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Calcium apatite and sodium alginate based composite material with ZnO microparticles doping

Among the various materials for orthopedic use, hydroxyapatite (HA) is the most common material, due to its chemical similarity to bone. However, the application is limited because of low mechanical properties (especially high brittleness), low water solubility, low antimicrobial activity. The introduction of trace metal ions, as well as biopolymers, can improve its properties. The present work is devoted to the synthesis and complex study of bioactive material based on nanostructured hydroxyapatite with the addition of sodium alginate and ZnO, which has properties close to bone tissue. The article presents information on the method of preparation and the results of the investigation of a new composite material based on HA and sodium alginate with the addition of ZnO microcrystals. In the research work next methods were used: scanning electron microscopy with the possibility of energy-dispersion analysis, transmission electron microscopy and IR spectroscopy. The morphology, the particles phase states are described, the regularities of the structural features of the new material are revealed. The main characteristics like, the ratio of Ca/P and functional groups correspond to the generally accepted parameters. An explanation of the grouping of ZnO microcrystals in the pores of the structure of a synthesized composite material is proposed.

Keywords: nanostructured biomaterials, hydroxyapatite with addition of sodium alginate and zinc oxide, biocomposite materials, biomaterials for medicine.

Introduction

Recently, there has been a significant increase in the number of studies aimed at developing new materials that promote the growth of biological tissue or its replacement [1]. To date, medicine, in particular orthopedics, has moved to minimally invasive methods of treatment. Accordingly, the time of tissue repair is reduced. Depending on the injury, various metals and metal oxides are used as bearing or fixing implants. These materials regenerate tissues to form a new bone or replace / maintain a completely damaged bone. Hydroxyapatite $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, (HA), which resembles the chemical composition of the inorganic part of human bone, is now widely used in medicine due to its positive properties of biocompatibility, osteoinductive and osteoconductive properties.

HA can form a direct chemical bond with adjacent bone tissue and facilitates rapid osseointegration of implants. Acceleration of the process of osteosynthesis in orthopedics is a key factor, as this leads to a reduction in the duration of treatment of patients, and consequently to a reduction in the economic costs of medical discussion [1–11].

It is known that HA can be synthesized by various methods and chemical routes. Doping with various elements is possible by adding salt solutions, during chemical synthesis, by electrochemical method or by ion implantation [2]. Introduction can affect not only the crystal structure and bio-mechanical properties, but also alter the behavior in vivo and in vitro. Basically, the HA is doped with elements contained in the biological bone, these are Li, Mg, Zn, Ag, Cu, Al, Zn, Mg, Sr, and Si ions. The addition of Zn, as studies have shown, has the best osseointegration, and also has good antibacterial properties. According to [11–17], HA with Zn addition has extensive properties such as load-bearing capacity and corrosion resistance, antibacterial and antifungal activity and applications, including better cell adhesion, differentiation of mesenchyme stem cells.

Based on the foregoing, the present work is devoted to the synthesis and complex study of a biocomposite material based on calcium apatite and sodium alginate with the addition of ZnO microparticles for further use in orthopedics.

Materials and methods of the experiment

The following chemicals «Merck» manufactured were used: calcium nitrate tetrahydrate $[\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}]$, ammonium hydrophosphate $[(\text{NH}_4)_2\text{HPO}_4]$, ammonium hydroxide $[\text{NH}_4\text{OH}]$, zinc nitrate

hexahydrate $[\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}]$ classification «Chemically clear» (purity 99 %), calcium chloride $[\text{CaCl}_2]$; sodium alginate (E401) with a molecular mass of 15 kDa (manufacturer of China).

The synthesis of HA was carried out by mixing 0.167 M $\text{Ca}(\text{NO}_3)_2$ and 0.1 M 6.6 g $(\text{NH}_4)_2\text{HPO}_4$. The molar ratio of Ca/P is 1.67, as in the case of stoichiometric HA. The formation of HA occurred when the ammonium hydrophosphate was added to the solution by dropping (1ml/min). A pH of about 11 was achieved by adding 90 ml of 25 % ammonia solution to the mixture with stirring.

To this suspension was added 2 % sodium alginate in a weight ratio of 1:1 and the mixture was sonicated to install UZDN at a power of 75 W for 5 minutes. The resulting product was dispersed in 0.125 M calcium chloride solution.

Synthesis of the biocomposite with the addition of ZnO was performed by electrochemical mixing by mixing 200 ml of a 0.2 M zinc nitrate hexahydrate solution and 4 ml of a 3 % sodium alginate solution while passing an alternating current (2A) for 30 minutes. Formation of ZnO compound began after addition of 15 ml of 25 % ammonia solution. After that, 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. 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 $\leq 63 \mu\text{m}$.

The HA with different additives was analyzed by scanning methods such as: scanning electron microscopy (SEM) using the JSM-6390LV microscope with the energy dispersive microanalysis system INCA Energy Penta FET X3, samples of samples were selected for which additional analysis was carried out in elemental contrast.

The functional groups were studied on an IR Fourier spectrometer FTIR-801 Simex, measuring range 500–4000 cm^{-1} with a resolution of 1 cm^{-1} .

Results and discussion

Figure 1 shows a micrograph of a pure HA powder. The HA powder has a different form, due to the process of sample preparation, namely the grinding of granules in a mortar. An EDS analysis of several sample's points was carried out to determine the elemental composition. The results are shown in Table 1. The Ca/P ratio was 2.12, the concentration change was insignificant for all elements except Al, which indicates a non-uniform Al distribution in the sample volume. The Al content is not significant, and ranges from 0.24 to 0.42 wt. %.

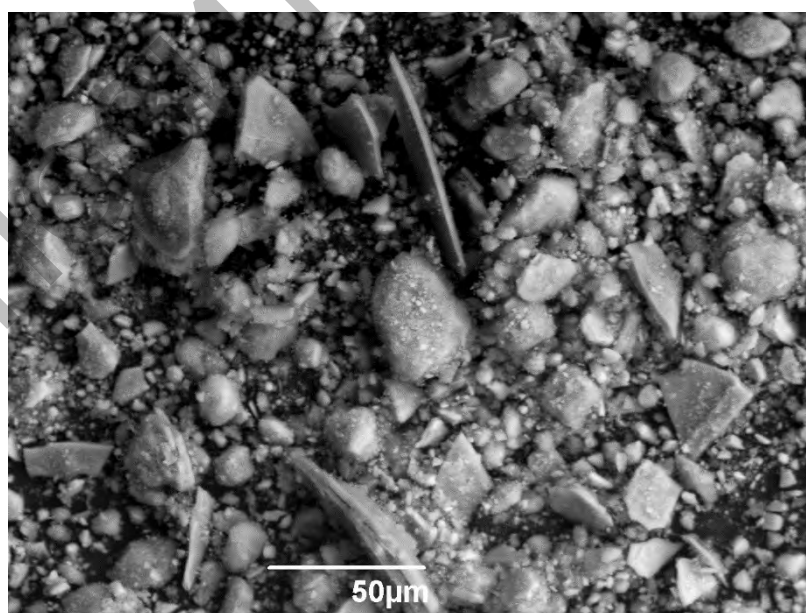


Figure 1. SEM image of pure HA powder

Results of EDS analysis on SEM, weight %

Spectrum	O	Al	P	Ca	Ca/P
Spectrum1	55.73	0.42	14.01	29.84	2.12
Spectrum 2	53.97	0.24	14.49	31.30	2.16
Spectrum 3	55.42	0.50	14.28	29.80	2.08
Average	55.04	0.38	14.26	30.31	2.12

Figure 2 shows a SEM image of a synthesized composite material HA/alginate/ZnO. The synthesized composite material has a bound, porous trabecular structure (similar to the structure of the cortical portion of the human bone), due to the addition of a sodium alginate polymer. ZnO microcrystallites are distributed evenly, located in the pores of the material. We explain this regularity by the peculiarity of synthesis of a composite material, namely, with the transmission of an alternating electric current, when local heating occurs.

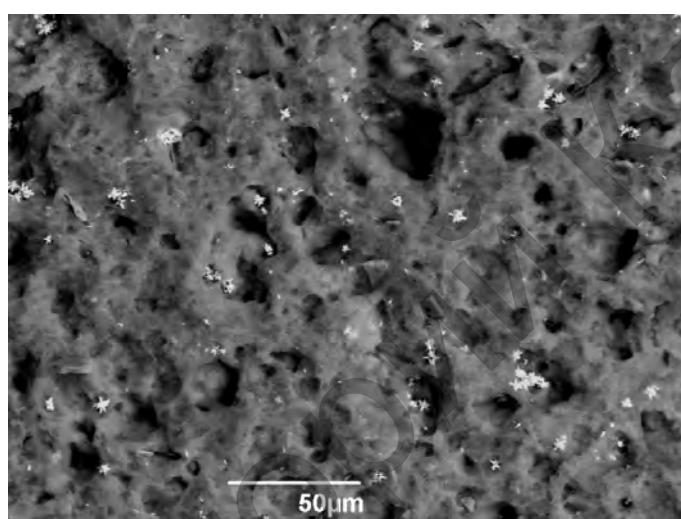


Figure 2. SEM image of the synthesized composite material HA/alginate/ZnO

Figure 3 shows the ZnO microcrystallites. Microcrystallites of ZnO is a spatially oriented group of single crystals of zinc, in the form of «snowflakes», 5 microns in size. This arrangement is an advantage, since in this case the addition of zinc oxide will have the maximum antibacterial effect, with little effect on the mechanical properties of the material.

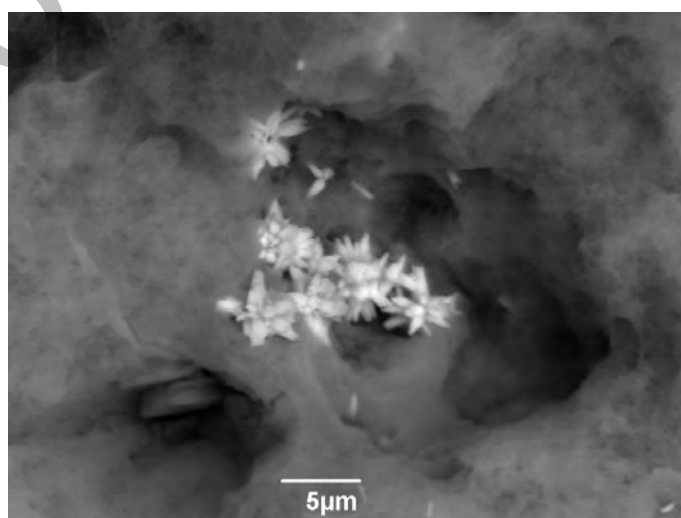


Figure 3. SEM image of ZnO microcrystalline

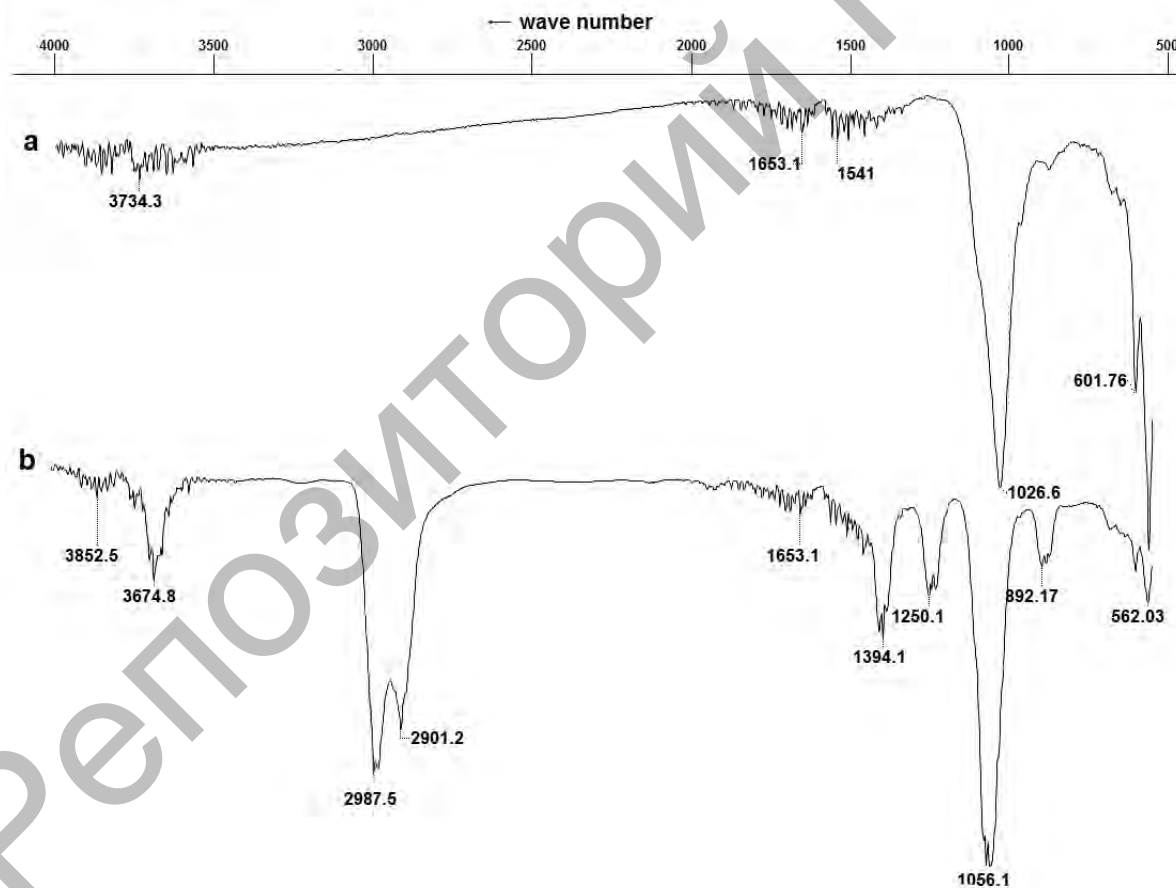
The EDS analysis carried out also for several sections of the synthesized biocomposite material, Table 2. With the injection of additional components, the elemental composition has also changed. There appeared such elements as: O, Na, Mg, Si, Cl, and Zn. All these elements are contained in the form of microelements in natural bone. The ratio of Ca/P slightly decreased to 2.03. The concentration of Zn varies from 7.08 to 24.28 % by weight, which is explained by a nonuniform distribution of Zn in the bulk of the sample. The change in concentration at different points of analysis of other elements is insignificant. The concentration of Al is insignificant, less than 0.55 % by weight.

Table 2

Results of EDS analysis of the biocomposite material HA/alginate/ZnO, % by weight

Spectrum	O	Na	Mg	Al	Si	P	Cl	Ca	Zn	Ca/P
Spectrum 1	40.69	4.69	0.85	0.49	9.98	5.95	1.91	11.78	23.66	1.97
Spectrum 2	38.80	3.99	0.72	0.40	10.67	6.61	1.83	12.71	24.28	1.92
Spectrum 3	48.40	4.09	0.87	0.55	11.10	8.01	2.52	17.38	7.08	2.16
Average	42.63	4.25	0.81	0.48	10.58	6.86	2.09	13.96	18.34	2.03

In Figure 4a is shown the IR spectrum of a pure HA powder. It is found that the main vibrational modes of the material synthesized by us (high-intensity lines 564–601 cm^{-1} , 962–1086 cm^{-1}) correspond to crystalline HA. The hydroxyl group is well pronounced (bands of 3600–4000 cm^{-1}).



a — pure HA; b — synthesized composite material HA/alginate/ZnO

Figure 4. IR-spectra

There is a significant change in the set of functional groups of HA with introducing sodium alginate and ZnO, as evidenced by the obtained data (see Fig. 4b). The displacement of the functional group $(\text{PO}_4)^{3-}$ from 1026.6 to 1056.1 cm^{-1} , as well as its considerable broadening, may indicate a violation of the geometry of the HA molecule and the formation of new chemical bonds with compounds of a polysaccharide nature. The

bands of stretching vibrations of C–H pertaining to amines ($2987.5\text{--}2901.2\text{ cm}^{-1}$) and 892.17 cm^{-1} are also observed. The bands of the group –OH are average intensive.

Conclusions

The synthesized biocomposite material HA/alginate/ZnO was studied. The Ca/P ratio corresponds to the generally accepted parameters. In the study, it was found that HA molecules are embedded in the crystal structure of ZnO and form a chemical bond with the substitution of the Ca atom. Further *in vitro* and *in vivo* researches are needed. The developed composite material is devoted for using in orthopedics as material for filling augments. The results indicate a high potential for the use of the synthesized material.

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ZnO микробөлшектері қосылған кальций апатиті және натрий альгинаты негізіндегі композициялық материал

Ортопедиялық қолдануға арналған әртүрлі материалдардың арасында гидроксипатит (ГА) сүйекке химиялық ұқсастығына байланысты ең көп таралған материал болып табылады. Алайда механикалық қасиеттерінің (әсіресе морт сыңғыштығы), суда ерігіштігі, микробқа қарсы белсенділігі сияқты қасиеттерінің төмендігіне байланысты, қолданылу дәрежесі шектеулі. Металл иондарын, сондай-ақ биополимерлерді енгізу оның қасиеттерін жақсартуға алады. Осы жұмыс сүйек тініне жақын қасиеттері бар натрий алигатымен және ZnO қосымен наноқұрылымды гидроксипатит негізінде биоадаптивті материалды синтездеуге және кешенді зерттеуге арналған. Мақалада ZnO микрокристалдарын қосу арқылы ГА және натрий алигатына негізделген жаңа композициялық материалдарды зерттеудің және оларды зерттеудің нәтижелері туралы ақпарат берілген. Зерттеу жұмысында келесі әдістер қолданылды: электронды-микроскопия, энергия-дисперсиялық талдау, трансмиссиялық электронды микроскопия және ИҚ-спектроскопия. Морфология, бөлшектердің фазалық күйі сипатталған, жаңа материалдың құрылымдық ерекшеліктерінің заңдылықтары анықталған. Синтезделген материалдың негізгі қасиеттері, Са/P қатынасы және функционалдық топтары жалпы қабылданған параметрлерге сәйкес келеді. Синтезделген композициялық материал құрылымының кеуектерінде ZnO микрокристалдарының топтасуы туралы болжам ұсынылды.

Кілт сөздер: наноқұрылымды биоматериалдар, натрий алигаты және мырыш тотығы қосылған гидроксипатит, биокомпозиттік материалдар, медицинаға арналған биоматериалдар.

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Композитный материал на основе апатита кальция и альгината натрия с добавлением микрочастиц ZnO

Среди различных материалов для ортопедического применения гидроксипатит (ГА) является самым распространенным из-за его химического сходства с костью. Однако различные свойства, такие как посредственные механические свойства (особенно высокая хрупкость), низкая растворимость в воде, малая антимикробная активность, ограничивают масштабы его применения до определенного уровня. Введение ионов металлов, а также биополимеров может способствовать улучшению его свойств. Настоящая работа посвящена синтезу и комплексному исследованию биоадаптивного материала на основе наноструктурированного гидроксипатита, с добавлением альгината натрия и ZnO, имеющего свойства, близкие к костной ткани. Авторами представлена информация о методе получения и результатах исследования нового композитного материала на основе ГА и альгината натрия с добавлением микрокристаллов ZnO. В исследовании применены методы растровой электронной микроскопии с возможностью энергодисперсионного анализа, просвечивающей электронной микроскопии и ИК спектроскопии. Описана морфология, фазовое состояние частиц, выявлены закономерности структурных особенностей нового материала. Основные характеристики, такие как соотношение Са/P и функциональных групп, соответствуют общепринятым параметрам. Предложено объяснение группирования микрокристаллов ZnO в порах структуры синтезированного композитного материала.

Ключевые слова: наноструктурированные биоматериалы, гидроксипатит с добавлением альгината натрия и оксида цинка, биокомпозитные материалы, биоматериалы для медицины.