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Obtaining of Biologically Soluble Membranes Based on Polymeric Nanofibres and Hydroxyapatite of Calcium

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Abstract

In this paper, the possibility of obtaining a synthetic hydroxyapatite of calcium from a biological waste material is shown. The characteristics influencing the synthesis process are studied. Based on the results of the X-ray analysis and the obtained electron microscope images, it can be concluded that the synthesized HAP has a Ca/P ratio of 1.5 and with crystals with an average size of 2 microns. In work, experiments on obtaining biologically soluble films based on nanoscale polymer fibers and calcium hydroxyapatite were carried out. As a result, the main parameters of the process for the electroforming of nano-sized fibers with HAP are determined. The proposed method allows the laying of strictly directed nanofibers from a polymer with a diameter of 50 to 500 nm. The use of different types of electrodes makes it possible to vary the size of nanofibers. The characteristics such as solution viscosity, high voltage and optimum parameters were selected, which allowed obtaining films from biologically soluble polymer nanofibers and HAP. Also, experiments were conducted to introduce medicines into the film structure.

1. Introduction

In modern medical practice in the field of surgery and dentistry to replace or repair damaged areas of bone tissue, are widely used materials based on calcium phosphates. Calcium phosphates are the most important inorganic components of biological solid tissues. Hydroxyapatite calcium (HAP) is present in the composition of bone tissue, teeth and tendons, which gives functionality and the necessary structure of the organs.

The treatment of various injuries and medical disease often entails surgical intervention. Bone fractures are usually treated with metal plates, joints are replaced with artificial endoprostheses (thigh or knee), and lost teeth are replaced with metal implants. Phosphates of calcium have an excellent biocompatibility, that is, they are practically not rejected by the human body. This depends on the fact that calcium phosphates are present in the human body in dissolved or solid form [1, 2–8]. Calcium phosphates are used as a substitute for bone in orthopedics for the treatment of bone defects and dentistry [1]. The ideal implant is, the patient's own spongy bone, mixed with the plasma of his blood, is not available in sufficient quantities, then completely synthetic materials are used. Synthesized materials must undergo sterilization, which should not affect the biological properties of the substitute. Today, chemically synthetic materials are used to replace bones based on HAP and its composites [1, 6, 7]. Crystalline hydroxyapatite can be synthesized in various ways, among which solid-phase synthesis methods are distinguished. Most often the synthesis of calcium phosphates is carried out from aqueous solutions using the processes of hydrolysis and precipitation.

Over the past three decades, scientists and engineers have focused on improving the old and creating of new technologies, which are a combination of different technologies. In turn, combining the technology of three-dimensional printing, the method of electroforming nanoscale fibers and the methodology of synthesis of HAP is of

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great interest. Three dimensional printing or 3D printing have several advantages over traditional production technologies. Integration of nanotechnology with 3D printing has a huge potential to complement existing nanotechnologies and create completely new nanocomposites. The paper shows the possibility of combining two different technologies. Such as the electroforming of nanofibers by means of high-voltage action. This technique is discussed in detail in [9]. The authors show the conditions for the realization of the process of electroforming.

2. Experimental

Synthetic HAP was obtained from biologically waste material. The egg shell containing $CaCO_3$ is annealed for 2 to 3 h at a temperature of 900–1000 °C. During annealing, the organic component of the shell burns off, and the resulting residue contains CaO as a fine powder without impurities. Then, the formed calcium oxide is loaded into the enameled reactor with a magnetic stirrer. 6–9% solution of orthophosphoric acid is injected into the reactor with constant stirring and heating from 60 to 80 °C to accelerate the course of chemical reactions. It was experimentally determined that for complete passage of the reaction it is necessary to melt the suspension within 1–2 h after the introduction of the acid. The amount of acid is added depending on the stoichiometric ratios. The synthesis process is monitored by maintaining the pH value in the range 6–8 throughout the reaction to obtain calcium hydroxyapatite with the required Ca/P ratio = 1.67, which corresponds to the stoichiometric ratio of calcium hydroxyapatite present in the bone structure of the human. The resulting solution is exposed to ultrasound for 1 to 2 h at a frequency of 32–36 kHz to ensure uniform size of the hydroxyapatite crystals.

After the end of the ultrasound influence, the resulting suspension merges into a settling tank where, without additional heating, happens ending of the formation of a calcium hydroxyapatite structure within 48 h. As a result, calcium hydroxiapatite with a purity of more than 95% and a particle size of less than 1 μm remains in the settling tank.

The resulting calcium oxide powder has been dissolved in a solution of orthophosphoric acid. The solution was exposed by an ultrasonic treatment to ensure the dispersion of the powder. The images obtained with an electron microscope are shown in Fig. 1.

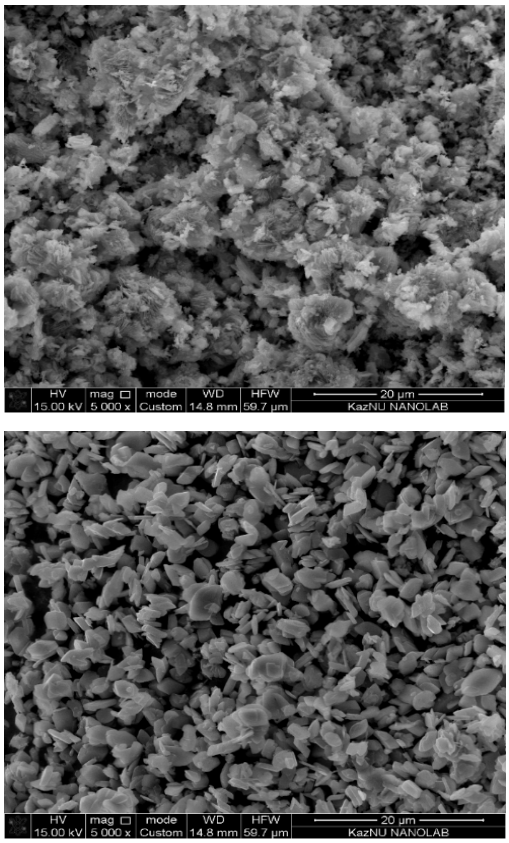

Fig. 1. SEM of obtained HAP.

From Fig. 1 can be seen crystals of size of 1–2 microns, it is worth to note that studies of obtained crystalline calcium hydroxyapatite, show that external conditions such as pH, composition and concentration of reagents and impurities, order and speed of mixing, temperature, time of the experiment strongly affect the crystallization process and the chemical composition of the synthesized powder. The resulting powdered material is a micron-sized fine powder (1–2 μm).

The determination of the phase composition of the synthesized samples of HAP was carried out by comparing the results of X-ray diffraction analysis of the obtained samples.

The X-ray phase analysis of the obtained HAP is shown in Fig. 2.

Based on the X-ray diffraction analysis data it was found that the sample contains a single phase of calcium hydroxyapatite.

Imperfections of structure due to the presence of vacancies, impurities of implementation and substitution, and determined by them distortions in the crystal lattice became energetically advantageous for the formation of HAP in the hexagonal syngony. Therefore, the presence of microimpurities and other defects in the structure of biological apatite determines its characteristics and affects the

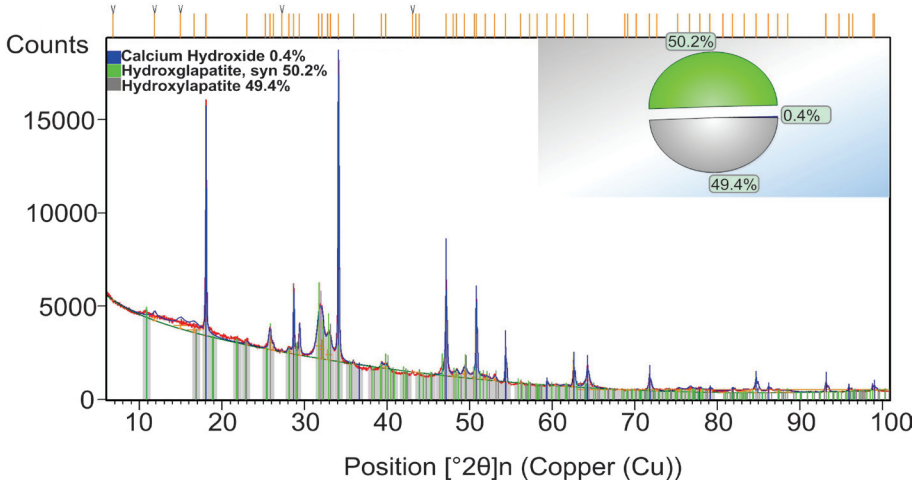

Fig. 2. X-ray pattern of the HAP sample.

physico-chemical and chemical-biological properties. Atoms of impurity atoms can be located randomly in the structure of hydroxyapatite [10]. To describe the structure of HAP, it is convenient to formulate the ideal stoichiometric formula of $Ca_{10}(PO_4)_6(OH)_2$, taking into account the different positions occupied by calcium atoms in the lattice of HAP as $Ca_{10}(PO_4)_6(OH)_2$, and also calculating the Ca/P ratio, which for our samples was 1.5. This feature is typical for the whole class of calcium apatites and can be considered using the example of a unit cell of the HAP (Fig. 3) [11]. In a unit cell ten calcium atoms occupy crystallographic two non-equivalent positions: The Ca (1) – 40% atoms combine phosphorus-oxygen tetrahedras, which in turn form "columns" along the *c* axis, on the walls of which calcium ions Ca (2) – 60% [12] are located. On the axis of the formed channel from Ca atoms (2) there are OH groups (Fig. 3). Each Ca (1) atom forms CaO_9 complexes through oxygen atoms of tetrahedrons $(PO_4)^{-3}$. The Ca(2) atoms include the formation of CaO_6OH complexes together with the OH group [13]. It should be noted that in a variety of processes for the synthesis of hydroxyapatite samples, the concentration of the initial reagents is chosen so that the ratio of Ca/P is equal to the stoichiometric ratio of 1.67, since in the mineral constituent of the bone tissue the ratio of Ca/P is close to this value and can vary from 1.5 up to 1.9 [14]. In addition, according to the results of various studies, it is noted that HAP with a ratio of Ca/P = 1.67 are more stable in regard to thermal action and dissolution, and ceramics based on such materials have the best performance in terms of mechanical characteristics of density, hardness, etc. The aim of this work is to search for optimal parameters for the synthesis of nanocrystalline HAP using the egg shell as a source of calcium, and to study the properties and characteristics of the synthesized samples.

Synthesized HAP was used to produce films based on nanoscale polymer fibers and HAP obtained by electroforming.

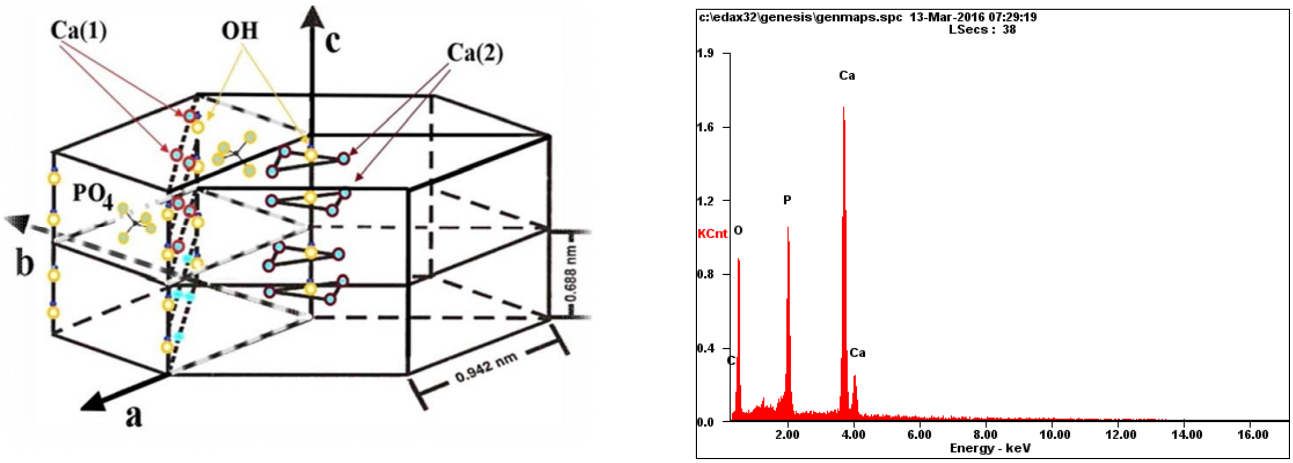

Fig. 3. Elementary cell of HAP [15] and EDX of HAP.

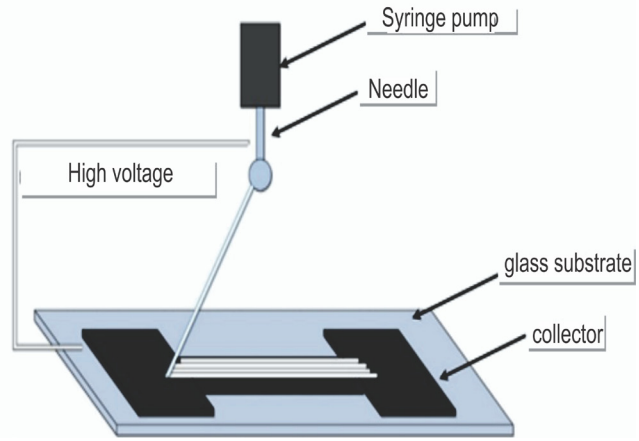

Fig. 4. Schematic representation of the experimental setup.

Figure 4 shows a schematic representation of the installation. High voltage was applied to two electrodes, one of which was on a glass plate, and the second was a needle.

The solutions were prepared under the same conditions, but at different concentrations. Polycaprolactone (PCL) was dissolved in acetone at 50 °C under stirring in a magnetic stirrer for 20 min. HAP and alendronate (ALN) were added after complete dissolution of the polymer. The use of alendronate was determined by characteristics which are used for the prevention and treatment of certain types of bone loss. It belongs to a class of drugs that act on the bone activity of cells. In the class of drugs are called – bisphosphonates.

Data on the composition and the ratio of electroforming solution that consists from polymer, HAP and ALN are given in Table. The electroforming process is more effective at a polymer and HAP concentration with a ratio of 80/20. The same conditions were used to produce polymer fibers in all experiments. The high voltage applied to the needle and collector was equal to 1.5 kV per centimeter. The rate of the syringe pump was 1.5 ml/h. Various forms of the collector were used to control the electric field. There are two methods that can be used to control the jet in an electric field.

First, since electrostatic charges are distributed along the jet, an external electric field can be used to control the jet. As early as 1902, one has manipulated the electric field to control the jet. Even a slight change in the profile of the electric field had an effect on the deposition of the fibers. Secondly, different geometric shapes can be used to control the jet.

Table

Composition and ratio of electroforming solution

Composition	Ratio, %	Solvent ratio, %
PCL/HA/ALN	80/19/1	20
PCL/HA	80/20	12
PCL/HA	75/25	20
PCL/HA	85/15	20
PCL/HA	85/15	12
PCL/HA/ALN	84/15/1	20
PCL/HA	75/25	15
PCL/HA/ALN	80/19/1	12

In allocation of the electric field profile of two parallel metal plates, the fibers will be sequentially deposited through the gap from the tip of one electrode to the other. Since the profile of the electric field between the tip of the needle and the collector affects the jet, which in turn can be used to create aligned or patterned fibers.

As a result of the work, the authors obtained nano-sized films from various types of polymers. The method of SEM was used to study the morphology of synthesized materials and to estimate the particle sizes of samples before and after annealing. Figure 5 shows the images obtained with an electron microscope with three different types of substrate electrodes. In the photographs, the orientation of the fibers, whose size is about 200 nm, is visible. The size of the resulting film depends only on the size of the first electrode, in our experiment we obtained films of size of 5 × 5 cm.

The images obtained with an electron microscope are shown in Fig. 5. Agglomerates of HAP are clearly visible on polymer fibers, which shows the possibility of using this technology for producing polymer fibers with HAP for different parts of medicine. Biologically soluble polymer matrices with HAP crystals are of great interest from the point of view of their use in medicine as matrices for the growth of cellular structures, targeted delivery of drugs.

Conclusions

A method for obtaining nanocrystalline calcium hydroxyapatite has been developed. In this work, calcium hydroxyapatite was synthesized by various methods of chemical deposition using a biological source – the egg shell of birds, which contains 94% calcium carbonate. The egg shell was heat treated at 900 °C, followed by the addition of an aqueous solution of orthophosphoric acid in ultrasonic bath.

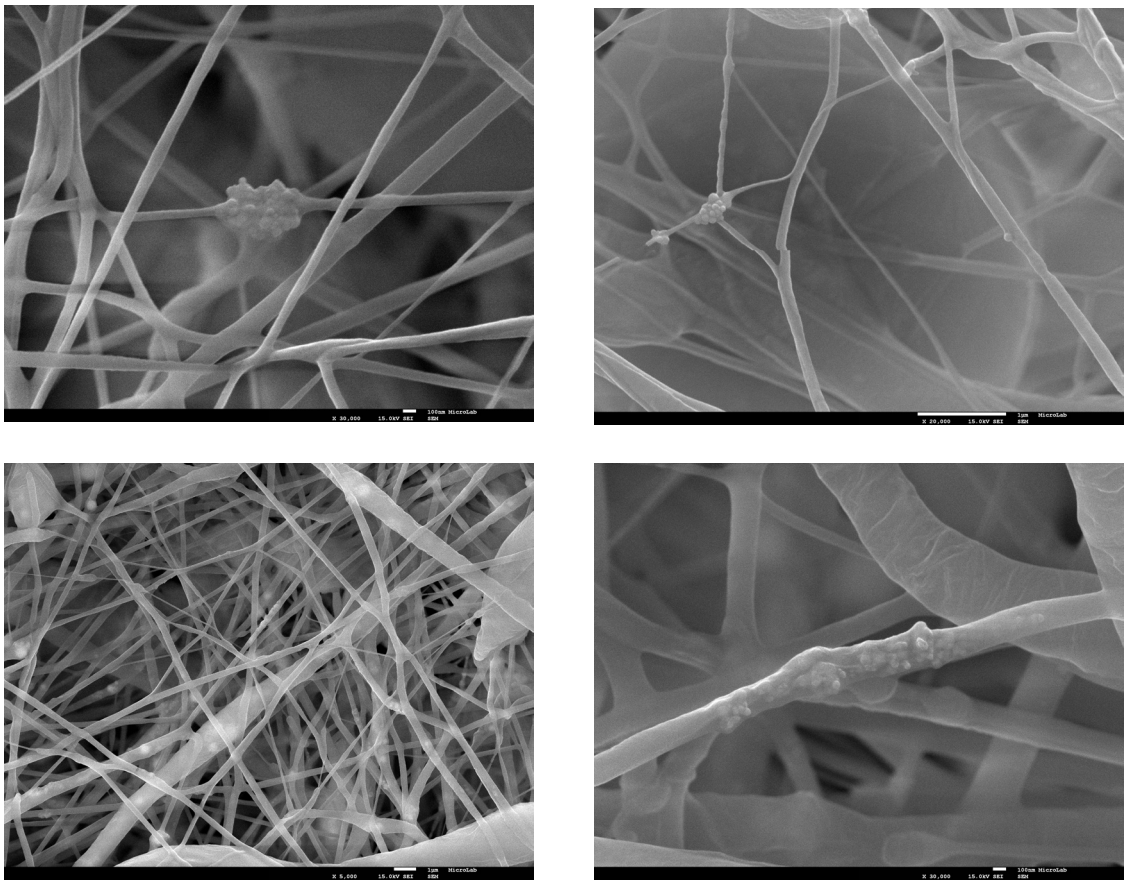

Fig. 5. SEM membranes based on polymeric nanofibres and hydroxyapatite of calcium.

Studies of crystalline calcium hydroxyapatite show that external conditions such as pH, composition and concentration of reagents and impurities, the order and rate of mixing, temperature, and time of the experiment strongly influence the crystallization process and the chemical composition of the synthesized powder. The resulting powdery material is a micron-dispersed powder of micron sizes (1–2 μm). The process of obtaining polymer films by the method of electrospinning was studied. The possibility of obtaining biologically soluble matrices based on polymeric nanofibers and HAP crystals is shown.

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