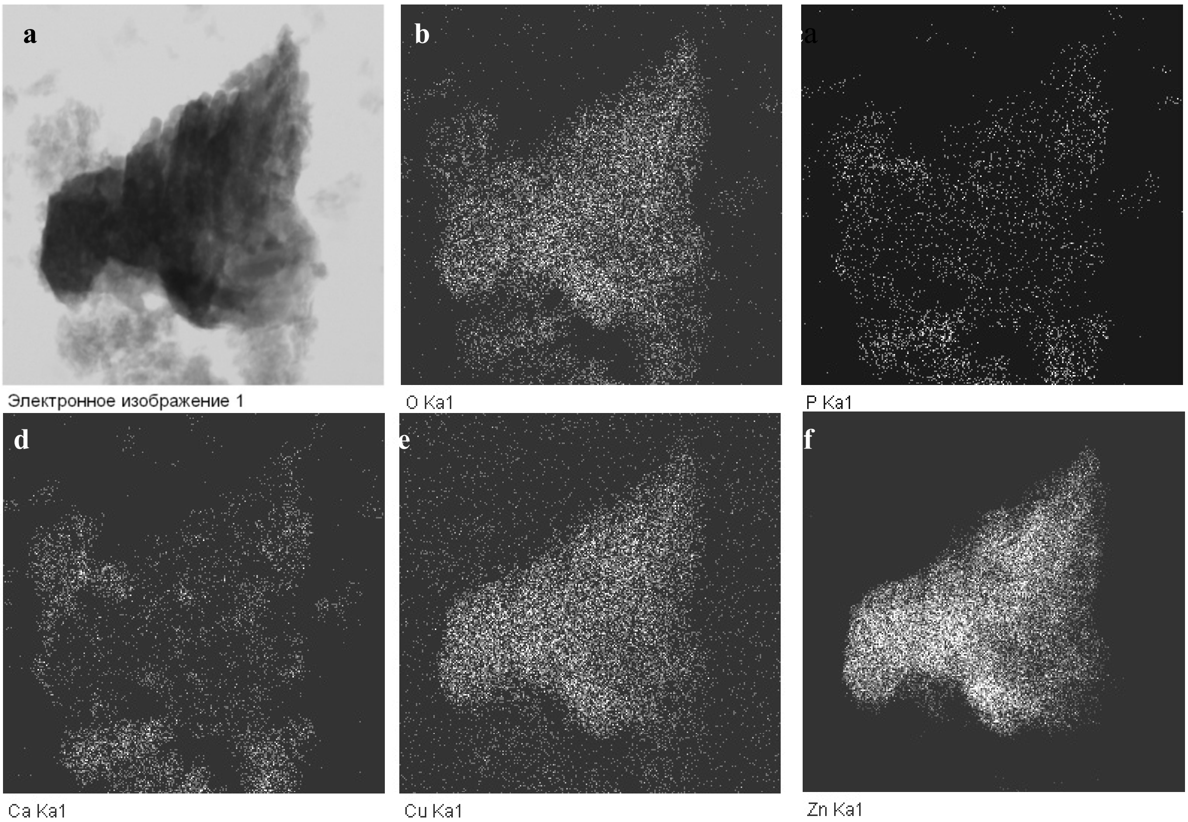


a b

a) microstructure of HAP doped by ZnO, x100; b) crystallites of ZnO, x3000

Figure 1. SEM images of the microstructure of ZnO doped HAP

From the obtained data, it can be seen that Zn is presented in the HAP composition with different concentrations of Zn at the EDS analysis points. Thus, the concentration of Zn varies from 10.35 to 43.28 % by weight. The average value of the Zn concentration is 25.63 %. The concentration of oxygen decreases, because part of it goes on the formation of crystallites ZnO. The Ca/P ratio is 1.88, which is close to the stoichiometric HAP, and indicates good biocompatibility. To explain such a significant change in the Zn concentration, mapping was performed on the TEM (see Fig. 2).



a — TEM image of ZnO doped HAP; b — distribution (map) for O; c — for P; d — for Ca; e — for Cu; f — for Zn

Figure 2. TEM image of the nanoparticle ZnO

For the mapping analysis, a ZnO nanoparticle was chosen. Analyzing the distribution of elements shows that P and Ca also persented in the ZnO nanoparticle. This, in turn, may indicate that the HAP molecules enter into a chemical bond with Zn, by the replacement of the Ca atom. The presence of Cu is explained by the method of investigation (application of a solution onto a copper mesh).

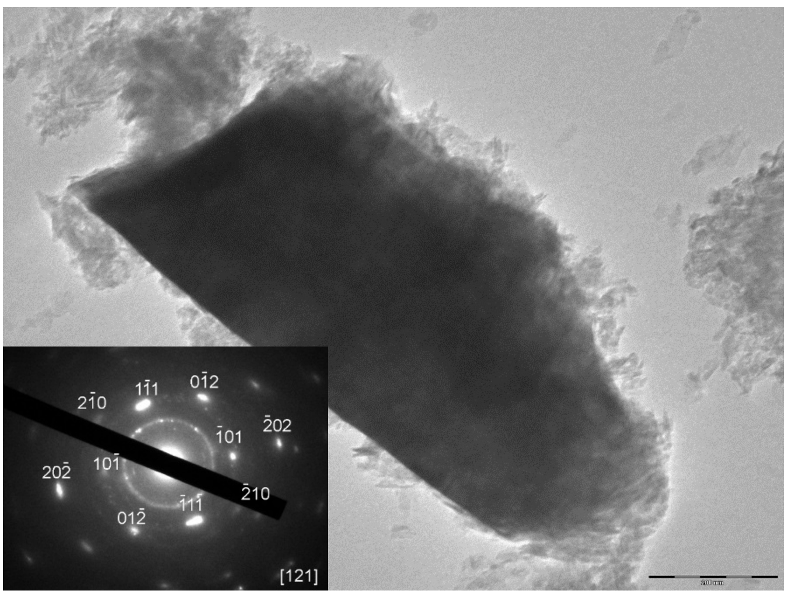


Figure 3. TEM image of the ZnO nanoparticle, scale of the ruler 200 nm

Analysis of ZnO particles using transmission electron microscopy (TEM) with high resolution (Fig. 3) shows that the structure of ZnO is hexagonal with lattice parameters a = 3.2427, b = 3.2427, c = 5.1499.

Conclusion

Synthesized HAP powder with ZnO addition was studied. The Ca/P ratio for some samples corresponds to the generally accepted parameters, close to bone tissue. In the study, it was found that HAP molecules are embedded in the crystal structure of ZnO and form a chemical bond with the substitution of the Ca atom. The microcrystals of ZnO are distributed evenly, it is found that the crystallites consist of single crystals crystallized in the form of a 6-star. That property should be profitable for antibacterial characteristics of composite material.This work, the first in the proposed series of works devoted to the study of a composite material having properties close to bone tissue.The investigations will be continued.

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References

1 Bhowmick, A., Pramanik, N., Manna, P.J., Mitra, T., Selvaraj, T.K., Gnanamani, A. & et al. (2015). Development of porous and antimicrobial CTS–PEG–HAP–ZnOnano-composites for bone tissue engineering. *RSC Advances, 5*(120), 99385–99393. doi:10.1039/c5ra16755h.

2 Kanhed, S., Awasthi, S., Midha, S., Nair, J., Nisar, A., Patel, A.K. & et al. (2018). Microporous Hydroxyapatite Ceramic Composites as Tissue Engineering Scaffolds: An Experimental and Computational Study. *AdvancedEngineeringMaterials,* 1701062. doi:10.1002/adem.201701062.

3 Saxena, V., Hasan, A., & Pandey, L.M. (2017). Effect of Zn/ZnO integration with hydroxyapatite: A review. *MaterialsTechnology, 33*(2), 79–92. doi:10.1080/10667857.2017.1377972.

4 Dorozhkin, S. V., & Epple, M. (2002). Biological and Medical Significance of Calcium Phosphates. AngewandteChemie International Edition, 41(17), 3130–3146. doi:10.1002/1521-3773(20020902)41:173.0.co;2-1.

5 Sukhodub, L. (2014). Metal Ions Doped Chitosan Nanoparticles. *Journal of Nano- and Electronic Physics, 6(4),* 04034-1-04034-6.

6 Sukhodub, L.B, Khrystian, G.E., Sukhodub, L., Shulga, N.M., Meshkov, A.M., Kazmirchuk, V.V., & et al. (2016). Composite Materials Based On Zinc Sulfide And Zinc Oxide: Structural And Biocidal Properties. *Annals of Mechnikov Institute, 4,* 34–39.

7 Ito, A., Otsuka, M., Kawamura, H., Ikeuchi, M., Ohgushi, H., Sogo, Y., & Ichinose, N. (2005). Zinc-containing tricalcium phosphate and related materials for promoting bone formation. *CurrentAppliedPhysics, 5*(5), 402–406. doi:10.1016/j.cap.2004.10.006.

8 Mieshkov, A.M., Grebenik, L.I., Ivahnuk, T.V., & Sukhodub, L.F. (2016). Antibacterial Properties of the Nanoparticles with the Zinc Sulfide Quantum Dots. *3rd International Conference on Nanotechnologies and Biomedical Engineering IFMBE Proceedings,* 267–270. doi:10.1007/978-981-287-736-9_65.

9 Pogrebjak, A.D., Krivets, A.S., Dyadyura, K.A., Maksakova, O.V., Shaimardanov, Z.K., & Peplinska, B. (2016). Research of the relief and element composition of the surface coatings based on hydroxyapatite implants from titanium alloys. *2016 International Conferenceon Nanomaterials: Application & Properties (NAP)*. doi:10.1109/nap.2016.775729.

10 Pogrebnjak, A.D., Krivets, A.S., Shaimardanov, Z.K., Syrnev, B.V., Plotnikov, S.V., & Maksakova, O.V. (2016). Surface properties, technology of fabrication and prospects of application of medical implants from porous titanium obtained by powder metallurgy. *2016 International Conferenceon Nanomaterials: Application & Properties (NAP)*. doi:10.1109/nap.2016.7757292.

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ZnO қосылған наноқұрылымды гидроксиапатиттің физика-химиялық қасиеттері

Асептикалық қабынудың бактериялық қабынуға (остеомиелит) өтпес үшін биоапатит негізінде синтетикалық биоматериалдарға түрлі қабынуға қарсы әсері бар қоспалар енгізу арқылы өзгерту өзекті мәселе болып табылады. Осы мәселені шешуі биологиялық сүйек тінінің минералды бөлігнде гидроксиапатит (ГАП) бар мырыш, күміс және марганец сияқты із металл иондарын қосу болуы мүмкін. Оның бактерияға қарсы қасиеттерін мырыш иондарын қосу арттырады. Бұл жұмыс сүйек тінінің қасиеттеріне жақын ZnO қосылған наноқұрылымды гидроксиапатит негізіндегі биоадаптивті материал синтездеуге және кешенді зерттеуге арналған. Мақала ZnO қосылған ГАП ұнтақ композициялық материал дайындау және зерттеудегі жаңа нәтижелерді ұсынады. Зерттеу жұмыстары элементтік құрамын салыстыру мүмкіндігімен жабдықталған трансмиссиялық электрондық микроскопия, энергия-дисперстік талдау мүмкіндігі бар сканерлеу электронды микроскопия арқылы жүргізілді. Бөлшектердің морфологиясы мен фазалық жағдайы зерттелді. Ca/P қатынасы жалпы қабылданған параметрлерге сәйкес келеді. Зерттеу барысында ГАП молекулалары мырышпен кальций атомын алмастыра отырып, химиялық байланыс түзетіні анықталды.

Кілт сөздер: наноқұрылымды биоматериалдарды, мырыш оксиді қосылған гидроксиапатит, биокомпозициялық материалдар.

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Физико-химические свойства наноструктурированного гидроксиапатита, легированного ZnO

Модификация синтетических биоматериалов на основе биоапатита различными добавками, которые, в свою очередь, имели бы антибактериальный и антисептический эффект для уменьшения риска перетекания асептического воспаления в бактериальное (остеомиелит), является актуальной задачей. Решением данного вопроса может быть включение следовых ионов металлов, таких как цинк, серебро и марганец, в гидроксиапатит (ГАП), которые присутствуют в биологическом костном минерале. Добавление ионов цинка дает возможность улучшить его биологические характеристики, так как последние исследования показывают его антибактериальные свойства. Статья посвящена синтезу и комплексному исследованию биоадаптивного материала на основе наноструктурированного гидроксиапатита с добавлением ZnO, имеющего свойства, близкие к костной ткани. Авторами представлены новые результаты по получению и исследованию композитного материала порошка ГАП с добавлением ZnO. Исследование проводилось методами растровой электронной микроскопии с возможностью энергодисперсионного анализа, просвечивающей электронной микроскопии с возможностью картирования элементного состава. Изучены морфология, фазовое состояние частиц. Соотношение Ca/P соответствует общепринятым параметрам. В ходе исследования выявлено, что в структуру кристалла ZnO внедряются молекулы ГАП и образуют химическую связь с замещением атома Ca.

Ключевые слова: наноструктурированные биоматериалы, гидроксиапатит с добавлением оксида цинка, биокомпозитные материалы.