Microstructure of spherical granules based on hydroxyapatite and wollastonite

A P Solonenko¹, E S Chikanova¹², A E Shevchenko¹, D A Polonyankin³

¹Omsk State Medical University, Lenina str., 12, Omsk 644099, Russia
²Dostoevsky Omsk State University, Mira ave., 55A, Omsk 644077, Russia
³Omsk State Technical University, Mira ave., 11, Omsk 644050, Russia

E-mail: annsolonenko@gmail.com

Abstract. Spherical granules are one of the preferred forms of biomaterials for bone defects healing. Chemical composition and microstructure of these materials are largely determines their behavior in vivo. Synthesis and properties investigation of polydisperse porous composite granular materials containing several bioactive components are the important task of modern biomedical chemistry. In this work, the microstructure of organomineral granules based on hydroxyapatite, wollastonite, and gelatine, and the products of their calcination were studied. It was found that as a result of removal of the polymer binder from the ceramic particles surface, nanopores were disclosed, and the subsequent crystallites sintering provide the granules shape stability.

1. Introduction
Granules are one of the biomaterials types demanded in bone tissue engineering. There are granules in the form of cylindrical or cubic blocks (Fig. 1 a, b), blocks of irregular shape (Fig. 1 c) and spherical particles (Fig. 1 d) [1-3]. The latter seem to be more preferable from a practical point of view due to the absence of sharp parts and the dense filling ability for the defect with any complex shape owing to application of particles with different diameters.

Figure 1. Granules from biocompatible calcium salts in the form of (a) porous cubic blocks, (b) cylinders [1], (c) particles with irregular shape («TriCaFor» [2]), (d) spheres («OsteoSet» [3])

A number of methods for spherical granules producing have been proposed. Among them are the powders agglomeration, blocks crushing with the subsequent rolling, spray drying, electrospinning,
hydrothermal synthesis, dispersing and suspension technology [4]. Depending on the chosen method, granules with diameters from several microns to several millimetres, dense or with open porosity up to 70% can be obtained. Value of porosity and presence of the pores with various sizes in granules is an important component of osteoinduction, because these characteristics provide the penetration of biological fluid (blood) and osteogenic cells into the material and the newly bone tissue growth.

One of the technically available method for porous granules producing is a suspension technology based on the effect of immiscible liquids, described in [5]. For this technique ceramic powders of various compositions can be used (mono- and/or multicomponent). Thus, it is reported about the production of spherical granules from hydroxyapatite (HA, \( \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \)) [6], octacalcium phosphate [7], brushite [7]. Materials based on HA with fluorapatite admixture (\( \text{Ca}_{10}(\text{PO}_4)_6\text{F}_2 \)) (up to 10 wt.%) [5], as well as HA mixed with calcium carbonate (from 20 to 80 wt.% each of the components) [9] are reported. Such combinations of bioactive substances with different dissolution rates allow to create materials that in different ways interact with the biological environment at the implantation site and have greater or lesser chemical and mechanical stability.

So, further investigations of multicomponent granular biomaterials for medicine are of relevance and have a great importance.

2. Task definition

The task of this work is investigation of the microstructure of spherical ceramic granules based on hydroxyapatite and wollastonite, obtained by suspension technology.

3. Materials and methods

In this work, granules were synthesized by the suspension technology [10]. Synthetic ceramic powders with a variable content of hydroxyapatite and wollastonite (WT, \( \text{CaSiO}_3 \)) [11] were used in the granules synthesis. A weighed portion of the powder with HA/WT mass ratio 80/20, 60/40, 50/50, 40/60, 20/80 or 0/100 was mixed with a given volume of 10% gelatine water solution, thoroughly stirred and this suspension was dropped into a container with vegetable oil, stirred with a magnetic stirrer. Spherical granules were formed in this system due to centrifugal forces from the drops of ceramic powder suspension in gelatine. Hard particles were filtered, washed from oil with ethyl alcohol and dried at 100 °C on air. Resulted granules are a composite of gelatine with HA and WT. Synthetic granules shown in Fig. 2.

![Figure 2. General view of granules (a) and a micrograph obtained on a microscope «JCM-5700», JEOL (b).](image)

Further, samples of granules with various compositions were calcined at 1250 °C with the duration of 1 hour for polymer-binder (gelatine) removing and hardening.
Granules microstructure was investigated by scanning electron microscopy (SEM) on a «JCM-5700» (JEOL) electron microscope at 15 kV accelerating voltage and a magnification from 50 to 10000 times. Nitrogen-free X-ray energy dispersive spectrometer «JED-2300» (JEOL) was used for the elemental analysis.

Granules porosity was determined by the pycnometric method. Samples were placed in a pycnometer, filled with distilled water and left for 24 hours. After that water was added to the pycnometer mark and it was weighed. Weight of the same pycnometer containing only distilled water was also determined. Based on these data, the true density value was calculated. Porosity was determined from the mean and true density values.

4. Results
Micrographs of the external and internal structure of granules, which are a composite of gelatin with HA and WT, are shown on the Fig. 3 a, b. It can be seen that the granules surface (Fig. 3 a) is irregular, rough, with ledges and cavities of various shapes and sizes. At that time, all the ledges and edges of the ceramic particles are smoothed, apparently due to the gelatin layer covering them. On the composite granules split, in their inner part (Fig. 3 b), the grain structure and the boundaries of the initial powders particles are visible.

Micrographs of granules after calcination and polymer-binder removing are shown in Fig. 3 c, d. In calcined materials the microstructure transformation is noticeable in comparison with the initial samples (Fig. 3 a, b). Thus, the forms smoothed out by the gelatine disappear and the boundaries of agglomerates from sintered HA and WT crystallites become visible on the granules surface. The same structure is observed in the inner part of the granules (Fig. 3 d). In addition, a large number of pores (micro- and nanosizes) in the ceramic framework are visible.

![Figure 3](image_url)

Figure. 3. Micrographs of the external surface and internal structure of the granules with proportion HA/WT 80/20 before (a, b) and after calcination (c, d)

In addition to micro- and nanometer-sized pores between ceramic crystallites and their agglomerates, the SEM method established the presence in the granules structure larger pores reaching hundreds of
microns in diameter (Fig. 4).

The values of porosity of granules with various compositions are shown in Table 1. It can be seen that the open porosity of each granules composition increases on 6 - 8 % after calcination.

| HA/WT (wt.%) | 80/20   | 50/50   | 20/80   |
|-------------|---------|---------|---------|
| P₁, %       | 41 ± 3  | 59 ± 2  | 50 ± 4  |
| P₂, %       | 49 ± 3  | 65 ± 3  | 58 ± 3  |

* P₁ - open porosity of initial granules, P₂ - open porosity of calcined granules

A quantitative elemental analysis of the granules surface layer before and after calcination was also performed. The results are presented in Table 2. According to the experimental data, all initial samples contain Ca, P, Si, O, N, C. At the same time, Ca, P and Si are found in small amounts, while the contents of C and N (constituents of gelatine) reach up to 30 - 40 wt.%. This may indicate the presence of an organic film on the investigated surface. After heat treatment, only Ca, P, Si, and O are found in all granules, because of complete gelatine removing and the retention of ceramic matrix from HA and WT.

| HA/WT (wt.%) | Element | 80/20 | 50/50 | 20/80 |
|-------------|---------|-------|-------|-------|
|             | The element content in the sample (wt.%) |
| Ca          | 1.18 / 37.6 | 0.16 / 33.9 | 2.04 / 31.4 |
| P           | 0.80 / 14.5 | 0.63 / 8.5  | 0.62 / 3.3  |
| Si          | 0.47 / 3.7  | 1.51 / 10.7 | 2.85 / 17.0 |
| O           | 45.54 / 44.2 | 47.33 / 46.9 | 43.65 / 48.3 |
| N           | 22.80 / -   | 9.96 / -    | 14.52 / -   |
| C           | 29.21 / -   | 40.41 / -   | 36.32 / -   |

* To the left of the line - the element content in the initial composite granules, to the right of the line - the element content in the granules calcined at 1250 °C

5. Discussion

In this work, a suspension technology based on the principle of immiscible liquids is used to obtain spherical granules from ceramic powders. Vegetable oil (dispersion medium) and an aqueous solution of gelatine mixed with ceramic powder (dispersed phase) were used as initial reagents. Composite granules from salt particles bonded together by gelatine, can be obtained upon synthesis completion at the stage of material washing from oil and drying in air. Their heat treatment leads to a mineral
composition due to polymer burnout. Changes in the granules structure at this stage of synthesis have been studied and presented in this article.

It was established by SEM (according to elemental analysis data) that gelatine in composite granules, obtained as an intermediate product of synthesis, connects and covers the particles of the initial ceramic powders with a thin film. As a result of the granules drying, pores and channels that go deep into the particles are formed. Due to their presence, the materials have a sufficiently high open porosity (about 50 % for each compositions, Table 1). The biocompatibility of all granules components (gelatine, HA and WT) provides the principal possibility of these composites application for biomedical purposes, in particular to fill the bone defects.

Heat treatment of organomineral granules in the air environment leads to gelatine removing from the materials composition. The thermolysis of gelatine occurs in the temperature range 300 - 500 °C. Calcination at 1250 °C provides sintering of the initial powders particles, that indicated by the rounded shapes in the structure of the mineral granules. Due to this, the materials are not destroyed and their spherical shape saved when the binder polymer is removed. In addition, after calcination, the open porosity of the granules increases on 6 - 8 % for each composition. Obviously, this is due to the appearance of nanopores in the ceramic matrix, which are identified by the SEM.

6. Conclusion

The microstructure of a spherical granules, which are a composite of gelatine and ceramic powders based on a mixture of hydroxyapatite and wollastonite, as well as the products of their calcination at 1250 °C, was investigated. It was found that in the initial organomineral materials, gelatine covers the crystallites of phosphates and calcium silicates with a thin layer, binding them together. At the stage of drying, due to the polymer drying, pores and channels that go deep into the particle’s volume are formed in the granules. The open porosity of the calcined granules is on 6 - 8 % higher than this characteristic of the initial composite samples, because of the nanopores appearance in the ceramic matrix between salt crystallites as a result of gelatine removing. The sintering of calcium phosphates and calcium silicates particles that occurs during the calcination provide the stability of the granules form.

The biocompatibility of all granules components (gelatine, HA and WT) provides the principal possibility of these composites application for biomedical purposes, in particular to fill the bone defects.

References

[1] Hayashi K, Kishida R, Tsuchiya A and Ishikawa K 2019 Materials Today Bio 4 100031.
[2] Ushakov R V, Aivazov T G, Ushakov A R 2014 Practicing dentist 3 46.
[3] Howlin R P, Brayford M J, Webb J S, Cooper J J, Aiken S S and Stoodley P 2014 Antimicrob. Agents Chemother. 59 111.
[4] Barinov S M, Komlev V S 2005 Calcium phosphate based bioceramics (Moscow: Nauka)
[5] Komlev V S, Barinov S M, Girardin E, Oscarsson S, Rosengren A, Rustichelli F and Orlovskii V P 2003 Sci. Technol. Adv. Mater. 4 503.
[6] Yang J H, Kim J H, You C K, Rattray T R and Kwon T Y 2011 J. Biomed. Mater. Res. B 99 150.
[7] Komlev V S, Barinov S M, Bozo I L, Deev R V, Eremin I I, Fedotov A Yu, Gurin A N, Khromova N V, Kopnin P B, Kuvshinova E A et al. 2011 ACS Appl. Mater. Interfaces 6 16610.
[8] Tas A C 2011 J. Am. Ceram. Soc. 94 3722.
[9] Smirnov V V, Goldberg M A, Komlev V S, Barinov S M Patent RF, no 2555348, 2014.
[10] Shevchenko A E, Solonenko A P, Blesman A I, Polonyankin D A and Chikanova E S 2021 J. Phys. Conf. Ser. 1791 012119
[11] Solonenko A P, Blesman A I, Polonyankin D A, Gorbunov V A 2018 Russ. J. Inorg. Chem. 63 993.

Acknowledgments

This work was funded by the Ministry of Health, Russian Federation (project «Experimental validation of the effectiveness of physical and biochemical factors influence on the processes of connective tissue reparative regeneration and specialization» within the framework of the basic part of the state assignment of the Ministry of Health, Russian Federation on years 2021 - 2023).