


Fabrication of silver/beta-tricalcium phosphate particle by a simple liquid chemical reduction method

Chao Guo , Juan Xue, Ying Tan, Yinsheng Dong

School of Materials Science and Engineering and Jiangsu Key Laboratory of Advanced Metallic Materials, Southeast University, Nanjing 211189, People's Republic of China

✉ E-mail: 101010759@seu.edu.cn

Published in Micro & Nano Letters; Received on 22nd January 2016; Revised on 29th April 2016; Accepted on 29th April 2016

Using beta-tricalcium phosphate (beta-TCP), silver nitrate and glucose as the carrier, silver resource and reducing agent, silver/beta-TCP particle was successfully fabricated by a novel liquid chemical reduction method to overcome the infection problem of beta-TCP. The X-ray diffraction pattern of the as-obtained sample confirmed the existence of metallic silver in the particle. The addition of silver nanoparticle affected the thermal stability of beta-TCP and prevented the transformation from beta-TCP to alpha-TCP. Transmission electron microscopy images and the corresponding calculation result proved the mean size and size distribution of silver nanoparticles in the silver/beta-TCP particle could be controlled by varying the molar ratio between beta-TCP and silver nitrate. Moreover, the mean size and size distribution of silver nanoparticles also could be changed by using different stabilisers. The prepared silver/beta-TCP particle is a potential biomaterial to replace pure beta-TCP particle and utilise for coating material and other applications.

1. Introduction: Owing to the excellent biocompatibility, biodegradability, biosafety and osteoinductivity, beta-tricalcium phosphate (beta-TCP) is widely utilised as biomaterial, including bone implant, tooth implant and coating material [1–4]. However, the infection problem with the implantation limits its further application. Therefore, many antibacterial metallic elements such as silver, zinc and copper have been doped into beta-TCP to enhance its antibacterial activity and overcome bacterial infection [5, 6].

Depositing antibacterial metallic nanoparticle on the surface of beta-TCP is an alternative method, which can add more antibacterial material and get stronger antibacterial ability compared with the doping method. Loher *et al.* [7] fabricated the silver/beta-TCP nanoparticle by a flame-spray synthesis method using silver carboxylate, calcium carboxylate and tributyl phosphate as silver, calcium and phosphor precursor, respectively. Gerber *et al.* [8] gained the silver/beta-TCP nanoparticle by the same method, but used silver acetate and calcium hydroxide as silver precursor and calcium precursor. This method can get quite small silver nanoparticles (1–2 nm), but need a flame-spray equipment. Bergmann *et al.* [9] prepared the silver/beta-TCP nanoparticle by an adsorption process while Liu *et al.* [10] mixed silver nanoparticle with beta-TCP by a high-temperature melting method. These two methods need prepared silver nanoparticles and may yield the aggregation of silver nanoparticles again. Therefore, it is necessary to propose other silver/beta-TCP fabrication method.

In this Letter, silver/beta-TCP particle is fabricated by a simple liquid chemical reduction method. Compared with those reported methods, this novel method has its significant advantages. On the one hand, silver/beta-TCP is gained by a very simple liquid chemical reduction reaction and no special equipment such as flame-spray equipment is needed in the preparation. On the other hand, silver nanoparticles are fabricated on the surface of beta-TCP particle during the chemical reduction reaction and separate from each other. Moreover, silver nanoparticles are gained by nucleating and growing on the surface of beta-TCP particle in this method, therefore, the properties of prepared silver nanoparticles such as their mean size and size distribution can be controlled by adjusting the quantity of beta-TCP particle. Except the fabrication method of silver/beta-TCP particle, the thermal stability of the particle and some factors affecting the size and distribution of silver

nanoparticles are also investigated. In addition, a potential application of silver/beta-TCP particle is proposed in this Letter.

2. Material and methods: Calcium hydroxide was purchased from Shanghai LingFeng Chemical Reagent Co., Ltd., China. Phosphoric acid was purchased from Shanghai Chemical Reagent Co., Ltd., China. Silver nitrate, soluble starch, polyvinyl pyrrolidone (PVP) and glucose were purchased from Sinopharm Chemical Reagent Co., Ltd., China. Polyethylene glycol (PEG) was purchased from Xilong Chemical Industry Incorporated Co., Ltd., China. Ammonia water was purchased from Shanghai Zhongshi Chemical Corporation, China. All the reagents were of analytical grade.

Beta-TCP particle was obtained by a precipitation method similar to our preparation method of hydroxyapatite (HA) [11]. First, calcium hydroxide solution was dropwise added into phosphoric acid solution to generate the neutralisation reaction. Then, the reaction solution was separated by centrifugation. The precipitation was carried out and ball-milled for 3 h. Finally, the ball-milled powder was dried in the oven and sintered at 900°C to form beta-TCP particle.

Silver/beta-TCP particle was fabricated by the liquid chemical reduction method. First, a silver ammonia solution was obtained by adding a suitable amount of ammonia water into the 0.05 mol/L silver nitrate solution. 3 wt% PVP solution and beta-TCP particle were sequentially added into the as-obtained silver ammonia solution under constant stirring at 60°C. Then, 10 wt% glucose solution was added to deposit silver nanoparticles on the surface of beta-TCP. Finally, silver/beta-TCP particle was gained after separating, washing with distilled water and drying at room temperature. Moreover, PVP was replaced by soluble starch or PEG to discover the effect of stabiliser type.

Phase composition of silver/beta-TCP particle was confirmed by X-ray diffraction (XRD, SHIMADZU XD-3A). The morphology and elemental analysis of silver/beta-TCP particle were gained by transmission electron microscopy (TEM, JEOL JEM2000EX) and the attached energy-dispersive spectrometer. The mean size and size distribution of silver nanoparticles were calculated with TEM images using Nano Measure software.

3. Results and discussions: Figs. 1a–d show typical XRD patterns of three silver/beta-TCP particles and pure beta-TCP particle. It can

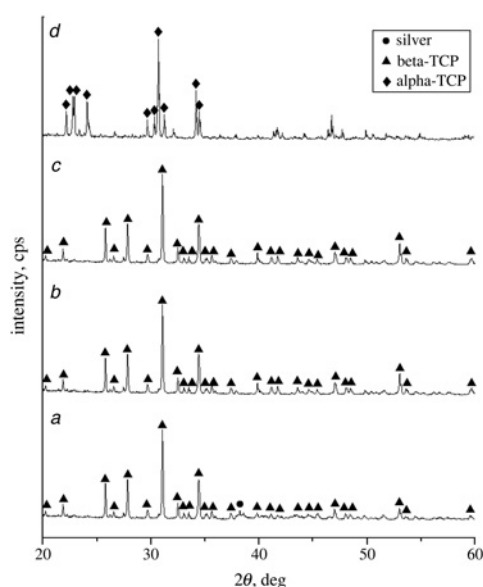


Fig. 1 Typical XRD patterns of three silver/beta-TCP particles (a, b, c) and pure beta-TCP particle (d)

a Unsintered silver/beta-TCP particle

b Silver/beta-TCP particle after being sintered at 1200°C for 2 h

c Silver/beta-TCP particle after being sintered at 1350°C for 2 h

d Pure beta-TCP particle after being sintered at 1350°C for 2 h

be found that the unsintered silver/beta-TCP particle is composed of metallic silver and beta-TCP, indicating that the silver nanoparticle has been successfully deposited on the surface of beta-TCP particle by our liquid chemical reduction method. The result that beta-TCP particles' colour changed from white to brown also suggests the formation of silver nanoparticle. Meanwhile, no other phase appears in the pattern, proving that silver nitrate was completely reduced to metallic silver and no phase transformation or decompose of beta-TCP happened during the generation of silver nanoparticle. However, silver phase disappears while beta-TCP exists in the XRD patterns when the silver/beta-TCP particle was sintered at 1200°C or at 1350°C for 2 h. In contrast, pure beta-TCP transformed to alpha-TCP after being sintered at 1350°C for 2 h as expected, because the normal phase transformation temperature is about 1150°C. The above result demonstrates that the addition of silver changes the thermal stability of beta-TCP and prevents the phase transformation from beta-TCP to alpha-TCP. This result is consistent with the results of Bergmann *et al.* [9] and Gokcekaya *et al.* [12]. In their research, only beta-TCP phase can be discovered in the XRD pattern after being

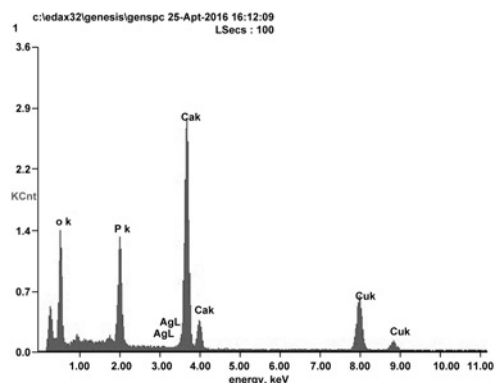


Fig. 2 EDX analysis result of silver/beta-TCP particle after being sintered at 1200°C for 2 h

sintered at 1200°C. Bergmann *et al.* [9] also confirmed the existence of silver by inductive coupled plasma atomic emission spectroscopy (ICP-AES). They assumed silver reacted with beta-TCP to form $\text{AgCa}_{10}(\text{PO}_4)_7$ and could not be discovered in the XRD pattern because of the similar XRD peak diagrams between $\text{AgCa}_{10}(\text{PO}_4)_7$ and beta-TCP. Gokcekaya *et al.* [12]

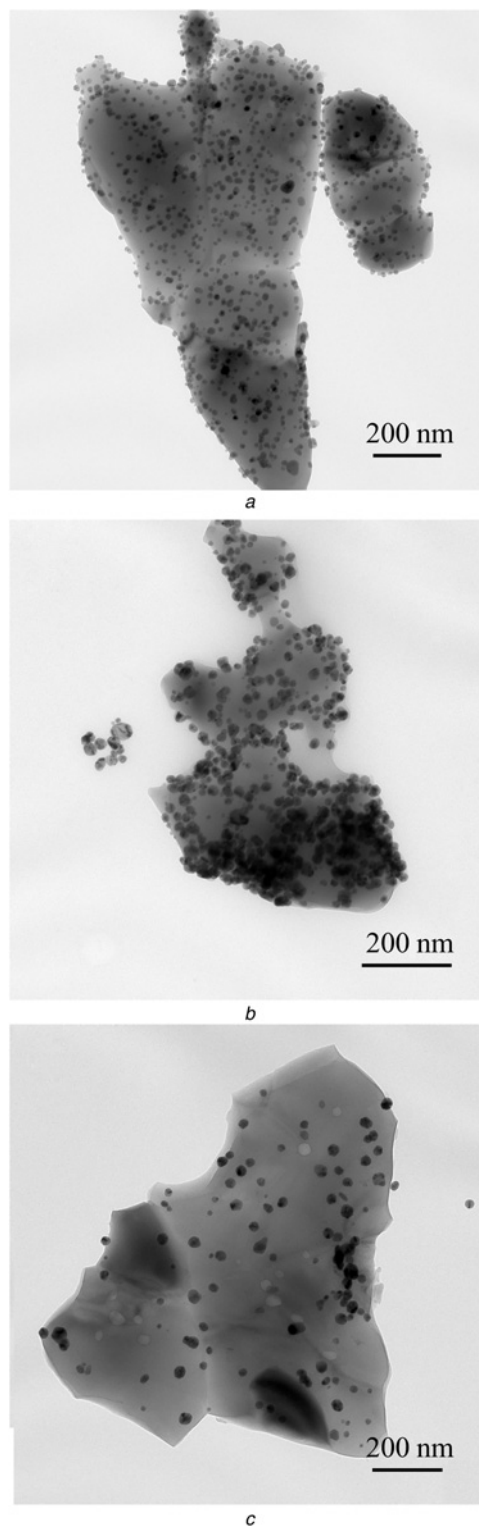


Fig. 3 Typical TEM images of silver/beta-TCP particles with a molar ratio between beta-TCP and silver nitrate of

a Ten using PVP as stabiliser

b Four using PVP as stabiliser

c Ten using PEG as stabiliser

Table 1 Mean size and size distribution of silver nanoparticles in the silver/beta-TCP particles

Molar ratio between beta-TCP and silver nitrate	Stabiliser	Mean size of silver nanoparticles, nm	Size distribution of silver nanoparticles, nm
15:1	PVP	7	3–12
10:1	PVP	13	4–20
10:1	soluble starch	10	2–18
10:1	PEG	15	9–28
6:1	PVP	19	8–31
4:1	PVP	21	9–31

suggested that the substitution of silver in beta-TCP was the main reason for the change in thermal stability of beta-TCP. In our investigation, the sample changed its colour from brown to yellow after being sintered at 400°C and finally changed back to white after being sintered at 1200°C. The result by energy dispersive X-ray (EDX) analysis further indicates the existence of silver element (Fig. 2). According to silver element's weak peak in Fig. 2, we suggest that most silver nanoparticles evaporated in the high temperature (above 1000°C) and resulted in the disappearance of XRD peak and the colour change of the sample; meanwhile, the happen of chemical reaction between silver and beta-TCP such as the formation of $\text{AgCa}_{10}(\text{PO}_4)_7$ and silver-substituted beta-TCP led to the change in thermal stability of beta-TCP. Meanwhile, our research proved that the addition of silver nanoparticle can maintain the thermal stability of beta-TCP at 1350°C, higher than the previous reports [9, 12]. The higher thermal stability of beta-TCP will benefit those potential applications in which silver/beta-TCP particle needs to sinter again, such as tooth implant and bone scaffold of tissue engineering.

Figs. 3a–c illustrate some typical TEM images of silver/beta-TCP particle. It can be seen that most beta-TCP particles are near rod-like and agglomerate with each other; the mean diameter of these particles is about 100–200 nm. In contrast, most silver nanoparticles deposited on the surface of beta-TCP particles are spherical with an average diameter far less than 100 nm and separate from each

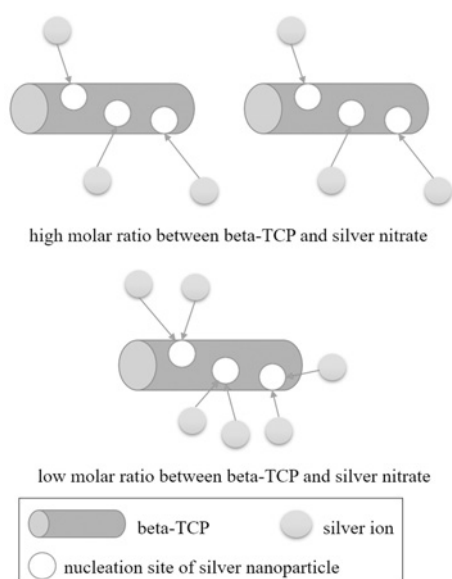


Fig. 4 Schematic diagram of the growths of silver nanoparticles with different molar ratios between beta-TCP and silver nitrate

other. The TEM images demonstrate beta-TCP can be utilised as the carrier of silver nanoparticles and effectively prevent the agglomeration of silver nanoparticles. Moreover, the result suggests that either molar ratio between beta-TCP and silver nitrate or stabiliser type has a significant effect on the mean size and size distribution of the deposited silver nanoparticles.

As given in Table 1, the molar ratio between beta-TCP and silver nitrate influences the mean size of silver nanoparticles in silver/beta-TCP particles. When decreasing the molar ratio from 15:1 to 4:1, the mean size of silver nanoparticles increases from 7 to 21 nm. Meanwhile, the size distribution changes from 3–12 to 9–31 nm. During the liquid chemical reduction process, beta-TCP supplies the surface active sites to facilitate the heterogeneous nucleation of silver nanoparticles. Therefore, the number of nucleated silver nanoparticles is not dependent on the amount of silver nitrate and corresponding silver ions, but dependent on the amount of beta-TCP (Fig. 4). With the molar ratio between beta-TCP and silver nitrate decreasing, nucleated silver continues to grow, generating the increase in the mean size of silver nanoparticles. The above result suggests that the size and distribution of silver nanoparticles can be easily controlled by varying the molar ratio between beta-TCP and silver resource.

From Table 1, it is evident that the stabiliser type also has a strong effect on the mean size and size distribution of silver nanoparticles in silver/beta-TCP particles. Among the three stabilisers, soluble starch has the best stabilising effect while PVP has a significantly stronger stabilising effect than PEG. The special properties of soluble starch such as template function, size selectivity [13] and reducing function [14] may contribute its best stabilising effect. This result is similar to our previous research result of the silver/HA nanoparticle [15] and indicates that different stabilisers can change the mean size and size distribution of silver nanoparticles in the silver/beta-TCP particles.

Fig. 5 gives a potential application of the as-prepared silver/beta-TCP particle. As we all know, poly (lactic acid) (PLA) is a common biomaterial, has been widely used as sutures, orthopaedic fixation devices, drug delivery matrices and porous scaffolds for tissue engineering because of its good biocompatibility and biodegradability. However, some problems often happen during the biodegradation of PLA. As represented in Fig. 5, few mineralisation products can deposit on the surface of PLA matrix and change to HA due to PLA's weak bioactivity; meanwhile, massive oligomers may appear around the PLA matrix in a short degradation time because of the well-known acid autocatalysis effect, especially in the case that the PLA matrix is a large solid without pore. The appearance of oligomers will cause a significant decrease in pH value of the surrounding solution and influence the growth of human cell. In addition, PLA has an ordinary antibacterial property, the matrix may be attacked by bacteria and bring infection. Our as-prepared silver/beta-TCP particle can be utilised as the modified coating of the PLA matrix to overcome the above problems. Compared with

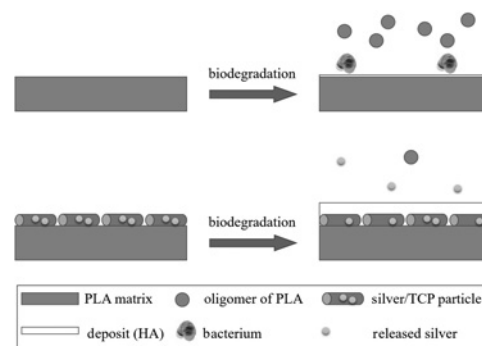


Fig. 5 Schematic diagram of a potential application (the coating of PLA matrix) of the as-prepared silver/beta-TCP particle

the raw material, a thicker HA layer will be deposited on the surface of the PLA matrix because of the strong bioactivity of beta-TCP; fewer oligomers will produce from the PLA matrix in the degradation because the basic products from silver/beta-TCP coating can neutralise the acidity of oligomers. Finally, the release of silver nanoparticles will bring a continuous antibacterial ability and prevent the reproduction of bacteria. The exact effect of silver/beta-TCP coating on PLA matrix will be discussed in our subsequent research.

4. Conclusion: A particle composed of silver nanoparticle and beta-TCP was fabricated by the liquid chemical reduction method using silver nitrate and beta-TCP particle as the silver resource and the carrier. Thermal stability of beta-TCP was changed by adding silver nanoparticle. The mean size of silver nanoparticles could be decreased by decreasing the molar ratio between silver nitrate and beta-TCP. The silver/beta-TCP particle with minimal silver nanoparticle mean size could be fabricated using soluble starch as the stabiliser. Silver/beta-TCP particle can be utilised as a coating material to enhance the surface property of PLA.

5. Acknowledgments: This work was supported by Jiangsu Key Laboratory of Advanced Metallic Materials (BM2007204).

6 References

- [1] Shayegan A., Petein M., Abbeele A.V.: 'The use of beta-tricalcium phosphate, white MTA, white Portland cement and calcium hydroxide for direct pulp capping of primary pig teeth', *Dent. Traumatol.*, 2009, **25**, (4), pp. 413–419
- [2] Tatsukawa E., Gonda Y., Kamitakahara M., *ET AL.*: 'Promotion of normal healing of bone defects under estrogen deficiency by implantation of beta-tricalcium phosphate composed of rod-shaped particles', *J. Orthop. Res.*, 2014, **32**, (2), pp. 189–196
- [3] Chen Z.T., Mao X.L., Tan L.L., *ET AL.*: 'Osteoimmunomodulatory properties of magnesium scaffolds coated with beta-tricalcium phosphate', *Biomaterials*, 2014, **35**, (30), pp. 8553–8565
- [4] Stefanic M., Krnel K., Kosmac T.: 'Novel method for the synthesis of a beta-tricalcium phosphate coating on a zirconia implant', *J. Eur. Ceram. Soc.*, 2013, **33**, (15–16), pp. 3455–3465
- [5] Matsumoto N., Sato K., Yoshida K., *ET AL.*: 'Preparation and characterization of β -tricalcium phosphate co-doped with monovalent and divalent antibacterial metal ions', *Acta. Biomater.*, 2009, **5**, (8), pp. 3157–3464
- [6] Ewald A., Hösel D., Patel S., *ET AL.*: 'Silver-doped calcium phosphate cements with antimicrobial activity', *Acta. Biomater.*, 2011, **7**, (11), pp. 4064–4070
- [7] Loher S., Schneider O.D., Maienfisch T., *ET AL.*: 'Micro-organism-triggered release of silver nanoparticles from biodegradable oxide carriers allows preparation of self-sterilizing polymer surfaces', *Small*, 2008, **4**, (6), pp. 824–832
- [8] Gerber L.C., Mohn D., Fortunato G., *ET AL.*: 'Incorporation of reactive silver-tricalcium phosphate nanoparticles into polyamide 6 allows preparation of self-disinfecting fibers', *Polym. Eng. Sci.*, 2011, **51**, (1), pp. 71–77
- [9] Bergmann C., Schwenke A., Sajti L., *ET AL.*: 'Temperature-dependent morphology changes of noble metal tricalcium phosphate-nanocomposites', *Ceram. Int.*, 2014, **40**, (6), pp. 7931–7939
- [10] Liu S., Fan C., Jin F., *ET AL.*: 'Preparation and antibacterial activities of porous silver-doped β -tricalcium phosphate bioceramics', *Int. J. Appl. Ceram. Technol.*, 2015, **12**, (2), pp. 294–299
- [11] Zhang Y.Y., Dong Y.S.: 'Effect of surfactant on morphology of hydroxyapatite', *React. Inorg. Met.-Org. Nano-Met. Chem.*, 2015, **45**, (3), pp. 411–415
- [12] Gokcekaya O., Ueda K., Narushima T., *ET AL.*: 'Synthesis and characterization of Ag-containing calcium phosphates with various Ca/P ratios', *Mater. Sci. Eng. C*, 2015, **53**, pp. 111–119
- [13] Raveendran P., Fu J., Wallen S.L.: 'Completely 'green' synthesis and stabilization of metal nanoparticles', *J. Am. Chem. Soc.*, 2003, **125**, (46), pp. 13940–13941
- [14] Vigneshwaran N., Nachane R.P., Balasubramanya R.H., *ET AL.*: 'A novel one-pot 'green' synthesis of stable silver nanoparticles using soluble starch', *Carbohydr. Res.*, 2006, **341**, (12), pp. 2012–2018
- [15] Guo C., Shao F.F., Zhang Y.Y., *ET AL.*: 'Preparation of silver/hydroxyapatite nanoparticles using hydroxyapatite nanoparticles with different calcination temperatures', *Micro. Nano. Lett.*, 2012, **7**, (9), pp. 904–906