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PRODUCING ANTIBACTERIAL SILVER-DOPED HYDROXYAPATITE POWDERS WITH CHEMICAL PRECIPITATION AND RESHAPING IN A SPRAY DRYER

IZDELAVA S SREBROM DOPIRANEGA PROTIBAKTERIJSKEGA PRAHU HIDROKSIAPATITA S KEMIJSKIM IZLOČANJEM IN PREOBLIKOVANJEM V RAZPRŠILNEM SUŠILNIKU

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Hydroxyapatite is used for fixing orthopedic prostheses and it has reached a significant level of clinical application. It is important to prevent the initial bacterial colonization of the existing colonies. Silver has been known to exhibit a strong cytotoxicity towards a broad range of microorganisms. In this study, the aim was to produce antibacterial silver-doped hydroxyapatite powders and to reshape them in a spray dryer. Mole fraction of silver 1 % was added to the hydroxyapatite structure with the ion-exchange technique. *E. coli* bacteria were used for investigating the powder antibacterial specimens. Different temperatures and a pressure of 1.5 bar were used for shaping them in a spray dryer. Scanning electron microscopy (SEM), X-ray diffraction (XRD), Energy-dispersive X-ray spectroscopy (EDX) and a bacterial test were used to characterize the powder specimens.

Keywords: hydroxyapatite, spray dryer, silver-doped hydroxyapatite, antibacterial HAP

Hidroksiapatit se uporablja za pritrditev ortopedskih protez in se množično uporablja na klinikah. Pomembno je preprečiti začetno bakterijsko kolonizacijo obstoječih kolonij. Za srebro je poznano, da izkazuje močno citotoksičnost v širokem spektru mikroorganizmov. Namen te študije je bila izdelava protibakterijskega, s srebrom dopiranega prahu iz hidroksiapatita in njegovo preoblikovanje v pršilnem sušilniku. Molski delež srebra 1 % je bil dodan v strukturo hidroksiapatita z metodo izmenjave ionov. Za preiskavo protibakterijskega prahu je bila uporabljena bakterija *E. coli*. Za preoblikovanje v pršilnem sušilniku so bile uporabljene različne temperature in tlak 1,5 bar. Za karakterizacijo vzorcev prahu so bile uporabljene: vrstična elektronska mikroskopija (SEM), rentgenska difrakcija (XRD), energijsko disperzijska rentgenska spektroskopija (EDX) in bakterijski preizkus.

Ključne besede: hidroksiapatit, razpršilni sušilnik, s srebrom dopiran hidroksiapatit, protiobakterijski sintetični hidroksiapatit

1 INTRODUCTION

Inorganic biomaterials based on calcium orthophosphate have a wide range of applications in medicine. Among them, synthetic hydroxyapatite (HAP, $Ca_{10}(PO_4)_6(OH)_2$) is the most promising one because of its biocompatibility, bioactivity and osteoconductivity. Hydroxyapatite has been used to fill a variety of bone defects in orthopedic and maxillofacial surgeries and in dentistry.[1] Hydroxyapatite [HAP, $Ca_{10}(PO_4)_6(OH)_2$] is the main mineral constituent of a human bone.[2] This material does not possess acceptable mechanical properties to be used as a bulk biomaterial; however, it does demonstrate a significant potential to be used as a coating on metallic orthopedic and dental prostheses.[3]

Post-surgical infections associated with the presence of implant materials are found with up to 5 % of patients; the problem usually requires their removal. Microorganism adhesions on implant surfaces represent the initial crucial reason for an infection and lead to the formation of a biofilm, whose microorganisms are more resistant to antimicrobial agents. To solve the problem of contaminating the hydroxyapatite, it has been proposed to use antimicrobial agents such as antibiotics, fluorine and biocide metal ions. The main concerns with antibiotics are the development of resistant microorganisms and the fact that the adsorbed antibiotics are quickly washed out by the body fluids so that they cannot prevent post-surgical infections in the long term. Metal ions (Ag^+, Cu^{2+} and Zn^{2+}) are widely used in medicine as antimicrobial agents. Silver ions, in particular, show an oligodynamic effect with a minimum development of the microorganism resistance.[4–6]

Compared with the other heavy-metal ions, silver has demonstrated a high antimicrobial activity while maintaining a relatively low cytotoxicity.[6–8] Silver ions are highly active ions that are strongly bound to the electron donor groups containing sulphur, oxygen or nitrogen. In the case of the biological molecule components such as thio, amino, imidazole, carboxylate and phosphate, the groups contain these electron donors.[7] Studies have shown that Ag^+ ions are able to penetrate a bacterial cell wall and cause DNA to transform to a condensed form that reacts with the thiol-group proteins resulting in a cell death. It was also found that silver ions are able to interfere with the replication process. It has been demon-

strated that the higher the level of silver incorporated into a material, the better is the antimicrobial effect, but it comes at the cost of an increased cytotoxicity.[8] Therefore, it is a good idea to incorporate a secondary chemical to alleviate the potential negative effects, while maintaining optimum antimicrobial properties of Ag.[7] Silver-doped ceramics have a high chemical durability and antibacterial activity.[3] Several *in vitro* studies reported that the silver ions in the HAP coatings play an important role in preventing or minimizing the initial bacterial adhesion.[7]

Spray drying can be defined as a transformation of a material from the fluid state into a dried particulate form by spraying the feed into a hot-drying gas medium.[9] The uniformity of the shape and the diameter of the particles is the main advantage of spray drying.[10] The aims of spray drying are the granulation of the solid phase in spherical, monodispersed, high-density aggregates and a quick, effective recovery of the production.[11]

In this study, we have produced antibacterial silver-doped hydroxyapatite powders and reshaped them in a spray dryer. 1 % silver was added to the hydroxyapatite structure with the ion-exchange technique. We have investigated the effect of adding silver to a hydroxyapatite powder.

2 EXPERIMENTAL PROCEDURE

In this study, the chemical-precipitation method was chosen for producing silver-doped hydroxyapatite. Calcium nitrate tetra hydrate ($Ca(NO_3)_{2.4}H_2O$, extra pure, Merck), orthophosphoric acid (H_3PO_4, 85 %, Merck), ammonium hydroxide (NH_4OH, 28–30 %, Merck) and silver nitrate ($AgNO_3$, crystalline, 99.9+ %, Alfa Aesar) were used as raw materials. Calcium nitrate, orthophosphoric acid and silver nitrate were dissolved separately in deionised water. Dissolved silver nitrate was added into the calcium nitrate solution. Finally, orthophosphoric acid was added. Ammonium hydroxide was added to fix the pH to 10–11. The pH affected the hydroxyapatite crystallinity.[12] The solution was mixed for 24 hours. After the precipitation, hydroxyapatite was washed with deionised water when filtered from the solution, then it was dried in a furnace at the temperature of 105 °C. The powders were sintered at the temperature of 1050 °C for 1 h under the 10 °C/min sintering regime. Dried powders were prepared for drying with deionised water. The inlet temperatures of 175 °C, 190 °C and 205 °C and a pressure of 1.5 bar were used for drying. 1 % silver was added to the hydroxyapatite structure. The plate-count test and *E. coli* colonies were used for the antibacterial activity.

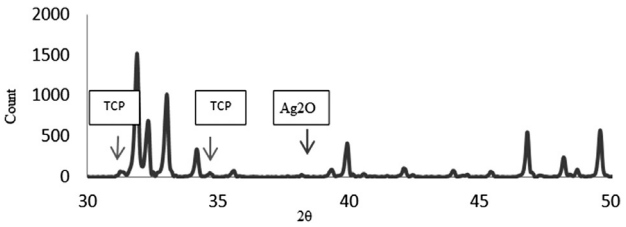

Figure 1: XRD peaks after sintering (TCP: tricalcium phosphate, Ag_2O: silver oxide)

Slika 1: XRD-vrhovi po sintranju (TCP: trikalcij fosfat, Ag_2O: srebrov oksid)

3 RESULT AND DISCUSSION

3.1 XRD results

It is seen in **Figure 1** that tricalcium phosphate and silver oxide were in the powder structure. Hydroxyapatite decomposes when heated to form β-TCP.[11] The

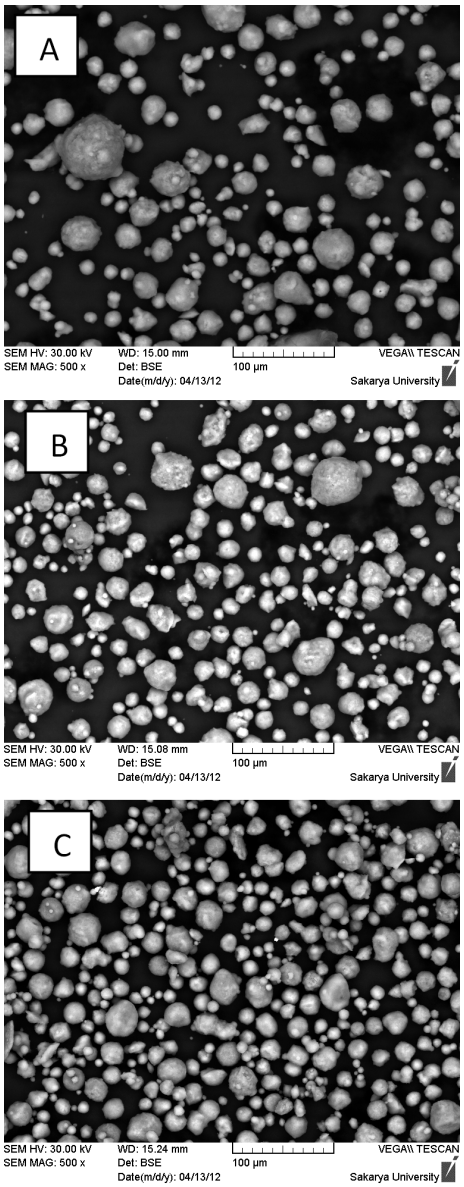

Figure 2: SEM images of the powders after spray drying at the inlet temperatures: a) 175 °C, b) 190 °C, c) 205 °C

Slika 2: SEM-posnetki prahov po razpršilnem sušenju pri vstopni temperaturi: a) 175 °C, b) 190 °C, c) 205 °C

secondary phases such as α-tricalcium phosphate and β-tricalcium phosphate were also found in the coatings to a small extent. The phases that are undesired such as tetra-tricalcium phosphate (TTCP) and calcium oxide (CaO) were absent. In the samples that contained Ag_2O, a characteristic Ag_2O (101) peak was observed as well as the peak shifts, indicating an effective incorporation of Ag_2O into the apatite structure.[13,14]

Metallic silver precipitates silver oxide according to the potential-pH diagram in an aqueous solution.[15] All the other peaks are hydroxyapatite peaks.

3.2 SEM results

However, the shapes of the powders are not totally spherical, though they are close to a spherical shape. The shape is an important phenomenon for the flowability and apparent density. When the inlet temperature was 205 °C, all the products were dried with the heat energy. At the other temperatures (175 °C and 190 °C), the problem was that the powders tended to stick together because of the moisture. It is seen in **Figure 2** that the temperature is an important factor affecting the particle size. Using high heat energy, particles were dried very quickly. Hence, the particle size decreased.

3.3 Particle-size-analysis results

It is seen in **Figure 3** that the average particle size was 37 µm for A and 28 µm for B. The particle size decreased with the increasing temperature. High energy provided quick drying and small particles were produced. Powder diameters after spray drying were recorded in **Table 1**.

Table 1: Mean diameters after spray drying (analyzed with a Microtrac S3500)

Tabela 1: Srednji premer zrn po razpršilnem sušenju (analizirano z Microtrac S3500)

Mean diameters	190 °C inlet temperature	205 °C inlet temperature
d10	19.89 µm	16.91 µm
d50	37.08 µm	28.52 µm
d90	67.13 µm	65.40 µm

It is clear from **Table 1** that the higher temperature led to a smaller particle size for all the mean diameters (d10, d50, d90).

3.4 Antibacterial-test results

Figure 4a shows that there are 63+ bacterial colonies in the medium and there are no bacterial colonies in the medium in **Figure 4b**. The hydroxyapatite powders with a 1 % silver addition show a 100 % antibacterial activity. Silver inhibited the growth of bacterial colonies. On the other hand, the amount of silver is important with respect to biocompatibility as an increased amount of silver

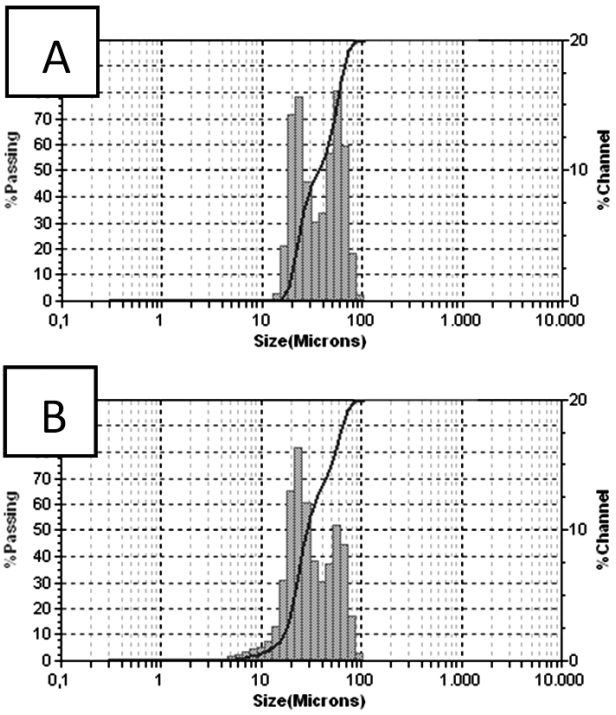

Figure 3: Analysis of the particle-size distribution after spray drying (inlet temperatures: a) 190 °C, b) 205 °C)

Slika 3: Šušenje po razprševanju. Analiza razporeditve velikosti zrn (vstopna temperatura: a) 190 °C, b) 205 °C).

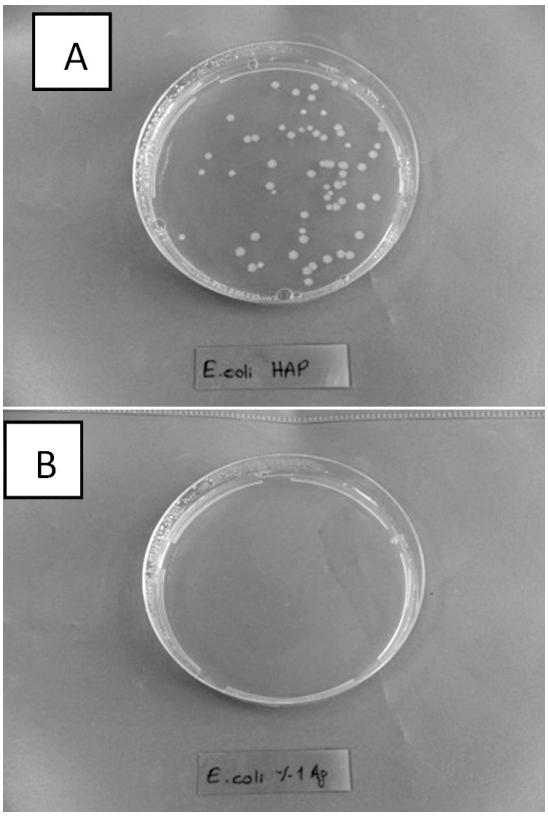

Figure 4: After the antibacterial test: a) no additive to hydroxyapatite, b) 1 % mol fraction Ag added to hydroxyapatite

Slika 4: Po protibakterijskem preizkusu: a) hidroksiapatit brez dodatkov, b) hidroksiapatit z dodanim molskim deležem 1 % Ag

causes a decrease in biocompatibility. A high content of silver leads to a poor biocompatibility because the alkaline phosphatase activity of osteoblasts increases.[16,17]

It was reported that the toxicity of Ag ions affected the basic metabolic cellular functions common to all the specialized mammalian cells. A concentration- and time-dependent depletion of the intracellular ATP content was attributed to the presence of Ag ions, thereby compromising the cell energy charge that precedes the cell death. Therefore, it is prudent to incorporate a minimum amount of Ag on the implant surfaces to adequately reduce the bacterial adhesion as well as minimizing the tissue cytotoxicity.[18]

4 CONCLUSION

HA coating materials have been well studied as osteogenic enhancing materials and are effectively used in several medical and dental applications. In this study, the silver-doped hydroxyapatite was produced and reshaped in a spray dryer. The shaping is very important in the coating application because the shape affects the fluidity.

The hydroxyapatite structure decomposed into hydroxyapatite and tricalcium phosphate phases at a high temperature. It was seen that the hydroxyapatite powders including silver show a 100 % antibacterial effect. So, we were able to effectively enhance the coating with sustainable, long-term antimicrobial properties, while minimizing the negative cytoplasmic effects on the osteoblast cells.

5 REFERENCES

[1] H. F. Lin, L. Chun-Jen, C. Ko-Shao, Thermal reconstruction behavior of the quenched hydroxyapatite powder during reheating in air, Materials Science and Engineering: C, 13 (**2000**) 1–2, 97–104

[2] C. F. Koch, S. Johnson, D. Kumar, Pulsed laser deposition of hydroxyapatite thin films, Materials Science and Engineering: C, 27 (**2007**) 3, 484–494

[3] S. Johnson, M. Haluska, R. J. Narayan, In situ annealing of hydroxyapatite thin films, Materials Science and Engineering: C, 26 (**2006**) 8, 1312–1316

[4] V. Stanić, D. Janaćković, S. Dimitrijević, S. B. Tanasković, M. Mitrić, M. S. Pavlović, A. Krstić, D. Jovanović, S. Raičević, Synthesis of Antimicrobial Monophase Silver-Doped Hydroxyapatite Nanopowders for Bone Tissue Engineering, Applied Surface Science, 257 (**2011**) 9, 4510–4518

[5] L. Harris, R. Richards, Staphylococci and implant surfaces: a review, Injury, 37 (**2006**), S3–S14

[6] A. Montali, Antibacterial coating systems, Injury, 37 (**2006**), S81–S86

[7] G. A. Fielding, M. R. Bandyopadhyay, A. S. Bose, Antibacterial and biological characteristics of silver containing and strontium doped plasma sprayed hydroxyapatite coatings, Acta Biomaterialia, 8 (**2012**) 8, 3144–3152

[8] K. Gross, Hydroxyapatite – Synthesis of Hydroxyapatite Powders, Journal of Biomedical Materials Research, 62 (**2002**) 2, 228–236

[9] V. Stanic, D. Janackovic, S. Dimitrijevic, Synthesis of antimicrobial monophase silver-doped hydroxyapatite nanopowders for bone tissue engineering, Applied Surface Science, 257 (**2011**) 9, 4510–4518

[10] H. Jeon, S. Yi, S. Oh, Preparation and antibacterial effects of Ag-SiO_2 thin films by sol-gel method, Journal of Materials Science, 42 (**2007**) 24, 10085–10089

[11] A. L. R. Rattes, W. P. Oliveira, Spray drying conditions and encapsulating composition effects on formation and properties of sodium diclofenac microparticles, Powder Technology, 171 (**2007**) 1, 7–14

[12] J. M. Gac, L. Gradon, A distributed parameter model for the spray drying of multicomponent droplets with a crust formation, Advanced Powder Technology, 24 (**2013**) 1, 324–330

[13] M. Serantoni, P. Piancastelli, A. L. Costa, L. Esposito, Improvements in the production of Yb:YAG transparent ceramic materials: Spray drying optimisation, Optical Materials, 34 (**2012**) 6, 995–1001

[14] A. K. Nayak, Hydroxyapatite Synthesis Methodologies: An Overview, International Journal of ChemTech Research, 2 (**2010**) 2, 903–907

[15] V. V. Skorokhod, S. M. Solonin, V. A. Dubok, L. L. Kolomiets, Decomposition Activation of Hydroxyapatite in Contact with β-Tricalcium Phosphate, Powder Metallurgy and Metal Ceramics, 49 (**2010**), 324–329

[16] M. Diaz, F. Barba, M. Miranda, F. Guitian, R. Torrecillas, J. S. Moya, Synthesis and Antimicrobial Activity of a Silver-Hydroxyapatite Nanocomposite, Journal of Nanomaterials, (**2009**), doi: 10.1155/2009/498505

[17] J. Qu, X. Lu, D. Li, Silver/hydroxyapatite composite coatings on porous titanium surfaces by sol-gel method, Journal of Biomedical Materials Research Part B: Applied Biomaterials, 97B (**2011**) 1, 4048

[18] W. Chen, Y. Liu, H. S. Courtney, M. Bettenga, C. M. Agrawal, In vitro anti-bacterial and biological properties of magnetron co-sputtered silver-containing hydroxyapatite coating, Biomaterials, 27 (**2006**) 32, 5512–5517