Bioceramic Microspheres Based on $\text{Si}_3\text{N}_4\text{--Ca}_3(\text{PO}_4)_2$

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This article describes a material prepared for biocements for medical use in human body. Biocements are used, for example, for bone implants or as a filling of bone cavities. The main method of preparation of Si_3N_4 microspheres in this article is based on flame synthesis. Different compositions of Si_3N_4 and $\text{Ca}_3(\text{PO}_4)_2$ powder mixtures were prepared and synthesized in $\text{CH}_4 + \text{O}_2$ flame. The aim was to characterize the influence of the proportion of Si_3N_4 and $\text{Ca}_3(\text{PO}_4)_2$ and the preparation of microspheres on their resulting chemical and crystalline phase composition and to determine the effect of these changes on the biological characteristics of the obtained microspheres.

Keywords: bioceramics, microspheres, biological properties, Si_3N_4 , $\text{Ca}_3(\text{PO}_4)_2$, flame synthesis

1 Introduction

Biomaterials are nowadays more used in tissue implant medicine. The most used materials for implants are metals and their alloys, polymers, and ceramics. The attributes of these materials are different, so their use for body implants is also different. Metal implants have variety of uses for bone implants, but are often the subject of corrosion, causing allergic reactions and rejection of surrounding tissues. Their mechanical durability and weight are considerably higher than those of the original bone. Their price is also very high due to expensive ingredients in alloys. Therefore, ceramics are possible alternative to solve this kind of problems. The ceramic materials used in bone implants have very strict criteria. Durable structure and mechanical properties similar to the human bone are desired. It needs to be capable of bioactivity for binding the implant to the rest of the tissue in its surroundings. Biocompatibility of ceramics is flawless in comparison to metal implants. Ceramics doesn't cause corrosion, allergic reaction, etc. Advantages of the application of bioceramic materials are the porosity of the material, as well as strength and bioactive surface, price, weight, organism acceptance, and application possibility as powder form. The most commonly used ceramic materials in orthopedics are oxide ceramics (Al_2O_3 and ZrO_2), due to their excellent biocompatibility. However, among the biocompatible materials was Si_3N_4 ceramics, which can additionally promote cell adhesion, normal proliferation, and differentiation [1, 2]. While burnished Al_2O_3 and ZrO_2 are used in the overall replacement of lumbar [3–4] and knee joints [5], the use of Si_3N_4 is attractive for improved porous structure adaptation and good mechanical properties. Si_3N_4 in porous form can promote direct and natural bone formation, which is required for sustained biological fixation to the host bone. Its use in orthopedics as a porous surface implant, which allows the possibility of tissue growth, has a great potential. Biological applications often require additional properties, such as bioactivity, which allows a stronger connection to the host tissue, different size, and shape of the implant (complex structures, light machinability, etc.), mechanical, physical, or chemical properties. Also, porous spherical shape particles with size up to a few tens of micrometers can significantly increase the possibilities of bioapplication of silicon nitride.

This paper deals with the characterization of Si_3N_4 microspheres (prepared by flame synthesis) with the addition of a bioactive component in the form of $\text{Ca}_3(\text{PO}_4)_2$ in terms of their structure and biological properties.

2 Experiment

For sample preparation, powders Si_3N_4 (UBE-SN-E10 from UBE INDUSTRIES) and $\text{Ca}_3(\text{PO}_4)_2$ (Lachema) were used. The Si_3N_4 E10 powder is typical for its high purity, uniform particle size, and high level of α -phase. Three mixtures of different composition were prepared from these powders (Table 1).

Each of these powder mixtures was homogenized with a wet way on a attritor mill for 4 h (isopropanol, 500 rpm). After homogenization, the isopropyl alcohol was removed from the mixture by rotary evaporation, and the mixture was dried for 24 h in an oven at 80 °C. This mixture was dosed into a gas burner (methane–oxygen), where it melts at a temperature of up to 2800 °C. The dosing rate of the powder into the flame is also related to the time delay of the powder in the flame, which is at the level of several milliseconds. In this experiment, a dosing rate of –3.5 hPa was used, which corresponds to about 2.3 g/min. The powder melts and forms droplets, which are then cooled out at a high speed in a water bath. The microspheres produced by melt droplets are very porous due to the high temperature of the flame, which also causes the decomposition of some components and the formation of gases in the volume of the microspheres.

Table 1. Composition of the starting mixtures for preparation of microspheres by flame synthesis

Sample	Si_3N_4 [vol. %]	$\text{Ca}_3(\text{PO}_4)_2$ [vol. %]
SNCP7030	70	30
SNCP5050	50	50
SNCP3070	30	70

Table 2. Particle size distribution depending on the composition of the starting mixture

Sample	(30:70)	(50:50)	(70:30)
Q3(x) [%]	size [μm]	size [μm]	size [μm]
10,00	0,62	0,32	0,32
50,00	23,42	6,78	15,42
90,00	124,81	181,53	141,56

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3 Discussion

The microspheres were prepared using flame synthesis conditions, and only their composition was changed, i.e., the ratio of Si_3N_4 and $\text{Ca}_3(\text{PO}_4)_2$ was modified in ratios of 30:70, 50:50, and 70:30. The influence of the change in component ratios was reflected in the structure, size, and biological properties of microspheres.

3.1 Size of Microspheres. Table 2 shows that the size of the microspheres depends on the composition of the incoming powder. It consists of particles smaller than $1\text{ }\mu\text{m}$ and particles with a diameter of several tens of micrometers. The results show that the used powder dosing method is not ideal for this mixture. The solution of this problem could be the granulation of the powder, which would increase the flowability of the powder and prevent the agglomeration of its particles. Into the flame, particles will enter with the same volume. In Table 2, you can see that for many small particles in the 50:50 mixtures, 10% of particles were below $32\text{ }\mu\text{m}$, and most likely it is only inlet powder, which has a particle size of $d_{50} = 0.2\text{ }\mu\text{m}$. On the other hand, the produced microspheres have also large particles (in the mixture (50:50), having 10% of particles above $181\text{ }\mu\text{m}$), which were likely formed by the agglomerate of the incoming powder, which was then pulled down into the flame.

The components whose ratios we have changed in the initial mixture determine the ratio of the melt, which is made of $\text{Ca}_3(\text{PO}_4)_2$, and the porosity forming component Si_3N_4 as you can see on the Figure 1. In the mixture with a higher ratio of

$\text{Ca}_3(\text{PO}_4)_2$, a large amount of melt and microspheres were formed in the flame into a drop and solidified in a water bath in the form of smooth microspheres without visible surface pores. Meanwhile, the increasing amount of Si_3N_4 resulted in the shape to be more irregular, because there is not enough melt for forming microspheres. The required structure, which was almost the spherical shape, while having a high porosity, was obtained at a ratio of 50:50. For a perfect spherical shape, the ratio should be studied further.

3.2 Pore Structure. Pores in the structure of microspheres are very important for their similarity to human bone, for large active surface to join the tissue with bioceramic and for binding the bioactive substance to the surface of the microspheres. The pores in the microspheres ($>100\text{ }\mu\text{m}$) are not only formed by gas, which is produced by the decomposition of the pore-forming component, but also by small balls with smooth surface and spherical shape (due to the surface tension of the forming melt in the solid phase environment), which are incorporated into the structure (Figure 3). These small microspheres ($<30\text{ }\mu\text{m}$) then apparently fell out of the microspheres surface during the cutting of microspheres or during grinding and form pores. We can compare the formation of pores mainly in Figure 2C, where the ratio of Si_3N_4 was higher, in which if it decomposed, pores with only a small amount of melt within the microspheres will formed. Non-spherical pores or pores were absent at all, whereas in Figure 2A, where the quantity of melt was the highest, we can observe the second pore formation process by creating small balls inside the

Figure 1. Surface structure of microspheres prepared from the incoming mixture with different ratios of its components: (A) 30:70, (B) 50:50, and (C) 70:30

Figure 2. Structure of the microsphere volume prepared from the incoming mixture with different ratios of compounds: (A) 30:70, (B) 50:50, and (C) 70:30

Figure 3. Photo of microspheres surface with different magnifications

Table 3. Parameter of porosity

Ratio of substance	Whole volume of pores [cc/g]	Volume of micropores [cc/g] / [%]	Volume of macropores [cc/g] / [%]	Whole porosity [%]
30:70	0,89	0,34 / 38,2	0,55 / 61,8	56,33
50:50	1,146	0,284 / 24,78	0,862 / 75,22	65,45
70:30	1,25	0,38 / 30,4	0,87 / 69,6	63,24

microspheres. These balls usually contain one central large pore and, in some cases, create micropores on the surface. For desiring homogeneous pore distribution, good homogenization components are needed in the starting mixture. In our case, it is a mixture of two different powders (one of these directly decomposes without melt formation), which can cause the separation of two phases even in the volume of the larger agglomerate by this method of dosing mixture into the flame. The result of such phase separation is a microsphere, in which smaller microspheres are trapped, as can be seen in Figure 2.

3.3 Pore Size. The ratio of components also altered the pore size and their volume percent in microspheres. From Table 3, we can say that the highest porosity was reached at a ratio 50:50, where the ratio of the polarizing component and the melt was the closest to the ratio we were looking for. The lowest porosity was in the sample with the 30:70 ratio.

3.4 Phase Composition. RTG analysis confirmed that the Si_3N_4 , $\text{Ca}_{10}(\text{PO}_4)_6\text{O}$, and $\text{Ca}_3(\text{PO}_4)_2$ phases were present in all samples. It also shows that the samples contain a considerable amount of amorphous phase that copies the amount of $\text{Ca}_3(\text{PO}_4)_2$ in the initial mixture.

3.5 Biological Properties

3.5.1 Determination of Viability. The viability was determined at these microspheres. Medium (DMEM) with 10% fetal calf serum (FCS) (Lonza) was added to the material in a ratio of 1 mL of medium to 100 mg of material. The material in the medium was then incubated for 24 h at 37 °C and 5% CO_2 and referred to as the first leach. The same amount of medium was then added again, and the material was incubated for 24 h at 37 °C (second leach). The third 24-h incubation with the medium was referred to as the third leach. MRC5 cells (human lung fibroblasts) were seeded in a 96-well plate with 50,000 cells in one well and incubated for 24 h at 37 °C and 5% CO_2 . After 24 h, 100 μL of leach was added to the cells, and the cells were incubated for 24 h at 37 °C and 5% CO_2 . After 24 h of incubation with the material, we did test of viability like the protocol of distributor (CellTiter-Blue cell viability assay, Promega). The CellTiter-Blue reagent was added to the medium and cells, and it was incubated for 3 h at 37 °C. After 3 h, the fluorescence was measured at A590 on a Synergy Multi-mode reader (BioTek). The background values were subtracted from the measured values, and all samples were related to the negative control. The graph shows the percentage of cell viability. The results were analyzed by a two-sample *T*-test (Student's *T* test) with a *P*-value < 0.05 considered to be significant.

3.5.2 Determination of Bioactivity. The bioactivity of the samples was determined by the procedure detailed written in the work [6]. Samples were placed in the prepared simulated body fluid (SBF) and left at rest for 4 weeks in a constant temperature incubator (36.5 °C). Bioactivity should be manifested by the formation of HA on the surface of the samples and thus by lowering the concentration of Ca^{2+} and PO_4^{3-} ions in the SBF solution after luffing. The concentration of Ca^{2+} and PO_4^{3-} ions is therefore determined by the inductively coupled plasma–optical emission spectrometry (5100 SVDV ICP–OES, Agilent)-induced plasma emission spectroscopy method [6].

3.5.3 Results of Biological Properties. The cell viability results are shown in Figures 4 and 5 and we can see that the studied material is biotoxic. Figure 4 shows the cell viability results after the addition of the first leachate (FCS) from each sample to the MRC5 cells. The high viability in the case of a 50:50 mixture after the first leaching (87.9%) cannot be explained now; however, because the cell viability in all the following extracts in all three samples was below 70%, we consider the prepared material to be unsuitable from the viewpoint of biotoxicity.

The bioactivity test was performed according to ref. 6. SEM analysis did not confirm the formation of hydroxyapatite on the surface of microspheres. At the same time, the smooth surface of the microsphere (30:70, high ratio of melt) changed after the SBF test. The damaged surface shows signs of corrosion and after the bioactivity test changes into a broken surface with a high percentage of microcracks (see Figure 6).

The change of the concentration of Ca^{2+} and PO_4^{3-} ions in the SBF solution after leaching is shown in Figure 6. The increased concentration of Ca^{2+} is inconsistent with the expected one. Comparing this Ca^{2+} concentration to Ca^{2+} concentration in the original SBF, we can say that calcium phosphate phases ($\text{Ca}_3(\text{PO}_4)_2$ and $\text{Ca}_{10}(\text{PO}_4)_6\text{O}$) were dissolved in the SBF

Figure 4. Viability of cells MRC5 in the first leach of samples

Figure 5. Viability of cells MRC5 in the third leach of samples

Figure 6. SEM analysis of microspheres (30:70) surface after the bioactivity test

solution during the dissolution test. The concentration of dissolved Ca^{2+} ions in the leachates increases, according to the amount of $\text{Ca}_3(\text{PO}_4)_2$ in the starting mixture. Conversely, PO_4^{3-} concentrations in leaches decreased radically (compared to original concentration in the SBF solution) in all samples, approximately at the same level, regardless of the amount of $\text{Ca}_3(\text{PO}_4)_2$ in the starting mixture.

ICP-OES analysis justifies new fiber structures after the SBF test. The largest fiber formation was observed in the sample, where is the ratio of components is 70:30, and in a lower degree at sample with a ratio of 30:70. For microspheres with a ratio of 50:50, no formation of these fibers was observed. Analysis of the chemical composition of the resulting fibers in microspheres (70:30) is shown in Figure 7. There is a high

Figure 7. Change of Ca^{2+} a PO_4^{2-} concentration in the SBF solution after leaching

element	At. %			
	1	2	3	4
P	74	86	83	65
S	19,6	5,0	7,6	3,1
Cs	3,12	8,23	8,5	3,2
Si	1,8	0	0	28,3

Figure 9. The elemental analyses in the point 3 from Figure 8

proportion of phosphorus, which is consistent with the low concentration of PO_4^{3-} ions in the SBF solution after luvation (Figure 6). It is highly probable that it is a phosphorus and sulfur-rich phase that comes from the SBF solution. In the elemental analysis, Cs was found, in which we can explain the biotoxicity of the samples (Figures 8 and 9) [6].

4 Conclusion

The results showed that the ratio of $\text{Si}_3\text{N}_4\text{--Ca}_3(\text{PO}_4)_2$ in the initial mixture has a significant effect on the resulting structure of microspheres prepared by flame synthesis. This ratio affects the size and shape of the microspheres and their porosity. A higher ratio of $\text{Ca}_3(\text{PO}_4)_2$ (ratio of 30:70) in the mixture causes the formation of smooth, spherical particles with a lower porosity due to the small porosity component. The mixture with a high amount of $\text{Ca}_3(\text{PO}_4)_2$ formed non-spherical particles, and the porosity is also lower due to insufficient amount of melt. The ideal ratio is 50:50, when the formed particles are not perfectly spherical, but combine spherical shape and high porosity. Homogeneous structures will be achieved by sufficient homogenization of the incoming mixture, which can be achieved by granulation of the incoming powder. The results suggest that the decomposition of Si_3N_4 is not the only cause of pore formation. The presence of Si_3N_4 decomposition–oxidation products (Si , $\text{Si}_2\text{N}_2\text{O}$, and SiO_2) has not been confirmed, and there is still an open question as to whether decomposition–oxidation of Si_3N_4 really occurs. The prepared material is biotoxic due to the presence of cesium. The origin of cesium is not known yet. If the origin of cesium contamination is eliminated, there is real assumption that the material will have sufficient viability.

Figure 8. The formation of new fiber structures after a test in SBF of microspheres with a ratio of incoming powder of 70:30 (high amount of Si_3N_4)

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