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Stability of Osteopathic Biomaterial with Magnetic Additions of *in Vitro* Experiment

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ABSTRACT

Background: Along with advantages, such as high biocompatibility and absence of infection risks, composite materials have low mechanical durability. Therefore, the development of materials with improved quality for different schemes of potential use in osteoplastic surgery is actively carried out all over the world. **Methods:** To study the properties of control materials of their specific surface area, X-ray analysis and chemical analysis are realized with the usage of photoelectric colorimetry method. As a model medium for the in vitro experiments 0.9 % physiological saline solution was used. **Results:** Relative stability of the studied materials in inorganic model medium was established through a change of some physicochemical characteristics before and after the in vitro experiments for two days. **Conclusion:** The use of two physicochemical methodical approaches for obtaining samples of two types of materials, which were similar with initial characteristics and adsorption activity but with different velocity of solubility in inorganic model medium, was obtained.

KEYWORDS

Biogenic Hydroxyapatite, Magnetic Additions, Bioresorption, Phase Composition, Specific Surface Area

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INTRODUCTION

As a result of injuries and diseases, such as osteomyelitis, osteoporosis, osteosarcoma, millions of people all over the world suffer bone tissue damages. Therefore, there is a continuous problem concerned with the reconstruction of lost biological material in traumatology and orthopedics [1, 2].

Nowadays, allografts and autografts, which are thermally treated for removing the mineral component, are widely used. Bone allografts are distinguished by slow osteointegration, risk of transmission from donor to recipient of various diseases of bacterial or viral etiology, possibility for spreading the reaction of histoincompatibility, chronic granulomatous inflammation, high cost, and religious restrictions. To minimize the risks, the allografts are intensively treated with the an expected result of which osteoinductive properties and mechanical strength (near 50 %) of the implants significantly are aimed to decrease, but the risk of infection and immune response of a patient is still not entirely removed. The usage of autografts for transplantation is limited by the amount of excluded material (20 cm^3), leading to an increase in operation time, blood loss, and prolonged hospitalization. Moreover, chronic neuropathic pains, infectious processes, rapid resorption, and degradation until not complete restoring a bone defect occurre in 20 % of cases under the extraction of autologous bone [1-3].

In general, if there is a concern about the transplantation of tissue substances, biochemical, immunological and tissue compatibility should be always taken into account with the satisfaction boundary of the basic needs of the identity of the antigens that are included in the composition of proteins. These factors are important, and they determine the depth of the concept for materials' biocompatibility [4-6].

A characteristic feature of material interaction with biological systems plays a determining role in its structure. In connection with this ceramics related to such class of materials the structure is determined as a defining parameter, and it is optimally suited to work as an implant. At the same time, one of the disadvantages of all bioceramic materials is a low crack resistance compared to metals, which is almost entirely missed in the plasticity and complex in processing during surgery [4, 7-8].

In the system of biogenic hydroxyapatite (BHA) +

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Fe_3O_4 nanomagnetite acts as a magneto-sensitive component with a reactive surface, which potentially allows to realize the chemical design and to construct required architecture of multifunctional nanocomposite, whereas hydroxyapatite plays the role of a thin stabilizer layer [5, 9-11].

Thus, the development of magnetic nanoparticles, which are safe for use in biology and medicine, remains being an unsolved problem at the present time.

METHODS

As an object of research we used a biogenic hydroxyapatite (BHA) – osteoapatite ceramic powder - material that was taken for medical using and obtained in accordance with TU U 33.1-22965991.002-2001. Microgranules (60-160 μm) of above-mentioned material by ferromagnetic particles were performed by using two methods of physicochemical mixing [12] with subsequent thermolysis at low-temperature in a protective carbon-containing media at 500 ºC (below the Curie point for magnetite – 572 ºC) for 2 hours. The phase composition of the powder materials was controlled by X-ray diffractometer «DRON-3.0» under Co-K_α radiation.

The specific surface area of the materials was determined by method of thermal desorption of nitrogen on the «MPP2» device. Total iron content was determined by photoelectrocalorimetric method on the «FEC-56M» device. The amount of total carbon was determined by using express carbon analyzer «AN-7529». The amount of bioresorption was determined by placing the samples in the inorganic model medium, namely, 0,9 % sodium saline («Novofarm Biosynthesis», Ukraine) for 2 days in thermostatic conditions under temperature that is similar to normal temperature of the human body (36.6–37 ºC). Adsorption activity of powders by methylen blue was determined according to the method prescribed in GOST 4453-74 before and after checking the bioresorption.

RESULTS

Osteopathic material for medical using was obtained by mechanical mixing of BHA microgranules with iron oxalate in the hydrosuspension (physico-mechanical method) and added directly in the obtaining process of iron oxalate under the interaction of the dihydrate of oxalic acid with a solution of iron sulphate in the stabilization of a mixture of isopropanol and sucrose (chemical method) [12]. To obtain a BHA doped nanomagnetite obtained from the oxalate salt , prepared materials after drying were yielded by carrying out a low-temperatured thermolysis in a protective carbon-containing medium. This heat treatment will also provide a stable phase composition of these composite systems (Fig.1).

X-ray diffraction data of these composite systems showed the presence of not only Fe_3O_4, Fe_2O_3, FeO, but also of lightly increased (compared to BHA) number of carbon-containing compounds, in particular, FeC

Fig. 1. The samples of studied osteoapatite powder materials after thermolysis in a protective carbon-containing medium: a) material obtained by chemical method; b) material obtained physico-mechanical method

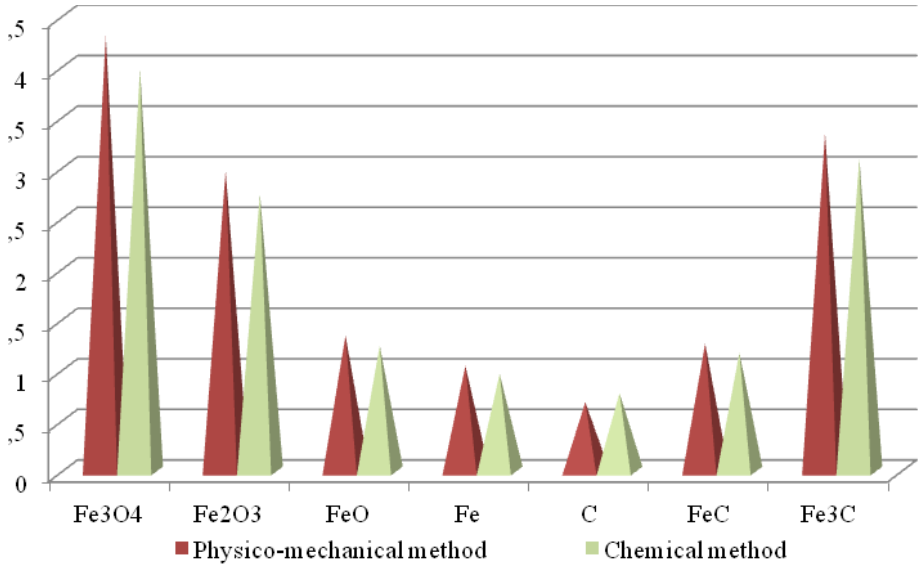

Fig. 2. The results of chemical analysis of the composite system of the BHA + Fe3O4, obtained by the different methods

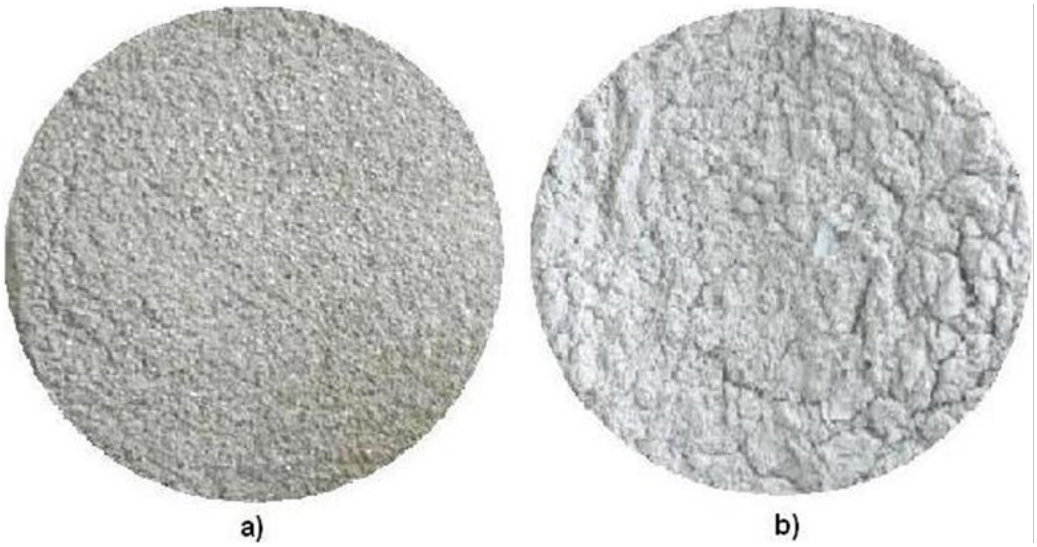

Fig. 3. The samples of studied osteoapatite powder materials after thermolysis in a protective carbon-containing medium and after 2 days in thermostatic conditions at 36,6-37 °C: a) material obtained by chemical method; b) material obtained physico-mechanical method

and Fe – C. Results of chemical analysis (% mas.) with taking into account the molecular weight of the previously mentioned chemical compounds is shown in Fig. 2.

Bioresorption of the materials obtained by chemical and physical-mechanical methods after 2 days in thermostatic conditions was amounted to 0,229 and 0,130 %mas./day respectively. It can be assumed that this difference is partly a result of heterogeneity of the chemical composition, as well as of the difference in specific surface area of the samples: 6,03 m^2/g in the first case and 5,23 m^2/g – in the second [13].

Chemical analysis of the material, which was obtained by direct BHA introduction in the process of deposition of iron oxalate (Fig. 3.a), showed a decrease in the number of total carbon (from 0.77 to 0.71% mas.) and total iron (from 0.96 to 0.89 % mas.) in their solid residues before and after the *in vitro* experiment. In the composition of the powder on the filter 0,96 % mas. of total iron and 0.82 % mas. of total carbon were detected. X-ray analysis of this sample confirmed the above-mentioned results. Since the end of the experiment on the diffraction pattern of the material a decrease in the intensity of the lines of carbon-containing compounds was observed (C, FeC, Fe–C and Fe3C) , reaching up to their disappearance.

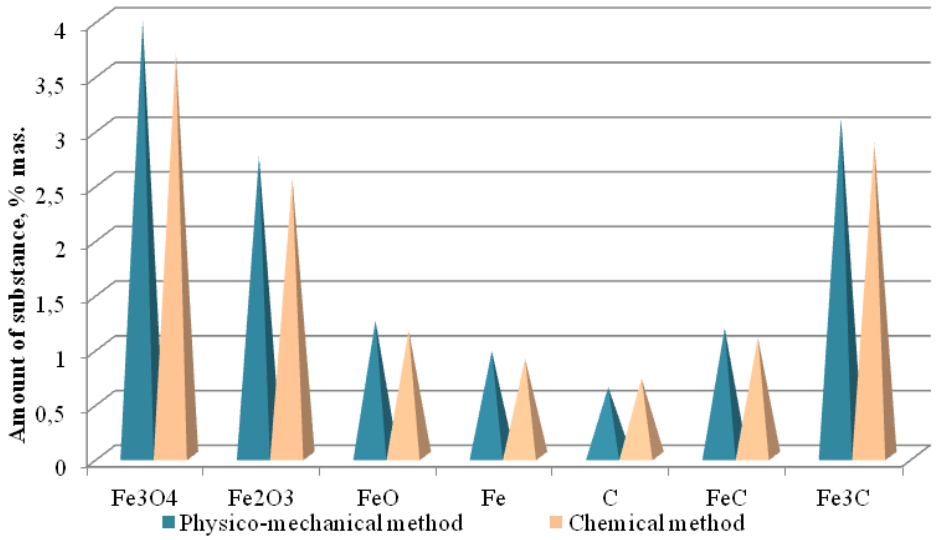

Fig.4. The results of chemical analysis of the composite system of the BHA + Fe3O4 obtained by various methods, after in vitro experiment

According to the results of chemical analysis the amount of total carbon in the solid residue material, which was obtained by mechanical mixing BHA with prepared iron oxalate (Fig.3.b), was reduced from 0,68 to 0.63 % mas., as well as an amount of total iron reduced from 1.04 to 0.96 % mas. after the in vitro experiment. In the powder on a filter 0.68 % mas. of total carbon and 0.91 % mas. of total iron were detected. In addition, the diffraction pattern of this material had the similar trends as in the previous case.

The results of chemical analysis in consideration of molecular masses for above-mentioned chemical compounds after determining the bioresorption are shown in Fig. 4.

The specific surface area of the sample obtained by chemical method after *in vitro* experiment (Fig. 3.a) decreased to 6.00 m^2/g while the value for the sample obtained by physico-mechanical method (Fig. 3.b) increased to 8.72 m^2/g.

The adsorption activity of methylene blue was studied for these samples before (Fig. 1) and after (Fig. 3*) in vitro* experiment. For powder obtained by physico-mechanical method ,adsorption value of methylene blue on the samples before and after testing of dissolution rate decreases from 117.47 to 117.03 mg/g. A sample of the material obtained by direct implantation of BHA in the process of deposition of iron oxalate, on the contrary, showed an increase of adsorption activity with 113,74 mg/g before *in vitro* experiment , reaching 118,86 mg/g (after it).

DISCUSSION AND CONCLUSION

Based on medical recommendations for implantable materials a number of doped additions for investigated samples did not exceed 2 % mass. on Fe.

According to the results of chemical analysis, a total content of Fe for these biomaterials was less than 1 % mass. To adjust result of chemical analysis with the results of X-ray phase analysis, the amount of iron compounds was calculated as a percentage by using their molecular masses (Fig. 2). The significant presence of magnetite, pure iron, and its carbide phases in the sample was obtained by chemical vapor deposition, defining dark gray color (Fig.1.a). A slight decrease in a number of these compounds leads to clearing of the material obtained by a mechanical method (Fig.1.b). Based on these data and visual observation, it can be affirmed that the process of mixing is more successfully passed in case of application of the chemical method.

During the *in vitro* experiments the previously described sample (Fig.1.a) showed slight signs of water repellency. In the material obtained by the physico-mechanical method (Fig.1.b) such evidence was absent. It can be explained by the presence of different amount of C and Fe_3C phases.

The diffractogram of the material, which was obtained by chemical vapor deposition before and after *in vitro* experiment, was identical in tendency with the sample obtained by a physico-mechanical method. It can be assumed that in this case the process of thermolysis

was less active than in the previous(the release of CO_2) one; and carbon particles, reacting with the iron particles, remained in the sample. After placing the sample in a modeling medium, these carbon-containing compounds through its hydrophobicity were on the surface, and , first of all, under filtration their removal occurred in reflection with results of chemical analysis (Fig. 4). Presence of a bigger amount of Fe_2O_3, FeO fraction, and smaller amount of C in the material, which was obtained by mechanical mixing with prepared iron oxalate, explains the differences in a color of samples before (Fig.1) and after (Fig.3) *in vitro* experiment [14, 15].

Taking into account small differences in specific surface area and chemical composition of samples before and after determining the resorption rate, an investigation of adsorption activity for methylene blue was conducted. The material, which was obtained by adding BHA directly in the process of deposition of iron, oxalates after removal of small hydrophobic particles, adsorbing 118.86 mg/g, whereas the initial sample – 113.74 mg/g. It can be explaned by the decrease of specific surface area. Adsorption activity of material, obtained physico-mechanical method before and after *in vitro* experiment almost were not changed(up 117.47 and 117,03 mg/g, respectively).

Thus, adding one technological operation is associated with the removal of hydrophobic compounds by filtration of the sample obtained by the chemical method. It is possible to obtain a material with improved chemical characteristics. Taking into account approximately equal adsorption activity of materials, two methods as well as the significant difference in their bioresorbtion velocity were obtained. Thus, two potentially suitable biomaterials with the different characteristics for osteoplastic surgery can be prepared.

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CONFLICT OF INTEREST

Authors confirm that this article content has no conflicts of interest.

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