

Bioactive materials for bone regeneration based on zinc-modified hydroxyapatite

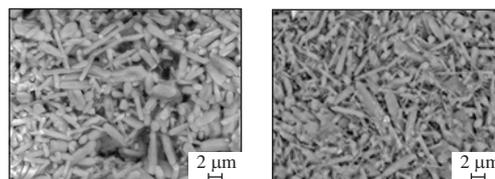
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Samples of zinc-modified hydroxyapatite (HA) $\text{Ca}_{(10-x)}\text{Zn}_x(\text{PO}_4)_6(\text{OH})_2$ with different zinc content ($x = 0.1$ and 0.5) were synthesized by the liquid-phase method. It was found that the addition of zinc affects the original hydroxyapatite structure and changes the parameters of the crystal lattice. The materials obtained show antibacterial activity.



SEM images of $\text{Zn}_{0.1}\text{HA}$ and $\text{Zn}_{0.5}\text{HA}$

Keywords: zinc-modified hydroxyapatite, phase composition, antibacterial activity, biocompatible materials, bone remodeling.

Creating biomaterials for implantation purposes should follow the principle of reproduction. The biomaterials composition, morphology and microarchitecture have to replicate the unique properties of natural bone tissue since those strongly affect the regeneration processes.^{1,2} Bone hardness and stiffness are provided by calcium phosphates in the form of crystalline hydroxyapatite (HA) and amorphous calcium phosphate. The ratio of the crystalline structure to amorphous in bone depends on many factors. In bone tissue, HA contains impurities of Zn^{2+} , Mg^{2+} , Na^+ , *etc.*^{3,4} HA modified with metal ions, are the main inorganic constituents of bone tissue approved for application as components of biocomposites and coatings for surgical implants.³

In this regard, this work aims to elucidate how zinc ions affect the biocompatibility of zinc-modified HA. This ion was chosen to modify HA because zinc is an essential trace element necessary for the normal course of many biochemical processes. Also, zinc ions in certain concentrations exhibit antibacterial activity.^{4–9} The zinc concentration introduced into the HA structure was selected following the data in the vast majority of studies on zinc-modified HA, where the zinc content was in the concentration range of 0.1–4 at%. However, the best results regarding biocompatibility, osteoconductivity and antimicrobial activity were achieved at a Zn^{2+} content of ~1–2 at%.¹⁰

The synthesis of zinc-modified HA samples (Zn_xHA) was accomplished according to the standard procedure.[†] The elemental analysis data confirmed the quality of the material obtained. The phase composition of the samples was determined by X-ray diffraction (XRD) analysis. One of the main

requirements for hydroxyapatite-based bioceramics used in medical practice for bone tissue restoration is bioresorbability.^{13,14} Therefore, the bioresorbability of the samples was also evaluated.[‡]

According to XRD data, the samples can be considered apatite in the hexagonal system. With an increase in the amount of substitutional Zn in the HA structure from 1 to 5 at%, the average crystallite size decreases from 68 to 60 nm, while the average crystallite size of stoichiometric HA was ~57 nm. The XRD patterns of HA powders show a slight shift in the diffraction maxima and a decrease in their intensity with increasing zinc content (Figure 1).

Table 1 shows the dependences of the lattice parameters of Zn_xHA powders on the amount of introduced substitutional zinc ions. One can see that zinc incorporation in the HA structure in

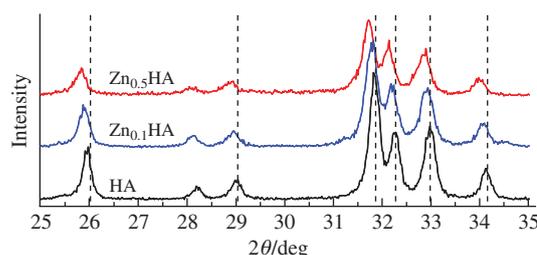


Figure 1 XRD patterns for HA, $\text{Zn}_{0.1}\text{HA}$ and $\text{Zn}_{0.5}\text{HA}$.

Table 1 Lattice parameters (a , b and c), unit cell volume (V) and coherent scattering region (CSR) size for Zn_xHA samples according to XRD data.

Sample	$a/\text{Å}$	$b/\text{Å}$	$c/\text{Å}$	$V/\text{Å}^3$	CSR/nm
HA	9.435	9.435	6.887	530	57
$\text{Zn}_{0.1}\text{HA}$	9.435	9.438	6.856	529	68
$\text{Zn}_{0.5}\text{HA}$	9.425	9.435	6.851	527	60

[‡] The samples' bioresorbability was determined by the concentration of Ca^{2+} ions in physiological solution (0.9% NaCl) at 25 and 37 °C using complexometric titration with an ammonia buffer (pH 9–10) in the presence of Eriochrome Black T as an indicator.

[†] As the initial components for the liquid-phase microwave-assisted synthesis of Zn_xHA , we used solutions of calcium nitrate (0.5 mol dm^{-3}) and ammonium hydrogen phosphate (0.3 mol dm^{-3}), observing the Ca/P molar ratio of 5/3, characteristic of HA.^{3,11,12} By varying the amount of zinc nitrate during the synthesis, we obtained several samples with different content of zinc, $\text{Ca}_{(10-x)}\text{Zn}_x(\text{PO}_4)_6(\text{OH})_2$, where $x = 0.1$ and 0.5 . Elemental analysis was performed on a Quantax 70 attachment for energy dispersive microanalysis. X-ray diffraction analysis of the samples was carried out on a Shimadzu XRD 6000 diffractometer in the 2θ range of 20–140° using Cu $K\alpha$ radiation. The POWDER CELL 2.4 full-profile analysis software was used to identify the phases.

Table 2 Results of elemental analysis for the Zn_xHA samples.

Sample	Content of elements (%)				
	O	Ca	P	Zn	(Ca + Zn)/P
Zn _{0.1} HA	67.5	18.7	13.7	0.2	1.4
Zn _{0.5} HA	67.8	18.6	12.6	0.7	1.6

an amount of up to 5 at% leads to a slight decrease in the unit cell parameters: from 9.435 and 6.887 Å to 9.425 and 6.851 Å for parameters *a* and *c*, respectively. This decrease may be due to the smaller radius of the substitutional Zn²⁺ ion compared to the radius of Ca²⁺. A decrease in the crystal lattice parameters in all directions leads to a decrease in the unit cell volume.

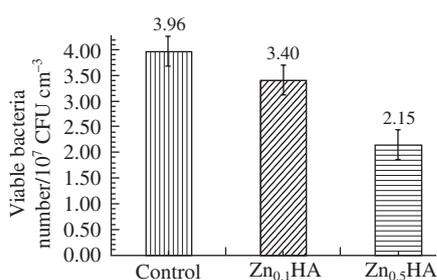
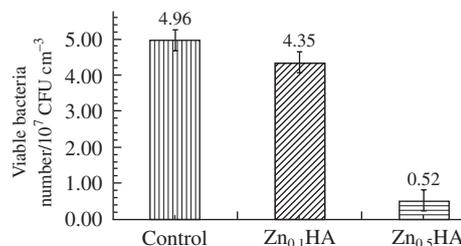
From the elemental composition (Table 2) of the synthesized Zn_xHA powders according to the results of energy dispersive X-ray microanalysis, it follows that the (Ca + Zn)/P atomic ratio is 1.4–1.6, which is consistent with the Ca/P ratio of 1.4–2.0 in bone tissue.

During the synthesis, increasing Zn²⁺ ion content resulted in Zn_xHA samples with slightly less solubility in physiological solution than unmodified HA. The solubility of Zn_xHA samples is significantly higher at 37 °C than at 25 °C (Table 3). It can be concluded that the addition of zinc significantly affects the solubility of materials. Probably, this tendency is because zinc, embedded in the crystal lattice, creates a large number of defects, which subsequently accelerates its destruction.

To evaluate the antibacterial activity against gram-positive and gram-negative microorganisms, we determined the number of cells by sowing on solid nutrient media (Koch's method) according to the standard technique modified recently by us.¹⁵ The antibacterial activity was different among the samples submitted for the study. The Zn_{0.1}HA sample had no suppressive effect on the number of *Escherichia coli* (Figure 2) and *Staphylococcus aureus* (Figure 3). In the liquid medium with this sample, the number of bacteria was slightly less than in the control sample, but the difference was not statistically significant (*p* > 0.05). On the contrary, Zn_{0.5}HA exhibited the highest antibacterial activity, remarkably reducing the number of bacteria in a statistically significant way compared to the control sample (*p* < 0.001). Also, in previous experiments, it was revealed that the antibacterial activity exhibited by the Zn_{0.5}HA sample did not differ from that of unmodified HA. It was found that the degree of antimicrobial activity of zinc-modified HA against gram-positive and gram-negative bacteria is different. It is slightly higher against gram-positive than gram-negative bacteria.

Table 3 Solubility of HA and Zn_xHA samples in physiological solution (pH 7) at different temperatures.

Sample	Solubility/mol dm ⁻³	
	25 °C	37 °C
HA	(1.19 ± 0.08) × 10 ⁻³	(0.98 ± 0.08) × 10 ⁻³
Zn _{0.1} HA	(0.52 ± 0.08) × 10 ⁻³	(0.86 ± 0.07) × 10 ⁻³
Zn _{0.5} HA	(0.45 ± 0.04) × 10 ⁻³	(0.59 ± 0.08) × 10 ⁻³

**Figure 2** Antimicrobial activity of the Zn_xHA samples against *Escherichia coli*.**Figure 3** Antimicrobial activity of the Zn_xHA samples against *Staphylococcus aureus*.

Presumably, this effect is due to the greater overall resistance of gram-negative bacteria to antibacterial agents.

Modification of HA with metal ions seems to be the key to achieving powerful antimicrobial activity. Such materials can counteract even very resistant microorganisms adapted to common antibiotics. In this case, the toxic side effects of such material will be significantly limited.^{16–19} The simultaneous release of ions with different mechanisms of action can not only prevent the adaptation of bacteria and fungi but also expand the antimicrobial activity spectrum against a larger number of pathogenic microbial strains.^{8,19–22} Biological tests of the samples have shown that HA modified with zinc ions exhibit antimicrobial activity, which can reduce or prevent the development of concomitant infections when used as components of bone implants.

In conclusion, samples of zinc-modified HA Ca_(10-x)Zn_x(PO₄)₆(OH)₂ with different zinc content (*x* = 0.1 and 0.5) were synthesized by the liquid-phase method. The samples obtained represent the hexagonal phase of HA Ca₁₀(PO₄)₆(OH)₂ with zinc included in the structure of HA. The elemental composition of the synthesized Zn_xHA powders according to the results of energy dispersive X-ray microanalysis shows that the (Ca + Zn)/P atomic ratio is 1.4 and 1.6 for Zn_{0.1}HA and Zn_{0.5}HA, respectively, which is consistent with the Ca/P atomic ratio of 1.4–2.0 in bone tissue. When investigating the dissolution of HA in physiological saline, it was found that an increase in the zinc content in HA leads to a decrease in its solubility compared to unmodified HA. The antibacterial activity study showed that HA modified with zinc ions demonstrate activity against both gram-negative and gram-positive bacteria. At the same time, the antimicrobial activity against gram-positive bacteria is somewhat higher than against gram-negative ones. The antibacterial effect of the obtained materials can reduce or prevent concomitant infections when used as components of bone implants. Thus, we believe that the microwave-assisted synthesis of HA modified with metal ions can be used to obtain biocompatible materials for bone implants.

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