Review

Nanostructured Calcium Phosphates: Preparation and Their Application in Biomedicine

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Abstract

Due to the similar chemical properties to the inorganic component in calcified tissues, synthetic calcium phosphate has been considered as ideal biomaterials with excellent biocompatibility. Nanostructured calcium phosphate materials play an important role in the formation of hard tissues in nature. It is reported that calcium phosphates materials in nano-size can mimic the dimensions of constituent components of calcified tissues. Recently, the synthesis and application of nanostructured calcium phosphate materials have become a very hot field. Lots of methods have been reported to prepare nanostructured calcium phosphate, and various morphologies including nanoparticles, plate-like nanocrystals, nano-needles, whiskers/fibres/wires, mesoporous, nanotubes, nano-blades, and powders with three-dimensional (3-D) structures have been obtained. More studies of nanostructured calcium phosphates are expected in biomedical area, such as tissue engineering scaffolds, drug/gene delivery systems and multifunctional systems. In this article, the synthesis and application of nanostructured calcium phosphates are reviewed and discussed.

Keywords: Calcium Phosphate, Nanostructure, Biomedicine, Hydroxyapatite, Tissue Engineering, Drug Delivery, Gene Delivery.

1. Introduction

Calcium phosphates are abundant in nature, especially in calcified tissue of vertebrate. The inorganic mineral in bone was determined and clarified as carbonated apatite in early 20th century [1]. The constituting building blocks of bone are composites of biological apatite and molecules of collagen [2, 3]. Calcified tissues, such as bone or dentin contain ~70 wt. % apatite and ~20 wt. % collagen [4,5]. The formation of mineral is the process of in vivo biomineralization, which are built up of mineralized collagen fibrils [6, 7]. The mineral particles in calcified tissue were studied, and described as plate-like in shape. The thickness of the platelets ranges from 2 to 7 nm, the length from 15 to 200 nm and the width from 10 to 80 nm [2, 8]. These mineral particles are combined with collagen into self-assembled complex hierarchical structure in calcified tissue to achieve a remarkable mechanical performance [9].

Many kinds of materials of calcium phosphates have been prepared. The synthesis and properties of different kinds of calcium phosphates are changed. The standard abbreviations and the chemical formulas of these materials are shown as follows: monocalcium phosphate monohydrate (MCPM, $Ca(H_2PO_4)_2 \cdot H_2O$), monocalcium phosphate anhydrous (MCPA, $Ca(H_2PO_4)_2$), dicalcium phosphate dehydrate (DCPD, $CaHPO_4 \cdot 2H_2O$), dicalcium phosphate anhydrous (DCPA, $CaHPO_4$), octacalcium phosphate (OCP, $Ca_8(HPO_4)_2(PO_4)_4 \cdot 5H2O$), α-tricalcium phosphate (α-TCP, $\alpha\text{-}Ca_3(PO_4)_2$), β-tricalcium phosphate (β-TCP, $\beta\text{-}Ca_3(PO4)_2$), amorphous calcium phosphate (ACP, $Ca_xH_y(PO_4)_z \cdot nH_2O$, n=3–4.5, 15-20% H_2O), calcium-deficient hydroxyapatite (CDHA, $Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}$), hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$), fluorapatite (FA, $Ca_{10}(PO_4)_6F_2$) and tetracalcium phosphate (TTCP, $Ca_4(PO_4)_2O$). The detailed information on calcium phosphates, their synthesis, structure, chemistry, properties and biomedical applications have been comprehensively reviewed recently [10-12]. Due to the high solubility, acidity and basicity, the calcium phosphates materials which have a Ca/P molar ratio less than 1 and more than 2 are not suitable for implantation

into the body [11]. Among the known calcium phosphates compounds, OCP, ACP, α/β-TCP and HA, are significantly useful for biomedical applications. Calcium phosphates are biocompatible, not recognized as foreign materials in the body, and can integrate into living tissue by the same processes active in bone remodeling [13].

Nowadays, the development of nanotechnology has benefited calcium phosphates as biomaterials. It is reported that calcium phosphates materials in nano-size can mimic the dimensions of constituent components of calcified tissues, and be utilized as biomaterials due to their excellent biocompatibility [14]. Lots of method have been reported to prepare nanostructured calcium phosphates, such as co-precipitation, sol–gel synthesis, hydrothermal reaction, mechanical milling, etc., by which calcium phosphate nanoparticles with various shapes and sizes can be obtained [15-21].

Herein, an overview of calcium phosphate materials as biomaterials is given. In this review, the nano-effects of calcium phosphates materials, synthesis of nanoscale calcium phosphates, as well as applications of nanostructured calcium phosphate in biomedicine have been discussed.

2. Nano-effects of calcium phosphates materials

Due to the similar dimensions to the inorganic components of calcified tissues, calcium phosphates materials in nano-size are expected to have better bioactivity compared with conventional materials [22]. The advantages of synthetic calcium phosphates materials in nano-size include higher biocompatibility, good biodegradability in situ, and excellent osteoconductive and osteoinductive capabilities [23, 24].

Calcium phosphates materials in nano-size have higher specific surface area and surface roughness compared to conventional calcium phosphates materials. Therefore, nanosized calcium phosphates materials have stronger interaction with organic materials. For example, nanophase HA appeared to have 11% more proteins of fetal bovine serum adsorbed per 1 cm^2 than conventional HA [25]. HA nanoparticles blended in polyacrylonitrile fibers were found to result in their degree of crystallinity rising by about 5% [26]. The sinterability of conventional calcium phosphate ceramics made from conventional powders is poor, and the resorption of ceramics is quite different from that of bone mineral [27]. Meanwhile, due to greater specific surface area, calcium phosphates materials in nano-size show improved sinterability and enhanced densification, which may improve fracture toughness, as well as other mechanical properties [24, 28]. With high biocompatibility, good surface properties, good sinterability and ability of interaction with organic molecules, synthetic nanostructured calcium phosphates materials have promising applied potential in biomedicine.

3 Synthesis of nanoscale calcium phosphate

The bioactivity, biocompatibility, stability and mechanical properties of calcium phosphate materials are usually determined by its composition, structure, morphology and crystallite size [29]. Calcium phosphate with different morphologies including nanoparticles [30, 31], plate-like nanocrystals [32], nano-needles [33], whiskers/fibres/wires [34-36], mesoporous [35], nanotubes [37], nano-blades [38, 39], and powders with three-dimensional (3-D) structures have been prepared [40-42]. The performance of calcium phosphates in applications depends greatly on its morphologies and chemical compositions. To optimize and achieve better performances, controlling the structure and size of calcium phosphate materials is still a hot filed.

3.1 Calcium phosphate nanoparticles

Calcium phosphate nanoparticles have been prepared by a variety of techniques such as mechanochemical synthesis, combustion preparation, and wet chemistry techniques [43-45]. The products obtained using method of wet chemistry techniques have controlled size and good dispersing property. The precipitation in solution is an easy method for fabrication of calcium phosphate nanoparticles, and chemical agents such as citric acid, amino acids and ethylenediaminetetraacetic acid (EDTA) have been used to mediate the structure of calcium phosphate nanoparticles [46-48].

Recently, biocompatible block copolymers have been used to control the synthesis of calcium phosphate nanoparticles. For example, calcium phosphate hybrid porous nanospheres have been prepared through a facile PLGA-mPEG/PLA-mPEG assisted route at a relatively low temperature [49-51]. The micelles formed by amphiphilic block copolymer of PLGA-mPEG/ PLA-mPEG act as templates and calcium phosphate is combined with the micelle via the electrostatic interaction between Ca^{2+} ions and polymer segments.

As a typical solution-based method, the hydrothermal approach has proven to be an effective and convenient process to prepare calcium phosphate nanoparticles. There are many advantages of this method, including easily controllable reaction conditions, relatively large scale and high crystallinity in terms of quantity and quality of the desired products [28]. Zhang et al. [29] reported the synthesis of HA nano- and microstructures using water as a reaction medium through a simple hydrothermal process. Our research group have prepared HA nanorods with relatively uniform sizes and high crystallinity via a hydrothermal strategy [52]. The shape and size of the sample can be adjusted by regulating the reaction conditions. Through hydrothermal treatment, the HA nanorods with larger sizes and higher crystallinity are obtained compared with those prepared at a low temperature.

3.2 3-D nanostructured calcium phosphate

Morphologies, 3-D architectures and chemical compositions influence on the performance of calcium

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phosphate materials in their applications. Recently, the fabrication and properties of 3-D architectured calcium phosphate materials have attracted great interests.

It was demonstrated that calcium phosphate hollow nanostructures could be prepared through biomineralization in the presence of polyelectrolyte [54]. Wang et al. synthesized HA microspheres using polyelectrolyte as the morphology directing-agent [55], and poly(styrene sulfonate) (PSS) was used as a modifier with the concentration varied ranging from 0 to 9.6 wt% during the hydrothermal synthesis of carbonated HA to achieve the controlled morphology and particle size. The presence of PSS drastically changed the growth pattern of HA crystallites. The concentration of PSS was elucidated as an important factor for the formation of HA microspheres of different sizes and hierarchical structures. 3-D architectured calcium phosphate materials were fabricated by the templating method. Schmidt and Tjandra et al. [56, 57] coated calcium phosphate on lipsome micelles/block copolymer templates to form nanoshells, and the thickness of shells was controllable by adjusting the addition time of calcium and phosphate salt, and hollow calcium phosphate nanospheres were obtained after calcination. The hard templates were also used to form 3-D architectured calcium phosphate materials. Lin et al. [58] reported that HA nanoparticles, nanowires and hollow nanostructured microspheres were successfully synthesized on a large scale via a facile hydrothermal treatment of similarly structured hard-precursors of $CaCO_3$ nanoparticles, xonotlite nanowires and hollow $CaCO_3$ microspheres in Na_3PO_4 solution in the absence of any surfactants, organic solvent or organic template-directing reagents, respectively. By using calcium carbonate nano-ellipsoidal particles as hard templates, our group has successfully fabricated HA nanostructured hollow ellipsoidal capsules with a large specific surface area, which exhibited high ibuprofen loading capacity and good releasing property (Fig. 1) [53]. It is a general strategy to delicately control the morphologies of HA materials from simple morphologies to complicated 3D architectures using the hard-template with similar morphologies and architectures.

Beside, surfactants are also usually used as the controlling reagent to prepare 3-D architectured calcium phosphate materials. Cheng and Liu et al. [59, 60] prepared flower-liked HA microspheres using EDTA as the template-directing reagent under hydrothermal treatment and microwave irradiation. The microwave-assisted method has been successfully used for the synthesis of monetite with flowerlike and bundlelike morphologies using $CaCl_2 \cdot 2.5H_2O$ and NaH_2PO_4 in the presence of sodium dodecyl sulfate (SDS) in mixed solvents of water and EG [61]. When the product was immersed in NaOH solution, the monetite could transform into HA, and the flowerlike structure could be maintained. Block copolymer was also used to control the synthesis of 3-D architectured calcium phosphate materials. Wang et al. [62] reported that flower-like nanostructured

HA hollow spheres assembled with nanosheets with a hierarchical morphology were fabricated via a rapid microwave-assisted hydrothermal route. The presence and concentration of block copolymer poly(lactide)-block-poly(ethylene glycol) (PLA-PEG) were important parameters for the formation of the hollow structure.

3.3 Biomimic calcium phosphate nanomaterials

The performance of calcium phosphate in biomedical applications depends greatly on its morphologies, architectures and chemical compositions. Therefore, efforts have been done to develop biomimic calcium phosphate nanomaterials. Up to now, the major efforts to develop biomimic HA mainly involved macromolecular and surfactant controlled self-assembling [63-68], and biomineralization [69-73]. Our research group has developed a surfactant-free solvothermal method using $CaCl_2$ and $NaH_2PO_4 \cdot H_2O$ in ternary solvents of water, ethylene glycol (EG) and N,N-dimethylfomamide (DMF) for the preparation of a variety of HA nanowire/nanotube ordered arrays and their fabrics with biomimic structures including HA sheets with brush-like ends, nanotube arrays and their fabrics, nanowire arrays and their fabrics (Fig. 2) [37]. The solvents have obvious effects on the morphology and crystal phase of the product. One advantage of this method is that the hard template and surfactant are not needed, avoiding the procedures and cost for their removal in the product. The as-prepared HA nanowire/nanotube ordered arrays and their fabrics show similarity in structure to the natural hard tissues, and may be potentially useful in biomedical areas.

Lin et al, reported single-crystalline of biomimetic HA porous microspheres with co-substituted essential trace elements (Na, Mg, K, F, Cl and CO_3^{2-}) of natural bone synthesized via a facile hydrothermal process at a low temperature [74]. The morphology images of the control sample and synthetic powders CS-HAp1-4 via hydrothermal treatment at 120 $^{\circ}$C for 24 h are presented in Fig. 3. The biomimetic HA porous microspheres were assembled from the nanosheets with thickness of about 60 nm, widths and lengths of up to 2 μm. The novel 3-D architectures resulted in favourable drug loading and release property, and the co-substituted essential trace elements enhanced the degradability of the obtained products in comparison with pure HA nano-particles.

Electrospinning has been recognized as an efficient technique for fabricating polymer nanofibers which can be widely used in biomedical areas [75]. Calcium phosphate nanofibers were obtained by electrospinning using solution containing polyvinyl alcohol [76,77]. Our research group reported a simple method for the preparation of HA/PVP composite nanofibers, 3-D fabrics with different shapes and aligned nanofiber arrays by electrospinning (Fig. 4) [78]. Then, the single-phase HA fabrics, tubular morphologies or aligned nanofiber arrays were obtained through thermal treatment of corresponding composite precursors. One of the advantages is that it is facile to obtain HA nanofibrous scaffold with designed
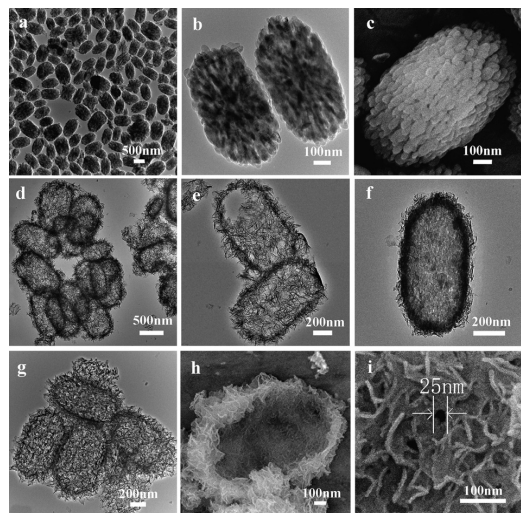
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Fig. 1 TEM and SEM micrographs of (a)–(c) CaCO$_3$ cores; (d)–(i) HAp nanostructured hollow ellipsoidal capsules prepared using dilute H$_3$PO$_4$: (d) and (e) H$_3$PO$_4$: H$_2$O = 1 : 300; (f) H$_3$PO$_4$: H$_2$O = 1 : 100; (g)–(i) H$_3$PO$_4$: H$_2$O =1:100. Reprinted from Ref. [53].

Fig. 2 SEM (a–c) and TEM (d–f) micrographs of the HAp sample prepared using CaCl$_2$ and NaH$_2$PO$_4$•H$_2$O in mixed solvents of H$_2$O/EG/DMF (H$_2$O/EG = 1 : 1) by solvothermal treatment at 200 °C for 24 h. The insets of (e and f) are the SAED patterns and HRTEM image. Reprinted from Ref. [37].

Fig. 3 Morphology images of the control sample (A) and the synthetic CS-HAp1 (B), S-HAp2 (C), CS-HAp3 (D) and CS-HAp4 (E, F) porous microspheres. Reprinted from Ref. [74].

Fig. 4 Scheme of the strategy for fabrication of HAp/PVP composite nanofibers and fabrics (A) and the process of formation of HAp nanofibers (B). Reprinted from Ref. [78].

structure. Biomineralization was also used to prepared biomimic calcium phosphate nanostructure combined with the method of electrospinning. Our group reported ACP/PDLLA composite nanofibers prepared by electrospinning [79]. ACP nanoparticles with diameters ranging from 20 to 80 nm were synthesized using a simple precipitation method. The hybrid materials showed good ability of biomineralization and cytocompatibility in vitro.

4. Application of nanostructured calcium phosphate

4.1 Hard tissue engineering

Because of excellent biocompatibility, calcium phosphate based materials have been used in hard tissue repair for decades [80]. The chemical properties of calc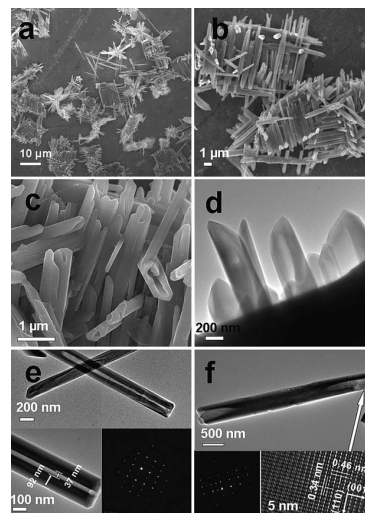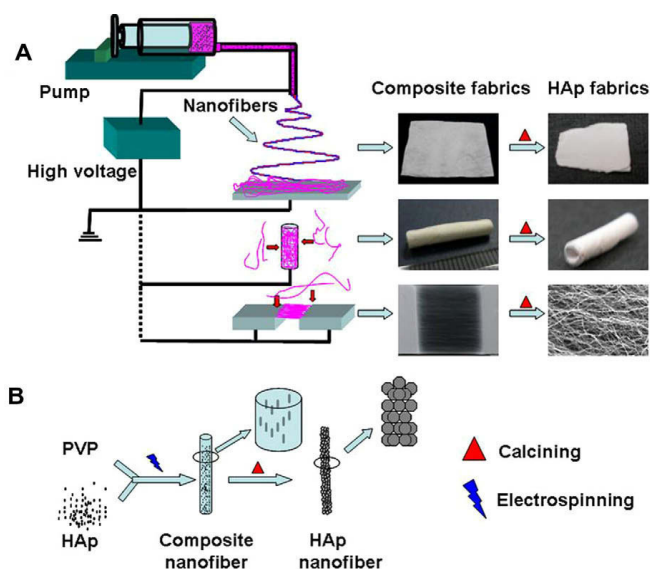ium phosphates are similar to the inorganic component in bone tissue. Thus, synthetic calcium phosphates with strong affinity to host hard tissues offer a great advantage in hard tissue repair.

However, the low mechanical properties of traditional calcium phosphate ceramics restrict their use [28]. Recent novel ceramics sintered with nano-sized grains of calcium phosphate have reignited interest in load-bearing applications. Compared with traditional calcium phosphate based materials, nano-scaled calcium phosphate materials have shown many advantages, such as improved sinterability, enhanced mechanical properties and better bioactivity. Bose et al. [81] reported nanostructured HA ceramics with average grain sizes ranging from 168 ± 86 nm to 1.16 ± 0.17 μm were processed using microwave sintering. The HA compact with smaller sized grains
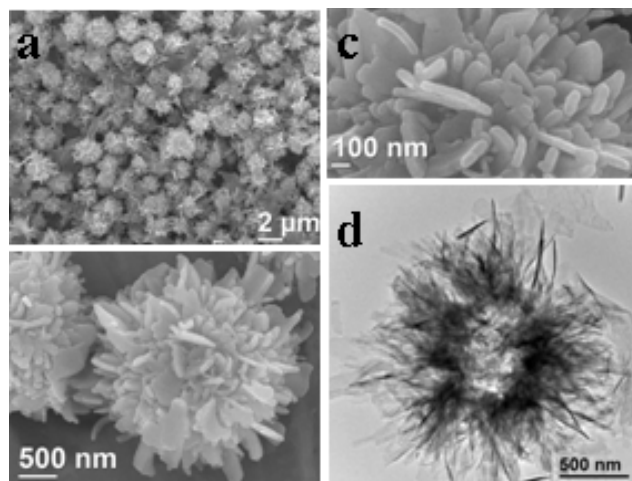
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Fig. 5 SEM and TEM micrographs of the sample prepared using 3.5 g/L PLA-PEG and 20 mM [Ca2+] by microwave heating at 120oC for 60 min. Reprinted from Ref. [62].

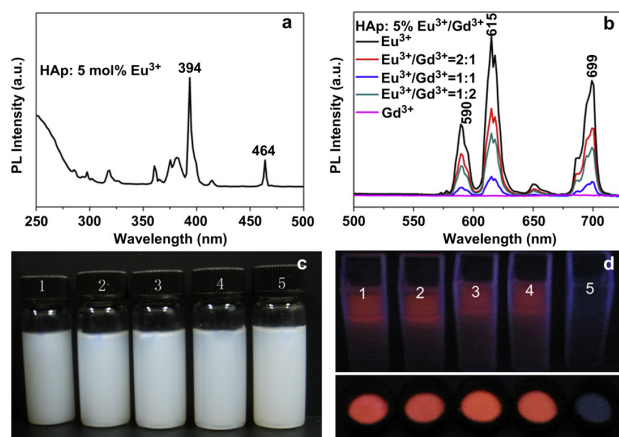**Fig. 6** Luciferase activity in COS-7 cells transfected with pGL3-control vector complexed with calcium phosphate/PLGA-mPEG hybrid porous nanospheres. The amount of DNA in the legend was for a well of 96-well plate. The final concentration of Ca2+ was 5 mM. The amount of Calcium phosphate/PLGAmPEG hybrid porous nanospheres was 0.2 mg per well. Custom Calcium phosphate transfection was carried out according to the literature. Reprinted from Ref. [49]

Fig. 7 Room-temperature excitation spectrum (a) and PL emission spectra (b) of Eu3+/Gd3+-HAp nanorods; (c) the photograph of dispersed solutions of Eu3+/Gd3+-HAp nanorods in deionized water; and (d) the PL photograph of Eu3t/Gd3teHAp nanorods powder and their dispersed solutions under irradiation by UV lamp (365 nm). The doping concentrations of Eu3+ and Gd3+ were 5 mol% relative to Ca2+, and the ratios of Eu3+ to Gd3+ in (c) and (d) from 1 to 5 were 1:0, 2:1, 1:1, 1:2 and 0:1. Reprinted from Ref [98].

showed the higher compressive strength. The decrease in strength due to increase in grain size was less prominent in HA than what would be predicted by the classical Hall–Petch equation. An increase in bone cell adhesion and proliferation with decreasing grain size was observed. Lin et al. [82] reported that the maximal values of the bending strength, elastic module, Vickers hardness and compressive strength of the samples fabricated from nano-size β-TCP powders were more than two-times those of bioceramics obtained from micro-size β-TCP powders. The degradability of β-TCP ceramics sintered from nanosized powders was just about one-quarter of that sintered from microsized powders, and the degradability of could be adjusted by the particle sizes. Beside the ceramic materials, porous 3-D nanocomposites of calcium phosphate and polymer can also be employed in bone repair. Cui et al. [83,84] developed nanosized
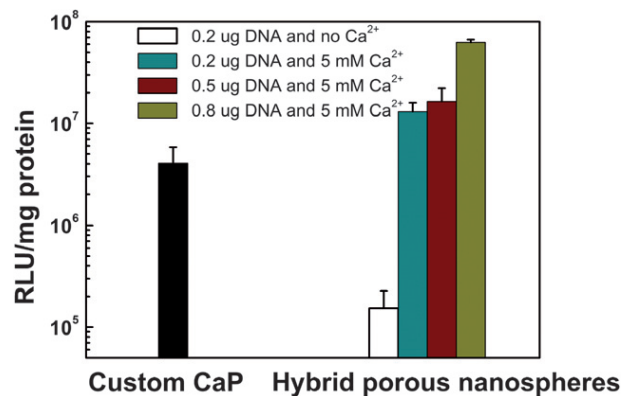
hierarchical self-assembly of mineralized collagen nano-fibrils that mimicked the nanostructure of bones. HA crystals grew on the surfaces of the collagen fibrils. The mineralized collagen fibrils aligned parallel to each other to form mineralized collagen fibers. The investigation and simulation of naturally occurring fibril structures can offer some new ideas in the design and fabrication of new functional materials for applications such as bone grafts or for use as scaffolds in tissue engineering and biomimetic engineering materials [84]. Li et al. [85] reported porous 3-D nanocomposites of n-HA/PA66 as tissue engineering scaffold material prepared by co-precipitation method. The composite had excellent mechanical properties close to that of natural bone. The porous material had not only macropores but also micropores on the walls of the macropores. Such biomaterial with well-controlled composition and porous structure can be a good bone repair materials and can provide a standard scaffold for investigating the cell/material interaction in tissue engineering.

Our group has reported HA fabrics which can be used as the bone tissue engineering scaffold. The single-phase HA fabrics, tubular morphologies or aligned nanofiber arrays were obtained through thermal treatment of corresponding electrospun HA/PVP composite nanofibers [78]. The as-prepared HA fabric was used as the substrate for MSCs culture. The results exhibited that the HA scaffold was biocompatible, and the cells could attach well on the HA fabric and combine tightly with HA fibers. In addition, the ACP/PDLLA composite nanofibers were also fabricated through electrospinnig [79]. The ACP/PDLLA composite nanofiber matrix showed a good biocompatibility when osteoblast-like MG63 cells were seeded. The ACP/PDLLA composite nanofibers exhibited a fast mineralization behavior in the simulated body fluid.

4.2 Drug/gene delivery

Bone not only acts as a reservoir for calcium and phosphate, but also stores growth factors, fatty acids and

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is involved in buffering the blood by controlled release of alkaline salts [86]. Calcium phosphate as the inorganic constituent in bone is biocompatible, therefore, synthetic calcium phosphate materials are ideal biomaterials for drug/gene delivery.

Our group reported a flower-like nanostructured HA hollow microspheres fabricated via a rapid microwave-assisted hydrothermal route, and explored its application as anticancer drug carrier for cellular delivery of mitoxantrone (Fig. 5) [62]. The materials exhibited sustained drug release behavior in vitro, and the intracellular drug distribution tests indicated that the MIT loaded in carriers could enter the cells efficiently. Cai et al.[87] reported hollow-structured calcium phosphate nanospheres which could be transformed into pin-shaped crystallites under ultrasonic treatment. The release of encapsulated compounds could be on/off triggered and the kinetics was precisely regulated by the power density, duty cycle and application time of ultrasound.

It was reported that calcium ions increased the in vitro transfection efficiency of pDNA–cationic liposome complexes from three- to 20-fold.[88] In the review by Maitra, calcium ions were described to play an important role in endosomal escape, cytosolic stability and enhanced nuclear uptake of DNA through nuclear pore complexes. The special role of exogenous calcium ions to overcome obstacles in practical realization of this field suggests that calcium phosphate nanoparticles can be designated as second-generation nonviral vectors for gene therapy [89].

Early methods of gene transfer using calcium phosphate materials, involved coprecipitating DNA with calcium phosphate [90]. But irregular particle morphology and large size impeded using calcium phosphate precipitate as a carrier for DNA. This group reported the preparation of calcium phosphate/PLGA-mPEG hybrid porous nanospheres and their application in gene delivery [49]. Calcium phosphate/PLGA-mPEG hybrid porous nanospheres exhibited very high DNA loading capacity (approximately 40–150 times higher than that of the mesoporous silica vectors reported) and good transfection efficiency. Unlike the traditional calcium phosphate transfection procedure in which calcium phosphate usually precipitates in the presence of DNA molecules, the reported method consisted of two steps: preparation of calcium phosphate nanostructured vectors in the absence of DNA, and then loading and transfection of DNA. HA nanorods were also used as the gene carrier (Fig. 6) [52]. The results demonstrated a significant enhancement of DNA adsorptive capacity of HA nanorods compared with traditional DNA adsorbents. And adsorbed DNA could be desorbed reversibly from HA nanorods with high eluted percentage.

4.3 Multifunctional nanostructured calcium phosphate

The development of multifunctional nanostructured systems holds a promise for the future of clinical treatments to enhance therapeutic efficacy [91, 92]. It is highly desirable to develop novel multifunctional nanostructured systems that can achieve simultaneous in vivo imaging and treatment. Due to the biocompatible nature, HA nanostructures may serve as the ideal candidate for both bio-imaging and drug delivery. Recently, the research on dual or multifunctional nanostructured HA systems for biomedical applications has become a hot topic [93-97].

Our group reported a facile method for the preparation of ACP/PLA-mPEG hybrid nanoparticles which were successfully used as the precursor for preparation of ACP porous nanospheres [99]. Photoluminescence function of ACP porous nanospheres was achieved by europium doping. The experimental results of photoluminescence, cytotoxicity as well as in vitro drug loading and release showed that the as-prepared Eu3+:ACP porous nanospheres were biocompatible and bioactive with favorable properties of photoluminescence, drug loading and drug release, implying Eu^{3+}:ACP porous nanospheres are a new kind of promising biomaterial with bi-functions of both luminescence and drug release. Multifunctional Eu^{3+}/Gd^{3+} dual-doped HA nanorods were prepared by a rapid microwave-assisted method [98]. The dual-doping of Eu^{3+}/Gd^{3+} endowed HA nanorods with photoluminescent and magnetic multifunctions. The PL intensity and magnetization of doped HA nanorods could be adjusted by varying Eu^{3+} and Gd^{3+} concentrations (Fig. 7). The as-prepared Eu^{3+}/Gd^{3+} doped HA nanorods exhibited inappreciable toxicity to the cells in vitro, and showed a high drug adsorption capacity and sustained drug release using ibuprofen as a model drug. The noninvasive visualization of nude mice with subcutaneous injection indicated that the Eu^{3+}/Gd^{3+} doped HA nanorods with the photoluminescent function are suitable for in vivo imaging. The Eu^{3+}/Gd^{3+} dual-doped HA nanorods are promising for applications in the biomedical fields such as multifunctional drug delivery systems with imaging-guidance. Furthermore, Adair et al. [100-102] reported calcium phosphate nanocomposite particles that encapsulated both fluorophores and chemotherapeutics, within a 20-30 nm diameter, pH-responsive, nonagglomerating, nontoxic calcium phosphate nanoparticle matrix. Encapsulation of imaging agents and drugs in calcium phosphate nanoparticles has a potential as a nontoxic, bioresorbable vehicle for drug delivery to cells and tumors.

5 Conclusion

To optimize and achieve better performances, controlling the structure and size of nanostructured calcium phosphate materials has become a hot field. Up to now, many methods have been reported to prepare nanostructured calcium phosphates, such as co-precipitation, sol–gel synthesis, hydrothermal reaction, mechanical milling, etc. Nanostructured calcium phosphate materials with a variety of morphologies including nanorods, nanosheets, nanoparticles, biomimic structure, novel 3-D structures, have been

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successfully obtained. The performances of calcium phosphates in application depend greatly on their morphologies, structure and chemical compositions. These nanostructured calcium phosphates are promising biomaterials for applications in tissue engineering scaffolds, drug/gene delivery and other medical areas. Furthermore, it is highly desirable to design and prepare novel nanostructured multifunctional systems based on calcium phosphates to achieve simultaneous in vitro/vivo imaging and treatment.

Acknowledgements

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