pH effect on the characteristics of mineralized self-assembled polymeric nanocomposites as controlled drug release carriers

Kyoung Dan SON and Young-Jin KIM[†]

Department of Biomedical Engineering, Catholic University of Daegu, Gyeongsan 712-702, Republic of Korea

The use of polymer/inorganic material composites as controlled drug delivery carriers can be promising in the future for the reason of their excellent mechanical properties, drug loading efficiency and controlled release behavior. To develop a controlled drug release carrier, mineralized self-assembled polymeric nanocomposites (POCA nanocomposites) containing caffeic acid (CA) were prepared simply in the presence of Pluronic F-68 and β -cyclodextrin (β -CD) at different pH. The solution pH value greatly affected the morphological structure of nanocomposites. POCA7, POCA8 and POCA9 exhibited plate-shaped morphology, however, POCA10 showed only the sphere-like structure. The size of nanocomposites significantly decreased with increasing the solution pH value from 238 ± 19 nm (POCA7) to 61 ± 4 nm (POCA10). The amount of CA loaded on the nanocomposites was also considerably affected by the solution pH value. In addition, these nanocomposites exhibited slow, long-term and controlled release rate in DPBS, and the release rate of CA from the nanocomposites could be adjusted by varying the pH value of the release medium, implying that the POCA nanocomposites might be potentially applicable in controlled drug release systems.

©2015 The Ceramic Society of Japan. All rights reserved.

Key-words : Polymeric nanocomposite, Mineralization, Hydroxyapatite crystal, Controlled release, Drug delivery

[Received April 17, 2015; Accepted June 19, 2015]

1. Introduction

Controlled drug delivery systems are one of the most promising applications for human health care.¹⁾ An excellent drug delivery carrier should have biocompatibility, zero premature release and a proper rate of drug release. Therefore, biopolymers including chitosan and sodium alginate have frequently been used as raw materials for the design of drug delivery systems due to their excellent properties such as non-toxicity, biodegradability and environmental sensitivity.²⁾ However, biopolymer-based drug carriers have the problems of weak mechanical properties and burst drug release at the beginning. These problems are owing to the weak interaction between the biopolymers and drugs, and the quick disintegration of the biopolymer carriers during the release process.

Recently, the preparation of polymer/inorganic material nanocomposites as controlled drug delivery carriers has attracted a great deal of attention because it is an effective means to improve the properties of polymer carriers. Among the numerous inorganic materials, hydroxyapatite (HA) has been mainly used for the preparation of nanocomposites as drug carriers for the reason of their excellent biocompatibility and bioactivity.¹⁾⁻⁴⁾ The strong interfacial interactions between polymer and HA via electrostatic interaction and hydrogen bonding could improve the mechanical properties, drug loading efficiency and controlled drug release behavior of carriers. In addition, HA is absorbable in specific cellular environments (endosome/lysosome) as non-toxic ionic species.⁵⁾ While the release of drugs from HA has been proved to be very fast because of the weak interaction between the drugs and HA particles, the combination of polymer and HA seems to be a practicable way to extend the release of drugs.⁶⁾ In particular, the employment of controlled mineralization technology using

self-assembled polymer templates would lead to the successful development of biocompatible nanocarriers for controlled drug release.

Poly(ethylene glycol) (PEG)-poly(propylene glycol) (PPG)-poly(ethylene glycol) (PEG) triblock copolymers, known as Pluronic, are amphiphilic polymers, which have been extensively used in drug delivery systems.⁷⁾ They tend to self-assemble into spherical micelles in aqueous environments with PPO forming the hydrophobic core and PEG forming the hydrophilic corona. The hydrophobic core can load various drug molecules and the biocompatible hydrophilic corona facilitates long circulation of the micelles in the blood stream. Moreover, to enhance micelle stability, binary mixed systems consisting of Pluronic F-127 and β -cyclodextrin (β -CD)-linked copolymers have been also explored.⁸⁾ β -CD is a cyclic oligosaccharide containing seven α -(1,4) linked glucopyranosyl units, which possesses a hollow truncated cone structure composed of a hydrophilic exterior and a hydrophobic internal cavity.^{9),10)} This molecular structure is capable of forming inclusion complexes, so-called guest-host compounds, with aliphatic molecules such as PEG and PPG.⁸⁾ The attractive property of β -CD is not only inclusion complexation with guest molecules but also many hydroxyl groups of the glucose units. These hydroxyl groups can promote the formation of intermolecular interaction between β -CD and hydrophilic molecules.

With their high area to volume ratio, mineralized self-assembled polymeric nanocomposites are expected to be excellent materials for biomedical applications, in which the characteristics of nanocomposites have been influenced by the change of mineralization conditions.^{2),11),12)} The major purpose of this study was to investigate the effect of pH on the characteristics of mineralized self-assembled polymeric nanocomposites (POCA nanocomposites) containing caffeic acid (CA) such as morphology and drug loading efficiency. For this reason, biocompatible POCA nanocomposites were synthesized by wet chemical pre-

[†] Corresponding author: Y.-J. Kim; E-mail: yjkim@cu.ac.kr

[‡] Preface for this article: [DOI http://dx.doi.org/10.2109/jcersj2.123.P9-1](http://dx.doi.org/10.2109/jcersj2.123.P9-1)

cipitation method in the presence of PEG–PPG–PEG triblock copolymer (Pluronic F-68) and β -cyclodextrin (β -CD) as polymer templates at different pH. The prepared nanocomposites were systematically examined by considering their morphologies, compositions, chemical structures, crystalline phases, thermal properties and CA loading capacities. In addition, the cumulative CA release profiles from the nanocomposites were investigated using in vitro release assay.

2. Experimental

2.1 Materials

Poly(ethylene glycol)-*block*-poly(propylene glycol)-*block*-poly(ethylene glycol) (PEG–PPG–PEG triblock copolymer, Pluronic F-68, $M_n = 8,400$), β -cyclodextrin (β -CD), calcium nitrate tetrahydrate [$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$], ammonium phosphate dibasic [$(\text{NH}_4)_2\text{HPO}_4$], caffeic acid (CA), 1 N hydrochloride (HCl), ammonium hydroxide solution (NH_4OH), Dulbecco's phosphate buffered saline (DPBS, CaCl_2 and MgCl_2 free) and were purchased from Sigma–Aldrich Co. and used without further purification. Other reagents and solvents were commercially available and were used as received.

2.2 Synthesis of POCA nanocomposites

A synthesis of CA-loaded POCA nanocomposites is as follows. 30 mL of 0.2 w/v% β -CD solution was first added to 30 mL of 0.2 w/v% Pluronic F-68 solution. To this solution, 2 w/v% CA solution was added dropwise for preparing self-assembled polymer micelle nanotemplate solution containing drug. Then, 10 mL of 0.1 M $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ solution was added dropwise, and pH was adjusted to 7, 8, 9 or 10 by the addition of HCl or NH_4OH . To this solution, a determined amount ($\text{Ca}/\text{P} = 1.67$) of 0.1 M $(\text{NH}_4)_2\text{HPO}_4$ was added dropwise for 1 h. The final concentration of CA in the reaction solutions was 3 w/w% based on the weight of polymers and HA precursors [$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and $(\text{NH}_4)_2\text{HPO}_4$]. The mixture was stirred at 40°C under air to induce the nucleation and growth of HA crystals in the polymer micelle nanotemplates. After 24 h, the resultant POCAs were washed with distilled water several times and freeze dried.

2.3 Characterization of POCA nanocomposites

The morphologies of POCA nanocomposites were observed by a field emission-scanning electronic microscope (FE-SEM, JSM-6335F, JEOL, Japan) and transmission electron microscopy (TEM, H-7600, Hitachi, Japan). The particle size distribution was determined by the dynamic light scattering (DLS) technique using a Zetasizer Nano ZS (Malvern Instruments, UK). UV–visible spectra were recorded on a Hitachi U-2900 spectrophotometer (Japan). Fourier transform infrared (FT-IR) spectra of the samples were obtained with an ALPHA spectrometer (Bruker Optics, USA) in the wavenumber range of 400–4000 cm^{-1} .

X-ray diffraction (XRD) measurements were carried out to characterize the crystalline phase of POCAs with a Panalytical X-ray diffractometer X'Pert Pro (Netherlands) with $\text{Cu K}\alpha$ radiation at 40 kV/30 mA. The diffractograms were scanned in a 2θ range of 20–60° at a rate of 2°/min. From the XRD data, the crystallinity of the POCAs was calculated according to the following equation:¹³⁾

$$\text{Crystallinity (\%)} = [1 - (V_{112/300}/I_{300})] \times 100$$

where $V_{112/300}$ is the intensity of the hollow between (112) and (300) peaks and I_{300} is the intensity of the (300) peak.

Ca/P molar ratio of nanocomposites was analyzed with X-ray fluorescence spectroscopy (XRF, ZSX Primus II, Rigaku, Japan).

The thermal stability of nanocomposites was evaluated by thermogravimetric analysis (TGA, Q500, TA Instruments, USA). The TGA measurements were carried out under nitrogen atmosphere at a heating rate of 5°C/min from 30 to 1,000°C, in which all of the samples were dried in vacuo at 100°C for 48 h prior to the measurement.

2.4 In vitro release of CA from POCA nanocomposites

CA release studies were carried out in a thermostatical shaking incubator (BioShaker MRB-022UP, Taitec Co., Japan). A weighted amount (40 mg) of POCA10 was first immersed into 40 mL of 0.01 M DPBS (pH = 7, 8 or 9) at 37°C. The supernatants were taken from the solution after 10, 30, 60, 90, 120, 180, 300, 540 and 900 min. The amount of released CA was determined by measuring the absorption of the samples at 285 nm using a UV–visible spectrophotometer. The percentage of released CA was then calculated based on the initial weight of CA incorporated in the POCA10. The effect of temperature (20, 37 and 45°C) and NaCl concentration (0.01, 0.1 and 1 M) on the release behavior of CA from the nanocomposites was also investigated in 0.1 M DPBS (pH 7.4).

3. Results and discussion

3.1 Morphology of POCA nanocomposites

Polymer/hydroxyapatite (HA) nanocomposites have been widely used as delivery carriers for various drugs and proteins because of enhanced bioavailability, drug loading capacity and safety of drugs.^{3),14),15)} In addition, even though the release of drugs from the polymer or HA nanoparticles was initially very fast, polymer/HA nanocomposites exhibited prolonged release of drugs.⁶⁾ Therefore, attempts have been focused on the synthesis of nanocomposites showing improved dispersibility and controlled drug release behavior.^{1)–3)} The equilibrium adsorption of drugs and the solution pH value affected the adsorption/desorption (release) behavior of drug molecules.¹⁶⁾

In this study, the caffeic acid (CA)-loaded polymeric nanocomposites (POCA nanocomposites) were prepared by rapid precipitation from water in the presence of self-associated Pluronic F-68 and β -CD at different pH. **Figure 1** shows the morphological structure of nanocomposites. The resulting nanocomposites synthesized at pH values of 7 (POCA7), 8 (POCA 8) and 9 (POCA9) exhibited plate-shaped morphology with a size of several hundred nanometers. The size of nanocomposites significantly decreased with increasing the solution pH value, and the spherical particles were observed at pH 10 (POCA10). This result may be related to the interaction between polymers and Ca^{2+} ions, and the driving force available for the crystal growth in the reaction. The driving force can be given by the associated interfacial/surface free energy change.¹²⁾ β -CD is a polar and water soluble cyclic oligosaccharide with OH side groups, which can be bound by Ca^{2+} ions in alkaline medium. Moreover, Pluronic F-68 was also able to form complexes with Ca^{2+} ions and enhance the homogenous mixing of Ca^{2+} ions due to the ether oxygen groups with rich electrons in its chain.¹⁷⁾ Then, polymer- Ca^{2+} complexes can strongly interact with the surface of PO_4^{3-} ions to nucleate the POCAs. Thus, these interactions between Ca^{2+} and polymers are one of the influence factors on the nucleation and growth of the spherical HA crystals during the mineralization process.¹⁸⁾ At this point, the growth of HA crystals are classified into the two regimes based on the driving force of reaction.¹²⁾ At low pH, the growing interface can move only laterally for layer-by-layer growth because of low driving force

Fig. 1. SEM micrographs of POCA nanocomposites synthesized at different pH: (a) pH 7 (POCA7), (b) pH 8 (POCA8), (c) pH 9 (POCA9) and (d) pH 10 (POCA10).

available for the motion of the growing interface, in which the driving force can be given by the associated volume free energy change. In the meantime, the interface can move by high driving force leading to a three-dimensional continuous growth at high pH.

The nanocomposites were also characterized with a TEM to confirm the morphological structures, which showed the plate-shaped (POCA7, POCA8 and POCA9) and sphere-like (POCA10) structures (**Fig. 2**). This is in good agreement with the result of SEM observation. According to the previous report, initial pH values influenced the morphology of HA particles by changing the direction of crystal growth.¹⁹⁾ The crystal growth units of HA particles are composed of the coordination anions $\text{Ca-P}_6\text{O}_{24}$ on the vertical axis, and the coordination cations OH-Ca_6 on two horizontal axes. The amount of OH^- was little at below pH 9 and thus OH-Ca_6 on horizontal axes formed slowly, while the vertical axis could get enough Ca^{2+} to develop the coordination anions $\text{Ca-P}_6\text{O}_{24}$. As a result, the plate-shaped or rod-like nanocomposites were obtained. However, OH-Ca_6 was formed quickly at pH 10 because of the present of large numbers of OH^- and the surface of POCA nucleus has a positive charge. In addition, since the negatively charged side groups of polymers can provide binding sites for the Ca^{2+} ions present in the solution, the formation of positively charged OH-Ca_6 is accelerated.¹⁸⁾ These positively charged surface sites will combine with negatively charged $\text{Ca-P}_6\text{O}_{24}$ at the horizontal and vertical axes with the same speed initiating the growth of the spherical nanocomposites.

The nanocomposites can offer benefits of appropriate size for accumulation of drugs at the tumor site by enhanced permeability and retention (EPR) effect, stable aqueous dispersion by using surface modification and protection of drugs from environmental degradation.²⁰⁾ Therefore, the suspensions of POCAs were prepared for the determination of particle size. As shown in **Fig. 3**

Fig. 2. TEM micrographs of POCA nanocomposites synthesized at different pH: (a) pH 7 (POCA7), (b) pH 8 (POCA8), (c) pH 9 (POCA9) and (d) pH 10 (POCA10).

and **Table 1**, the pH value considerably affected the average particle size of nanocomposites. The average particle size decreased with increasing the solution pH value from 238 ± 19 nm (POCA7) to 61 ± 4 nm (POCA10) as mentioned in the results of SEM and TEM observations. Moreover, the POCA10 exhibited very narrow size distribution. This means that these nanocomposites are the optimum carriers for delivering drugs because they can easily pass through cell barriers and preferentially

Fig. 3. Particle size distribution of POCA nanocomposites synthesized at different pH.

Fig. 4. FT-IR spectra of (a) commercial HA, (b) POCA7, (c) POCA8, (d) POCA9 and (e) POCA10.

Table 1. Characteristics of Mineralized Self-assembled POCA Nanocomposites Synthesized at Different pH

Sample	Mean diameter (nm) ^{a)}	Caffeic acid loading content (w/w%) ^{b)}	Crystallinity (%)	Ca/P Molar ratio
POCA7	238 ± 19	0	29.6 ± 1.2	1.50 ± 0.03
POCA8	217 ± 17	0	29.8 ± 1.5	1.54 ± 0.01
POCA9	131 ± 7	0.2 ± 0.01	18.4 ± 1.3	1.53 ± 0.02
POCA10	61 ± 4	1.3 ± 0.11	8.4 ± 0.8	1.70 ± 0.02

a) Determined by dynamic light scattering (DLS) technique at 25°C. b) Weight content of CA in POCA nanocomposites, determined by UV–visible spectrophotometer.

accumulate at the tumor sites based on the EPR effects. That is to say, smaller nanocomposites can prolong circulation in the blood stream and permeate cell barriers more rapidly and fenestrate vasculature in cancers.²¹⁾

3.2 Physicochemical properties of POCA nanoparticles

FT-IR analysis was carried out for identifying the functional groups present in the POCA nanocomposites, which in turn provided information about the constitution and phase composition of the products synthesized at different pH. All of the samples exhibited characteristic absorption bands for the vibrational modes of PO_4^{3-} appeared at around 1090, 1022, 961, 593 and 558 cm^{-1} , and the bands at 3336 and 1641 cm^{-1} associated with OH of polymers and absorbed H_2O as shown in Fig. 4.^{14),18),22)} In addition, the POCAs showed absorption bands assigned to OH^- of HA at 3565 and 629 cm^{-1} . Furthermore, in the case of POCA10, new absorption peak was found at 1422 cm^{-1} , which was attributed to the substitution of CO_2^{3-} ions in the place of PO_4^{3-} ions and confirmed the substitution of CO_2^{3-} in apatite structure.¹⁸⁾ These CO_2^{3-} ions were formed by the reaction of CO_2 present in the atmosphere with OH^- ions of reaction medium. These data suggest that the POCAs were mainly composed of HA phase and the chemical structure of products was not changed with pH. However, the characteristic absorption bands ascribed to CA were not observed because of significant overlapping with the absorption bands of polymers and HA, and thus the incorporation of CA was not clearly identified.

To confirm the incorporation of CA into the nanocomposites, the amount of CA loaded on the nanocomposites was determined by UV–visible spectroscopy. As shown in Table 1, even though CA was hardly inserted into the nanocomposites at below pH 9, the CA loading content of POCA10 was $1.3 \pm 0.11\text{ w/w\%}$ based

Fig. 5. Difference UV–visible spectra of POCA nanocomposites synthesized at different pH. Each spectrum was obtained by subtracting the spectrum of nanocomposites synthesized in the absence of CA from that in the presence of CA.

on the weight of nanocomposites and the CA loading efficiency was 43.3%. This is probably due to the limitation on the crystal growth of nanocomposites along the horizontal axes at below pH 9 as mentioned above. A difference in the UV absorption spectra of POCAs synthesized at different pH was also observed using 0.1 w/v% solutions of all samples to verify the introduction of CA into the nanocomposites (Fig. 5). As a result, the POCA10 only revealed the significant increase of specific absorption peak at around 285 nm ascribed to CA moiety.

The crystalline phases of the POCA nanocomposites were investigated by means of XRD (Fig. 6). The XRD patterns of synthesized POCAs showed the peaks attributed to the HA crystalline phase at around 22.6, 25.9, 28.2, 31.8, 32.1, 33.5,

Fig. 6. X-ray diffraction patterns of (a) POCA7, (b) POCA8, (c) POCA9 and (d) POCA10.

34.2, 39.8, 46.7, 49.6 and 53.4°, which reflected characteristic of the (111), (002), (210), (211), (112), (300), (202), (310), (222), (213) and (004) planes.¹⁸⁾ This XRD pattern was matched with the structural data of HA described in the Powder Diffraction File (JCPDS 09-432). However, all the peaks were broad diffraction peaks indicating a poorly crystallized HA phase. The diffraction peaks became slightly wider and less intense with increasing the pH value. This is owing to the complex formation of HA with amorphous polymer. Moreover, the isomorphous substitution of PO_4^{3-} by CO_3^{2-} derived from the absorption of CO_2 in the air during preparation process of the nanocomposites affected the decrease of crystallinity. The formation of submicron crystallites in the powders also caused the reduction of crystallinity and the XRD peaks would be broadened.

The crystallinity of synthesized POCA was calculated from the XRD data, which diminished with the increment of pH value from 29.6 ± 1.2 to 8.4 ± 0.8 as shown in Table 1. This result is related to the augmentation of polymer amount included in the nanocomposites with increasing pH value and the complex formation between HA and amorphous polymer. It was already found that the HA nanoparticles synthesized with β -CD exhibited only a broad diffraction peak and the HA phase was poorly crystallized.¹⁸⁾ In addition, the composition of POCA was determined by XRF. The Ca/P molar ratio of POCA was ranged from 1.50 for POCA7 to 1.70 for POCA10, which was deeply affected by the pH value (Table 1). These results mean that calcium-deficient HA (CDHA) crystals can be formed in the presence of polymer templates at low pH since the accumulation of coordination cations OH-Ca_6 on the horizontal axes is possibly restricted.

Thermal property of the synthesized POCA nanocomposites was investigated. Figure 7 shows the typical TGA curve that the amount of weight loss is plotted against the temperature. All of the samples synthesized in this study exhibited the same pattern of weight loss comprised of three steps. The first step takes place between 30°C and about 150°C. This step mainly is assigned to the evaporation of absorbed water and the second step in temperature range of 200–450°C is maybe imputed to the elimination of crystalline water and the decomposition of polymers and CA.^{18),23)} Additionally, the third step is probably the loss of constitution water of POCA, which can change to oxyapatite $[\text{Ca}_{10}(\text{PO}_4)_6\text{O}]$. The total weight loss at 1,000°C increased from 10.1 to 17.3% by the change of pH value owing to the enhancement of polymers and CA content included in

Fig. 7. TGA curves of (a) POCA7, (b) POCA8, (c) POCA9 and (d) POCA10.

Fig. 8. The cumulative release profiles of CA from POCA10 in different pH of 0.01 M DPBS at 37°C.

the nanocomposites. The decomposition temperatures of β -CD, Pluronic F-68 and CA are about 308, 292 and 241°C, resulting that the heightened weight loss of POCA10 is attributed to the decomposition of polymers and CA. This is a result of the complex formation via electrostatic interaction and hydrogen bonding between polymers and HA and CA.

3.3 CA release behaviors of POCA nanocomposites

The ideal controlled drug delivery system should have a high loading capacity of the drug, and it should release the drug with a reasonable rate to reach the concentration required to show efficacy within a relatively short time, and then, the drug release rate should maintain at an appropriate level for a prolonged period. Given that the physiological pH in the blood stream is 7.4 and the pH value of intracellular lysosome is 4.5,²⁴⁾ CA release experiments from POCA10 were performed in DPBS (pH = 5, 7, 9) at 37°C for different periods of time. In the initial stage the release of CA from POCA10 was fast as shown in Fig. 8, which implied the release of CA molecules present close to the surface of nanocomposites. It was followed by slow and steady release of CA owing to the strong intermolecular interaction between CA molecules, polymer templates and inorganic material. In addition, the release rate of CA notably depended on the pH value of release medium, meaning that the increase of pH value accel-

Fig. 9. The cumulative release profiles of CA from POCA10 in 0.01 M DPBS (pH = 7.4) at different temperature.

Fig. 10. The cumulative release profiles of CA from POCA10 in 0.01 M DPBS (pH = 7.4) containing different concentration of NaCl at 37 °C.

erated the release of CA from the nanocomposites because acidic condition of solution could induce rapid HA dissolution.²⁵⁾ Consequently, this dissolution of HA layers certainly removed the diffusion barrier, thereby enhancing the CA release rate.

We found that the temperature and concentration of NaCl in DPBS also could influence the release rate of CA. High temperature caused increased release rate of CA, which could be explained by improved diffusion coefficient of CA molecules and activated dissolution of HA layers with increasing the temperature (Fig. 9).²⁶⁾ Moreover, cumulative released amount of CA at higher concentration of NaCl slightly enhanced compared to that of low concentration of NaCl, which might be due to the faster fluid exchange between inside and outside of the nanocomposites driven by osmotic pressure gradient under high salt concentration (Fig. 10).¹⁵⁾ These results suggest that the diffusion of CA molecules can be controlled by temperature and salt concentration.

4. Conclusion

The combination of polymer and HA seems to be a practicable way to extend the release of drugs. In addition, an increase in the surface area of nanocomposites can lead to the improvement of drug loading capacity of carriers. In the present study, a novel and simple reaction for the preparation of CA-loaded polymer/HA nanocomposites was successfully developed by the use of self-associated Pluronic F-68 and β -CD as polymer templates. The

pH value of solution greatly affected the morphological structure and CA loading efficiency of products. The nanocomposites synthesized at pH 10 in the presence of polymer templates (POCA10) had sphere-like structure and very narrow size distribution, which exhibited significantly improved CA loading efficiency compared with those of the nanocomposites synthesized at below pH 9. These results may be due to the move of growing interface of HA crystals to itself leading to a three-dimensional continuous growth at high pH. In addition, CA-loaded polymer/HA nanocomposites showed a slow, long-term and controlled release rate in DPBS. Based on these results, the POCA nanocomposites can contribute to the development of a new generation of controlled drug release carriers.

Acknowledgements This work was supported by research grants from the Catholic University of Daegu in 2013.

References

- 1) T. Cao, W. Tang, J. Zhao, L. Qin and C. Lan, *J. Bionics Eng.*, **11**, 125–133 (2014).
- 2) J. Zhang, Q. Wang and A. Wang, *Acta Biomater.*, **6**, 445–454 (2010).
- 3) Q.-L. Tang, Y.-J. Zhu, J. Wu, F. Chen and S.-W. Cao, *Nanomed.-Nanotechnol. Biol. Med.*, **7**, 428–434 (2011).
- 4) W. Zhu, L. Wan, C. Zhang, Y. Gao, X. Zheng, T. Jiang and S. Wang, *Mater. Sci. Eng., C*, **34**, 78–85 (2014).
- 5) Y. Kakizawa, S. Furukawa and K. Kataoka, *J. Controlled Release*, **97**, 345–356 (2004).
- 6) Y. Mizushima, T. Ikoma, J. Tanaka, K. Hoshi, T. Ishihara, Y. Ogawa and A. Ueno, *J. Controlled Release*, **110**, 260–265 (2006).
- 7) N. Jindal and S. K. Mehta, *Colloids Surf., B*, **129**, 100–106 (2015).
- 8) X. Li, Y. Yu, Q. Ji and L. Qiu, *Nanomed.-Nanotechnol. Biol. Med.*, **11**, 175–184 (2015).
- 9) X. Q. Guo, L. X. Song, F. Y. Du, Z. Dang and M. Wang, *J. Phys. Chem. B*, **115**, 1139–1144 (2011).
- 10) Z. Aytac, H. S. Sen, E. Durgun and T. Uyar, *Colloids Surf., B*, **128**, 331–338 (2015).
- 11) G. Devanand Venkatasubbu, S. Ramasamy, G. S. Avadhani, V. Ramakrishnan and J. Kumar, *Powder Technol.*, **235**, 437–442 (2013).
- 12) B. Viswanath and N. Ravishankar, *Biomaterials*, **29**, 4855–4863 (2008).
- 13) Y. X. Pang and X. Bao, *J. Eur. Ceram. Soc.*, **23**, 1697–1704 (2003).
- 14) F. Ye, H. Guo, H. Zhang and X. He, *Acta Biomater.*, **6**, 2212–2218 (2010).
- 15) J. Tang, J.-Y. Chen, J. Liu, M. Luo, Y.-J. Wang, X.-W. Wei, X. Gao, B.-L. Wang, Y.-B. Liu, T. Yi, A.-P. Tong, X.-R. Song, Y.-M. Xie, Y. Zhao, M. Xiang, Y. Huang and Y. Zheng, *Int. J. Pharm.*, **431**, 210–221 (2012).
- 16) S. Wang, X. Wang, H. Xu, H. Abe, Z. Tan, Y. Zhao, J. Guo, M. Naito, H. Ichikawa and Y. Fukumori, *Adv. Powder Technol.*, **21**, 268–272 (2010).
- 17) Y. H. Tseng, C. S. Kuo, Y. Y. Li and C. P. Huang, *Mater. Sci. Eng., C*, **29**, 819–822 (2009).
- 18) X. Xiaohao, R. Liu, C. Qiu, D. Zhu and F. Liu, *Mater. Sci. Eng., C*, **29**, 785–790 (2009).
- 19) P. Wang, C. Li, H. Gong, X. Jiang, H. Wang and K. Li, *Powder Technol.*, **203**, 315–321 (2010).
- 20) Y. Cheng, A. C. Samia, J. D. Meyers, I. Panagopoulos, B. Fei and C. Burda, *J. Am. Chem. Soc.*, **130**, 10643–10647 (2008).
- 21) L. L. Ma, M. D. Feldman, J. M. Tam, A. S. Paranjape, K. K. Cheruku, T. A. Larson, J. O. Tam, D. R. Ingram, V. Paramita, J. W. Villard, J. T. Jenkins, T. Wang, G. D. Clarke, R. Asmis, K. Sokolov, B. Chandrasekar, T. E. Milner and K. P. Johnston,

- ACS Nano*, **3**, 2686–2696 (2009).
- 22) A. Šturcová, P. Schmidt and J. Dybal, *J. Colloid Interface Sci.*, **352**, 415–423 (2010).
- 23) F. Hapiot, S. Tilloy and E. Monflier, *Chem. Rev.*, **106**, 767–781 (2006).
- 24) E. I. Alevizou and E. C. Voutsas, *J. Chem. Thermodyn.*, **62**, 69–78 (2013).
- 25) H. J. Lee, S. E. Kim, I. K. Kwon, C. Park, C. Kim, J. Yang and S. C. Lee, *Chem. Commun.*, **46**, 377–379 (2010).
- 26) M. Öner, E. Yetiz, E. Ay and U. Uysal, *Ceram. Int.*, **37**, 2117–2125 (2011).