Silicon substitution in the calcium phosphate bioceramics

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Abstract

Silicon (Si) substitution in the crystal structures of calcium phosphate (CaP) ceramics such as hydroxyapatite (HA) and tricalcium phosphate (TCP) generates materials with superior biological performance to stoichiometric counterparts. Si, an essential trace element required for healthy bone and connective tissues, influences the biological activity of CaP materials by modifying material properties and by direct effects on the physiological processes in skeletal tissue. The synthesis of Si substituted HA (Si-HA), Si substituted α-TCP (Si-α-TCP), and multiphase systems are reviewed. The biological performance of these Si substituted CaP materials in comparison to stoichiometric counterparts is discussed. Si substitution promotes biological activity by the transformation of the material surface to a biologically equivalent apatite by increasing the solubility of the material, by generating a more electronegative surface and by creating a finer microstructure. When Si is included in the TCP structure, recrystallization to a carbonated HA is mediated by serum proteins and osteoblast-like cells. Release of Si complexes to the extracellular media and the presence of Si at the material surface may induce additional dose-dependent stimulatory effects on cells of the bone and cartilage tissue systems.

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1. Introduction

Due to the high demand for synthetic biomaterials to assist and replace skeletal tissues, and the high failure rate of these medical implants, a great deal of research focuses

on improving the strength of the implant–tissue interface, and in the design of implants that degrade in concert with the natural healing process [1].

Hard skeletal tissue is a complex composite consisting of cells embedded within a mineralized organic matrix. Bone mineral is calcium phosphate (CaP) based with a structural similarity to hydroxyapatite (HA; Ca₁₀(PO₄)₆(OH)₂) [2]. On account of this similarity, synthetic stoichiometric HA has
been extensively utilized as a skeletal replacement material. However stoichiometric HA has a limited ability to form an interface with, and to stimulate the development of, new bone tissue. Also, stoichiometric HA does not degrade significantly but rather remains as a permanent fixture susceptible to long-term failure [3]. In contrast, the mineral found in bone is not a stoichiometric compound, but exhibits variable deficiencies in Ca, P and OH [2]. Various substitutions exist in bone mineral, in particular carbonate ions that are found at up to 8 wt%, as well as elements such as Na, Mg, K, Sr, Zn, Ba, Cu, Al, Fe, F, Cl and silicon (Si) that occur at trace (<1 wt%) levels [1,4]. These substitutions in the apatite structure play important roles in the biological activity of both bone mineral and CaP-based implant materials that incorporate elemental substitutions, by influencing the solubility, surface chemistry and morphology of the material. Si in particular has been found to be essential for normal bone and cartilage growth and development. Synthetic CaP-based materials that include trace levels of Si in their structures demonstrate markedly increased biological performance in comparison to stoichiometric counterparts [5]. This increase in biological performance can be attributed to Si-induced changes in the material properties and also to the direct effects of Si in physiological processes of the bone and connective tissue systems.

2. Si in bone and cartilage physiology

Apart from oxygen, Si is the most abundant element in the earth’s crust. The presence of Si in mammalian systems is quite variable. Si is present at a level of ~1 ppm in the serum, 2–10 ppm in the liver, kidney, lung and muscle, 100 ppm in the bone and ligaments and 200–600 ppm in cartilage and other connective tissues [6]. In the examination of a variety of connective tissues using chemical methods, Si was found in high levels of 200–550 ppm bound to extracellular matrix compounds such as hyaluronic acid, chondroitin sulfate, dermatan sulfate, and heparan sulfate in connective tissues such as cartilage and the umbilical cord [6]. The high concentration of Si observed in extracellular matrix components implies a role for Si as a biological cross-linking agent that contributes to the architecture and resilience of connective tissue [6].

Si was first implicated in the early biomineralization process when electron microprobe examination of bones showed Si localized at active calcification sites in the bones of young mice and rats [7]. Si levels were observed to be highest (0.5 wt%) at the earliest stages of calcification, when Ca/P ratios are low (Ca/P = 0.7) while Si levels were observed to fall below the detection limit with more advanced mineralization as the Ca/P of bone increased towards that of HA, Ca/P = 1.67 [7]. The presence of aqueous Si has been shown to act directly in the mineralization process, where aqueous Si, in the form of orthosilicic acid (Si(OH)4), is able to induce the precipitation of HA from electrolyte solutions in the presence of proteins that normally inhibit its precipitation [8,9].

The role of Si as an essential element for higher biological organisms (i.e. other than primitive diatoms, sponges and plants such as bamboo) was discovered in the 1970s through the deficiency studies of Carlisle [10,11], Schwarz [12] and Seaborne [13]. Carlisle [10] performed an initial Si deficiency study that demonstrated the significant dependence of healthy skeletal development on Si. Chicks were fed a diet consisting of casein and corn with a very low Si content of 2 μg/g with half of the animals supplemented with 100 mg/g Si as sodium metasilicate. At 26 days the average mass of Si deprived chicks was 76 g, while for Si supplemented chicks the mass was 116 g. Deformities in the comb, skin, and bones were observed in the low Si chicks. Chicks deprived of dietary Si incurred fifteen times lower levels of Si in serum that were associated with deformation to the ends of tibia, femur and metatarsus [11]. Lower levels of collagen were observed in the cartilage of Si deficient animals, with no significant difference in the level of non-collagenous proteins [11]. In a similar study by Schwartz and Milne, rats raised with a low Si diet and environment were compared with rats supplemented with sodium metasilicate as a fresh aqueous solution at 50 mg Si/100 g of diet. The Si supplemented animals showed a 33.8% increase in the growth rate compared to the Si deficient animals [12]. In the Si deficient animals deformities were observed in the skull and tooth enamel and a lower water content of the bones coincided with a lower glycosaminoglycan content [12]. On the basis of these studies, Carlisle determined that Si should be categorized as an essential trace element for metabolic processes associated with development of bone and connective tissues and she has written several reviews, which give an overview of her findings [14–16].

A recent comparison of dietary Si intake with bone mineral density (BMD) in humans found that BMD was positively and significantly linked to dietary Si intake in men and premenopausal women [17]. BMD data was obtained from one lumbar spine and four hip sites, and adjustments were made for confounding factors known to affect nutrient absorption or BMD. Increased BMD was correlated to increased dietary Si intake at all hip measurement sites for men and premenopausal women (but not postmenopausal women), with BMD differences as large as 10% observed between the highest (>40 mg Si/day) and lowest (<14 mg Si/day) levels of Si intake.

Dietary Si supplementation also shows stimulatory effects on cartilage synthesis and may inhibit the physiological resorption process. In calves receiving 23 weeks of supplementation with a Si stabilized orthosilicic acid, a 70% increase in the blood serum levels of Si were observed, coinciding with an increased collagen concentration in the cartilage [18]. Studies using dietary supplementation of Si with ovariectomized mice indicate Si has roles in the remodeling process of bone [19,20]. Ovariectomized or control-operated (sham) animals were supplemented with either 0.1 or 1 mg/kg/day Si as orthosilicic acid in drinking water, or 10 μg/kg/day β-estradiol for a 1-month period.
Trabecular bone volume was decreased by 48% in untreated, ovariectomized animals, compared to sham operated, however for the treatment groups receiving either aqueous Si or estradiol, an inhibition of resorption was observed with a 21% decrease in the osteoclast area [19]. In a similar study, ovariectomized or sham-operated rats were fed a Si deficient diet, with half of each group supplemented with 35 mg/g dietary Si [20]. Si deficiency in ovariectomized animals lead to a decrease in the concentration of plasma osteopontin and sialic acid, and an increase in total helical peptides [20].

Aqueous Si has been shown to enhance osteoblast proliferation, differentiation and collagen production, and to have dose dependent effects on osteoclast cells under in vitro conditions. Following supplementation of cell cultures of human osteoblasts with orthosilicic acid at levels from 0 to 50 μM (0–1.4 ppm Si) a 1.8 fold increase in collagen type I synthesis was observed with 1.5 and 1.2 times increases in alkaline phosphatase and osteocalcin activities, implying an associated increase in osteogenesis [21]. Dosing homogenous human osteoblast cultures with 0.1–100 ppm aqueous Si for 48 h leads to a dose-dependent increase in osteoblast proliferation and cell differentiation through up-regulation of transforming growth factor beta (TGF-β) [22]. The dissolution products of Bioglass, which contain high levels of aqueous Si, stimulate osteogenesis and collagen synthesis [23]. Treating cultures with the ionic products of Bioglass, significantly increased cell proliferation, differentiation, collagen secretion and the viability of osteoblasts derived from rat calvaria in an in vitro culture [24]. Supplementation of an osteoblast culture with the ionic products from Bioglass showed significant effects on the gene expression of human osteoblast cells with notable increases to genes that encode proteins involved in osteoblast proliferation, extracellular matrix remodeling, and cell matrix attachment [23]. Similarly, the ionic products of pseudowollastonite (CaSiO3) have been observed to enhance osteoblast activities [25]. In vitro experiments on rat osteoclast cultures dosed with aqueous Si derived from Si-TCP materials show a dose-dependent effect of Si on osteoblast and osteoclast cells [26]. Osteoblasts are stimulated with aqueous Si levels from 0 to 100 ppm [26]. Osteoclasts show a more complex response, with levels below 30 ppm stimulating the development of osteoclasts, while higher levels of Si inhibit osteoclast development and resorption [26].

3. Si substitution in CaPs

The synthesis and characterization of Si substituted HA (Si-HA) and Si substituted z-tricalcium phosphate (Si-z-TCP) has been the focus of many research efforts [27–42]. Both Si-HA and Si-TCP based materials exhibit enhanced bone apposition, bone in-growth and cell-mediated degradation in comparison to stoichiometric HA controls. The synthesis of Si-HA and Si-z-TCP has focused on wet chemical methods where Si is introduced as a chemical carrier such as tetra ethyl or propyl ortho-silicate (TEOS or TPOS), Si IV acetate (Si(COOCH3)4) [27–32], or as some form of nano-particulate silica during the precipitation or firing of an amorphous CaP or nanocrystalline HA [33–37]. Solid state [39] and hydrothermal methods [41] of preparation have also been investigated. The materials are typically sintered at temperatures between 700 and 1200 °C. TriCaPs, unlike HA, do not precipitate from solution but rather are created from decomposition reactions at temperatures exceeding 700 °C [4]. A calcium-deficient HA or amorphous CaP material with a Ca/P ratio between 1.5 and 1.67 will decompose into β-TCP or a biphasic system of β-TCP and HA with sintering between 700 and 1125 °C [4]. Sintering above 1125 °C, the β-polymorph of TCP is the stable phase [4]. However, the presence of Si stabilizes the α-TCP polymorph, which can form at lower sintering temperatures of 700 °C [33–36].

The phase composition of the materials are highly dependent on the Ca/(P + Si) and Ca/P ratio of the system, the level of Si addition, the method of introducing Si to the CaP and sintering conditions, most notably the sintering temperature. Based on changes to lattice parameters with Si inclusion, and considerations of atomic radii, the simplest model of incorporation is that both Si-HA and Si-z-TCP accept substitutions of SiO4−4 for PO4−3 groups. To avoid a large cost in energy, local charge neutrality is mandated, and this requires some other defect to be associated with the PO4−3 to compensate for the charge deficit. There are many possible mechanisms for charge compensation and which predominates depends on the thermodynamic conditions during the preparation of the materials. Mechanisms that have been considered include O vacancies and excess Ca, which can each compensate for two Si substitutions. Also, an excess of H in Si-HA and Si-z-TCP can charge compensate. For Si-HA, OH vacancies can also provide compensation [28,30,34,35,43]. Si-HA is an interesting case because excess Ca, H and O or OH vacancies can charge compensate. Which of the mechanisms dominates depends on the availability of water during the manufacture, and it appears that different types of material can result [32,44–47]. This notwithstanding, chemical formulæ have been proposed for Si-HA and Si-z-TCP assuming that the charge compensation is OH vacancies and excess Ca, which can each compensate for two Si substitutions. Also, an excess of H in Si-HA and Si-z-TCP can charge compensate. For Si-HA, OH vacancies can also provide compensation [28,30,34,35,43]. Si-HA is an interesting case because excess Ca, H and O or OH vacancies can charge compensate. Which of the mechanisms dominates depends on the availability of water during the manufacture, and it appears that different types of material can result [32,44–47]. This notwithstanding, chemical formulæ have been proposed for Si-HA and Si-z-TCP assuming that the charge compensation is OH and O vacancies. These formulæ are Ca3(PO4)x(SiO4)3−x(OH)1−x and Ca3(P1−xSiO4−x/2)2, for Si-HA and Si-z-TCP, respectively [28,29,36,45].

Below a limiting Si concentration, single-phase Si substituted forms of HA and z-TCP share the same crystallographic space groups as their stoichiometric counterparts, with characteristic changes to lattice parameters occurring with Si substitution [28,29,36,45]. The space groups and lattice parameters of HA, Si-HA, z-TCP and Si-z-TCP are summarized in Table 1.

Different phase mixtures of Si-HA, α, and β-TCP, Si-z-TCP and other phases such as silicocarnitite and amorphous compounds are produced depending on the sintering temperature, the Ca/P and Ca/(P + Si) ratio of the system,
and the level of Si addition. Single-phase Si-HA of variable doping can be produced by maintaining a constant Ca/(P+Si) ratio of 1.67–1.75 as Si is added to the system at levels up to 1.97 wt% [33]. Correspondingly, it is possible to produce a single-phase Si-\(\alpha\)-TCP material by fixing the Ca/(P+Si) ratio at 1.50 as Si is added between 0.6 and 0.9 wt% [36]. If the Ca/P ratio is fixed with Si addition the system is mixed phase [34]. For example, for a Ca/P ratio fixed at 1.67 with Si additions up to 8 wt%, a mixed-phase system of Si-TCP, Si-HA, \(\beta\)-TCP and an amorphous phase of variable Si-Ca-P composition results [34]. The development of this phase composition also depends on the delivery of Si. When Si is introduced as an organometallic carrier such as TEOS or silicon acetate rather than a carrier such as TEOS or silicon acetate rather than a nanophase SiO\(_2\), a lower phase composition of \(\alpha\) or Si-TCP is observed and silicacarnotite is observed at higher doping levels [27]. This implies a role for a SiO\(_2\)-HA interface in the stabilization of a Si-\(\alpha\)-TCP phase originating from the reaction of HA with Si [35,43]. An alternative explanation is the inhibiting effect of CO\(_3\)\(^{-}\) on Si doping of HA might predominate: OH vacancy, SiO\(_3\)OH formation, or Si\(_2\)O\(_7\) formation due to a Si-HA interface of biological interest but rather the interface of the solid with a biological medium rich in water. Simulation of such a complex system is beyond the capability of the methods at present, but a first step has been taken by investigating the interaction of one or two H\(_2\)O molecules with the surface [54]. There is a strong interaction of H\(_2\)O with all the HA surfaces considered in natural bone. Ca loss from the surfaces in aqueous media, and especially the nature of HA platelets and protein action takes place leading to bone resorption and deposition. The \(ab\) initio methods can be used to simulate the material–vacuum interface, and studies of HA and \(\alpha\)-TCP surfaces have appeared [51,54]. If a bulk crystal is cleaved to produce a free surface, the atoms near the surface respond by relaxing their positions. The surface reconstruction of the CaPs that have been studied tends to neutralize charged surfaces, but the phosphate groups at the surface have under-coordinated oxygens, which lead to fairly reactive surfaces. HA surfaces have been studied most extensively [54] and there are several distinct high-index surfaces depending on the type of cut made. The surface energies can be estimated and their relative stability predicted. However, it is not so much the solid–vacuum interface that is of biological interest but rather the interface of the solid with a biological medium rich in water. Simulation of such a complex system is beyond the capability of the methods at present, but a first step has been taken by investigating the interaction of one or two H\(_2\)O molecules with the surface [54]. There is a strong interaction of H\(_2\)O with all the HA surfaces considered because of the exposed undercoordinated O atoms, and dissociation takes place in most cases. The HA (0 1 0)-type surfaces were the most reactive, and (0 0 1) the least, which may be related to the morphology of HA crystallites in aqueous media, and especially the nature of HA platelets found in natural bone. Ca loss from the surfaces in exchange for two H from the medium was also found to be very favorable energetically, which may have interesting consequences on the bioactivity of the materials [54].

4. Theoretical studies of Si substitution in CaPs

The nature of the structure of CaPs, in which covalently bound PO\(_4\)\(^3\)\(^-\) units are stacked in a columnar form and ionically bonded to Ca\(^2+\) has allowed theoretical computation of their structures using density functional theory (DFT) [49–51]. These are notable studies that use first principles or so-called \(ab\) initio methods based on density functional theory and pseudopotentials [52,53]. These studies have been used to simulate bulk and surface properties, and the effects of Si doping of HA and TCP. When appropriate questions are addressed, and great care is taken, the DFT methods have predictive capacity and these studies have revealed details of the energetics and structure on an atomic scale.

The simulations indicate which of the charge compensation mechanisms for Si doping of HA might predominate: OH vacancy, SiO\(_3\)OH formation, or Si\(_2\)O\(_7\) formation due to an O vacancy. They conclude that there is not likely a unique Si-HA material but different types depending on the details of the preparation conditions. The same is probably true of HA itself for the availability of water during the manufacture will affect the water content of the material and the fraction of OH sites occupied [4,47], and perhaps also of Si-\(\alpha\)-TCP.

The atomic arrangements at the surfaces of these biomaterials is of interest because the interface between the solid material and the biological medium is where cell and protein action takes place leading to bone resorption and deposition. The \(ab\) initio methods can be used to simulate the material–vacuum interface, and studies of HA and \(\alpha\)-TCP surfaces have appeared [51,54]. If a bulk crystal is cleaved to produce a free surface, the atoms near the surfaces respond by relaxing their positions. The surface reconstruction of the CaPs that have been studied tends to neutralize charged surfaces, but the phosphate groups at the surface have under-coordinated oxygens, which lead to fairly reactive surfaces. HA surfaces have been studied most extensively [54] and there are several distinct high-index surfaces depending on the type of cut made. The surface energies can be estimated and their relative stability predicted. However, it is not so much the solid–vacuum interface that is of biological interest but rather the interface of the solid with a biological medium rich in water. Simulation of such a complex system is beyond the capability of the methods at present, but a first step has been taken by investigating the interaction of one or two H\(_2\)O molecules with the surface [54]. There is a strong interaction of H\(_2\)O with all the HA surfaces considered because of the exposed undercoordinated O atoms, and dissociation takes place in most cases. The HA (0 1 0)-type surfaces were the most reactive, and (0 0 1) the least, which may be related to the morphology of HA crystallites in aqueous media, and especially the nature of HA platelets found in natural bone. Ca loss from the surfaces in exchange for two H from the medium was also found to be very favorable energetically, which may have interesting consequences on the bioactivity of the materials [54].
The ab initio simulation methods are rather new themselves and their application to biomaterials is just beginning. However, the studies reported so far suggest that the methods form a useful tool in material science when used in conjunction with experiment, and when well-chosen questions regarding the atomic scale structure are posed.

5. Comparative biological activity of Si substituted CaP bioceramics

Given the significant roles of Si in the enhancement of bone growth, it is not surprising that bioceramics that incorporate Si into their composition realize higher bioactivity. These include materials with very high Si levels such as Bioglass (Na–Ca–P–Si glasses of variable composition) [55] and Pseudowollastonite (CaSiO₃) [25,56] as well as CaP-based materials with trace levels of Si doping such as Si-HA and Si-TCP [57,58].

The superior biological performance of Si-HA and Si-TCP implant materials has been well documented [3,26,57–62]. An in vivo study comparing the biological activity of Si-HA and HA granules reports a 14.5% increase in bone in-growth in Si-HA versus HA controls [61]. This study showed that the morphology of apatite deposits and the sequence of events at the interfaces of Si-HA and HA implants and bone were different [61]. The formation of fibrils of organized collagen was observed at the bone/Si-HA interface after 6 weeks, while for undoped HA these structures were observed after 12 weeks, suggesting a role for Si in the remodeling process [61]. A 12-week, small animal model study by Hing et al. [57] showed that levels of bone apposition, in-growth and adaptive remodeling were remarkably affected by the Si content of the Si-HA, with the 0.8 wt% Si showing the optimal response for both bone forming and bone resorbing cells. Mastrogiacoma et al. [58] have investigated the performance of Si-TCP-based implant scaffolds in a long term, large animal in an in vivo study. Mastrogiacoma et al. [58] found that only 10–20% of the original Si-TCP-based scaffold remained after 1 year, and that the scaffolds were essentially completely resorbed after 2 years, and were replaced by newly formed highly mineralized laminar bone tissue, as shown in Fig. 1. In contrast, a stoichiometric HA scaffold with a similar porosity remained nearly completely intact after 5 years [58].

In vitro studies help to understand specific material cell interactions on Si-HA and Si-TCP. Both Si-TCP and Si-HA materials have been shown to support the development of osteoclasts from mononuclear precursors and exhibit resorption of the material by osteoclast cells [26,60,62]. Si-TCP and Si-HA materials also show increased osteogenesis of osteoblast-like cells with corresponding increased new matrix formation in vitro [59,62].

The safety of the presence of Si in an implanted bone biomaterial has been confirmed by Lai et al. [63] who measure the secretion of Si from a resorbable Si containing glass by tracing Si in the blood, and urine during 24 h periods for 7 months after implantation. Si was found to leave the body primarily through the urine at an average rate of 1.8 mg/day [63]. Bone, kidney, liver, lung, lymph nodes and spleen were analyzed for Si and found to have no increase aside from the bone surrounding the implant side, concluding that the Si from the resorbable implant is harmlessly secreted through the urine [63].

6. Influence of Si in the biological response to an implant

When a biomaterial is implanted into a biological system, dynamic reactions occur at the material–tissue interface that have been shown to determine the degree and conformation of specific proteins which influence recruitment and activation of cells and the stimulation of new tissue development [55,64,65]. Precipitation of a biologically equivalent carbonated HA (biomimetic precipitation) at the surface of an implant has been consistently associated with the bioactivity [60] of a wide variety of implant types [55,64,67–71]. The increase in bioactivity of a material on which a biomimetic precipitation occurs has been associated with increased adsorption and incorporation of biological moieties that are thought to serve as attachment sites for cells [64]. Biomimetic precipitation is associated with better performance under in vivo conditions [72]. Materials that promote biomimetic precipitation have been shown to preferentially adsorb fibronectin RGD protein, and in addition to enhance the attachment function of fibronectin to the conditioned surface, leading to enhanced osteoblast attachment, proliferation and differentiation [73]. It is the initial reaction of the material with the liquid media, occurring within 2–48 h of incubation wherein protein adsorption occurs to recruit cells that further prepare and condition the surface [55,64,65]. As an example of the association between biomimetic precipitation and biological activity, a chemical surface treatment of Si to generate Si-OH [68] or the application of a negative potential to a Si wafer [67], will induce the precipitation of an apatite-like phase in physiological media, creating a material that osteoblasts will mineralize [67,68]. The limited bioactivity of stoichiometric HA ceramics is in fact attributed to a stability in media that does not encourage the surface conditioning process of biomimetic precipitation [64].

The ability of a CaP material to induce a biomimetic precipitation has been correlated to phases with more soluble surfaces such as β-TCP [74], however, the chemistry of the surface also influences biomimetic precipitation and a base CaP precursor phase has been shown to not be a prerequisite [70]. In fact, biomimetic precipitation occurs on a variety of materials such as Bioglass [75], sol–gel processed silica and titania [69], and polymers such as polyethylene oxide [71]. Biomimetic precipitation is encouraged by factors such as more soluble CaP-based materials [64,76], the development of a negative surface charge associated with the disassociation of chemical
groups such as Ti-OH or Si-OH in media [67,68,70], the specific chemistry of the surface [77], and a nano-crystalline microstructure [78].

Biomimetic precipitation has been observed at the surface of Si-HA and Si-z-TCP materials [26,76,79]. Full conversion to a biologically equivalent HA is observed in some Si-z-TCP materials incubated in simulated body fluid electrolyte [79]. The surface of Si-z-TCP films shows increased OPN protein affinity for the surface corresponding with visualization of a precipitation reaction at the surface [80].

It appears likely that Si promotes biomimetic precipitation on Si-z-TCP and Si-HA by a combination of increasing the solubility of the materials via creation of defects in the lattice [34,76,81,82], by generating a more electronegative surface [83] and by generating a smaller grain size with more triple point junctions per unit area, facilitating increased dissolution at the surface [76]. Atomic force microscopy studies of Si-HA and HA materials indicate that Si-HA surfaces using COO\(^{-}\)-terminated tips have shown larger van der Waals force components, more electronegative surfaces and increased surface adhesion on Si-HA materials [83]. AFM studies with RGD protein functionalized tips on Si-TCP materials show higher adhesion for Si-TCP surfaces that undergo a rapid biomimetic precipitation, and these samples show an associated higher biological activity [80]. This study also shows more electronegative surfaces with higher Si additions to the system, however these are not necessarily indicative of a better biological response [80]. The substitution of SiO\(_4^{2-}\) for PO\(_4^{3-}\) is believed to facilitate the generation of a more negative surface for Si containing materials [80,83].

It is well recognized that numerous biological components such as proteins, glycoproteins and polysaccharides, have a significant influence on the initiation or inhibition of nucleation and growth and remarkably effect the morphology of CaP precipitates under \textit{in vitro} and \textit{in vivo} conditions [84–87]. Comparisons between HA and Si-HA under \textit{in vitro} and \textit{in vivo} conditions indicate that while the amount of dissolution under the two environments does not differ, the mechanism of dissolution and the morphology of the crystallites forming on HA and Si-HA are different [88]. Under \textit{in vitro} conditions nodular apatite deposits are formed, while under \textit{in vivo} conditions, plate-like crystals grow [88], suggesting an influence from proteins in the \textit{in vivo} environment. Si-z-TCP shows remarkable sensitivity to different aqueous environments such as simulated body fluid (SBF) electrolyte, SBF containing serum proteins, and full \textit{in vitro} cell culture, which contains SBF electrolyte, serum proteins and cells which produce specific proteins. Fig. 2 shows Si-z-TCP, HA and Si-HA materials incubated under various conditions. Si-z-TCP shows little reactivity in a SBF-type environment that is cell and serum free (Fig. 2C), while a progressively more rapid transformation to a
carbonated-HA like phase occurs with incubation in SBF containing serum proteins (Fig. 2B), and ultimately in the presence of osteoblast-like cells in an in vitro culture a complete transformation of Si-z-TCP to plate-like HA occurs, Fig. 2A and D. In comparison to Si-z-TCP, HA and even Si-HA studied under identical conditions show very limited reactivity, Fig. 2E.

The presence of Si at the surface of a material may also enhance osteogenesis through a direct chemical mechanism. Alumina, a biologically inert ceramic, was doped with Si to investigate the effects on bone in-growth, osteogenesis, cell differentiation and remodeling, in comparison to an undoped control [89]. For low doping (0.5 mol%) osseous remodeling of the implant was observed, supporting a primary chemical role of Si in resorption. In samples with increased doping to 5 mol%, tissues appeared more differentiated [89].

The release of aqueous Si complexes to the extracellular media is an additional factor that may promote biological activity to Si-containing implants. Fig. 3 demonstrates the unique, rapid release of Si from multi-phase Si-z-TCP bioceramics compared to HA and β-TCP materials with 24–72 h of incubation in an electrolytic fluid mimicking the ionic composition of human serum (Earl’s buffered salt solution, Sigma-Aldrich). As discussed previously, aqueous Si has dose-dependent effects on osteoblast differentiation, proliferation and collagen synthesis, osteoclast formation and the resorption process, as well as implications for the formation of the extracellular matrix and the biomineralization process. The release of Si complexes in the form of Si(OH)₄ from the implant may therefore stimulate tissue regeneration and effect the remodeling process.

Currently, two different Si-substituted CaPs have been used in bone substitute applications. Single-phase Si-HA materials are manufactured commercially by Apatech Ltd. [90] under the tradename Actifuse™. Multiphase Si-stabilized CaPs, composed primarily of Si-z-TCP, were manufactured commercially by Millenium Biologix...
Corporation [91] under the trade name Skelite™. Actifuse™ and Skelite™ are both prepared in microporous scaffold and/or granule formats intended for filling bone defects in non-load-bearing applications.

7. Conclusions

It is clear that Si plays important and significant roles in the bone and cartilage systems, acting on the physiological system most prominently during the growth and development of the skeletal system of higher organisms. Si has also been shown to influence cartilage synthesis and the integrity of the extracellular matrix. Direct effects of Si on the biomineralization process are also observed. Si has also shown to have effects on the differentiation, proliferation and collagen synthesis of osteoblasts, as well as dose-dependent effects on the remodeling process and osteoclast development and resorption activities.

Si-substituted CaP materials have increased biological activity which can be attributed to a number of different factors which presumably act synergistically. Si substitution in CaP facilitating precipitation of a biologically equivalent HA to the implant surface, as well as to direct effects of Si released to the extracellular media or present at the material surface. Si promotes biomimetic precipitation by increasing the solubility of the material through the creation of crystalline defects with substitution for PO₄³⁻ and associated charge compensation mechanism, by generating a more electronegative surface with the exchange of SiO₄⁴⁻ for PO₄³⁻ and by creating a nano-crystalline material.

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