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REVIEW ARTICLE

Silicate-doped hydroxyapatite and its promotive effect on bone mineralization

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ABSTRACT: Bone defect is one of the most common diseases in clinic. Existing therapeutic approaches have encountered many problems, such as lack of autogenous allogeneic bone and immunological rejection to allogeneic implant. Synthetic hydroxyapatite (HA) provided solutions for bone repair, since the HA is the main inorganic component of animals' bone. Although HA has good biocompatibility, but only the limited osteogenic capability, which is of significance for modern bone repair materials. Si is an essential trace element in bone tissue, and it has been demonstrated to be able to promote bone formation. Therefore, silicate-doped hydroxyapatite (Si–HA) may serve as a promising material for bone repair, and promote bone regeneration in the repair. The current review discusses development of Si–HA, focusing on its preparation and characterization, *in vitro* and *in vivo* evaluations of the material, positive effect of Si–HA on promoting bone formation in clinical applications, and molecular mechanism investigation of such promotive effect.

KEYWORDS: silicate-doped hydroxyapatite (Si–HA); osteogenesis; collagen biosynthesis; bone mineralization

Contents

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1 Introduction

Hydroxyapatite (HA) is the major inorganic component of animals' hard tissues, such as bone and teeth [1–3]. In 1950s–1960s, researchers paid attention to HA in animals and investigated HA and biomineralization in bone, enamel, cartilage and so on [1,4–5], as well as started to synthesize HA [6–8]. In 1960s, crystal structure of HA was analyzed and resolved [9–10]. In the decades that followed, HA was investigated more widely and thoroughly, many HA-involved materials and products were developed and some of them have been proceeded into clinical application, including nanosized HA, element-doped HA, HA coating, template-mediated HA, HA/organic composite and so on [11–16]. Moreover, application of HA have also

been expanded to other field of biomedical materials, for example, drug carrier and gene transfection material [17–18], thus enriched content of HA research and enhanced its application value.

Element-doped HA is a modified HA with specific site being doped with trace element, including Zn, F, Sr, Mg and so on. There are three types of element-doped HA according to the doping site: doping at Ca^{2+} site, doping at PO_4^{3-} site, and doping at channel-ion (OH^-). Element doping could bring special biological functionalities to HA, thereby improving its applications in bone tissue engineering and other biomedical fields. For example, Zn-doped HA could increase bone density and promote bone formation [19–20], and is able to down-regulate inflammatory response that would induce aseptic loosening of the implant [21–22]; Se-doped HA could both promote proliferation of normal osteocytes and inhibit bone tumor [13].

Silicon is an essential trace element existing in many organs of animals, although there is very little amount of Si needed [23]. As early as 1970s, the role of Si in bone mineralization was investigated. Carlisle et al. observed assembly of Si at active calcification sites of infantile rat and mouse (0–28 days) by electron microprobe analysis. Ca content was very low at the earliest calcification stage in these sites, and both Ca and Si contents gradually increased along with the progress of the calcification. As the completion stage of the calcification, bone tissue formed and Si content decreased evidently. When the percentage of Ca approached that in the HA, Si content has dropped to detection limit of electron microprobe analysis [24]. Carlisle et al. further found that the intake amount of Si is very important to bone formation. Increased Si intake could increase the ash content of tibia of low-calcium-fed rat by 35%, indicating Si could promote bone mineralization [25]. Related studies also found that Si is able to increase extracellular matrix of connective tissue, polysaccharide content of the matrix and the contents of hydroxyproline, total protein and collagen [26].

Si in animal mainly exists in connective tissues (such as tendon, skin and so on) and bone. Lack of Si may lead to bone diseases and joint diseases. Carlisle et al. found that compared to normal fed chicken, low-silicon-fed chicken had shorter and smaller leg bones with thinner cortex, as well as femur and tibia that much easier to fracture. The weights of the low-silicon-fed chicken were also much lower than the normal ones [27]. Schwartz et al. found that Si deficient diet made rats contracted diseases of skull and peripheral bones deformities, poor formation of joints and defective endochondral bone growth. The contents of articular cartilage and collagen were also reduced, and there were low concentrations of minerals (Ca, Mg, Zn, Mn, etc.) in the femur and vertebrae [28].

Therefore, modification of HA by Si doping could introduce novel biological functionalities to such traditional biomaterial and play more important roles in bone repair. In recent decades, many studies were focused on Si-doped HA (Si–HA), which mainly involved Si in the form of orthosilicate, and related products have been being developed.

2 Preparation and characterization of Si–HA

Some of Si-containing compounds can serve as silicon source in the preparation of Si–HA, for example, tetraethyl orthosilicate ($Si(OC_2H_5)_4$) and silicon acetate ($Si(CH_3COO)_4$). Wet chemical methods were most commonly used synthetic routes, such as precipitation method and hydrothermal method, since the silicon source needs to hydrolyze to produce orthosilicate ions. The reaction mechanism was proposed by as:

$$10Ca^{2+} + (6-x)PO_4^{3-} + xSiO_4^{4-} + (2-x)OH^- \rightarrow Ca_{10}(PO_4)_{6-x}(SiO_4)_x(OH)_{2-x}\square_x \quad (1)$$

wherein, $0 \leqslant x \leqslant 2$. In the preparation reaction, SiO_4^{4-} is supposed to substitute PO_4^{3-} in the lattice, as shown in Fig. 1 [11]. Accordingly, a portion of hydroxyl group would be lost to retain charge balance within the molecule, thus resulting in vacancy at the position of the hydroxyl group (represented by the symbol "□" in Eq. (1)).

Si–HA prepared by wet chemical method was firstly reported by Gibson et al. Si–HA with the Si content of 0.4 wt.% was prepared by a neutralization precipitation reaction of $Ca(OH)_2$ and H_3PO_4 and silicon acetate was used as the silicon source. Si doping did not affect the X-ray diffraction (XRD) patterns for both as-prepared and 1200°C sintered samples at such low doping level [29].

However, higher Si doping content could affect the crystallinity of Si–HA and increase the amount of amorphous phase [30], as shown in Fig. 2 [11]. Accordingly, crystal size of Si–HA decreases with the increase of Si content [11,30]. Tian et al. prepared Si–HA with Si content ranged from 0.5 wt.% to 2.0 wt.% by using tetraethoxysilane (TEOS) as the silicon source via a wet

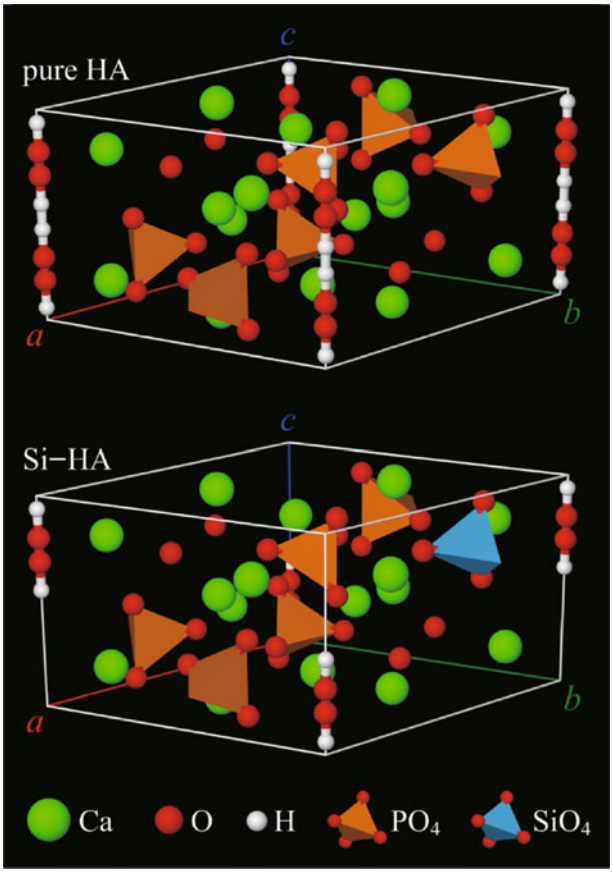

Fig. 1 Schematic presentation of the cell lattice comparison between pure HA and Si–HA. The loss of the hydroxyl group indicates the decrease of occupancy, since the substitution of SiO_4^{4-} for PO_4^{3-}. (Reproduced with permission from Ref. [11], Copyright 2012 IOP Publishing Ltd.)

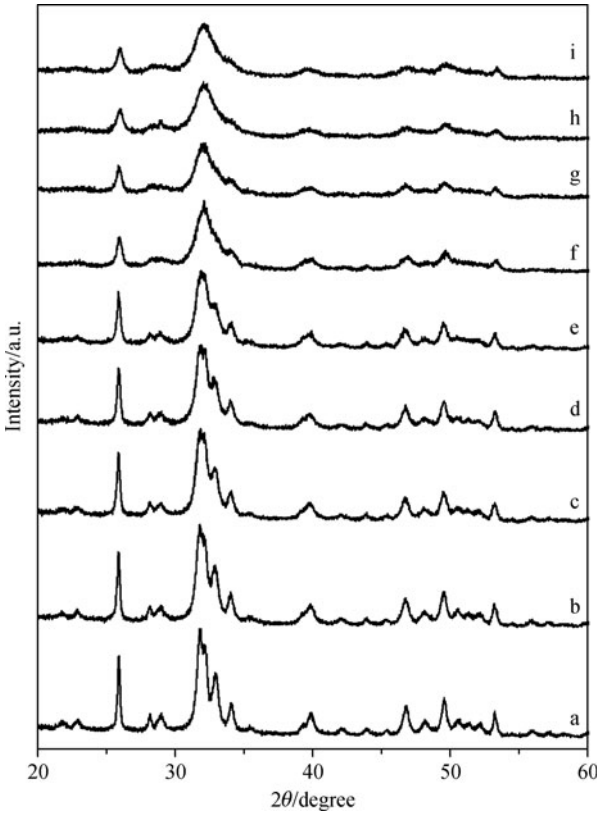

Fig. 2 XRD patterns of as-prepared Si–HA samples: pure HA (a); 0.4 wt.% Si–HA (b); 0.8 wt.% Si–HA (c); 1.2 wt.% Si–HA (d); 1.6 wt.% Si–HA (e); 2 wt.% Si–HA (f); 3 wt.% Si–HA (g); 4 wt.% Si–HA (h); 5 wt.% Si–HA (i). (Reproduced with permission from Ref. [11], Copyright 2012 IOP Publishing Ltd.)

mechanochemical method, the average particle size gradually decreased from 0.93 μm of pure HA to 0.44 μm of 2.0 wt.% Si-containing Si–HA [31].

Si doping also takes effects on the sintering properties of Si–HA. Similar to as-prepared material, Si doping showed a negative effect on the particle size of sintered Si–HA [32]. Gibson et al. also demonstrated that densification temperature of Si–HA in the sintering was raised with the increase of Si content [32]. As shown in Fig. 3, Si doping would influence phase composition of sintered Si–HA by modifying its phase transition temperature. It is reported that when Si content exceeded 2 wt.%, Si–HA largely or even completely transformed to the β-tricalcium phosphate (β-TCP) phase without any α-tricalcium phosphate (α-TCP) after a sintering at 1250°C, indicating the decomposition temperature of HA was decreased, but the phase transition temperature from β-TCP to α-TCP was increased by Si doping [11].

Crystalline structure of Si–HA is inevitably influenced by Si doping, such as modifications of lattice parameters and atomic site occupancy. Many related studies were performed to analyze such modifications via Rietveld refinement of XRD data. However, many quite different, even opposite study results on the modification of the lattice parameters were reported. Table 1 lists some refinement conclusions [11,29,31,33–36], which were largely determined by the preparation method, the raw materials, and the refinement strategy. Anisotropic features of particle size and microstrain were recently considered in the Rietveld refinement of Si–HA. The refinement quality could be improved by comprehensively considering these factors. It is reported that the calculated anisotropic particle sizes corresponded to transmission electron microscopy (TEM) observations of Si–HA, and the microstrains were also demonstrated to possess anisotropic distributions [11]. All the Rietveld refinements concerning atomic occupancy indicated that hydroxyl site occupancy within Si–HA lattice decreased, which could also be demonstrated by Fourier transform infrared spectroscopy (FTIR) that

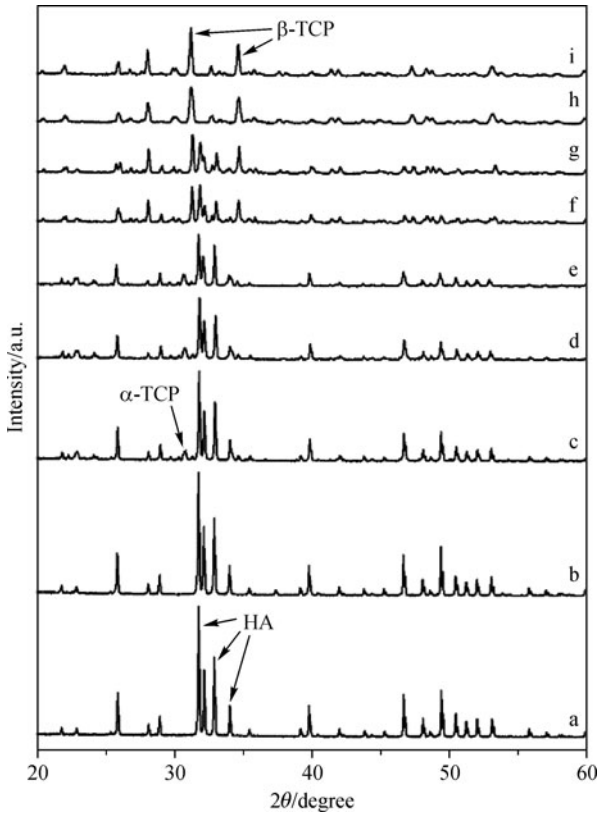

Fig. 3 XRD patterns of 1250°C sintered Si–HA samples: pure HA (a); 0.4 wt.% Si–HA (b); 0.8 wt.% Si–HA (c); 1.2 wt.% Si–HA (d); 1.6 wt.% Si–HA (e); 2 wt.% Si–HA (f); 3 wt.% Si–HA (g); 4 wt.% Si–HA (h); 5 wt.% Si–HA (i). (Reproduced with permission from Ref. [11], Copyright 2012 IOP Publishing Ltd.)

absorption bands at about 3569 and 631 cm^{-1} became weaker as Si doping content increased [11].

Some other studies were performed to characterize Si–HA in terms of surface energy, solubility and so on. Botelho et al. investigated surface Zeta potential of Si–HA and indicated that the surface charge of Si–HA was much lower than the pure HA at physiological pH = 7.4. Therefore, Si–HA was in favor of Ca^{2+} adsorption and easier to form amorphous apatite layer on the surface, thus showing a better bioactivity [37].

3 *In vitro* and *in vivo* studies of Si–HA

As a biomaterial, Si–HA ought to possess good biocompatibility. In addition to this, Si–HA should be able to take a promotive effect on formation of bone according to its original design, since the doped Si has been proved to be indispensable and positive for bone mineralization, as mentioned above. In recent decade, many studies were focused on biological evaluations of Si–HA *in vitro* and *in vivo*.

In vitro studies showed good adhesion and proliferation of cells on Si–HA materials, including osteoblast [38–39], fibroblast [40] and so on. Figure 4 shows proliferation of human bone mesenchymal stem cells (bMSCs) on sintered Si–HA discs determined by water-soluble tetrazolium salt-8 (WST-8) assay, and Fig. 5 is scanning electron microscopy (SEM) observation of human bMSCs viability on the same discs. Cell morphology observations suggested similar conclusion that no difference was found between cells on Si–HA and pure HA [40]. There were also reported that Si–HA with specific Si contents exhibited better biocompatibility than the pure HA via total DNA quantification or growth activity evaluation of osteoblastic cells [41–42].

Other than traditional bioinert materials, bioactivity is a significant property for modern biomaterials. As shown in Fig. 6, compared to pure HA, Si–HA was reported to effectively up-regulate osteogenic-related gene expressions of osteoblastic cells *in vitro*, including alkaline phosphatase (ALP), bone morphogenetic protein 2 (BMP-2) and type I collage (Col I), thus suggesting a good osteogenesis ability of Si–HA [41]. However, it does not mean the more Si doped, the better Si–HA is. Botelho et al. investigated human osteoblast responses to Si–HA, and suggested that the expression of osteoblast markers (including Col I, ALP, osteocalcin and bone mineral formation) on 0.8 wt.% Si–HA were higher than those on 1.5 wt.% Si–HA [39].

In addition, *in vitro* studies of Si–HA were also performed in simulated body fluid (SBF) to evaluate the

Table 1 Crystal lattice variations of Si–HA with the increase of Si content [11]

Si source	Ca source	P source	Preparation method	*a* axis	*c* axis	Cell volume	Refs.
STA	CH	PA	PPN	decrease	increase	slight decrease	[29]
TEOS	CN	AP	HT	decrease	increase	slight decrease	[33]
STA	CH	PA	PPN	slight decrease	increase	increase	[34]
TEOS	CH	DAP	PPN	increase	increase	increase	[31]
STA	CN	DAP	PPN	increase	irregular	increase	[35]
STA	CH	PA	PPN	decrease	increase	increase	[36]

Note: STA, $Si(CH_3COO)_4$; TEOS, $Si(OCH_2CH_3)_4$; CH, $Ca(OH)_2$; CN, $Ca(NO_3)_2$; PA, H_3PO_4; AP, $(NH_4)_3PO_4$; DAP, $(NH_4)_2HPO_4$; PPN, precipitation method; HT, hydrothermal method.

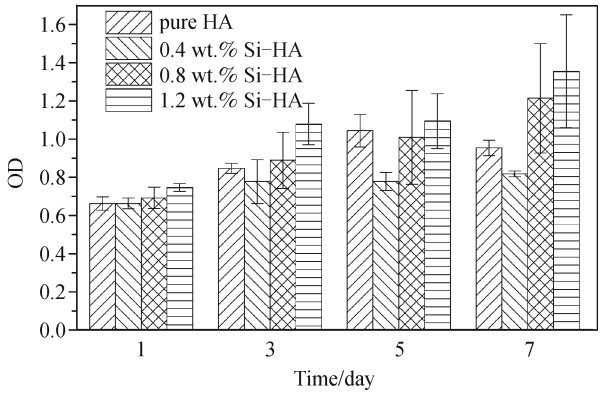

Fig. 4 Proliferation of human bMSCs on sintered Si–HA discs determined by WST-8 assay.

formation of apatite deposits. Such evaluation in SBF was considered to be useful for predicting *in vivo* bioactivity of a biomaterial and has been accepted by many R&D activities [43–45]. *In vitro* bioactivity studies on Si–HA using SBF demonstrated that the formation of apatite layer on the surface of Si–HA was significantly improved with respect to pure HA [46].

In vivo studies further demonstrated good osteogenesis activity of Si–HA. Hing et al. investigated the effects of Si–HA on bone formation rate and quality *in vivo* by using porous scaffolds with Si content that did not exceed 1.5 wt.% [47]. The scaffolds were fabricated by a foaming technique and then sintered at 1250°C to form the porous structure, and the phase compositions were demonstrated

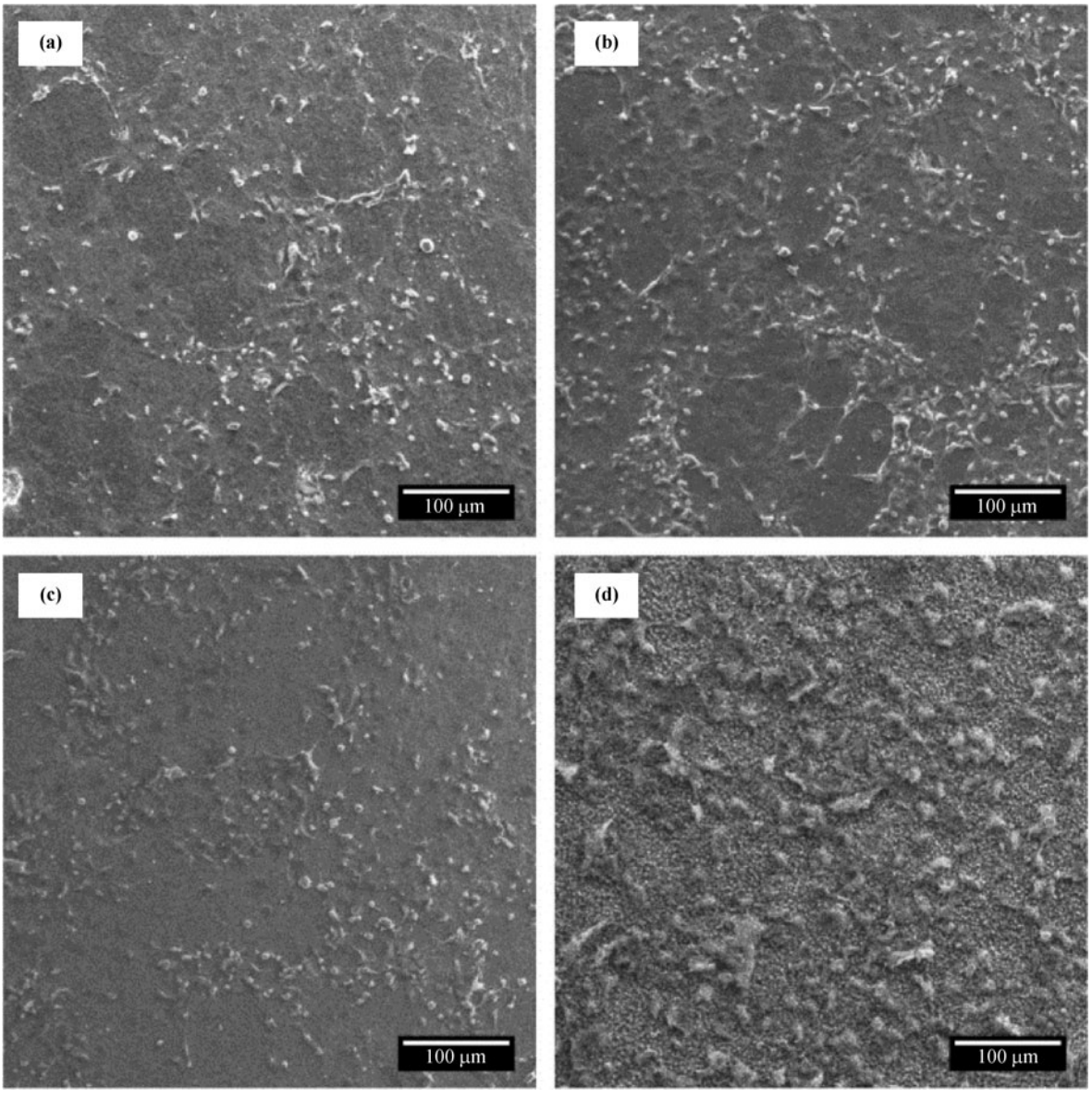

Fig. 5 SEM observation of the viability of human bMSCs on sintered Si–HA discs: **(a)** pure HA; **(b)** 0.4 wt.% Si–HA; **(c)** 0.8 wt.% Si–HA; **(d)** 1.2 wt.% Si–HA.

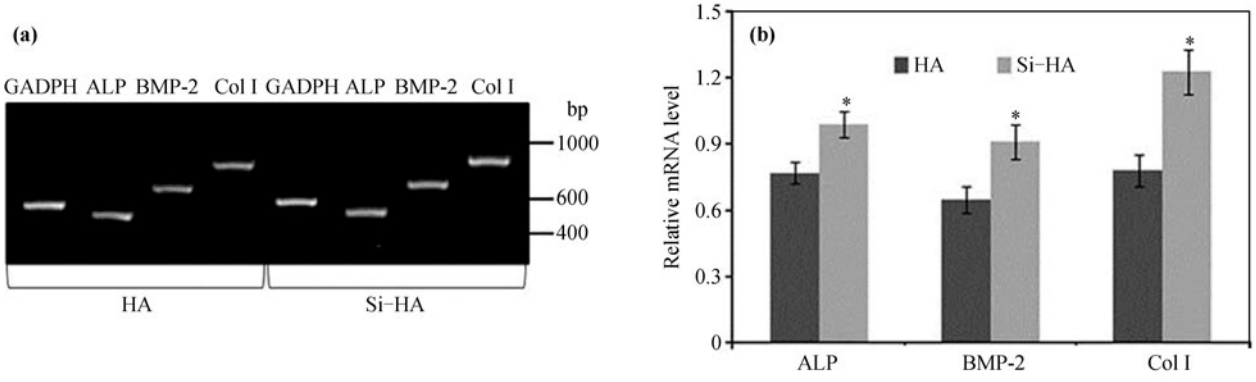

Fig. 6 Reverse-transcriptase polymerase chain reaction (RT-PCR) results of the expression of osteogenic-associated genes for 14 days on the surface of pure HA and Si–HA. **(a)** Agarose gel electrophoresis results. **(b)** Corresponding densitometric analysis. (Reproduced with permission from Ref. [41], Copyright 2010 John Wiley and Sons)

to be HA phase by XRD and doped Si was in the form of SiO_4^{4-} proved by FTIR. *In vivo* studies were performed for periods of 1, 3, 6 and 12 weeks. Volume of bone in-growth was obtained via histological observation and histomorphometric quantification to evaluate the osteogenesis activities. As the results shown in Fig. 7, 0.8 wt.% Si–HA exhibited the best effect on promoting bone in-growth and was considered to be an optimum level of Si doping for bone defect repair [47].

Another *in vivo* study was performed to investigate the interaction between the host and Si–HA implant via microcosmic observation of remodeling process on bone-implant interface by ultramicrotomy and TEM [48]. Porter et al. implanted sintered granules of HA and Si–HA into femoral condyle of sheep for 6 and 12 weeks. The animals were sacrificed at each time point and 70–90 nm thick sections of bone/implant interface were studied by TEM and selected area electron diffraction (SAED), as well as scanning transmission electron microscopy (STEM) combined with energy dispersive X-ray spectroscopy (EDX). The results indicated that remodeling of bone surrounding the Si–HA was promoted compared to pure HA, since formation of organized collagen fibrils at the bone/Si–HA interface was earlier than that around pure HA implant, and there were many more nodular aggregates observed near Si–HA implants than that around pure HA ones. Furthermore, TEM observations of bone/Si–HA interface revealed that trabecular bone integrated with Si–HA and collagen fibrils also formed mechanical interlocks with Si–HA, indicating a favorable osteoconductivity of Si–HA.

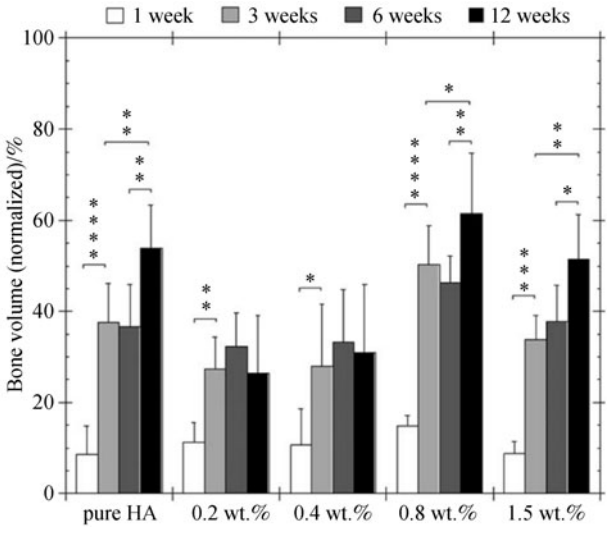

Fig. 7 Normalized volumes of new bone within pure HA and Si–HA scaffolds *in vivo* (data: mean, error bars = SD, $*P < 0.05$, $**P < 0.005$, $***P < 0.0005$, $****P < 0.0001$). (Reproduced with permission from Ref. [47], Copyright 2006 Elsevier)

4 Clinical studies of Si–HA products

Thanks to the beneficial effect on bone formation, Si–HA has been rapidly translated into clinical practice. It was reported that some implants made of Si–HA have been employed in many orthopedic surgeries, such as spinal fusion, bone defect repair and so on, and good results were achieved.

It was reported that synthetic Si–HA ceramic implant has been used for spinal fusion. Lumbar spinal fusion surgeries were performed for 57 levels of 42 patients. Follow-up of 24 months showed that 76.5% of levels exhibited fusion at the 24th month, but ectopic bone formation or osteolysis was not observed. Besides, both back and leg pain scores were significantly improved, which were not second to other bone grafts [49].

Nagineni et al. assessed spinal fusion outcomes by using Si–HA for 108 patients and their 204 individual spinal

levels, covering each level of the spine and both anterior and posterior operations. The statistical result showed that 90% of the patients demonstrated radiographical fusion at 12-month follow-up. Cervical spine achieved highest fusion rate, followed by thoracic and lumbar spines. Radiographical loosening due to infection or nonunion was not observed, and there was no subsequent revision for nonunion [50].

In the field of bone defect repair, Si–HA granules were reported to be used as filler for benign bone defects in children. Clinical follow-up and radiological observations showed good healing results without intraoperative and short-term complications. After a few weeks of healing, all the patients were able to bear full weight. Cancellous bone reconstruction occurred at the defect for all the patients, and there was no osteolysis observed radiologically [51].

Nowadays, there are only a few Si–HA products and related clinical studies were seldom reported. Existing literatures show that Si–HA presented good repair effects in orthopedic applications. Use of Si–HA was considered to effectively eliminate, or at least reduce the need of autogenous bone graft.

5 Mechanism of promotive effect of Si–HA on bone mineralization

Many studies have revealed that Si–HA is beneficial to bone formation. However, related mechanism is not clear. Such mechanism is a complex and systematic study, which relates to physicochemical properties the material, cell-material interactions from cellular to molecular levels, target and pathway identification, and so on.

Porter et al. investigated the dissolution of Si–HA and suggested that Si doping formed small crystals and thereby produced more grain boundaries and triple junctions (where three grains meet), which accelerated the dissolution of Si–HA and was beneficial to the incorporation of the host tissue and the Si–HA implant [52–54].

Up-regulated expression of osteogenic-related genes by Si–HA has been demonstrated *in vitro* as mentioned above, but lack identified molecular mechanism. There has been a theory about the effect of SiO_4^{4-} on promoting the biosynthesis of the collagen. It was proposed that the major action of Si was to enhance bioactive of prolyl 4-hydroxylase (EC 1.14.11.2, P4H), a key enzyme in biosynthesis of collagen [55]. On rough endoplasmic reticulum (RER), P4H hydroxylates proline of preprocollagen (peptide chains) into hydroxyproline (a characteristic amino acid of collagen) to form procollagen [56]. In such hydroxylation, Fe^{2+} is an important cofactor. However, the binding site of Fe^{2+} could be competitively bound by Al^{3+}, thus inhibiting enzyme activity. SiO_4^{4-} is able to react with Al^{3+} to form aluminosilicate, so as to relieve the inhibitory effect on P4H activity [55].

We investigated the effect of Si–HA on P4H by enzyme-linked immunosorbent assay (ELISA) *in vitro*. Human bMSCs were seeded on to sintered Si–HA discs with different Si content. Total protein was extracted at the 1st and 7th day, and P4H was quantitatively determined by ELISA. The results shown in Fig. 8 indicated that Si–HA with certain Si content (0.8 wt.%) could increase P4H concentration in human bMSCs.

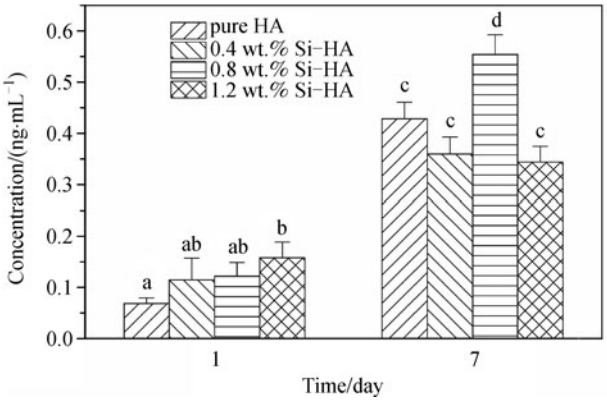

Fig. 8 ELISA determination of P4H concentration in human bMSCs seeded on sintered Si–HA discs.

Therefore, the promotive effect of Si–HA on bone mineralization could be summarized as shown in Fig. 9. Firstly, SiO_4^{4-} dissolved from Si–HA enters cell via endocytosis or non-specific ion channel. Then SiO_4^{4-} reaches RER and relieves inhibition of Al^{3+} on P4H activity. Finally, the activated P4H hydroxylates proline of preprocollagen into hydroxyproline to form procollagen, followed by further modification in Golgi apparatus and extracellular procedures processed by some other enzymes to form mature collagen fibrils.

The effect of SiO_4^{4-} on the enhancement of P4H activity is one probable pathway that Si–HA promotes bone mineralization. Besides collagen synthesis, alkaline phosphatase activity and osteocalcin expression were also demonstrated to be enhanced by orthosilicate acid in human osteoblast-like cells [57]. However, further investigations are required to identify more detail and comprehensive molecular mechanisms.

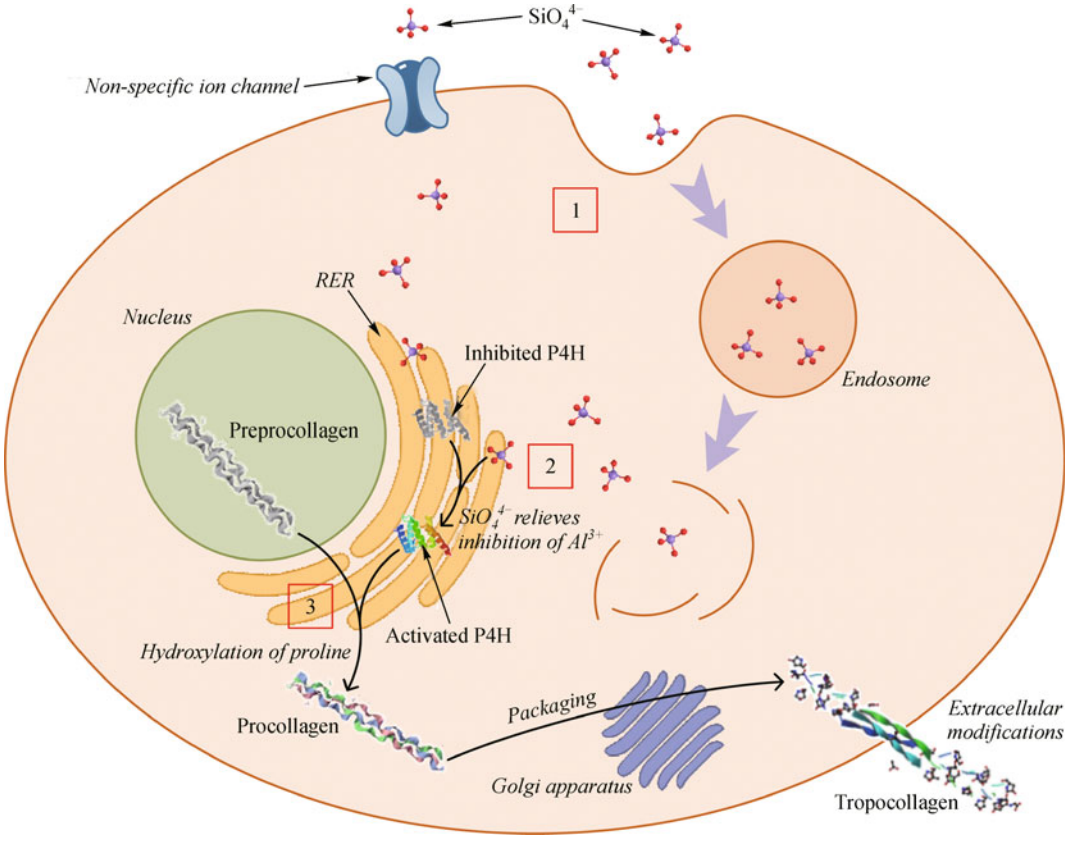

Fig. 9 Schematic diagram of a probable P4H pathway that Si–HA promotes biosynthesis of collagen.

6 Summary and outlook

Si–HA has been prepared by various synthetic routes using appropriate Si source. Si substitutes PO_4^{3-} tetrahedron in the form of SiO_4^{4-}. Phase of Si–HA with low Si percentage is consistent with that of HA, but higher Si doping decreases crystallinity and introduces impurity phase to the final product. Crystalline structure and sintering properties of Si–HA are also influenced by Si content. Crystal growth process and doping mechanism of SiO_4^{4-} need further investigations.

Many *in vitro* and *in vivo* studies demonstrated good biocompatibility and osteogenesis activity of Si–HA. Si–HA with certain Si content (usually considered to be 0.8 wt.%) is capable of enhancing the expression of osteogenic related genes. *In vivo* studies demonstrated that Si–HA could promote bone in-growth and form good osteointegration with the host bone tissue.

Implants made of Si–HA have been applied in clinical practices. Good repair effects were achieved for both spinal fusion and bone defect filling. Si–HA was considered to be able to reduce, or even substitute the use of autogenous bone graft.

The molecular mechanism of the promotive effect of Si–HA on bone mineralization is not identified. The positive effect of SiO_4^{4-} on P4H activity is probably a pathway. Modern molecular biology techniques, for example, DNA array could be employed to screen out up- or down-regulation of gene expressions, so as to explore more systematic and comprehensive molecular mechanism.

Abbreviations

ALP	alkaline phosphatase
BMP-2	bone morphogenetic protein 2
bMSC	bone mesenchymal stem cell
Col I	type I collage
EDX	energy dispersive X-ray spectroscopy
ELISA	enzyme-linked immunosorbent assay
FTIR	Fourier transform infrared spectroscopy
HA	hydroxyapatite
RER	rough endoplasmic reticulum
RT-PCR	reverse-transcriptase polymerase chain reaction
SAED	selected area electron diffraction
SBF	simulated body fluid
SEM	scanning electron microscopy

Si–HA	Si-doped HA
STEM	scanning transmission electron microscopy
α-TCP	α-tricalcium phosphate
β-TCP	β-tricalcium phosphate
TEM	transmission electron microscopy
TEOS	tetraethoxysilane
WST-8	water-soluble tetrazolium salt-8
XRD	X-ray diffraction

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