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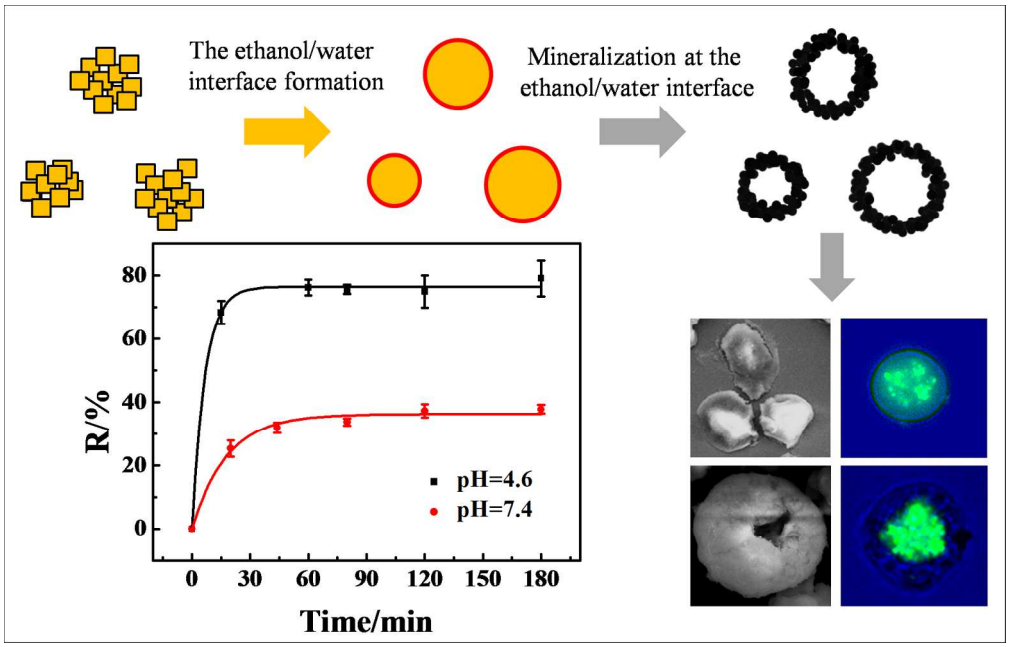

A crystallization – dissolution – interface mineralization (CDIM) method was reported to synthesize calcium carbonate (CaC) and calcium phosphate (CaP) inorganic microcapsules with good biocompatibility and good pH sensitive, which are promising in drug delivery.
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ARTICLE

Inorganic microcapsules mineralized at the interface of water droplets in ethanol solution and their application as drug carriers

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This paper reported a crystallization – dissolution – interface mineralization (CDIM) method on synthesizing calcium carbonate (CaC) and calcium phosphate (CaP) inorganic microcapsules with good biocompatibility and good pH sensitivity. The method is based on mineralization at ethanol/water interface. The microcapsules were formed in few seconds and do not need post treatment for removing the templates. The diameters of microcapsules can be controlled by the size of crystal clusters regulated by stirring time. Carboxyfluorescein(CF) molecules as model drugs were encapsulated inside the capsules after coating with Fe^{III}-polyphenol tannic acid (TA) films. The pH sensitive carboxyfluorescein molecule releasing behavior was investigated. The lower pH caused faster and thorough release of CF. CDIM method can be applied for fabricating other inorganic microcapsules, which holds a great potential on drug delivery.

1.Introduction

Inorganic microcapsules have attracted tremendous attentions recently because of their applications in a wide range of areas, such as targeted drug delivery[1, 2], wastewater treatment[3], micro reactors[4, 5], micro templates[6], autonomous motor[7], lithium ion battery[8, 9], sensors[10, 11] and catalysts[12]. With many significant and substantial efforts, facile and effective methods have been developed for the fabrication of various inorganic microcapsules, which can be divided into two categories, i.e., template-free synthesis, and template-based synthesis. The template-free synthesis is commonly based on Ostwald ripening[13-15]. Wang *et al.*[15] fabricated Fe_3O_4 microcapsules by inside-out Ostwald ripening as an anode material for lithium-ion batteries. The hard templates include particles of Cu_2O[16], SiO_2[17], $MnCO_3$[18], $Co_{0.33}Mn_{0.67}CO_3$[19], silica[20], polystyrene[21] and carbon spheres[22, 23], etc. Park *et al.*[20] prepared uniform hollow metal oxide particles using silica templates through calcinating silica@coordination polymer. Zhang *et al.*[24] fabricated TiO_2 microcapsules using carbon spheres as templates for lithium storage. The soft templates are bubbles[25], emulsions[26], vesicles[27], micelles[28], and yeasts [29, 30]. Luo *et al.*[25] reported a one-pot synthesis of metal sulfide microcapsules using CO_2 and NH_3 bubbles as soft templates. Xu *et al.*[27] used cetyltrimethylammonium bromide (CTAB) multilamellar vesicles as soft templates to synthesize multishelled Cu_2O microcapsules. The template-based synthesis is the most commonly used way to prepare microcapsules, but it requires tedious post-treatment which limits its applications and developments. It remains a significant challenge to develop a simple, effective, and suitable for massive production process for microcapsule fabrication.

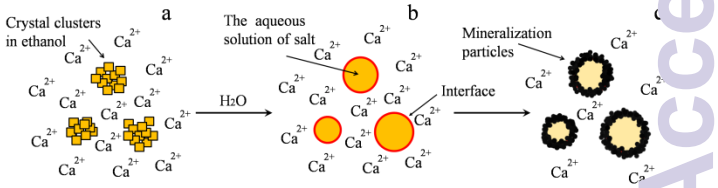

Fig. 1 The schematic of CDIM method for inorganic microcapsule formation. a) crystallization of salt, b) dissolution upon addition of water, c) interfacial mineralization to form microcapsules.

Herein, we presented a novel and effective strategy to prepare microcapsules without post-treatment. We call it crystallization – dissolution – interface mineralization (CDIM) method. The crystal clusters of Na_2CO_3/K_2HPO_4 are formed in ethanol by adding saturated aqueous Na_2CO_3/K_2HPO_4 solution into ethanol. Because of the attraction of water by ethanol, the saturated Na_2CO_3/K_2HPO_4 solution becomes over saturated to result in crystals, as schematically shown in Fig. 1a. Upon adding additional water into the mixed solution, the crystals in ethanol are dissolved to form liquid droplets in the ethanol solution (Fig. 1b). If the ethanol contains CaI_2, the mineralization occurs at the interface of salt water droplet and its surrounding ethanol (Fig. 1c). To the best of our knowledge, there is no report on fabricating microcapsules using CDIM method with the interface of water/ethanol. The calcium carbonate (CaC) and calcium phosphate (CaP) inorganic microcapsules were successfully fabricated using this method, which were then tested for drug release triggered by pH after coating with Fe^{III}-polyphenol tannic

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Electronic Supplementary Information (ESI) available: XRD patterns of Na_2CO_3, K_2HPO_4, CaC and CaP samples, and the calibration curve of maximum emission intensity against concentration of fluorescein with different pH. See DOI: 10.1039/x0xx00000x

acid (TA) film. CDIM method can be used for fabrication of various inorganic microcapsules.

2.Experimental

2.1.Materials

Calcium iodide (CaI_2, 98%) and 5(6)-Carboxyfluorescein ($C_{21}H_{12}O_7$, 95%) were purchased from Aladdin (China). Fluorescence latex beads (carboxylate-modified polystyrene, 0.03 μm, 2.5%) were purchased from Sigma (China). Dipotassium phosphate ($K_2HPO_4{\cdot}3H_2O$, 99%), dibasic sodium phosphate ($Na_2HPO_4{\cdot}2H_2O$, 99%) and sodium carbonate (Na_2CO_3, 99%) were purchased from Xilong chemicals (China). Citric acid ($C_6H_8O_7{\cdot}H_2O$, 99.5%) was purchased from Tianjin Fengchuan (China). Ethanol (C_2H_5OH, 99.7%) was purchased from Tianjin Tianli chemicals (China). Ferric chloride ($FeCl_3$, 97.0%) was purchased from Sinopharm chemicals (China). Tannic acid ($C_{76}H_{52}O_{46}$) was purchased from Sigma-Aldrich (China). The solutions were prepared with ultrapure water (18.2 MΩ • cm).

2.2.Preparation of CaC and CaP microcapsules

The details on preparing CaC microcapsules are described as below. 500 μL saturated Na_2CO_3 aqueous solution (1 M at 20 °C) was injected into 50 mL ethanol under magnetic stirring about 1400 rpm for 3 h to prepare Na_2CO_3 crystal clusters in ethanol. After that 1 mL Na_2CO_3 crystals in ethanol and 1 mL 0.2 M CaI_2 ethanol solution were mixed homogeneously. The CaC microcapsules were formed in 1200 ms by the addition of 2 mL water. The precipitates were washed by centrifuging with ethanol for 3 times. The CaP microcapsules were synthesized as follow. 500 μL saturated K_2HPO_4 aqueous solution (4 M at 20 °C) was injected into 50 mL ethanol under magnetic stirring about 1400 rpm for 6 h to prepare K_2HPO_4 crystal clusters in ethanol. Then 1 mL K_2HPO_4 crystals in ethanol and 1 mL 0.7 M CaI_2 ethanol solution were mixed well. After adding 2 mL water, the CaP microcapsules were formed in 300 ms. The precipitates were washed by centrifuging with ultrapure water for 3 times.

2.3.The observation of microcapsule formation under microscope

The forming processes of CaC and CaP microcapsules were observed by microscope, and videos were recorded. 25 μL ethanol containing crystal clusters, 25 μL CaI_2 ethanol solution and 50 μL water were injected into a sealed cell in a proper order as described in section 2.2.

2.4.Model drug loading and in vitro release

The Fe^{III}-TA coating reaction was described elsewhere.[31] In brief, 30 μL 2 mg/mL carboxyfluorescein (CF), 1 mL sodium dihydrogen phosphate-citrate buffer solution and 200 μL 9 mg/mL CaP microcapsules were mixed together. 1 mL 1 mg/mL (3.7 mM) $FeCl_3$ and 1 mL 4 mg/mL (2.4 mM) TA were added into the mixture solution for 30 min in order to form TA films on the surface of capsule. Then the supernatant liquid was taken away. Fe^{III}-TA coating was repeated for several times. The releasing behavior of CF controlled by pH (4.6 and 7.4) was performed using fluorescence spectrophotometer.

2.5.Characterizations

The morphology of the CaC and CaP microcapsules was characterized with a scanning electron microscopy (Quanta 200 FEG, Netherlands) at an accelerating voltage of 20 kV. The sample was prepared by casting purified products onto a piece of silicon wafer. Powder X-ray diffraction (XRD) was measured in the reflection mode (Cu K radiation) on a diffractometer (D/Max-RB, Japan). Fluorescence microscopy images were obtained using a Nikon Eclipse 80i fluorescence microscope. Fluorescence measurements were performed on a fluorescence spectrophotometer (Fluoro Max-4, US).

3.Results and discussion

The crystal clusters of Na_2CO_3 and K_2HPO_4 were prepared by adding 500 μL Na_2CO_3/K_2HPO_4 saturated solution into 50 mL ethanol. Due to the extraction of water by ethanol, Na_2CO_3 and K_2HPO_4 were crystallized in the ethanol. Their XRD patterns are shown in Fig. S1, which confirms that the crystallized samples are Na_2CO_3 crystals according to standard data (PDF: 19-1130), and K_2HPO_4 crystals according to standard data (PDF: 25-0639), respectively. Their SEM images are shown in Fig. 1a and f. The diameter is ~ 1.3 μm for Na_2CO_3 crystal and ~ 3.2 μm for K_2HPO_4 crystals respectively. After mineralization, CaC microcapsules contain mainly amorphous $CaCO_3$ with a small amount of vaterite according to XRD data in Fig. S2c. While CaP microcapsules are a mixture of brushite (PDF: 0[illegible]-0077) and calcium hydrogen phosphate hydroxide (PDF: 09-0432) from XRD patterns in Fig. S2d. SEM images of CaC and CaP microcapsules are shown in Fig. 2b and g respectively. The walls of CaC microcapsules are too thin to keep their original shape in the vacuum chamber as shown in Fig. 2b. By measuring the folded wrinkles, the wall thickness is ~ 1 μm. On the contrary, the CaP microcapsules are more rigid with the wall thickness of ~ 7 μm as shown in Fig. 2g. The hollow nature of the microcapsules is confirmed by the open hole of zoom-in image of one microcapsule in the top inset of Fig. 2g. The hollow nature of microcapsules was also studied using fluorescent microscope. Small amount of green fluorescent latex beads were mixed inside the $NaCO_3$ and K_2HPO_4 crystals, which were then used for forming microcapsules. The bright field and fluorescence images of CaC microcapsule are shown in Fig. 2c and d respectively. After merging these two images, a clear green core and dark ring can be seen in Fig. 2e, which confirmed its capsule structure. Similarly, the bright field, fluorescence images of CaP microcapsules, and their merged image are shown in Fig. 2h, i, and j respectively. It is noted that its wall is much thicker than that of CaC microcapsule, which is consistent with the SEM results.

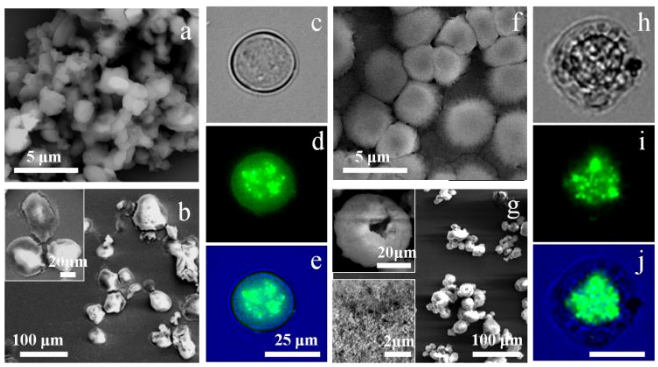

Fig. 2 The SEM images of Na_2CO_3 (a), K_2HPO_4 (f) crystals, CaC microcapsules (b) and CaP microcapsules (g). Insets in image b and g are the enlarged images at a higher magnification. The bright filed (c and h), fluorescence (d and i), and merged images (e and f) of CaC and CaP microcapsules containing latex beads.

Above experimental results confirms the validity of CDIM method on inorganic microcapsule fabrication. In the following context, the parameters influencing the microcapsule formation were studied. The concentration of CaI_2 was adjusted to be 0.05 M, 0.2 M and 0.7 M for CaC microcapsules, respectively. The microcapsule formed only at 0.2 M CaI_2. Similarly 0.7 M CaI_2 was chosen for CaP microcapsule formation. The diameters of CaC and CaP microcapsules were related to the size of Na_2CO_3 and K_2HPO_4 crystal clusters. The stirring time was controlled during the crystallization step. The diameter of microcapsules was analyzed by imageJ. The stirring time of 10 min and 180 min were studied for CaC microcapsules. There is almost no difference between 10 and 180 minute stirring. The microcapsule shown in Fig. 3a was formed with 180 minute stirring for the crystallization. The diameter of microcapsule is ~ 27 μm. In terms of CaP microcapsules, the stirring time was studied with 10 min and 1 h. Their corresponding capsules are ~50 and 24 μm in diameter, respectively as shown in Fig. 3b. With the stirring time over 1 h, there is no influence on microcapsule size. The discrepancy of stirring time infuluence on CaC and CaP microcapsules comes from the nature of Na_2CO_3 and K_2HPO_4 crystal clusters, i.e., Na_2CO_3 is easier to break into small clusters compared with K_2HPO_4 crystals.

The forming processes of CaC and CaP microcapsules were recorded by the camera equipped in the microscope. ImageJ software was used to convert video into frame images, as shown in Fig. 4. The videos of CaC and CaP microcapsules forming processes were provided in supporting information, which were slowed down by 50 and 100 times respectively. The crystal clusters of Na_2CO_3 and K_2HPO_4 are shown in Fig. 4a and e. Upon the addition of water, crystals were dissolved as shown in Fig. 4b and f. In Fig. 4 b, c, d, g and h, a clear interface can be seen. CaC microcapsules were fabricated in 1200 ms, whilst CaP microcapsule formation only took 300 ms. In Fig. 4 d, there is some crystals in the microcapsules because water and ethanol was not mixed uniformly in a limited space under microscope without stirring. Normally all the crystals disappeared after microcapsule formation. The process is similar to Kirkendall Effect, which was used to fabricate hollow metal nanostructures[32,33]. However, in our case the temperature is the room temperature rather than ~ 180 ℃, and the interface crystalization took less than 1.2 s which is much faster than several hours for fabricating metal capsules using Kirkendall effect[33]. The Kirkendall effect involves diffusion of metal atoms, therefore needs high temperature and long time. While CDIM method involves metal ions diffusion in the liquid phase, resulting in microcapsule formation in short time and low temperature.

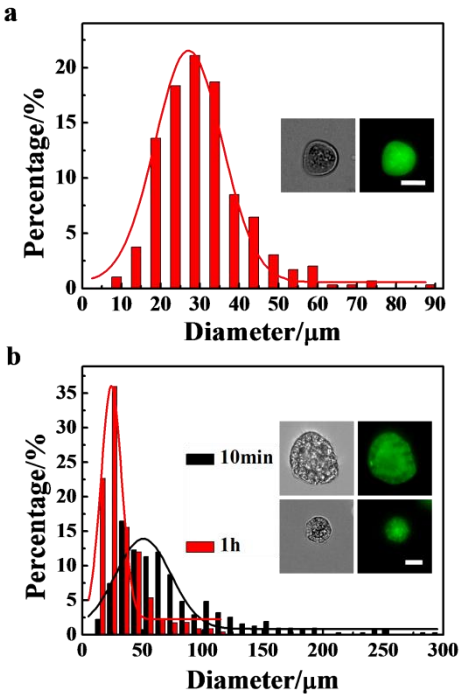

Fig. 3 The diameter distribution and fitted curves of microcapsule against stirring time for the formation of Na_2CO_3 (a), and K_2HPO_4 (b) crystal clusters. Insets are the typical bright filed (left) and fluorescence (right) microscope images of CaC and CaP microcapsules after corresponding stirring time. The scale bar is 20 μm.

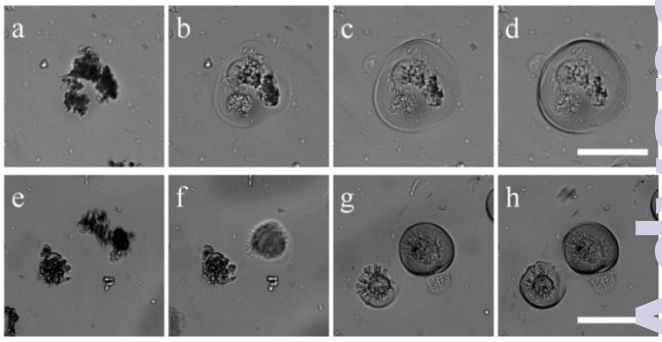

Fig. 4 The images of CaC microcapsule formation (a: 0 ms, b: 400 ms, c: 800 ms and d: 1200 ms) and CaP microcapsule formation (e: 0 ms, f: 100 ms, g: 200 ms and h: 300 ms). The scale bar is 50 μm.

The fabricated microcapsule is porous structure, as shown in bottom inset in Fig. 2g. Therefore Fe^{III}-TA films were used to coat

the capsule surface in order to keep encapsulated CF inside the capsules. In order to confirm the Fe^{III}-TA films on capsule surface, FTIR spectra of CaP, Fe^{III}-TA, and CaP with Fe^{III}-TA films were carried out, as shown in curve 1, 2 and 3 of Fig. 5a respectively. The peaks at 555 cm^{-1}, 590 cm^{-1}, 1030 cm^{-1} and 1103 cm^{-1} in curve 1 of Fig. 5a are attributed to PO_4^{3-} in pure CaP microcapsules. In curve 2 of Fig. 5a, the peak at 1195 cm^{-1} is due to aromatic C-O-C antisymmetric stretching vibrations, and peaks at 1440 cm^{-1}, 1490 cm^{-1} and 1603 cm^{-1} correspond to aromatic rings in TA molecules. Curve 3 of Fig. 5a contains almost all the characteristic peaks of pure CaP capsules and Fe^{III}-TA films, which indicates the successful coating of Fe^{III}-TA films on the capsule surface. The optical image of Fe^{III}-TA films coated CaP microcapsule is shown in Fig. 5b. After dissolving CaP in pH 3 solution, the Fe^{III}-TA film was left, as shown in Fig. 5c. It also confirmed that films were successfully coated.

The good points of CaP microcapsules as drug carriers are biocompatible and pH responsive, since they have been proved as bioactive implants[34-36] and drug carrier[37-39].The CF releasing from Fe^{III}-TA films coated CaP microcapsules was studied as function of pH values. In Fig. 6, the releasing behavior controlled by buffer solution with different pH (4.6 and 7.4) was performed by fluorescence spectrophotometer. Because the CF is sensitive to pH, the data in Fig. 6 have been corrected according the curve in Fig. S2. The data for the controlled drug releasing within 3 h are fit very well with an exponential equation (1).

$$R = a(1 - e^{-bt}) \quad (1)$$

where R is the percentage of CF releasing, α is the maximum releasing percentage and b is the parameter corresponding to the releasing rate.

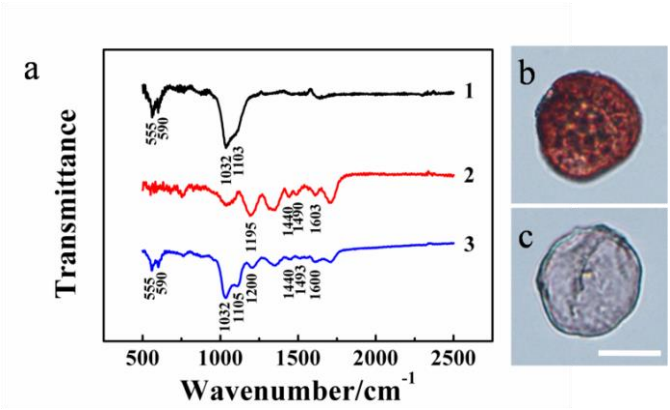

Fig. 5 a) The FTIR spectra of CaP capsules (curve 1), Fe^{III}-TA films (curve 2), and Fe^{III}-TA films coated CaP capsules (curve 3). The optical images of Fe^{III}-TA film coated CaP microcapsule before b) and after c) dissolving CaP in pH 3 buffers. The scale bar is 20 μm.

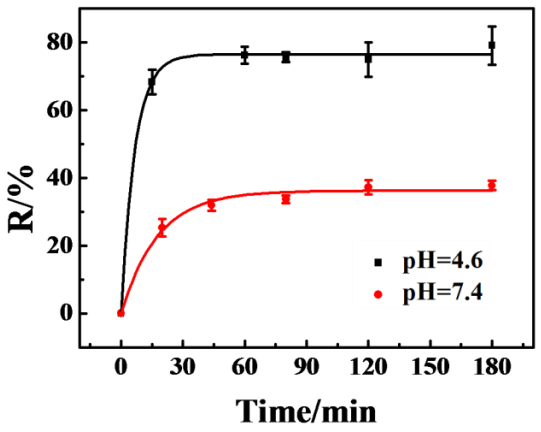

Fig. 6 The release curves of CaP microcapsules coated with Fe^{III} - T films at different pH.

In Fig. 6, the maximized releasing percentages are 76.5% and 36.2% at pH 4.6 and 7.4 respectively. The b value is 0.15 and 0.06 at pH 4.6 and 7.4 respectively. All the data indicate a faster and more thorough release of CF at lower pH solution, which is due to the dissolution of CaP wall.

4.Conclusions

The novel CDIM method was developed for fabricating inorganic microcapsules. It was successfully exploited to synthesize CaC and CaP microcapsules. The microcapsules are biocompatible and pH responsive, which makes them good candidates as drug carriers. When pH values were 7.4 and 4.6, the maximum release percentage of CF from CaP microcapsules are about 36.2% and 76.5%, respectively, which implies that CaP microcapsules are promising in drug delivery. The CDIM method has a great potential for massive production of inorganic microcapsules.

Acknowledgements

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Notes and references

1 Y. Chen, H. R. Chen, D. P. Zeng, Y. B. Tian, F. Chen, J. W. Feng and J. L. Shi, *Acs Nano*, 2010, **4**, 6001-6013.
2 Y. Wang and H. Gu, *Advanced materials*, 2015, **27**, 576-585.
3 B. Wang, H. Wu, L. Yu, R. Xu, T. T. Lim and X. W. Lou, *Advanced materials*, 2012, **24**, 1111-1116.
4 Y. Yamada, M. Mizutani, T. Nakamura and K. Yano, *Chemistry of Materials*, 2010, **22**, 1695-1703.
5 A. M. Yashchenok, M. Delcea, K. Videnova, E. A. Jares-Erijman, T. M. Jovin, M. Konrad, H. Mohwald and A. G. Skirtach, *Angew Chem Int Edit*, 2010, **49**, 8116-8120.

6 J. Li, Z. Jiang, H. Wu, L. Zhang, L. Long and Y. Jiang, *Soft Matter*, 2010, **6**, 542-550.
7 Y. J. Wu, Z. G. Wu, X. K. Lin, Q. He and J. B. Li, *Acs Nano*, 2012, **6**, 10910-10916.
8 J. Y. Wang, N. L. Yang, H. J. Tang, Z. H. Dong, Q. Jin, M. Yang, D. Kisailus, H. J. Zhao, Z. Y. Tang and D. Wang, *Angew Chem Int Edit*, 2013, **52**, 6417-6420.
9 L. Zhou, D. Y. Zhao and X. W. Lou, *Angew Chem Int Edit*, 2012, **51**, 239-241.
10 S. Ci, T. Huang, Z. Wen, S. Cui, S. Mao, D. A. Steeber and J. Chen, *Biosensors & bioelectronics*, 2014, **54**, 251-257.
11 X. Y. Lai, J. Li, B. A. Korgel, Z. H. Dong, Z. M. Li, F. B. Su, J. A. Du and D. Wang, *Angew Chem Int Edit*, 2011, **50**, 2738-2741.
12 S. Mandal, M. Sathish, G. Saravanan, K. K. R. Datta, Q. M. Ji, J. P. Hill, H. Abe, I. Honma and K. Ariga, *J Am Chem Soc*, 2010, **132**, 14415-14417.
13 L. Cao, D. H. Chen and R. A. Caruso, *Angew Chem Int Edit*, 2013, **52**, 10986-10991.
14 A. Q. Pan, H. B. Wu, L. Yu and X. W. Lou, *Angew Chem Int Edit*, 2013, **52**, 2226-2230.
15 B. Wang, H. B. Wu, L. Zhang and X. W. Lou, *Angew Chem Int Edit*, 2013, **52**, 4165-4168.
16 M. L. Pang, J. Y. Hu and H. C. Zeng, *J Am Chem Soc*, 2010, **132**, 10771-10785.
17 L. Yu, H. B. Wu and X. W. Lou, *Advanced materials*, 2013, **25**, 2296-2300.
18 W. Liu, J. Liu, K. F. Chen, S. M. Ji, Y. L. Wan, Y. C. Zhou, D. F. Xue, P. Hodgson and Y. C. Li, *Chem-Eur J*, 2014, **20**, 824-830.
19 L. Zhou, D. Y. Zhao and X. W. Lou, *Advanced materials*, 2012, **24**, 745-748.
20 J. U. Park, H. J. Lee, W. Cho, C. Jo and M. Oh, *Advanced materials*, 2011, **23**, 3161-3164.
21 S. X. Li, J. B. Cai, X. Q. Wu, F. Y. Zheng, X. F. Lin, W. J. Liang, J. Chen, J. Z. Zheng, Z. H. Lai, T. J. Chen and L. C. Zhu, *Appl Catal B-Environ*, 2014, **160**, 279-285.
22 Z. H. Dong, X. Y. Lai, J. E. Halpert, N. L. Yang, L. X. Yi, J. Zhai, D. Wang, Z. Y. Tang and L. Jiang, *Advanced materials*, 2012, **24**, 1046-1049.
23 G. Q. Zhang and X. W. Lou, *Angew Chem Int Edit*, 2014, **53**, 9041-9044.
24 G. Zhang, H. B. Wu, T. Song, U. Paik and X. W. D. Lou, *Angewandte*, 2014, **53**, 1-5.
25 M. Luo, Y. Liu, J. C. Hu, J. L. Li, J. Liu and R. M. Richards, *Appl Catal B-Environ*, 2012, **125**, 180-188.
26 X. Wang, W. Zhou, J. Cao, W. Liu and S. Zhu, *Journal of colloid and interface science*, 2012, **372**, 24-31.
27 H. L. Xu and W. Z. Wang, *Angew Chem Int Edit*, 2007, **46**, 1489-1492.
28 M. Sasidharan and K. Nakashima, *Accounts Chem Res*, 2014, **47**, 157-167.
29 D. Z. Ni, L. Wang, Y. H. Sun, Z. R. Guan, S. Yang and K. B. Zhou, *Angew Chem Int Edit*, 2010, **49**, 4223-4227.
30 M. J. Huang and Y. J. Wang, *J Mater Chem*, 2012, **22**, 626-630.
31 H. Ejima, J. J. Richardson, K. Liang, J. P. Best, M. P. van Koeverden, G. K. Such, J. W. Cui and F. Caruso, *Science*, 2013, **341**, 154-157.
32 Y. D. Yin, R. M. Rioux, C. K. Erdonmez, S. Hughes, G. A. Somorjai, A. P. Alivisatos, Science, 2004, **304**, 711-714.
33 X. Liang, X. Wang, Y. Zhuang, B. Xu, S. Kuang, and Y. D. Li, J Am Chem Soc, 2008, **130**, 2736-2737.
34 C. Schopper, D. Moser, W. Goriwoda, F. Ziya-Ghazvini, E. Spassova, G. Lagogiannis, A. Auterith, R. Ewers, *Clin Oral Implan Res,* 2005, **16**, 357-368.
35 A. F. Lemos, J. M. F. Ferreira, *Mat Sci Eng C-Bio S,* 2000, **11**, 35-40.
36 C. Schiller, C. Rasche, M. Wehmoller, F. Beckmann, H. Eufinger, M. Epple, S. Weihe, *Biomaterials*, 2004, **25**, 1239-1247.
37 W. Wei, G. H. Ma, G. Hu, D. Yu, T. Mcleish, Z. G. Su, Z. Y. Shen, *J Am Chem Soc*, 2008, **130**, 15808-15810.
38 B. P. Bastakoti, S. Guragain, Y. Yokoyama, S. Yusa, K. Nakashima, *Langmuir*, 2011, **27**, 379-384.
39 M. P. Ginebra, T. Traykova, J. A. Planell, *J Control Release*, 2006, **113**, 102-110.