Synthesis and physicochemical investigation of hydroxyapatite and wollastonite composite granules

A E Shevchenko¹,², A P Solonenko¹, A I Blesman², D A Polonyankin², E S Chikanova¹

¹Omsk State Medical University, Lenina str., 12, Omsk 644099, Russia
²Omsk State Technical University, Mira ave., 11, Omsk 644050, Russia

E-mail: annsolonenko@gmail.com

Abstract. Creation of osteoinductive materials based on biocompatible synthetic salts, with a sufficiently high strength and porosity, with the shape which allow filling bone defects with various shapes and sizes is an actual task of modern biomaterial science. In this work, a series of granular materials were obtained from synthetic powders with various proportions of hydroxyapatite and wollastonite using a suspension technology based on the principle of immiscible liquids. Ceramic particles have a spherical shape, microhardness at least 17 HV and open porosity about 50 %. These materials can be used for reparation of bone defects of various locations, shapes and sizes in surgical dentistry, traumatology and orthopaedics.

1. Introduction

In modern medicine, a large number of synthetic materials are used for restoration of human tissues and organs. These group of materials include artificial heart valves, lenses and eye prostheses, auditory ossicles, coronary stents, soft tissue patches, catheters, cranial plates, filling materials and artificial joints. Bone tissue engineering is also actively developing. Materials that allow not only restore the physical integrity of the organ, but also conduct effective therapy of the defect resulting from injuries, diseases and / or surgical interventions in order to completely regeneration its natural form and properties are being developed. It is believed that such materials should act as a supporting structure and gradually dissolve in surrounding body fluids, being a source of calcium and phosphorus involved in bone tissue formation de novo [1-3]. Therefore, they must have biological compatibility, interconnected porosity and controlled kinetics of resorption in the human body, as well as strength, which can withstand physiological stresses in the transition period [3, 4].

To date, a large number of biomaterials variations based on calcium phosphates (CP), in particular hydroxyapatite (HA, Ca₁₀(PO₄)₆(OH)₂), a synthetic analogue of the mineral component of human bone tissue, have been created and studied. Various dense ceramics, cements, porous matrices, granules, etc. are known. However, in a number of clinical cases, spherical granules are the most preferred variant of biomaterial form. This is due to the possibility of their use for loose filling of defects of various shapes and sizes. At the same time, it is possible to create the necessary density of material in bone cavity by using particles of various sizes and introducing drugs into the inter- or intragranular space. In addition, the spherical shape of the material improves its technological characteristics, prevents the removing and adhesion of particles [5].

A number of methods have been proposed for producing granules. These include crushing blocks, spray drying, quenching in liquids, hydrothermal synthesis [6]. An interesting method for producing porous spherical HA granules is a suspension technology based on the principle of immiscible liquids [7]. In this case, as a rule, a suspension of HA powder and a binder component (gelatin, collagen, chitosan,
etc.) is obtained, which is then introduced into a dispersing medium (for example, vegetable oil) with constant stirring. Granules are formed under the surface forces tension action. However, it is known that HA is a very low soluble compound (IP = 10^{-117} \text{[3]}) and, accordingly, materials which is made from HA are characterized by an extremely low rate of resorption in the human body. This problem can be solved by doping HA with more soluble substances, for example calcium silicates (CS). Materials based on CS, in particular wollastonite (WT, \(\beta\)-CaSiO\(_3\)), have high biocompatibility, do not cause negative body reactions, act as a filling agent in the place of bone defect, stimulate the new bone tissue formation due to the gradual dissolution and release of calcium ions into the surrounding liquid media [8]. In this regard, the creation of biomaterials, in particular granules, from mixtures of HA and WT is actual task.

2. Task definition
The task of this work is the synthesis and physicochemical study of granules from ceramic powders with various proportions of hydroxyapatite and wollastonite.

3. Materials and methods
Ceramic powders with different contents of HA and BT synthesized according to the technique presented in [9] were used for granules production. Some characteristics of ceramic powder composition and particles micrographs are presented in Table 1 and in Fig. 1.

| HA/WT proportion, wt. % | \(\text{Ca/(P+Si)}\) | \(D_{\text{median}}, \mu m\) \(^1\) | \(D_{\text{(HA)}}, \mu m\) \(^2\) | \(D_{\text{(WT)}}, \text{nm}\) \(^3\) |
|------------------------|-----------------|-----------------|-----------------|-----------------|
| 80/20                  | 1.57 ± 0.02     | 87.7            | 12.4            | -               |
| 60/40                  | 1.39 ± 0.02     | 99.0            | 11.9            | 9.5             |
| 50/50                  | 1.30 ± 0.05     | 103.6           | 11.4            | 12.2            |
| 40/60                  | 1.24 ± 0.04     | 110.7           | 11.0            | 11.0            |
| 20/80                  | 1.10 ± 0.01     | 133.8           | 12.2            | 10.8            |
| 0/100                  | 0.99 ± 0.01     | 133.3           | -               | 13.1            |

\(^1\) \(D_{\text{median}}\) – median diameter of powder particles, 
\(^2\) \(D_{\text{(HA)}}\) – HA crystallite diameter, 
\(^3\) \(D_{\text{(WT)}}\) – WT crystallite diameter.

For granules production 1 g of a synthetic powder with a certain weight ratio HA/WT was mixed with 1.25 - 2.0 mL 15% gelatin solution in distilled water, heated to about 40 °C, and quickly mixed in a homogeneous mass. The prepared creamy suspension was introduced dropwise into vegetable oil and stirred using a magnetic stirrer at 850 rpm speed. The granules were cooled, washed with ethyl alcohol to remove oil, and dried at 25 °C for 24 hours. After that samples were dried at 100 °C for 2 hours and analyzed with a group of physicochemical methods.
FTIR-spectra of the granulated materials were recorded on FTIR-spectrometer «FT-801» (Simex). Samples were studied in tablets form with KBr. Spectra were collected in the region from 500 to 4000 cm⁻¹ with 4 cm⁻¹ scanning resolution and 25 times of scanning for each sample. The program «ZaIR 3.5» (Simex) was used to obtain and process of the spectra.

The morphology, structure and sizes of granulated particles was studied by SEM. Samples analysis was carried out by using a «JCM-5700» microscope (JEOL) equipped with an energy dispersive X-ray spectrometer «JED-2300» (JEOL) for elemental analysis in a high vacuum mode.

The sizes of granules formed as a result of synthesis from powders with various composition were determined during sieve analysis. Studied samples were scattered on a series of laboratory sieves with mesh sizes from 100 μm to 5 mm.

The microhardness of samples was measured by the Vickers method on microhardness tester «PMT-3M» (LOMO) according to state standard GOST R ISO 6507-4-2009. The granules were pre-polished to obtain a flat surface on one side.

To determine the middle density, the granules were weighed on an analytical weighing-machine and their diameter was measured using a micrometer. The density value was calculated as the ratio of sample mass to its volume, based on the assumption of spherical shape of the particles. True density and porosity were 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.

The gelatin content in the granules was determined by the samples weight loss after its calcination on air in muffle furnace «EKPS-5» (Smolensk SKTB SPU) at 400 °C for 2 hours. The mineral phases fraction (totaly HA and WT) was determined from the initial and calcined samples masses.

4. Results

According to the described method, granules were obtained from all the synthetic ceramic powders with different mass contents of HA and WT. Shape of the particles closes to spherical. Granules view is shown in Fig. 2. Diameters of granules vary from 150 μm to 5 mm. The main fraction has size 1 - 2 mm (about 50 wt. % in each sample).

![Figure 2. Granules from composite HA/WT powder](image-url)

Micrographs of granules presented in Fig. 3. It can be seen from Fig. 3b that globules surface is rough. It contains ledges and cavities of various heights. Obviously, they appeare when the micrometer particles of the initial ceramic powder were bonded with a polymer-binder (gelatine). As a result of gelatine drying, pores of various sizes and depth appear in granules (Fig. 3 a, c). Basically, the pores are through and go deep into the sample volume from the surface, forming a system of channels. Large
and massive cavities are found in the centre of bulk granules (Fig. 3 c). In case of materials biomedical application, these inner pores can act as a drugs store.

Figure 3. Micrographs of granules with 40/60 wt. % HA and WT: general view (a), surface structure (b), inner structure (c, d)

Figure 4. IR-spectra of initial components of granules (a) and granulated materials with different weight ratio HA/WT (b)

The composition of the starting compounds (HA, WT, gelatine) (Fig. 4 a) and granules obtained from ceramic powders with variable salt content (Fig. 4 b) were studied by FTIR-spectroscopy. It was found that synthetic apatite spectrum contains well-resolved bands at 565 and 603 cm⁻¹, corresponding to the
stretching vibrations mode of O–P–O bonds in the salt structure; bands at 962, 1040, and 1090 cm\(^{-1}\) caused by symmetric deformation vibrations of P–O bonds in the PO\(_4\)-tetrahedra; absorption maxima at 630 and 3570 cm\(^{-1}\) caused by vibrations of O–H groups, and bands at 1630 and 3450 cm\(^{-1}\) associated with vibrations of H–O–H bonds [10].

In WT spectrum at 565 cm\(^{-1}\) the modes of O–Si–O deformation vibrations and Ca–O stretching vibrations in CaO\(_6\) octahedra appear; the doublet in the region of 600 - 800 cm\(^{-1}\) corresponds to symmetric stretching vibrations of siloxane bridges (Si–O–Si); the group of peaks in wave number range 850 - 1100 cm\(^{-1}\) refers to asymmetric stretching vibrations of Si–O bonds in SiO\(_4\)-tetrahedra; the shoulder at 1200 cm\(^{-1}\) belongs to stretching asymmetric vibrations of Si–O–Si fragment connecting silicate chains in \(\beta\)-CaSiO\(_3\) [11].

The gelatin spectrum includes bands in region 1260 - 1400 cm\(^{-1}\) caused by vibrations of carboxyl groups in type I gelatin; at 1550 cm\(^{-1}\) the band of N–H bonds deformation vibrations in amide II is recorded, and the absorption maximum in region 3270 - 3370 cm\(^{-1}\) corresponds to N–H bonds stretching vibrations; at 1650 cm\(^{-1}\) the bands of N–H bending vibrations and C=O, O–H stretching vibrations appear; the doublet at 2880 and 2940 cm\(^{-1}\) corresponds to C–H and N–H stretching vibrations [12].

The IR spectra of the granules contain all the main bands characteristic for their components.

### Table 2. Some characteristics of granules

| HA/WT ratio in powder, wt. % | \(W_{HA+WT}\), wt.% | \(W_{Gel}\), wt.% | HV 0.02 | \(\rho_{mid}\), g/cm\(^3\) | \(\rho_{true}\), g/cm\(^3\) | P, % |
|-----------------------------|---------------------|-------------------|---------|--------------------------|--------------------------|-----|
| 80 / 20                     | 77.6 ± 1.1          | 18.4 ± 0.7        | 17.0 ± 0.4 | 1.13 ± 0.03             | 1.91 ± 0.11             | 41 ± 3 |
| 60 / 40                     | 77.1 ± 4.3          | 18.7 ± 0.5        | 22.2 ± 0.4 | 1.09 ± 0.03             | 2.25 ± 0.07             | 52 ± 2 |
| 50 / 50                     | 85.2 ± 2.8          | 13.2 ± 0.6        | 26.7 ± 0.4 | 1.07 ± 0.03             | 2.61 ± 0.12             | 59 ± 2 |
| 40 / 60                     | 76.6 ± 5.3          | 20.4 ± 0.3        | 24.1 ± 0.3 | 1.01 ± 0.04             | 2.27 ± 0.14             | 55 ± 3 |
| 20 / 80                     | 79.9 ± 2.9          | 14.2 ± 1.0        | 22.7 ± 0.4 | 1.23 ± 0.03             | 2.47 ± 0.20             | 50 ± 4 |
| 0 / 100                     | 82.8 ± 0.8          | 15.4 ± 0.7        | 16.9 ± 0.4 | 1.20 ± 0.03             | 2.53 ± 0.21             | 53 ± 4 |

\(W_{HA+WT}\) – salt overall content in granules,
\(W_{Gel}\) – gelatin content in granules,
HV 0.02 – Vickers’ microhardness,
\(\rho_{mid}\), \(\rho_{true}\) – middle and true density of granules,
P – granules porosity.

According to thermal analysis data, all granules contain from 13 to 20 wt. % of gelatin. The rest is the mineral component, i.e. HA and WT mixed in various proportions (Table 2).

It was found that all samples have at least 17 HV microhardness (Table 2). This parameter increases with increasing WT content in the samples to 50 wt. %, and then reduced.

The middle and true density of granules are at least 1.9 and 1.0 g/cm\(^3\), respectively (Table 2). The particles wetted with aqueous solutions sink. It should prevent the entrainment of the material from the implantation site.

All samples have about 50 % of open porosity. This value is in good agreement with the results of granules analysis by SEM.

### 5. Discussion

In the scientific field of biomaterials development, great attention is paid to materials’ composition, porosity and strength, which provide the possibility of gradual dissolution, intergrowth of blood vessels and new bone tissue, and also allow making mechanical manipulations without disturbance of the integrity and form of the artificial substance. In this regard, it is currently believed that the material should consist of biocompatible substances, should have a high porosity provided by the system of pore with various diameters, and should be strength enough.
In this work, using a suspension technology based on the principle of immiscible liquids from synthetic mixtures of biocompatible salts - HA and WT - a series of granulated materials containing about 80 wt. % of the mineral composite component and the polymer biodegradable binder (gelatine) were obtained. Different proportions of calcium phosphate and calcium silicate should provide different rates of material resorption and, consequently, the release into the environment of calcium, phosphate and silicate ions involved in the de novo bone mineral construction processes. The presence of gelatine in the granules’ composition determines their mechanical strength and microhardness, which for each of the obtained materials is at least 17 HV (Table 2). These values are comparable with the results of similar studies [13] and allow providing the necessary manipulations with the material in clinical practice without disturbing the particles shape and size. Different sizes of granules obtained in a single synthesis operation (in other words, samples polydispersity) will allow to achieve complete intraoperative filling of a bone defect with any complex shape. The high porosity of the materials, including the presence of an internal cavity in the granules, also makes it possible to use them for prolonged delivery of medicinal substances.

6. Conclusion
The synthesis of granules from ceramic powders with a variable content of HA and WT was carried out. According to the results of FTIR-spectroscopy, granules contain Ca$_{10}$(PO$_4$)$_6$(OH)$_2$, $\beta$-Ca$_2$SiO$_4$ and gelatine, i.e. during synthesis operations no transformations of starting components occur. The resulting granules have a shape close to spherical, open porosity about 50 % and at least 17 HV microhardness.
These materials could be promising drugs for filling bone defects with any complex shape.

References
[1] Galindo T G P, Chai Y and Tagaya M 2019 Journal of Nanomaterials 2019 23
[2] Dorozhkin S V 2015 Materials Science and Engineering 55 272-326
[3] Barinov S M 2010 Russian chemical reviews 79 15-32
[4] Jeong J, Kim J H, Shim J H, Hwang N S and Heo C Y 2019 Biomaterial Research 23 11
[5] Yang J H, Kim J H, You C K, Rautray T R and Kwon T Y 2011 Journal of Biomedical Materials Research Part B 99 150-7
[6] Paul W, Sharma C P 1999 Journal of Materials Science: Materials in Medicine 7 383-8
[7] Komlev V S, Barinov S M, Girardin E, Oscarsson S, Rosengren A, Rustichelli F and Orlovskii V P 2003 Science and Technology of Advanced Materials 4 503-8
[8] Wu C, Chang J 2013 Biomedical Materials 8 12
[9] Solonenko A P, Blesman A I, Polonyankin D A and Gorbunov V A 2018 Russian journal of inorganic chemistry 63 993-1000
[10] Shi J, Klocke A, Zhang M and Bismayer Y 2005 European Journal of Mineralogy 17 769-75
[11] Handke M 1985 Fourier and computerized infrared spectroscopy 553 395-6
[12] Hossana M J, Gafurb M A, Kadirb M R and Karima M M 2014 International Journal of Engineering & Technology IJET-IJENS 14 24-32
[13] Kaya I, Sahin M C, Cingoz I D, Aydin N, Atar M, Kizmazoglu C, Kavuncu S and Aydin H E 2019 Turkish Journal of Medical Sciences 49 922-7