Low-temperature bioresorbable composite material magnesium-hydroxyapatit

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Abstract. This work is focused on the development of «Magnesium (Mg) – Hydroxyapatite (HA)” composite for bioresorbable implant obtained by spark plasma sintering method (SPS). The structural-phase state of the samples was studied using the X-ray phase analysis method and scanning microscopy (SEM). It is essential to understand the mechanical behaviour of these materials: compressive strength were determined for material characterization. The obtained composite materials were investigated in the solution simulating the extracellular fluid of the human body (SBF) and NaCl solution, and there was shown the allocation of ions of calcium and magnesium and the formation of calcium-phosphate layer (CFL) on the surface of sintered samples and demonstrated. It follow that the material Mg–HA has a biocompatibility in vitro.

1. Introduction

Surgical intervention leads to extensive postoperative defects and, as a result, to functional insufficiency of the musculoskeletal system. A traditional approach is to replace a bone defect with an implant. The main purpose of this developing direction is the creation of an implant material, which must appropriate the following requirements:

- biocompatibility
- bioactivity
- corrosion resistance
- strength

Magnesium (Mg) exhibits excellent biocompatibility with the human body, the strength properties are most similar to the human bone strength properties, that is way, Mg is a perspective material for creating biodegradable implants. However, its corrosion performance at the current stage of development is still not good enough for increasingly diverse practical applications [1]. Thus, in order to reduce the corrosion rate and increase the time of implant degradation until the bone tissue is fully restored, various approaches are being discussed [2-7]. On the one hand, the authors of [2-6] propose to increase the corrosion resistance of magnesium implants by creating alloys - adding to magnesium alloying elements such as: Al, Zn, Mn, Zr, Gd, Sr. On the other hand, to prolong the time of implant degradation helps purification of magnesium from harmful impurities and anodizing, proposed [6]. In addition, to temporarily slow down the decomposition, appropriate types of coatings can be applied to the surface of a magnesium implant [7].

Considerable efforts were directed to the development of ceramic materials based on calcium hydroxyapatite (HA, Ca_{10}(PO_4)_6(OH)_2), the analog on the phase and chemical composition of the
mineral component of human bone tissue [8-11]. Materials based on HA do not have a negative impact in the human body, unlike a number of metals and polymers, and biologically active in relation to the formation of bone apatite. But the known hydroxyapatite ceramic materials have rather low mechanical characteristics in order to perceive physical exertion in many necessary situations with a high degree of reliability.

The aim of the work was to create a porous composite based on HA with reinforcing substance-magnesium (Mg) in the matrix of the material. To retain the initial properties - bioactivity of hydroxyapatite and biodegradability of magnesium, and close values of the strength characteristics with bone tissue, this composite can only be obtained by using powder metallurgy methods.

In the conditions of the modern world and rapidly developing technologies, the goals of this development are not just to reduce the corrosion rate of implants and increase the strength of the composite obtained by powder metallurgy methods, but to create such a material that will increase the rate of bone splicing, the time of bone tissue regeneration, contributing to the formation of healthy callus by replacing the implant with live bone tissue.

2. Experimental procedure

2.1. Sample preparation

As a matrix of the composite, the powder of the mineral component of bone tissue – hydroxyapatite – \( \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \) powder obtained by the sol-gel method was used, Magnesium powder (Mg) was used as a reinforcing filler. Visually, magnesium powder has a silver color, and HA powder has a pure white color. The diffraction pattern of HA using X-ray diffractometer "SHIMADZU–6000" presented at figure 1 is typical for the structure of Apatite. All lines of the spectrum of individual stoichiometric hydroxyapatite are reproduced on the diffractogram of synthetic HA. Peaks of extraneous phases (CaCO\(_3\), CaO and Ca\(_3\)(PO\(_4\))\(_2\)) are absent, which indicates the formation of stoichiometric HA during synthesis.

![Figure 1. X-ray pattern of the HA powder](image)

The particle size distribution was carried out on a laser particle analyzer ANALYSIS22. The analysis showed the average particle size of magnesium is about 100 \( \mu m \) (figure 1-2). Using a Jeol JSM scanning electron microscope, an image of the particles was obtained and the size of agglomerates of synthesized HA range is 80 \( \mu m \) (figure 2-3).

The pycnometric density of these powders was measured at the Helium pycnometer – AccuPyc II 1340. The density of magnesium powder is 1,729\( g/cm^3 \), which is consistent with the theoretical density, the density of HA is 3,472 \( g/cm^3 \).
Pure powders of the following components were prepared: HA and Mg; the mixture was mixed by a mechanical activation method; grinding of powders in a ML-1 ball vibratory mill. The mixture consisted of 9g HA and 1g Mg (volume ratio of 70% HA and 30% Mg), steel cups with steel balls were used; grinding was carried out in an Ar atmosphere in order to exclude the formation of magnesium oxide. Duration of grinding was 15 minutes. The size and shape of the powder particles after grinding is presented in figure 4.

As can be seen from the above figure, the size of the mixture after grinding average 8μm, but there is also an area with nanoscale particles. Using the method of low-temperature adsorption of Brunauer-Emmet-Teller nitrogen, the specific surface area of the powders was measured, the data are given in the table 1:
Table 1. Specific surface area of powders

| Powder     | $S_{sp}$ m$^2$/g |
|------------|------------------|
| HA         | 20.9 ± 0.0243    |
| Mixture    | 49.0 ± 0.1023    |

We can observe an increase in the surface values of the powders after grinding, this indicates that there is a grinding of the material. Grinding is always strongly associated with activation, because under the influence of external forces, the energy reserve of the crushed substance increases – the growth of its surface energy.

2.2. Sintering of the composite by the SPS method

The next step in the experiment was to test the possibility of obtaining a composite “Mg–HA” using the spark-plasma sintering method. For this, a mechanically activated mixture was prepared; it was poured into a graphite matrix of 10mm in size, with graphite paper and tungsten foil, in order to eliminate interaction with graphite. Sintering of samples was carried out on the LABOX–152VHD unit at different temperatures: 400, 500 and 600 °C in order to establish the optimal parameters of compaction, heating 20 °C per minute, pressure – 25 MPa, holding time – 10 min. At this stage mixture of this composite with 70% HA–30% Mg by volume were sintering to determine the effect of the amount of reinforcing substance (Mg) on the structure of the composite as a whole and the effect of the relative amount of HA on porosity of the obtained samples after sintering. The microstructure of the samples is shown in figure 5. Magnesium particles are elongated and rounded grains ranging in size from 50 to 60 microns, evenly spaced in a porous ceramic matrix, the interfacial boundary is clearly traced:

![Microstructure of composite](image)

Figure 5. Electron image in the back-scattered electrons of the microstructure of the sample of the composite “Mg–HA” obtained by the SPS method: a) the composition of the composite is 70% HA–30% Mg by volume, magnification × 100; b) 70% HA–30% Mg by volume with magnification × 500.

3. Results and discussion

3.1. The study of the allocation of ions of calcium and magnesium in the solutions modeling the extracellular fluid of the body.

Determination of solubility values is an important step in the study of the properties of biomaterials based on calcium phosphate, as it allows to assess the ability of materials to resorption, i.e. dissolution in body fluids. Table 2 shows the results of determining the solubility of Mg-HA composite samples in saline and SBF solution at a human body temperature of 37 °C by inductively coupled plasma atomic emission spectrometry.
Table 2. Positive values of elements concentration in solutions for the first day.

| Solution | Ca\(^{2+}\) | Mg\(^{2+}\) | Na\(^+\) |
|----------|-------------|-------------|---------|
| NaCl     | -           | -           | 11,79 mlg/l |
| SBF      | 0,17 mlg/l  | 0,18 mlg/l  | 10,28 mlg/l |

According to the above table it can be seen that in the first day in the physiological solution NaCl there is no dissolution of the composite, calcium and magnesium cations are not released into the solution. On the surface of the composite adsorption of ions Ca\(^{2+}\) and Mg\(^{2+}\) from SBF solution is faster than on the surface of the composite of NaCl solution; this is apparently due to the increase in the acidic properties of the surface and the appearance of phase boundaries, whereby the diffusion and adsorption of ions from SBF solution to the surface of the sample is faster. It can be concluded that the dissolution of the composite in SBF solution and the formation of Mg\(^{2+}\) cations on the surface of the sample contributes to the active formation of new structures.

3.2. Study of the formation of calcium phosphate layer on the surface of the sintered samples during exposure in SBF solution and NaCl.

The morphology of the surface of the substrates with the formed CFL was investigated by scanning electron microscopy (figure 6-7), the results of biomimetic experience were evaluated by the presence of CFL.

![Figure 6. Microstructure of the area of composite Mg – HA before holding in SBF solution at magnification ×100](image)

![Figure 7. Microstructure of the area composite Mg– HA after holding in SBF solution for 30 days](image)

In the first case we can observe the formed spherical structure of the deposited particles on the surface of the composite Mg– HA after aging in SBF solution due to creation of a biomimetic layer – occurs a re-crystallization of hydroxyapatite on the surface of the material. It follow that the material Mg–HA has a biocompatibility in vitro. The composition of the formed CFL is a non-stoichiometric precipitated hydroxyapatite (calcium deficiency) with the formula Ca\(_{10-x}\)(HPO\(_4\))\(_x\)(PO\(_4\))\(_{6-x}\)(OH)\(_{2-x}\). As the porous developed surface of the composite, on which the adsorption of molecules and the formation of a phosphate layer occur, brings about the porous structure of the particles and the process of their growth. Formation of CFL on substrates occurs after 3 days of their aging in SBF-solution. By
28 days, the presence of CFL becomes apparent, the size of the grains of calcium phosphates on the surface layer of the newly formed CFL is 1-2 microns (figure 7).

The results of XRD of the sample area Mg – HA after exposure to SBF solution are shown in figure 8. The weight and atomic percentages of the chemical elements in the spectra are given in table 3:

Table 3. Content of chemical elements on the part of the composite Mg – HA after holding in the SBF solution for two spectra

| Spectra   | Spectra 1 | Spectra 2 |
|-----------|-----------|-----------|
|           | at.%      | at.%      |
| O         | 52.7      | 47.5      |
| Na        | 2.6       | 1.0       |
| Mg        | 4.3       | 2.7       |
| P         | 16.4      | 17.9      |
| Cl        | 3.0       | 0.9       |
| Ca        | 21.1      | 29.9      |
| Total     | 100       | 100       |

The ratio of calcium to phosphorus is close to stoichiometric and equal to Ca/P=1.65-1.66, according to two spectra, this is evidence of recrystallization of hydroxyapatite on the surface of the composite Mg – HA. In addition to there is the presence of ions Na,Cl and Mg, suppose the formation of crystallites NaCl and MgO on the surface.

In the second case on the surface of the composite sample after aging in NaCl solution have felt magnesium oxide crystals, in comparison with SBF solution (figure 9). It was found that MgO crystals deposited on the surface of the sample have a thin lamellar morphology and a size of 1-2 microns. Such particles cover the entire surface of the material:
Magnesium oxide MgO has antibacterial properties and is one of the main components of bio-glass with excellent thermal [12] and bone cements with optimal setting time and mechanical properties [13]. In addition, MgO is capable of complete degradation in the human body, resulting in the formation of the same products as in the biodegradation of Mg in vivo. The resulting MgO layer reacts with the solution to form a Mg(OH)_2 protective film that can resist erosion in the NaCl solution.

3.3. Mechanical testing of samples

The strength values of the samples after SPS are given in the table 4

| Sample | Load, kgf | σ₀, MPa | σᵧ, MPa |
|--------|-----------|---------|---------|
| 400 °C | 595,51 | 74±4 | 66,08 |
| 500 °C | 2291,15 | 286±14 | 91,65 |
| 600 °C | 1177,71 | 147±7 | 67,43 |

In various types of deformation, the compact bone substance forming the middle part of the tubular bones has a compression strength in the range from 100 MPa up to 250 MPa, one of the main tasks of obtaining the composite Mg – HA is as follows the maximum approximation of the mechanical properties of the material to the properties of the natural bone, in order to eliminate the resorption of human bone. From this point of view, the optimum sintering temperature is 500 °C, the tensile strength of the sample is 286 MPa. At a temperature of 400 °C, the "typical" strength of ceramic materials is preserved, it can be concluded that at this temperature there was no sintering of the composite. Table 5 shows the values of the open porosity of the samples after sintering at different temperatures:

| Sample | Σρ, g/cm³ | ρgeo, g/cm³ | ρpic, g/cm³ | % ρgeo | % ρrelative | % open por. |
|--------|-----------|-------------|-------------|---------|-------------|-------------|
| 400°C  | 2,9       | 1,72        | 2,3         | 58,2    | 77,8        | 19,6        |
| 500°C  | 2,9       | 1,72        | 2,1         | 58,4    | 71,9        | 13,5        |
| 600°C  | 2,9       | 1,71        | 2,14        | 58,2    | 72,4        | 14,2        |

The porosity of the compact bone substance is about 13-18% from the total volume of bone tissue, the composite Mg – HA obtained by SPS method gives rise to close initial porosity to the porosity of compact bone substance, it is a significant indicator for increasing the rate of germination of bone vessels into the composite material followed by optimize accelerating bone tissue regeneration.

4. Conclusion

This study focused on the development of a composite "Magnesium – Hydroxiapatite" for a bioresorbable implant using spark plasma sintering method. During the experiments, the following conclusion was drown: the samples, sintered at a temperature of 500 °C had the highest compressive strength – 286 MPa, it is a good indicator of mechanical properties of the material, the open porosity after sintering is about – 14%, the structural-phase state of the composite Mg – HA was studied.

An important step in the analysis of the properties of the composite material was the study of the formation of a calcium phosphate layer on the surface of sintered samples after holding in a solution of SBF and NaCl. During the experiment, the formation of a calcium phosphate layer on the surface of the sample in SBF solution was established (figure 7), which indicates material biocompatibility in vitro and the ability to initiate the passive formation of the mineral component of solid tissues.

The formation of an MgO layer on the surface of the composite (figure 8) is facilitated by the dissolution and isolation of Mg²⁺ cations into the SBF solution, magnesium oxide has antibacterial
properties and is capable of complete degradation in the human body, resulting in the same products as in the biodegradation of Mg in vivo. Many studies show a positive effect of magnesium biodegradation products on osteogenesis, but the mechanism of their action is not yet clear [3]. According to one theory, certain enzymes are adsorbed on the surface of such material from the biological environment, which stimulate the growth of bone cells, thereby causing the healing process. This is preceded by ion exchange reactions on the surface of the introduction and the appearance of a layer of magnesium oxide.

Further implementation of this development will be continued, the ultimate goal of the work is to obtain such material that can offer an alternative solution to existing problems in osseosynthesis, to exclude the re-operation and reduce the period of rehabilitation of the patient.

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